

Running head: Indirect evolutionary responses to climate change

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**Climate change triggers morphological and life-history evolution in response to predators**

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## Abstract

Although climate change is expected to reorganize entire communities, this restructuring might reflect either direct ecological or evolutionary responses to abiotic conditions or indirect effects mediated through altered species interactions. We tested the hypothesis that changes in trophic interaction strength due to altered predator abundance have a cascading evolutionary response in a prey species (*Daphnia pulex*). Using a multiyear / multigenerational field experiment, we manipulated 12 open aquatic mesocosms to simulate hydrological conditions under climate change. After a three-year press manipulation, we collected *Daphnia pulex* from each pond and raised them in a common garden. Using quantitative genetic methods, we measured a series of quantitative traits every other day on 108 individuals for eight weeks. There was a significant decrease in tail spine length and population growth rate in groups exposed to the most extreme future climate scenarios. Structural equation models demonstrated that trait changes were best explained as an indirect effect of climate change treatments mediated through changes in predator abundance. Our results suggest climate change can trigger a cascade of ecological and evolutionary forces by reducing predator density, which in turn acts as a selective force leading to evolutionary change in prey morphology and life history.

*Keywords:* Climate change, evolution, predation pressure, trophic structure, *Daphnia pulex*

## 50 **Introduction**

51 Global climate change is expected to lead to major shifts in the composition of communities and  
52 the structure of populations (Tylianakis et al. 2008, Kratina et al. 2012). These shifts often occur  
53 because of individualistic species responses to direct abiotic factors such as temperature (Menge  
54 et al. 2008) or precipitation (Hart and Gotelli 2011). The resulting assemblage may exhibit  
55 altered trophic structure (Urban et al. 2012b) which could trigger additional change. What  
56 remains unclear is how the direct responses of one species to climate change lead to indirect  
57 effects on other community members, and whether these effects are strictly ecological or have an  
58 evolutionary basis. Complex community-level shifts in response to climate change are  
59 themselves potential selective agents (Harmon et al. 2009) and could trigger evolutionary  
60 responses in a focal species.

61

62 Direct responses to abiotic factors associated with climate change are important (Lavergne et al.  
63 2010, Hoffmann and Sgro 2011), and there are well-documented examples of direct evolutionary  
64 responses of populations to elevated CO<sub>2</sub> (Collins and Bell 2004), temperature (Jump et al.  
65 2008), and pH (Lohbeck et al. 2012). However, populations may have complex responses to  
66 climate change that are driven by direct changes in abiotic factors (e.g. temperature,  
67 precipitation) and by indirect changes in the density of predators or competitors (Harmon et al.  
68 2009, Urban et al. 2012a). For example laboratory populations of *Daphnia magna* evolved  
69 differently in response to increased temperature in isolated populations versus those that were  
70 exposed to an entire assemblage of competitors and predators (Van Doorslaer et al. 2010).

71

72 Although terrestrial communities have been the primary research focus, climate change will also  
73 have important impacts in aquatic systems (Kratina et al. 2012). Climate change is expected to  
74 lead not only to increases in air temperature, but also to changes in precipitation intensity and  
75 drought stress (Frumhoff et al. 2007). Freshwater food webs will be strongly affected by the  
76 resulting changes in hydrology, particularly in ephemeral habitats such as vernal ponds (Brooks  
77 2009). One key taxon in many aquatic food webs is *Daphnia pulex* (Branchiopoda: Cladocera) a  
78 filter-feeding crustacean that is abundant in many temporary and permanent aquatic habitats  
79 (Lynch 1980). In laboratory studies, *Daphnia* life history and morphology respond directly to  
80 changes in temperature (VanDoorslaer et al. 2010), as well as to the presence of predators,  
81 which can cause rapid evolution in growth and spine length (Spitze 1991, Fisk et al. 2007).

82  
83 Here we present the results of a three year field experiment in which simulated changes in the  
84 hydrology of vernal ponds reduce the abundance of aquatic predators, leading to evolutionary  
85 change over several generations in the morphology and life history of *Daphnia pulex*. Using a  
86 multi-generation field study and a common garden experiment, we show that increased  
87 evapotranspiration and drought stress, both predicted with climate change (Brooks 2009), reduce  
88 the abundance of invertebrate predators, which triggers rapid evolutionary shifts in the spine  
89 length and intrinsic rate of increase of *D. pulex*. Our results demonstrate that climate change can  
90 initiate a cascading response by reducing the abundance of predators therefore altering trophic  
91 interaction strength, which leads to evolutionary change in the morphology and life history of  
92 prey populations.

93

94 **Materials and Methods**

95 *Study organism and system*

96 *Daphnia pulex* (Branchiopoda: Cladocera) is a filter-feeding zooplankter that consumes bacteria  
97 and phytoplankton and is common in a wide variety of temporary and permanent aquatic  
98 habitats. *D. pulex* individuals usually survive for 16 instars (Lynch 1980) and exhibit a sigmoid  
99 growth curve for body size, with rapid growth to first reproduction between the fourth and sixth  
100 instars, and then slower growth in later instars as body size approaches an asymptote (Green  
101 1956, Lynch 1980). In our system, *D. pulex* is obligately parthenogenetic (Hebert and Finston  
102 2001), with mature diploid females clonally producing daughters every instar (Green 1956).  
103 Female body size, clutch size, and neonate size are all correlated within isofemale lines, but there  
104 is substantial variation in traits among clones (Green 1956). Because *D. pulex* undergoes  
105 frequent cycles of clonal reproduction, differential survival of clonal lineages can lead to highly  
106 adapted local populations (Haag et al. 2006).

107

108 *Effects of climate change on vernal ponds:*

109 Vernal ponds are fishless habitats that fill in the spring (vernal) or fall (autumnal) and hold water  
110 for at least 4 months, but are seasonally dry. Regional climate change models predict a warming  
111 of 3.5° to 6.5° F by 2100 under low-emission scenarios and an even larger increase under high-  
112 emission scenarios (Frumhoff et al. 2007). Climate change scenarios for the northeastern United  
113 States also predict an increased water budget in the winter and spring and increased deficit in the  
114 summer and fall (Frumhoff et al. 2007). Precipitation events are likely to become more variable,  
115 with longer periods of drought followed by more intense deluges (Hayhoe et al. 2008). Two  
116 effects of climate change will alter the hydroperiod of vernal ponds: increased  
117 evapotranspiration, and more variable precipitation. More variable spring and summer rainfall

118 combined with greater evapotranspiration will increase the drying frequency and the variance of  
119 water level (Brooks 2009).

120

### 121 *Experimental design*

122 In May of 2008, we used a small excavator to dig 81 ponds within a 500 × 500 m area at the  
123 University of Vermont Jericho Research Forest (44.45° N, 73.00° W). Ponds were dispersed and  
124 constructed along a network of logging roads to facilitate access and reduce spatial  
125 autocorrelation. We cleared ponds of roots and rocks and smoothed them into shape using hand  
126 tools until they were approximately 1.5 m in diameter and 60 cm deep. We lined each pond with  
127 3 × 3 m sheets of 0.254 mm thick black plastic, covered the bottom of the ponds with a layer of  
128 dirt and leaf litter, and filled them with water from an on-site spring-fed well. The final depth of  
129 the full ponds was approximately 45 cm, with a starting volume of approximately 5000 L. We  
130 inoculated each pond on 16 June 2008 with 1L of water from a mixture of 81 plankton tows and  
131 243 dip-net samples collected from a large local vernal pond. The average number of *Daphnia*  
132 *pulex* that was introduced into each pond was ~150 individuals.

133

134 We simultaneously varied the rate at which a pond lost water (water loss rate), and the frequency  
135 and intensity of rainfall events (drought severity) to experimentally generate a range of  
136 hydrology profiles that represented current to future climates. Our experiment had nine levels  
137 each of water loss rate and drought severity applied to 81 artificial ponds in a fully-crossed  
138 response-surface design. Our treatments mimicked two aspects of projected climate change:  
139 increased evapotranspiration (Yu et al. 2002) and increased variability in precipitation (Hayhoe

140 et al. 2008). We simulated a model of linear rate of water loss, allowing ponds to hold water  
141 from between 50 and 180 days, to mimic changes in evapotranspiration (Yu et al. 2002). Each  
142 treatment level represented an increase in pond hydroperiod of 15 days. This factor combined  
143 evaporation from increased temperature (Hayhoe et al. 2008) and increased water usage by trees  
144 (transpiration; Bonan 2008). The precipitation treatment was a single parameter with two levels  
145 tied together: as rainfall probability decreased, rainfall intensity increased. We estimated rainfall  
146 probabilities from the past 51 years of rainfall data (April to September from 1957 to 2007)  
147 recorded at Burlington International Airport (44.47° N, -73.15°). The mean daily probability of  
148 rainfall over those 6 months was 0.39, with a minimum of 0.18 and a maximum of 0.53. We  
149 created nine treatment levels that ranged evenly between 0.4 (the 51-year mean) and 0.03 (high  
150 potential for an extreme drought). Levels 1 - 5 were within current norms, and the remaining 4  
151 levels represented extreme drought frequencies that Vermont has not yet experienced. Tied to  
152 each rainfall probability was a probability of an intense rainfall event that ranged from 0.01 to  
153 0.1. An intense rainfall event was defined as any event in the 95th percentile of all rainfall events  
154 since 1957. Using the two parameters of water loss rate and drought severity, we created a  
155 simple model of vernal pond hydrology:

$$v_t = v_{t-1} + (rain_t - et_t)$$

156 The pond volume  $v$  at time  $t$  is the volume at the previous time step plus rainfall ( $rain_t$ ,  
157 determined by drought severity) minus evapotranspiration ( $et_t$ , determined by the water loss  
158 rate). We calculated the initial volume of each pond as one half of an ovoid sphere with a short  
159 radius of 42 cm and a long radius of 150 cm. The model was used to calculate the daily water  
160 balance by first randomly determining whether it would rain that day with the probability  $p_i$  of  
161 rainfall in drought severity level  $i$ . If the algorithm specified rain, the rainfall event was

162 characterized as an intense storm event with probability  $s_i$ . Depending on the outcome, the model  
163 drew randomly from a gamma distribution fit to the past 51 years of data for whichever month  
164 the simulation was in (Apr – Sept). If an extreme rainfall event was selected in the model, then  
165 the amount was specified by a random draw from the distribution of previous extreme events.  
166 Thus, intense rainfall events were based on a statistical distribution, but we simulated future  
167 climates by making intense rainfall events more frequent.

### 168 *Common garden design*

169 After three years of treatment application, we sampled the most extreme combinations of the  
170 experimental parameter space to create a  $2 \times 2$  factorial ANOVA design. Although the ponds  
171 were not identical replicates, the hydrological profiles within the 4 clusters were very similar to  
172 one another. We collected 20 individual *Daphnia pulex* from each pond in late August 2010 from  
173 dip-net sweeps placed in a collection tray and live collected with an eye dropper. Field collected  
174 individuals were isolated in 250 ml glass jars. We established 240 isofemale lines, raised in  
175 filtered water that we changed every 5 days. All isofemales were raised in a Percival growth  
176 chamber on a 14:10 day:night cycle and a 23:18 day:night temperature regime (Spitze 1993).

177

178 In order to minimize maternal effects (Bernardo 1996), we raised lines over several generations  
179 in the growth chamber before beginning the life history and growth measurements on January 1<sup>st</sup>  
180 2011. Using a spectrophotometer, we diluted a stock of live *Nannochloropsis* (green algae,  
181 Carolina Biological Supply) food solution to 4 mg C/L and fed the isofemale lines every other  
182 day; this feeding regime ensured there was no reduction in fecundity due to food limitation  
183 (Lampert 1978). We randomly selected 3 isofemale lines per pond from the available 20 and  
184 isolated three offspring per isofemale line from their first clutch (3 clones / isofemale  $\times$  3



185 isofemales/ pond  $\times$  3 ponds / treatment  $\times$  2 levels of drying rate  $\times$  2 levels of rainfall = 108  
186 replicates) and photographed individuals every other day to measure morphological characters.  
187 We measured clutch size by counting live-born offspring in each jar, after which we removed  
188 them. We recorded all life history data on these individuals. Because all individuals were raised  
189 through multiple generations in a common garden, differences in average measured traits should  
190 reflect genetic differences among populations, not maternal (Bernardo 1996) or early-  
191 environment effects (Spitze 1991, Conner and Hartl 2004, Hansen et al. 2012). The common  
192 garden experiment ran from 1 January to 14 February 2011, and we measured a total of 251  
193 individuals. If an individual did not survive to produce 3 clutches, we started a new clone from  
194 the stock population. Mortality and clutch size measurements were used in a life-table analysis to  
195 estimate  $r$ .

196

### 197 *Trait measurements*

198 We measured both morphological and life-history traits. Morphological traits were measured  
199 based on photos we took every other day of every individual. We used ImageJ software  
200 (Abràmoff et al. 2004) and a stage micrometer to measure three morphological traits at each time  
201 step based on photos. These traits were tail spine length (Havel and Dodson 1984, Ebert 1991),  
202 body size not including tail spine (Ebert et al. 1993), and head width (Havel and Dodson 1984).  
203 We also estimated three life history traits: clutch size (Spitze 1991) from counts of live born  
204 offspring, body size at first reproduction (Spitze 1991) from photos, and intrinsic population  
205 growth rate  $r$  (Dodson & Havel 1988) from a life-table analysis. To calculate  $r$ , we constructed a  
206 standard life table (Stearns 1992) for each population based on the reproduction and survivorship  
207 of individually raised clones. We estimated population-level level  $l_x$  (stage specific survivorship)

208 and  $m_x$  (stage specific fecundity). We then used the *optim()* function in R 2.10 (Team 2012) to  
209 solve the Euler-Lotka equation (Stearns 1992) for  $r$ :

210

$$1 = \sum_{x=0}^{x=w} e^{-rx} l_x m_x$$

### 211 *Covariate measurement*

212 We measured both biotic and abiotic covariates to test against *Daphnia* traits in a structural  
213 equation model (SEM). The abiotic variables measured weekly each summer were: pH,  
214 conductivity, dissolved O<sub>2</sub>, air temperature, water temperature, and light availability. We  
215 examined these variables to confirm that their distributions were stationary through time, and  
216 then calculated a single average for each pond across weekly samples over three years. We also  
217 collected soil cores from each pond to assess the potential effects of different allochthonous  
218 inputs of leaf litter. These cores were dried, sorted by tree species, and weighed. We used the  
219 cumulative weight for each tree species as a predictor variable. Finally, we measured the average  
220 predator density for all taxa that preyed on *D. pulex*. Predators were sampled with two cross  
221 sectional sweeps with a 10.2 × x 15.2 cm dip-net. We calculated the mean logarithm of predator  
222 abundance by summing the total number of predators on *D. pulex* from each sampling period and  
223 then taking the mean of the natural log of these abundances. These values were then averaged  
224 across each year to give mean log predator abundance for the duration of the study.

225

### 226 *Community composition*

227 Twenty-seven different genera of aquatic animals were observed in the ponds over the course of  
228 three years. These taxa were almost all arthropods except for two anuran species: the green frog

229 (*Rana clamitans*) and the wood frog (*Rana sylvatica*). The typical pond community consisted of  
230 four to ten different genera at any one sampling period. Three taxa of zooplankton other than  
231 *Daphnia pulex* were commonly observed: *Ceriodaphnia spp.*, Cyclopoid copepods, and  
232 Podocopid ostracods. Non-predatory taxa were either Diptera (larvae of mosquitoes, non-biting  
233 midges, or Dixid midges) or adult Coleoptera (family Hydrophilidae). The most common  
234 *Daphnia* predators were phantom midge larva (*Chaoborus spp.*), predaceous diving beetles  
235 (Dytiscidae: *Agabus spp* and *Accilius spp.*), and dragon fly nymphs (genus *Anax*), all of which  
236 are known to feed on *Daphnia pulex* (Kehl and Dettner 2003). We excluded water striders (genus  
237 *Gerris*), and Megaloptera larvae (Family Corydalidae, genus *Chauliodes*) from analysis as  
238 potential predators because we did not have evidence they specifically consume *Daphnia*.

239

#### 240 *Statistical analysis*

241 To test for direct effects of treatments on life history and morphology, we used a mixed model  
242 with nested random factors in R 2.14 (R Core Team 2012) with water loss rate and drought  
243 severity as fixed effects, and clone nested within isofemale line nested within pond as random  
244 effects (n = 108). Because the New England populations of *D. pulex* are obligately parthenogenic  
245 (Hebert and Finston 2001), we also used mixed models to partition phenotypic variance from the  
246 nested clonal design (Conner and Hartl 2004) to calculate broad-sense heritabilities. If  
247 experimental populations consisted of only one clone, there would be zero additive genetic  
248 variation and a heritability of zero. Population level traits such as *r* and traits such as clutch size  
249 and somatic growth rate for which sample sizes were not fully balanced due to mortality could  
250 not be analyzed with a mixed model. In these cases, we calculated pond level averages for each  
251 trait, treated each pond as replicate, and performed a two factor ANOVA (n = 12), with water

252 loss rate and drought severity as the two crossed treatments. We also tested all traits against all  
253 measured covariates using linear regression, and tested for significant relationships between  
254 covariates and treatments using ANOVA. When we detected significant relationships among  
255 traits, treatments and covariates, we used a structural equation model (SEM) to tease apart  
256 indirect and direct relationships (Shipley 2004). Because of small sample sizes and the need for a  
257 continuous predictor variable for SEM analysis, we created a single continuous predictor  
258 variable from the sequential measurements of pond depth from each census. We calculated a  
259 pond coefficient of variation (C.V.) based on the weekly measurements of pond depth ( $C.V. = \sigma /$   
260  $\mu$ , where  $\sigma$  and  $\mu$  were estimated from eq. 6 and 7 in Ives et al. 2003 for a univariate time series).

261

262 In a full factorial ANOVA, water loss rate and drought severity accounted for 99% of the  
263 variation in pond C.V. (44% attributable to water loss rate, 55% to drought severity, and the  
264 remainder to error and interactions). Thus, pond C.V. effectively captured the variation imposed  
265 by the two experimental treatments as a single continuous variable for fitting an SEM. The SEM  
266 analysis consisted of 18 different models, all specified and run in the software package lavaan  
267 (Rosseel 2012). We tested a null model that included only traits, and a model with just pond C.V.  
268 directly affecting the traits. The remaining 16 models included one of the 8 possible covariates in  
269 two variations. The first model variation included pond C.V. directly linked only to a covariate;  
270 therefore it could only indirectly affect the response variable. The second model included a direct  
271 link to both the covariate and the two response variables. Multiple model selection criteria (BIC,  
272 AIC and AICc) were used to determine the best-fitting model (Burnham and Anderson 2010).

273

## 274 **Results**

### 275 *Direct response of traits to experimental treatments*

276 Of the seven measured traits four showed at least one significant response based on ANOVA and  
277 mixed models: tail spine length at first instar (hereafter tail spine length), population growth rate  
278 ( $r$ ), body size at first reproduction, and average clutch size (Table 1). Tail spine length and  $r$  both  
279 responded significantly to water loss rate and drought severity treatments (Figure 1). Tail spine  
280 length and  $r$  were largest in treatments with low water loss rates and low drought intensity  
281 (simulation of current climate conditions). We quantified reductions in tail spine length and  $r$  as  
282  $1 - \bar{x}_{Future}/\bar{x}_{Current}$ , one minus the trait mean measured under future climate scenarios (high  
283 water loss rate, high drought severity) divided by the trait mean measured under current climate  
284 scenarios (low water loss rate, low drought severity). Tail spine length was 15% shorter in high  
285 water loss rate and high drought treatments (simulation of future climate conditions; Figure 1,  
286 Figure 2). Similarly,  $r$  was 18% lower in the high water loss rate and high drought treatments  
287 (Figure 1). We calculated broad-sense heritabilities for tail spine to ensure that ponds had  
288 existing additive genetic variance. Broad-sense heritabilities for tail spine length ranged from  
289 18.5% to 74.8% with a mean of 44.3%, which is comparable to other published estimates for the  
290 heritability of morphological characters in *Daphnia* (Ebert et al. 1993). Size at first reproduction  
291 was significantly lower in high drought severity treatments ( $\bar{x} = 1.26$  mm,  $n = 12$ , d.f. = 1,  $F = 6$ ,  
292  $p < 0.05$ ) compared to low drought severity treatments ( $\bar{x} = 1.38$  mm). The average clutch size  
293 was significantly larger in high water level treatments ( $\bar{x} = 11.3$  daughters / clutch,  $n = 12$ , d.f.  
294 = 1,  $F = 9.75$ ,  $p < 0.05$ ) compared to low water level treatments ( $\bar{x} = 9.5$  daughters / clutch). All  
295 other traits showed no significant response.

296

297 *Response of traits to measured covariates*

298 Both tail spine length ( $R^2 = 77\%$ ,  $p < 0.05$ ) and  $r$  ( $R^2 = 69\%$ ,  $p < 0.05$ ) were significantly  
299 correlated with mean predator abundance (Figure 3A). No other *Daphnia* traits were  
300 significantly correlated with predator abundance. No traits were significantly correlated with any  
301 of the other environmental covariates (Figure 4). The most parsimonious SEM model was one  
302 that included predators having a direct effect on traits, mediated through pond C.V. The second  
303 most parsimonious model included direct effects of pond C.V. on traits, but those direct effects  
304 were not significant (Figure 3B,  $\Delta\text{BIC} > 2$ ). The next closest models in BIC value included pH or  
305 did not include any covariate. These models all had substantially larger  $\Delta\text{BIC}$  values ( $\Delta\text{BIC} >$   
306 22), implying strong support for the best-fitting model that included direct effects of predators  
307 and an indirect effect of pond C.V. (Table 1).

308

309 **Discussion**

310 Although the importance of eco-evolutionary feedbacks is becoming increasingly recognized  
311 (Ellner et al. 2011, Urban et al. 2012a, Walsh et al. 2012), ecologists have often neglected rapid  
312 evolutionary responses and have mostly emphasized simple ecological responses of assemblages  
313 to abiotic conditions that are expected with future climate change. Here we have provided  
314 evidence from a multi-generation field experiment that abiotic climate change can restructure  
315 ecological communities via shifts in the abundance of predators, and that the new community  
316 structure is itself an evolutionary selective force. *Daphnia* populations in experimental ponds  
317 responded strongly to predator abundance (Figure 1, Figure 2), but not to measured changes in  
318 abiotic factors associated with simulated climate change (Figure 4))

319

320 Although some traits showed a clear selective response to predation (Figure 1), there was no  
321 significant response in somatic growth rate or body size (Table 1), which some other  
322 investigators have observed (Spitze 1991). The *Daphnia* in this experiment were all originally  
323 collected from the same vernal pond, and perhaps there was little additive genetic variance due to  
324 genetic isolation of this single source (Haag et al. 2006). However strong founder effects caused  
325 by the presence of only a few clones colonizing a pond (Allen et al. 2010) seem unlikely in this  
326 experiment because the ponds were seeded with ~150 individuals each, and because the system  
327 was always open to external colonization during the 3 years of the experiment. *Daphnia* can  
328 respond rapidly to selection (Hairston et al. 1999), which is consistent with the strong effects  
329 measured in response to experimental alterations of hydroperiod (Figures 1 and 2), and traits  
330 responded in the direction consistent with previous experimental studies (Spitze 1991) and with  
331 the predictions of life history theory (Taylor and Gabriel 1992).

332

333 *D. pulex* with longer tail spines are less vulnerable to predation (Havel and Dodson 1984,  
334 Dodson and Havel 1988), possibly due to increases in predator handling time or changes in prey  
335 buoyancy (Lüning 1992). Predator abundances are predicted to decrease as habitat variability  
336 increases because of habitat preferences in colonization and longer development times  
337 (Schneider 1997). In response to climate change manipulations, the evolved trait changes  
338 measured in *D. pulex* were comparable to those found in laboratory experiments on the response  
339 of *D. pulex* to predators (Spitze 1991, Lüning 1992). In those earlier studies, first-instar tail  
340 spines of *D. pulex* populations that were exposed to multiple predators in the laboratory  
341 increased in length by 12% (Spitze 1991). In some studies, increases in tail spine length are an  
342 induced, phenotypically plastic, response to predators (Lüning 1992). However, in this study,

343 *Daphnia* collected from the field were reared through multiple generations in laboratory  
344 conditions in the complete absence of predators or water-borne chemical cues associated with  
345 predators. Moreover, neck teeth in *Daphnia pulex* are the characteristic sign of a phenotypically  
346 plastic response to kairomones (Riessen 1999), but they were never observed in our laboratory-  
347 reared populations.

348  
349 Life-history theory predicts that, in the presence of predators that feed selectively on small-size  
350 classes (Spitze et al. 1991), prey populations should evolve delayed reproduction, increased  
351 investment in early somatic growth, and greater fecundity at later instars. These life-history shifts  
352 may lead to increases in  $r$  (Brett 1992, Taylor and Gabriel 1992). Population growth rates were  
353 greatest in ponds with the highest predator densities. Results of the common garden experiment  
354 were also consistent with other predictions of life-history theory and previous *Daphnia*  
355 laboratory studies: with decreasing predation pressure, body size at first clutch decreased, and  
356 clutch sizes of older age classes decreased (Spitze 1991, Brett 1992).

357  
358 Two lines of evidence suggest that the morphological and life history changes in *Daphnia*  
359 lineages from different ponds reflect evolutionary responses to predators, rather than  
360 evolutionary responses to altered abiotic conditions. First, none of the measured abiotic variables  
361 in each pond (pH, conductivity, dissolved O<sub>2</sub>, water temperature, and light availability) was  
362 correlated with *Daphnia* spine length and  $r$  (Figure 4). In contrast, average predator abundance  
363 (the 3-year average of weekly measurements of the logarithm of the abundance of all predatory  
364 taxa in a pond) was highly correlated and explained most of the variation among ponds in tail  
365 spine length (77%) and  $r$  (69%; Figure 3A). Second, the best-fitting SEM model included direct



366 effects of predators but did not include direct effects of the experimental treatments on the  
367 response variables (Figure 3B). This SEM model fit the data substantially better than an  
368 alternative model that included only treatment effects and no predator covariate ( $\Delta\text{BIC} = 33$ ) and  
369 better than a null model that included only correlations between the response variables tail spine  
370 length and  $r$  ( $\Delta\text{BIC} = 80$ ). Collectively, these analyses suggest that observed trait differences are  
371 genetically based evolutionary responses reflecting altered interactions with predators, rather  
372 than direct responses to altered abiotic conditions.

373

374 Collectively, our results suggest that climate change can trigger a cascading response in which  
375 both altered abiotic conditions and species interactions can affect populations through ecological  
376 and evolutionary pathways. The indirect effects of altered species interactions such as predation,  
377 parasitism, and competition may be just as important as the direct effects of altered temperature,  
378 precipitation, and concentrations of greenhouse gasses on the response of species and  
379 populations to global climate change.

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383

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520 **Table 1. F-ratios of ANOVA models for seven morphological and life history traits.**

<i>Factor</i>	<i>Spine Length</i>	<i>Body size</i>	<i>Head width</i>	<i>Clutch size</i>	<i>Growth rate</i>	<i>Size at first reproduction</i>	<i>r</i>
Water loss rate	<b>36.3</b>	0.51	0.04	<b>9.75</b>	0.26	0.94	<b>5.38</b>
Drought severity	<b>42.1</b>	0.003	0.13	2.53	0.18	<b>5.99</b>	<b>8.68</b>
WLR * DS	0.42	0.47	0.42	<b>7.52</b>	0.07	0.00	0.00

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523 Table 1. F-ratios from a two-way ANOVA with interactions for pond level averages of all  
524 measured traits; significant effects ( $p < 0.05$ ) are in bold, with 1 degree of freedom for each  
525 model term and 8 residual degrees of freedom.

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536 **Figure Legends**

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538 *Figure 1.* Evolutionary changes in morphology (tail spine length) and life history ( $r$ , the intrinsic  
539 rate of increase) of *Daphnia pulex* in response to experimental manipulations of pond hydrology  
540 representing different scenarios of climate change. Each solid point is the response of the  
541 population from a single pond in an orthogonal 2-factor field experiment in which water loss rate  
542 and drought severity were manipulated for 3 consecutive years. Open circles represent the grand  
543 mean of each treatment combination. The longest tail spines and highest  $r$  values were measured  
544 in ponds that mimic current climate (low water loss rate and low drought severity) and have the  
545 greatest number of predators.

546

547 *Figure 2.* Representative tail spine lengths of first instar *D. pulex* collected from four  
548 experimental climate change treatments, and reared for 3 clonal generations in common garden  
549 conditions. Tail spine length exhibited an additive response to the climate change treatments and  
550 is greatest in the low water-loss-rate, low drought-severity treatment combination.

551

552 *Figure 3.* A) Linear regression of average *D. pulex* tail spine length (mm) and intrinsic rate of  
553 increase ( $r$ ; individuals/individual•day) versus daily average log predator abundance ( $R^2 = 0.77$ ,  
554  $P = 0.0001$  for tail spine and  $R^2 = 0.69$ ,  $P = 0.0008$  for  $r$ ). B.) The best-supported SEM analysis  
555 model for evolutionary changes in tail spine length and intrinsic rate of increase ( $r$ ) of *Daphnia*  
556 *pulex* populations exposed to a 3-year climate change experiment. Solid arrows represent  
557 statistically significant SEMs; dashed arrows represent non-significant SEMs. The width of each

558 arrow is proportional to the standardized model coefficient (red arrows = negative effects, green  
559 arrows = positive effects). Pond C.V. is a continuous composite continuous variable based on the  
560 experimental treatments.

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562 *Figure 4.* Spine length and r plotted against other potential abiotic and biotic covariates. Each  
563 point represents the average for a single experimental pond (n = 12)/ Covariate measures are  
564 pond level averages calculated over the 3 years of treatment application. No significant  
565 relationship was found among any other variables, indicated by dashed lines. Open and closed  
566 circles indicate water loss rate, blue and red colors indicate drought severity.

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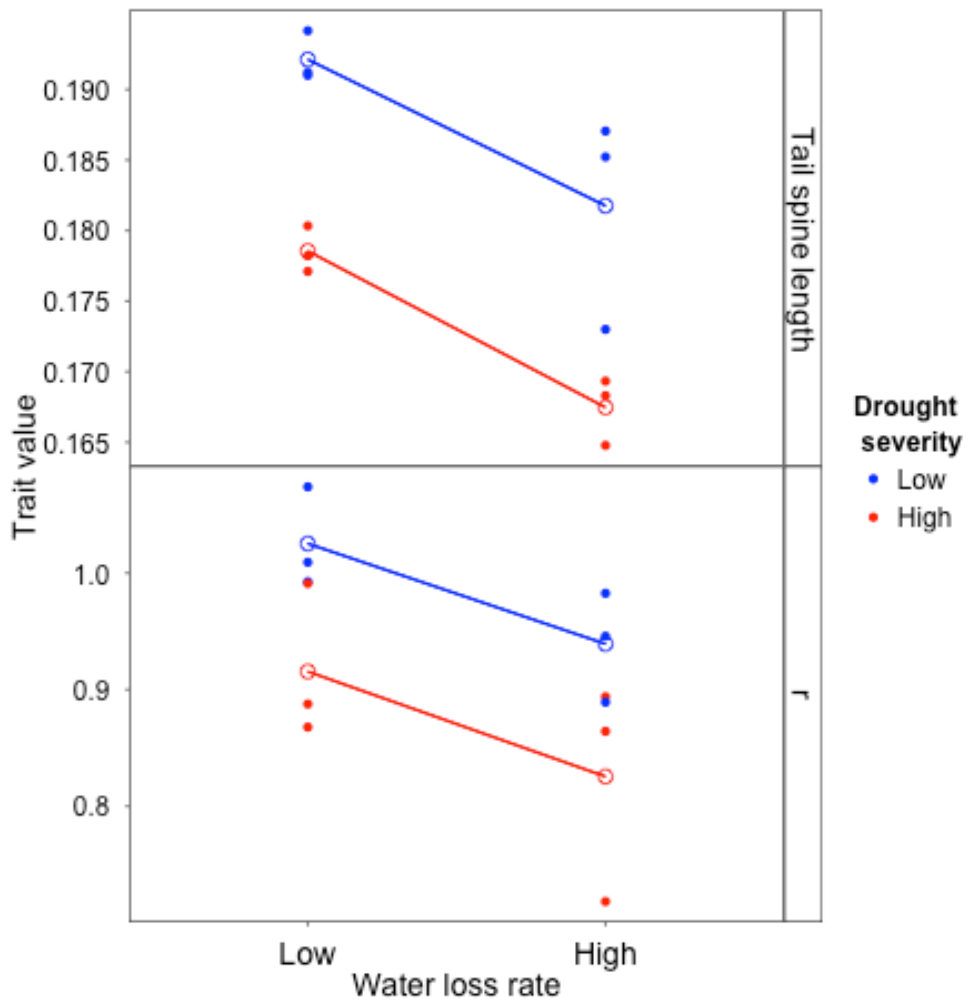
Figure 1

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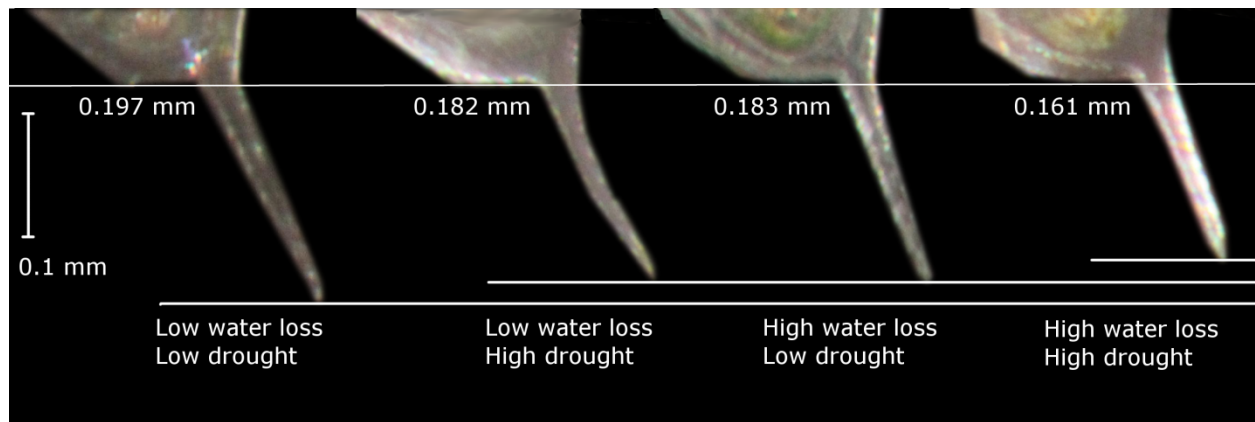
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**Figure 2**

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Figure 3

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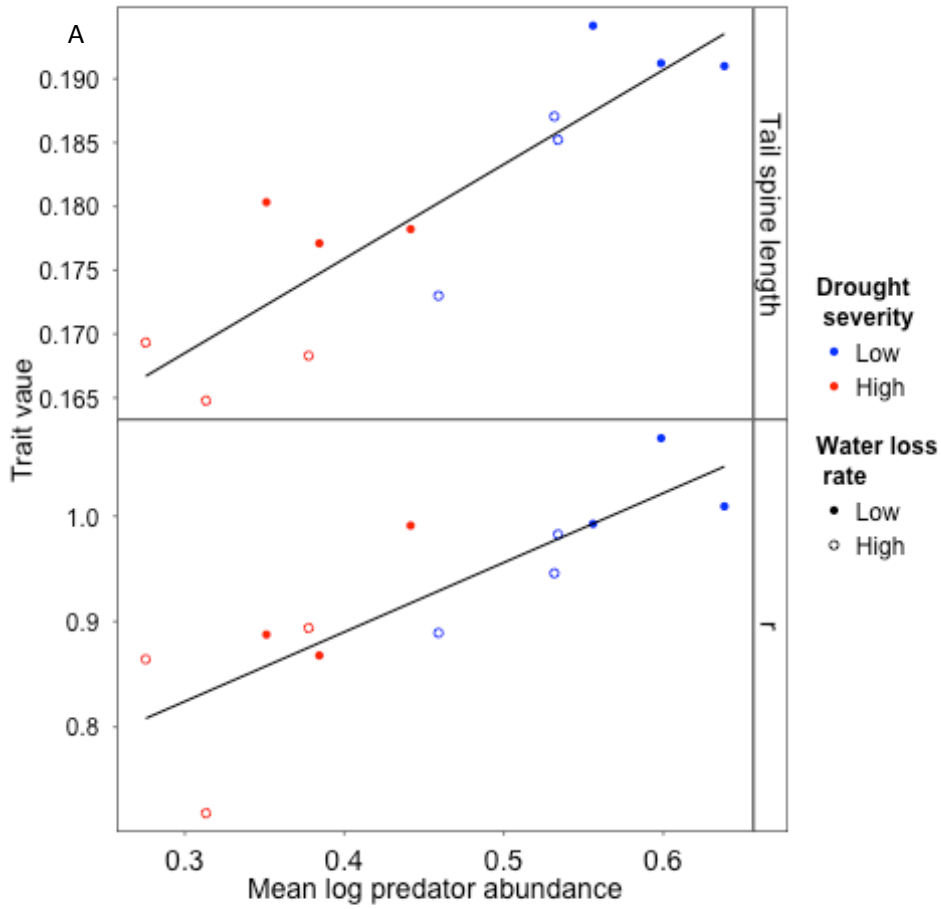
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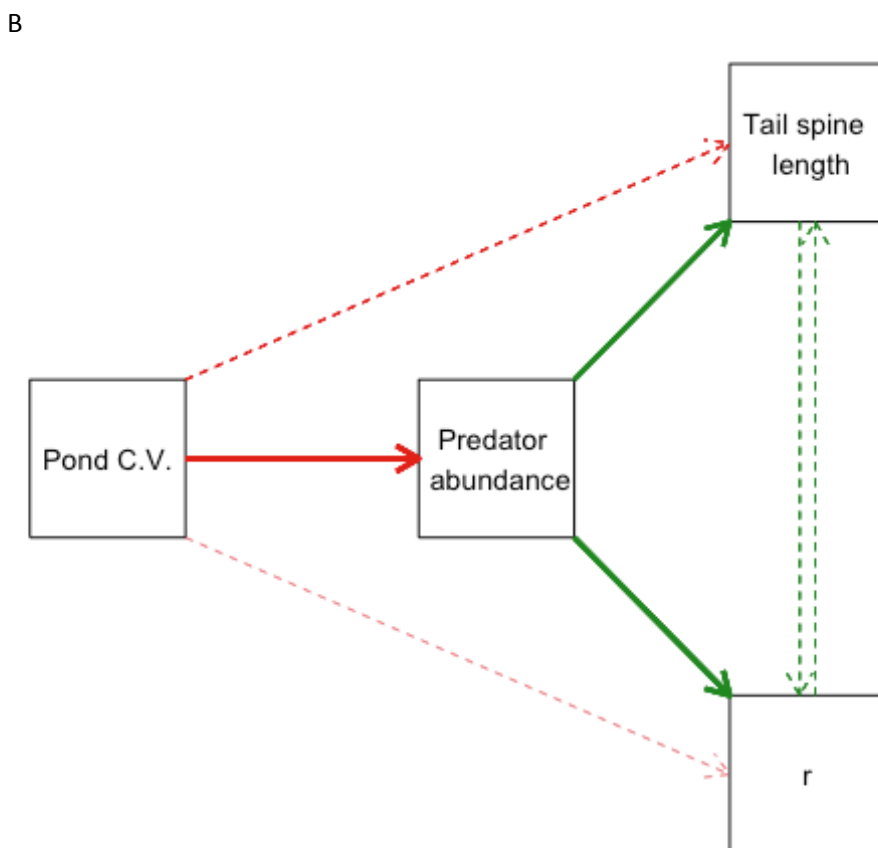
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Figure 4

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