

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 CO₂ (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 2×2 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 1st
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 O₂, 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 μ , where σ and μ 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 ΔBIC 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 O₂, 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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389 **Literature Cited**

- 390 Abràmoff, M. D., P. J. Magalhães, and S. J. Ram. 2004. Image processing with ImageJ.
391 Biophotonics international 11:36–42.
- 392 Allen, M. R., R. A. Thum, and C. E. Cáceres. 2010. Does local adaptation to resources explain
393 genetic differentiation among *Daphnia* populations? *Molecular Ecology* 19:3076–3087.
- 394 Bernardo, J. 1996. Maternal effects in animal ecology. *American Zoologist* 36:83.
- 395 Bonan, G. B. 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of
396 forests. *Science* 320:1444–1449.
- 397 Brett, M. 1992. *Chaoborus* and fish-mediated influences on *Daphnia longispina* population
398 structure, dynamics and life history strategies. *Oecologia* 89:69–77.
- 399 Brooks, R. T. 2009. Potential impacts of global climate change on the hydrology and ecology of
400 ephemeral freshwater systems of the forests of the northeastern United States. *Climatic*
401 *Change* 95:469–483.
- 402 Burnham, K. P., and D. Anderson. 2010. *Model Selection and Multi-Model Inference*, 3rd
403 edition. Springer-Verlag, New York.
- 404 Collins, S., and G. Bell. 2004. Phenotypic consequences of 1,000 generations of selection at
405 elevated CO₂ in a green alga. *Nature* 431:566.
- 406 Conner, J., and D. Hartl. 2004. *A primer of ecological genetics*. Sinauer Associates Incorporated,
407 Sunderland.

- 408 Dodson, S. I., and J. E. Havel. 1988. Indirect prey effects: some morphological and life history
409 responses of *Daphnia pulex* exposed to *Notonecta undulata*. Limnology and
410 Oceanography:1274–1285.
- 411 Ebert, D. 1991. The effect of size at birth, maturation threshold and genetic differences on the
412 life-history of *Daphnia magna*. Oecologia 86:243–250.
- 413 Ebert, D., L. Yampolsky, and S. C. Stearns. 1993. Genetics of life-history in *Daphnia magna* 1.
414 Heritabilities at 2 food levels. Heredity 70:335–343.
- 415 Ellner, S. P., M. A. Geber, and N. G. Hairston. 2011. Does rapid evolution matter? Measuring
416 the rate of contemporary evolution and its impacts on ecological dynamics. Ecology Letters
417 14:603–14.
- 418 Fisk, D. L., L. C. Latta, R. a Knapp, and M. E. Pfrender. 2007. Rapid evolution in response to
419 introduced predators I: rates and patterns of morphological and life-history trait divergence.
420 BMC Evolutionary Biology 7:22.
- 421 Frumhoff, P., J. McCarthy, J. Melillo, S. Moser, and D. Wuebbles. 2007. Climate Change in the
422 U.S. Northeast. Union of Concerned Scientists, Cambridge.
- 423 Green, J. 1956. Growth, size, and reproduction in *Daphnia* (Crustacea: Cladocera). Journal of
424 Zoology 126:173–204.
- 425 Haag, C. R., M. Riek, J. W. Hottinger, V. I. Pajunen, and D. Ebert. 2006. Founder events as
426 determinants of within-island and among-island genetic structure of *Daphnia*
427 metapopulations. Heredity 96:150–158.

- 428 Hairston, N. G., W. Lampert, C. E. Cáceres, C. L. Holtmeier, L. J. Weider, U. Gaedke, J. M.
429 Fischer, J. A. Fox, and D. M. Post. 1999. Rapid evolution revealed by dormant eggs. *Nature*
430 401:446–47.
- 431 Hansen, M. M., I. Olivieri, D. M. Waller, and E. E. Nielsen. 2012. Monitoring adaptive genetic
432 responses to environmental change. *Molecular Ecology* 21:1311–1329.
- 433 Harmon, J. P., N. A. Moran, and A. R. Ives. 2009. Species response to environmental change:
434 impacts of food web interactions and evolution. *Science* 323:1347–1350.
- 435 Hart, E. M., and N. J. Gotelli. 2011. The effects of climate change on density-dependent
436 population dynamics of aquatic invertebrates. *Oikos* 120:1227–1234.
- 437 Havel, J. E., and S. I. Dodson. 1984. *Chaoborus* predation on typical and spined morphs of
438 *Daphnia pulex*: behavioral observations. *Limnology and Oceanography* 29:487–494.
- 439 Hayhoe, K., C. Wake, B. Anderson, X.-Z. Liang, E. Maurer, J. Zhu, J. Bradbury, A. DeGaetano,
440 A. Stoner, and D. Wuebbles. 2008. Regional climate change projections for the Northeast,
441 USA. *Mitigation and Adaptation Strategies for Global Change* 13:425–436.
- 442 Hebert, P. D., and T. L. Finston. 2001. Macrogeographic patterns of breeding system diversity in
443 the *Daphnia pulex* group from the United States and Mexico. *Heredity* 87:153–61.
- 444 Hoffmann, A. A., and C. M. Sgro. 2011. Climate change and evolutionary adaptation. *Nature*
445 470:479–485.

- 446 Ives, A. R., B. Dennis, K. L. Cottingham, and S. R. Carpenter. 2003. Estimating community
447 stability and ecological interactions from time-series data. *Ecological Monographs* 73:301–
448 330.
- 449 Jump, A. S., J. Penuelas, L. Rico, E. Ramallo, M. Estiarte, J. A. Martinez-Izquierdo, and F.
450 Lloret. 2008. Simulated climate change provokes rapid genetic change in the Mediterranean
451 shrub *Fumana thymifolia*. *Global Change Biology* 14:637–643.
- 452 Kehl, S., and K. Dettner. 2003. Predation by pioneer water beetles (Coleoptera, Dytiscidae) from
453 sandpit ponds, based on crop-content analysis and laboratory experiments. *Archiv für*
454 *Hydrobiologie* 158:109–126.
- 455 Kratina, P., H. S. Greig, P. L. Thompson, T. S. Carvalho-Pereira, and J. B. Shurin. 2012.
456 Warming modifies trophic cascades and eutrophication in experimental freshwater
457 communities. *Ecology* 93:1421–30.
- 458 Lampert, W. 1978. A field study on the dependence of the fecundity of *Daphnia spec.* on food
459 concentration. *Oecologia* 36:363–369.
- 460 Lavergne, S., N. Moquet, O. Ronce, and W. Thuiller. 2010. Biodiversity and climate change:
461 Integrating evolutionary and ecological responses of species and communities. *Annual*
462 *Review of Ecology, Evolution, and Systematics* 41:321–350.
- 463 Lohbeck, K. T., U. Riebesell, and T. B. H. Reusch. 2012. Adaptive evolution of a key
464 phytoplankton species to ocean acidification. *Nature Geoscience* 5:346–351.

- 465 Lynch, M. 1980. The evolution of cladoceran life histories. *Quarterly Review of Biology* 55:23–
466 42.
- 467 Lüning, J. 1992. Phenotypic plasticity of *Daphnia pulex* in the presence of invertebrate predators:
468 morphological and life history responses. *Oecologia* 92:383–390.
- 469 Menge, B. A., F. Chan, and J. Lubchenco. 2008. Response of a rocky intertidal ecosystem
470 engineer and community dominant to climate change. *Ecology Letters* 11:151–162.
- 471 R Core Development Team. 2012. R: A language and environment for statistical computing. R
472 Foundation for Statistical Computing, Vienna, Austria.
- 473 Riessen, H. P. 1999. *Chaoborus* predation and delayed reproduction in *Daphnia*: a demographic
474 modeling approach. *Evolutionary Ecology* 13:339–363.
- 475 Rosseel, Y. 2012. lavaan: An R Package for Structural Equation Modeling. *Journal of Statistical*
476 *Software* 48:1–36.
- 477 Schneider, D. 1997. Predation and food web structure along a habitat duration gradient.
478 *Oecologia* 110:567–575.
- 479 Shipley, B. 2004. *Cause and Correlation in Biology A User’s Guide to Path Analysis , Structural*
480 *Equations and Causal Inference*. Cambridge University Press. . Cambridge University Press,
481 Cambridge.
- 482 Spitze, K. 1991. *Chaoborus* predation and life-history evolution in *Daphnia pulex*: temporal
483 pattern of population diversity, fitness, and mean life history. *Evolution* 45:82–92.

- 484 Spitze, K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic
485 variation. *Genetics* 135:367–374.
- 486 Spitze, K., J. Burnson, and M. Lynch. 1991. The covariance structure of life-history characters in
487 *Daphnia pulex*. *Evolution* 45:1081–1090.
- 488 Stearns, S. 1992. *The evolution of life histories*. Oxford University Press, New York.
- 489 Taylor, B. E., and W. Gabriel. 1992. To grow or not to grow: optimal resource allocation for
490 *Daphnia*. *American Naturalist* 139:248–266.
- 491 Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and
492 species interactions in terrestrial ecosystems. *Ecology Letters* 11:1351–1363.
- 493 Urban, M. C., L. De Meester, M. Vellend, R. Stoks, and J. Vanoverbeke. 2012a. A crucial step
494 toward realism: responses to climate change from an evolving metacommunity perspective.
495 *Evolutionary Applications* 5:154–167.
- 496 Urban, M. C., J. J. Tewksbury, and K. S. Sheldon. 2012b. On a collision course: competition and
497 dispersal differences create no-analogue communities and cause extinctions during climate
498 change. *Proceedings of the Royal Society B Biological Sciences* 279:2072–80.
- 499 VanDoorslaer, W., R. Stoks, I. Swillen, H. Feuchtmayr, D. Atkinson, B. Moss, and L. De
500 Meester. 2010. Experimental thermal microevolution in community-embedded *Daphnia*
501 populations. *Climate research* 43:81–89.

502 Walsh, M. R., J. P. DeLong, T. C. Hanley, and D. M. Post. 2012. A cascade of evolutionary
503 change alters consumer-resource dynamics and ecosystem function. *Proceedings of the*
504 *Royal Society B Biological Sciences* 279:3184–3192.

505 Yu, P. S., T. C. Yang, and C. C. Chou. 2002. Effects of climate change on evapotranspiration
506 from paddy fields in southern Taiwan. *Climatic change* 54:165–179.

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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	36.3	0.51	0.04	9.75	0.26	0.94	5.38
Drought severity	42.1	0.003	0.13	2.53	0.18	5.99	8.68
WLR * DS	0.42	0.47	0.42	7.52	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.

561

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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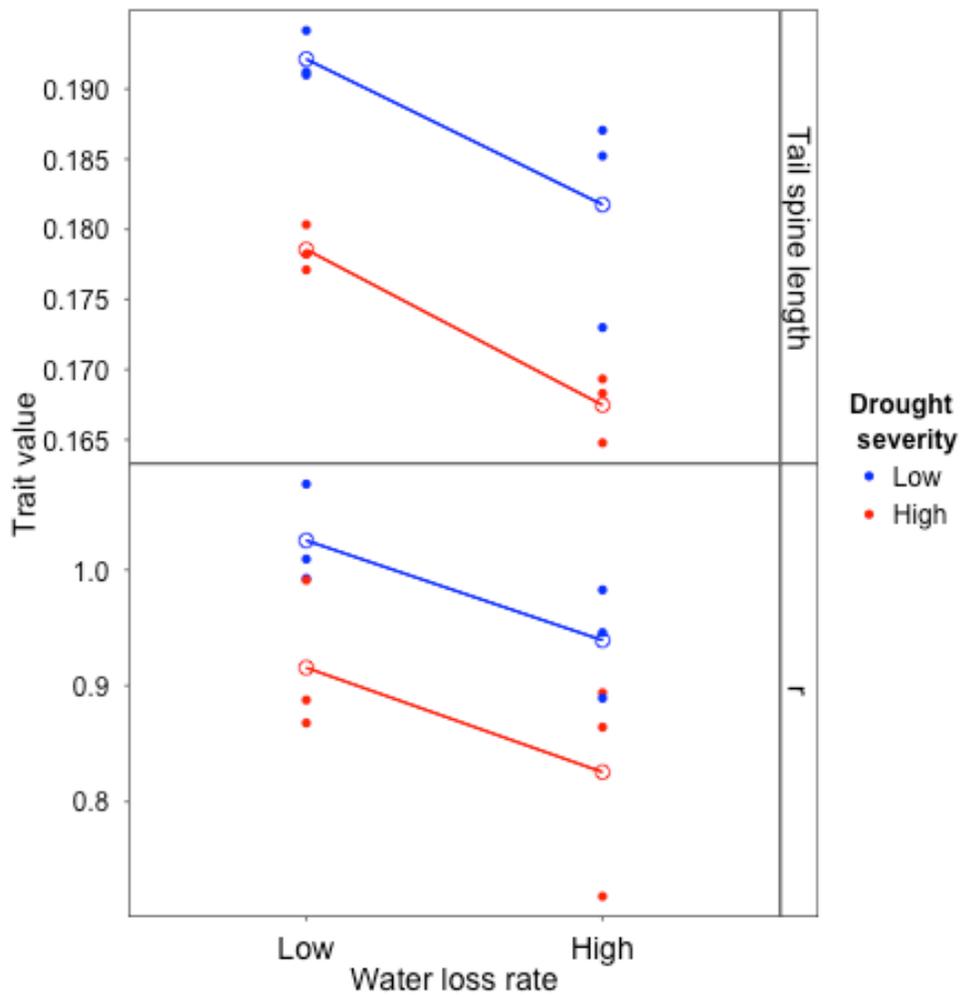
Figure 1

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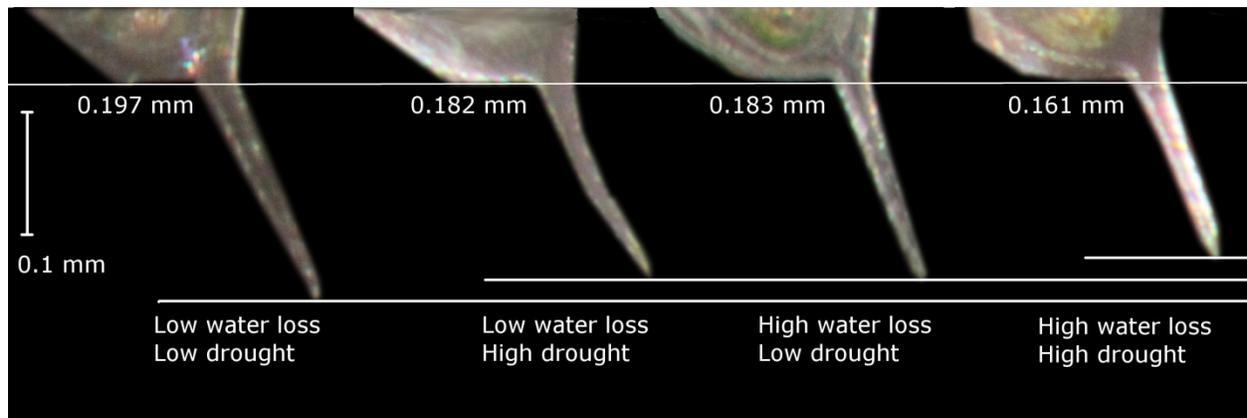
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Figure 2

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Figure 3

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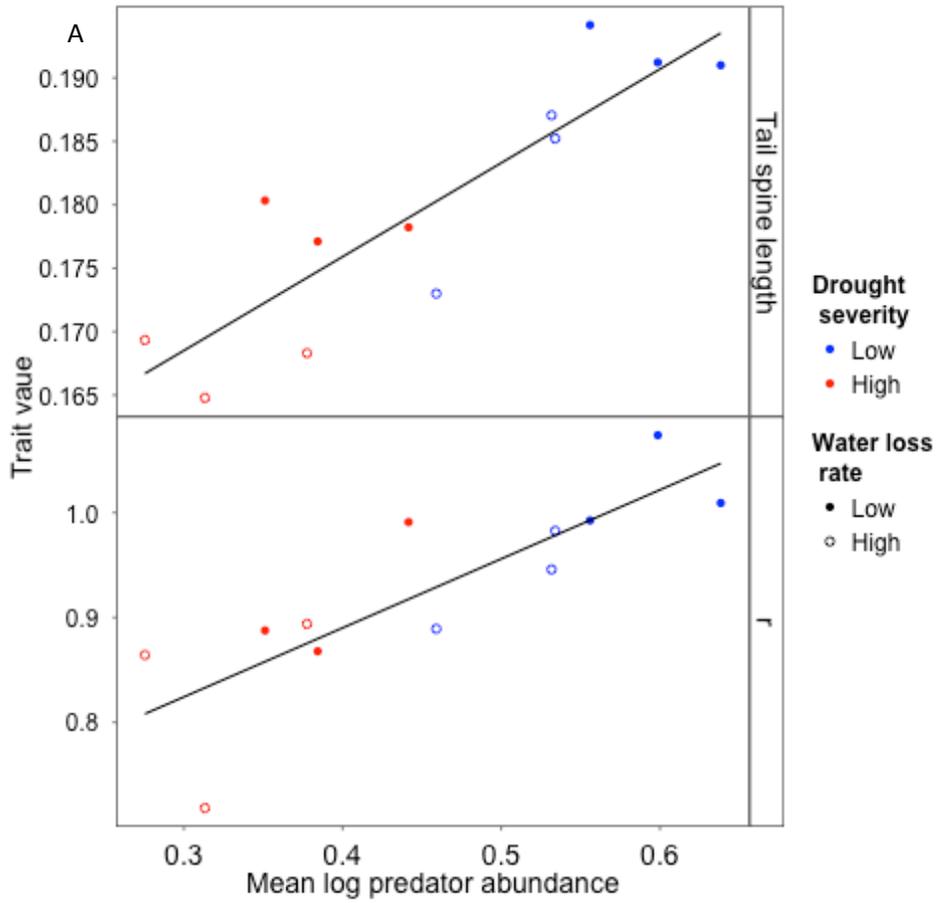
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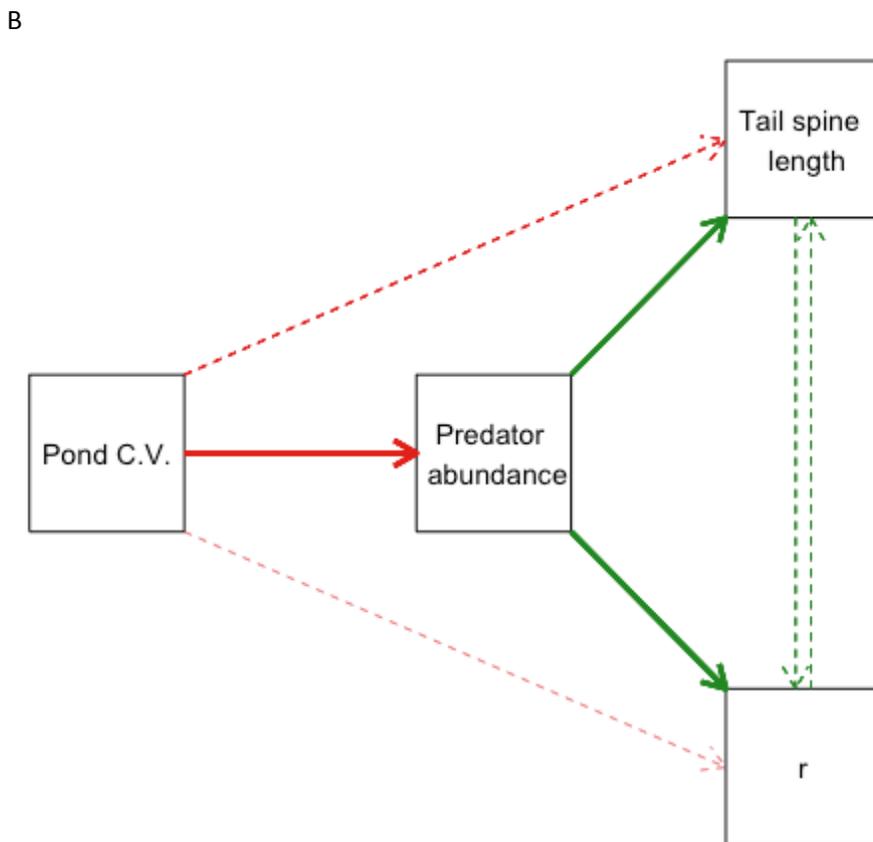
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Figure 4

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