Title: Thermal environment and ecological interactions modulate the importance of evolution in response to warming

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Abstract

Understanding the interaction between evolutionary history, the abiotic environment, and biotic interactions is critical for a more nuanced understanding of how communities respond to anthropogenic stressors. We leveraged a long term experiment manipulating temperature in mesocosms containing communities of phytoplankton and zooplankton to examine how evolution in response to long-term community warming could affect consumer-resource dynamics at different temperatures. We showed that both the current thermal environment, as well as the presence of interspecific interactions can affect the importance of prior evolution in response to warming, and that this evolution also influences the outcomes of current ecological dynamics. For each consumer–resource pair, the effects of evolution were temperature-dependent, but both the effects and the temperature dependence itself additionally depended upon the identity of evolving species in each pair. The evolution that we observed in one resource species, *Chlamydomonas reinhardtii*, appeared to confer to it a double advantage; it was more fit across all temperatures, while simultaneously reducing the success of the consumer, but with the key result that fitness gains were largest at intermediate temperatures when the consumer was present. Thus we found support for the "hotter is better" hypothesis for this resource species, but especially at moderate or intermediate temperatures, and that hotter was worse for the consumer. In the second species pair, patterns were more complex, but warm-origin populations of both the resource and the consumer generally failed to show improved fitness. Overall, our results show that evolution altered resource and consumer fitness, but that these effects were dependent the current combination of abiotic and biotic conditions.

Key words: consumer-resource interactions, eco-evolutionary dynamics, global climate warming, predator-prey interactions, temperature dependence, thermal adaptation

Introduction

Global climate change has emerged as a critical challenge to species and ecological communities (Parmesan 2006, Radchuk et al. 2019). Multiple mechanisms by which species and communities respond to increasing temperatures (e.g., range shifts, plastic changes to behavior, phenology, etc.) interact to determine the degree to which species and communities are affected by climate change (O'Connor et al. 2012, Gaitán-Espitia and Hobday 2021). Because temperature represents a strong selective agent for populations, evolution may constitute one important mechanism by which species respond to warming, especially for dispersal-limited populations or species with rapid generation times (O'Donnell et al. 2018). Evolution in response to warming has been experimentally documented in a variety of organisms, including for instance, green algae (Padfield et al. 2016, Schaum et al. 2017), arthropods (Gilchrist and Huey 1999, Geerts et al. 2015, Hangartner and Hoffmann 2016), and larger organisms, such as lizards (Logan et al. 2014). While less frequently studied, evolution in natural populations in response to warming has been observed as well (Higgins et al. 2014, Geerts et al. 2015, Johansson et al. 2016, Johansson and Laurila 2017, Diamond et al. 2017). Because evolution in response to abiotic factors, like climate warming, is frequently explored in single populations, the implications of evolution in communities responding to habitat warming remain understudied. Nonetheless, given that many of the species most likely to evolve in response to warming are critical for ecosystem functioning and services (*e.g.,* water quality, carbon sequestration)(O'Donnell et al. 2018), we can expect that evolutionary responses to warming will have important consequences for both ecological and human systems (Rudman et al. 2017).

In general, intuition suggests that when evolution improves thermal performance at warm temperatures, populations or species should be more successful in warmer environmental conditions (Stoks et al. 2014) relative to colder conditions, although multiple hypotheses exist for how warming

63 evolution may alter the populations' thermal response curves [e.g., "hotter is better", "biochemical
64 adaptation", and "partial compensation" (Liu et al. 2022)]. Evidence from natural populations, however,
65 demonstrates that the performance of populations that evolve in response to warming can be variable.
66 In some cases responses match theoretical expectations of shifts in thermal tolerance towards warmer
67 temperatures (Diamond et al. 2017), while in other cases they exhibit unexpected results, which can also
68 depend upon the life history parameter measured (Johansson et al. 2016). Such unexpected results may,
69 in part, be explained by life history tradeoffs that can emerge between coping with acute thermal stress
70 and chronic exposure (Rezende et al. 2014). For instance, evolution may favor increased survival under
71 chronic warm temperatures at the expense of critical thermal maximum temperature during acute
72 thermal stress (Johansson and Laurila 2017). Thus, both the type of thermal stress experienced by
73 populations (i.e., increasing mean temperature vs. frequent acute fluctuations), as well as current
74 thermal environment (e.g., temperature and duration of thermal experiments) may be important for
75 interpreting empirical patterns and understanding the implications of evolution that occurs in response
76 to warming. Physiological responses to temperature (e.g., changes to metabolism) provide an additional
77 clue to explain why variable responses may be observed in populations evolving to warming. In
78 particular, many traits exhibit unimodal responses to temperature, such that effects of increased
79 temperature can appear vastly different depending upon where in the temperature response curve one
80 examines the response. Thus an additional wrinkle is introduced by the choices of the experimenter.

81 The presence of additional interacting species may introduce further factors influencing
82 evolutionary outcomes. The effects of evolution can depend in important ways on the current ecological
83 conditions in populations experiencing warming. When species are embedded in complex communities,
84 the opportunities for additional costs, constraints, or benefits of evolution can emerge. For instance,
85 there can be trait tradeoffs or novel selective pressures present that arise out of additional ecological
86 (or evolutionary) changes that occur as communities respond to warming. Such trait tradeoffs can drive

differences in evolutionary responses compared to evolution in single populations (Van Doorslaer et al. 2009a, 2010). Selection that occurs within an ecological community is therefore likely to qualitatively change predictions about thermal adaptation derived from single populations (Angilletta Jr. et al. 2006), such that individual species' responses to temperature can be insufficient to explain changes to community composition (Tabi et al. 2020). For example, previous research has demonstrated that locally adapted populations of consumer and resource species from different latitudes have contrasting eco-evolutionary dynamics at two temperatures (De Block et al. 2013).

In consequence, we expect that when shifts in thermal performance occur in traits that affect how species interact, such as resource acquisition or metabolism, it could affect overall fitness of populations that are simultaneously involved in interspecific interactions and responding to warming. Specifically, 1) different life history traits within a species can have different thermal dependencies, 2) consumer and resource species have documented differences in these thermal dependencies, and 3) evolutionary potential can differ across trophic levels. Therefore, it follows that evolution in response to warming has the potential to alter consumer-resource dynamics, while also being reciprocally influenced by them, and that the effects of evolution may additionally depend upon current thermal environment. If this prediction is correct, we expect more complex outcomes when considering consumer-resource interactions where changes in thermal performance for both the consumer and the resource are simultaneously possible. For example, one possibility that emerges is cryptic eco-evolutionary dynamics where evolution in both species yields a "moving target" that mimics purely ecological dynamics. In this case, evolution may alter the thermal performance of the interacting species in similar ways, such that the outcomes of the interaction remain apparently unchanged, but are actually being maintained by the evolutionary response. A second possibility is that the effects of the interaction on species' fitness may depend upon the current thermal environment. This might occur if, for example, their thermal tolerances evolve to different degrees or if only one interacting species evolves. The variety of possible

111 responses become complex, but studying evolutionary responses in this complexity is necessary to
112 better understand how evolution can alter the responses of populations and communities to habitat
113 warming. Additionally, although responses are sometimes examined at multiple temperatures, most
114 studies are typically of short duration and do not consider the effects of evolution at longer timescales
115 to quantify changes to fitness. Thus, considering evolutionary responses in more complex communities
116 acknowledges importance of community ecology in driving evolutionary responses to anthropogenic
117 environmental change.

118 To examine both the influence of ecological interactions on evolutionary responses to warming
119 as well as understanding the long-term effects of evolution on the ecology of interacting species, we
120 used populations of two resource species (i.e., freshwater green algae, *Chlamydomonas reinhardtii* and
121 *Desmodesmus* sp.) and a consumer species (i.e., *Daphnia pulex*) differing in thermal origin. We leveraged
122 a long-term experiment of manipulated warming in mesocosm plankton communities to understand the
123 consequences for consumer-resource interactions of evolution in response to long-term warming in
124 communities. We hypothesized that the fitness effects of this evolution would be dependent upon
125 current thermal environment and the presence of ecological interactions. Specifically, we asked, 1)
126 whether biotic interactions affect the importance of evolutionary history across a range of
127 temperatures, and 2) whether temperature affects the importance of evolutionary history.

128 **Methods**

129 *Source and maintenance of organisms prior to experimentation*

130 Organisms originated from a long-running mesocosm experiment in Dorset, England. This
131 mesocosm experiment has been the subject of multiple previous publications (Yvon-Durocher et al.
132 2015, Schaum et al. 2017). Briefly, at the time of sampling in 2019, open-air experimental ponds of 1 m^3
133 water of mixed freshwater phytoplankton and zooplankton had been continuously maintained since

134 2005 (i.e., approximately 14 years). Half of the mesocosms were kept at ambient temperature
135 conditions, while half experienced a consistent temperature increase of +4 °C above ambient
136 temperatures (n = 8 for both at the time of our sampling). Temperature in all ponds fluctuated
137 seasonally. Mesocosm communities initially contained the same mixture of phytoplankton and
138 zooplankton, but previous work has demonstrated significant changes to community structure in the
139 warmed mesocosms, as well as evolution in thermal tolerance in warmed populations of at least one
140 phytoplankton species, *Chlamydomonas reinhardtii* (Yvon-Durocher et al. 2015, Schaum et al. 2017)(Fig.
141 1).

142 We sampled two phytoplankton resource species, the algae *C. reinhardtii* and *Desmodesmus* sp.
143 from each treatment in January 2019, as well as a consumer species, *Daphnia pulex* in September 2019
144 (for further details see Álvarez-Codesal et al. 2023). The discrepancy in dates occurred because we were
145 unsuccessful in isolation of consumer populations on the first attempt. Sorting flow cytometry isolated
146 single algal cells and PacBio Sequel 2 long-read sequencing using full-length 18S fusion primers (forward:
147 NSF4/18 = CTGGTTGATYCTGCCAGT; reverse: EukR = TGATCCTTCTGCAGGTTCACCTAC) confirmed species
148 identity and genotypic differences between ambient-origin and warm-origin populations of the algae
149 (Comeau et al. 2017). We identified one unique clone for each of the two species, *C. reinhardtii* and
150 *Desmodesmus* sp. The identity of the consumer populations originating from each of 5 individuals
151 originally isolated from the experiment was confirmed morphologically by a taxonomist (however,
152 genotypic differences were not verified by sequencing for the *Daphnia*). All species reproduced
153 asexually, enabling us to maintain clonal populations of each genotype (algae) or putative clone
154 (*Daphnia*) in semi-continuous laboratory batch cultures until the start of the experiments. To avoid
155 confounding effects of plasticity or maternal effects, we maintained populations of these species in
156 common garden conditions—20 °C with 12:12 light-dark cycle—for over six months (many generations)
157 prior to subsequent experimentation. All organisms grew in autoclave-sterilized COMBO growth

158 medium. *Daphnia* grew in modified low-phosphorus COMBO (Kilham et al. 1998) that reduces bacterial
159 load. Aeration maintained culture health and growth and algal populations were in the exponential
160 growth phase at the start of the experiment. Additionally, *D. pulex* grew on a laboratory culture of
161 *Ankistrodesmus* sp. (sourced from Sciento UK) to ensure that daphnids were not acclimated to the algal
162 species used in the experiments. *Daphnia* cultures received 50 mL of dense *Ankistrodesmus* once per
163 week as a food source. Periodic filtering of adult and juvenile daphids into fresh medium removed
164 metabolic waste products and resting eggs.

165 *Experimental design*

166 Our experimental design crossed long-term evolutionary history—either to "ambient" or
167 "warm" temperature conditions— of each consumer–resource pair with 4 growth temperatures (Fig. 1).
168 In this way, we sought to examine how the effects of evolution in response to warming in a consumer–
169 resource pair depend upon the current thermal environment. The experiment occurred in 4 incubators
170 that varied in temperature: 14, 18, 22, and 26 °C. These temperatures encompass much of the observed
171 growing season activity range for the consumer from this system, with the presumed approximate
172 thermal optimum of reproduction of the ambient-origin population of 20 °C serving as the mid-point
173 across the range (Craddock 1976). Non-consumption controls additionally allowed us to verify the
174 importance of algal evolutionary history in the absence of consumers. We conducted the experiment for
175 each consumer–resource pair in two temporal blocks (for tractability and to avoid pseudoreplication at
176 the incubator level). Thus, we had 6 evolutionary history–consumption treatments crossed with 4
177 growth temperatures in each temporal block for each consumer–resource pair (n = 4 per treatment,
178 yielding a total of 96 microcosms within temporal block). At the start of each experimental block, we
179 estimated abundance of the ambient-origin and warm-origin resources using a hemocytometer to count
180 cells under a compound microscope (NIKON Eclipse Ni). We then standardized approximate starting
181 abundances of the two populations, however starting densities thus differed somewhat between blocks

182 within a resource species and between resource species. We randomized microcosm position within
183 each incubator. The experiment ran a total of 4 times (n = 8 for treatment across 2 temporal blocks,
184 yielding N = 192 per consumer–resource pair and N = 384 across both pairs).

185 To each sterile microcosm we added 100 mL of sterile COMBO medium and 100 mL of density-
186 standardized resource from either the ambient-origin or warm-origin population (either *C. reinhardtii* or
187 *Desmodesmus* sp.). To treatments containing *Daphnia*, we then added 5 adult daphnids either of
188 ambient-origin or warm-origin to each microcosm (previously starved for 24-hours, filtered, and rinsed
189 in sterile water to remove residual *Ankistrodesmus*), such that the evolutionary origin of the resource
190 was crossed with that of the consumer. Thus our evolutionary history–consumption treatments
191 repeated at 4 temperatures were: 1) ambient-origin resource alone, 2) warm-origin resource alone, 3)
192 both ambient-origin resource and consumer, 4) ambient-origin resource + warm-origin consumer, 5)
193 warm-origin resource + ambient-origin consumer, and 6) both warm-origin resource and consumer.

194 We confirmed the survival of the *Daphnia* on the second day of the experiment. The experiment
195 ran for 3 weeks, or approximately 42–100 generations of algae and 2–4 generations of *Daphnia*. A
196 failure in the first 24 hours of the experiment for 26 °C incubator resulted in the incubator temperature
197 declining to 1 °C overnight for the first temporal block using *C. reinhardtii*. We therefore discarded these
198 microcosms and reassembled them approximately 12 hours after the initial experimental set-up using
199 the same sources of algae and *Daphnia*.

200 *Estimation of equilibrium densities and interaction strength*

201 Responses included equilibrium density (per ml) of the resource species, as well as a census of
202 adult and larval *Daphnia* present in each microcosm. After 3 weeks, we first filtered and preserved all
203 *Daphnia* adults and juveniles from a microcosm in 95% ethanol for later enumeration under a dissecting
204 scope in two size categories, adults and juveniles. We next preserved two 150 µL samples of well-mixed

205 algae suspension from each microcosm using 15 µL of a 1% sorbitol solution in 96-well plates. Incubation
206 for 1 hour in the dark ensured sorbitol penetration to prevent cell lysis before sample storage at - 80 °C.
207 Flow cytometry (BD FACSCanto II high-throughput sampler) then estimated cell densities. We used
208 averaged $\log_{10}$-transformed densities of the two technical flow cytometry replicates measured from
209 each microcosm in further analyses (see below). Parameters of flow cytometric lasers were as follows:
210 FSC: 136, SSC: 258, FITC: 484, PE: 432, PerCP-Cy5: 297, PE-Cy7: 570, APC: 440, APC-H7: 472, V450: 373
211 and V500: 484. For *Desmodesmus* sp., because flow cytometry measures particles, we report our results
212 as coenobia (i.e., small colonial cellular aggregates) rather than converting from aggregates to cells.
213 Coenobia are the units with which consumers interact, making them an appropriate unit for this species.
214 Safranin (Sigma-Aldrich) stained the *Daphnia* for simpler visualization of the preserved samples during
215 counting under a stereo microscope (Leica S9i), which ensured that molts were not counted
216 erroneously.

217 We used interaction strength to quantify the effect of consumers on resource abundance. To
218 calculate interaction strength (IS), using Equation 1 we calculated an inverse form of the log-response
219 ratio (also called the Dynamic Index) (Berlow et al. 2003; Schaum et al. 2018):

220 $$IS = \frac{ln(\frac{\overline{N}}{D_i})}{t}$$ Equation 1

221 Where $\overline{N}$ is the final mean algal abundance of the no consumption (algae-alone) controls for each
222 population, ambient-origin or warm-origin, for each resource species, *C. reinhardtii* or *Desmodesmus*
223 sp., D_i is the final algal abundance of replicate i from a given population in the presence of consumers,
224 and t is the length of the experiment (equal to 21 days for our experiment). With the inverse log-
225 response ratio, larger values of IS indicate stronger effects of consumers on resource abundance.

226 *Analyses*

227 We $\log_{10}$-transformed abundances to better meet assumptions of normality in the subsequent
228 analyses. In all models, a block effect and all block interactions accounted for differences related to
229 temporal blocks. We conducted analyses to examine how current thermal environment and biotic
230 interactions determined the importance of past evolutionary history to warming in governing fitness (as
231 assessed by abundance across multiple generations of interactions) of resources and consumers. A
232 single MANOVA including evolutionary history treatments with and without consumers present would
233 obfuscate differences that might occur in the interaction between evolutionary history and temperature
234 when the algae grew alone or with consumers (in part through spurious significance related to the
235 presence or absence of *Daphnia* as a response in the two types of treatments). Therefore, to examine
236 how current biotic interactions impact the importance of evolutionary history, we conducted separate
237 analyses on the $\log_{10}$-transformed abundance per ml of algae when consumers were present or absent.
238 In all models, the interaction between the main effects (evolutionary history and temperature) assessed
239 whether the effects or importance of evolutionary history depend upon current thermal environment.
240 Thus, we first conducted an analysis of variance (ANOVA) for each algal species grown alone (because
241 they were tested independently). These analyses tested whether algal evolutionary history interacted
242 significantly ($\alpha = 0.05$) with temperature when consumers were absent. The model included the
243 evolutionary history (2 levels—ambient-origin or warm-origin resources) and temperature (4 levels—
244 14, 18, 22, and 26 °C test temperature) and the interaction, in addition to the block effect and block
245 interactions, with final algal density as the response.

246 We next conducted a separate multivariate analysis of variance (MANOVA) for each consumer–
247 resource pair. Responses included the $\log_{10}$-transformed abundance per ml of the resource, interaction
248 strength, and the $\log_{10}$-transformed abundances per microcosm of adult and juvenile *Daphnia*. Main
249 effects of the model included evolutionary history treatment (4 levels—ambient-origin resource and
250 consumers, ambient-origin resource and warm-origin consumers, warm-origin resource + ambient-

251 origin consumers, and warm-origin resource and consumers), temperature (4 levels—14, 18, 22, and 26
252 °C test temperature), and the interaction between temperature and evolutionary history. Univariate
253 tests assessed significance at $\alpha = 0.05$ for individual responses. Orthogonal contrasts further
254 disentangled the drivers underlying the evolutionary history treatment and determined specifically if 1)
255 resource evolution, 2) consumer evolution, or 3) the interaction between evolutionary history of the
256 resource and consumer (i.e., coevolution) affected responses. All analyses were conducted in SAS 9.4
257 (SAS Institute 2011).

258 **Results**

259 Evolutionary history in response to warming in complex communities, current temperature, and
260 their interaction had highly significant effects for both consumer–resource pairs, but the relative
261 importance of resource, consumer, or coevolution differed between the pairs (see Tables 1 and 2 for
262 specific results from algae-alone ANOVAs and consumer–resource MANOVAs). We describe specific
263 effects below using results from ANOVAs for each response variable.

264 *Current thermal environment influences the importance of prior resource evolution in response to*
265 *warming*

266 Resource evolution in warmed complex communities affected both algal species when grown
267 alone, but the effects of evolutionary history were opposite in direction and differed in strength (Table
268 1). Warm-origin *Chlamydomonas reinhardtii* had higher abundance than the ambient-origin genotype at
269 all temperatures with no significant interaction between resource evolutionary history and current
270 thermal environment (effect of evolutionary history in algae-alone ANOVAs: $F_1 = 158.47$, $p < 0.0001$;
271 interaction between evolutionary history and temperature: NS) (Fig. 2A). For *Desmodesmus* sp., the
272 effect of evolutionary history depended upon temperature, with higher abundance of the warm-origin
273 genotype at 14 °C, but lower abundance than the ambient-adapted genotype at the remaining three

temperatures (effect of evolutionary history in algae-alone ANOVAs: $F_1 = 7.02$, $p = 0.0109$; interaction between evolutionary history and temperature: $F_3 = 5.86$, $p = 0.0017$) (Fig. 2B).

Interspecific interactions modulate how current thermal environment interacts with prior evolution in response to warming

Effects of interspecific interactions on resource abundance

The presence of the consumer *Daphnia pulex* altered the effects of resource evolutionary history for both resources, but in opposite directions (Tables 2–4). For *C. reinhardtii*, where no significant interaction between resource evolutionary history and temperature occurred when grown alone, the presence of the consumer resulted in a significant evolutionary history by temperature interaction driven by resource evolution (consumer–resource ANOVA resource evolution contrast: $F_1 = 318.47$, $p < 0.0001$). Specifically, when consumers were present, the importance of resource evolutionary history in driving differences in algal abundance was higher at intermediate temperatures (18 and 22 °C) than at the two temperature extremes (14 and 26 °C)—in other words, while temperature did not affect the direction of the response to evolutionary history, it increased the magnitude of the effect size at intermediate compared to extreme temperatures (effect of evolutionary history in consumer–resource ANOVA: $F_3 = 107.54$, $p < 0.0001$; interaction between evolutionary history and temperature: $F_9 = 4.93$, $p < 0.0001$) (Fig. 2C). On the other hand, for *Desmodesmus* sp., the presence of consumers rendered the interaction between resource evolutionary history and temperature non-significant (effect of evolutionary history in consumer–resource ANOVA: $F_3 = 3.54$, $p = 0.0175$; interaction between evolutionary history and temperature: NS) (Fig. 2D). Here, when consumers were present, resource evolution decreased algal abundance, reversing the pattern seen at 14 °C in the absence of consumers (consumer–resource ANOVA resource evolution contrast: $F_1 = 10.57$, $p = 0.0016$) (Fig. 2D).

297 *Effects of evolutionary history on interaction strength*

298 The importance of resource evolutionary history on algae abundance was reflected in the
299 changes to interaction strength for each consumer–resource pair (Tables 3 and 4). For the *D. pulex*–*C.*
300 *reinhardtii* pair, resource evolution reduced interaction strength (consumer–resource ANOVA resource
301 evolution contrast: $F_1 = 54.90$, $p < 0.0001$), but primarily at intermediate temperatures (interaction
302 between evolutionary history and temperature: $F_9 = 5.16$, $p < 0.0001$) (Fig. 2E). For the *D. pulex*–
303 *Desmodesmus* sp. pair, however, resource evolution increased interaction strength, independent of
304 temperature (consumer–resource ANOVA resource evolution contrast: $F_1 = 5.86$, $p = 0.0174$; interaction
305 between evolutionary history and temperature: NS) (Fig 2F).

306 *Consumer responses to evolutionary history and temperature*

307 For the consumer, *Daphnia pulex*, the effects of evolutionary history differed depending upon
308 with which resource it was paired. When paired with *C. reinhardtii*, resource evolution significantly
309 reduced the abundance of adult and juvenile *D. pulex* (adult resource evolution contrast: $F_1 = 5.50$, $p =$
310 0.0211; juvenile resource evolution contrast: $F_1 = 5.07$, $p = 0.0266$; Table 2, Fig. 3A,B). In this pairing,
311 neither consumer evolution nor coevolution affected the abundance of *D. pulex* and the effects of
312 resource evolution were independent of temperature (consumer–resource ANOVA interaction between
313 evolutionary history and temperature: NS).

314 When growing instead on the resource *Desmodesmus* sp., the effects of consumer evolution
315 became apparent. In this case, warm-origin consumers had overall lower abundance than the ambient-
316 origin population (adult consumer evolution contrast: $F_1 = 15.87$, $p = 0.0001$; juvenile consumer
317 evolution contrast: $F_1 = 9.77$, $p = 0.0024$; Table 4, Fig. 3C,D). There was no direct effect of *Desmodesmus*
318 evolution, but coevolution between the resource and consumer affected consumer abundance (adult

319 coevolution contrast: $F_1 = 8.52$, $p = 0.0044$; juvenile coevolution contrast: $F_1 = 12.55$, $p = 0.0006$). The
320 effects of evolutionary history were also dependent upon current thermal environment when growing
321 on *Desmodesmus* sp. (evolutionary history-temperature interaction for adult and juvenile daphnids
322 respectively: $F_9 = 2.09$, $p = 0.0380$ and $F_9 = 2.21$, $p = 0.0281$; Table 4, Fig. 3C,D). For *D. pulex* growing on
323 *Desmodesmus* sp., the effects of consumer evolution were most evident at 14 °C, where evolved *D.*
324 *pulex* adults ("Evolved consumer" and "Both evolved" treatments) had significantly reduced abundance
325 regardless of *Desmodesmus* evolutionary history compared to the ambient-origin *D. pulex* ("Neither
326 evolved" and "Evolved resource" treatments). The effects of coevolution between the consumer and
327 resource were strongest at 26 °C, with warm-adapted adult *D. pulex* abundance being significantly
328 reduced only in the presence of warm-adapted *Desmodesmus* sp. ("Both evolved" treatment) (Fig. 3B).
329 In contrast to the temperature-dependence observed for the resource, *C. reinhardtii*, evolutionary
330 history was less important for consumer abundance at intermediate temperatures.

331 **Discussion**

332 Understanding the interaction between evolutionary history, the abiotic environment, and
333 biotic interactions is critical for a more nuanced understanding of how communities respond to
334 anthropogenic stressors. Here we showed that both the current thermal environment, as well as the
335 presence of interspecific interactions can affect the importance of prior evolution in response to
336 warming in complex communities, and that this evolution also influences the outcomes of current
337 ecological dynamics. For each consumer–resource pair, the effects of evolution were temperature-
338 dependent, but both the effects and the temperature-dependence itself additionally depended upon
339 the identity of evolving species in each pair.

340 *The importance of evolutionary history depends upon current thermal environment*

341 Evolution in warmed communities had significant effects on two algal resource species when
342 grown alone, but these effects were opposite in direction and differed in magnitude. In one species, *C.*
343 *reinhardtii*, prior evolution in warmed communities increased fitness (as assessed by abundance) across
344 all temperatures, a result that is broadly consistent with the "hotter is better" hypothesis (Liu et al.
345 2022), although our tested temperature range (determined by the thermal tolerance and activity of the
346 consumer) precluded us from definitively evaluating competing proposed evolutionary responses. The
347 positive direction of change for *Chlamydomonas reinhardtii* agrees with a previous experiment
348 examining the short-term effects of thermal evolution of *C. reinhardtii* independently isolated from
349 these same mesocosms (Schaum et al. 2017). In the second alga, *Desmodesmus* sp., the effects of
350 evolutionary history were dependent upon the current thermal environment. For this species, fitness
351 was lower in the warm-origin genotype at three of the four tested temperatures, but the pattern was
352 reversed at 14 °C, the lowest temperature in our range. This reversal of direction of effect across
353 temperatures suggests the possibility of a response that is harmful in part of the thermal range, but
354 possibly advantageous in another. The pattern of the result is surprising however, as the expectation is
355 that warm-origin populations would perform better at warm temperatures compared to colder
356 temperatures if the evolutionary response was directly driven by the experimentally imposed
357 temperature treatment.

358 Evaluating the mechanisms underlying this result was impossible given that we only successfully
359 isolated one genotype for each species from each of the ambient and warm mesocosms, however, these
360 results may be consistent with one or more aspects of our system. First, our algal genotypes were
361 isolated from the experimental ponds during the winter season, so this particular genotype may be
362 more successful in the winter conditions experienced by the experimentally warmed ponds. Typical
363 winter conditions in Dorset ranged from approximately 3–10 °C with a mean of around 6 °C (Yvon-
364 Durocher et al. 2015), thus the additional 4 °C of warming experimentally imposed upon these ponds

raised the winter temperature to within a few degrees of our 14 °C treatment. We did not examine the effects of evolutionary history at any temperature lower than 14 °C, however, so we are unable to compare the relative fitness of each genotype at typical winter temperatures compared to 14 °C. Conversely, the warm-origin genotype that we isolated may be maladaptive, with lower fitness than the ambient-origin genotype under most conditions. Anthropogenic warming is anticipated to create an extinction debt in many communities (Cotto et al. 2017), with poorly adapted species expected to be lost over time, especially as a result of stochastic processes combined with low dispersal (Kuussaari et al. 2009). It is possible that we have captured part of this process, with a maladapted genotype "trapped" in the warm mesocosms. Another possibility is that the selection occurred in response to an agent that emerged secondary to the experimentally imposed temperature treatment. Our manipulative approach deconfounds other climatic variables that may affect evolutionary trajectory in natural populations, but our populations were nonetheless potentially evolving in response to a number of factors (e.g., community composition) that had diverged over the course of this long-running experiment (Yvon-Durocher et al. 2011, 2015, Dossena et al. 2012). Thus, while we can ultimately link our responses to the experimentally-imposed warming treatment, populations are potentially evolving to a number of simultaneous and related stressors (terHorst et al. 2018). While this situation reflects the expectation for natural communities responding to habitat warming—namely that responses will encompass altered biotic interactions as well as direct responses to changed abiotic factors—it is therefore impossible for us to definitively disentangle whether the lowered fitness that we observed in warm-origin populations of *Desmodesmus* sp. was the result of maladaptive evolutionary response or evolutionary response to conditions not captured in our subsequent experiments.

Current ecological context modulates the temperature-dependence of the effects of evolutionary history

387 Our understanding of how evolution is altered in complex communities is still limited. However,
388 previous studies suggest that evolution to environmental stressors, like habitat warming, that occurs in
389 populations embedded within communities can yield different outcomes compared to evolution in
390 single populations (Van Doorslaer et al. 2009b, 2010, terHorst et al. 2018, Hiltunen et al. 2018, De
391 Meester et al. 2019). Our experiment complements a growing body of evidence that shows that
392 evolutionary responses to warming do occur in complex communities (De Meester et al. 2019), and that
393 such evolution has important consequences for both the direct responses to evolutionary stressors as
394 well as for the interspecific interactions that ultimately shape communities.

395 The inclusion of consumer–resource interactions also introduced an additional layer of
396 complexity necessary to understand how the importance of evolutionary history is affected by
397 temperature. Organisms exhibit a mixture of unimodal and linear responses of traits to temperature,
398 and interacting organisms differ in both the ecological and evolutionary responses of these traits. The
399 interactions themselves may therefore depend upon temperature, influencing the outcomes of
400 evolutionary change evaluated across temperatures (Barton and Yvon-Durocher 2019). For both
401 consumer–resource pairs in our study system, the presence of the consumer altered the interaction
402 between evolutionary history and temperature, indicating that examining the effects of past evolution
403 in response to environmental change can be highly dependent on the current ecological conditions.

404 In the case of the *Daphnia pulex–C. reinhardtii* pair, the importance of evolutionary history was
405 only temperature-dependent when the consumer was present. For this pair, the magnitude of the effect
406 evolutionary history on algal abundance was higher at intermediate temperatures compared to
407 temperature extremes, adding nuance to the result that "hotter is better", making it instead "hotter is
408 best at moderate or intermediate temperatures". Consumer evolution was unimportant when *D. pulex*
409 was paired with *C. reinhardtii*—warm-origin *C. reinhardtii* significantly reduced the abundance of the

410 consumer regardless of temperature. Thus, “hotter is better” for this resource translates into “hotter is
411 worse” for the consumer. We noted that the warm-origin genotype exhibited a mucilaginous extra-
412 cellular matrix that might be expected to reduce ingestion rates for filter-feeding consumers like *D.*
413 *pulex*. These results are also consistent with observations of an increased carbon to phosphorus ratio
414 (i.e*.,* reduced resource quality) observed previously in warm-adapted *C. reinhardtii* independently
415 isolated from these experimental mesocosms (Yvon-Durocher et al. 2017). Such a reduction in resource
416 quality would be expected to negatively affect consumer performance (Leal et al. 2017), but an analysis
417 of nutrient content of the algae was outside the scope of our experiment. It is noteworthy, however,
418 that despite the lack of a significant interaction between evolutionary history and temperature, the
419 importance of resource evolution was strongest at 22 °C, the temperature at which *D. pulex* abundance
420 was highest, and weakest at 26 °C, where the daphnids struggled to persist. The evolution that we
421 observed in *C. reinhardtii* thus appeared to confer a double advantage to the resource, making it both
422 more fit, while simultaneously reducing the success of the consumer, possibly the reason why we
423 observed larger effect sizes for resource evolutionary history in the presence of the consumer, especially
424 at intermediate temperatures (i.e., effect size more than doubled on a $\log_{10}$ scale).

425 The effects of consumers for *Desmodesmus* sp. were opposite to those for *C. reinhardtii*. Here,
426 the presence of the consumer eliminated the temperature-dependence seen when *Desmodesmus* sp.
427 grew alone, and even reversed the direction of the effect of evolutionary history at 14 °C. When paired
428 with the consumer, this subtlety across temperatures was lost, and warm-origin *Desmodesmus* sp.
429 underperformed relative to ambient-origin *Desmodesmus* regardless of temperature. One possible
430 explanation for the apparently reduced fitness of the warm-origin genotype could be that it served as a
431 superior food source for the *Daphnia*. The two *Desmodesmus* sp. lineages have different propensities to
432 remain as single cells or aggregate into 2, 3, or 4-celled coenobia, with the warm-origin lineage occurring
433 more frequently as 1 or 2-cell aggregates and the ambient-origin lineage more frequently as larger

434 aggregates (unpublished data). This difference in average particle size could affect the filtering efficiency
435 of the *Daphnia*, making the warm-origin genotype potentially more accessible to the consumer and
436 intensifying the fitness disadvantage of the warm-origin genotype in the presence of consumers. If this
437 were the case, we would expect to see that the *Daphnia* achieved higher abundances when consuming
438 the warm-origin algae. In fact, *Desmodesmus* sp. evolutionary history had contrasting effects on the
439 consumer's fitness depending upon consumer thermal origin (i.e., the effect of coevolution on *D. pulex*
440 abundance in this pair). Ambient-origin consumers exhibited a trend towards slightly higher fitness with
441 warm-origin resource, but warm-origin *Daphnia* had lowest fitness when paired with warm-origin
442 *Desmodesmus*. Thus, the effect does not appear to be consistent with increased consumption by the
443 *Daphnia*. Additionally, in this pair, the *Daphnia* experienced temperature-dependent effects of
444 evolutionary history. Here, effects were strongest at the two temperature extremes, with warm-origin
445 *Daphnia* having lowest abundance at these temperatures, and especially at 26 °C and when paired with
446 warm-origin *Desmodesmus* sp.

447 We examined the temperature-dependent consequences of evolution for two consumer–
448 resource pairs over multiple generations of interactions and found evidence for adaptive evolution in
449 response to warming in one of the two species pairs examined. For the *D. pulex*–*C. reinhardtii* pair, we
450 observed adaptive evolution consistent with the "hotter is better" hypothesis for the resource, but at
451 the same time observed that hotter was worse for the consumer, likely due to the fact that hotter was
452 worse for the interaction from the perspective of the consumer. This result may seem surprising, but not
453 only is evolutionary response to warming not expected in all species (Hoffmann and Sgró 2011), it also
454 remains possible that the fitness consequences of the response depend upon the length of the
455 temperature exposure. We focused on the effects of constant temperature over multiple generations,
456 however, the semi-natural mesocosms from which we isolated these genotypes additionally fluctuate in
457 temperature both diurnally and seasonally. These fluctuations may be an important component of the

458 selection imposed by the experimental warming treatment (Diamond 2017), and organisms in warmed
459 ponds may experience more frequent and intense, but shorter, periods of thermal stress. A more
460 complete understanding of the interaction between current thermal environment and evolutionary
461 history, therefore, will require an improved understanding of how populations respond to fluctuating
462 temperatures.

463 **Conclusions**

464 Our work demonstrates that local thermal environment and biotic interactions can together
465 modulate the importance of past thermal evolutionary history. For example, in some cases, evolution in
466 response to warming can be beneficial for resources but detrimental for consumers, suggesting that we
467 must account for the effects of warming on ingestion rates (see e.g., Barneche et al. 2021). In other
468 cases, the picture is more complex and changes across a temperature range. This topic deserves further
469 attention to improve our understanding of how populations and communities evolve to habitat
470 warming, and how this then feeds back to drive the effects of the evolution. We have built upon
471 previous studies that have primarily used space-for-time latitudinal substitutions in comparisons of
472 different thermally adapted populations (e.g., Sanford et al. 2003, De Block et al. 2013, Dinh Van et al.
473 2013, Tran et al. 2016) and argue that future studies should consider both the current biotic and thermal
474 context of their experiments, while definitely attributing evolutionary response to a manipulated factor
475 of interest. These community properties can affect outcomes, not just in terms of the magnitude of the
476 effect size, but even in direction of the effect. It is therefore of the utmost importance that we account
477 for these interactions in our collective understanding. Evolution can drive responses in unexpected
478 directions, but it is essential that we consider evolutionary responses to anthropogenic change in the
479 context of both the current abiotic and biotic conditions to understand the full extent and direction of
480 its effects.

Acknowledgements

The French ANR through LabEx TULIP (ANR-10-LABX-41) and the FRAGCLIM Consolidator Grant, funded by the European Research Council under the European Union's Horizon 2020 research and innovation programme (Grant Agreement Number 726176) supported this research. We thank X anonymous referees for their feedback.

Author contributions

CAF and JMM conceived and designed the study. CAF, SAC, AG, and EB isolated organisms and collected data. CAF analyzed the data. CAF wrote the first draft of the manuscript. CAF, JMM, SAC, and EB jointly revised the manuscript. The authors declare no competing financial interests.

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624 Table 1. Results from univariate ANOVAs (n = 8) examining the effects of resource evolution when
625 growing alone for *Chlamydomonas reinhardtii* and *Desmodesmus* sp. The models included an evolution
626 treatment and temperature as two main effects, as well as a temporal blocking variable and all
627 interactions. Here, the evolution treatment included just the evolutionary history of the resource
628 species, either ambient-origin or warm-origin. The interaction between evolution treatment and
629 temperature evaluated any temperature-dependence of the effects of evolution. Significant results are
630 in bold.

C. reinhardtii			
	d.f.	F-value	p-value
Evolution	1	**158.47**	**< 0.0001**
Temperature	3	**12.56**	**< 0.0001**
Temporal block	1	**54.12**	**< 0.0001**
Evolution–temperature	3	0.67	0.5776
Temperature–block	3	**15.43**	**< 0.0001**
Evolution treatment– block	1	**127.63**	**< 0.0001**
Evolution treatment–temperature–block	3	**5.96**	**0.0015**
Desmodesmus sp.			
	d.f.	F-value	p-value
Evolution	1	**7.02**	**0.0109**
Temperature	3	**47.41**	**< 0.0001**
Temporal block	1	3.94	0.0529
Evolution– temperature	3	**5.86**	**0.0017**
Temperature–block	3	**5.19**	**0.0035**
Evolution treatment–block	1	**42.75**	**< 0.0001**
Evolution treatment–temperature–block	3	**3.10**	**0.0353**

631

632 Table 2. MANOVA (n = 8) results examining the effects of evolution of both the consumer and the
633 resource in complex communities in response to warming for two consumer–resource pairs, *C.*
634 *reinhardtii* and *D. pulex*, and *Desmodesmus* sp. and *D. pulex*. The models included an evolution
635 treatment and temperature as two main effects, as well as a temporal blocking variable and all
636 interactions. Here, the evolution treatment included the four possible combinations crossing the
637 ambient- or warm-origin populations of both resources and consumers, but these analyses did not
638 include algae-alone controls. The interaction between the evolution treatment and temperature
639 evaluated any temperature-dependence of the effects of evolution. Orthogonal contrasts evaluated the
640 effects of 1) resource evolution, 2) consumer evolution, and 3) coevolution between the resource and
641 the consumer. Reported are Wilks' Lambda F-values and associated p-values. Significant results are in
642 bold.

C. reinhardtii–D. pulex			
	d.f.	F-value	p-value
Evolution	12, 243.7	**70495.6**	**< 0.0001**
Temperature	12, 243.7	**45724.4**	**< 0.0001**
Temporal block	4, 92	**1.82E10**	**< 0.0001**
Evolution–temperature	36, 346.5	**1163.05**	**< 0.0001**
Temperature–block	12, 243.7	**51809.9**	**< 0.0001**
Evolution treatment– block	12, 243.7	**63495.4**	**< 0.0001**
Evolution treatment–temperature–block	36, 346.5	**1863.05**	**< 0.0001**
Resource evolution contrast	4, 92	**4.93E10**	**< 0.0001**
Consumer evolution contrast	3, 93	1.72	0.1683
Coevolution contrast	3, 93	1.05	0.3729
Desmodesmus sp.–*D. pulex*			
	d.f.	F-value	p-value
Evolution	12, 243.7	**578.58**	**< 0.0001**
Temperature	12, 243.7	**563.64**	**< 0.0001**
Temporal block	4, 92	**2818757**	**< 0.0001**
Evolution–temperature	36, 346.5	**56.96**	**< 0.0001**
Temperature–block	12, 243.7	**363.01**	**< 0.0001**
Evolution treatment– block	12, 243.7	**633.42**	**< 0.0001**
Evolution treatment–temperature–block	36, 346.5	**61.15**	**< 0.0001**
Resource evolution contrast	4, 92	**55119.7**	**< 0.0001**
Consumer evolution contrast	3, 93	**52771.4**	**< 0.0001**
Coevolution contrast	3, 93	**55945.4**	**< 0.0001**

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Table 3. *Chlamydomonas reinhardtii* and *Daphnia pulex* univariate ANOVA (n = 8) results examining the effects of evolution of both the consumer and the resource in complex communities in response to warming. The model included an evolution treatment and temperature as two main effects, as well as a temporal blocking variable and all interactions. Here, the evolution treatment included the four possible combinations crossing the ambient- or warm-origin populations of both resources and consumers, but these analyses did not include algae-alone controls. The interaction between the evolution treatment and temperature evaluated any temperature-dependence of the effects of evolution. Orthogonal contrasts evaluated the effects of 1) resource evolution, 2) consumer evolution, and 3) coevolution between the resource and the consumer. Reported are Type III SS F-values and p-values for the model (due to one missing value causing a slightly unbalanced design). Significant results are in bold.

	***C. reinhardtii* $\log_{10}$ abundance**			**Interaction strength**			***D. pulex adult* $\log_{10}$ abundance**			***D. pulex* juvenile $\log_{10}$ abundance**		
	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value
Evolution	3	**107.54**	**< 0.0001**	3	**19.64**	**< 0.0001**	3	2.35	0.0772	3	1.80	0.1529
Temperature	3	**17.48**	**< 0.0001**	3	**48.61**	**< 0.0001**	3	**6.37**	**0.0006**	3	**7.48**	**0.0001**
Temporal block	1	**162.18**	**< 0.0001**	1	**359.69**	**< 0.0001**	1	0.10	0.7532	1	0.00	0.9543
Evolution–temperature	9	**4.39**	**< 0.0001**	9	**5.16**	**< 0.0001**	9	1.20	0.3067	9	0.53	0.8469
Temperature–block	3	**12.52**	**< 0.0001**	3	**25.83**	**< 0.0001**	3	**5.08**	**0.0026**	3	1.55	0.2062
Evolution–block	3	**4.00**	**0.0099**	3	**11.85**	**< 0.0001**	3	**3.51**	**0.0183**	3	2.31	0.0815
Evolution–temperature–block	9	1.63	0.1170	9	**3.52**	**0.0009**	9	1.40	0.2010	9	1.07	0.3949
Resource evolution contrast	1	**318.47**	**< 0.0001**	1	**54.90**	**< 0.0001**	1	**5.50**	**0.0211**	1	**5.07**	**0.0266**
Consumer evolution contrast	1	1.36	0.2456	1	1.36	0.2456	1	1.48	0.2262	1	0.29	0.5904
Coevolution contrast	1	2.53	0.1150	1	2.53	0.1150	1	0.01	0.9339	1	0.00	0.9740

Table 4. *Desmodesmus* sp. and *Daphnia pulex* univariate ANOVA (n = 8) results examining the effects of evolution of both the consumer and the resource in complex communities in response to warming. The model included an evolution treatment and temperature as two main effects, as well as a temporal blocking variable and all interactions. Here, the evolution treatment included the four possible combinations crossing the ambient- or warm-origin populations of both resources and consumers, but these analyses did not include algae-alone controls. The interaction between the evolution treatment and temperature evaluated any temperature-dependence of the effects of evolution. Orthogonal contrasts evaluated the effects of 1) resource evolution, 2) consumer evolution, and 3) coevolution between the resource and the consumer. Reported are Type III SS F-values and p-values for the model (due to one missing value causing a slightly unbalanced design). Significant results are in bold.

	***Desmodesmus* sp. $\log_{10}$ abundance**			**Interaction strength**			***D. pulex* adult $\log_{10}$ abundance**			***D. pulex* juvenile $\log_{10}$ abundance**		
	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value	d.f.	F-value	p-value
Evolution	3	**3.54**	**0.0175**	3	2.50	0.0645	3	**8.26**	**< 0.0001**	3	**8.66**	**< 0.0001**
Temperature	3	**11.26**	**< 0.0001**	3	**13.15**	**< 0.0001**	3	**14.08**	**< 0.0001**	3	**33.57**	**< 0.0001**
Temporal block	1	**47.32**	**< 0.0001**	1	0.88	0.3513	1	**17.64**	**< 0.0001**	1	**83.67**	**< 0.0001**
Evolution–temperature	9	0.73	0.6804	9	0.77	0.6466	9	**2.09**	**0.0380**	9	**2.21**	**0.0281**
Temperature–block	3	**5.39**	**0.0018**	3	**5.96**	**0.0009**	3	**4.62**	**0.0046**	3	**54.08**	**< 0.0001**
Evolution–block	3	**14.12**	**< 0.0001**	3	**10.25**	**< 0.0001**	3	2.66	0.0528	3	**4.92**	**0.0032**
Evolution–temperature–block	9	0.45	0.9066	9	0.33	0.9636	9	1.33	0.2309	9	**2.10**	**0.0368**
Resource evolution contrast	1	**10.57**	**0.0016**	1	**5.86**	**0.0174**	1	0.51	0.4752	1	3.61	0.0605
Consumer evolution contrast	1	0.07	0.7925	1	1.16	0.2846	1	**15.87**	**0.0001**	1	**9.77**	**0.0024**
Coevolution contrast	1	0.01	0.9385	1	0.58	0.4496	1	**8.52**	**0.0044**	1	**12.55**	**0.0006**

Figure 1. Experimental design in which populations from two resource species and one consumer species were first collected from a long-running warming experiment using complex communities of plankton in outdoor mesocosms. After isolation and a period of laboratory common garden, for each consumer–resource pair, subsequent experiments examined the interaction between evolutionary history of resources and consumers and current thermal environment on population abundance.

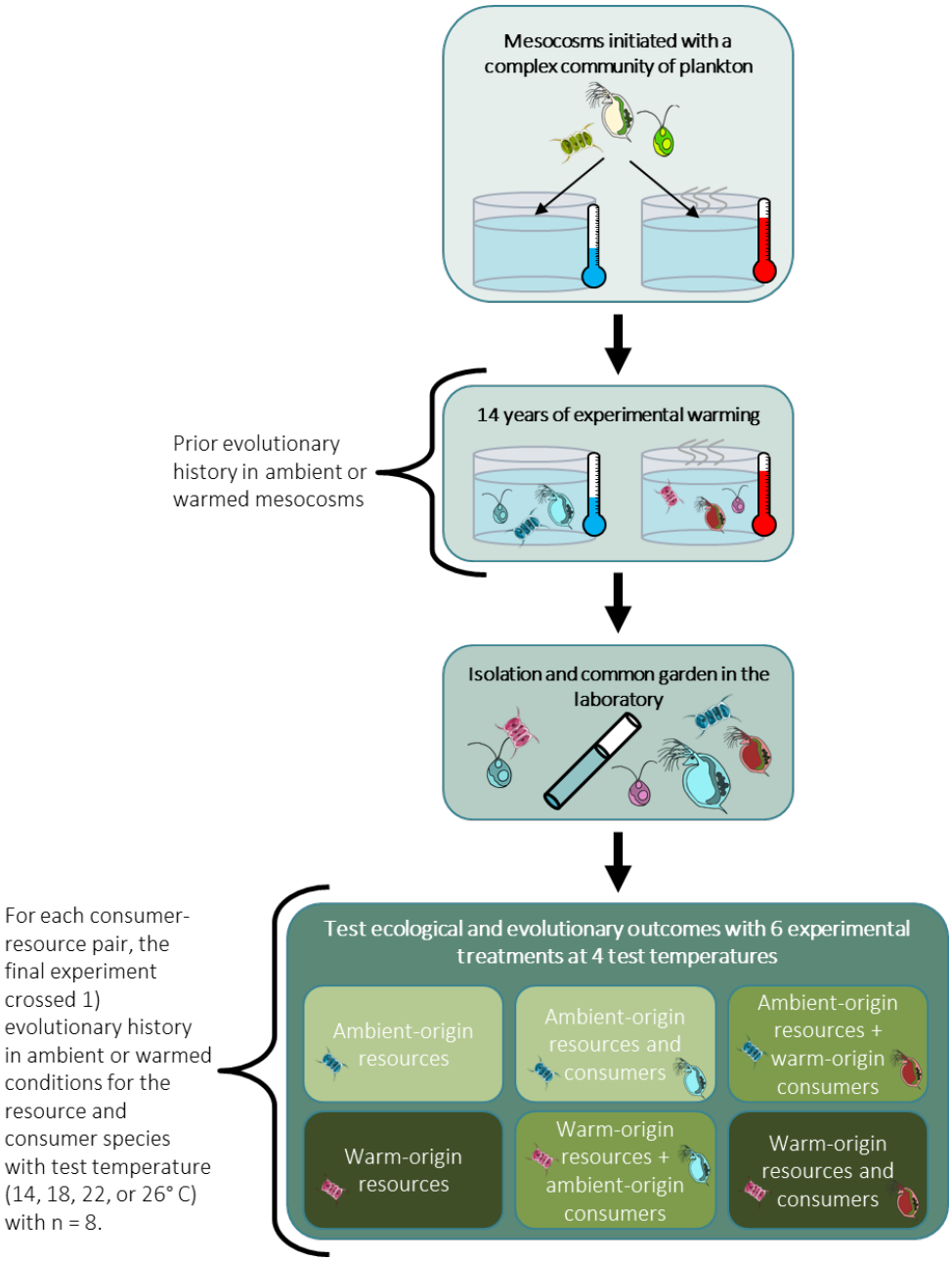

Figure 2A–F. The effects of evolution in warmed communities on the $\log_{10}$-abundance of *C. reinhardtii* and *Desmodesmus* sp. depend upon current thermal environment and whether consumers are absent (A, B) or present (C, D), with related effects to the consumer–resource interaction strength (E, F). Evolutionary history treatments are shown on the x-axis and color of the line indicates the test temperature. Circles are the means (n = 8) of each treatment with the error bars showing ± the SEM.

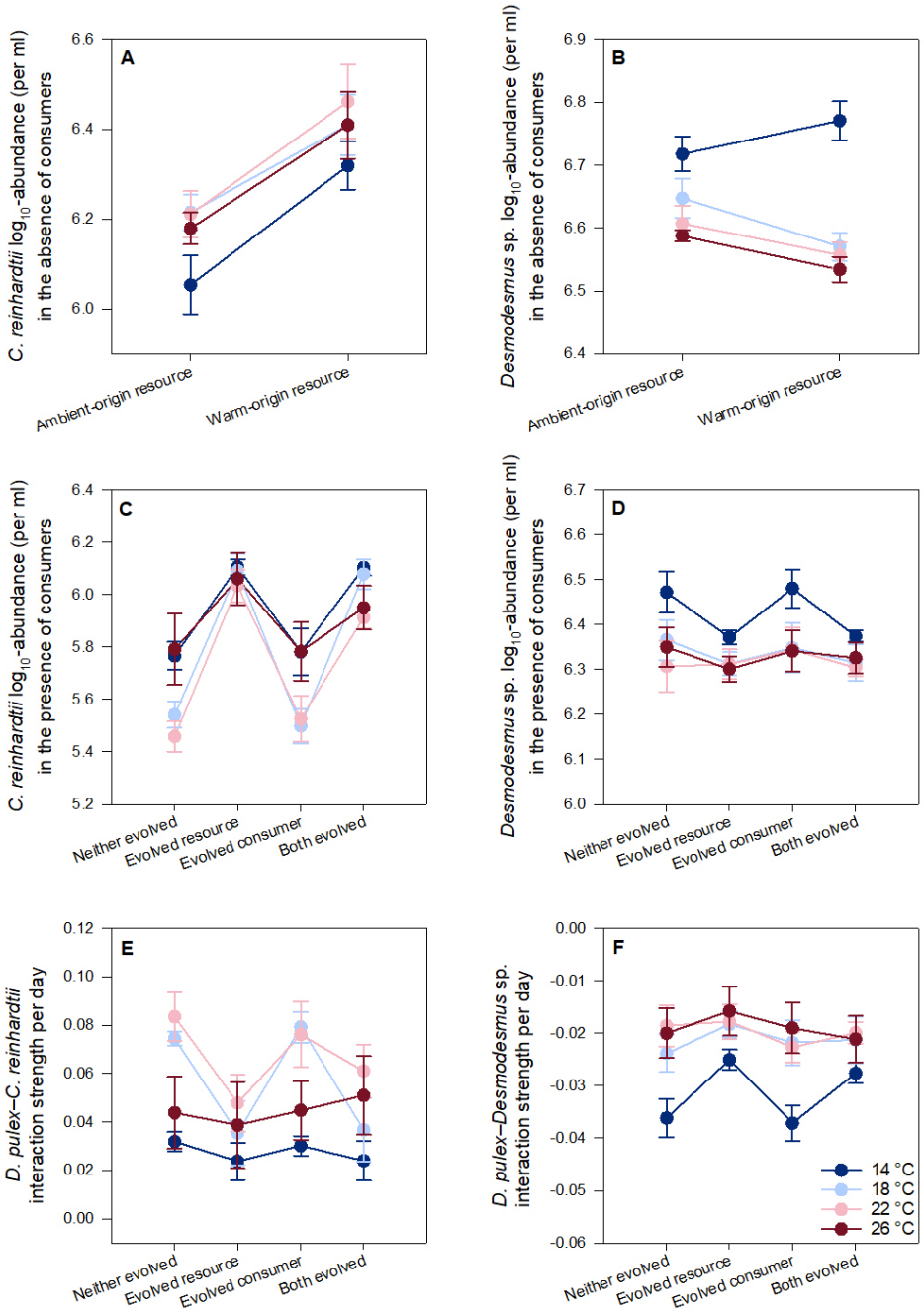
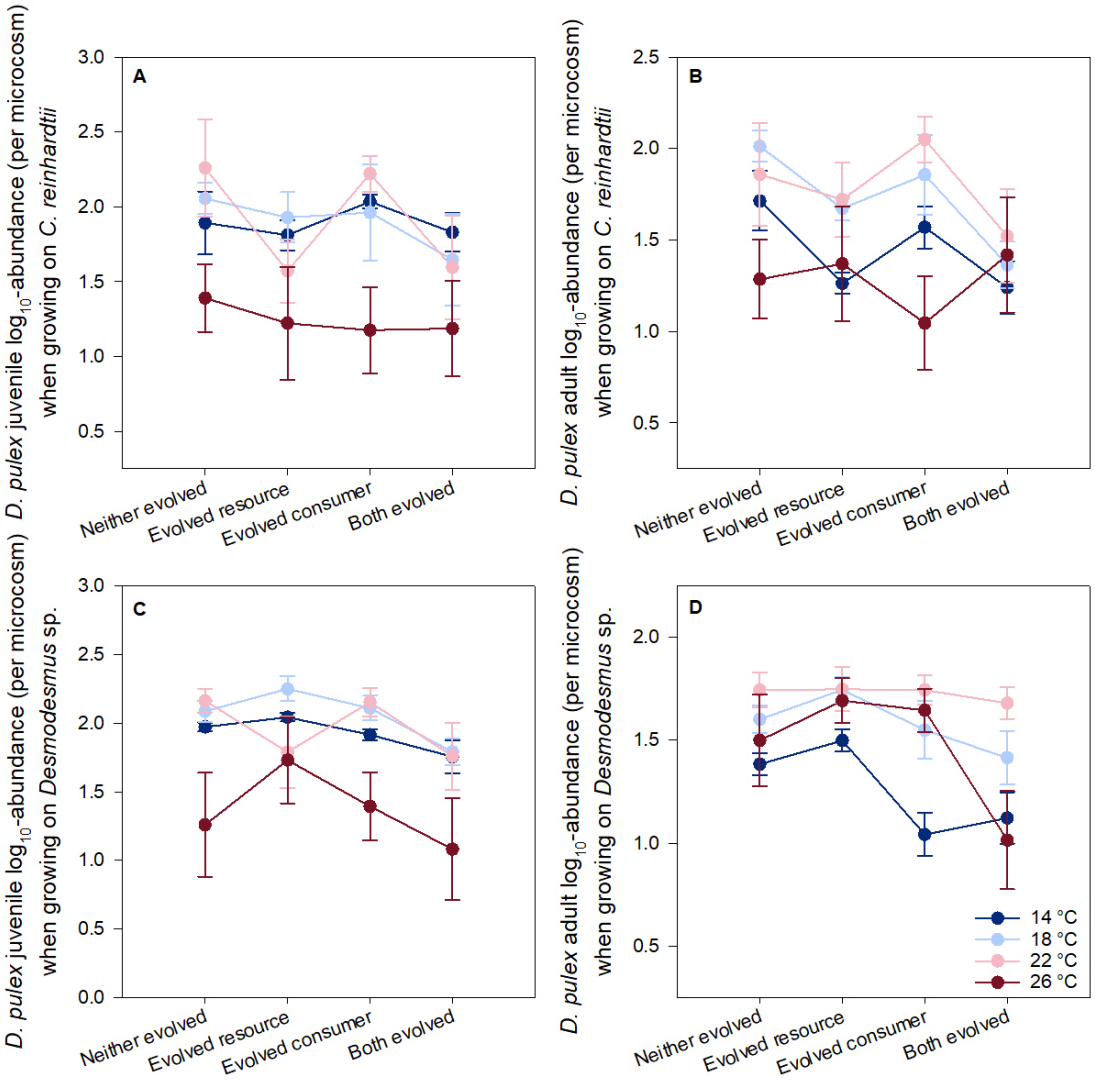
Figure 3A–D. The effects of evolution in warmed communities on the $\log_{10}$-abundance of *D. pulex* juveniles (A) and adults (B) growing on *C. reinhardtii* and on *Desmodesmus* sp. (C, D) depend upon current thermal environment. In all plots, evolutionary history treatments are shown on the x-axis and color of the line indicates the test temperature. Circles are the means (n = 8) of each treatment with the error bars showing ± the SEM.