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8 Running Title: CT<sub>max</sub> variation in wetlands

9

10 **Variation in upper thermal tolerance among 19 species from temperate wetlands**

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28 ABSTRACT

29 Communities usually possess a multitude of interconnected trophic interactions within  
30 food webs. Their regulation generally depends on a balance between bottom-up and top-  
31 down effects. However, if sensitivity to temperature varies among species, rising  
32 temperatures may change trophic interactions via direct and indirect effects. We examined  
33 the critical thermal maximum ( $CT_{max}$ ) of 19 species from temperate wetlands (insect  
34 predators, amphibian larvae, zooplankton and amphipods) and determined if they vary in  
35 their sensitivity to warming temperatures.  $CT_{max}$  differed between the groups, with predatory  
36 insects having higher  $CT_{max}$  than amphibians (both herbivorous larval anurans and predatory  
37 larval salamanders), amphipods and zooplankton. In a scenario of global warming, these  
38 differences in thermal tolerance may affect top-down and bottom-up processes, particularly  
39 considering that insect predators are more likely to maintain or improve their performance at  
40 higher temperatures, which could lead to increased predation rates on the herbivores in the  
41 food web. Further studies are needed to understand how the energy flows through  
42 communities, how species' energy budgets may change and whether other physiological and  
43 behavioral responses (such as phenotypic plasticity and thermoregulation) can buffer or  
44 increase these changes in the top-down regulation of wetland food webs.

45

46 Keywords: critical thermal maximum; top-down regulation; amphibians; insects;  
47 zooplankton; climate change

48 1. INTRODUCTION

49 Predicting the impacts of climate change and understanding species' responses to  
50 accelerating environmental changes has been a major challenge for the scientific community.  
51 There is increasing evidence that species' phenologies and distributions are changing in  
52 response to current climate change (e.g., Parmesan and Yohe 2003, Parmesan 2006).  
53 Renewed interest in thermal physiology has produced large databases that enable a global  
54 perspective of species' physiological limits and their relation to the environment (e.g.,  
55 Deutsch et al., 2008; Duarte et al., 2012; Katzenberger et al., 2014; Sunday et al., 2014;).  
56 These data have given new insights into how species (and some communities) may respond  
57 to current and future climate change and are providing a general assessment of which taxa  
58 may be the most vulnerable.

59 In spite of increasing information on the responses of species and populations to  
60 climate change, robust models of ecological systems are still badly needed to forecast the  
61 future state of communities and ecosystems under long-term environmental changes (Jochum  
62 et al., 2012; Shurin et al., 2012). However, the multitude of factors that affect ecosystems and  
63 their complex interactions have hindered the development of these models. Predicted  
64 environmental changes include an increase in the frequency of heat waves and other extreme  
65 events (Diffenbaugh and Ashfaq, 2010; Schär et al., 2004). Shifts in UV radiation,  
66 precipitation and temperature patterns are expected to vary geographically (IPCC, 2013).  
67 Furthermore, several aspects of the environment are simultaneously affected by geochemical  
68 cycles and changes in these cycles may influence local environmental conditions  
69 (Christensen et al., 2006; Vinebrooke et al., 2004). Apart from the physical environment data,  
70 models should also incorporate information about species' physiological and life-history  
71 traits. Differential evolutionary responses of species' physiological traits to climate change  
72 may also promote shifts in community interactions, food web dynamics and ecosystem

73 processes (Coulson et al., 2011; Gilman et al., 2010). However, current experimental  
74 evidence is limited (Jochum et al., 2012). Moreover, the direct effects on species' physiology  
75 and demographics may be further altered by indirect effects via ecological interactions in  
76 food webs (Bothwell et al., 1994; Ockendon et al., 2014; Suttle et al., 2007).

77         The critical thermal maximum ( $CT_{max}$ ) is the temperature at which an organism loses  
78 its ability to avoid the conditions that will lead to its death (Cowles and Bogert, 1944).  
79 Thermal performance curves are usually asymmetrical, with the interval between the  
80 optimum temperature and the critical thermal maximum commonly characterized by a steep  
81 decline in performance (Huey and Kingsolver, 1989). However, determining optimum  
82 temperature is usually methodologically more difficult than determining  $CT_{max}$  (e.g.,  
83 Katzenberger et al. 2014). Since optimum temperature and  $CT_{max}$  have been established as  
84 co-adaptive traits (Angilletta, 2009; Huey et al., 2009), a species with high  $CT_{max}$  is expected  
85 to also have a high optimum temperature. Hence, determining interspecific variation in  $CT_{max}$   
86 and warming tolerance (the difference between  $CT_{max}$  and current environmental  
87 temperatures) is a simple way to assess species vulnerability to the direct effects of climate  
88 change (Deutsch et al., 2008; Tewksbury et al., 2008).

89         Current global warming may promote changes in species interactions and community  
90 structure (Dell et al. 2011), particularly when species have contrasting thermal niches. A  
91 reduction in available niche space for species with low heat tolerance can potentially enhance  
92 density-dependent interactions, whereas species with high heat tolerance could benefit from  
93 the release of competitive pressures (Diamond et al., 2017). Warm-adapted consumers are  
94 also expected to exert increased top-down pressure, leading to a reduction in the biomass of  
95 species from the lower trophic levels if the latter are less heat tolerant (Urban et al., 2017).  
96 Species asymmetries in thermal responses may also affect the dynamics of consumer–  
97 producer interactions due to differences in activation energies (the life-dinner principle)

98 (Dawkins and Krebs, 1979; Dell et al., 2014, 2011). Top predators may be closer to their  
99 tolerance limits (Pincebourde et al., 2008; Twomey et al., 2012), with carnivores having peak  
100 performances at temperatures 10°C lower than herbivores (Dell et al., 2014; Voigt et al.,  
101 2003). However, much more information is needed to establish a comprehensive  
102 generalization of thermal tolerance across trophic levels.

103         Differences in thermal tolerance across trophic levels may also affect top-down and  
104 bottom-up effects in food webs (Relyea and Ricklefs, 2018). Warming can influence trophic  
105 interactions and alter the relative importance of these top-down and bottom-up effects by  
106 increasing the metabolic requirements of species (Dillon et al., 2010). Several studies have  
107 demonstrated that top-down effects increase with warming, strengthening the trophic cascade  
108 from consumers to producers (Hoekman, 2010; Jochum et al., 2012; Kratina et al., 2012;  
109 O'Connor et al., 2009; Shurin et al., 2012). This occurs when the metabolic requirements of  
110 consumers increase faster with temperature than that of producers (Allen et al., 2005),  
111 resulting in an increase in consumer activities (Dillon et al., 2010; Hoekman, 2010; O'Connor  
112 et al., 2009; Shurin et al., 2012). However, metabolic demands can increase faster than  
113 feeding rates (Rall et al., 2010) and increased feeding rates may also lead to resource  
114 competition. Moreover, decreased food intake may also reduce growth rates, reduce optimal  
115 temperatures, and reduce upper thermal limits for growth (Huey and Kingsolver, 2019).  
116 Therefore, in the long-term, consumers may suffer from reduced fitness, lower abundance  
117 and reduced biomass when compared to producers, thereby weakening the top-down effects  
118 (O'Connor et al., 2011). In addition, warming tends to favor organisms that compete better  
119 for nutrients (Falkowski and Oliver, 2007) and smaller organisms (Daufresne et al., 2009;  
120 Yvon-Durocher et al., 2011; Yvon-Durocher and Allen, 2012). Both of these factors should  
121 influence community size structure and alter species composition (Yvon-Durocher et al.,  
122 2011).

123 We aimed to understand how the current climate-change scenario may affect a  
124 temporary freshwater wetland community by determining the critical thermal maxima of  
125 species from different trophic levels. The species include primary consumers (tadpoles,  
126 zooplankton and amphipods) and secondary and tertiary consumers (salamander larvae and  
127 predatory insects, respectively; Fig. 1). These taxa have been used to study the dynamics of  
128 food web structure and the mechanisms controlling the bottom-up and top-down processes in  
129 temperate wetlands for several decades (Jones et al., 2016; Leibold and Wilbur, 1992; Stoler  
130 and Relyea, 2016; Wilbur, 1997).  $CT_{max}$  values may be phylogenetically constrained (Huey,  
131 1982; Huey et al., 2009; Kellermann et al., 2012), although they are adaptively associated  
132 with environmental temperatures even when controlling for phylogeny (e.g., Duarte et al.  
133 2012). Hence, we expected to find differences in thermal physiology among higher  
134 taxonomic groups (e.g., Duarte et al. 2012; Sunday et al. 2014) and among trophic levels.  
135 Based on previous studies, we expected predatory insect species to have higher  $CT_{max}$  values  
136 than amphibians (e.g., Sunday et al. 2014). We also expected body mass to be a good  
137 predictor of upper thermal resistance across species of ectotherms (Klockmann et al., 2017),  
138 although this relationship may not be evident within species (Duarte et al., 2012).

139

## 140 2. METHODS

### 141 2.1. *Field collection and animal husbandry*

142 In spring 2010, we collected 11 species of amphibians (egg masses), four species of  
143 aquatic insects, three species of amphipods and one species of zooplankton (Cladocera) from  
144 natural ponds and wetlands. Each species was collected at a single location (Tables 1-2). All  
145 animals were brought to the Pymatuning Laboratory of Ecology (University of Pittsburgh), in  
146 northwest Pennsylvania, USA. Microenvironmental pond temperatures were measured in  
147 seven locations by placing HOBO Pendant® temperature dataloggers in most collection sites

148 at the deepest part of the pond (Table 2). When thermal stratification occurs, usually during  
149 sunny days with little to no wind (Boeckman and Bidwell, 2015), these measurements are  
150 assumed to represent the minimum environmental temperatures to which the animals are  
151 exposed during the time period considered; other locations within the pond (shallower  
152 locations or higher in the water column) presumably had higher temperatures (Bancroft et al.,  
153 2008; Boeckman and Bidwell, 2015; Oberle et al., 2019; Song et al., 2013). Water  
154 temperature was recorded every 5–15 min during the period in which most species in the  
155 community were present.

156 The species we used belong to different trophic levels of a wetland food web,  
157 including herbivores, detritivores, and predators (Figure 1). Most of these species overlap in  
158 distribution so they can co-occur (at least partially) and interact. Since feeding preferences of  
159 anuran larvae depend on species and may include multiple resources, such as periphyton,  
160 zooplankton, phytoplankton and detritus (Altig et al., 2007; Arribas et al., 2015; Carreira et  
161 al., 2016; Montaña et al., 2019), we considered several potential energy pathways for this  
162 group. In the laboratory, the zooplankton and amphipod species were kept in plastic  
163 containers (40 x 25 x 20 cm) with approximately 10 L, to which an aliquot of filtered (397-  
164 mm net) local pond water was added as source of algae. Aquatic insects were kept  
165 individually in 500-mL plastic cups (filled with 400 mL of water) and fed tadpoles every 2 d.  
166 Outdoor pools were filled with aged well water to accommodate the amphibian eggs. All egg  
167 masses from the same species were placed together in the same pool. Tadpoles were then fed  
168 rabbit pellets *ad libitum* and allowed to grow until reaching the desired developmental stage.  
169 Moreover, salamander larvae were fed zooplankton *ad libitum*.

170

171 *2.2. Critical thermal maximum assessment*

172           Sets of amphibian aquatic larvae (anuran tadpoles and larval salamanders) were  
173 brought indoors to acclimate for the experiment. Amphibian larvae were tested at a  
174 developmental stage where they were able to swim freely and begin feeding independently.  
175 In most anuran species, this occurs after reaching Gosner stage 25 (Gosner, 1960). Other  
176 organisms were tested at the same developmental stage as they were when collected (Table  
177 3). Amphibian larvae, insects, amphipods and zooplankton were kept at an acclimation  
178 temperature of 20°C (approximately the average temperature experienced in the outdoor  
179 pools), with a 12L:12D photoperiod, for four days, as in previous studies (Duarte et al., 2012;  
180 Gutiérrez-Pesquera et al., 2016; Simon et al., 2015). This allowed to the animals to acclimate  
181 to the lab temperature and stabilize their  $CT_{max}$  (Allen et al., 2012; Brattstrom, 1968;  
182 Buchanan et al., 1988; Hutchison, 1961). After the acclimation period, the species were tested  
183 for their  $CT_{max}$  (Brattstrom, 1968; Hutchison, 1961) using Hutchison’s dynamic method  
184 (Lutterschmidt and Hutchison, 1997a).

185           The  $CT_{max}$  trials used a water bath which consisted of a 250-mL container filled with  
186 200 mL of dechlorinated water at 20 °C placed within a larger 2-L container, set upon a  
187 magnetic stirrer hotplate. Water temperature was measured in the smaller container, whereas  
188 the magnetic stirrer was placed in the larger container to avoid perturbing the organisms. We  
189 exposed the organisms to a constant heating rate of 1.0 °C min<sup>-1</sup>, as in previous studies  
190 (Duarte et al., 2012; Simon et al., 2015). This heating rate is fast enough to avoid acclimation  
191 during the  $CT_{max}$  trials but also slow enough to avoid both heat shock and a significant lag  
192 between water and body temperatures (Lutterschmidt and Hutchison, 1997a, 1997b). While  
193 approaching their upper thermal limit, organism first lose their righting response, then go  
194 through a stage of complete immobility before reaching the onset of spasms, which precedes  
195 death (Lutterschmidt and Hutchison, 1997a, 1997b). For tadpoles, the onset of spasms was  
196 considered the experimental endpoint. In the case of the insects, amphipods and zooplankton,



197 since spasms could not be observed during the experiment, we used complete immobility as  
198 the endpoint of the critical thermal tolerance experiments. Once  $CT_{max}$  was reached, we  
199 placed all organisms into cooler water (20°C) to allow for complete recovery. Those  
200 individuals unable to recover were excluded for the analyses. All experiments were approved  
201 by the Institutional Animal Care and Use Committee from the University of Pittsburgh  
202 (Protocol #12050451).

203

### 204 2.3. *Statistical analysis*

205 To determine how the upper thermal limits varied across species, we conducted a  
206 generalized linear model (log-linked gamma distribution) using  $CT_{max}$  as the dependent  
207 variable, species as a categorical factor and mass as a covariate (including the interaction  
208 between species and mass). Although it is recommended to incorporate phylogenetic  
209 information (Felsenstein, 1985; Garland Jr. et al., 1992), we could not implement a PGLS  
210 analysis since the number of species (n=19) is below the recommended threshold of >20  
211 (Blomberg et al., 2003). Therefore, to account for the non-independence of species, we also  
212 conducted a generalized linear mixed model (log-linked gamma distribution), that included  
213 the taxonomic levels (from species to phylum) as nested, random effects (Seebacher et al.,  
214 2015). We then compared both models using the Akaike information criterion (AIC) (Akaike,  
215 1974), to determine if the inclusion of higher taxonomic levels improved our model. Next, we  
216 conducted Tukey HSD post-hoc tests to see which species differed from each other. To  
217 examine the potential link between body size and  $CT_{max}$ , we also assessed the relationship  
218 between  $CT_{max}$  and mass, within each species, using Pearson's correlation coefficient.

219 Our second analysis examined the effects of trophic level on  $CT_{max}$ . For each species,  
220 trophic level was determined as the longest chain length from a consumer to a basal species,  
221 plus one (Pimm, 1980). The obtained trophic levels were then used as a grouping variable in

222 subsequent analysis (Table 3). Amphipods and zooplankton were grouped together  
223 (Zoo/Apod group) since they represent the main phytoplankton and periphyton consumers.  
224 Similar to the previous analyses, we conducted two models to determine how the upper  
225 thermal limits varied across trophic levels: 1) a generalized linear model (log-linked gamma  
226 distribution) using  $CT_{max}$  as the dependent variable, trophic group as a categorical factor and  
227 mass as a covariate (including the interaction between trophic group and mass); and 2) a  
228 generalized linear mixed model (log-linked gamma distribution), that included the taxonomic  
229 levels (from species to phylum) as nested, random effects. Again, both models were  
230 compared using AIC, to determine if the model can be improved by including taxonomic  
231 levels as random factors. Tukey HSD post-hoc tests were then conducted to see which trophic  
232 groups differed from each other. All analyses were performed in R version.3.6.5 (R  
233 Development core team, 2020) and conducted at the significance level of  $\alpha=0.05$ .

234

### 235 3. RESULTS

236 Critical thermal maximum differed among species ( $F_{18,163}=306.3$ ,  $p<0.001$ , Table 3),  
237 with a significant positive effect of body mass ( $F_{1,163}=4.8$ ,  $p=0.030$ ), as well as a significant  
238 interaction between species and body mass ( $F_{17,163}=1.9$ ,  $p=0.019$ ). The addition of taxonomy  
239 as a random factor did not improve the model (without taxonomy,  $AIC=312.5$ ; with  
240 taxonomy,  $AIC= 326.5$ ) and results were similar for the effects of species, body mass and  
241 their interaction on  $CT_{max}$  (Table 4). When assessing the correlation between  $CT_{max}$  and body  
242 mass within each species, only *R. pipiens* (Pearson's  $r=0.677$ ,  $p=0.006$ ) and *P. triseriata*  
243 (Pearson's  $r=0.644$ ,  $p=0.01$ ) had a positive correlation while *E. simplicollis* (Pearson's  $r=-$   
244  $0.937$ ,  $p=0.018$ ) had a negative correlation.

245 The  $CT_{max}$  values (means  $\pm 1$  SE) differed between trophic levels ( $F_{3,192}=337.3$ ,  
246  $p<0.001$ ), with predatory insects ( $45.2$  °C  $\pm 0.1$ ) showing higher values than larval anurans

247 (39.3 °C ± 0.1), larval salamanders (37.5 °C ± 0.1) and zooplankton/amphipods (39.5 °C ±  
248 0.2, all  $p < 0.001$ ). Anurans and zooplankton/amphipods did not differ in their  $CT_{max}$   
249 ( $p = 0.886$ ); however, larval salamanders presented lower  $CT_{max}$  values than either of these  
250 two groups ( $p < 0.001$ ). However, there was no relation between  $CT_{max}$  and either body mass  
251 ( $F_{1,192} = 0.02$ ,  $p = 0.885$ ) or the interaction of trophic level with body mass ( $F_{3,192} = 0.8$ ,  $p = 0.506$ )  
252 in this model. The inclusion of taxonomy as a random factor improved the model (without  
253 taxonomy,  $AIC = 573.5$ ; with taxonomy,  $AIC = 345.7$ ) and also allowed us to identify a  
254 significant effect of body mass on  $CT_{max}$  but not of the interaction between trophic level and  
255 body mass (Table 5). However, there is collinearity among taxonomy and trophic level; since  
256 the predators feeding at the highest trophic level (i.e. 5) were all insects. Although these  
257 insect species were also amongst the largest in size, their  $CT_{max}$  is higher than amphibian  
258 species of similar body mass (Table 3).

259

#### 260 4. DISCUSSION

261 In our tests of  $CT_{max}$  among 19 species, we discovered that thermal responses differed  
262 among trophic levels, with insect predators exhibiting much higher  $CT_{max}$  values than the  
263 lower trophic levels represented by larval anurans, larval salamanders, zooplankton, and  
264 amphipods. These findings are similar to a previous study on terrestrial insect communities  
265 (Franken et al., 2018) but differ from others on marine aquatic communities (Noyola Regil et  
266 al., 2015; Vinagre et al., 2019, 2018), where differences in thermal sensitivities of top  
267 predators versus other consumers were not evident. Our  $CT_{max}$  estimates are within the range  
268 published for tadpoles (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016; Katzenberger et  
269 al., 2018; Miller and Packard, 1977), cladocerans (Brans et al. 2017), and predatory insect  
270 species (Dallas and Rivers-Moore, 2012; Martin et al., 1976; May, 1978; Op de Beeck et al.,  
271 2017). The collinearity between taxonomy and trophic level hinders the interpretation of

272 which of these factors (or the combination of both) is more important in determining  $CT_{max}$ .  
273 Therefore, further conclusions on this topic should be taken carefully. Nevertheless, this does  
274 not change the observed  $CT_{max}$  pattern for this community with low species richness.

275         Body mass was a good predictor of  $CT_{max}$  and the insect species that presented the  
276 highest  $CT_{max}$  were amongst the largest species in this study (Table 3). While we  
277 incorporated phylogeny in our analyses because mass can be confounded with phylogeny, its  
278 inclusion did not improve the model fit. The insect species with the highest  $CT_{max}$ , *Erythemis*  
279 *simplicollis*, had a similar body size to several anuran larvae with much lower  $CT_{max}$ , which  
280 suggests that while mass matters, other factors may also affect  $CT_{max}$  values. Moreover, 16 of  
281 the 19 species showed no relation between body mass and  $CT_{max}$ , perhaps due to the range of  
282 body mass within species being too small. A disparity in the effect of size on  $CT_{max}$  between  
283 intraspecific and community-level analysis was also found in some tropical ant communities  
284 (Nowrouzi et al., 2018). Among the aquatic insect families, Notonectidae and Libellulidae are  
285 known to exhibit high thermal tolerance (Dallas and Rivers-Moore, 2012), indicating that  
286 those phylogenetic lines may be well adapted to cope with higher environmental  
287 temperatures. Moreover, there is a general understanding that the body size of many  
288 organisms is declining due to climate warming (Ohlberger, 2013) and that body size may  
289 constrain their thermal tolerance (Klockmann et al., 2017; Peck et al., 2009).

290         Air breathing may ameliorate the effects of low oxygen availability in the water  
291 associated with high environmental temperatures. As a result, the ability of our aquatic  
292 species to breathe air could play an important role in tolerating high temperatures. Terrestrial  
293 (air-breathing) arthropods (Franken et al., 2018) have higher  $CT_{max}$  values than other aquatic  
294 arthropods (Vinagre et al. 2019). Among our four insect species, the *L. americanus* and  
295 *Notonecta* sp. get their oxygen from atmospheric air whereas the larvae of *A. longipes* and *E.*  
296 *simplicollis* use tracheal gills to obtain the oxygen dissolved in the water (Triplehorn et al.,

297 2005). Despite these two different methods of obtaining oxygen, all four insect species had  
298 high thermal tolerance. Moreover, many species of tadpoles (including some species studied  
299 here), larval salamanders, and some species of snails can engage in a “bubble-sucking”  
300 behaviour, which allows these species to circumvent water’s surface tension and also breathe  
301 air (Schwenk and Phillips, 2020).

302         Although a complete thermal performance curve would provide a full assessment of  
303 thermal tolerance, the biological significance of  $CT_{max}$  and its relationship with optimum  
304 temperature allows us to infer species vulnerability to global warming (Angilletta, 2009;  
305 Huey et al., 2012, 2009). The IPCC (2013) predicts an increase in average air temperature of  
306 4°C in the region during the next 80 years, with an associated increase in the frequency and  
307 duration of heat waves (Schär et al., 2004). While current environmental temperatures are not  
308 close to the upper thermal limits of the 19 species, some of these species already experience  
309 maximum environmental temperatures very close or above their optimum temperature for  
310 locomotor performance (Katzenberger, 2014). Moreover, if aquatic habitats suffer similar  
311 thermal changes to those predicted in air temperature models, many species will probably be  
312 exposed to temperatures that approach their physiological limits, where performance steeply  
313 declines due to the asymmetrical nature of the thermal performance curves (Huey and  
314 Kingsolver, 1989). Amelioration of the possible direct negative effects of increasing  
315 environmental temperatures will depend on species’ abilities to avoid harmful temperatures  
316 (e.g., behavioural thermoregulation) or to change their physiological limits by either  
317 phenotypic plasticity or rapid evolution (e.g., Logan et al. 2014; Seebacher et al. 2015).  
318 However, since  $CT_{max}$  can be phylogenetically constrained (Araújo et al., 2013; Huey, 1982;  
319 Kellermann et al., 2012) and if species with low  $CT_{max}$  cannot disperse, their reliance on  
320 behavioral thermoregulation and other mechanisms might prompt energetic imbalance and  
321 other physiological costs (Sinervo et al., 2010), including performance reduction (Gilbert and

322 Miles 2017). Moreover, faster pond desiccation associated with higher environmental  
323 temperatures may affect organism developmental rates and incur in additional stress (Gomez-  
324 Mestre et al., 2013).

325         Several studies have demonstrated shifts in community structure associated with  
326 climate change and its effect on the strength of top-down and bottom-up processes (Anderson  
327 and Piatt, 1999; Kratina et al., 2012; Litzow and Ciannelli, 2007; O'Connor et al., 2009;  
328 Shurin et al., 2012; Yvon-Durocher et al., 2010). Metabolic theory predicts that top predators  
329 should be most vulnerable to environmental warming, due to energetic and morphological  
330 constraints on trophic position (Arim et al., 2007; O'Gorman et al., 2019). Some microcosm  
331 experiments support this prediction (Petchey et al., 1999). Such effects could lead to  
332 truncated food chains because of greater local extinction rates by top predators. However,  
333 phenological mismatches between organisms and their food (Both et al., 2009), potential  
334 changes in species feeding preferences (Carreira et al., 2016) and different thermal  
335 physiology limits among the community components (this study; Peck et al. 2009), may also  
336 affect the importance of top-down and bottom-up processes. Additional changes in preferred  
337 body temperature and other thermal physiology traits may occur as a result of low resource  
338 availability (Gilbert and Miles, 2016), further affecting community dynamics.

339         Predicting impacts on the food web may also depend on population location. Several  
340 studies show intraspecific variation in thermal physiology parameters across populations  
341 (Brattstrom, 1968; Huang and Tu, 2008; Hutchison, 1961; Richter-Boix et al., 2015;  
342 Terblanche et al., 2006; Winne and Keck, 2005), although this variation in  $CT_{max}$  was  
343 generally much lower than the variation we found (approx. 6°C) between predatory insects  
344 and the other trophic levels. However, whether this indicates that some populations are less  
345 vulnerable to global warming than others still depends on how close each population's  $CT_{max}$   
346 is to each population's environmental temperatures. Nevertheless, variation in the thermal

347 profiles among populations can be quite significant (Table 2; Duarte et al. 2012; Richter-Boix  
348 et al. 2015; Gutiérrez-Pesquera et al. 2016). Hence, predicted effects of global warming on  
349 this food web may vary substantially according to habitat characteristics and location,  
350 ranging from no impact to strong impacts on the community structure and the strength of top-  
351 down/bottom up processes.

352

## 353 5. CONCLUSIONS

354 Our study indicates that the insect predators have a substantially higher  $CT_{max}$  ( $> 5^{\circ}C$ )  
355 than all other consumers. We cannot unconfound whether this is due to trophic level,  
356 phylogeny, body mass or their interaction, and future studies on communities with high  
357 species richness could provide further insights on this topic. Regardless, in warmer  
358 environmental conditions, these predators are more likely to improve or maintain their  
359 performance at higher temperatures, which could lead to a stronger top-down regulation.  
360 However, predictions based solely on physiological data, although important, may not tell the  
361 full story regarding each species' ability to cope with global warming. Thermal physiology  
362 should be complemented with additional data on behavioral responses, phenotypic plasticity  
363 of traits and evolutionary potential. A better understanding of trait variation across  
364 populations is also instrumental to improve the extrapolation potential of multi-species  
365 studies (Dowd et al., 2015; Herrando-Pérez et al., 2019). In addition, current knowledge  
366 regarding the impact of temperature changes at a community level is still limited (Kratina et  
367 al., 2012; Montoya and Raffaelli, 2010; Urban et al., 2017; Walther, 2010). Long-term  
368 monitoring of freshwater communities and experimental approaches under natural or semi-  
369 natural conditions (e.g., mesocosms) with diverse food webs are needed to determine if these  
370 physiological results translate to field observations. In this way, we can more fully

371 understand the temporal and spatial dynamics of these freshwater food webs under the  
372 current and future global warming.

373

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381

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672 Table 1. Collection sites, number of organisms used (N), life-stage and food web groups of the 19 studied species from a freshwater community.

<b>Species</b>	<b>Common name</b>	<b>N<sup>‡</sup></b>	<b>Food Web Group</b>	<b>Life-stage</b>	<b>Pond</b>
<i>Ambystoma laterale</i>	Blue-spotted salamander	12 (10)	amphibian	larvae	Edwin S. George Reserve
<i>Anaxyrus americanus</i>	American toad	15 (12)	amphibian	larvae	Oberdick
<i>Hyla versicolor</i>	Gray treefrog	15 (9)	amphibian	larvae	Mallard Pond B
<i>Pseudacris crucifer</i>	Spring peeper	15 (10)	amphibian	larvae	Trailer Pond
<i>Pseudacris feriarum</i>	Upland chorus frog	15 (20)	amphibian	larvae	Seven Island Reserve
<i>Pseudacris triseriata</i>	Western chorus frog	15 (23)	amphibian	larvae	Edwin S. George Reserve
<i>Rana catesbeiana</i>	American bullfrog	15 (10)	amphibian	larvae	Love Pond
<i>Rana clamitans</i>	Green frog	15 (11)	amphibian	larvae	Oberdick
<i>Rana palustris</i>	Pickerel frog	10 (5)	amphibian	larvae	Seven Island Reserve
<i>Rana pipiens</i>	Northern leopard frog	15 (7)	amphibian	larvae	Mallard Pond B
<i>Rana sylvatica</i>	Wood frog	15 (10)	amphibian	larvae	Mallard Pond A
<i>Anax longipes</i>	Comet darner	15	insect	larvae	Edwin S. George Reserve
<i>Erythemis simplicollis</i>	Common pondhawk	5	insect	larvae	Geneva Pond A
<i>Lethocerus americanus</i>	Giant water bug	5	insect	adult	Geneva Pond B
<i>Noctonecta</i> sp.	Common backswimmer	5	insect	adult	Geneva Pond A
<i>Daphnia magna</i>	Waterflea	5	zooplankton	adult	Love Pond
<i>Hyaella</i> sp.	Sideswimmer (scud)	5	amphipod	adult	Lake LeBoeuf
<i>Hyaella spinicauda</i>	Sideswimmer (scud)	5	amphipod	adult	Lake LeBoeuf
<i>Hyaella wellborni</i>	Sideswimmer (scud)	5	amphipod	adult	Lake LeBoeuf

673 <sup>‡</sup> For amphibians, number of clutches collected is shown in parentheses.

675 Table 2. Location, altitude (meters) and thermal profile (in °C) of the ponds where the 19 studied species were collected. The time period considered  
676 encompasses the months of 2010 (number of days in parentheses), from the collection of organisms and placing of dataloggers until the water  
677 bodies dried out or most of the species in the community were absent.

<b>Pond</b>	<b>Location</b>	<b>Latitude/ Longitude</b>	<b>Alt<sup>£</sup></b>	<b>T<sub>max</sub></b>	<b>T<sub>avg</sub></b>	<b>T<sub>min</sub></b>	<b>DTF<sub>max</sub></b>	<b>DTF<sub>avg</sub></b>	<b>DTF<sub>min</sub></b>	<b>period</b>
E.S. George Res.	Livingston County, MI	42°27'30.03"N, 84° 0'41.20"W	304	20.8	14.7	6.7	5.2	1.8	0.1	April-June (66)
Geneva Pond A	Crawford County, PA	41°35'18.02"N, 80°14'41.01"W	325	-	-	-	-	-	-	-
Geneva Pond B	Crawford County, PA	41°35'17.10"N, 80°14'29.91"W	327	-	-	-	-	-	-	-
Lake LeBoeuf	Crawford County, PA	41°55'59.64"N, 79°58'58.01"W	357	-	-	-	-	-	-	-
Love Pond	Crawford County, PA	41°41'08.60"N, 80°30'48.26"W	311	31.0	24.6	10.7	10.7	1.7	0.3	May-October (126)
Mallard Pond A	Crawford County, PA	41°41'30.09"N, 80°30'02.91"W	318	28.9	17.0	7.1	15.4	7.2	1.1	April-June (60)
Mallard Pond B	Crawford County, PA	41°41'27.96"N, 80°29'57.07"W	317	29.5	18.1	7.0	13.8	4.4	0.8	April-July (87)
Oberdick	Crawford County, PA	41°41'16.33"N, 80°25'33.89"W	333	25.7	20.2	9.7	8.3	3.2	0.4	April-October (187)
Seven Island Res.	Blount County, TN	35°37'35.96"N, 83°41'00.53"W	482	27.3	20.7	14.1	7.0	4.7	1.9	April-May (34)
Trailer Pond <sup>¥</sup>	Crawford County, PA	41°34'08.60"N, 80°27'09.03"W	371	26.1	16.3	5.6	8.8	4.0	0.6	April-July (104)

678 <sup>¥</sup> data from Katzenberger et al. 2018

679 <sup>£</sup> Alt, altitude in meters; T<sub>max</sub>, maximum environmental temperature; T<sub>avg</sub>, average environmental temperature; T<sub>min</sub>, minimum environmental  
680 temperature; DTF<sub>max</sub>, maximum diel temperature fluctuation; DTF<sub>avg</sub>, average diel temperature fluctuation; DTF<sub>min</sub>, minimum diel temperature  
681 fluctuation.

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683

684 Table 3. Critical thermal maximum ( $CT_{max}$ ;  $^{\circ}C \pm SE$ ), mass ( $mg \pm SE$ ), food web group,  
 685 trophic level, and Tukey HSD results for 19 species from a temperate wetland food web.  
 686 Each letter of Tukey HSD corresponds to a potential grouping of species according to the  
 687 post-hoc test results. Order of species was changed to ease visualization of Tukey HSD  
 688 grouping.

689

Species	Food Web Group	Trophic Level	Tukey HSD grouping	$CT_{max}$ ( $^{\circ}C$ )	Mass (mg)
<i>Ambystoma laterale</i>	amphibian	4	a	$37.5 \pm 0.1$	$79.1 \pm 7.7$
<i>Rana pipiens</i>	amphibian	3	ab	$38.0 \pm 0.1$	$119.3 \pm 13.4$
<i>Rana sylvatica</i>	amphibian	3	abc	$38.2 \pm 0.1$	$214.2 \pm 16.1$
<i>Rana catesbeiana</i>	amphibian	3	d	$38.7 \pm 0.1$	$43.9 \pm 3.8$
<i>Hyalella spinicauda</i>	amphipod	2	cde	$38.9 \pm 0.1$	$0.302 \pm 0.008$
<i>Hyalella wellborni</i>	amphipod	2	de	$39.2 \pm 0.2$	$0.316 \pm 0.032$
<i>Daphnia magna</i>	zooplankton*	2	de	$39.2 \pm 0.2$	$0.179 \pm 0.018^{\text{¥}}$
<i>Pseudacris crucifer</i>	amphibian	3	de	$39.3 \pm 0.1$	$84.6 \pm 11.1$
<i>Pseudacris feriarum</i>	amphibian	3	de	$39.2 \pm 0.1$	$133.6 \pm 8.8$
<i>Pseudacris triseriata</i>	amphibian	3	de	$39.2 \pm 0.1$	$129.0 \pm 6.4$
<i>Rana palustris</i>	amphibian	3	bcde	$39.4 \pm 0.1$	$875.9 \pm 105.3$
<i>Rana clamitans</i>	amphibian	3	ef	$39.7 \pm 0.1$	$37.6 \pm 3.4$
<i>Hyla versicolor</i>	amphibian	3	f	$40.3 \pm 0.1$	$134.2 \pm 14.1$
<i>Hyalella</i> sp.	amphipod	2	fg	$40.5 \pm 0.1$	$0.405 \pm 0.047$
<i>Anaxyrus americanus</i>	amphibian	3	g	$41.0 \pm 0.2$	$56.0 \pm 4.1$
<i>Noctonecta</i> sp.	insect	5	h	$43.9 \pm 0.2$	$113.2 \pm 5.5$
<i>Anax longipes</i>	insect	5	hi	$45.2 \pm 0.1$	$2249.9 \pm 58.9$
<i>Lethocerus americanus</i>	insect	5	hi	$45.9 \pm 0.2$	$900.6 \pm 168.4$
<i>Erythemis simplicollis</i>	insect	5	i	$46.0 \pm 0.1$	$247.1 \pm 20.2$

690 \* *Daphnia magna* was grouped with amphipods for community analysis (Zoo/Apod group).<sup>¥</sup>

691 Mass values for *Daphnia magna* were obtained from Simcic and Brancelj (1997).

692

693 Table 4. Generalized linear mixed model (log-link gamma distribution) to determine the  
 694 effects of species, body mass and their interaction on  $CT_{max}$ . Taxonomic levels from species  
 695 to phylum were included as nested, random effects to account for the non-independence of  
 696 related species.  
 697

<b>Fixed effects</b>	<b>df</b>	<b>Sum sq</b>	<b>Mean Sq</b>	<b>F</b>	<b><i>p</i></b>
species	18	0.6346	0.0353	306.3	< 0.001
mass	1	0.0006	0.0006	4.8	0.030
species*mass	17	0.0038	0.0002	1.9	0.019
<b>Random effects</b>		<b>Variance ± SD</b>			
phylum		0.00000 ± 0.00000			
class		0.00000 ± 0.00000			
order		0.00000 ± 0.00000			
family		0.00000 ± 0.00000			
genus		0.00000 ± 0.00000			
species		0.00000 ± 0.00000			
Residual		0.00012 ± 0.01073			

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700

701 Table 5. Generalized linear mixed model (log-link gamma distribution) to determine the  
 702 effects of trophic level, body mass and their interaction on  $CT_{max}$ . Taxonomic levels from  
 703 species to phylum were included as nested, random effects to account for the non-  
 704 independence of related species.  
 705

<b>Fixed effects</b>	<b>df</b>	<b>Sum sq</b>	<b>Mean Sq</b>	<b>F</b>	<b><i>p</i></b>
trophic	3	0.0820	0.0273	150.2	< 0.001
mass	1	0.0011	0.0011	5.8	0.017
trophic*mass	3	0.0003	0.0001	0.6	0.636
<b>Random effects</b>		<b>Variance ± SD</b>			
phylum		<0.00001 ± <0.00001			
class		<0.00001 ± 0.00004			
order		<0.00001 ± 0.00005			
family		<0.00001 ± 0.00011			
genus		0.00002 ± 0.00426			
species		0.00010 ± 0.00977			
Residual		0.00018 ± 0.01349			

706



707 Figure 1. Simplified wetland food web with trophic relations between the studied species  
708 (modified from Wilbur 1997). Solid arrows indicate the primary direction of energy flow.  
709 Dotted arrows indicate potential energy flow pathways, depending on tadpole species (Altig et  
710 al., 2007; Arribas et al., 2015; Montaña et al., 2019). Dashed arrow indicates a secondary  
711 energy flow that occurs when salamander larvae grow bigger than some tadpole species and  
712 are then able to consume them. A) predatory insects: *Anax longipes*, *Erythemis simplicollis*,  
713 *Lethocerus americanus* and *Noctonecta* sp.. B) larval salamanders: *Ambystoma laterale*. C)  
714 larval anurans: *Anaxyrus americanus*, *Hyla versicolor*, *Pseudacris crucifer*, *P. feriarum*, *P.*  
715 *triseriata*, *Rana catesbeiana*, *R. clamitans*, *R. palustris*, *R. pipiens* and *R. sylvatica*. D)  
716 zooplaknton: *Daphnia magna*. E) Amphipods: *Hyaella* sp., *H. spinicauda* and *H. wellborni*.  
717

718 Figure 2. Boxplot (median and quartiles) of critical thermal maximum ( $CT_{max}$ ) of the studied  
719 species. Species were grouped according to their taxonomy. Amphipods and zooplankton  
720 were grouped together (Zoo/Apod group) since they represent the main phytoplankton  
721 consumers. Dashed lines indicate the average  $CT_{max}$  for each group and the respective  
722 standard deviation. Species: ALO, *Anax longipes*; ESI, *Erythemis simplicollis*; LAM,  
723 *Lethocerus americanus*; NOC, *Noctonecta* sp.; ALA, *Ambystoma laterale*; AAM, *Anaxyrus*  
724 *americanus*; HVE, *Hyla versicolor*; PCR, *Pseudacris crucifer*; PFE, *P. feriarum*; PTR, *P.*  
725 *triseriata*; LCA, *Rana catesbeiana*; RCL, *R. clamitans*; RPA, *R. palustris*; RPI, *R. pipiens*;  
726 RSY, *R. sylvatica*; DMA, *Daphnia magna*; HYA, *Hyaella* sp.; HSP, *H. spinicauda*; HWE,  
727 *H. wellborni*.

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