1 **Trophic interactions modify the temperature dependence of community biomass and**

2 **ecosystem function**

3

4 **Running head: ecological effects of temperature**

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1

Abstract

Aquatic ecosystems worldwide continue to experience unprecedented warming and ecological change. Warming increases metabolic rates of animals, plants and microbes, accelerating their use of energy and materials, their population growth and interaction rates. At a much larger biological scale, warming accelerates ecosystem level processes, elevating fluxes of carbon and oxygen between biota and the atmosphere. Though these general effects of temperature at finer and broader biological scales are widely observed, they can lead to contradictory predictions for how warming affects the structure and function of ecological communities at the intermediate scale of biological organization. We experimentally tested the hypothesis that the presence of predators and their associated species interactions modify the temperature-dependence of net ecosystem oxygen production and respiration. We tracked a series of independent freshwater ecosystems (370-L) over 9 weeks, and we found that at higher temperatures, cascading effects of predators on zooplankton prey and algae were stronger than at lower temperatures. When grazing was weak or absent, standing phytoplankton biomass declined by 85-95% (< 1-fold) over the temperature gradient (19-30 °C), and by 3-fold when grazers were present and lacked predators. Surprisingly, these temperature-dependent species interactions and consequent community biomass shifts occurred without signs of species loss or community collapse, and only modestly affected the temperature dependence of net ecosystem oxygen fluxes. The exponential increases in net ecosystem oxygen production and consumption were relatively insensitive to differences in trophic interactions among ecosystems. Furthermore, monotonic declines in phytoplankton standing stock suggested no threshold effects of warming across systems. We conclude that local changes in community structure, including temperature-dependent trophic cascades, may be compatible with prevailing and predictable effects of temperature on ecosystem functions related to fundamental effects of temperature on metabolism.

Introduction

Temperature affects metabolic rates of all organisms, thereby affecting ecological patterns and processes across scales of organization – from individuals to ecosystems. Increasing temperature accelerates major metabolic processes that drive net ecosystem production and ecosystem respiration in aquatic and terrestrial ecosystems [1-4]. Highly conserved metabolic processes - photosynthesis and aerobic respiration [5] - power somatic growth, maintenance and activity in aerobic organisms. As a result, the effects of temperature on cellular photosynthesis and respiration have accurately described the exponential increases in ecosystem-scale ecosystem productivity (NEP) and respiration (ER) in aquatic systems across macroecological thermal gradients, after accounting for body size, nutrient content and light availability [4,6,7]. The ecological importance of temperature-dependent *per capita* metabolic rates has supported the use of metabolic models to understand and predict ecological change from local to global scales [3,4,8]. This has been a general theme in the metabolic theory of ecology (MTE). Models that associate change in ecosystem scale metabolism (e.g., oxygen or carbon flux) with individual-level oxygen production and respiration, but bypass the complexity of population and community dynamics at intermediate biological scales, provide much needed predictability for how climate change affects ecosystem functions when ecosystems are compared across broad spatial or temporal thermal gradients [2,4,9].

Reconciling the high explanatory power of general temperature-dependent metabolic scaling models at macroecological scales with the well-documented contingencies of how temperature affects community level outcomes of population dynamics and species interactions at intermediate scales has been challenging [10-17]. Whether at macro-ecological or community (e.g., single-site) scales, ecosystem-level functions (ER, NEP) or biomass is simply the sum of *per capita* function (respiration, net photosynthesis) and biomass. Metabolic theory models applied at macro-ecological scales assume that the relationship

76   between temperature and community-level distributions of body sizes and traits is constant in

77   time, or that communities are at stable state so that descriptions of community structure apply

78   to future states of the community under the same abiotic conditions [10-12]. Yet, at local

79   scales, species interactions can influence biomass of primary producers, and the strength and

80   outcomes of species interactions reflect dynamical processes that are often sensitive to

81   temperature [13-15]. For example, the presence of fish in experimental aquatic ponds reversed

82   a negative effect of temperature on algal biomass to a positive effect, mediated by trophic

83   interactions between fish, zooplankton and phytoplankton [16], under otherwise constant

84   consistent abiotic conditions across ponds. Understanding how temperature dependent species

85   interactions affect biomass, size distributions and traits remains a challenge. For example,

86   why do temperature-dependent species interactions influence the effects of temperature on

87   community properties such as biomass, abundance and body size, but have little or no

88   apparent effect on the variation in ecosystem functions (NEP, ER) over macro-ecological

89   scales? This challenge is central to efforts to apply general models of metabolic temperature

90   dependence to communities [17,18]. This paradox between macroecological patterns – which

91   can be consistent with direct scaling of *per capita* thermal responses - and results of smaller

92   scale, short term experiments that allow population dynamics to play out over intermediate

93   time scales, leads to the suggestion that general metabolic scaling models that do not consider

94   the complexities associated with species interactions do not apply at the local scales [19].

95   Reconciling these apparently divergent patterns is critical to improving understanding and

96   projections of how shifting global thermal regimes affect ecological patterns and processes

97   across scales and achieving a more unified understanding of ecology across scales.

98   One way to reconcile the apparent context dependence of empirical results under

99   controlled conditions with the generality of temperature dependence of ecosystem function at

100   broader scales is to consider how the direct and indirect effects of temperature on population

101   dynamics interact. Direct effects of temperature on *per capita* metabolic rates cause

102   organismal photosynthesis and respiration rates to increase exponentially when temperatures

103   increase as long as resources are not limiting in algae and animals, up to an optimal

104   temperature. This relationship between temperature and fundamental metabolic rates

105   (photosynthesis and respiration) is referred to as general metabolic scaling [1]. For any single

106   phenotype, performance above some optimal temperature declines due to stress responses and

107   metabolic scaling no longer explains the effects of temperature on performance. However, in

108   multi-species communities the signal of metabolic scaling is likely to dominate over a broad

109   range of temperatures if species with distinct thermal phenotypes can compensate for each

110   other along the thermal gradient [10,20]. Warming is also associated with other biological

111   changes that affect species interactions, such as reductions in body size (the temperature size

112   rule, [21-23]), fecundity, and attack rates (Fig 1) [24-27], and these changes can feed back to

113   influence community-level biomass and productivity [28-30].

114

115   **Figure 1.** A) Temperature and predation directly and indirectly affect population density and

116   metabolic rates in aquatic communities. In our experimental communities, predation directly

117   (solid lines) affects the abundance, size and species composition of prey, and predation by

118   notonectids on grazers leads to an indirect effect (dashed line) called a trophic cascade on

119   algal abundance. Temperature directly affects *per capita* metabolic rates (solid lines), and

120   indirectly affects algal abundance (dashed lines) by increasing grazing rates, and may have

121   stronger effects on heterotrophic metabolic rates relative to algal metabolic rates (thicker lines

122   represent a stronger direct effect of temperature). Other indirect effects of temperature are

123   possible. B) Experimental communities varied in their trophic structure. Ten communities

124   included algae only (A), 10 comprised algae + grazers (AG), and 10 included algae + grazers

125   + predators (AGP). We sampled net ecosystem oxygen production (NEP), ecosystem

126   respiration (ER) and total phytoplankton biomass (M_B) weekly for 8 weeks.

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The temperature-dependence of consumer-resource interactions – mediated by
dynamics of two or more populations - has received substantial attention in this context,
because these *trophic* interactions can influence many aspects of community structure and
ecosystem function, including biomass, abundance, species composition and stability
[10,12,32,34]. Trophic species interactions appear to strengthen with warming [16,29,31].
Series of trophic interactions, called *trophic cascades* (Fig 1), link predator populations to the
abundance, biomass and ecosystem functions of primary producers [32,33]. The strength of
trophic cascades depends on predator and prey body sizes and primary production [34].
Considering the prevalence of consumer-resource interactions and trophic cascades in aquatic
systems [35] begs the question, how is it that population-level responses to temperature
[28,36] do not appear to cause major variation or context dependence in macroecological
relationships between subcellular metabolic processes (photosynthesis, respiration) and
ecosystem processes (NEP, ER)?

Here, we aimed to resolve the paradox between apparent direct effects of temperature
on ecosystem functions (NEP, ER) that emerge when comparing communities across larger
gradients and the potentially more complex effects of temperature at the population and
community scales. We experimentally tested the hypothesis that temperature-dependent
trophic interactions in a trophic cascade alter the effect of temperature on community
properties such as biomass, abundance and body size, but have little or no effect on the
variation in ecosystem functions (NEP, ER) over a temperature gradient. In freshwater
plankton communities, we compared the effects of temperature on community properties
typically measured in warming experiments (e.g., biomass, density, body size) with the
effects of temperature typically compared in macroecological studies (e.g., NEP, ER). We
controlled variation in biotic and abiotic conditions other than temperature and trophic
structure (presence of grazers and predators) (Fig 1). We quantified ecosystem function

154   (NEP, ER) and community structure (biomass, abundance) in ecosystems with algae only (A),

155   algae and grazers (AG), or algae, grazers and predators (AGP) across an experimental

156   temperature gradient of 10 °C. We found that exponential effects of temperature on algal

157   biomass were greater than effects of temperature on NEP and ER, suggesting that even large

158   changes in community structure do not necessarily lead to large changes in how temperature

159   affects NEP and ER.

160

161   **Hypotheses**

162   We drew on the Metabolic Theory of Ecology (MTE) frame our hypotheses and

163   predictions for how temperature affects NEP and ER via *per capita* metabolic temperature

164   dependence and indirect effects of temperature at the community scale. We first briefly

165   outline the framework, and then express our specific hypotheses. MTE relates whole-

166   organism metabolic rates (b_i, gO$_2$/hr) and related biological functions for organism i to body

167   size (m_i, g) and body temperature (T, in Kelvin) [1,37,38]:

168          $$b_i = b_0 e^{-E_a/kT} m_i^a$$ Eqn 1

169   in which activation energy (E_a, in eV) captures the exponential effect of temperature on *per*

170   *capita* metabolic rate, k is the Boltzmann constant (eV/K), b_0 is a normalization constant

171   independent of body size and temperature that includes the effects of temperature-

172   independent traits on metabolic rate (gO$_2$/g$^\alpha$/hr). The allometric scaling factor α relates

173   metabolic rate to body size [see Methods: Models and Hypotheses for justification of Eqn 1

174   over alternative models].

175   The effects of temperature on ecosystem metabolic rates (B_R), such as NEP or ER

176   (gO$_2$/hr) reflect the sum of all *per capita* photosynthesis rates by autotrophs and respiration

177   rates by autotrophs and heterotrophs as well as shifts in abundance, body size and

178   acclimation. These models implicitly assume ample and constant supply of resources. Note

179   that NEP and ER can be quantified in this way as positive numbers, and we do this – using

180   their absolute values – in our analyses. Following Barneche et al (2014), we capture direct

181   and indirect effects of temperature on ecosystem scale metabolic rates in the following

182   equation (see Barneche et al 2014 for derivation):

183   $$B_R = b_0(T_C) \, e^{-E_R\left(1/_{kT} - 1/_{kT_C}\right)} M_B \langle m_B^{\alpha-1} \rangle. \qquad\qquad \text{Eqn 2}$$

184   The term $e^{-E_R\left(1/_{kT} - 1/_{kT_C}\right)}$ captures the temperature dependence E_R (eV) of ecosystem-level

185   metabolic rate B_R. Equation 2 represents a 'first order metabolic scaling' prediction that

186   ecosystem scale mass-normalized metabolic rates (e.g., NEP) vary proportionally with the

187   temperature dependence of the underlying metabolic processes (e.g., photosynthesis).

188   Observed temperatures T are related to an arbitrarily chosen reference temperature T_c. This

189   centering causes the normalization constant $b_0(T_C)$ to be for metabolic performance at

190   temperature T_c.

191   When considering the indirect effects of temperature on ecosystem oxygen production

192   and respiration, we can consider how each term in Eqn 2 may vary with temperature. To

193   account for changes in total biomass, body size or relative abundance of phenotypes (traits)

194   associated with temperature, we use the term $M_B \langle m_i^{\alpha-1} \rangle_B$. The total biomass, M_B (g) in

195   ecosystem volume V, which equals the sum of mass m_i for all individuals i to J ($M_B =$

196   $\frac{1}{V}\sum_{i=1}^{J} m_i$). The term $\langle m_i^{\alpha-1} \rangle$ is the average of all individual metabolic biomasses, $\langle m_i^{\alpha-1} \rangle =$

197   $\left(\sum_{i=1}^{J} m_i^{\alpha}\right)/\left(\sum_{i=1}^{J} m_i\right)$, corrected for the greater contribution to total mass-specific metabolic

198   biomass by small individuals resulting from the allometric scaling (α) of oxygen production

199   and consumption with body size [2,10]. This 'mass correction' is necessary, because if

200   community biomass is comprised of one large individual, that biomass will [produce and]

201   consume less oxygen per gram biomass in a given time period than if the same total biomass

202   were comprised of many small individuals; in other words, $M_B \langle m_B^{\alpha-1} \rangle$ increases as m_i. If

203   thermal traits acclimate or species composition shifts with temperature, this term would

204   capture that change. Therefore Eqn 2 can capture direct effects of temperature on community

205   metabolism via changes in *per capita* metabolic rate (E_R) and via changes in biomass, size

206   distribution and phenotypes.

207

208   ***Hypothesis 1: The relationship between algal biomass and temperature is modified by the***

209   ***number of trophic levels.*** Via strong trophic interactions, predators can change the standing

210   biomass of primary producers in communities. Total algal biomass (M_B) can be expressed in

211   terms of temperature, traits and size distributions:

212    $$M_B = \frac{B_R\, e^{E_R\left(1/kT - 1/kT_C\right)}}{b_0(T_C)\langle m_B^{\alpha-1}\rangle}.$$ Eqn 3

213   If we assume that $B_R * (b_0(T_C) * \langle m_B^{\alpha-1}\rangle)^{-1}$ is independent of temperature, we predict that

214   algal biomass M_B declines with temperature by E_R, in this case $E_R = -E_{NEP}$. This prediction has

215   been supported empirically in a single species algae system [39], and in that system the

216   predicted decline in total biomass was robust to changes in cell size. However, it is unlikely

217   that grazers and temperature would not alter the abundance and size of algae, altering $\langle m_B^{\alpha-1}\rangle$

218   among trophic treatments [40], and also the traits of algae, and thereby modifying $b_0(T_C)$

219   among trophic treatments [41]. A fuller integration of how temperature and trophic treatment

220   affect these terms for multispecies assemblages would require theoretical development that is

221   beyond the scope of this paper, but we use the equation here to highlight why we expect

222   trophic structure and temperature to affect algal biomass. To test this hypothesis, and the

223   alternative that $B_R * (b_0(T_C) * \langle m_B^{\alpha-1}\rangle)^{-1}$ is independent of temperature, we linearized Eqn 3

224   for analysis by log transforming (Methods: Eqn 7) and then we compared $\ln(M_B)$ trends with

225   temperature across ecosystems with and without a trophic cascade (AGP vs AG ecosystems).

226

227   ***Hypothesis 2: Increasing temperature strengthens the trophic cascade***. We estimated the

228   strength of the trophic cascade as the log ratio of primary producer biomass in the presence of

229   predators (AGP) *vs* in predator-free environments (AG) [42]. We predicted that predators

230   would reduce the abundance of zooplankton through predation, and shift zooplankton

231   composition to smaller sizes and less-edible species, typical of classic freshwater trophic

232   cascades [43]. We also predicted that these trophic interactions would strengthen with higher

233   temperatures due to the effect of temperature on *per capita* zooplankton grazing rates. We can

234   relate algal biomass among treatments using Eqn 3 for primary producer biomass in the

235   presence of predators (AGP) and grazers only (AG), simplifying and taking the natural log to

236   yield (see Methods, eqns 7-10, for details):

237   $$ln\left(\frac{M_{B.AGP}}{M_{B.AG}}\right) = \frac{\ln(b_0(T_C)_{AG}) + ln(\langle m_B^{\alpha-1}\rangle_{AG}) - \frac{E_{b.ag} - E_{m.ag}}{kT}}{\ln(b_0(T_C)_{AGP}) + ln(\langle m_B^{\alpha-1}\rangle_{AGP}) - \frac{E_{b.agp} - E_{m.agp}}{kT}} \qquad \text{Eqn 4}$$

238   Numerous experiments have demonstrated that the strength of the trophic cascade ($\log\frac{M_{B.AGP}}{M_{B.AG}}$,

239   increases with increases in temperature of a few degrees [29,44,45], and theoretical work

240   suggests that strengthening of this interaction under warming is expected for a greater range

241   of consumer-resource parameter values than would predict declines in the trophic cascade

242   [15]. We therefore predict the trophic cascade strength will increase with temperature in our

243   experiment. But, as shown in Eqn 4, because trophic interactions could affect the realized

244   temperature dependence via several possible mechanisms – shifts in body size, traits, etc, - it

245   is not possible to predict *a priori* the temperature dependence of the trophic cascade, in terms

246   of the differences and ratios of the temperature dependence terms in Eqn 4 (see Methods). A

247   'first order' metabolic scaling prediction would be no change in trophic cascade strength,

248   because the model would assume that the temperature dependences of mass and the

249   normalization constant ($E_{b.ag}$, $E_{b.agp}$, $E_{m.ag}$, and $E_{m.agp}$) all equal 0. We expected the indirect

250   effects of predators on algae to be mediated by changes in zooplankton density and/or body

251   size. Zooplankton attributes are not explicitly modeled in Eqn 4, but could contribute to

252   temperature dependence of algal cell size and trait distributions. Reduced zooplankton size or

253   density in the presence of predators could lead to different indirect effects of temperature on

254   algal cell size and traits in the presence vs absence of predators. We tested the prediction that

10

255   temperature dependence of zooplankton size and density are different from zero (Methods:

256   Statistical Analysis).

257

258   ***Hypothesis 3: Temperature dependence of net ecosystem production and respiration***

259   ***depends on the strength of the trophic cascade.*** We test this by using Equation 2 to model

260   ecosystem-scale NEP and ER, but we allow $b_o(T_C)$ to vary not only with temperature but with

261   trophic structure (Z_j). We expect that trophic structure will influence the number and size of

262   individuals, and thereby affect $M_B \langle m_B^{\alpha-1} \rangle$. Alternatively, trophic structure may not modify the

263   relationship between temperature and B_R, if $b_o(T_C)$ and $\langle m_B^{\alpha-1} \rangle$ are independent of

264   temperature. We can test these alternate predictions by comparing models with and without

265     $b_o(T_C)$ and $\langle m_B^{\alpha-1} \rangle$ terms that depend on ecosystem temperature and trophic structure.

266

267   For each hypothesis, we used linear mixed effects models (LMMs) to test 'first order'

268   metabolic scaling models for the appropriate model (equation 2, 3 or 4) that included $b_o(T_C)$

269   as independent of the ecosystem's temperature or trophic structure [Methods: Statistical

270   Analyses]. We tested alternate models that allowed $b_o(T_C)$ to vary with ecosystem temperature

271   and/or trophic structure. If the simpler, first order models are best supported, we would infer

272   that indirect effects of temperature do not overwhelm the signals of direct metabolic scaling

273   effects on ecosystem functions, consistent with inferences drawn in macroecological studies.

274   To estimate intercepts and temperature dependence terms (e.g., E_R), we summed coefficient

275   values and estimated uncertainties in these aggregated parameters from best models

276   (Methods: Statistical Analysis). For each hypothesis, we tested two measures of ecosystem

277   temperature: mean temperature over the 9-week experiment, which captures differences

278   among systems, or weekly mean temperature, which captures differences within ecosystems

279   over time. Our data do not permit testing specific predictions about size distributions or trait

280   shifts, but support for models with variation in $b_o(T_C)$ and $\langle m_B^{\alpha-1} \rangle$ among treatments would

281   suggest these mechanisms as likely explanations.

282

283   **Results**

284   **Hypothesis 1:** As temperature increased across ecosystems, phytoplankton biomass,

285   estimated as the concentration of chlorophyll *a* in the water column, declined (Fig 2A).

286   Trophic interactions modified the effect of temperature on chlorophyll *a* concentration (Fig

287   2A, Table 1). This inference is supported by the inclusion of a main effect for trophic

288   structure (Z_j) in the best model (Table 1) and an estimate for the temperature dependence of

289   chlorophyll *a* concentration with confidence intervals that exclude 0 (Fig 3). Phytoplankton

290   biomass declined much more strongly with temperature in algae-grazer (predator-free)

291   communities, with a decline of over three orders of magnitude in phytoplankton biomass

292   standing stock over the 10 °C temperature gradient (Fig 2A). In the algae-grazer-predator

293   treatments, phytoplankton biomass declined with a slope indistinguishable from that in the

294   algae only treatments (Fig 3). We did not observe shifts in taxonomic composition with

295   temperature (Fig S3).

296

297   **Figure 2: A**) Estimated phytoplankton biomass (chlorophyll *a* concentration) declined with

298   increasing temperature and varied with trophic structure (A, AG, AGP). Lines are estimated

299   effects of temperature on phytoplankton biomass based on linear mixed effects models (Eqn

300   11) for equation 3 with temperature dependent in model terms for the intercept and slope

301   (Table 1). From the best model, the intercept and slope of each line was estimated by pooling

302   terms for the intercept and temperature dependence in eqn 11 (see Methods, Eqn 13). All

303   observations for phytoplankton biomass are shown in Fig 6. **B**) Strength of the trophic

304   cascade at a given temperature was estimated by taking the log ratio of algal biomass

305   (estimated as chlorophyll *a* concentration) in the presence of predators and grazers (AGP) vs

306   the algal abundance in the presence of grazers only (AG) (Eqn 4, Table 2). Lines represent

fixed effects of temperature from the full model (Table 2), centered on the grand mean of all

recorded ecosystem temperatures (Eqn 12). Gray shading and symbols indicate the week,

from week 2 (July 10) to week 9 (August 28) 2012.

Table 1. Model selection results for linear mixed effects models of phytoplankton

biomass. The full statistical model (Methods: Eqn 11) related ln(chlorophyll a) to ecosystem

trophic structure (Z_j) and average ecosystem temperature over the entire experimental period

(T_M), while accounting for effects of temperature variation over time (weekly average

temperature (T_{wj})) and with ecosystem identity as a random effect. We compared models

using likelihood ratios (LogLik), AICc, Akaike weights (w) and delta AICc weights. The

model was fit to 240 observations in 30 groups. The full model (modelPBF) includes all

terms, and models representing alternate hypotheses excluded terms indicated by 'NA'.

Values indicate model-estimated coefficients. Coefficients were pooled (Methods: Statistical

Analysis) to estimate slopes and intercepts for Fig 2 and 3.

	Int	Z_j	T_{wj}	T_M	$T_{wj}*Z_j$	T_M*Z_j	T_M*T_{wj}	df	logLik	AICc	d	w
modPBF	2.05	+	-0.52	1.30	1.34	+	+	12	-155.37	336.11	0.00	9.982260e-01
modPB8	2.05	+	-0.66	1.30	NA	+	+	11	-162.86	348.87	12.76	1.690617e-03
modPB7	2.05	+	-0.96	1.30	NA	NA	+	9	-168.05	354.89	18.78	8.342130e-05
modPB4	1.50	NA	-0.96	1.70	0.96	NA	NA	6	-207.94	428.24	92.13	9.830504e-21
modPB6	1.91	+	-0.66	NA	NA	+	NA	8	-206.58	429.79	93.68	4.538012e-21
modPB3	1.50	NA	-0.96	1.71	NA	NA	NA	5	-211.73	433.72	97.62	6.341539e-22
modPB5	1.91	+	-0.96	NA	NA	NA	NA	6	-211.45	435.26	99.16	2.937427e-22
modPB2	1.50	NA	-0.96	NA	NA	NA	NA	4	-218.40	444.97	108.87	2.287011e-24
modPB1	1.90	+	NA	NA	NA	NA	NA	5	-257.21	524.68	188.57	1.124143e-41
modPB0	1.49	NA	NA	NA	NA	NA	NA	3	-264.15	534.41	198.30	8.684503e-44

Figure 3. Comparison of estimated temperature dependences of phytoplankton biomass (M_B),

net ecosystem oxygen production (NEP), ecosystem respiration (ER) for communities with

algae only (A), algae and grazers (AG) and algae, grazers and predators (AGP). Composite

estimates of temperature dependences as shown in Figures 2A and 3 (following Methods, Eqn

13). No temperature dependence is indicated by the dashed line, and the vertical gray dotted

328   lines indicate 0.65 and 0.32 eV, expected temperature dependences of photosynthesis and

329   respiration, and -0.65 and -0.32 as expectations for the temperature dependence of

330   phytoplankton total biomass.

331

332   **Hypothesis 2:** Consistent with our second hypothesis, and the patterns observed for

333   phytoplankton biomass, there was a strong trophic cascade in the warm ecosystems by the end

334   of the experiment (Fig 2B). The trophic cascade became apparent after the first weeks of the

335   experiment, and strengthened over time and with temperature (Fig 2B) (Table 2). The best

336   model included a term for mean ecosystem temperature (T_M), as well as week (T_w), and a

337   week x temperature interaction. By week 9, the trophic cascade increased exponentially with

338   temperature (Fig 2B) to an estimated $E_{TC} = 0.77$ (estimated from model fixed effects shown in

339   Table 2 plus random effect).

340

341   **Table 2. Model selection results for trophic cascade analysis**. We used linear mixed effects

342   models with terms for average temperature for ecosystem j in week w (T_{wj}), week 2-9 (Wk)

343   and their interaction. We treated the power level (e.g., 100W, 200W, etc), our temperature

344   treatment, as a random effect to account for repeated measures on ecosystems over time. We

345   compared models using likelihood ratios (LogLik), AICc, Akaike weights (w) and delta AICc

346   weights. The model was fit to 79 observations in 10 groups. The full model (TCFull) includes

347   all terms, and models representing alternate hypotheses excluded terms indicated by 'NA'.

348   Coefficients were pooled (Methods) to estimate slopes and intercepts for Fig 2.

	Int	T_{wj}	Wk	T_{wj}*Wk	df	logLik	AICc	d	w
TCFull	0.19	-0.01	0.12	0.11	6	-60.78	134.73	0.00	0.769299027
TCmodc	0.02	0.74	0.14	NA	5	-63.26	137.33	2.60	0.209484257
TCmode	0.56	NA	0.04	NA	4	-67.36	143.27	8.54	0.010776915
TCmodf	0.79	NA	NA	NA	3	-68.80	143.92	9.19	0.007786805
TCmodd	0.79	-0.05	NA	NA	4	-68.76	146.07	11.34	0.002652997

349

352   We find additional evidence of temperature-dependent trophic interactions in the

353   responses of the zooplankton grazer assemblages to warming and predation. Total

354   zooplankton density declined with increasing temperature (Table 3; E_{ZP} = 1.28 95% CI: 0.19

355   – 2.39, z = 2.31, P = 0.021 based on regression with negative binomial distribution and

356   likelihood ratio tests; Fig 4). Predators reduced density of *Daphnia*, the dominant grazer

357   (linear regression with Poisson distributed errors: estimate: = -1.14 95% CI: -1.91, -0.36, z = -

358   2.87, P < 0.01) and density declined with increasing temperature (Table 4) although the

359   temperature term retained in the best model was not significant (E_D = 0.88 95% CI: -0.59,

360   2.35, z = 1.17, P = 0.24). Copepod density declined with temperature (E_C = 2.21, z = 3.12, P

361   = 0.002), and not in response to predation (best model did not include a predation term, Table

362   6). We measured zooplankton standard length for 641 individuals of all ages. Mean length

363   was 0.72 cm, and sizes ranged from 0.34 – 1.94 cm. We did not observe a decline in body size

364   with temperature (best model did not include a temperature term, Table S2), as would be

365   expected by a hypothesis based on the temperature size rule. Predation reduced total

366   zooplankton body size, driven by size shifts in *Daphnia* (Table S3).

367

368

369   **Table 3: Zooplankton density**. Results of model selection for zooplankton abundance in

370   ecosystems with grazers (AG) and with grazers and predators (AGP). We used negative

371   binomial regressions with ecosystem identity as a random effect [Methods: Statistical

372   Analysis]. Models included terms for weekly average temperature (T_{wj}), ecosystem trophic

373   treatment (Z_j) and their interaction, and a random effect for ecosystem identity. We modeled

374   120 observations in 20 groups (ecosystems). We compared models using likelihood ratios

375   (LogLik), AICc, Akaike weights (w) and delta AICc weights. NA indicates that the term was

376   not included in the model.

	Int	T_{wj}	Z_j	df	logLik	AICc	d	w
nbinommod1b	-47.41	1.28	NA	5	-367.00	744.53	0.00	0.49557915
nbinommod1	-48.76	1.32	+	6	-366.37	745.47	0.95	0.30895745
nbinommod1c	2.95	NA	NA	4	-369.46	747.26	2.74	0.12621312
nbinommod1a	3.07	NA	+	5	-368.97	748.46	3.94	0.06925027

377

378

379   **Table 4: *Daphnia* density**: Results of model selection for *Daphnia* abundance in ecosystems

380   with grazers and with grazers and predators. We used Poisson regressions with ecosystem

381   identity as a random effect [Methods: Statistical Analysis]. Models included terms for weekly

382   average temperature (T_{wj}), ecosystem trophic treatment (Z_j) and their interaction, and a

383   random effect for ecosystem identity. We compared models using likelihood ratios (LogLik),

384   AICc, Akaike weights (w) and delta AICc weights. We modeled 120 observations in 20

385   groups (ecosystems). NA indicates that the term was not included in the model.

	Int	T_{wj}	Z_j	$T_{wj}*Z_j$	df	logLik	AICc	d	w
poismod.Db	-34.88	0.88	+	NA	4	-115.75	239.85	0.00	0.63118106
poismod.Da	-38.64	0.97	+	+	5	-115.73	241.98	2.13	0.21709779
poismod.Dd	-0.97	NA	NA	NA	2	-119.71	243.52	3.67	0.10060719
poismod.Dc	-31.32	0.77	NA	NA	3	-119.33	244.87	5.03	0.05111395

386

387   **Table 5: Copepod density**: Results of model selection for copepod *spp* abundance in

388   ecosystems with grazers and with grazers and predators. We used Poisson regressions with

389   ecosystem identity as a random effect [Methods: Statistical Analysis]. Models included terms

390   for weekly average temperature (T_{wj}), ecosystem trophic treatment (Z_j) and their interaction,

391   and a random effect for ecosystem identity. We compared models using likelihood ratios

392   (LogLik), AICc, Akaike weights (w) and delta AICc weights. We modeled 120 observations

393   in 20 groups (ecosystems). NA indicates that the term was not included in the model.

	Int	T_{wj}	Z_j	$T_{wj}*Z_j$	df	logLik	AICc	d	w
poismod.Cc	-87.63	2.21	NA	NA	4	-135.11	278.56	0.00	0.67753474
poismod.Cb	-87.50	2.21	+	NA	5	-135.10	280.72	2.16	0.23026158
poismod.Ca	-96.20	2.43	+	+	6	-135.04	282.83	4.27	0.07997327
poismod.Cd	-0.34	NA	NA	NA	3	-140.19	286.59	8.03	0.01223041

Figure 4: A) Total zooplankton density (ind / 10L), comprising *Daphnia* and copepod taxa, declined with increasing temperature but not with predator presence. B) *Daphnia* density (ind/L) declined with temperature and with predators (gray line, vs black line indicating trend with no predators) (Table 4), and C) copepod *spp* density (ind/L) declined with temperature but not predators (Table 5). Lines are regression fits with negative bionomial (total zooplankton) or Poisson (*Daphnia*, copepods) zero-inflated error structures with ecosystem as a random effect for ecosystems with predators (gray dashed line) and without predators (black solid line). Each datapoint is an observed total zooplankton density for crustacean taxa (*Daphnia* and copepods) in each ecosystem on a sampling date.

Hypothesis 3: Across ecosystems higher temperatures increased net ecosystem oxygen production (NEP) and respiration (ER) (Tables 6, 7; Fig 5). The LMM for NEP (Table 6) suggests that ecosystem temperature and trophic structure interact to influence ecosystem oxygen fluxes, yet their estimated temperature dependences did not appear to differ when confidence intervals were compared (Fig 3). The estimated across-system temperature dependence of NEP was the strongest in algae-only communities (Fig 5), and confidence intervals for the temperature dependence term include 0 for the systems with predators (Fig 3). Ecosystem respiration (ER) increased with temperature across ecosystems (Fig 5), and this effect did depend on trophic structure (Table 7). The estimated temperature dependence on NEP and ER was strongest in the algae-only systems, and weakest in systems with predators

417 (Fig 3).

418

419 **Table 6: Results of model comparisons for effects of temperature and time on net**

420 **ecosystem productivity (NEP)** based on AIC weight (w) and δAIC_C values. Nested versions

421 of the full model (Eqn 11, Methods). Response variables are modelled as functions of

422 temperature T_{wj} for each tank j on week w relative to the mean temperature T_M for tank j over

423 all weeks (T in Kelvin), and trophic structure (Zj). Models included a random effect for the

424 experimental unit – tanks with and without predators receiving the same power inputs. See

425 Methods for additional details on modeling. The model was fit to 219 observations in 30

426 groups. The full model (modNEPF) includes all terms, and models representing alternate

427 hypotheses excluded terms indicated by 'NA'. Values indicate model-estimated coefficients.

428 Coefficients were pooled (Methods: Statistical Analysis) to estimate slopes and intercepts for

429 Fig 3 and 5.

	Int	Z_J	T_{wJ}	T_M	$T_{wJ}*Z_J$	T_M*Z_J	$T_{wJ}*T_M$	df	logLik	AICc	d	w
modNEP8	-6.42	+	0.29	-1.40	NA	+	+	11	-266.47	556.21	0.00	3.878747e-01
modNEPF	-6.42	+	0.37	-1.42	0.84	+	+	12	-265.54	556.60	0.39	3.192963e-01
modNEP7	-6.41	+	0.03	-1.39	NA	NA	+	9	-269.68	558.22	2.01	1.421535e-01
modNEP3	-6.15	NA	0.02	-0.96	NA	NA	NA	5	-274.36	559.01	2.80	9.557409e-02
modNEP4	-6.15	NA	0.02	-0.96	0.61	NA	NA	6	-273.86	560.12	3.91	5.487563e-02
modNEP0	-6.15	NA	NA	NA	NA	NA	NA	3	-283.15	572.41	16.20	1.179389e-04
modNEP2	-6.15	NA	0.03	NA	NA	NA	NA	4	-283.13	574.44	18.23	4.265400e-05
modNEP1	-6.26	+	NA	NA	NA	NA	NA	5	-282.25	574.78	18.57	3.596974e-05
modNEP6	-6.26	+	0.27	NA	NA	+	NA	8	-279.83	576.34	20.13	1.647426e-05
modNEP5	-6.26	+	0.03	NA	NA	NA	NA	6	-282.23	576.85	20.64	1.278556e-05

430

431

432 **Table 7: Results of model comparison for effects of temperature and time on ecosystem**

433 **respiration (ER)** based on AIC weight (w) and δAIC_C values. Nested versions of the full

434 model (Eqn 11, Methods). Response variables are modelled as functions of temperature T_{wj}

435 for each tank j on week w relative to the mean temperature T_M for tank j over all weeks (T in

436 Kelvin), and trophic structure (Z_j). Models included a random effect for the experimental unit

437 – tanks with and without predators receiving the same power inputs. The model was fit to 240

observations in 30 groups. See Methods for additional details on modeling.

	Int	Z_J	T_{wJ}	T_M	$T_{wJ}*T_M$	$T_{wJ}*Z_J$	T_M*Z_J	df	logLik	AICc	d	w
modER7	-6.09	+	0.11	-1.32	NA	NA	+	9	-185.88	390.54	0.00	6.013204e-01
modER8	-6.09	+	0.02	-1.32	NA	+	+	11	-184.58	392.31	1.77	2.479721e-01
modERF	-6.09	+	0.06	-1.32	0.42	+	+	12	-183.97	393.31	2.77	1.506867e-01
modER3	-5.79	NA	0.11	-0.67	NA	NA	NA	5	-201.37	413.00	22.46	7.978045e-06
modER4	-5.79	NA	0.11	-0.68	0.50	NA	NA	6	-200.45	413.27	22.73	6.977684e-06
modER1	-5.94	+	NA	NA	NA	NA	NA	5	-202.46	415.18	24.64	2.685333e-06
modER5	-5.94	+	0.11	NA	NA	NA	NA	6	-201.75	415.85	25.31	1.916292e-06
modER6	-5.94	+	0.02	NA	NA	+	NA	8	-200.49	417.60	27.06	8.013775e-07
modER0	-5.79	NA	NA	NA	NA	NA	NA	3	-207.04	420.17	29.63	2.211271e-07
modER2	-5.79	NA	0.11	NA	NA	NA	NA	4	-206.32	420.82	30.28	1.602501e-07

Figure 5: The effect of mean ecosystem temperature on A) phytoplankton biomass, B) net ecosystem productivity (NEP), and C) net ecosystem respiration (ER) for three community types that varied in their trophic interactions: i) algae-only (A), ii) algae + grazers (AG), and iii) algae + grazers + notonectid predators (AGP). Black lines indicate the among-ecosystem effects of temperature, modelled by equation 5 using hierarchical regressions fit to among-ecosystem variation in temperature, after taking into account within-group variation temperature effects (light lines) (Table 2), and may be compared with predicted effects of temperature and species interactions depicted in Figure 1. Activation energies were estimated by best model or best model set (Table 1, Supplementary Material 2). Temperature in Celsius is shown for comparison only, models were fit to inverse temperature. A similar figure showing all measured datapoints to which models were fitted is shown in the appendix (Figure S3). Temperatures within tanks declined over time (Fig S1.1C).

In addition to the variation among ecosystems in temperature that was the main focus of our analysis, the biotic and abiotic conditions in experimental ecosystems varied over time. Temperature varied within experimental ecosystems over time (Fig S1). Overall, temperatures declined between the beginning and the end of the experiment, with some variation among

459   weeks reflecting weather conditions. Phytoplankton community composition shifted over time

460   (Fig S2), but visual inspection of the species at each time point indicated no specific taxa

461   driving the changes, and there was no association between phytoplankton species composition

462   and temperature (Fig S3). Chlorophyll *a* concentration declined over time in all treatments

463   (Fig S4). A linear mixed effects model indicated that this decline was weakest in the algae-

464   only treatments (Table S6). Visual inspection of trends (Fig S4) suggests that this decline over

465   time was driven by the decline in all tanks in weeks 8 and 9, following a major rain event and

466   drop in all tank temperatures (Fig S1). When we reanalyzed the temporal trend for just weeks

467   2-7, excluding weeks 8-9, the trend in chlorophyll *a* over time approached 0 in A and AGP

468   treatments, but still persisted in AG treatments (Table S5).

469   Effects of temporal temperature variation on phytoplankton biomass within

470   ecosystems differed starkly from effects of temperature among ecosystems (Fig 6). Within

471   ecosystems, higher temperatures were associated with higher phytoplankton standing stocks

472   (Fig 6A), opposite to the trend with temperature among ecosystems. Net ecosystem oxygen

473   production (NEP) and ecosystem respiration (ER) varied with temperature within ecosystems,

474   and there is some evidence that this temperature effect interacted with both the trophic

475   structure treatment (Table 5, modelNEP8; Table 6, model ER8).

476

477

478   **Figure 6.** The effect of ecosystem temperature (T_{wj}) on A) phytoplankton biomass, B) net

479   ecosystem productivity (NEP), and C) net ecosystem respiration (ER) for three community

480   types that varied in their trophic interactions: i) algae-only (A), ii) algae + grazers (AG), and

481   iii) algae + grazers + notonectid predators (AGP). There were 10 ecosystems (*j*) in each

482   trophic treatment, and each ecosystem was sampled 8 times (once per week from weeks 2 –

483   9). Each week is indicated by a shape, and ecosystem identity within weeks are distinguished

484   by shades of gray. In a single model (Eqn 12), we considered effects of temperature within

485 ecosystems over time, as well as among ecosystem variation in mean temperature (Fig 2, 5).

486 Blue lines are fit to the 8 observations (points) from each ecosystem, and their slope indicates

487 within-ecosystem temperature effects estimated from best models in Tables 2, 6 and 7. Black

488 lines indicate the modelled among-ecosystem effects of temperature (Tables 1, 6, 7; Fig 2, 4).

489 Temperature in Celsius is shown for comparison only, models were fit to inverse temperature.

490 Temperatures within tanks declined over time (Fig S1).

491

492

493 **Discussion**

494   Temperature affects the metabolic rates of all organisms, and *per capita* responses to

495 temperature of many co-occurring individuals add up to nothing less than the biological

496 component of ecosystem scale carbon and oxygen flux. Understanding biological responses to

497 temperature change across scales of organization (cells to the biosphere) is a major challenge

498 in ecological research. Meeting this challenge requires joining theoretical frameworks and

499 synthesizing empirical evidence of temperature effects across scales and systems. Despite

500 much progress, there remains a gap between patterns that emerge in community-level

501 experiments and the multi-scale theoretical framework (MTE) that links temperature

502 dependent metabolism to larger scale patterns for temperature dependence. Here, we aimed to

503 test the hypothesis that the effects of temperature on ecosystem processes that reflect

504 metabolic temperature dependence are not highly sensitive to local differences in trophic

505 structure of a community (e.g., presence or absence of a predator). This question draws upon

506 ideas supported by the metabolic theory of ecology and community ecology theory predicting

507 that species interactions modify the effects of temperature on community structure and

508 function. We found that in aquatic ecosystems characterized by the presence or absence of

509 predator-prey species interactions, temperature-dependent trophic cascades only modestly

510 altered the effects of temperature on net ecosystem oxygen production and consumption (NEP

511 and ER). We found that higher average temperatures increased NEP and ER while total

512 phytoplankton biomass declined, and all ecosystem level temperature responses were stronger

513 than expected for *per capita* temperature dependent oxygen production or consumption.

514   Our first hypothesis was based on the expectation that our experimental systems would

515 include trophic interactions that altered phytoplankton standing stock, and possibly interacted

516 with temperature to influence algal size distributions or other traits. We found that trophic

517 structure did modify the effect of temperature on phytoplankton biomass, failing to reject our

518 first hypothesis. The decline in phytoplankton standing stocks that we observed with warming

519 across ecosystems is consistent with theoretical expectations that in closed systems with

520 limited resources, increases in *per capita* metabolic rates with temperature could lead to

521 declines in standing stocks [15,18,39,46]. The observed temperature dependence of

522 phytoplankton standing stocks was highest in the communities with grazers but no predators,

523 suggesting that temperature dependent grazing can exacerbate the temperature dependence of

524 algal standing stocks. Overall, the temperature-dependence of phytoplankton standing stocks

525 greatly exceeded expectations based on temperature dependence of *per capita* photosynthesis

526 or respiration rates (Fig 3). Our hypothesis (Eqn 3) allowed for changes in phytoplankton

527 standing stocks to be explained by direct effects of temperature on *per capita* metabolism, as

528 well as effects of temperature on thermal traits, density or body size distributions. We suggest

529 that change in *per capita* metabolic response and density were the primary components of this

530 change. We did not observe clear shifts in the species composition of the phytoplankton

531 assemblage with temperature; still, we do not have high resolution data on phytoplankton cell

532 size or traits, so we cannot reject these mechanisms as contributors to the patterns we observe.

533   Our second hypothesis, based on recent experimental results in other freshwater and

534 grassland systems, was that the trophic cascade would get stronger as ecosystem temperatures

535 warmed. We found support for this hypothesis in our system, providing the first evidence that

536 trophic cascade strength increases continuously with temperature. Prior to our study, evidence

537   of stronger trophic cascades with warming were from experiments that test two temperature

538   levels, an ambient and a simulated future scenario of ~ +3 °C [29,47,48]. We show here that

539   this pattern continues over a thermal range of 10 °C. The indirect effects of predators on

540   phytoplankton biomass appears to have been mediated by predation on the dominant grazer,

541   *Daphnia*. Predators reduced *Daphnia* density and thereby shifted grazer assemblages toward

542   the less effective copepod grazers at all temperatures. This trophic cascade, mediated by shifts

543   in grazer composition as well as total density, is a classic food web motif in freshwater

544   systems [43]. Interestingly, at warmer temperatures grazer density was lowest, yet we still

545   observed declines in biomass of phytoplankton. This pattern could reflect higher *per capita*

546   grazing by the remaining grazer individuals. Algal productivity rates are an important element

547   of trophic cascade strength [15,34], and higher NEP at warmer temperatures would contribute

548   to a stronger trophic cascade, even as grazer density declines. As with hypothesis 1, we infer

549   that the effect of temperature on the trophic cascade strength reflects not only the effect of

550   temperature on *per capita* metabolic rate but also shifts in algal traits or body sizes, or both.

551   We tested a third hypothesis that the effects of temperature on biomass and trophic

552   cascade strength would lead to distinct relationships between temperature and NEP and ER

553   for each trophic treatment type (e.g., with vs without predators). We found that the effect of

554   temperature on phytoplankton standing stock was much greater than the effects of

555   temperature on NEP or ER. For NEP and ER, there was support for a model with and

556   interaction between trophic structure and mean temperature, but for NEP a model without the

557   interaction was ranked quite highly (Table 6), and confidence intervals for the pooled

558   estimated temperature dependence do not indicate differences among trophic treatments.

559   Therefore, the strong effects of temperature on community structure (biomass, trophic

560   cascade strength) did not translate directly to net ecosystem flux rates.

561   The estimated temperature dependences of NEP and ER were greater than expected

562   based on temperature dependent *per capita*, mass normalized respiration and photosynthesis

metabolic rates. It is well established that temperature dependence of aerobic respiration is approximately $E_R = \sim 0.65$ eV, and that this value explains the temperature dependence of mass normalized ecosystem metabolism at the ecosystem scale [2-4]. The temperature dependence of photosynthesis at suboptimal temperatures appears to be $E_{PS} = \sim 0.32$ eV for algal systems, and this can emerge at population [39] and ecosystem scales [4] in aquatic systems, suggesting $E_{NEP} = \sim 0.32$ eV, but other studies have found evidence for stronger or weaker values of E_{NEP}, ranging from 0 to 1.2 eV at population and ecosystem scales [10,20,49]. Across our experimental temperature gradient, we observed values of $E_R > \sim 0.65$ eV for both NEP and ER, though confidence intervals for ER did include this value (Fig 3) for algae-only ecosystems. These results led us to reject the 'first order metabolic theory' hypotheses that temperature dependence of ecosystem functions scales directly with general temperature dependence of metabolism. Our results further suggest that changes in species interactions within communities, such as loss or gain of a predator species, could alter the responses of net ecosystem fluxes to temperature changes.

Temperature had a stronger effect on phytoplankton standing stock than on NEP. This difference in phytoplankton biomass and oxygen-flux responses to temperature could reflect several processes operating at different scales of organization. First, we expect that *per capita* rates of oxygen flux increase with warming, so that a given biomass of phytoplankton can be more productive at warmer temperatures if resources are not limiting [4,46,50]. Patterns at the ecosystem scale could deviate from expectations based on direct metabolic scaling of *per capita* metabolism if size distributions shift toward smaller cells, as is common with warming as described by the temperature size rule [23,51]. The allometric scaling of metabolic rate with body size (Eqn 2) predicts greater oxygen flux for a given total biomass comprised of small individuals. The distribution of thermal tolerance phenotypes may have shifted within the phytoplankton communities. Three months is sufficient time for evolutionary change [52]. We did not see clear evidence of shifts in species composition with temperature, and it is

589 likely that the species we collected to inoculate our ecosystems were able to tolerate our

590 experimental conditions because we collected them from a shallow lake in Vancouver in

591 which the water temperature likely tracks summertime air temperatures, therefore

592 experiencing temperatures between 19 – 30 °C. Our experimental ecosystems likely did not

593 expose zooplankton to temperature outside what they would have experienced in a natural

594 system, and we therefore assume they were adapted to these conditions.

595   In addition to the effects of temperature on *per capita* metabolism and size structure,

596 at the ecosystem scale, effective resource supply may have changed with temperature,

597 violating an implicit assumption of equations 1-4. Even though these were closed ecosystems

598 with regard to external influxes of nutrients, and they experienced the same light conditions,

599 internal nutrient processes could have varied with temperature in ways that made nutrients

600 more available in warmer ecosystems. For example, our ecosystems did not include a benthic

601 habitat that can store nutrients and organic material and slow down nutrient cycling.

602 Heterotrophic microbial processes responsible for rapid nutrient turnover would be

603 accelerated by temperature perhaps making available nutrients in warmer systems more than

604 in colder systems. Another potential, and speculative, explanation for higher productivity than

605 expected in warmer ecosystems is that some algae species are capable of biological nitrogen

606 fixation [53] and this activity is more feasible at higher temperatures. These two biological

607 processes that are themselves temperature dependent could create a resource gradient in

608 parallel with the temperature gradient [15,50], leading to higher than expected NEP at warmer

609 temperatures relative to the same ecosystem at cooler temperatures.

610   Although there was no benthic sediment in our ecosystems, algae likely colonized the

611 sides and bottom of the tanks. Benthic algae may also have contributed to NEP and ER

612 estimates in our systems [54]. We did not observe notable amounts of accumulated benthic

613 algae, but even small amounts could have contributed to total ecosystem fluxes and led to

614 covariation in total biomass with temperature. If the ratio of phytoplankton to benthic algae

615   was temperature-dependent [54], our primary producer biomass estimates may have

616   increasingly under-represented total algal biomass at higher temperatures. To be conservative,

617   we did not present mass-normalized NEP estimates because we could not normalize to any

618   benthic algal metabolic biomass. Covariation between biomass and temperature is common

619   across geographic variation in temperature [12,20,53] and therefore present in other estimates

620   of NEP across broad spatial scales when biomass cannot be estimated well.

621   Across mean ecosystem temperatures of 19 – 30 °C, we observed no sign of

622   ecosystem collapse or threshold responses to warming. Changes in community structure and

623   the increase in trophic control along the temperature gradient appear to be exponential and

624   monotonic over the 10 °C gradient (Eqn 2), suggesting that linear (or additive) models of

625   temperature effects in most warming experiments, which only test two or three temperatures,

626   may underestimate warming effects over broader thermal gradients. We observed little

627   evidence of abrupt transitions that might be expected if thermal stress responses by individual

628   phenotypes drove ecosystem scale responses. We did observe declines in grazer density with

629   warming even in the absence of predators, suggesting there were direct or indirect negative

630   effects of temperature on grazers. But we did not see clear shifts in algal species composition

631   among treatments, suggesting that no species group was exposed to temperatures above its

632   critical thermal maximum. Another challenging aspect of warming experiments at the

633   population and community scales is interpreting patterns in the context of transient dynamics.

634   Our ecosystems certainly did not reach long term states, because varying weather conditions

635   and multi-week generation times of zooplankton would have precluded that. Still, we did not

636   observe signs of transient dynamics in these communities over time such as population cycles

637   or abrupt changes.

638   In our systems, algal biomass and zooplankton abundance in food webs were more

639   resistant to temperature in the presence of longer food chains. Predators reduced zooplankton

640   density and caused a clear trophic cascade. Trophic control, and therefore any mitigating

641 effects of predators on biomass change, was weak at low temperatures and increasingly strong

642 at higher temperatures (A vs AG treatment, Fig 3). This pattern is consistent with previous

643 findings that ecosystem functions in systems with two (or even numbers) of trophic levels

644 tend to be more sensitive to warming than systems with odd numbers, due to cascading effects

645 of predation on primary producers [48]. Additionally, in our experiment, predators were not

646 dynamically responsive; they did not have time to reproduce during the experiment.

647 Consequently, they represent mortality for zooplankton that may have varied with

648 temperature effects on *per capita* predation rates by predators, but not demographic response.

649 In many systems, predators are subsidized by other habitats and food sources, and their

650 populations are not dynamically coupled to prey. In fact, this decoupling has been shown to

651 be important in thermally stratified lakes [57]. Inferences drawn based on this experiment

652 about how species interactions affect community and ecosystem responses are restricted to

653 systems with dynamics in the primary producers and primary consumers, with predation-

654 related mortality imposed by a third trophic level through per capita consumption effects but

655 not population dynamics of the predators.

656　　　The growing literature of experimental tests of how warming affects interacting

657 species aims to reduce uncertainty in projected ecological changes associated with climate

658 change. Warming experiments have shown a wide variety of consequences for species

659 interactions, from shifts in community composition, strengthening top-down control, and

660 shifts in body size [16,18,54]. We have shown that these shifts do alter the effects on the

661 temperature dependence of net ecosystem oxygen production and consumption as modeled by

662 the metabolic theory of ecology, but that these models may be extended to consider

663 community level changes. By measuring community and ecosystem responses over a broad

664 thermal gradient under controlled conditions, we have provided empirical evidence that large

665 effects of temperature on community biomass can occur in the context of less strong effects of

666 temperature on net ecosystem function. This is a step toward closing the gap between patterns

667 observed across ecosystems that appear to reflect effects of temperature on metabolic rates,

668 and observations at intermediate scales that temperature can have large effects on the

669 abundance of species. Taken together, these results suggest our efforts to predict community

670 change with warming may benefit from the general metabolic scaling theory framework to

671 understand even local-scale effects of temperature change at the community level.

672

673

674

675 **Methods**

676 **Experimental Design and Set-up**

677 We assembled freshwater food webs in 30 outdoor mesocosms (370 L tanks) at the University

678 of British Columbia, Vancouver, Canada (49°14'52" N, 132°13'57" W). Mesocosms were

679 filled with municipal water on June 26th, 2012, heaters were added, and filled tanks were left

680 for one week to allow chlorine to evaporate before organisms were introduced. We

681 experimentally manipulated temperature (10 levels) and trophic structures (algae-only (A),

682 algae + grazer (AG), and algae + grazer + predator (AGP), Fig. 1B). There was one tank per

683 temperature per trophic treatment; statistical power was derived from the regression design

684 rather than replication within treatment levels (see Methods: Statistical Analysis). We

685 monitored temperature continuously, and sampled biotic variables once per week for 9 weeks.

686 Tanks were arranged randomly in space with regard to treatment. The spatially randomized

687 assignment of temperature and trophic treatments eliminated systematic variation in

688 negligible allochthonous carbon inputs.

689   On July 2, 2012, mesocosms were inoculated with pondwater (1L) from the UBC Pond

690 Facility, containing living algae, collected and filtered through a 64-µm sieve to remove

691 zooplankton and larvae. Three days later, we collected zooplankton at Trout Lake,

692 Vancouver, B.C. (49°15'23" N, 123°03'44" W), with a vertical tow net (64-µm mesh).

693   Zooplankton were mixed in buckets to homogenize species composition, were introduced to

694   mesocosm temperatures over a 12-hour gradual acclimation period to avoid stress associated

695   with an abrupt temperature change, and dead organisms were removed. Initial experimental

696   communities consisted of 25 phytoplankton taxa (Table S1), and those with zooplankton

697   included predominantly 2 zooplankton taxa (cladocerans *Daphnia* s*p.,* and calanoid copepod

698   *Eurytemora sp.*) and, rarely, cyclopoid copepods. To ensure colonization of grazing

699   zooplankton, in addition to the random aliquot of zooplankton added to each zooplankton

700   ecosystem (all algae-grazer and algae-grazer-predator ecosystems), we added two individuals

701   of *Daphnia* sp. and ten *Eurytemora* sp. Thus each zooplankton community began with *at least*

702   12 grazing zooplankton individuals. We introduced 2 individual notonectid predators

703   (*Notonecta undulata*), collected from ponds at the UBC Pond Facility, on July 4th, 2012 to 10

704   algae-grazer-predator tanks. Notonectids generate trophic cascades by suppressing

705   zooplankton [58]. Notonectids did not reproduce during the experiment, and we replaced dead

706   notonectids during the experiment with similar-sized individuals from the same source

707   population.

708   We added 160-µg $NaNO_3$ L^{-1} and 10-µg KH_2PO_4 L^{-1} to each tank (16:1 N:P) on July

709   3rd, 2012. These quantities of nutrients represent typical deposition inputs to similar lakes

710   [59]. Water was heated with submersible aquarium heaters (50, 100, 150, 200, 250, 300, 350,

711   400, 450 Watt) to increase temperature above ambient daily temperature. Temperatures were

712   recorded hourly using Thermochron iButton dataloggers. Data loggers were suspended in the

713   middle of the tanks, approximately halfway between the surface and the bottom. Temperature

714   differences among tanks were consistent throughout the course of the experiment (Fig S1).

715   Heaters were placed at the bottom of the mesocosms. Mesocosms were covered with two

716   layers of window screen to minimize colonization by other invertebrates. Water levels were

717   maintained by natural precipitation and weekly additions to maintain volume.

718

Plankton Sampling and Analysis

We sampled phytoplankton, chlorophyll *a*, zooplankton, and oxygen concentrations weekly until August 28th, 2012. We sampled algal assemblages in 100-mL water samples collected from ~40-cm below the surface. We counted and identified cells using the Utermöhl sedimentation method [60] and identified algae species or taxon level by inverted microscopy. We estimated chlorophyll *a* concentration using a Trilogy fluorometer (Turner Designs). Chlorophyll *a* concentration can be used as a proxy for biomass, and though the ratio between chlorophyll *a* and total biomass can itself vary with temperature, size and species composition [61,62], the chlorophyll *a* concentration represents biomass allocated to photosynthesis and NEP, our measure of ecosystem function. We measured oxygen concentrations *in situ* using YSI-85 oxygen sensor (Yellow Springs Instruments, Yellow Springs, Ohio, USA).

We collected zooplankton samples using a 'depth integrated zooplankton sampler'. The device is a cylinder 4 cm in diameter and 60 cm in length with a cap at one end. We mixed mesocosm water gently, then submerged vertically the sampler, sealed it, removed it and dumped water in to a bucket. We repeated until we had removed 10 L of water, which was then filtered through a 64-µm to collect zooplankton, and then the filtered water was returned to mesocosms. Plankton was fixed with Lugol's iodine solution (5%). Under 10x magnification, we counted and identified zooplankton to genus and measured standard length for all development stages in weeks 4-8.

Estimation of biomass and oxygen fluxes

We estimated whole ecosystem oxygen fluxes using the dissolved oxygen (DO) change technique [63]. Oxygen production during the daytime is the product of photosynthesis minus respiration (net ecosystem production, or NEP), and oxygen depletion during the night is the result of respiration (ER). We compared DO concentrations measured over 24 hours (dawn, dusk and the following dawn). Comparison of oxygen concentrations at

745 dawn, dusk and dawn of the following day (Eqn 5) can indicate not only the cumulative biotic

746 NEP and ER fluxes during that time interval, but also differences in water temperature that

747 affect oxygen concentrations in water. At standard pressure, which is appropriate for our

748 experiment near sea level, oxygen saturation can change by approximately 1 mg/L with a

749 change in temperature of 5 °C, described by:

750 $$[O_2]_E = e^{([O_2]_{water} - [O_2]_{sat} * \ln(T + 45.93))},$$ Eqn 5

751 where $[O_2]_{water}$ is the O_2 concentration of water, $[O_2]_{sat}$ is the concentration the water would

752 have if it were at equilibrium with the atmosphere (390 µatm), T is temperature of the

753 observation (°C) [64]. For the differences in temperature we observed, corrections were on

754 the order of mean $0.0002 \pm$ sd 0.0008 µmol O_2 / L / hr for NPP, and mean $0.0008 \pm$ sd 0.0003

755 µmol O_2 / L / hr for ER. Because these values are within 25% of our total observed changes in

756 oxygen during those periods (mean $0.003 \pm$ sd 0.001 µmol O_2 / L / hr for NEP and mean

757 $0.003 \pm$ sd 0.002 µmol O_2 / L / hr for ER), we included the correction in our analyses.

758 Overall, the conclusions based on model selection did not depend strongly on the use of the

759 correction.

760   We estimated NEP and ER by converting changes in observed O_2 (mg L^{-1}) between

761 daytime observation times (t_{dawn}, t_{dusk}) and overnight observations (t_{dusk}, t_{dawn2}) to micromolar

762 concentration ($z = 31.25$ µmol/1 mg), and correcting for changes in estimated equilibrium

763 oxygen concentration ($[O_2]_E$) (Eqn 5) due to changes in saturation state with temperature at

764 each time:

765 $$NEP = \left[\frac{([O_2]_{dusk} - [O_2]_{dawn}) - ([O_2]_{Edusk} - [O_2]_{Edawn})}{z * (t_{dusk} - t_{dawn1}} \right]$$ Eqn 6a

766 $$ER = \left[\frac{([O_2]_{dawn2} - [O_2]_{dusk}) - ([O_2]_{Edawn2} - [O_2]_{Edusk})}{z * (t_{dawn2} - t_{dusk})} \right].$$ Eqn 6b

767

768 **Model and hypothesis development**

769 The expression of temperature effects on a *per capita* metabolic rate b_i – in our case, oxygen

770 production via photosynthesis or consumption via respiration - in this model is a special case

771 of a more complex equation that allows each species to follow a thermal performance curve

772 (TPC), often described by a modified Sharpe-Schoolfield equation [10,20,65], in which an

773 individual's or population's performance declines at high temperatures above some optimal

774 temperature. We do not use this TPC model here for two reasons: we do not expect

775 photosynthesis or respiration to exceed optimal operating temperatures in our system for most

776 taxa based on the fact that we collected them locally from a lake and habitat type (shallow

777 pond) near the experimental site. We model our system using equations based on Eqn 2. We

778 believe this simpler exponential model is a suitable hypothesis for cross-system comparison in

779 which community phenotypes or taxonomic composition may turnover along the thermal

780 gradient [17,23]. We do not have thermal performance data for the many species in our

781 communities that would allow fitting of thermal performance curves within communities to

782 test an alternate approach.

783

784 We modeled M_B (Eqn 3) by including a term for trophic treatment (Z_j) in the intercept term

785 (Eqn 3 rearranged and log transformed):

786 $$\ln(M_B) = \ln\left(\frac{B_R}{Z_j * b_0(T_C)\langle m_B^{\alpha-1}\rangle}\right) + E_{MB}\left(\frac{1}{kT_j} - \frac{1}{kT_C}\right)$$ Eqn 7

787

788 We derived the expression for the trophic cascade by relating algal biomass in the

789 AGP and AG treatments:

790 $$\frac{M_{B.AGP}}{M_{B.AG}} = \frac{\frac{B_{R.AGP}\, e^{E_R\left(\frac{1}{kT} - \frac{1}{kT_C}\right)}}{(b_0(T_C))_{AGP}\langle m_B^{\alpha-1}\rangle_{AGP}}}{\frac{B_{R.AG}\, e^{E_R\left(\frac{1}{kT} - \frac{1}{kT_C}\right)}}{(b_0(T_C))_{AG}\langle m_B^{\alpha-1}\rangle_{AG}}}$$ Eqn 8

791

792   We then simplified and added temperature dependence of mass (E_m) and normalization

793   constants (E_b). In the absence of additional information about their functional forms, we used

794   general Arrhenius functions, but we note that other functions could be used if appropriate.

795   Consequently, the ratio of M_B with and without predators may vary with temperature

796   according to the relative temperature dependences of thermal traits and size distributions:

797
$$\frac{M_{B.AGP}}{M_{B.AG}} \propto \frac{b_0(T_C)_{AG}e^{-E_{b.ag}/kT}\langle m_B^{\alpha-1}\rangle_{AG}e^{-E_{m.ag}/kT}}{b_0(T_C)_{AGP}e^{-E_{b.agp}/kT}\langle m_B^{\alpha-1}\rangle_{AGP}e^{-E_{m.agp}/kT}} \qquad \text{Eqn 9}$$

798

799   and the strength of the trophic cascade may therefore be expected to decline with a

800   temperature dependence that reflects the temperature dependences of mass and normalized

801   performance for each trophic treatment:

802
$$ln\left(\frac{M_{B.AGP}}{M_{B.AG}}\right) \propto \frac{\ln(b_0(T_C)_{AG})+ln(\langle m_B^{\alpha-1}\rangle_{AG})-\frac{E_{b.ag}-E_{m.ag}}{kT}}{\ln(b_0(T_C)_{AGP})+ln(\langle m_B^{\alpha-1}\rangle_{AGP})-\frac{E_{b.agp}-E_{m.agp}}{kT}} \qquad \text{Eqn 10}$$

803

804   We modeled zooplankton density (N / L) as a function of mean weekly ecosystem

805   temperature T_{wj} and ecosystem trophic structure Z_j, with ecosystem identity as a random

806   effect.

807

808   **Statistical Analysis**

809   We tested our hypotheses about whether effects of temperature on metabolism are modified at

810   the ecosystem level by species interactions using a regression experimental design involving

811   30 independent ecosystems (Fig S1). We maintained ecosystems at distinct temperatures in a

812   regression design with mean ecosystem temperatures T_{wj} ranging from 19.7 ($\pm$ 3.15) °C to

813   26.1 ($\pm$ 3.59) °C (Fig S1). The regression design allowed us to estimate slopes (e.g., E_R, Eqn

814   2) of response variables along a continuous temperature gradient for different trophic

815   structures (A, AG, AGP) by log-transforming equation 2 and fitting linear models to log

816   transformed response variables the continuous temperature gradient. We chose the regression

817   design, though unreplicated within temperature levels, because it allowed us to compare

818   activation energies (E_R, Eqn 2) fitted over a broad range of temperatures; an important test of

819   thermal responses that is not possible with designs with only 2 or even three temperature

820   levels. Regression designs, even without replication within levels, gain statistical power from

821   the range of x-levels tested [66,67].

822   We used a mixed effects model (lme function in the nlme package of R) to examine

823   the main and interactive effects of temperature (a continuous fixed factor) and trophic

824   structure (a categorical fixed factor) on net ecosystem oxygen production (NEP), net

825   ecosystem oxygen consumption (ER), and chlorophyll *a* concentration with a random

826   intercept for individual ecosystems. We used a within-subject mean centering approach to

827   distinguish temperature effects into those associated with an ecosystem's average temperature

828   (T_j) over the entire experimental period (a 'between-ecosystem' effect) from effects variation

829   in temperature over time (T_{wj}) (a 'within-ecosystem temperature' effect) [68]. The response

830   variable (Y) for each ecosystem *j* in week *w* was modelled as a continuous response to

831   variation in inverted ecosystem temperature ($1/kT_{wj}$) and trophic treatment (Z_j):

832    $\ln(Y_{wj})$

833
$$= \beta_{0.j(w)} + \beta_1 \left(1/kT_{wj} - 1/k\bar{T}_j \right) + \beta_2 \left(1/k\bar{T}_j \right) + \beta_3 \left(1/kT_{wj} - 1/k\bar{T}_j \right)\left(1/k\bar{T}_j \right) + \beta_4 Z_j$$

834
$$+ \beta_5 Z_j \left(1/k\bar{T}_j \right) + \beta_6 Z_j \left(1/k\bar{T}_j - 1/k\bar{T}_j \right) + u_j + e_{wj}$$

835

Eqn 11

836   where $\beta_{0.j(i)}$ represents an intercept allowed to vary randomly among ecosystems. The terms

837   in the full model (Eqn 11) are: the between-ecosystem effect of temperature (β_2), estimated as

838   the slope of ln(Y*wj*) on the mean temperature over all weeks for ecosystem *j*, expressed as

839   inverse temperature $\left(1/k\bar{T}_j\right)$; the within-ecosystem ($\beta_1$) effect of temperature variation over

840   time estimated as the slope of ln(Y_{wj}) *vs* centered weekly temperature $\left(1/k\bar{T}_{wj}\right) - \left(1/k\bar{T}_j\right)$;

841   interaction (β_3) between within-ecosystem temporal variation in temperature and the

842   experimental temperature treatment; trophic species interactions (β_4), and interactions

843   between species interactions and overall mean (β_5) and weekly temperature (β_6).

844   To test our hypothesis that species interactions modify temperature dependence (E_R,

845   Eqn 2) of response variables (Y), we compared models with and without trophic level terms

846   (β_4) and interactions between Z_j and temperature (β_5, β_6). We also tested models without

847   temperature terms for within-system variation (β_4). In total, the model set included 9 models

848   (Table 1). Response variables were ln-transformed prior to analyses to achieve normal

849   distributions and to linearize temperature effects for analysis and to fit E_R values from Eqn 2.

850   When modelling, we centered temperature treatment (1/kT$_j$) on the grand mean of all

851   temperatures observations $\bar{T}$ (not shown in Eqn 11) to reduce correlations between slope and

852   intercept terms [69].

853   To test the effect of temperature on trophic cascade strength, we used the following

854   statistical model:

855    $$\ln\left(TC_{ij}\right) = \beta_{0.p(w)} + \beta_1 * \left(1/kT_{wp} - 1/k\bar{T}_p\right) + \beta_2 * w + \beta_3 * \left(1/kT_{wp} - 1/k\bar{T}_p\right) * w +$$

856    $$u_p + e_{wp} \hspace{4cm} \text{Eqn 12,}$$

857   in which the effect of temperature on trophic cascade strength in each temperature treatment *j*

858   was modeled for each week *w* and for the temperature of the tanks, with random effects u_j

859   were assigned for each power treatment (*p*).

860   We ranked models using Akaike's Information Criterion weights (using the MuMin

861   package in R), adjusted for small sample sizes (AIC$_C$). When two or more models were

862   considered comparable or equivalent (δAIC$_C$ < 2) we reported all models meeting this

863   criterion and report averaged coefficients. We estimated the realized temperature dependence

864   of our response variables (slopes) and intercepts for among-ecosystem responses to

865   temperatures by first rearranging Eqn 12 to group coefficients by temperature term.

866    $\ln(Y_{jw}) = \beta_{0.j(w)} + \left(\beta_1 + \beta_3 * \left(\frac{1}{k\bar{T}_j}\right) + \beta_6 * Z_j\right)\left(\frac{1}{kT_{wj}} - \frac{1}{k\bar{T}_j}\right) + (\beta_2 + \beta_5 * Z_j) *$

867    $\left(\frac{1}{k\bar{T}_j}\right) + \beta_4 * Z_j + u_j + e_{wj}.$

Eqn 13

868   The pooled coefficients $(\beta_2 + \beta_5 * Z_j)$ include the temperature dependence of ecosystem

869   responses plus any variation with trophic structure, and gives the slope of lines plotted in

870   figures 2A and 5. The intercept is set by $\beta_{0.j(w)} + \beta_4 * Z_j$, and the remaining coefficient gives

871   within-tank variation as plotted in Figure 6. We estimated confidence intervals for composite

872   terms following [70]. We used R statistical software (R v. 1.0.44 R Developmental Core

873   Team 2006). Our models controlled for the effect of temperature variation over time on

874   ecosystem fluxes and biomass within systems.

875   We determined the effects of temperature and predator presence on zooplankton

876   abundance data using generalized linear mixed effects models with tank as a random effect

877   modeled on a negative binomial regression distribution to account overdispersed Poisson

878   distributed count data (using the glmmADMB package in R c 3.5.0).

879

885

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886

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