1 **A temperature dependent trophic cascade modifies temperature dependence of**

2 **ecosystem function**

3

4 **Running head: Food chain length modifies effects of warming**

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24 Research paper

25

Abstract

Ecological communities and their ecosystem functions are sensitive to temperature, and aquatic habitats worldwide continue to experience unprecedented warming. Understanding ecological effects of warming requires linking empirical evidence to theories that allow projection to unobserved conditions. Metabolic scaling theory and its tests suggest that warming accelerates ecosystem functions (e.g., oxygen flux), yet this prediction apparently contradicts community-level studies suggesting warming is a stressor that can reduce ecosystem function. We sought to reconcile these predictions with an experimental test of the hypothesis that cascading trophic interactions modify the temperature-dependence of community structure and ecosystem fluxes. In a series of independent freshwater ecosystems exposed to a thermal gradient, we found that warmer temperatures strengthened the trophic cascade increased and indirectly changed community structure by altering grazer species composition and phytoplankton biomass. Temperature-driven community shifts only modestly affected the temperature dependence of net ecosystem oxygen fluxes. Over the 10 °C thermal gradient, NPP and ER increased ~2.7-fold among ecosystems, while standing phytoplankton biomass declined by 85-95%. The exponential increase in oxygen flux over the thermal gradient, as well as monotonic declines in phytoplankton standing stock, suggested no threshold effects of warming across systems. We also observed temperature variation over time, within ecosystems. For phytoplankton biomass, temporal variation had the opposite effect to spatial variation, suggesting that within-community temporal change in community structure was not predicted by space-for-time substitution. We conclude that food chain length can modify effects of temperature on ecosystem fluxes, but that temperature can still have continuous and positive effects on ecosystem fluxes, consistent with patterns based on large-scale, macroecological comparisons. Changes in community structure, including temperature dependent trophic cascades, may be compatible with prevailing and predictable

51   effects of temperature on ecosystem functions related to fundamental effects of temperature

52   on metabolism.

53

Introduction

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55   Understanding how warming associated with climate change affects species

56   interactions and communities is one of the most pressing current ecological challenges. Two

57   leading conceptual frameworks, the Metabolic Scaling Theory (MST) and community

58   ecology, produce very different predictions for community-scale responses to environmental

59   temperature. Metabolic scaling theory predicts that the relative temperature dependence of

60   major metabolic functions explains a large fraction of variation in ecosystem fluxes and

61   biomass stocks (López-Urrutia *et al.*, 2006; Enquist *et al.*, 2007; Wohlers *et al.*, 2009; Yvon-

62   Durocher *et al.*, 2010; O'Connor *et al.*, 2011; Yvon-Durocher *et al.*, 2012; Barneche *et al.*,

63   2014). This signal of temperature dependence of fundamental metabolic rates emerges over

64   broad climate gradients and comparisons among independent ecosystems (López-Urrutia *et*

65   *al.*, 2006; Anderson-Teixeira *et al.*, 2008; Yvon-Durocher *et al.*, 2012) and suggests that

66   energetic constraints of two highly conserved metabolic processes (oxygenic photosynthesis

67   and aerobic respiration) may drive responses to environmental temperature change at the

68   ecosystem, community and population levels (Gillooly *et al.*, 2001; Yvon-Durocher *et al.*,

69   2010; 2012; Padfield *et al.*, 2016).

70   The metabolic scaling approach to understanding community level responses to

71   temperature change is challenged by an apparent incongruence between effects predicted by

72   the temperature-dependence of metabolic functions such as photosynthesis and respiration, on

73   the one hand, and the often large effects of temperature on species interactions, on the other.

74   Temperature affects species interactions via population and bioenergetic dynamics, often

75   producing non-intuitive patterns in species' abundances (Beisner & Peres-Neto, 2009;

76   O'Connor, 2009; Kordas *et al.*, 2011; Dell *et al.*, 2013; Gilbert *et al.*, 2014). Numerous

77   warming experiments have shown that warming alters the abundance of interacting

78   consumers and resources and the strength of top down control of community structure

79   (Hansson *et al.*, 2012; Shurin *et al.*, 2012). It has even been suggested that MST is not

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80   relevant to understanding community change in response to warming (Tilman *et al.*, 2004;

81   Brauer *et al.*, 2009). Whether demographic or bioenergetic changes at the community scale

82   are constrained by highly conserved metabolic thermal asymmetries, or represent additional

83   and potentially confounding effects of temperature on species interactions, remains

84   unresolved. This lack of resolution implies that we have no accepted framework for

85   determining which patterns observed in simple mesocosm experiments can be extrapolated to

86   projections for effects of climate change in nature.

87   If the effects of temperature on consumer-resource interactions are constrained by the

88   temperature dependence of photosynthesis and respiration (Allen *et al.*, 2005), net ecosystem

89   effects of warming may be largely independent of demography or species interactions. In

90   other words, though temperature-dependent trophic interactions can alter density, biomass and

91   species composition of consumers and primary producers (Beisner *et al.*, 1996; Petchey *et al.*,

92   1999; O'Connor *et al.*, 2009; Yvon-Durocher *et al.*, 2010; DeLong *et al.*, 2015), there is little

93   evidence that these community changes cause temperature-dependence of ecosystem-level

94   oxygen fluxes to deviate from expectations based on the temperature dependence of

95   photosynthesis and respiration at macroecological scales (López-Urrutia *et al.*, 2006;

96   Anderson-Teixeira *et al.*, 2008; Yvon-Durocher *et al.*, 2010; 2012). The best evidence to

97   support the importance of community shifts in modifying ecosystem level responses to

98   temperature comes from experimental tests of gross primary production of periphyton (in the

99   absence of grazing) across a thermal gradient in streams (Padfield *et al.*, 2017). These results

100   showed that species composition shifts can reduce the effect of temperature at the ecosystem

101   level, compensating for effects of temperature on total productivity. However, we do not yet

102   have evidence that trophic interactions can play such a role, although local-scale experiments

103   with a few species suggest that the strength or presence of strong top-down control by

104   consumers can influence ecosystem level energy flux (Schindler *et al.*, 1997); one likely

105   pathway, therefore, through which species interactions could modify the effect of temperature

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106   on net ecosystem fluxes is if trophic cascades increase in strength at higher temperatures. The

107   strength of trophic cascades depends on primary production and the magnitude of herbivore

108   density and/or behavioral responses to predation (Polis *et al.*, 2000; Schmitz *et al.*, 2003), in

109   addition to the activities and density of predators themselves. Temperature dependent trophic

110   cascades could therefore disrupt relationships between temperature and ecosystem fluxes

111   mediated by direct effects of temperature on per capita metabolic rates.

112   Here, we tested whether temperature-dependent top-down control on herbivore and

113   algal abundance and composition can alter the effect of temperature on ecosystem function

114   (oxygen flux, phytoplankton standing stock). We experimentally quantified change across a

115   broad temperature gradient (10 °C) and compared ecosystem and community states across this

116   gradient (photosynthesis and respiration). The purpose of the broad temperature gradient,

117   which exceeds forecasted warming over the coming century, is to test the functional response

118   of warming at the community and ecosystem scales to allow comparison with theoretical

119   predictions about this relationship. This question gets at the heart of the larger question of

120   whether energy and material fluxes in ecosystems can be adequately predicted at the local

121   scale in terms of temperature constraints on fundamental metabolic rates (photosynthesis,

122   respiration), or whether community dynamics render such metabolic-theory-derived

123   predictions insufficient to the point of not useful (Brown *et al.*, 2004; Tilman *et al.*, 2004;

124   Brauer *et al.*, 2009).

125

126   **MST framework and hypotheses**

127   We express our hypotheses in terms of testable relationships between temperature and

128   ecosystem function. One common approach to understanding how temperature affects

129   communities and ecosystems is to 'scale up' from individual physiological processes and

130   species-specific traits. This approach requires a high burden of information about each

131   species' thermal traits, and this need for detailed species-level information prohibits such

132   scaling-up for most ecosystems. The macro-ecological framework of metabolic scaling theory

133   provides an alternative approach, in which relatively little information (in this case,

134   temperature dependence of the fundamental and highly conserved metabolic processes

135   photosynthesis and respiration) is applied to whole systems with many individuals and species

136   to understand the aggregate functional response to temperature change. In this framework,

137   whole-organism metabolic rates (e.g., oxygen flux) and related biological functions for

138   organism *i* have been described as following a power-law dependence on body mass and

139   exponential (Boltzmann-Arrhenius) dependence on temperature (Gillooly *et al.*, 2001; Brown

140   *et al.*, 2004):

141   $$b_i = b_0 e^{-E_a/kT} m_i^a \qquad\qquad \text{Eqn 1a}$$

142   in which activation energy (E_a, in eV) captures temperature (T, in Kelvin) effects on per

143   capita metabolic response (b_i) for individual *i, k* is the Boltzmann constant (eV/K), b_0 is a

144   normalization constant independent of body size and temperature, m_i corresponds to the body

145   mass of an individual *i*, and *a* is the allometric scaling factor. This model is a special case of a

146   more complex equation that allows each species to follow a thermal performance curve, often

147   described by a modified version of the Sharpe-Schofield equation, in which performance

148   declines at high temperatures. However, for multi-species systems, the exponential model

149   performs well (Padfield *et al.*, 2017). This exponential model has been extended to produce a

150   first-order expectation for the effects of temperature on ecosystem-level rates (B_R):

151   $$B_R = b_0^R e^{-E_B/kT} M_B \langle m_B^{\alpha-1} \rangle, \qquad\qquad \text{Eqn 1b}$$

152   in which $-E_B$ is the ecosystem-level temperature dependence term for ecosystem rate R, M_B is

153   total biomass of the community, and $\langle m_B^{\alpha-1} \rangle$ is a weighted average biomass. Together,

154   $M_B \langle m_B^{\alpha-1} \rangle$ represents 'mass-corrected' biomass, which is a measure of the total metabolic

155   capacity of the biomass pool in the ecosystem (Yvon-Durocher & Allen, 2012). Here, we

156   tested the effects of temperature on independent (isolated) experimental ecosystems with

157   distinct thermal histories, sharing a common initial species pool. Following Eqn 1b, our

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158   hypotheses about how the mean temperatures of these independent ecosystems lead to

159   patterns in ecosystem function across systems can be stated as: for communities closed to

160   immigration and ecosystem closed to resource inputs other than light:

161   *i)*   The temperature dependence ($-E_B = -E_{NPP}$) of net primary production ($B_{NPP} = B_{NPP}$) is

162   predicted by the temperature dependence of photosynthesis ($E_{PS} = E_{NPP} = -0.32$ eV)

163   for autotroph-dominated communities,

164   *ii)*   Across systems, the temperature dependence $-E_{ER}$ of net ecosystem respiration (B_{ER})

165   is predicted by the temperature dependence of respiration ($E_{ER} = E_R = -0.65$ eV)

166   *iii)* These temperature dependence coefficients do not vary with food chain length (Fig.

167   1).

168   These hypotheses that net ecosystem fluxes vary with temperature with the activation energies

169   associated with per capita metabolic rates (photosynthesis and temperature) assume that

170   minimal changes occur in total mass-corrected autotrophic and heterotrophic metabolic

171   biomass over the thermal gradient within each trophic treatment (Yvon-Durocher *et al.*, 2010;

172   Yvon-Durocher & Allen, 2012; Yvon-Durocher *et al.*, 2012). Past experiments on species

173   interactions suggest this assumption is not supported, so, following community ecological

174   theory and empirical evidence, we consider two alternate hypotheses:

175   (*iv*) total phytoplankton biomass standing stock (M_B) declines with temperature and

176   this effect varies with food chain length reflecting a temperature-dependent trophic cascade.

177   (v) food chain length and temperature change the relative abundance of species and

178   trophic groups within each community, but these community changes do not affect net

179   ecosystem fluxes.

180

181   **Methods**

182   **Experimental Design**

183   We tested our hypotheses by manipulating temperature and food chain length in 30

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184   independent aquatic ecosystems (Fig. 1A-B). For each food chain length (algae-only, algae-

185   grazer or algae-grazer-predator), we maintained ecosystems at distinct temperatures in a

186   regression design with mean temperatures ranging from ~19 °C to ~31 °C (Fig. 1C). The

187   regression design allowed us to estimate slopes (E_A, Eqn 1b) of response variables along a

188   continuous temperature gradient (Cottingham *et al.*, 2005; Gotelli & Ellison, 2013) for

189   different trophic structures by log-transforming equation 1b and fitting linear models to the

190   continuous temperature gradient. The regression design was the right choice to compare

191   activation energies over a broad range of the x-variable (temperature); an important test of

192   thermal functional responses that is not possible with designs with only 2 or even three

193   temperature levels. The three-trophic-level treatment included predators that were not a

194   dynamic part of the system – they did not reproduce and their several month life span did not

195   allow for demographic responses. Therefore, inferences about trophic structure are restricted

196   to systems with dynamics in the primary producers and primary consumers, with fixed

197   predation-related mortality imposed by a third trophic level.

198   We designed an experiment to test these hypotheses by comparing replicate

199   communities across a thermal gradient. Each ecosystem experienced the same conditions

200   (weather, seasonal variation), and differed in the average temperature of the ecosystem (Fig.

201   1C). Our experiment was not designed to track and measure community dynamics over time –

202   we did not sample frequently enough for a robust test of temporal dynamics. Therefore, we

203   aim to test our hypotheses and draw inferences based on a comparison of 30 independent

204   ecosystems along the thermal gradient, rather than by detailed analysis of their temporal

205   trajectories. Considerations of how ecosystems responded to temperature variation over time

206   in this experiment would be confounded by temporal changes in community structure and

207   successional dynamics. We focus here on how the structure and function of ecosystems varies

208   with average temperature. Our approach thus uses a controlled experiment to mirror the

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209   application of temperature scaling models to whole ecosystem change over broad spatial

210   scales in comparative studies (López-Urrutia *et al.*, 2006; Anderson-Teixeira *et al.*, 2008;

211   Yvon-Durocher *et al.*, 2012).

212

213   **Experimental Food Webs**

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

215   of British Columbia, Vancouver, Canada (49°14'52" N, 132°13'57" W). From June 26th to

216   August 28th 2012, we experimentally manipulated temperature (10 levels) and food chain

217   length (3 levels: algae-only, algae + zooplankton, and algae + zooplankton + predator food

218   chains, Fig. 1A-B). Initially (experiment day 7) mesocosms were inoculated with pondwater

219   (1L) containing living algae, collected and filtered through a 64-µm sieve to remove

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

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

222   Zooplankton were mixed in buckets to homogenize species composition, acclimated

223   overnight to mesocosm temperatures and dead organisms removed. Initial experimental

224   communities consisted of 25 phytoplankton taxa (S1) and predominantly 2 zooplankton taxa

225   (the cladoceran *Daphnia* s*p.,* and calanoid copepod *Eurytemora* sp.), though a few cyclopoid

226   copepods were included. To ensure colonization of grazing zooplankton, two individuals of

227   *Daphnia* sp. and ten *Eurytemora* sp. were added to each consumer treatment (all 2- and 3-TL

228   ecosystems). We introduced 2 individual notonectid predators (*Notonecta undulata*) on July

229   3rd, 2012 (experiment day 7) to 10 3-TL tanks. Notonectids generate trophic cascades by

230   suppressing zooplankton (Mcardle & Lawton, 1979). Notonectids did not reproduce during

231   the experiment, and we replaced dead notonectids during the experiment with similar-sized

232   individuals from the same source population.

233

234   **Figure 1: A)** Experimental food web composition and **B)** experimental design for three

235   trophic level systems. Food chains contained Notonectid predators (N), zooplankton (Z)

236   grazers *Eurytemora* sp. and *Daphnia* sp., and phytoplankton (P). **C)** Ecosystem temperature

237   and food chain length were manipulated in a regression experimental design with 10

238   independent systems spanning a 10 °C temperature gradient. The temperature gradient was

239   repeated for each food chain length: 1-, 2- and 3-trophic levels. The experiment occurred over

240   one to many generations of grazers and phytoplankton, but within one (adult) life stage of

241   notonectid predators.

242

243
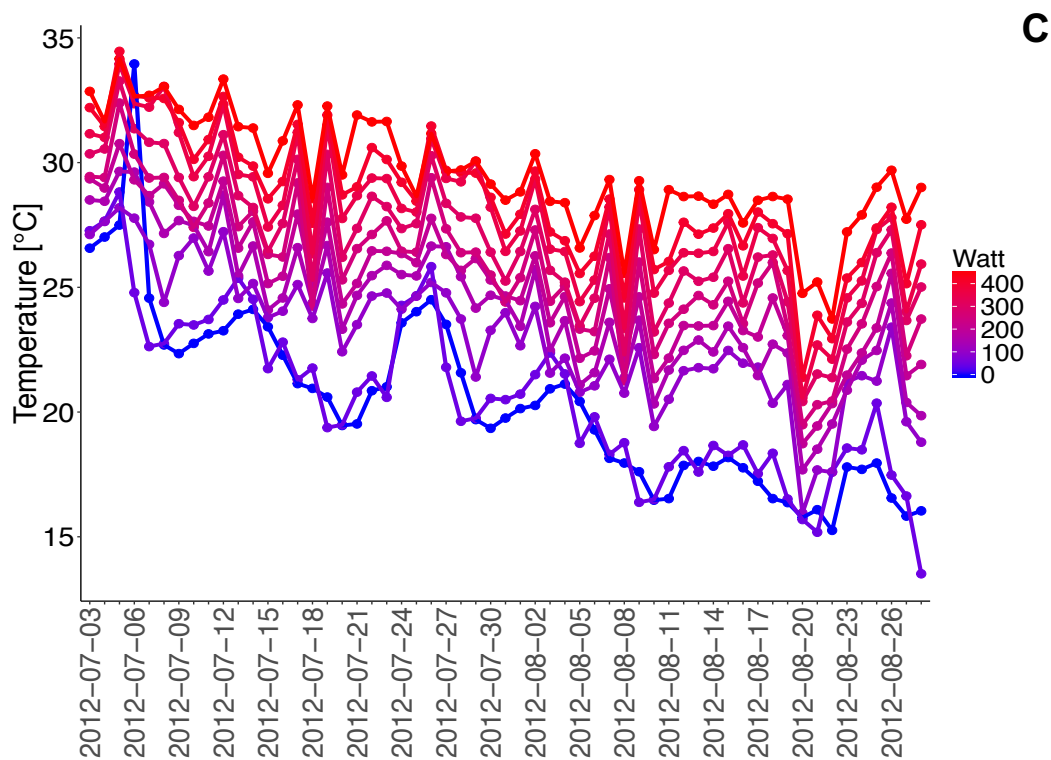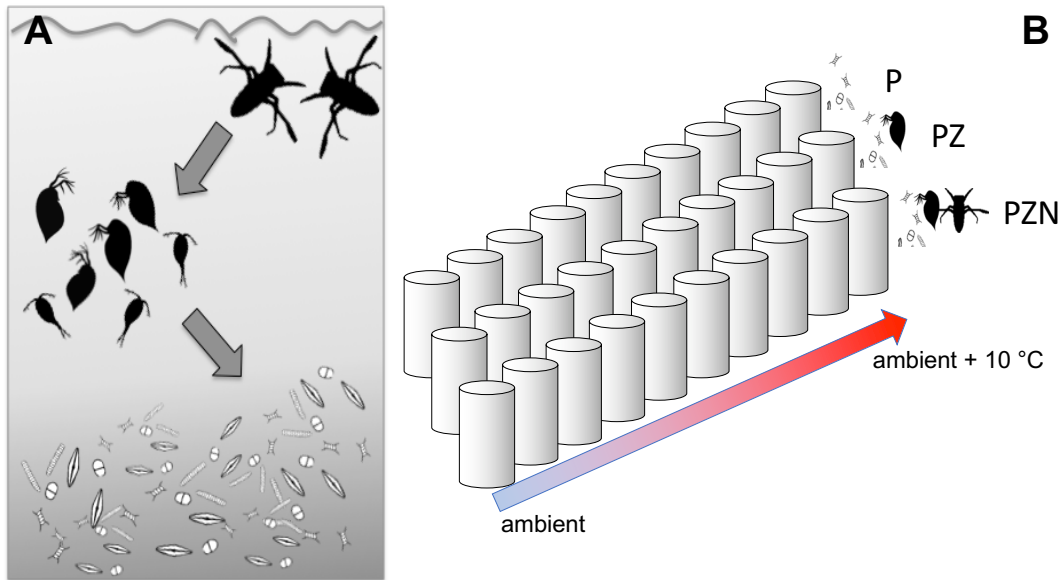
Abiotic and biotic conditions

245 Mesocosms were filled with municipal water and left for one week to allow chlorine to

246 evaporate before organisms were introduced (Kratina *et al.*, 2012). We added 160-µg $NaNO_3$

247 L^{-1} and 10-µg KH_2PO_4 L^{-1} to each tank (16:1 N:P) on experiment day 7. Water was heated

248 with submersible aquarium heaters (50, 100, 150, 200, 250, 300, 350, 400, 450 Watt) to

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249   increase temperature above ambient daily temperature. Temperature differences among tanks

250   were consistent throughout the course of the experiment (Fig. 1C). Mesocosms were covered

251   with two layers of window screen to minimize colonization by other invertebrates. Water

252   levels were maintained by natural precipitation and weekly additions to maintain volume. The

253   spatially randomized assignment of temperature and trophic treatments eliminated systematic

254   variation in negligible allochthonous carbon inputs.

255

256   **Plankton Sampling and Analysis**

257   Weekly, we sampled phytoplankton, chlorophyll *a*, zooplankton, and oxygen

258   concentrations. We sampled algal assemblages in 100-mL water samples collected from ~40-

259   cm below the surface. We counted and identified cells using the Utermöhl sedimentation

260   method (Utermöhl, 1958) and estimated chlorophyll *a* concentration using a Trilogy

261   fluorometer (Turner Designs). Phytoplankton were identified and counted to species or taxon

262   level by inverted microscopy. We collected depth-integrated zooplankton samples (10 L water

263   filtered through a 64-μm); the filtered water returned to mesocosms. Plankton was fixed with

264   Lugol's iodine solution (5%). Under 10x magnification, we counted and identified

265   zooplankton to genus, measured standard length for all development stages in week 8. We

266   measured oxygen concentrations using YSI-85 oxygen sensor (Yellow Springs Instruments,

267   Yellow Springs, Ohio, USA).

268

269   **Estimation of biomass and fluxes**

270   We estimated carbon biomass of phytoplankton (M_P) by converting chlorophyll *a*

271   concentrations using 0.05 μg chlorophyll *a* / μg C at 295K, and a temperature dependence of

272   this ratio of -0.001 °C^{-1} (Geider *et al.*, 1997). For zooplankton (grazers and notonectids), we

273   used length-weight ratios to convert length to carbon (S2). We did not estimate microbial or

274   periphyton biomass.

275   We estimated whole ecosystem oxygen fluxes using the dissolved oxygen (DO)

276   change technique (Marzolf *et al.*, 1994), correcting for temperature effects on oxygen

277   saturation state. The change in DO (ΔDO) was measured over 24 hours (dawn, dusk and the

278   following dawn) according to forecasted sunrise and sunset on measurement days (Hanson *et*

279   *al.*, 2003). We assumed sunrise represented minimum daily O_2 concentration, after which all

280   subsequent values were greater (Yvon-Durocher *et al.*, 2010; Kratina *et al.*, 2012; Yvon-

281   Durocher & Allen, 2012), and we assumed that sunset coincided with maximum O_2

282   concentration (DO) after which all subsequent values were lower. Physical oxygen flux (mg

283   m^{-2} d^{-1}) with the atmosphere was calculated as follows: $O_{2flux} = \exp(O_{2water} - O_{2sat})$ * ln(T +

284   45.93) - e, where O_{2water} is the O_2 concentration of water, O_{2sat} is the concentration the water

285   would have if it were at equilibrium with the atmosphere (390-μatm), T is the temperature

286   correction for O_2 saturation, e is the pressure coefficient for elevation of study area (here

287   0.03) (Atwood *et al.*, 2015). We estimated NPP and ER, in μmol O_2 L^{-1} d^{-1}, as follows:

288    $NPP = [\dfrac{(O_{2.dusk} - O_{2.dawn}) \times 1000}{32}]d^{-1}$ Eqn 3a

289    $ER = \left[\dfrac{24}{(t_{dawn} - t_{dusk})}\right] \times \left[\dfrac{((O_{2.dawn2} - O_{2.dusk}) * 1000)}{32}\right]$ Eqn 3b

290   in which O_2 is dissolved oxygen (mg L^{-1}), 32 μg O_2/μmol O_2, and measurement time t_i.

291   We processed and analysed the full set of response variables for weeks 4 – 9, except

292   for zooplankton size, excluding transient bloom conditions (weeks 1-3) that were not the

293   focus of this study.

294

Statistical Analysis

296   We used linear mixed effects models to describe relationships between ecosystem and

297   community response variables (Y) and temperature, a continuous predictor in our

298   experimental design. While temperature varied over time in the experimental ecosystems, so

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299   did other conditions including ecological succession and weather. Our hypotheses are about

300   how different average temperature conditions affect ecosystem functions, given normal

301   environmental variation within ecosystems. Therefore, we modelled among-system responses

302   to temperature, while also modelling within-system variation associated with temperature, and

303   other conditions, over time. To distinguish within-tank variation from among-tank effects of

304   temperature, we used a within-subject mean centering approach that decomposes the

305   environmental effects into those associated with the average environment experienced over

306   the experimental duration ('between-tank' effect), versus deviations of the environment in a

307   given temperature treatment ('within-tank temperature' effect) (van de Pol & Wright, 2009).

308   We first tested a random effects model, in which the response variable (Y) for each

309   ecosystem j in week i was modelled as a continuous response to variation in inverted

310   ecosystem temperature ($1/kT_{ij}$) and trophic level (TL_j)

$$\ln(Y_{ij}) = \beta_{0.j(i)} + \beta_1 \left(\frac{1}{kT_{ij}} - \frac{1}{k\bar{T}_j} \right) + \beta_2 \frac{1}{k\bar{T}_j} + \beta_3 * \left(\frac{1}{kT_{ij}} - \frac{1}{k\bar{T}_j} \right) * \left(\frac{1}{k\bar{T}_j} \right)$$

311
$$+ \beta_4 * TL_j + \beta_5 * \left(\frac{1}{k\bar{T}_j} \right) * TL_j + \beta_6 * \left(\frac{1}{kT_{ij}} - \frac{1}{k\bar{T}_j} \right) * TL_j$$

$$+ u_j + e_{ij}$$

    Eqn 4

312   where $\beta_{0,j(i)}$ represents an intercept allowed to vary randomly among tanks. The between-

313   ecosystem effect of temperature (β_2) is estimated as the slope of $\ln(Y_{ij})$ on the mean value of

314   inverse temperature for ecosystem j $\left(\frac{1}{k\bar{T}_j} \right)$ over all weeks. The within-subject (β_1) effect of

315   temperature variation over time is estimated as the slope of $\ln(Y_{ij})$ vs the deviation of the

316   mean temperature each week $\left(\frac{1}{k\bar{T}_{ij}} \right)$ relative to $\left(\frac{1}{k\bar{T}_j} \right)$. We also tested for an interaction

317   between within-treatment temporal variation in temperature and the experimental temperature

318   treatment (β_3). To test our hypothesis that trophic structure modifies the effect of temperature

319   on ecosystem function, we included the terms (β_6) and (β_5). We tested for effects of trophic

320   structure on ecosystem function, independent of temperature, with the term (β_4). Response

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321   variables were ln-transformed prior to analyses to achieve normal distributions and to

322   linearize temperature effects for analysis and comparison with MST predictions (Eqn 1b).

323   When modelling we centered temperature treatment $(1/kT_j)$ on the grand mean temperature $\bar{\bar{T}}$

324   (not shown in Eqn 4) to reduce correlations between slope and intercept terms. For each

325   response variable, we used ANOVA to determine the need for the random effect of tank by

326   first comparing the full model (Eqn 4) with the same model without the random effect.

327   To test our hypothesis that food chain length (1, 2 or 3 trophic levels) modifies the

328   estimated temperature dependence (E_a, Eqn 1b) of oxygen flux and biomass, we first

329   identified the best model for each response variable. We compared models with and without

330   trophic level terms (β_4) and interactions between TL and temperature (β_5, and β_6). We also

331   tested models without each of the temperature terms. In total, after testing for the random

332   effects structure, the model set included 9 models (Table 1). We ranked models using

333   Akaike's Information Criterion (AIC_C) weights, and compared models using likelihood ratio

334   tests (LRT). When two or models were considered comparable or equivalent ($\delta AIC < 2$) we

335   reported all models meeting this criterion and report averaged coefficients. We estimated

336   activation energy and intercepts for among tank responses to temperatures by first rearranging

337   Eqn 4 to group coefficients by temperature term (Eqn 4a):

338

$$\ln(Y_{ij}) = \beta_{0.j(i)} + \left(\beta_1 + \beta_3 * \left(\frac{1}{k\bar{T}_j}\right) + \beta_6 * TL_j\right)\left(\frac{1}{kT_{ij}} - \frac{1}{k\bar{T}_j}\right) + \left(\beta_2 + \beta_5 * TL_j\right) * \left(\frac{1}{k\bar{T}_j}\right)$$
$$+ \beta_4 * TL_j + u_j + e_{ij}$$

339   We estimated confidence intervals for composite terms following (Figueiras *et al.*, 1998).

340   To analyse the effects of time and temperature on the phytoplankton community composition,

341   we performed a Non-metric multidimensional scaling (NMDS). It is a rank-based ordination

342   technique which is robust towards data sets where abundances are highly diverse among taxa.

343   While NMDS does not allow assessing the effects of environmental gradients, it enables the

344   detection of temporal patterns in the data. We used the metaMDS function in R to measure

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345   the Bray-Curtis community dissimilarities. Species abundances were square root transformed.

346   We used R statistical software (R v. 1.0.44 R Developmental Core Team 2006) with packages

347   MuMIn, nlme, plyr, tidyverse, broom, reshape2, lubridate, hms and zoo.

348

349   **Table 1:** Candidate models. The full model, as written in Eqn 4 in the main text, is

350   reproduced here for convenience. The table indicates with an 'x' which terms were included

351   in each candidate model.

$$\ln(Y_{ij}) = \beta_{0.j(i)} + \beta_1\left(\frac{1}{kT_{ij}} - \frac{1}{k\overline{T}_j}\right) + \beta_2 \frac{1}{k\overline{T}_j} + \beta_3 * \left(\frac{1}{kT_{ij}} - \frac{1}{k\overline{T}_j}\right)*\left(\frac{1}{k\overline{T}_j}\right)$$

352

$$+ \beta_4 * TL_j + \beta_5 * \left(\frac{1}{k\overline{T}_j}\right)*TL_j + \beta_6 * \left(\frac{1}{kT_{ij}} - \frac{1}{k\overline{T}_j}\right)*TL_j$$

$$+ u_j + e_{ij}$$

Mod.	Model parameter							
	$\beta_{0.j(i)}$	β_1	β_2	β_3	β_4	β_5	β_6	μ_j
Full.r	x	x	x	x	x	x	x	x
Full	x	x	x	x	x	x	x	
8	x	x	x		x	x	x	
7	x	x	x		x	x		
6	x	x			x		x	
5	x	x			x			
4	x	x	x	x				
3	x	x	x					
2	x	x						
1	x				x			
0	x							

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Results

Temperature dependence of top-down control (hypotheses iv - v)

We first present results for hypotheses iv-v, because they demonstrate top-down control that was temperature dependent in our ecosystems. Total ecosystem oxygen fluxes varied with food chain length, after controlling for temperature, reflecting top down control of ecosystem functions, as indicated by significant variation in intercept terms and the β_4 term in the highest ranking models for NPP and ER (Fig. 2, Table 2). Net oxygen fluxes in systems with predators tended to be more similar to systems without consumers (Fig. 2A-F), indicating that strong grazer effects on oxygen fluxes were minimized in the presence of predators by top down control.

Predator-grazer-algae food chains had higher phytoplankton biomass (M_P) (Fig. 2G-I) than systems with grazers but no predators, similar to algae-only food chains. This classic trophic cascade became stronger as temperature increased (Fig. 2G-I, Table 1). By week 8, predators had shifted composition of zooplankton to less effective grazers (from *Daphnia* sp. to copepods, Fig. 3B, S3), though there was no clear effect of predators on zooplankton density in week 8. The proportion of inedible phytoplankton taxa (cyanobacteria) increased over time at higher temperatures, but this change was unaffected by food chain length (Fig. 4).

Phytoplankton biomass (M_P) declined with increasing temperature (Fig. 2G-I, Table 2). Across-system effects of temperature were steepest in 2-TL systems with zooplankton grazers and no predators, with a 30-fold decline as temperature increased across systems (Fig. 2, Table 3). The trophic cascade strengthened with temperature, and we therefore do not reject hypothesis *iv*. For ecosystem-level phytoplankton biomass ln(M_P), a model with random effect for tank (Full.R) performed better than the model without random effects (Full) (Full vs

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380   Full.R: p = < 0.001, S4), and subsequent analyses were done with random effects models.

381   Within-ecosystem trends in biomass associated with temperature differed starkly from effects

382   of temperature among ecosystems (Fig. 2). Over time, higher temperatures were associated

383   with higher phytoplankton standing stocks within systems, though interpretation of the

384   temperature effect is confounded by the decline in temperature over time likely associated

385   with other temporal changes.

386

387   **Figure 2:** Net ecosystem oxygen flux, phytoplankton standing stock, and estimated activation

388   energies varied over the experimental thermal gradient. **A-C)** Net primary production (NPP),

389   **D-F)** net ecosystem respiration (ER) and **G-I)** phytoplankton standing stocks (M_B) were

390   estimated once per week (for 6 weeks post bloom) in each replicate ecosystem (n = 30). For

391   each ecosystem (shade of grey), 6 points are shown, one point for each week (symbols). Bold

392   lines indicate hierarchical regressions fit to among-group variation in temperature, after taking

393   into account within-group variation temperature effects (light lines)(Table 2). Temperatures

394   within tanks declined over time (Fig. 1C). Activation energies and confidence intervals given

395   in Table 3.
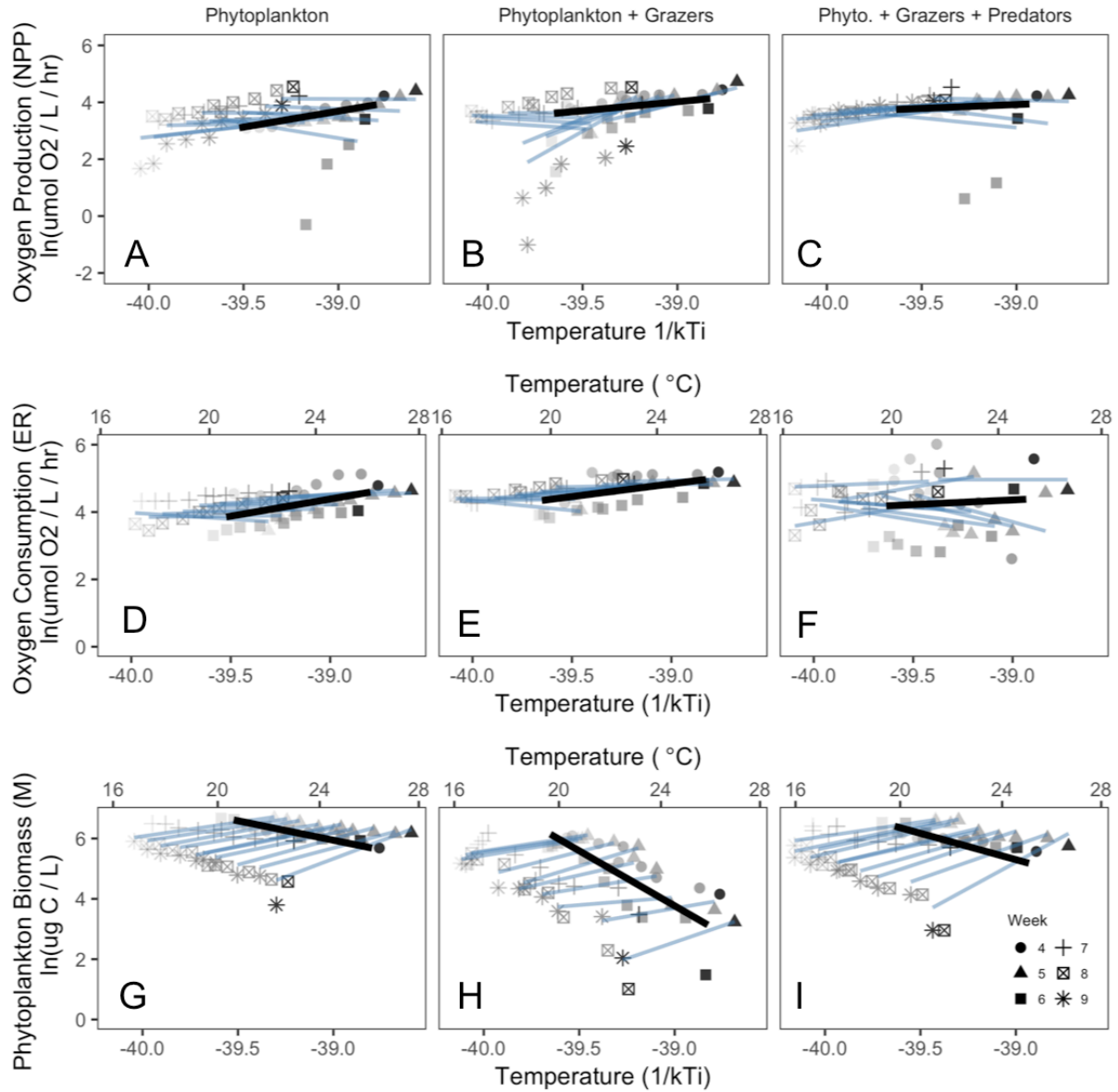
Table 2: Model comparisons for effects of temperature and food chain length on biological responses based on AIC weight (w) and δAIC values. Response variables are modelled as in Equation 4, as functions of temperature T_{ij} for each tank j on week i relative to the mean temperature T_{Mj} for tank j over all weeks (T in Kelvin), and food chain length (TL). Only models ranking in the top set (δAIC < 2) are shown.

Model	w	df	δ	loglik
Net Primary Productivity (NPP)				

8	$\ln(Y_{ij}) = \beta_{0.j(i)} + \beta_1\left(\dfrac{1}{kT_{ij}} - \dfrac{1}{k\overline{T}_j}\right) + \beta_2\,\dfrac{1}{k\overline{T}_j}$ $+ \beta_4 * TL_j + \beta_5 *\left(\dfrac{1}{k\overline{T}_j}\right)* TL_j + \beta_6 *\left(\dfrac{1}{kT_{ij}} - \dfrac{1}{k\overline{T}_j}\right)* TL_j$ $+ e_{ij}$	0.45	10	0.00	-189.77
3	$\ln(Y_{ij}) = \beta_{0.j(i)} + \beta_1\left(\dfrac{1}{kT_{ij}} - \dfrac{1}{k\overline{T}_j}\right) + \beta_2\,\dfrac{1}{k\overline{T}_j} + e_{ij}$	0.18	4	1.82	-197.28
Ecosystem Respiration (ER)					
7	$\ln(Y_{ij}) = \beta_{0.j(i)} + \beta_1\left(\dfrac{1}{kT_{ij}} - \dfrac{1}{k\overline{T}_j}\right) + \beta_2\,\dfrac{1}{k\overline{T}_j} *\left(\dfrac{1}{k\overline{T}_j}\right)$ $+ \beta_4 * TL_j + \beta_5 *\left(\dfrac{1}{k\overline{T}_j}\right)* TL_j + e_{ij}$	0.74	8	0.00	-116.10
Phytoplankton Biomass (Mp)					
7	$\ln(Y_{ij}) = \beta_{0.j(i)} + \beta_1\left(\dfrac{1}{kT_{ij}} - \dfrac{1}{k\overline{T}_j}\right) + \beta_2\,\dfrac{1}{k\overline{T}_j} *\left(\dfrac{1}{k\overline{T}_j}\right)$ $+ \beta_4 * TL_j + \beta_5 *\left(\dfrac{1}{k\overline{T}_j}\right)* TL_j + u_j + e_{ij}$	0.73	9	0.00	-120.08
Average Zooplankton body size					
6	$\beta_0 + \beta_4 *(TL) *taxon + \varepsilon_j$	0.62	5	0.00	-64.38
8	$\beta_0 + \beta_2 *(T_{Mj}) + \beta_4 *(TL) *taxon + \varepsilon_j$	0.25	6	1.82	-64.25
Zooplankton density					
5	$\beta_0 + \beta_2 *(T_{Mj}) + \varepsilon_j$	0.79	3	0.00	-10.80

403

Figure 3: In the 8th week of the experiment, zooplankton density declined with increasing temperature (A) but did not differ between ecosystems with (triangles) and without (circles) notonectid predators (Table 2). Daphnia size declined in the presence of predators, but copepod size did not, and there was no effect of temperature on body size in week 8 (Table 2).
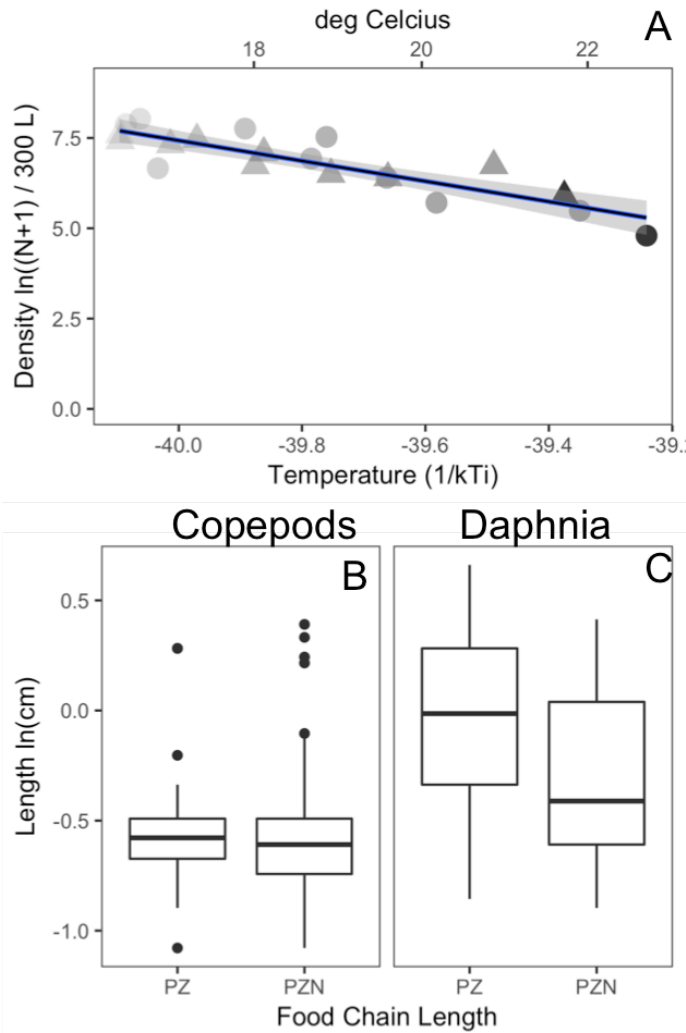
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412 **Figure 4.** Temperature and food chain length shifted algal community composition in

413 experimental ecosystems. Each point represents one ecosystem observed at one time, and

414 lighter shades are communities at higher temperatures as indicated by the inset legend with

415 mean temperature for each tank over the experimental period. Tanks sharing the same

416 temperature treatment are indicated by an ellipse. Mesocosm phytoplankton species diversity

417 mostly affected by time are the ones closest to the axes of the graph in the NMDS plot.

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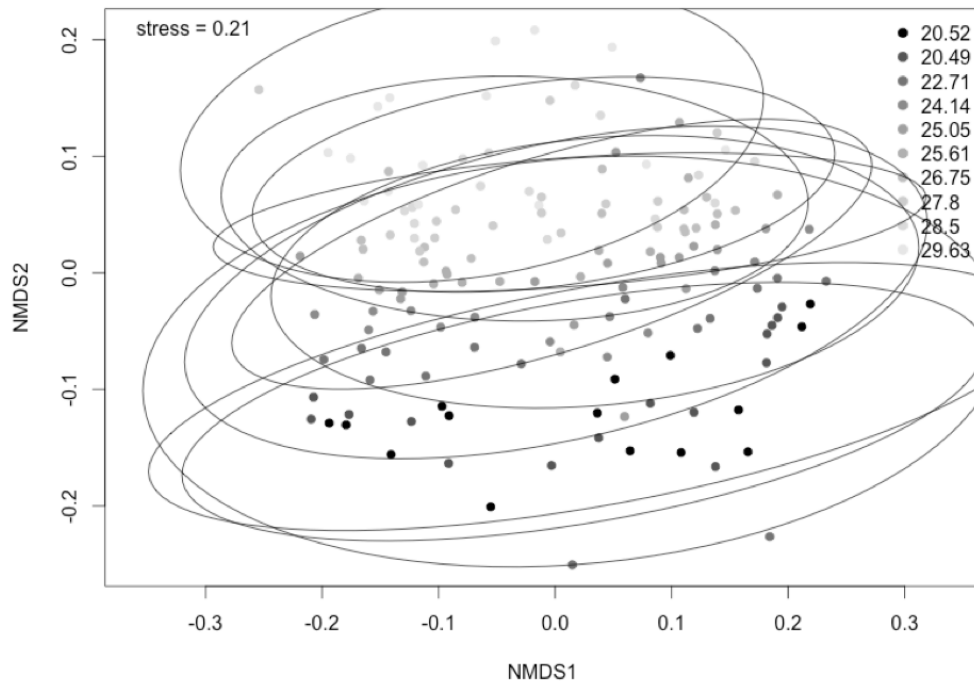
Trophic structure influenced temperature dependent ecosystem function (hypotheses *i - iii*)

421   Our results suggest that trophic structure alters the effect of temperature on ecosystem-

422   level oxygen flux. Across the experimental temperature gradient, net ecosystem oxygen

423   production (NPP) increased with temperature (bold lines, Fig. 3A-C), and varied with trophic

424   structure (Fig. 2A-C, Tables 2-3). Mean temperature dependence of NPP across ecosystems

425   was not statistically different than the expected E_{NPP} = -0.32 eV (consistent with hypothesis *i*),

426   though the confidence intervals are wide enough to include 0 for ecosystems with consumers,

427   and also the activation energy of respiration (-0.65 eV) (Table 3). In the absence of

428   zooplankton the estimated across-system temperature dependence was stronger than expected:

429   E_{NPP} = -1.12 eV ($\pm$ 1.02) (Table 3). For ecosystem-level ln(NPP), a model without a random

430   effect for tank (Full) performed just as well as a model with the random effect (Full.R) (Full

431   vs Full.R: p = 0.999, S5), and subsequent analyses were done with fixed effects models. The

432   best model included an interaction between trophic level and average temperature (Table 2),

433   suggesting that trophic structure alters across-system temperature dependence of NPP.

23

434   However, the difference in the coefficients of temperature dependence among trophic

435   treatments is not well resolved, due to the broad confidence intervals (Table 3).

436   Trophic structure also altered the across-system temperature dependence of ER,

437   weakening the effect of temperature when food chains were longer. Thus we reject hypothesis

438   *v*. Net ecosystem respiration (ER) increased with temperature across ecosystems, consistent

439   with the predicted E_R = -0.65 eV (consistent with hypothesis *ii*, Fig. 2D-F, Table 2), and with

440   confidence intervals that do not include the proposed temperature dependence of NPP for

441   short and long food chain systems (Fig. 2, Table 2). For tank-level ln(ER), a model without a

442   random effect for tank (Full) performed just as well as a model with the random effect

443   (Full.R) (Full vs Full.R: p = 0.859, S5), and subsequent analyses were done with fixed effects

444   models.

445   Within ecosystems, effects of temperature variation over time on oxygen fluxes were

446   weaker than or comparable to among-system effects (Fig. 2), and did not depend on

447   temperature treatment or trophic level (Table 2).

448

449   **Table 3. Estimates and formulae for activation energy terms.**

Resp.	Model	Trophic Structure	Slope (E_a)	Slope (E_a) est.
NPP	Avg(8,3)	P	β_2	-1.12 (-2.15, -0.10)
		PZ	$\beta_2 + \beta_{5.PZ}$	-0.63 (-1.53, 0.27)
		PZN	$\beta_2 + \beta_{5.PZN}$	-0.27 (-1.29, 0.74)
ER	7	P	β_2	-1.02 (-1.67, -0.36)
		PZ	$\beta_2 + \beta_{5.PZ}$	-0.76 (-1.31, -0.20)
		PZN	$\beta_2 + \beta_{5.PZN}$	-0.28 (-0.93, 0.37)
PB	7	P	β_2	1.23 (0.76, 1.81)

		PZ	$\beta_2 + \beta_{5.PZ}$	3.64 (3.21, 4.09)
		PZN	$\beta_2 + \beta_{5.PZN}$	1.75 (1.22, 2.27)
Size	Avg(6,8)	PZ	β_2	2.81 (2.03, 3.61)
		PZN	β_2	2.81 (2.03, 3.61)

450

Discussion

The growing literature of experimental tests of how warming affects interacting species aims to reduce uncertainty in projected ecological changes associated with climate change. Warming experiments have shown a wide variety of consequences for species interactions, from shifts in community composition, strengthening top-down control, and shifts in body size. However, how these responses to warming, in short-term experiments designed to test specific hypothesis, can be best related to projections for climate change impacts over broad scales of space, time and complexity is not always clear. One way to facilitate such projections is for experiments to test functional responses of ecological processes along thermal gradients, as we have done here, and to test for sensitivity of functional responses to ecological conditions. Empirically estimated functional responses that can be incorporated into theoretical models can support projections of change based on system dynamics, rather than direct extrapolation from experimental conditions, and are likely to prove the most useful in understanding ecological change with climate change (Cuddington *et al.*, 2013).

Here, we quantified the functional response of net ecosystem fluxes of oxygen and community structure to temperature, over a broad thermal gradient and in the context of metabolic scaling theory. Oxygen fluxes indicate the productivity of an ecosystem and are directly proportional to carbon fluxes and the potential of an ecosystem to be a carbon source or sink (López-Urrutia *et al.*, 2006). We found that increasing ecosystem average temperatures increased NPP and ER at the community scale, and these effects varied with

25

472   trophic structure of the local community. The exponential increase in NPP and ER with

473   warming was greatest for communities without consumers (algae only), and least pronounced

474   in communities with grazers and top predators. These results suggest that models relying on

475   functional responses of net ecosystem oxygen or carbon fluxes to temperature might be more

476   accurate when they can include coarse aspects of trophic structure such as the presence of

477   grazers and predators. Further, these results suggest that simplification of trophic structure

478   with environmental change (Estes *et al.*, 2011) could increase the responses of net ecosystem

479   fluxes to temperature changes.

480   The temperature gradient also affected key aspects of community structure across

481   independent ecosystems, and the response of phytoplankton biomass to temperature was

482   much greater than the effect of temperature on net ecosystem fluxes. These community-level

483   shifts associated with temperature are consistent with what other experimental studies have

484   reported over a much smaller range of temperatures (Beisner *et al.*, 1996; Hansson *et al.*,

485   2012; Shurin *et al.*, 2012). However, the large community changes over a thermal gradient did

486   not directly predict the effects of temperature on local ecosystem fluxes based on comparisons

487   across ecosystems and metabolic scaling models –variation in fluxes was several orders of

488   magnitude less over the thermal gradient than the variance in phytoplankton biomass. This

489   difference is likely in part attributable to changes in per capita phytoplankton productivity

490   associated with species composition shifts and temperature, and could also be attributed to

491   shifts in phytoplankton abundance relative to benthic algae (which we did not quantify).

492   We had hypothesized that ecosystem level NPP and ER would be predicted directly by

493   the temperature dependences of photosynthesis and respiration, and insensitive to trophic

494   structure, as they appear to be in many macro-ecological scale analyses (López-Urrutia *et al.*,

495   2006). However, we found that the temperature dependences varied with trophic structure

496   such that only the grazer-only food chain ecosystems were consistent with temperature

497   dependence of the underlying metabolic processes. Deviations from these expected activation

498   energies could be explained by temperature driven shifts in total biomass. Ecosystem NPP

499   reflected both changes in per capita photosynthesis as well as large changes in mass corrected

500   metabolic biomass (Yvon-Durocher & Allen, 2012), which we were not able to fully

501   characterize in this experiment. Though we did not observe notable amounts of accumulated

502   benthic algae in our tanks, even small amounts could have contributed to total ecosystem

503   fluxes and led to covariation in total biomass with temperature. If the ratio of phytoplankton

504   to benthic algae was temperature-dependent (Dossena *et al.*, 2012), our primary producer

505   biomass estimates may have increasingly under-represented total algal biomass at higher

506   temperatures. In our experiment, we would expect any contribution of benthic algae to NPP to

507   increase over time, and be strongest in weeks 8 and 9 after having had time to accumulate

508   biomass. However, there is no apparent shift in the slopes of NPP *vs* temperature as time

509   progresses (Fig. 2A), suggesting that accumulated benthic algal biomass did not confound our

510   estimate of NPP over the thermal gradient. Nonetheless, to be conservative, we did not

511   present mass-normalized NPP estimates because we could not normalize to any benthic algal

512   metabolic biomass. Covariation between biomass and temperature is common across

513   geographic variation in temperature (Michaletz *et al.*, 2014; Padfield *et al.*, 2017) and

514   therefore present in other estimates of NPP across broad spatial scales when biomass cannot

515   be estimated well.

516   Another possible reason for the deviation between observed and expected effects of

517   temperature on net oxygen production rates is that resource availability to phytoplankton may

518   have co-varied with temperature such that warmer tanks were less resource-limited. Some

519   cyanobacteria species that increased in our tanks can fix atmospheric nitrogen, but are

520   competitively inferior under conditions of high ambient nitrogen (Hecky & Kilham, 1988).

521   Nitrogen fixation requires the enzyme nitrogenase, which has a biphasic temperature

522   dependence (E_a = -2.18 eV below 22°C and E_a = -0.65 eV above 22°C (Ceuterick *et al.*,

523   1978)). If metabolically active, we speculate that these species may have supplied additional

27

524   bioavailable N to experimental systems at the warmer end of the thermal gradient (Anderson-

525   Teixeira *et al.*, 2008; Welter *et al.*, 2014). Nutrient limitation may have been eased at higher

526   temperatures by yet another mechanism: all food chains would have included microbial

527   assemblages that may have been recycling nutrients faster at higher temperatures, with

528   respiration-limited metabolic rates (López-Urrutia & Morán, 2007; Beveridge & Humphries,

529   2010). Fully understanding the effects of temperature on communities and their functions will

530   require including microbial and benthic functional groups.

531   We observed no sign of ecosystem collapse with warming. Changes in community

532   structure and the increase in trophic control along the temperature gradient appear to be

533   exponential and monotonic (Eqn 1b, Fig. 2), suggesting that observations made from only two

534   temperatures as is typical of many community-level warming experiments may extend to

535   broader thermal gradients using nonlinear (exponential) models (Fig. 2D, Fig. 3). In this

536   pattern, there is little evidence of abrupt transitions that might be expected if thermal stress

537   responses by individual phenotypes emerged at the ecosystem scale. While individual species

538   may experience thermal stress and decline in performance at high temperatures, in our

539   systems these effects were functionally compensated for by other species and increases in per

540   capita performance. The limits to what temperatures could be extrapolated to are not clear

541   from our data, but we would not expect NPP and ER to continue to increase as observed

542   beyond 35C.

543   Predators, as expected, reduced zooplankton density and body sizes, and caused a

544   clear trophic cascade. Trophic control, and therefore any mitigating effects of predators on

545   biomass change, was weak at low temperatures and increasingly strong at higher temperatures

546   (compare consumer-free control treatment with consumer treatments, Fig. 2D). Over the

547   temperature gradient, community (biomass, abundance) responses were less related to

548   temperature in systems with predators relative to grazer-algae systems. This pattern is

549   consistent with previous findings that systems with two (or even numbers) of trophic levels

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

551   of predation on primary producers (Hansson *et al.*, 2012; Shurin *et al.*, 2012). In our systems,

552   food webs with longer food chains were more resistant to community change with warming.

553   This result contradicts theories in which dynamically responsive predators can make three-

554   trophic-level systems less stable than shorter food chains (Hastings & Powell, 1991). In our

555   experiment, predators were not dynamically responsive. In this way, they represent mortality

556   for zooplankton that may have varied with temperature effects on per capita predation rates by

557   predators, but not demography. In many systems, predators are subsidized by other habitats

558   and food sources, and their populations are not dynamically coupled to prey; in fact, this

559   decoupling has been shown to be important in thermally stratified systems (Tunney *et al.*,

560   2014). However, our results cannot be extended directly to systems with local dynamic

561   predator population.

562   Metabolic scaling theory provides an ecological framework that can produce

563   functional responses that relate environmental temperature changes to ecological changes

564   (Padfield *et al.*, 2017). Still missing is a clear understanding of how metabolic temperature

565   dependence emerges at the community level in simple food webs, where effects of species

566   interactions, phenotypic plasticity, evolution and resource limitation can be strong and

567   dominate signals of environmental change (Padfield *et al.*, 2016; 2017). Despite recent

568   advances incorporating light limitation, evolutionary change, trophic interactions and other

569   factors into the metabolic scaling theory framework, empirical tests at the community level

570   such as ours can further shed light on whether there are reliable functional responses of

571   community-scale processes to temperature change. Our study suggests that for oxygen fluxes,

572   metabolic temperature dependence functions associated with MST might be used to model

573   changes with temperature across systems. Changes in species composition and community

574   structure occur within the context set by temperature constraints on energy fluxes via

575   fundamental metabolic processes(Bruno *et al.*, 2015; Padfield *et al.*, 2016; 2017). To extend

576   our findings to a conjecture about implications for climate change, we suggest that

577   conservation actions that maintain predators and top down control may also promote an

578   ecosystem that changes less with temperature than a system with a large abundance of

579   grazers. Taken together, these results suggest our efforts to predict community change with

580   warming may benefit from the general metabolic scaling theory framework to understand

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

582

583   ***Acknowledgments***

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