**Trophic interactions modify the temperature dependence of community biomass and ecosystem function**

**Running head: ecological effects of temperature**

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Research paper**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 and their population growth and interaction rates. At a much larger biological scale, warming also 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 over a range of temperatures from 19-30 °C. In a series of independent freshwater ecosystems (400-L) we found that at higher temperatures, cascading effects of predators on zooplankton prey and algae were stronger. Standing phytoplankton biomass declined by 85-95% (< 1-fold) over the temperature gradient when grazing was weak or absent, and by 3-fold when grazers were present and lacked predators. Surprisingly, these temperature-dependent species interactions and consequent community shifts only modestly affected the temperature dependence of net ecosystem oxygen fluxes. The exponential increase in net ecosystem oxygen production and consumption was 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], suggesting that the temperature dependence of these fundamental metabolic processes constrains higher order ecological responses to temperature. The ecological importance of temperature-dependent *per capita* metabolic rates has led to the use of metabolic models, and the development of an important theme in the metabolic theory of ecology (MTE) of general temperature dependence, to understand and predict ecological change across scales, from local to global [3,4,8]. 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, 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].

One challenge has been to reconcile 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 [10-16]. 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 model used macro-ecological scales to explain relationships between temperature and ecosystem function assume that the relationship between temperature and community-level distributions of body sizes and traits is constant in time, or at stable state so that descriptions of the community apply to future states of the community under the same abiotic conditions [17-19]. Yet, at local scales, species interactions can strongly influence community-level NEP, ER and primary producer abundance, and the strength and outcomes of species interactions reflect dynamical processes that are often sensitive to temperature [10,12,15] may vary over time until they reach steady state. The temperature dependence of species interactions and their consequences for how biomass, size distributions and traits vary under even constant abiotic conditions raises a challenge for the application of general temperature dependence models that assume individual metabolism to change at community scales without explicitly measuring [20,21]. For example, the presence of fish in experimental aquatic ponds reversed a negative effect of temperature on algal biomass to a positive effect, mediated by trophic interactions between fish, zooplankton and phytoplankton [22], under otherwise constant consistent abiotic conditions across ponds. This paradox between macroecological patterns – which can be consistent with direct scaling of per capita thermal responses - and results of smaller scale, short term experiments that allow population dynamics to play out over intermediate time scales, leads to the suggestion that general metabolic scaling models that do not consider the complexities associated with species interactions do not apply at the local scales. Reconciling these apparently divergent patterns is critical to improving understanding and projections of how shifting global thermal regimes affect ecological patterns and processes across scales and achieving a more unified understanding of ecology across scales.

One way to reconcile the apparent context dependence of empirical results under controlled conditions with the generality of temperature dependence of ecosystem function at broader scales is to consider how the direct and indirect effects of temperature on population dynamics interact. Direct effects of temperature on *per capita* metabolic rates cause organismal photosynthesis and respiration rates to increase exponentially as temperatures increase when resources are not limiting in algae and animals, up to an optimal temperature. This relationship between temperature and fundamental metabolic rates (photosynthesis and respiration) is referred to as general metabolic scaling [1]. For any single phenotype, performance above some optimal temperature declines due to stress responses and metabolic scaling no longer explains the effects of temperature. However, in multi-species communities the signal of metabolic scaling is likely to dominate over a broad range of temperatures if species’ with distinct thermal phenotypes can compensate for each other along the thermal gradient [17,23]. Warming is also associated with other biological changes that affect species interactions, such as reductions in body size (the temperature size rule, [24-26]), fecundity, and attack rates (Fig 1) [27-30].

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 [10,31]. Trophic species interactions appear to strengthen with warming [22,32,33]. Series of trophic interactions, called *trophic cascades* (Fig 1), link predator populations to the abundance, biomass and ecosystem functions of primary producers [34,35] and the strength of trophic cascades depends on body size, primary production [36]. Considering the multitude of indirect effects of temperature on population dynamics and the prevalence of consumer-resource interactions and trophic cascades in aquatic systems [37] begs the question, how is it that these locally dominant population-level responses to temperature [38,39] 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 over time. 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 temperature dependence of ecosystem functions (NEP, ER) over a temperature gradient (Fig 1). 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 consumers and predators) (Fig 1). We quantified ecosystem function (NEP, ER) and community structure (biomass, abundance) across an experimental temperature gradient of 10 °C. We found that exponential effects of temperature on algal biomass were greater than effects of temperature on NEP and ER, suggesting that even large changes in community structure do not necessarily lead to large changes in how temperature affects NEP and ER.

**Hypotheses**

We drew on the Metabolic Theory of Ecology (MTE) frame our hypotheses and predictions for how temperature affects NEP and ER via per capita metabolic temperature dependence and indirect effects of temperature at the community scale. We first briefly outline the framework, and then express our specific hypotheses. MTE relates whole-organism metabolic rates (*bi*, mgO2/g/hr) and related biological functions for organism *i* to body size (*mi*, g) and body temperature (*T*, in Kelvin) [1,40,41]:

Eqn 1

in which activation energy (*E*a, in eV) captures the exponential effect of temperature on per capita metabolic rate*, k* is the Boltzmann constant (eV/K), *b0* is a normalization constant independent of body size and temperature that includes the effects of temperature-independent traits on metabolic rate (dimensionless). The allometric scaling factor α relates metabolic rate to body size [see Methods: Models and Hypotheses for justification of Eqn 1 over alternative models].

The effects of temperature on ecosystem metabolic rates (*BR*), such as NEP or ER (mgO2/L/hr) reflect the sum of all *per capita* photosynthesis rates by autotrophs and respiration rates by autotrophs and heterotrophs as well as shifts in abundance, body size and acclimation. Note that NEP and ER can be quantified in this way as positive numbers, and we do this – using their absolute values – in our analyses. Following Barneche et al (2014), we capture these direct and indirect effects of temperature on ecosystem scale metabolic rates in the following equation (see Barneche et al 2014 for derivation):

. Eqn 2

The term captures the temperature dependence *ER* (eV) of ecosystem-level metabolic rate *BR*. Equation 2 represents a ‘first order metabolic scaling’ prediction that ecosystem scale mass-normalized metabolic rates (e.g., NEP) vary proportionally with the temperature dependence of the underlying metabolic processes. Observed temperatures T are related to an arbitrarily chosen reference temperature Tc. This centering causes the normalization constant to be for metabolic performance at temperature Tc. These studies assume or find that *b0(TC)* and *MB* do not vary with temperature in influential ways [1,17].

When considering the indirect effects of temperature on ecosystem oxygen production and respiration, we can consider how the other terms in Eqn 2 may vary with temperature. To account for changes in total biomass, body size or relative abundance of differently sized individuals associated with temperature, we use the term . The total biomass, *MB* (g/L) in ecosystem volume V, which equals the sum of mass *mi* for all individuals *i* to *J* in . The term is the average of all individual metabolic biomasses, , corrected for the greater contribution to total mass-specific metabolic biomass by small individuals resulting from the allometric scaling () of oxygen production and consumption with body size [2,17]. This ‘mass correction’ is necessary, because if community biomass is comprised of one large individual, that biomass will [produce and] consume less oxygen per gram biomass in a given time period than if the same total biomass were comprised of many small individuals; in other words, increases as *mi* declines and density increases. Finally, the term relates the normalizing constant *b0* to a particular temperature (here, Tc), allowing this to change with temperature. If thermal traits acclimate or species composition shifts with temperature, this term would capture that change.

***Hypothesis 1: Trophic interactions modify the effect of temperature dependent metabolic rates on total algal biomass.*** Total algal biomass (MB) can be expressed in terms of temperature, traits and size distributions by rearranging eqn 2:

Eqn 3

and linearizing for analysis by log transforming (Methods: Eqn 7). If we assume that is independent of temperature, we predict that algal biomass *MB* declines with temperature by *ER*, in this case *ER* = -*ENEP*. This prediction has been supported empirically in a single species algae system [42], and in that system the predicted decline in total abundance was robust to changes in cell size. However, it is unlikely that grazers and temperature would not alter the abundance and size of algae, altering among trophic treatments, and also the traits of algae, and thereby modifying among trophic treatments [43]. A fuller integration of how temperature and trophic treatment affect these terms would require theoretical development that is beyond the scope of this paper, but we use the equation here to highlight why we expect trophic structure and temperature to affect algal biomass. To test this hypothesis, we used eqn 7 to compare ln(*MB*) trends with temperature across ecosystems with and without a trophic cascade.

***Hypothesis 2: Increasing temperature strengthens the trophic cascade***. We estimated the strength of the trophic cascade as the ratio of primary producer biomass in the presence of predators (AGP) *vs* in predator-free environments (AG) (Shurin et al 2002). We predicted that predators would reduce the abundance of zooplankton through predation, and shift zooplankton composition to smaller sizes and less-edible species, typical of classic freshwater trophic cascades [44], and that these trophic interactions would strengthen with higher temperatures due to the effect of temperature on *per capita* grazing rates. We can relate algal biomass among treatments using Eqn 3 for primary producer biomass in the presence of predators (AGP) and grazers only (AG), simplifying and taking the log to yield (see Methods, eqns 7-10, for details):

Eqn 4

Numerous experiments have demonstrated that the strength of the trophic cascade (the ratio , increases with increases in temperature of a few degrees [16,45,46], and theoretical work suggest that strengthening of this interaction under warming is expected for a greater range of consumer-resource parameter values than would predict declines in the trophic cascade [10]. We therefore predict the trophic cascade will increase with temperature in our experiment. But, as shown in eqn 4, because trophic interactions could affect the realized temperature dependence via several possible mechanisms – shifts in body size, traits, etc, - it is not possible to predict *a priori* the exact temperature dependence value, in terms of the differences and ratios of the temperature dependence terms in Eqn 4. We expected the indirect effects of predators on algae to be mediated by changes in zooplankton density and/or body size. We tested this expectation by testing the prediction that temperature dependence of zooplankton size and density different from zero (Methods).

***Hypothesis 3: Temperature dependence of net ecosystem production and respiration depends on the strength of the trophic cascade.*** We test this by using Equation 2 to model our results, but we allow *bo*(*TC*) to vary not only with temperature but with trophic structure (Zj), and we expect that trophic structure will influence the number and size of individuals, and thereby affect . Alternatively, trophic structure may not modify the relationship between temperature and *BR*. We can test these alternate predictions by comparing models with and without *bo*(*TC*) and terms that depend on ecosystem temperature and trophic structure.

To summarize, for each hypothesis, we used linear mixed effects models (LMMs) to test ‘first order’ metabolic scaling models for the appropriate model (equation 2, 3 or 4) that included *bo*(*TC*) as independent of the ecosystem’s temperature or trophic structure [Methods: Statistical Analyses]. We tested alternate models that allowed *bo*(*TC*) to vary with ecosystem mean temperature, weekly temperature and/or trophic structure. If the simpler, first order models are best supported, we would infer that indirect effects of temperature do not overwhelm the signals of direct metabolic scaling effects on ecosystem functions, consistent with inferences drawn in macroecological studies. To estimate intercepts and temperature depdence terms (e.g., ER), we summed coefficient values and estimated uncertainties in these aggregated parameters from best models (Methods: Statistical Analysis). Our data do not permit testing predictions about size distributions or trait shifts, but support for models with variation in *bo*(*TC*)and among treatments would suggest these mechanisms as likely explanations.

Results

**Hypothesis 1:** As temperature increased across ecosystems, phytoplankton biomass, estimated as the concentration of *chlorophyll a* in the water column, declined (Fig 2A). Trophic interactions modified the effect of temperature on *chlorophyll a* concentration (Fig 2A, Table 1). This inference is supported by the inclusion of a main effect for trophic structure (Zj)in the best model (Table 1) and an estimate for the temperature dependence of chlorophyll *a* concentration with confidence intervals that exclude 0 (Fig 3). Phytoplankton biomass declined much more strongly with temperature in algae-grazer (predator-free) communities, with a decline of over three orders of magnitude in phytoplankton biomass standing stock over the 10 °C temperature gradient (Fig 2A). In the algae-grazer-predator treatments, phytoplankton biomass declined with a slope indistinguishable from that in the algae only treatments (Fig 3). We did not observe shifts in taxonomic composition with temperature (Fig S2).

**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 (Zj) and average ecosystem temperature over the entire experimental period (TM), while accounting for effects of temperature variation over time (weekly average temperature (Twj)) and with ecosystem identity as a random effect. We compared models using likelihood ratios (LogLik), AICc, Akaike weights (w) and delta AICc weights. The full model (modelPBF) includes all terms, and models representing alternate hypotheses excluded terms indicated by ‘NA’. Coefficients were pooled (Methods) to estimate slopes and intercepts for Fig 2 and 3.



**Hypothesis 2:** Consistent with our second hypothesis, and the patterns observed for phytoplankton biomass, there was a strong trophic cascade in the warm ecosystems by the end of the experiment (Fig 2B). The trophic cascade became apparent after the first weeks of the experiment, and strengthened over time and with temperature (Fig 2B) (Table 2). The best model included a term for mean ecosystem temperature (TM), as well as week (Tw), and a week x temperature interaction. By week 9, the log response ratio of chlorophyll *a* concentration with *vs* without predators increased exponentially with temperature (Fig 2B) to an estimated *ETC* = 0.77 (estimated from model fixed effects shown in Table 2 plus random effect, not shown).

**Table 2. Model selection results for trophic cascade analysis**. We used linear mixed effects models with terms for average temperature for ecosystem *j* in week *w* (Twj), week 2-9 (Wk) and their interaction. We treated the power level (e.g., 100W, 200W, etc), our temperature treatment, as a random effect to account for repeated measures on ecosystems over time. We compared models using likelihood ratios (LogLik), AICc, Akaike weights (w) and delta AICc weights. The full model (TCFull) includes all terms, and models representing alternate hypotheses excluded terms indicated by ‘NA’. Coefficients were pooled (Methods) to estimate slopes and intercepts for Fig 2.



We find additional evidence of temperature dependent trophic interactions in the responses of the zooplankton grazer assemblages to warming and predation. Total zooplankton density declined with increasing temperature (Table 3; *EZP* = 1.28 95% CI: 0.19 – 2.39, z = 2.31, *P* = 0.021 based on regression with negative binomial distribution and likelihood ratio tests; Fig 4). Predators reduced densityof *Daphnia*, the dominant grazer (linear regression with Poisson distributed errors: estimate: = -1.14 95% CI: -1.91, -0.36, z = -2.87, *P* < 0.01) and density declined with increasing temperature (Table 4) although the temperature term retained in the best model was not significant (*ED* = 0.88 95% CI: -0.59, 2.35, z = 1.17, *P* = 0.24). Copepod density declined with temperature (*EC* = 2.21, z = 3.12, *P* = 0.002), and not in response to predation (best model did not include a predation term, Table 6). We measured zooplankton standard length for 641 individuals of all ages. Mean length was 0.72 cm, and sizes ranged from 0.34 – 1.94 cm. We did not observe support for a decline in body size with temperature (best model did not include a temperature term, Table S2), as would be expected by a hypothesis based on the temperature size rule. Predation reduced total zooplankton body size, driven by size shifts in Daphnia (Table S3).

**Table 3: Zooplankton density**. Results of model selection for zooplankton abundance in ecosystems with grazers (AG) and with grazers and predators (AGP). We used negative binomial regressions with ecosystem identity as a random effect [Methods: Statistical Anlaysis]. Models included terms for weekly average temperature (Twj), ecosystem trophic treatment (Zj) and their interaction, and a random effect for ecosystem identity. We compared models using likelihood ratios (LogLik), AICc, Akaike weights (w) and delta AICc weights. NA indicates that the term was not included in the model.



### **Table 4:** **Daphnia density**: Results of model selection for *Daphnia* abundance in ecosystems with grazers and with grazers and predators. We used Poisson regressions with ecosystem identity as a random effect [Methods: Statistical Analysis]. Models included terms for weekly average temperature (Twj), ecosystem trophic treatment (Zj) and their interaction, and a random effect for ecosystem identity. We compared models using likelihood ratios (LogLik), AICc, Akaike weights (w) and delta AICc weights. NA indicates that the term was not included in the model.



### **Table 5:** **Copepod density**: Results of model selection for copepod *spp* abundance in ecosystems with grazers and with grazers and predators. We used Poisson regressions with ecosystem identity as a random effect [Methods: Statistical Analysis]. Models included terms for weekly average temperature (Twj), ecosystem trophic treatment (Zj) and their interaction, and a random effect for ecosystem identity. We compared models using likelihood ratios (LogLik), AICc, Akaike weights (w) and delta AICc weights. NA indicates that the term was not included in the model.



**Hypothesis 3:** Across ecosystems, and in the context of temperature-dependent trophic cascades and community taxonomic shifts, higher temperatures increased net ecosystem oxygen production (NEP) and respiration (ER) (Tables 6, 7; Fig 5). The lmm for NEP (Table 6) and ER (Table 7) suggested 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), though the estimated temperature dependence in systems with predators was weak or absent (Fig 3).

### **Table 6:** Results of model comparisons for effects of temperature and time on net ecosystem productivity (NEP) based on AIC weight (*w*) and δAICC values. Nested versions of the full model (Eqn 11, Methods). Response variables are modelled as functions of temperature Twjfor each tank *j* on week *w* relative to the mean temperature for tank *j* over all weeks (T in Kelvin), and trophic structure (Zj). Models included a random effect for the experimental unit – tanks with and without predators receiving the same power inputs. See Methods for additional details on modeling. NA indicates that the term was not included in the model.



Table 7: Results of model selection for effects of temperature and time on ecosystem respiration (ER) based on AIC weight (w) and δAICC values. Nested versions of the full model (Eqn 11, Methods). Response variables are modelled as functions of temperature Twj for each tank *j* on week *w* relative to the mean temperature for tank *j* over all weeks (T in Kelvin), and trophic structure (Zj). Models included a random effect for the experimental unit – tanks with and without predators receiving the same power inputs. See Methods for additional details on modeling.



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 on average between the beginning and the end of the experiment, with some variation among weeks reflecting weather conditions. Phytoplankton community composition shifted over time (Fig S2), but visual inspection of the species driving these shifts indicated no specific taxa driving the changes, and there was no association between phytoplankton species composition and temperature. Chlorophyll *a* concentration declined over time in all treatments (Fig S3). A linear mixed effects model indicated that this decline was weakest in the algae-only treatments (Table S6). Visual inspection of trends (Fig S3) suggests that this decline over time was driven by the decline in all tanks in weeks 8 and 9, following a major rain event and drop in all tank temperatures (Fig S1), rather than a more gradual decline as observed in the +grazer tanks. When we reanalyzed the temporal trend for just weeks 2-7, excluding weeks 8-9, the trend in chlorophyll *a* over time approached 0 in A and AGP treatments, but still declined in AG treatments (Table S5).

Effects of temporal temperature variation on phytoplankton biomass differed starkly from effects of temperature among ecosystems (Fig 6). Within ecosystems, higher temperatures were associated with higher phytoplankton standing stocks (Fig 6A). Net ecosystem oxygen production (NEP) varied with temperature within ecosystems, and there is some evidence that this temperature effect interacted with both the trophic structure treatment (Table 5, modelNEP8). Net ecosystem respiration varied within ecosystems over time, but this variation did not depend on temperature treatment or trophic level (Table 7).

**Discussion**

Temperature affects the metabolic rates of all organisms, and *per capita* responses to temperature of many co-occurring individuals add up to nothing less than the biological component of ecosystem scale carbon and oxygen flux. Understanding biological responses to temperature change across scales of organization (cells to the biosphere) is a major challenge in ecological research, requiring joining theoretical frameworks and empirical evidence across scales and systems. Here, we aimed to test the hypothesis that the effects of temperature on ecosystem processes that reflect metabolic temperature dependence are not highly sensitive to local differences in trophic structure of a community (e.g., presence or absence of a predator). This question draws upon ideas supported by the metabolic theory of ecology – a multi-scale theoretical framework that links temperature dependent metabolism to larger scale patterns -- and community ecology theory predicting that species interactions modify the effects of temperature on community structure and function. We found that in aquatic ecosystems characterized by the presence or absence of predator-prey species interactions, temperature-dependent trophic cascades only modestly altered the effects of temperature on net ecosystem oxygen production and consumption (NEP and ER). We found that higher average temperatures increased NEP and ER while total phytoplankton biomass declined.

Trophic structure did modify the effect of temperature on phytoplankton biomass, failing to reject our first hypothesis. This hypothesis was based on the expectation that our experimental systems would include trophic interactions that altered phytoplankton standing stock, and possibly interact with temperature to influence algal size distributions or other traits. The decline in phytoplankton standing stocks that we observed with warming across ecosystems is consistent with theoretical expectations that in closed systems with limited resources, increases in *per capita* metabolic rates with temperature could lead to declines in standing stocks [10,11,42,47]. The observed temperature dependence of phytoplankton standing stocks was highest in the communities with grazers but no predators, suggesting that temperature dependent grazing played a role in reducing algal standing stocks. Overall, the temperature dependence of phytoplankton standing stocks greatly exceeded expectations based on temperature dependence of per capita photosynthesis or respiration rates (Fig 3). Our hypothesis (eqn 3) allowed for changes in phytoplankton standing stocks to be explained by direct effects of temperature on per capita metabolism, as well as effects of temperature on thermal traits or body size distributions. We did not observe clear shifts in the species composition of the phytoplankton assemblage with temperature; still, we do not have high resolution data on phytoplankton cell size or traits, so we cannot reject these mechanisms as possible drivers of the patterns we observe.

Our second hypothesis, based on recent experimental results in other freshwater and grassland systems, was that the trophic cascade would get stronger as ecosystem temperatures warmed. We found support for this hypothesis in our system, providing the first evidence that trophic cascade strength increases continuously with temperature. Prior to our study, evidence of stronger trophic cascades with warming were from experiments that test two temperature levels, an ambient and a simulated future scenario of ~ +3C [16,48,49]. We show here that this pattern continues over a thermal range of 10 °C. The indirect effects of predators on phytoplankton biomass appears to have been mediated by predation on the dominant grazer, *Daphnia*. Predators by reducing *Daphnia* density, and thereby shifted grazer assemblages toward the less effective copepod grazers at all temperatures. This trophic cascade, mediated by shifts in grazer composition as well as total density, is a classic food web motif in freshwater systems [44]. Interestingly, at warmer temperatures grazer density was lowest, yet we still observed declines in biomass of phytoplankton. This pattern could reflect higher per capita grazing by the remaining grazer individuals. Algal productivity rates are an important element of trophic cascade strength [10,36], and higher NEP at warmer temperatures would contribute to a stronger trophic cascade, even as grazer density declines. As with hypothesis 1, we infer that the effect of temperature on the trophic cascade strength reflects not only the effect of temperature on per capita metabolic rate but also shifts in traits or algal body sizes, or both.

We tested a third hypothesis that the effects of temperature on biomass and trophic cascade strength would lead to distinct relationships between temperature and NEP and ER for each trophic treatment type (e.g., with vs without predators). We found that the effect of temperature on phytoplankton standing stock and zooplankton density was much greater than the effects of temperature on NEP or ER. For ER, the best model did not include a trophic treatment x temperature interaction term, suggesting that the effect of temperature on ER was the same for all ecosystem types (Table 7). For NEP, there was support for a model with this interaction, but a model without the interaction was ranked quite highly (Table 6), and confidence intervals for the pooled estimated temperature dependence to not indicate differences among trophic treatments. Therefore, the strong effects of temperature on community structure (biomass, trophic cascade strength) did not translate directly to net ecosystem flux rates.

The estimated temperature dependences of NEP and ER were greater than expected 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 *ER*= ~0.65 eV, and that this value emerges at the ecosystem scale, such that ecosystem respiration varies with temperature across systems as *EER* = ~0.65 eV [2-4]. The temperature dependence of photosynthesis at suboptimal temperatures appears to be *EPS*= ~0.32 eV for algal systems, and this can emerge at population [42] and ecosystem scales [4] in aquatic systems, suggesting *ENEP*= ~0.32 eV, but other studies have found evidence for stronger or weaker values of *ENEP,* ranging from 0 to 1.2 eV at population and ecosystem scales [17,23,50]. We observed values of *ER*> ~0.65 eV for both NEP and ER across ecosystems, though confidence intervals for ER did include this value (Fig 3) for algae-only food webs. These results led us to reject the ‘first order metabolic theory’ hypothesis that temperature dependence of ecosystem functions scales directly with general temperature dependence of metabolism and further suggests 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.

NEP did not vary with temperature as the inverse of the effect of temperature on phytoplankton biomass. 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 [4,11,51]. In addition, if size distributions shift toward smaller cells, as is common with warming [52,53], the allometric scaling of metabolic rate with body size (Eqn 2) predicts greater oxygen flux for a given biomass. At the ecosystem scale, effective resource supply may have changed with temperature. Even though these were closed ecosystems with regard to external influxes of nutrients, and they received the same light conditions, internal nutrient processes could have varied with temperature in ways that made nutrients more available in warmer ecosystems. For example, our ecosystems did not include a benthic habitat that can store nutrients and organic material and slow down nutrient cycling. Microbial processes would also be accelerated by temperature perhaps making available nutrients in warmer systems more than in colder systems. Another potential, and speculative, explanation for higher productivity than expected in warmer ecosystems is that some algae species are capable of biological nitrogen fixation [54] and this activity is more feasible at higher temperatures. These two biological processes that are themselves temperature dependent could create a resource gradient in parallel with the temperature gradient [10,51].

Benthic algae may also have contributed to NEP and ER estimates in our systems [55]. Though we did not observe notable amounts of accumulated benthic algae in our tanks, even small amounts could have contributed to total ecosystem fluxes and led to covariation in total biomass with temperature. If the ratio of phytoplankton to benthic algae was temperature-dependent [55], our primary producer biomass estimates may have increasingly under-represented total algal biomass at higher temperatures. To be conservative, we did not present mass-normalized NEP estimates because we could not normalize to any benthic algal metabolic biomass. Covariation between biomass and temperature is common across geographic variation in temperature [19,23,54] and therefore present in other estimates of NEP across broad spatial scales when biomass cannot be estimated well. We cannot distinguish among these explanations in our experiment, and we suspect they are all relevant.

We observed no sign of ecosystem collapse or threshold responses to warming. Changes in community structure and the increase in trophic control along the temperature gradient appear to be exponential and monotonic over the 10 °C gradient (Eqn 2), suggesting that linear (or additive) models of temperature effects in most warming experiments, which only test two or three temperatures, may underestimate warming effects over broader thermal gradients. We observed little evidence of abrupt transitions that might be expected if thermal stress responses by individual phenotypes drove ecosystem scale responses. We did observe declines in grazer density with warming even in the absence of predators, suggesting there were direct or indirect negative effects of temperature on grazers. But we did not see clear shifts in algal species composition among treatments, suggesting that no species group was exposed to temperatures above its critical thermal maximum. Another challenging aspect of warming experiments at the population and community scales is interpreting patterns in the context of transient dynamics. Our ecosystems certainly did not reach long term states, because varying weather conditions and multi-week generation times of zooplankton would have precluded that. Still, we did not observe signs of transient dynamics in these communities over time such as population cycles or abrupt changes.

In our systems, community biomass and abundance in food webs were more resistant to community change with warming and longer food chains. Predators reduced zooplankton density and caused a clear trophic cascade. Trophic control, and therefore any mitigating effects of predators on biomass change, was weak at low temperatures and increasingly strong at higher temperatures (A vs AG treatment, Fig 3). This pattern is consistent with previous findings that ecosystem functions in systems with two (or even numbers) of trophic levels tend to be more sensitive to warming than systems with odd numbers, due to cascading effects of predation on primary producers [56]. Yet, this result contradicts theories in which dynamically responsive predators can make three-trophic-level systems dynamically less stable than shorter food chains [57]. The difference between the prediction for instability in population dynamics and stability in ecosystem function may be explained by biodiversity in our systems and functional compensation among zooplankton or phytoplankton species [58]. Additionally, in our experiment, predators were not dynamically responsive; they did not have time to reproduce during the experiment. Consequently, they represent mortality for zooplankton that may have varied with temperature effects on per capita predation rates by predators, but not demographic response. In many systems, predators are subsidized by other habitats and food sources, and their populations are not dynamically coupled to prey. In fact, this decoupling has been shown to be important in thermally stratified lakes [59]. Inferences drawn based on this experiment about how species interactions affect community and ecosystem responses are restricted to systems with dynamics in the primary producers and primary consumers, with fixed predation-related mortality imposed by a third trophic level.

The growing literature of experimental tests of how warming affects interacting species aims to reduce uncertainty in projected changes associated with climate change. Warming experiments have shown a wide variety of effects on species interactions, from shifts in community composition, strengthening top-down control, and shifts in body size [47,55,60]. We have shown that these shifts do alter the effects on the temperature dependence of net ecosystem oxygen production and consumption. By measuring these responses over a broad thermal gradient, we have provided empirical evidence for nonlinear effects of temperature at the community and ecosystem level. Further, we have measured these changes in communities in which changes in abundance and community structure occurred within the context set by temperature constraints on energy fluxes via fundamental metabolic processes. Taken together, these results suggest our efforts to predict community change with warming may benefit from the general metabolic scaling theory framework to understand even local-scale effects of temperature change at the community level.

**Methods**

**Experimental Design and Set-up**

We assembled freshwater food webs in 30 outdoor mesocosms (370 L tanks) at the University of British Columbia, Vancouver, Canada (49°14’52” N, 132°13’57” W). Mesocosms were filled with municipal water on June 26th, 2012, heaters were added, and filled tanks were left for one week to allow chlorine to evaporate before organisms were introduced. We experimentally manipulated temperature (10 levels) and species interactions associated with different trophic structures (3 levels: algae-only (A), algae + grazer (AG), and algae + grazer + predator (AGP), Fig. 1B). There was one tank per temperature per trophic treatment; statistical power was derived from the regression design rather than replication within treatment levels (see Methods: Statistical Analysis). We monitored temperature continuously, and sampled biotic variables once per week for 9 weeks. Tanks were arranged randomly in space with regard to treatment. The spatially randomized assignment of temperature and trophic treatments eliminated systematic variation in negligible allochthonous carbon inputs.

At the beginning of the experiment tanks were allowed to sit for one week. After this first week, mesocosms were inoculated with pondwater (1L) from the UBC Pond Facility, containing living algae, collected and filtered through a 64-µm sieve to remove zooplankton and larvae. Three days later, we collected zooplankton at Trout Lake, Vancouver, B.C. (49°15’23” N, 123°03’44” W), with a vertical tow net (64-µm mesh). Zooplankton were mixed in buckets to homogenize species composition, were gradually introduced to mesocosm temperatures over a 12-hour gradual acclimation period to avoid stress associated with an abrupt temperature change, and dead organisms were removed. Initial experimental communities consisted of 25 phytoplankton taxa (Table S1), and those with zooplankton included predominantly 2 zooplankton taxa (the cladoceran *Daphnia* s*p.,* and calanoid copepod *Eurytemora* sp*.*) and, rarely, cyclopoid copepods. To ensure colonization of grazing zooplankton, in addition to the random aliquot of zooplankton added to each zooplankton ecosystem (all algae-grazer and algae-grazer-predator ecosystems), we added two individuals of *Daphnia* sp. and ten *Eurytemora* sp. Thus each zooplankton community began with *at least* 12 grazing zooplankton individuals. We introduced 2 individual notonectid predators (*Notonecta undulata*), collected from ponds at the UBC Pond Facility, on July 4th, 2012 (experiment day 8) to 10 algae-grazer-predator tanks. Notonectids generate trophic cascades by suppressing zooplankton [61]. Notonectids did not reproduce during the experiment, and we replaced dead notonectids during the experiment with similar-sized individuals from the same source population.

We added 160-µg NaNO3 L-1 and 10-µg KH2PO4 L-1 to each tank (16:1 N:P) on July 3rd, 2012. These quantities of nutrients represent typical deposition inputs to similar lakes [62]. Water was heated with submersible aquarium heaters (50, 100, 150, 200, 250, 300, 350, 400, 450 Watt) to increase temperature above ambient daily temperature. Temperatures were recorded hourly using Thermochron iButton dataloggers. Data loggers were suspended in the middle of the tanks, approximately halfway between the surface and the bottom. Temperature differences among tanks were consistent throughout the course of the experiment (Fig S1). Heaters were placed at the bottom of the mesocosms. Mesocosms were covered with two layers of window screen to minimize colonization by other invertebrates. Water levels were maintained by natural precipitation and weekly additions to maintain volume.

**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 [63] 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 [64,65], 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 4-0.

**Estimation of biomass and oxygen fluxes**

We estimated whole ecosystem oxygen fluxes using the dissolved oxygen (DO) change technique [66]. 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 dawn, dusk and dawn of the following day (Eqn 5) can indicate not only the cumulative biotic NEP and ER fluxes during that time interval, but also differences in water temperature that affect oxygen concentrations in water. At standard pressure, which is appropriate for our experiment near sea level, oxygen saturation can change by approximately 1 mg/L with a change in temperature of 5 °C, described by:

, Eqn 5

where [O2]water is the O2 concentration of water, [O2]sat is the concentration the water would have if it were at equilibrium with the atmosphere (390 µatm), T is temperature of the observation (°C) [67]. For the differences in temperature we observed, corrections were on the order of mean 0.0002 + sd 0.0008 µmol O2 / L / hr for NPP, and mean 0.0008 + sd 0.0003 µmol O2 / L / hr for ER. Because these values are within 25% of our total observed changes in oxygen during those periods (mean 0.003 + sd 0.001 µmol O2 / L / hr for NEP and mean 0.003 + sd 0.002 µmol O2 / L / hr for ER), we included the correction in our analyses. Overall, the conclusions based on model selection did not depend strongly on the use of the correction (results not shown).

We estimated NEP and ER by converting changes in observed O2 (mg L-1) between daytime observation times (*tdawn*, *tdusk*) and overnight observations (*tdusk*, *tdawn2*) to micromolar concentration (*z* = 31.25 µmol/1 mg), and correcting for changes in estimated equilibrium oxygen concentration () (Eqn 3) due to changes in saturation state with temperature at each time.:

Eqn 6a

. Eqn 6b

**Model and hypothesis development**

The expression of temperature effects on a *per capita* metabolic rate *bi* – in our case, oxygen production via photosynthesis or consumption via respiration - in this model is a special case of a more complex equation that allows each species to follow a thermal performance curve, often described by a modified Sharpe-Schoolfield equation [17,23,68], in which an individual’s or population’s performance declines at high temperatures above some optimal temperature. We do not use this TPC model here for two reasons: we do not expect photosynthesis or respiration to exceed optimal operating temperatures in our system for most taxa based on the fact that we collected them locally from a lake and habitat type (shallow pond) near the experimental site. We believe the simpler exponential is a suitable hypothesis for cross-system comparison [17,23], and we do not have thermal performance data for the many species in our communities that would allow fitting of thermal performance curves within communities.

We modeled MB (eqn 3) by including a term for trophic treatment (Zj) in the intercept term (eqn 3 rearranged and log transformed):

Eqn 7

We derived the expression for the trophic cascade by relating algal biomass in the AGP and AG treatments:

Eqn 8

We then simplified and added temperature dependence of mass and normalization constants to approximate their temperature dependence. In the absence of additional information about their functional forms, we used general Arrhenius functions, but we note that other functions could be used if appropriate. Consequently, the ratio of *MB* with and without predators may vary with temperature according to the relative temperature dependences of thermal traits and size distributions:

Eqn 9

and the strength of the trophic cascade may therefore be expected to decline with a temperature dependence that reflects the temperature dependences of mass and normalized performance for each trophic treatment:

Eqn 10

We modeled zooplankton density (N / L) as a function of mean weekly ecosystem temperature Twj and ecosystem trophic structure Zj, with ecosystem identity as a random effect.

**Statistical Analysis**

We tested our hypotheses about whether effects of temperature on metabolism are modified at the ecosystem level by species interactions using a regression experimental design involving 30 independent ecosystems (Fig S1). We maintained ecosystems at distinct temperatures in a regression design with mean ecosystem temperatures Twj ranging from 19.7 (+ 3.15) °C to 26.1 (+ 3.59) °C (Fig S1). The regression design allowed us to estimate slopes (e.g., *ER*, Eqn 2) of response variables along a continuous temperature gradient for different trophic structures (A, AG, AGP) by log-transforming equation 2 and fitting linear models to log transformed response variables the continuous temperature gradient. We chose the regression design, though unreplicated within temperature levels, because it allowed us to compare activation energies (*ER,* Eqn 2) fitted over a broad range of temperatures; an important test of thermal responses that is not possible with designs with only 2 or even three temperature levels. Regression designs, even without replication within levels, gain statistical power from the range of x-levels tested [69,70].

We used a mixed effects model (lme function in the nlme package of R) to examine the main and interactive effects of temperature (a continuous fixed factor) and trophic structure (a categorical fixed factor) on net ecosystem oxygen production (NEP), net ecosystem oxygen consumption (ER), and chlorophyll *a* concentration with a random intercept for individual ecosystems. We used a within-subject mean centering approach to distinguish temperature effects into those associated with an ecosystem’s average temperature (Tj) over the entire experimental period (a ‘between-ecosystem’ effect) from effects variation in temperature over time (Twj) (a ‘within-ecosystem temperature’ effect) [71]. The response variable (Y) for each ecosystem *j* in week *w* was modelled as a continuous response to variation in inverted ecosystem temperature (1/kTwj) and trophic treatment (*Z*j):

Eqn 11

where *β0.j(i)* represents an intercept allowed to vary randomly among ecosystems. The terms in the full model (Eqn 11) are: the between-ecosystem effect of temperature (*β2*), estimated as the slope of ln(Y*wj*) on the mean temperature over all weeks for ecosystem *j*, expressed as inverse temperature; the within-ecosystem (*β1*) effect of temperature variation over time estimated as the slope of ln(Y*wj*) *vs* centered weekly temperature; interaction (*β3*) between within-ecosystem temporal variation in temperature and the experimental temperature treatment; trophic species interactions (*β4*), and interactions between species interactions and overall mean (*β5*) and weekly temperature (*β6*).

To test our hypothesis that species interactions modify temperature dependence (*ER*, Eqn 2) of response variables (Y), we compared models with and without trophic level terms (*β4*)and interactions between *Zj* and temperature (*β5*, *β6*). We also tested models without temperature terms for within-system variation (*β4*). In total, the model set included 9 models (Table 1). Response variables were ln-transformed prior to analyses to achieve normal distributions and to linearize temperature effects for analysis and to fit *ER* values from Eqn 2. When modelling, we centered temperature treatment (1/kTj) on the grand mean of all temperatures observations (not shown in Eqn 11) to reduce correlations between slope and intercept terms [72].

To test the effect of temperature on trophic cascade strength, we used the following statistical model:

Eqn 12,

in which the effect of temperature on trophic cascade strength in each temperature treatment *j* was modeled for each week *w* and for the temperature of the tanks, with random effects *uj* were assigned for each power treatment (*p*).

We ranked models using Akaike’s Information Criterion weights (using the MuMin package in R), adjusted for small sample sizes (AICC). When two or more models were considered comparable or equivalent (δAICC < 2) we reported all models meeting this criterion and report averaged coefficients. We estimated *ER* and intercepts for among-ecosystem responses to temperatures by first rearranging Eqn 12 to group coefficients by temperature term.

Eqn 13

We estimated confidence intervals for composite terms following [73]. We used R statistical software (R v. 1.0.44 R Developmental Core Team 2006)*.* Our models controlled for the effect of temperature variation over time on ecosystem fluxes and biomass within systems.

We determined the effects of temperature and predator presence on zooplankton abundance data using generalized linear mixed effects models with tank as a random effect modeled on a negative binomial regression distribution to account overdispersed Poisson distributed count data (using the glmmADMB package in R).

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