**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. The metabolic theory of ecology predicts that warming increases net ecosystem oxygen fluxes by increasing per capita metabolic rates. In addition, temperature change can alter species interactions, which in turn influence ecosystem function. Surprisingly, the role of species interactions in modifying the general metabolic scaling effects of temperature at the ecosystem scale remains unclear. We experimentally tested the hypothesis that cascading trophic interactions modify the temperature-dependence of community structure and net ecosystem oxygen fluxes over a range of temperatures from 19-30 °C. In a series of independent freshwater ecosystems 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. 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 flux over the temperature gradient, as well as monotonic declines in phytoplankton standing stock, suggested no threshold effects of warming across systems. We conclude that species interactions can modify effects of temperature on primary producer biomass and net ecosystem oxygen consumption relative to first-order metabolic theory predictions, but even so, temperature can have continuous, 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 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 (Gillooly, Allen, Yvon Derochers). Highly conserved metabolic processes - photosynthesis and aerobic respiration (louca et al) - power somatic growth, maintenance and activity in aerobic organisms. As a result, the effects of temperature on photosynthesis and respiration translate to organism performance and population dynamics that in turn determine the rates and outcomes of processes such as carbon cycling, food web production and resilience (Barneche, Kerkhoff, michaletz?). The importance of temperature-dependent metabolism has led to the use of metabolic models to understand and predict ecological change across scales, from local to global [1-3].

Models that associate change in net metabolic processes at the ecosystem scale with individual-level oxygen production and respiration provide much needed predictability for how climate change affects ecosystem functions when ecosystems are compared across broad spatial or temporal thermal gradients (lopez-urrutia, michaletz, Anderson texiera). One challenge has been to reconcile the generality of temperature dependent metabolic scaling models with the well-documented effects of temperature on community level outcomes of species interactions. As higher temperatures increase metabolic rates and associated processes such as consumption, population growth and reproductive rates, these changes themselves influence species interactions in communities (Dell, Rall, O’Connor etc). For example, warming can strengthen the effects of predators on prey in *trophic* species interactions, strengthening top-down control of production and biomass in consumer-controlled systems (). Strong top down control can change NEP and ER relative to systems without strong effects of predators and grazers on community structure (schindler et al science).

A persistent challenge, therefore, is to relate general, simple models of metabolic scaling that explain variation in ecosystem properties with temperature at the macroecological scale (e.g., Brown et al 2004) to the growing theoretical and empirical evidence that at the scale of species interactions, temperature can cause changes in species traits, abundance and performance that do not appear consistent with the macroecological patterns (e.g., Kirk et al, Gilbert et al, O’Connor et al). Reconciling these apparently divergent patterns is critical to improving understanding and projections of how shifting global thermal regimes affect ecological patterns and processes.

Here, we aimed to resolve the paradox between apparent direct effects of temperature on ecosystem functions (NEP, ER, primary producer standing stocks) that emerge when comparing communities across larger gradients and the potentially more complex effects of temperature at the population and community scales over time. These ecosystem functions are typically monitored because they are indicative of total ecosystem state and function (). They primarily represent the stocks and fluxes mediated by primary producers (plants, algae), though respiratory fluxes also include heterotrophic metabolism. Macroecological comparisons of ecosystem function typically emphasize these functions, often achieving adequate explanatory power of models that do not include species interactions or community structure. However, at the local scales, it is well known that species interactions can strongly influence NEP, ER and primary producer abundance (). This paradox 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.

We experimentally tested the hypothesis that temperature-dependent trophic interactions alter the effect of temperature on three ecosystem functions (NEP, ER and phytoplankton standing stock), relative to ecosystems dominated by primary producers without strong trophic interactions. We measured the effect of temperature on ecosystem-level phytoplankton biomass, net ecosystem production and ecosystem respiration in replicate ecosystems without grazing and predators, and compared this temperature dependence to ecosystems with grazers, and with grazers and predators (Figure 1). We quantified changes in abundance, biomass, species composition and ecosystem oxygen flux across an experimental temperature gradient of 10 °C in freshwater plankton communities whose biomass structure and population dynamics reflected three scenarios of species interactions. In algae-only communities (A), phytoplankton biomass was expected to reflect competitive dynamics in the absence of grazing zooplankton. In algae+grazer communities (AG), phytoplankton biomass was expected to reflect top down control by grazers, and grazer abundance and composition was expected to reflect competitive interactions among grazers. In algae-grazer-predator (AGP) communities, top down control by predators was expected to limit zooplankton abundance and release phytoplankton from consumer control in a classic indirect interaction called a trophic cascade [14,19,20]. These three community types are common in nature, and transitions from systems with predators to systems without have been associated with dramatic changes to phytoplankton biomass and ecosystem level fluxes of oxygen and carbon [19,21,22].

Hypotheses

Hypothesis 1: Warming strengthens a trophic cascade

We predicted that grazing and predation (hereafter, ‘trophic interactions’) affect the amount of phytoplankton biomass, net ecosystem production and ecosystem respiration in our experimental ecosystems (intercepts in Figure 1). Our reference state for all trophic comparisons is the algae-only (A) treatment, without added grazers or predators. Grazing was expected to reduce algal abundance in the AG treatment, and predation on grazers was expected to reverse effects of grazing so that algal abundance in the AGP treatment was similar to the no-consumer (A) treatment (comparing intercepts in Figure 1). This pattern of predation reversing effects of grazers on plant biomass or production is called a ‘trophic cascade’, and is one important link between species composition and ecosystem function in terrestrial and aquatic ecosystems (Shurin et al, Estes, etc). The strength of a trophic cascade – or the magnitude of the indirect effect that predators can have on plant abundance – can vary with environmental conditions (). In our analysis, 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 their composition to smaller sizes and less-edible species, typical of classic freshwater trophic cascades (Brooks and Dodson), and that these trophic interactions would strengthen with higher temperatures. Because the species interactions in question are driven by predation rates and growth rates of phytoplankton (ref), we predicted an exponential increase in trophic cascade strength with temperature over our experimental temperature gradient.

Hypothesis 2: temperature dependence of biomass, net ecosystem production and respiration depends on the strength of the trophic cascade

Our primary focus in this study is understanding the temperature dependence of community biomass and ecosystem function. Drawing on recent empirical evidence and theory that temperature alters the strength of species interactions [5,6,14,23-25], we predicted that increasing temperature would strengthen the trophic cascade in our experimental aquatic communities (the ‘species interactions matter’ hypothesis, Fig 1). In the absence of consumers, increases in per capita rates of photosynthesis with temperature are expected to reduce standing stocks of algal biomass, due to the increased metabolic demand for individual productivity (Bernhardt, savage, Barneche et al 2016). This prediction assumes that resources and losses to consumption remain constant as temperature increases. If consumption by grazers increases with temperature, as we predict in our AG and AGP treatments, algal biomass responses to temperature could be very different in treatments with grazing than without (O’Connor et al 2011, Gilbert et al; Figure 1iii). In our trophic control hypothesis, we predict that the effects of temperature reflect changes in the strength of grazing with temperature and trophic treatment. In our first order metabolic theory hypothesis, we predict that increasing temperature causes a decline in phytoplankton abundance in all treatments, and that grazers are food limited so they simply respond to the temperature dependence of algae but do not affect it (Figure 1ii). We also tested a compensation hypothesis, which allows for algal species’ phenotypes to compensate for shifts in primary productivity of any single species (Loreau, Padfield). Compensation mechanisms include acclimation, adaptation, shifts in species dominance or in nature, immigration. In our system acclimation, adaptation, and shifts in dominance are possible, but not immigration. These mechanisms of phenotypic replacement along the thermal gradient could explain maintenance of biomass or function over a thermal gradient despite metabolic scaling (e.g., Enquist). The strength of a trophic cascade reflects the rate of resource productivity and the strength of top-down control on grazers and herbivory [6,20,26], so as these population-level rates of biomass production and consumption increase with temperature, the effect of releasing primary producers from consumer control is expected to be stronger (ref).

**Figure 1** Graphical illustration of hypotheses for how temperature affects primary producer biomass (A-C) and net ecosystem oxygen fluxes (D-F) for communities dominated by autotrophs (A), with autotrophs and grazers (AG) and with autotrophs, grazers and predators (AGP) (codes shown in panel A apply throughout). Net ecosystem oxygen fluxes refer to the absolute value of oxygen flux rate (production due to photosynthesis or oxygen consumption due to respiration). Exponential responses to temperature are plotted as linearized (log-transformed) and on 1/kT axes for later comparison with predicted slopes from Eqns 1 and 5 in the main text. In the first-order metabolic scaling hypotheses (E), net ecosystem oxygen flux (NEP and ER) in systems without heterotrophic consumers are expected to increase with temperature due to the temperature dependence of photosynthesis. In systems with heterotrophic consumers, oxygen production and oxygen consumption (if plotted separately) are expected to increase more steeply with temperature due to the stronger temperature dependence of respiration than photosynthesis. If species interactions modify metabolic scaling effects on ecosystem level biomass and oxygen flux (C, F), the temperature dependence is expected to be strongest in communities in which grazers are abundant and not limited by predators.

We then developed hypotheses for how the predicted changes in species interactions might influence temperature effects on net ecosystem primary production, ecosystem respiration and biomass standing stock. We drew on the Metabolic Theory of Ecology (MTE) and past empirical evidence in cross-system comparisons (YD 2012) to predict how temperature affects net ecosystem oxygen fluxes, an indication of major metabolic processes of photosynthesis and respiration. MTE relates whole-organism metabolic rates (*bi*) and related biological functions for organism *i* to body size (*mi*) and body temperature (*T*, in Kelvin) [12,27,28]:

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, and *a* is the allometric scaling factor that relates metabolic rate to body size. The expression of temperature effects on a 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 [7,8,29], in which an individual’s or population’s performance declines at high temperatures above some optimal temperature. We do not use this model here for two reasons: we do not expect photosynthesis or respiration to exceed optimal operating temperatures in our systems () so we believe the simpler exponential is a suitable hypothesis for cross-system comparison (following Yvon-Durochers, etc etc) [7,8], 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.

This exponential model has been extended to produce a ‘first-order’ expectation for the effects of temperature on ecosystem metabolic rates (*BR*), such as NEP or ER, that would reflect the sum of all *per capita* production rates by autotrophs and all consumption rates by autotrophs and heterotrophs in an ecosystem:

, Eqn 1b

in which *ER* is the ecosystem-level temperature dependence term for ecosystem rate *R*. 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 (Yvon-Durocher et al 2012, Allen et al 2005, L-U 2006, L-U 2008). The temperature dependence of photosynthesis at suboptimal temperatures appears to be *EPS*= ~0.32 eV, and this can emerge at population () and ecosystem scales (), 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 (michaletz, yvon durochers, Barnechek et al 2014). This hypothesis outlines a direct effect of environmental temperature on net ecosystem oxygen production and consumption mediated simply by the effects of temperature on per capita metabolic rate. To account for changes in total biomass, body size or relative abundance of species in the community with temperature, we use a mass term that accounts for shifts in the size structure of a community, following Allen et al 2005, Barneche etl al 2014: *MB* is total biomass (per unit volume?) [Mb = 1/V sum(mi)] of the community (autotrophs for NEP and all organisms for ER). We further include the term which is the estimated metabolic biomass for the average of all individual biomasses [] corrected for the greater contribution to total mass-specific metabolic biomass by small individuals. This ‘mass-corrected biomass’ estimate accounts for the allometric scaling (alpha) of oxygen production and consumption with body size (YD and Allen 2012, barneche). This 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, approaches 1 as *mii* declines and density increases. ogether, represents ‘mass-corrected’ biomass, which is a measure of the total metabolic capacity of biomass in an ecosystem [30]. [when does M(mba-1) approach Mb?]

Our ‘first-order metabolic scaling’ hypothesis is that water temperature affects total ecosystem oxygen fluxes (net ecosystem oxygen production and net ecosystem oxygen consumption) following equation 1b (Fig 1E).

If species interactions drive systematic changes in ‘mass-corrected’ biomass with temperature, these shifts could determine how temperature affects net ecosystem oxygen fluxes (the ‘species interactions matter’ hypothesis, Fig 1F). *add TSR here?* Alternatively, if compositional turnover or shifts in relative abundance within phytoplankton or grazer species composition occurs and maintains ecosystem function, this could lead to no change in net oxygen flux with temperature although species composition does (the ‘compensation’ hypothesis, Fig 1D) [8,31].

**Results**

We observed a trophic cascade on planktonic algal biomass that became apparent after the first weeks of the experiment, and strengthened over time and with temperature (Fig 2) consistent with our first prediction (Table 1, SX). The log response ratio of algal biomass with vs without predators increased exponentially with temperature (Fig 2) (*give coefficient)*. Support for also comes fromthe22B

In the absence of herbivores or predators, phytoplankton biomass declined with increasing temperature (Fig 3Ai), indicated by the inclusion of a main effect of temperature in the best model (Table 2) and an estimate for the activation energy that does not overlap with 0 (Fig. 3Ai). This decline with increasing temperature was much stronger in algae-grazer (predator-free) communities, with a decline over three orders of magnitude in phytoplankton biomass standing stock over the 10 °C temperature gradient (Fig 3Ai), likely reflecting temperature dependent grazing by zooplankton. These results are most consistent with the ‘species interactions matter’ hypothesis (Fig 1C) for phytoplankton biomass. Reject MTE or compensation?

Table 1. Model selection results for trophic cascade analysis. The full model is , see Methods for details; just put full table here.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Model Terms from Equation X** | | | | | | **Model comparison results** | | | |
|  | ***β0.j(i)*** | ***β1*** | ***β2*** | ***β3*** | ***μj*** | ***w*** | **df** | **δ** | **loglik** |
| full | x | x | x | x | x | 0.75 | 6 | 0.00 | -73.17 |
| Full - | x | x | x |  | x | 0.22 | 5 | 2.41 | -75.55 |

**Figure 2:** Mean overall **A**) phytoplankton biomass, estimated as the concentration of chlorophyll a, **B**) net ecosystem oxygen consumption, or ecosystem respiration (ER) and **C**) net ecosystem oxygen production (NEP) for ecosystems with algae only (A), algae + grazers (AG), and algae + grazers + predators (AGP). Trophic cascades describe the pattern in which predators control grazers’ impact on algae and render algal biomass or ecosystem function of a system with predators and grazers similar to one without consumers. Here data were pooled across temperatures and analysed with one-way ANOVA with week as a random factor, and significant differences (p < 0.05) indicated by \*. Full results in appendix.

Predators and increased temperature independently reduced total zooplankton density (Table 2); zooplankton density declined with increasing temperature (*EZP* = 1.28 95% CI: 0.19 – 2.39, z = 2.31, p = 0.021 based on regression with negative binomial distribution) (Fig S3.3). 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 although the temperature term retained in the best model was not significant based on the p-value (*ED* = 0.88 95% CI: -0.59, 2.35, z = 1.17, p = 0.24) (Tables S3.7, S3.8, Fig S3.5). 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, Tables S3.4, S3.5). We observed no significant effect of temperature or predation on the total zooplankton community body size distribution (model estimated mean *Daphnia* body size was 0.83 cm 95% CI: 0.79-0.88, and mean copepod size was 0.54 cm + 95% CI: 0.52-0.57; Fig S3.8).

**Figure 3:** The effect of mean ecosystem temperature on A) phytoplankton biomass, B) net ecosystem productivity (NEP), and C) net ecosystem respiration (ER) for three community types that varied in their trophic interactions: i) algae-only (A), ii) algae + grazers (AG), and iii) algae + grazers + notonectid predators (AGP). Black lines indicate the among-ecosystem effects of temperature, modelled by equation 5 using hierarchical regressions fit to among-ecosystem variation in temperature, after taking into account within-group variation temperature effects (light lines) (Table 1), and may be compared with predicted effects of temperature and species interactions depicted in Figure 1. Activation energies and confidence intervals estimated by best model or best model set (Table 1, Supplementary Material 2). Temperature in Celsius is shown for comparison only, models were fit to inverse temperature. For clarity here, the three trophic treatments are separated into three rows of panels. Response variables were estimated once per week for 9 weeks in each replicate ecosystem (n = 30). For each ecosystem (shade of grey), 6 points are shown, one point for each week (symbols). Temperatures within tanks declined over time (Fig S1.1C).

**Table 2:** Results of model comparisons for effects of temperature and time on trophic cascade strength based on AIC weight (*w*) and δAIC values. Nested versions of the full model (Eqn 5, Methods, Table S1.1), and only highest ranked models (δAIC < 2) are shown. Response variables are modelled as functions of temperature Tij for each tank *j* on week *i* relative to the mean temperature for tank *j* over all weeks (T in Kelvin), and food chain length (TL). Models included a random effect for the experimental unit – tanks with and without predators receiving the same power inputs. See Methods and Tables S2.1, S2.3, and S2.5 for additional details on modeling.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Model Terms from Equation 5** | | | | | | | | | | | **Model comparison results** | | | | | | | |
|  | | ***β0.j(i)*** | ***β1*** | ***β2*** | ***β3*** | ***β4*** | ***β5*** | ***β6*** | ***μj*** | ***w*** | | | **df** | | **δ** | | **loglik** |
| **Phytoplankton Biomass (Mb)** | | | | | | | | | | | | | | | | | |
| 8 | | x | x | x |  | x | x | x |  | 0.95 | | | 11 | | 0.00 | | -162.86 |
| 7 | | x | x | x |  | x | x |  |  | 0.05 | | | 9 | | 6.02 | | -168.05 |
| **Net Ecosystem Oxygen Production (NEP)** | | | | | | | | | | | | | | | | | |
| 8 | | x | x | x |  | x | x | x |  | 0.39 | | | 11 | | 0.00 | | -266.46 |
| Full | | x | x | x | x | x | x | x |  | 0.32 | | | 12 | | 0.39 | | -265.54 |
| **Net Ecosystem Respiration (ER)** | | | | | | | | | | | | | | | | | |
| 7 | | x | x | x |  | x | x |  |  | 0.812 | | | 9 | | 0.00 | | -158.72 |
| 8 | | x | x | x |  | x | x | x |  | 0.15 | | | 11 | | 3.39 | | -158.19 |
| **Zooplankton Density** | | | | | | | | | | | | | | | | | | |
| 1 |  | | | | | | | | | 0.50 | | 5 | | 0.00 | | -367.00 | | |
| 1b |  | | | | | | | | | 0.31 | | 6 | | 0.95 | | -366.37 | | |
|  | | | | | | | | | | | | | | | | | | |

Trophic interactions modified the effect of temperature on net ecosystem oxygen production (NEP) and respiration (ER) (Tables 1, 2, Fig 3, Supplementary material 2). Based on the inclusion of a temperature x trophic treatment interaction term in the best model for both NEP and ER (Table 1), we would reject the ‘first-order metabolic scaling’ hypothesis (Fig 1B, E) that effects of temperature on net ecosystem oxygen fluxes were independent of trophic interactions. The estimated across-system temperature dependence of NEP was the strongest (Fig 3 Bi) in algae-only communities, and confidence intervals for the temperature dependence term include 0 for the systems with predators. Net ecosystem respiration (ER) also increased with temperature across ecosystems (Fig 3 C), consistent with the predicted *ER*= -0.65 eV in systems with herbivores [and predators??], and only the activation energy estimate for systems with predators suggest a weak or absent effect of temperature. Still, our conclusion remains somewhat equivocal, because confidence intervals for activation energies overlap across trophic treatments (Fig 3B-C) so although model selection suggests trophic structure is informative, differences in activation energy estimates are not significant among trophic treatments.

In addition to the variation among ecosystems in temperature that was the main focus of our analysis (Methods), temperature varied within our experimental ecosystems over time (Fig S1C). Effects of temporal temperature variation on biomass differed starkly from effects of temperature among ecosystems (Fig 3A). Within ecosystems, higher temperatures were associated with higher phytoplankton standing stocks (light lines indicate within-system patterns in Fig 3), contrary to the among-ecosystem pattern (bold lines indicate among group pattern) of lower biomass at warmer temperatures. Within ecosystems, effects of temporal temperature variation depended on trophic structure treatment, with the strongest effects of within-ecosystem temperature variation apparent in the AG treatments (Fig 3Aii). Net ecosystem oxygen production varied with temperature within ecosystems, and this temperature effect interacted with both the species interaction treatment and the overall average tank temperature. Net ecosystem respiration varied within ecosystems over time, but this variation did not depend on temperature treatment or trophic level (Table 1).

**Discussion**

The joint effects of biodiversity loss and climate change are affecting ecosystems worldwide [32,33]. Biodiversity loss can change species interactions in a community {Estes:2011eo}, and the role of local species interactions in amplifying or moderating effects of temperature on ecosystem functions is a critical link between biodiversity at the community level and climate change impacts on individual and ecosystem level performance [34,35]. Here, we found that in a series of 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). These two ecosystem functions (NEP, ER) reflect the total metabolic activity of all aerobic organisms in the ecosystem. They are directly related to carbon storage and cycling by aquatic ecosystems [36], and they are expected to vary with temperature reflecting the effects of temperature on per capita metabolic rates (Eqn 1). We found that higher average temperatures increased net ecosystem oxygen production and consumption while total phytoplankton biomass declined. Local community structure and species interactions associated with a typical food web motif in freshwater food webs – the trophic cascade - modified how temperature affected oxygen flux, thereby linking changes in biodiversity ecological responses to climate warming. Predators reduced the grazing pressure imposed by the dominant grazer, *Daphnia*, by reducing its density, and thereby shifted grazer assemblages toward the less effective copepod grazers. This trophic cascade, mediated by shifts in grazer composition as well as total density, is a classic food web motif in freshwater systems [37].

These results also provide a key test of how we can use one of the more general ecological theories for temperature effects – the metabolic theory of ecology – to understand community-level responses to temperature change. The effects of temperature on ecosystem function varied with the types of species interactions dominant in each local community. The temperature dependences of net ecosystem fluxes were least pronounced in communities with grazers and predators. 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.

The metabolic theory of ecology predicts that highly conserved metabolic rates (respiration, photosynthesis) are sensitive to temperature in ways that emerge at scales of communities and ecosystems. Across broad spatial scales, and within experiments, ecosystem level fluxes have been shown to vary with temperature according to the temperature dependences of photosynthesis and respiration, irrespective of trophic structure and consistent with the ‘first order metabolic scaling’ hypothesis (Fig 1ii) [2,3,38]. Despite repeated support at macro-ecological scales, this ‘first-order metabolic scaling’ prediction has been rejected or challenged at local community and ecosystem scales [39,40]. Other studies have found that acclimation and adaptation can compensate for temperature effects on fundamental metabolic processes at the ecosystem scale [8,10,41], and additional theory has been developed to explain how the general temperature dependence of metabolic rate interacts with population and community level processes to influence the emergent responses to temperature, producing an expanded version of metabolic scaling theory that incorporates local ecological and evolutionary processes [4,5,7,42,43]. Here, we contribute to this line of inquiry with our finding that the way net oxygen fluxes varied over the temperature gradient in this controlled experiment depended on trophic structure of the community. Our models indicated the need for the species interaction x temperature model term. Still, we observed temperature dependences that were consistent with expected temperature dependence of the underlying metabolic processes of photosynthesis (*Ea* = 0.32 eV) and respiration (*Ea* = -0.65 eV), though our confidence intervals on estimates are wide enough to preclude strong inference about the exact values of the activation energies in this study.

We observed a much stronger decline in phytoplankton biomass with warming than the increase in oxygen fluxes, as indicated by higher slope values for biomass (Fig 3). 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 [3,5,44]. In addition, if size distributions shift toward smaller cells, as is common with warming [45,46], the allometric scaling of metabolic rate with body size (Eqn 1b) predicts greater oxygen flux for a given biomass. At the ecosystem scale, resource supply may change with temperature, if microbial assemblages, biological nitrogen fixation, and recycling processes accelerate [47], creating a resource gradient in parallel with the temperature gradient [6,44]. Additionally, benthic algae may contribute to NEP and ER estimates [48]. 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 [48], 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 [8,41,47] 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 1b, Figure 3), 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 (Fig 3D, Fig S3.3, S3.4). We observed little evidence of abrupt transitions that might be expected if thermal stress responses by individual phenotypes drove ecosystem scale responses. While individuals may experience thermal stress and decline in performance at high temperatures, in our systems these effects were functionally compensated for by other species and increases in per capita performance. The exponential effects of temperature on biomass and oxygen fluxes persisted for all species interaction scenarios. In other studies, in the absence of grazers, algal biomass tends to decline with increasing temperature when resources do not increase with temperature [5,17,49]. This is an expected consequence of increased mass-specific metabolic demand at higher temperatures, and could be exacerbated by temperature-dependent consumer control of phytoplankton biomass [5,6]. Though we did not observe signs of transient dynamics in these communities over time, we also cannot conclude that these systems had reached an equilibrium or stable state. Longer experiments have demonstrated continued shifts in community composition after months and years of warming [15,50].

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 3D). 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 [49]. Yet, this result contradicts theories in which dynamically responsive predators can make three-trophic-level systems dynamically less stable than shorter food chains [51]. 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 [31]. 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 [52]. 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 [9,14,48]. We have shown that these shifts do alter the effects on the temperature dependence of net ecosystem oxygen fluxes. 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 species composition and community structure occurred within the context set by temperature constraints on energy fluxes via fundamental metabolic processes. To extend our findings to a conjecture about implications for climate change, we suggest that conservation actions that maintain predators and top down control may also promote an ecosystem that changes less with temperature than a system with a large abundance of grazers. 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 Food Webs**

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 food chain lengths (3 levels: algae-only, algae + grazer, and algae + grazer + predator food chains, Fig. S1A-B). 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.

After 1 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 S1.2), 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 [53]. Notonectids did not reproduce during the experiment, and we replaced dead notonectids during the experiment with similar-sized individuals from the same source population.

**Temperature and Nutrient conditions**

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 [54]. 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. The 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 S1.1C). 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 [55] and 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 [56,57], the chlorophyll a concentration represents biomass allocated to photosynthesis and NPP, our measure of ecosystem function.

Phytoplankton were identified and counted to species or taxon level by inverted microscopy. 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 week 8. We measured oxygen concentrations using YSI-85 oxygen sensor (Yellow Springs Instruments, Yellow Springs, Ohio, USA).

**Estimation of biomass and oxygen fluxes**

We estimated whole ecosystem oxygen fluxes using the dissolved oxygen (DO) change technique [58]. 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 4) 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 3

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) [59]. 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 4a

. Eqn 4b

**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 S1.1). For each food chain length (algae-only (A), algae-grazer (AG) or algae-grazer-predator (AGP)), we maintained ecosystems at distinct temperatures in a regression design with mean temperatures ranging from 19.7 (+ 3.15) °C to 26.1 (+ 3.59) °C (Fig S1.1C). The regression design allowed us to estimate slopes (*Ea*, Eqn 1) of response variables along a continuous temperature gradient for different trophic structures by log-transforming equation 1b and fitting linear models to the continuous temperature gradient. We chose the regression design, though unreplicated within temperature levels, because it allowed us to compare activation energies (*Ea,* Eqn 1) fitted over a broad range of temperature; 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 [60,61].

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 food chain length (a categorical fixed factor) on net ecosystem oxygen production, net ecosystem oxygen consumption, 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 over the entire experimental period (a ‘between-ecosystem’ effect) from effects variation in temperature over time (a ‘within-ecosystem temperature’ effect) [62]. The response variable (Y) for each ecosystem *j* in week *i* was modelled as a continuous response to variation in inverted ecosystem temperature (1/kTij) and trophic level (TLj):

 Eqn 5

where *β0.j(i)* represents an intercept allowed to vary randomly among ecosystems. The terms in the full model (Eqn 5) are: the between-ecosystem effect of temperature (*β2*), estimated as the slope of ln(Y*ij*) 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*ij*) *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 (*Ea*, Eqn 1) of response variables (Y), we compared models with and without trophic level terms (*β4*)and interactions between *TL* 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 S1.1). Response variables were ln-transformed prior to analyses to achieve normal distributions and to linearize temperature effects for analysis and to fit *Ea* values from Eqn 1. When modelling, we centered temperature treatment (1/kTj) on the grand mean of all temperatures observations (not shown in Eqn 5) to reduce correlations between slope and intercept terms [63].

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

Eqn 6,

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 (*j*).

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 *Ea* and intercepts for among-ecosystem responses to temperatures by first rearranging Eqn 5 to group coefficients by temperature term (Eqn 5a).

 Eqn 5A

We estimated confidence intervals for composite terms following [64]. 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 zoolplankton 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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