Shifts in community size structure drive temperature invariance of secondary production in a stream-warming experiment

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Abstract. A central question at the interface of food-web and climate change research is how secondary production, or the formation of heterotroph biomass over time, will respond to rising temperatures. The metabolic theory of ecology (MTE) hypothesizes the temperature-invariance of secondary production, driven by matched and opposed forces that reduce biomass of heterotrophs while increasing their biomass turnover rate (production:biomass, or *P*:*B*) with warming. To test this prediction at the whole community level, we used a geothermal heat exchanger to experimentally warm a stream in southwest Iceland by 3.8°C for two years. We quantified invertebrate community biomass, production, and *P*:*B* in the experimental stream and a reference stream for one year prior to warming and two years during warming. As predicted, warming had a neutral effect on community production, but this result was not driven by opposing effects on community biomass and *P*:*B*. Instead, warming had a positive effect on both the biomass and production of larger-bodied, slower-growing taxa (e.g., larval black flies, dipteran predators, snails) and a negative effect on small-bodied taxa with relatively high growth rates (e.g., ostracods, larval chironomids). We attribute these divergent responses to differences in thermal preference between small- vs. large-bodied taxa. Although metabolic demand vs. resource supply must ultimately constrain community production, our results highlight the potential for idiosyncratic community responses to warming, driven by variation in thermal preference and body size within regional species pools.

Key words: biomass; body size; climate change; experimental warming; Iceland; metabolic theory of ecology; secondary production; streams; temperature.

Introduction

Climate warming is expected to affect freshwater ecosystems globally (Mulholland et al. 1997, Woodward et al. 2010*a*). Given that freshwater communities support high levels of biodiversity and are dominated by ectotherms (Dudgeon et al. 2006), higher temperatures will inevitably lead to changes in the structure of communities, as well as in the ecosystem services they provide. One pressing question is how secondary production, or the formation of heterotrophic biomass over time, will respond as freshwater ecosystems warm. Secondary production is an ecosystem flux that integrates a range of taxon-specific traits (i.e., biomass, growth rate, body size, reproduction, survivorship; Benke 1993, Huryn and Wallace 2000), all of which will respond to warming (Brown et al. 2004). While several studies have

incorporated temperature as a variable in empirical secondary production models (Huryn and Wallace 1986, Morin and Dumont 1994, Tumbiolo and Downing 1994), few have explicitly examined the effects of temperature on secondary production and biomass turnover in natural communities (but see Huryn 1990, Huryn et al. 1995). Nevertheless, understanding how higher temperatures will affect production of animal biomass is a prerequisite for predicting whole-community-level and ecosystem-level responses to climate warming.

The metabolic theory of ecology (MTE; Brown et al. 2004) provides a useful framework for making predictions about how temperature affects secondary production (Cross et al. 2015). The MTE relates biomass (*B*), body mass (*M*), and temperature as

$$B \propto [R]M^{0.25}e^{E/kT} \qquad (1)$$

where [*R*] is the supply of a limiting resource, *E* is the activation energy of cellular respiration, *k* is the Boltzmann constant, and *T* is the absolute temperature in K (constrained to a range of 0–40°C). From this equation,

biomass is predicted to increase with individual body size but decrease with temperature (Brown et al. 2004).

The MTE also predicts that biomass turnover (production : biomass, or P:B) is related to body size and temperature as

$$P : B \propto M^{-0.25} e^{-E/kT} \qquad (2)$$

This equation predicts that P:B will decrease with individual body size and increase with temperature. Because biomass is expected to decrease with increasing temperature (Eq. 1), temperature should have a neutral effect on secondary production (P)

$$P \propto [R] M^0 \qquad (3)$$

because

$$P \approx B \times P : B. \qquad (4)$$

Thus, assuming no change in resource supply [R], MTE predicts that secondary production should be equivalent across a wide range of body sizes and temperatures (Huryn and Benke 2007, Cross et al. 2015; Fig. 1).

To test these hypotheses at a spatial scale that is ecologically relevant to community-level energy fluxes, we experimentally warmed a spring-fed stream in southwest Iceland by 3.8°C for two years using a gravity-fed,
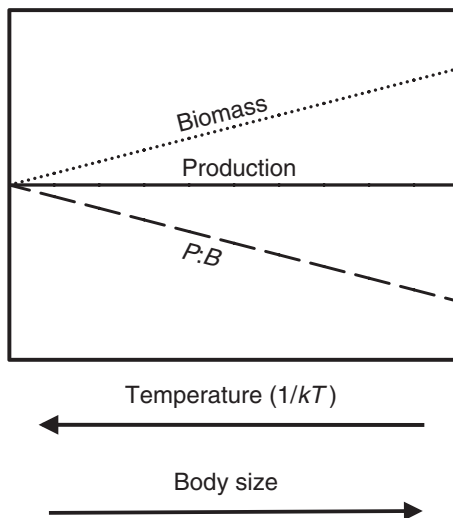

FIG. 1. Arrhenius plot showing predicted relationships between the reciprocal of temperature (as $1/kT$, where k is the Boltzmann constant and T is the absolute temperature in K), body size (M), biomass (B), production (P), and biomass turnover (P:B). Biomass (B) is predicted to scale with body size and temperature as $B \propto [R] M^{0.25} e^{E/kT}$, while biomass turnover (P:B) is predicted to scale as $P:B \propto M^{-0.25} e^{-E/kT}$. Because secondary production (P) can be calculated as the product of B and P:B, P should be both body size and temperature invariant, $P \propto [R] M^0$, under conditions of constant resource supply. Predictions are derived from Brown et al. (2004).

geothermal heat exchanger (Nelson et al., *in press*). We studied the experimental stream and a nearby reference stream for one year prior to warming and two years during warming. In a related study, we tested effects of this experimental warming on invertebrate community structure (Nelson et al., *in press*). Warming decreased invertebrate abundance, but changes in assemblage structure that favored large-bodied taxa resulted in no net effect on total invertebrate biomass (Nelson et al., *in press*). Here, our primary goal was to examine how the increase in mean annual stream temperature affected community-level invertebrate production, via its effects on biomass and P:B, under the assumption that resource supply remains unchanged with warming.

METHODS

Study sites and experimental warming

Our study was conducted in two first-order groundwater-fed streams in the Hengill geothermal region of southwest Iceland. The streams had comparable physical and chemical characteristics, similar average annual temperatures, and closely matched invertebrate communities (Ólafsson et al. 2010; Nelson et al., *in press*). Although unreplicated, the benefits of such paired catchment designs include their realism and scale, making them essential to investigating the dynamics and experimental responses of natural populations and communities (Carpenter et al. 1995, Schindler 1998, Oksanen 2001). The experimental stream is in the Hengill geothermal field (64°03′ N 021°18′ W, 380 m above sea level), while the reference stream is approximately 5 km from the experimental stream in the Ölkelduháls geothermal field (64°03′ N 021°12′ W, 360 m above sea level). We sampled each stream during a 12-month pre-warming period (YR1) beginning in October 2010. We began warming a 35-m reach of the experimental stream using a gravity-fed heat exchanger (O'Gorman et al. 2014; Nelson et al., *in press*) in October 2011. The cold experimental stream (mean annual temperature 5.8°C) runs parallel to a warm stream (mean annual temperature 22°C), with just 2 m separating the two streams at their closest point, before discharging into the Hengladalsá River. Water from the cold experimental stream was diverted into the heat exchanger submerged in the warm stream, warmed, and discharged back into the experimental stream. The warming manipulation resulted in an average temperature increase of approximately 3.8°C during the two years of warming (YR2 and YR3; Nelson et al., *in press*; see Appendix S1), a shift that is consistent with the upper range of global climate change predictions for the next century (IPCC 2013).

Invertebrate and organic matter sampling

We sampled invertebrates from each stream approximately monthly for three years between October 2010

and September 2013 using a Surber sampler (0.0225 m², 250-μm mesh). On each date, we collected five samples from random locations within each stream reach. Mineral substrata were scrubbed with a stiff brush to dislodge invertebrates and organic matter. Samples were preserved in 5% formaldehyde in the field and returned to the laboratory where they were separated into fine (0.25–1 mm) and coarse (>1 mm) fractions using nested sieves. Invertebrates were manually removed from both fractions with the aid of a dissecting microscope (10–15× magnification). Fine samples were subsampled (one-half to one-eighth) when necessary using a Folsom plankton splitter (Wildco, Yulee, Florida, USA). Invertebrates were identified to the lowest practical taxonomic level (genus in most cases) using Gíslason (1979), Cranston (1982), Wiederholm (1983), and Merritt et al. (2008). The body length of each individual was measured to the nearest millimeter, except for Chironomidae, which were measured to the nearest 250 μm. Biomass was estimated using length–mass relationships (Benke et al. 1999, Johnston and Cunjak 1999, Miserendino 2001, Tod and Schmid-Araya 2009). To determine if warming had an effect on resource quantity or quality and thus violated the assumptions of our predictions, we also quantified the biomass of organic matter and measured the quality of epilithic biofilm (the most dominant food source in the guts of invertebrates from the two streams) as C:nutrient ratios (see Appendix S2 for details).

Secondary production

We estimated annual secondary production (mg dry mass·m⁻²·yr⁻¹) using methods most appropriate for each taxon (see Appendix S3 for a list of taxa and methods used to estimate production). We used the instantaneous growth-rate method (Benke and Huryn 2007) for taxa with well-defined cohorts. This method requires measurements of population biomass and individual growth rates over time. For taxa showing synchronous cohort development, daily instantaneous growth rates (IGRs) were estimated using changes in average size based on data from the Surber samples. Briefly, for each month, the size-specific abundance data were resampled 1,000 times with replacement to generate vectors of mean size and biomass. Consecutive vectors of mean size (i.e., two consecutive months) were ln-transformed and regressed against the number of elapsed days to generate 1,000 estimates of IGR. We then multiplied the vector of IGR estimates by the vectors of mean biomass for two consecutive dates to generate 1,000 estimates of daily secondary production (Benke and Huryn 2007). Each vector of daily secondary production was multiplied by the number of days between sampling periods to generate vectors of interval production, which were then summed across months to generate 1,000 estimates of annual secondary production. Confidence intervals (95% CIs) were produced by discarding the upper and lower 2.5% of bootstrap values (Benke and Huryn 2007).

For taxa that did not exhibit well-defined cohorts, we estimated production using either the size-frequency method or from the product of mean annual biomass and appropriate P:B (P:B = 5 for all cases). For production estimates using the size-frequency method, we used bootstrapping and Monte Carlo methods to generate 1,000 estimates of biomass, production, and P:B. We corrected production estimates using our best estimates of cohort production intervals (CPI) based on size-frequency data and time series of abundance and biomass (Hamilton 1969, Benke 1979, Benke and Huryn 2007). For each iteration, CPIs were randomly selected within a restricted range. For production estimates using P:B relationships, we multiplied the values in the biomass vector by the selected P:B for that taxon. Production estimates derived from this method accounted for <5% of the total annual production (except YR3 in the experimental stream where Oligochaeta C comprised ~10% of total secondary production). Thus, assumptions of P:B relationships and their lack of variance have a negligible influence on our overall results. All biomass and production estimates were computed using R statistical software version 3.1.2 (R Core Team 2014).

Statistical approach

To assess differences in community biomass, annual secondary production, and annual P:B among years within each stream, we performed two-sample randomization tests on the 1,000 bootstrapped estimates of the variable of interest (Manly 1997, Benke and Huryn 2007). First, we calculated the difference in the means from the vectors of the two years being compared. We then created a vector containing all bootstrapped values from both years. We randomly sampled from this vector without replacement and randomly assigned values to one of two vectors and calculated the differences between paired samples. P values were calculated as the proportion of randomized differences greater than or equal to the observed mean difference. For individual taxa, we used the 95% confidence intervals of the bootstrapped estimates of the variable of interest to assess differences in biomass, production, and P:B between streams and among years (Benke and Huryn 2007). If confidence intervals overlapped, the difference in means was considered non-significant.

To assess differences in the experimental stream relative to the reference stream, we computed the differences (deltas) in the variable of interest between the bootstrapped estimates from the experimental and reference streams from the same year to generate 1,000 delta estimates. We then used two-sample randomization tests (see above) to determine significant differences among years. Finally, we calculated the magnitude of change in biomass, secondary production, and P:B from YR1 to YR3 and from YR1 to YR2 for each taxon. First, we calculated the response in the experimental stream relative to the reference stream as the relative percent change

TABLE 1. Total invertebrate biomass (B), secondary production (P), and production:biomass ($P{:}B$) in the experimental and reference streams during all three years of the study.

Stream	Biomass (mg DM/m²)		Production (mg DM·m⁻²·y⁻¹)			
	B	CI	P	CI	$P{:}B$	CI
Experimental						
YR1	1,676	1,459–1,914	12,239	9,931–14,714	8.0	7.2–9.0
YR2	1,370	1,229–1,521	9,233	8,046–10,628	8.3	7.2–10.0
YR3	1,688	1,515–1,854	11,172	9,477–13,247	7.8	7.1–8.7
Reference						
YR1	872	730–1,011	5,817	4,742–7,198	10.4	7.1–17.8
YR2	908	785–1,038	6,667	5,505–8,019	9.1	8.2–10.2
YR3	789	698–897	5,383	4,459–6,541	10.1	9.0–11.4

Notes: YR1, October 2010 to September 2011 (pre-warming); YR2, October 2011 to September 2012 (during warming); YR3, October 2012 to September 2013 (during warming). CI refers to the 95% confidence interval around the mean for the variable of interest.

$$\text{relative \% change} = [(X_{BR}/X_{BE}) - (X_{AR}/X_{AE})]/ (X_{AR}/X_{AE}) \times 100 \quad (5)$$

where X_{BR} is the mean variable of interest in the reference stream before warming of the experimental stream; X_{BE} is the mean variable of interest in the experimental stream before warming; X_{AR} is the mean variable of interest in the reference stream after warming; X_{AE} is the mean variable of interest in the experimental stream after warming. Second, we calculated the magnitude of change as a response ratio (RR) within each stream as

$$\text{RR} = \log_{10}(X_A/X_B) \quad (6)$$

where RR is the log(proportional change) in the variable of interest from before warming (X_B, YR1) to after warming (X_A, YR3). To examine whether taxon-specific changes in biomass or production were related to body size, we plotted RR vs. log(body mass) and used least squares regression to fit linear models to plots. To determine if these relationships were different between streams, we compared them using analysis of covariance (ANCOVA) with RR as the response variable, body mass as the covariate, and stream as the grouping factor. We also examined whether $P{:}B$ was related to body size by plotting the log(annual $P{:}B$) of each taxon in each stream during all three years vs. $\log_{10}$ body mass and used least squares regression to fit linear models to the plot.

RESULTS

Biomass, secondary production, and P:B

Estimates of mean invertebrate biomass, $P{:}B$, and annual community production for both the reference and experimental streams did not differ significantly among years (Table 1, Appendix S4). Community biomass in the experimental stream was nearly double that of the reference stream during all three years of the study, ranging from 1,370 to 1,688 mg dry mass/m²

(Table 1). Biomass in the reference stream ranged from 789 to 908 mg dry mass/m² (Table 1). In addition, experimental warming had no effect on relative biomass (i.e., the inter-stream difference; Table 2, Fig. 2a). Similarly, $P{:}B$ showed no significant differences among years within each stream (Table 1, Appendix S4) or any significant differences in the experimental stream relative to the reference stream over the course of the study (Table 2, Fig. 2b). Annual $P{:}B$ ranged from 7.8 to 8.3 in the experimental stream and from 9.1 to 10.4 in the reference stream (Table 1). Annual secondary production was also twofold higher in the experimental stream than in the reference stream (Table 1) and, like biomass and $P{:}B$, showed no significant differences among years within each stream (Table 1, Appendix S4). In the experimental stream, annual secondary production ranged from 9,233 to 12,239 mg dry mass·m⁻²·yr⁻¹, while in the reference stream, it ranged from 5,383 to 6,667 mg dry mass·m⁻²·yr⁻¹ (Table 1). Production in the experimental stream relative to the reference stream also did not change among years (Table 2, Fig. 2c).

Although warming had little apparent effect on total community biomass and production, it was associated with significant changes in the biomass and production of individual taxa and taxonomic groups. For example, the biomass and production of several non-biting midges (Chironomidae) decreased significantly in the

TABLE 2. Results (P values) from two-sample randomization tests on bootstrapped delta values ($X_E - X_R$). YR1 = October 2010 – September 2011; YR2 = October 2011 – September 2012; YR3 = October 2012 – September 2013.

	Deltas ($X_E - X_R$)		
	YR1 vs. YR2	YR1 vs. YR3	YR2 vs. YR3
Biomass	0.176	0.266	0.135
Production	0.141	0.238	0.152
$P{:}B$	0.245	0.213	0.218

Note: Deltas were calculated as $\Delta = X_E - X_R$, where X_E is the mean value in the experimental stream and X_R is the mean value in the reference stream.
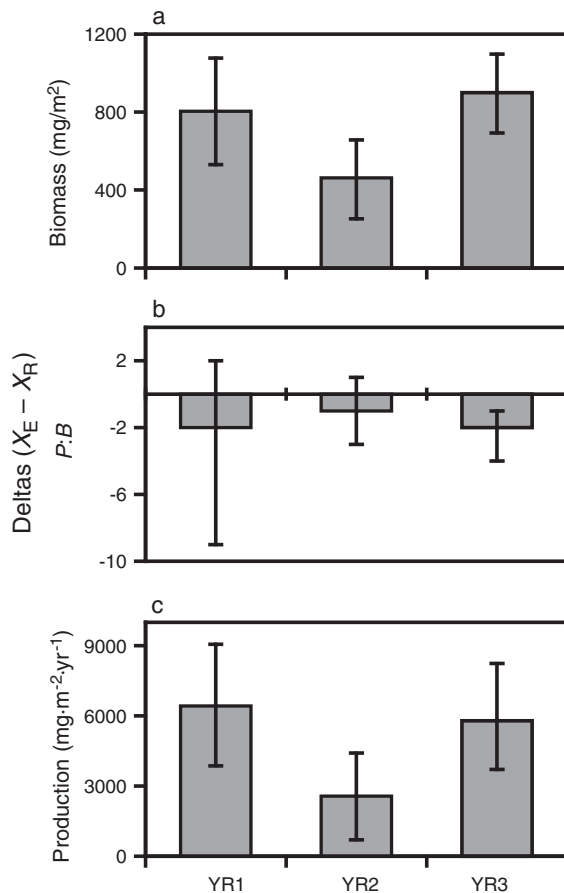
Fig. 2. Differences (deltas) between the experimental stream and reference stream in (a) biomass, (b) $P{:}B$, and (c) production during YR1, YR2, and YR3. Deltas were calculated as $\Delta = X_E - X_R$, where X_E is the mean value in the experimental stream and X_R is the mean value in the reference stream. Time periods YR1, October 2010–September 2011; YR2, October 2011–September 2012; YR3, October 2012–September 2013. Experimental warming began in October 2011. Error bars represent the 95% confidence intervals around the mean.

experimental stream (Fig. 3, Appendices S3, S5). Of the 11 chironomid taxa found in the reference and experimental streams during YR1 and YR3, five declined in both biomass and production by at least 50% during the warming period. In addition, the biomass and production of ostracods and other small-bodied taxa (e.g., copepods and the oligochaete, *Nais communis*) also decreased significantly in the experimental stream (Fig. 3, Appendices S3, S5). Conversely, the biomass and production of many other taxa increased in the experimental stream in response to warming (Fig. 3, Appendices S3, S5). Biomass of the snail *Radix balthica*, for example, increased by 597% in the experimental stream from YR1 to YR3, while its production increased by 831%. Other taxa responded with large increases in both biomass (the black fly *Simulium vittatum*, +1,566%; the predatory muscid fly *Limnophora riparia*, +1,227%) and production (*S. vitta-tum*, +1,878%; *L. riparia*, +409%). Annual $P{:}B$ increased

significantly for only three taxa: *S. vittatum*, the freshwater snail *Galba truncatula*, and the predatory oligochaete *Chaetogaster diaphanous* (Appendix S3). The only decrease in annual $P{:}B$ was shown for the black fly *Prosimulium ursinum* (Appendix S3).

Changes in biomass and production of taxa in the experimental stream were related to body size. Warming had a greater positive effect on the biomass and production of larger- vs. small-bodied taxa in the experimental stream (Fig. 4). Overall, the biomass of small-bodied taxa declined in the experimental stream from YR1 to YR3 (Fig. 4a, b), while the biomass of many large-bodied taxa increased (Fig. 4a, b). These patterns generally offset the predicted negative effect of warming on community-level biomass (Nelson et al., *in press*). Relationships between changes in annual production with warming and body size were similar to those found between biomass and body size (Fig. 4c, d). The decline in the production of small-bodied taxa offset the increased production of large-bodied taxa, resulting in no net change in community production (Table 1). While the relationships between changes in biomass and production and body size were significant in the experimental but not the reference stream, results from ANCOVA indicate that the slopes of these relationships were not significantly different between streams (biomass, df = 1,59, $F = 2.93$, $P = 0.09$; production, df = 1,59, $F = 2.19$, $P = 0.14$). There was no significant relationship between the magnitude of change in $P{:}B$ and body size for either stream. However, annual $P{:}B$ was negatively related to body size (Fig. 5), indicating that, overall, the smaller taxa that declined had higher turnover rates than the larger taxa that increased in abundance.

Discussion

Our experiment provided a novel test of a key MTE prediction: that secondary production is temperature invariant (Brown et al. 2004, Cross et al. 2015). Our results are consistent with this prediction, but occurred via unexpected mechanisms. Community-level biomass and production did not change with warming, despite shifts in body-size distributions among invertebrate taxa. This net-neutral effect of warming on production was likely driven by the presence of diverse life-history traits and thermal preferences among taxa within the regional species pool, resulting in increases in the relative biomass and production of large-bodied, slower-growing, warm-adapted taxa at the expense of more abundant, small, cold-adapted taxa with higher growth rates.

As predicted by the MTE, warming had a neutral effect on invertebrate secondary production. However, it is important to note that this effect was not a result of the predicted changes in total biomass or community $P{:}B$ (Fig. 1), but resulted from a broad range of taxon-specific responses. In previous work, we found that experimental warming significantly reduced invertebrate abundance but had no effect on invertebrate biomass
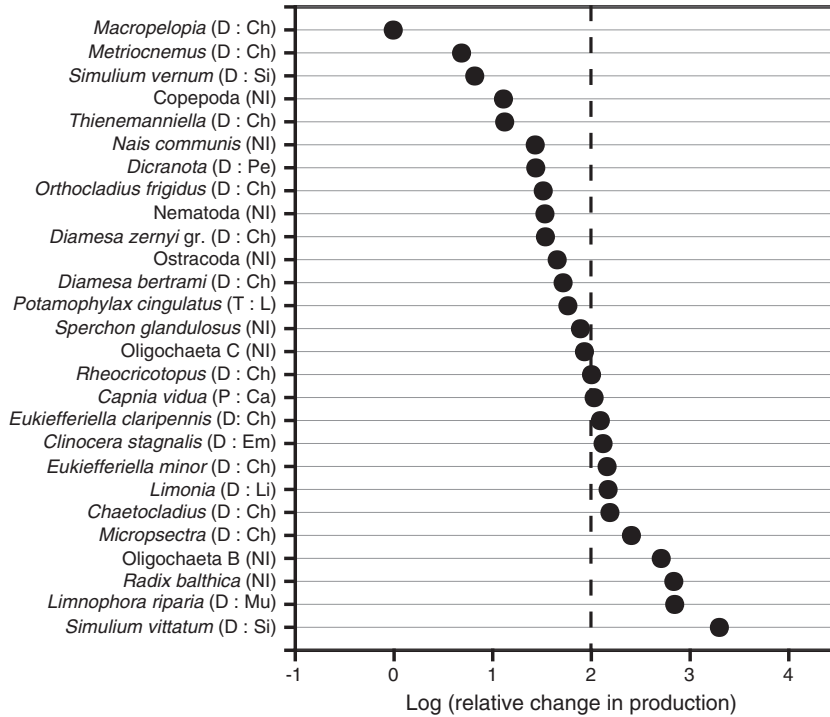
Fig. 3. Change in production (log[relative percent change + 100]) of invertebrate taxa in the experimental stream relative to the reference stream from YR1 to YR3. A value of 2 on the *x*-axis equates to no change (0%). Only taxa that occurred during both YR1 and YR3 in both streams are shown. Not shown are taxa that newly colonized the experimental stream during warming (i.e., *Galba truncatula* and *Chaetogaster diaphanous*) and *Prosimulium ursinum*, which was present only in the experimental stream. Abbreviations are D, Diptera; P, Plecoptera; T, Trichoptera; Ch, Chironomidae; Si, Simuliidae; L, Limnephilidae; Ca, Capniidae; Em, Empididae; Li, Limoniidae; Pe, Pediciidae; Mu, Muscidae; NI, non-insect. Oligochaetes B and C are morphospecies of aquatic oligochaetes.
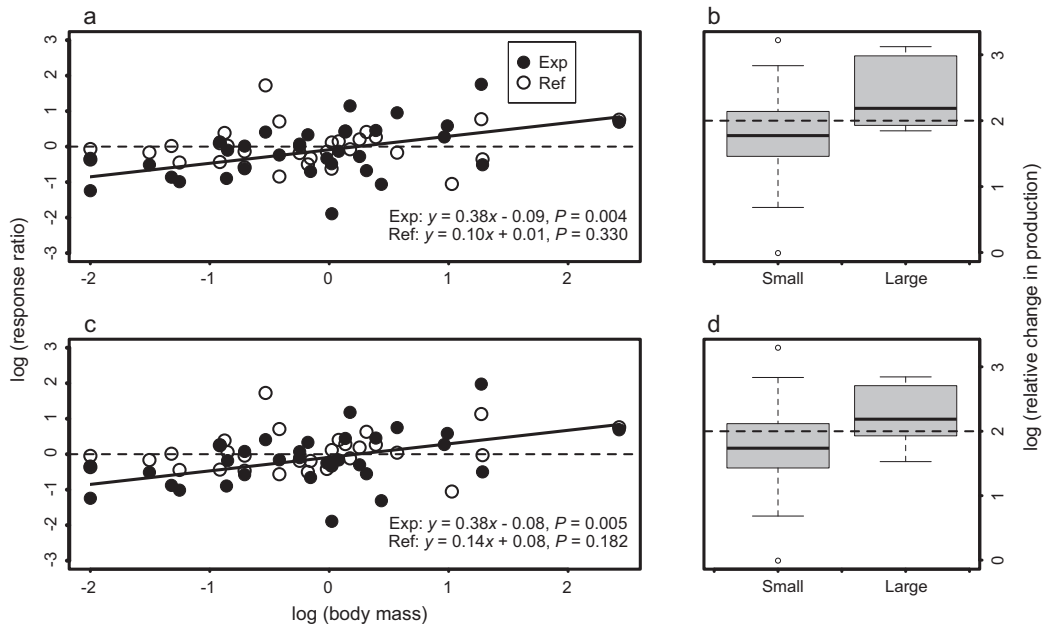

Fig. 4. (a, b) Biomass (measured in mg) and (c, d) production responses of invertebrate taxa to experimental warming between YR1 and YR3. The response ratio was calculated as $RR = \log(YR_1/YR_3)$, where RR is the log(percent change) in the variable of interest from pre (YR1) to post warming (YR3). Horizontal gray dashed lines indicate no change. Box plots show the log(percent change + 100) for small (≤the log[geometric mean body size]) and large (>log[geometric mean body size]) taxa in the experimental stream. Exp, experimental stream; Ref, reference stream.
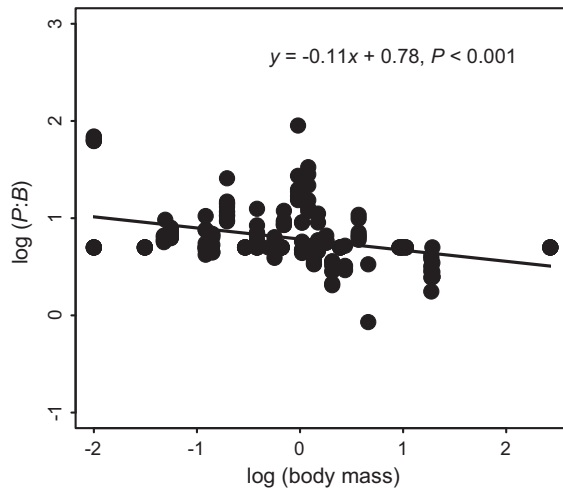
$$y = -0.11x + 0.78, P < 0.001$$

FIG. 5. Relationship between log(annual $P{:}B$) and $\log_{10}$ (body mass) (measured in mg) for all taxa in both streams during all three years of the study.

(Nelson et al., *in press*). Between, YR1 and YR3, the biomass of several taxa significantly decreased in the experimental stream, while other taxa showed increases (Nelson et al., *in press*; Appendix S5). Of the 31 taxa found in the experimental stream during both YR1 and YR3, 16 decreased in biomass and 12 increased in biomass (Nelson et al., *in press*; Appendix S5), resulting in no net change in community biomass. Thus, warming had a neutral effect on community biomass that was driven by variation in the responses of individual taxa.

Taxon-specific responses were also likely responsible for the temperature invariance of annual $P{:}B$ that we observed. Annual $P{:}B$ was predicted to increase with warming as increasing temperatures generally accelerate growth and developmental rates (Vannote and Sweeney 1980, Gillooly et al. 2002, Brown et al. 2004). With a few notable exceptions, however, the annual $P{:}B$ of taxa varied little among years. These exceptions include increases in $P{:}B$ for three taxonomically different species; two species that colonized the experimental stream during warming (*G. truncatula* and *C. diaphanous*) and one resident species (*S. vittatum*) that likely shifted from a univoltine life cycle to a bivoltine life cycle during warming (D. Nelson, *unpublished data*). Warming had either no or very little effect on voltinism and thus the annual $P{:}B$ of other taxa, indicating that the level of warming in our study, which reflects the level of warming possible within this century, was not sufficient to affect biomass turnover of most taxa. Moreover, as warming had a greater positive effect on larger taxa than smaller taxa within the species pool, it could be expected that community $P{:}B$ would decrease, since $P{:}B$ is usually negatively related to body size (Huryn and Benke 2007). In fact, the negative relationship between annual $P{:}B$ of individual taxa and body size in our streams was highly significant (Fig. 5). Thus, neutral effects of warming on $P{:}B$ ratios were also due to an increased proportion of

larger taxa offsetting or overriding the effects of increasing temperature on annual $P{:}B$ of any given taxon.

The taxon-specific responses to warming shown by our study were likely driven by variation in thermal niches among taxa. Ectotherm performance is tightly coupled to temperature (Huey and Stevenson 1979, Angilletta et al. 2002). Temperatures that diverge from the thermal optimum result in inefficient energy use, lower development and reproductive rates, reduced fecundity, and lower population sizes (Vannote and Sweeney 1980). Small shifts in temperature can thus change the relative abundances of species within communities. At our study site in western Iceland, invertebrate community structure shifts from primarily chironomid midges in cold streams to primarily snails, *S. vittatum* and brachyceran dipteran predators such as *L. riparia* in warm streams (Woodward et al. 2010*b*, O'Gorman et al. 2012, Hannesdóttir et al. 2013). In the warmest streams in the Hengill region of Iceland, for example, the snail *R. balthica* and *S. vittatum* can comprise >80% of macroinvertebrate abundance (Woodward et al. 2010*b*). Our study similarly showed that the biomass and production of chironomids decreased in the experimental stream after warming, while the biomass and production of *R. balthica*, *S. vittatum*, and *L. riparia* increased significantly. Our experimental results thus support the spatial patterns described along a natural gradient of temperature-acclimated streams (Woodward et al. 2010*b*, O'Gorman et al. 2012, Hannesdóttir et al. 2013) being driven by an interaction between ambient temperature and the diversity of thermal niches across the regional species pool.

While the taxon-specific responses observed in our study were strongly driven by thermal preference, they were also strongly related to body size. Overall, warming had a greater positive effect on larger taxa than smaller ones. This result conflicts with those of some empirical studies in which warming has been associated with reductions of mean body size and community size distributions that favor smaller organisms (Gardner et al. 2011, Sheridan and Bickford 2011, Dossena et al. 2012, O'Gorman et al. 2012). Furthermore, since warming places greater energetic stress on organisms, the performance of larger organisms is expected to decline more rapidly than that of smaller ones due to greater metabolic demands. However, our study illustrates that empirical patterns based on large-scale gradients and theoretical predictions do not always account for the variety of thermal preferences that exist within real communities, which may or may not be related to body size.

Indirect responses to warming that are mediated by community interactions may also be important in driving taxon-specific responses to warming. For example, the biomass and production of both the predacious dipteran *L. riparia* and the black fly *S. vittatum* increased significantly in the experimental stream during warming. *Limnophora riparia* is a specialized predator of larval black flies (Merritt and Wotton 1988, Wotton and Merritt 1988) and its dietary preference and thermal

preference suggest that increases in its production may have been a direct result of warming, a numerical response to the increased production of its prey (Merritt and Wotton 1988, Wotton and Merritt 1988), or both. In addition, the production of the predatory tanypod *Macropelopia* declined markedly during warming in the experimental stream. It is possible that this decline in production may have released its prey from predation, resulting in their increased biomass and production. However, it seems plausible that a combination of direct and indirect factors drove most taxon-specific responses to warming.

The MTE predicts a neutral effect of warming on secondary production if resource supply remains unchanged and production of consumers is limited by the availability of resources. Differences in the supply of resources (i.e., energy) to consumers should thus result in a change in the intercept but not the slope of the relationships between temperature and biomass and *P:B* (Cross et al. 2015). Thus, ecosystems receiving lower supplies of carbon or energy are predicted to support less consumer production than those receiving higher inputs. In our experiment, warming had no effect on the supply of seston or fine benthic organic matter (FBOM; Appendix S2), but biomass of primary producers increased approximately 20-fold during the growing season (Nelson et al., *in press*) and annual gross primary production increased ~170% (J. M. Hood, *unpublished manuscript*), suggesting an increase in resource supply due to warming. However, neither consumer biomass nor production increased in response to this apparent boost in resources. It is important to note, however, that the observed increase in primary producer biomass, and presumably primary production, was primarily due to the increase in biomass of a single taxon of filamentous green algae, *Ulva* (Nelson et al., *in press*). Although *Ulva* is palatable to some invertebrates (Guidone and McGlathery 2001, Kamermans et al. 2002, Guidone et al. 2010), gut-content analysis of invertebrates in the experimental stream indicates that it was rarely consumed and contributed <25% to total consumer production (D. Nelson, *unpublished data*). The presence of macroalgae, such as *Ulva*, however, can have significant non-trophic effects on invertebrate community structure and densities of many invertebrate groups (Dudley et al. 1986, Koksvik and Reinertsen 2008, Tonkin et al. 2014). Macroalgae provide food, trap detritus, and create substrate on which to attach; they also block sunlight and exclude insects by competing for space (Dudley et al. 1986). While increased *Ulva* biomass may seemingly provide more food or habitat for invertebrate consumers, it also has the potential to have neutral or even negative consequences for secondary productivity. Thus, predicting the interactive effects of temperature and resource supply on consumer production will require consideration of factors such as palatability and match or mismatch between consumers and potential resources (Cross et al. 2015).

While a change in resource quantity can still result in temperature-invariance of production, a change in resource quality (e.g. %C, %N, %P, or C : nutrient ratios) can alter nutrient supply rates to consumers, potentially interacting with the effects of temperature on secondary production (Makino et al. 2011, Persson et al. 2011, Cross et al. 2015). In our study, warming had variable effects on the quality of epilithic biofilm (which supports most consumer production in the reference and experimental streams) as measured by elemental content (Appendix S2). Warming, for example, increased the biofilm C content, but not the %N or %P relative to the reference stream (Appendix S2). Furthermore, biofilm C:P increased while biofilm C:N decreased from YR1 to YR3 in the experimental stream. These results suggest a potential change in resource quality with warming. However, while algal C:N and C:P are often considered measures of food quality for herbivores, such measures are relatively crude indices of nutritional value. Other biochemical and structural attributes of algae (e.g., protein and lipid content, cell wall thickness) also influence palatability, assimilation, and overall nutritional quality (Ahlgren et al. 1990). Further studies investigating the interactive effects of temperature and resource nutritional value could help to shed light on this important area of uncertainty (Cross et al. 2015).

Our experiment was designed to increase the mean temperature of the experimental stream by 3–4°C, a level of warming consistent with predictions over the next century (IPCC 2013). While we were successful in achieving this level of warming, we also inadvertently introduced higher variability to the diel and seasonal temperature cycles of the experimental stream. Furthermore, our levels of warming were not consistent from year to year. The mean annual temperature increase from YR1 to YR2 was 1°C lower than from YR1 to YR3 (mean annual temperature, YR1 5.8°C, YR2 9.1°C, YR3 10.1°C; Nelson et al., *in press*). Alterations to diel and seasonal temperature cycles, as well as shifts in mean temperature, may have significant effects on ectotherm physiology and phenology (Vannote and Sweeney 1980). While partitioning the relative effects of temperature variability vs. the shift in mean temperature on invertebrate production were beyond the scope of this experiment, we acknowledge that a combination of these factors (and perhaps others) could have had important influences on invertebrate life history and production in our study system.

In summary, our stream-warming experiment had a neutral effect on secondary production, as predicted by metabolic theory, but this result occurred via unanticipated species-level shifts that differentially affected community biomass and *P:B*. Our results demonstrate the importance of natural diversity in life-history traits and thermal preferences in dictating how ecological communities respond to warming. The responses of individual taxa to warming are complex and driven by factors that are often overlooked in theoretical studies. Our results demonstrate the importance of combining theory with knowledge of the regional species pool in predicting how natural communities will respond to future warming.

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