Warming Alters the Size Spectrum and Shifts the Distribution of Biomass in Aquatic Ecosystems

Running title: Warming Alters Community Size Structure

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ABSTRACT

Body size is one of the key determinants of community structure. The relationship between abundance and body size can explain how community biomass is partitioned among the biota of an ecosystem. We used an aquatic mesocosm experiment to determine how warming of ~4°C would affect the body size, biomass and taxonomic structure of planktonic communities. We found that warming increased the steepness of the slope of the community size spectrum, primarily by altering the phytoplankton size spectrum. Warming also reduced the mean and maximum body size of phytoplankton by approximately one order of magnitude. The observed shifts in phytoplankton size structure were reflected in large shifts in phytoplankton community composition, though zooplankton taxonomic composition was unaffected by warming. Furthermore, warming reduced community biomass and total phytoplankton biomass, although zooplankton biomass was unaffected. This resulted in an increase in the zooplankton to phytoplankton biomass ratio in the warmed mesocosms, which could be explained by faster turnover within the phytoplankton assemblages. Overall, warming shifted the distribution of phytoplankton body size towards smaller individuals with rapid turnover and low standing biomass, resulting in a reorganisation of the biomass structure of the food webs. These results indicate future environmental warming may have profound effects on the structure of aquatic communities.

INTRODUCTION

Body size can play a key role in determining community structure (Elton 1927, Lindeman 1942, Damuth 1981, Peters 1983, Brown *et al.* 2004, Petchey *et al.* 2008) because it influences ecological processes across multiple levels of organisation; from individuals (Peters 1983, Brown *et al.* 2004), and their interactions (Emmerson & Raffaelli 2004, Berlow *et al.* 2009), to populations (Damuth 1981, Jennings & Mackinson 2003, Reuman *et al.* 2008), communities and ecosystems (Petchey *et al.* 2008). Understanding how this "size-structure" might then be altered by human impacts is an important contemporary challenge for ecology given recent concerns over accelerating rates of biodiversity loss and climate change (Pimm 2009, Montoya & Raffaelli 2010).

The relationship between abundance and body size (equal to body mass; terms are interchangeable hereafter) is potentially a very powerful descriptor of how energy and nutrients are partitioned within the biomass of an ecosystem (White *et al.* 2007). It is also an emergent property of size structure at lower levels of organisation: for example, body size can be important for determining the presence and strength of trophic interactions between individuals because it constrains their metabolic requirements (Berlow *et al.* 2009). The trophic architecture of the community determines the amount of energy available to an organism of a given size, and therefore its population abundance (Damuth 1981). The relationship between abundance and body mass therefore integrates size-structure over many levels of organisation.

Since the pioneering work of Sheldon *et al.* (1972) the relationship between abundance and body size in pelagic food webs has typically been conceptualised as a frequency distribution of individual body sizes (Sheldon *et al.* 1972). This relationship has been dubbed the “size spectrum” (Kerr & Dickie 2001). The negative slopes of size spectra describe how quickly abundance decreases with size, and have often been used to assess the ecological status of ecosystems impacted by fisheries (Rice & Gislason 1995) and, more recently, agricultural practices in terrestrial ecosystems (Mulder & Elser 2009). For example, steep size spectra with negative slopes in marine ecosystems are indicative of over-fishing because the relative abundance of large organisms is suppressed by size-selective harvesting (Pauly *et al.* 1998).

Understanding how the distribution of biomass in aquatic ecosystems might respond to warming is crucial for predicting the robustness and functioning of these ecosystems in the future warmer climate. New evidence suggests that “*reduced body size is the third universal response to global warming*, *besides range, and phenological shifts*” (Daufresne *et al.* 2009). Changes in the size-structure of communities in response to warming are now being documented across a range of ecosystem types and spatial scales. For instance, experiments on aquatic micro-organisms have found that warmed communities tend to be dominated by smaller bacteria (Daufresne *et al.* 2009). Macroecological studies across latitudinal temperature gradients (Moran *et al.* 2010), and paleoecological studies (Finkel *et al.* 2005) in the open ocean have revealed an increased prevalence of small phytoplankton in warmer oceanic regions. These studies suggest that the underlying size structure of aquatic ecosystems might not be robust to global warming (Finkel *et al.* 2005, Falkowski & Oliver 2007, Daufresne *et al.* 2009, Winder *et al.* 2009, Finkel *et al.* 2010, Moran *et al.* 2010).

However, these studies have tended to focus on the effects of warming on restricted subsets of species (e.g. diatoms or phytoplankton) within an ecosystem (Finkel *et al.* 2005, Winder *et al.* 2009) and documented changes in body size across latitudinal gradients where other factors (i.e. nutrient limitation) are potentially confounded with temperature (Moran *et al.* 2010). At present, we still lack sufficient data documenting the effects of warming *per se* on the size-structure of entire local communities comprising multiple trophic levels to be able to isolate its effects at this level of biological organisation.

Here we attempt to address this current knowledge gap by measuring for the first time the consequences of experimental warming on the community size structure and distribution of biomass of entire planktonic food webs from 20 replicated freshwater mesocosms. These experimental systems were maintained at either ambient temperature (n = 10), or ~ 4ºC above ambient (n = 10), in line with warming scenarios predicted for temperate latitudes by the end of the 21st century (IPCC 2007). Mesocosm scale experiments such as these afford the opportunity to isolate the effects of temperature from other potentially confounding variables (e.g. spatial gradients in available nutrients) on the structure of entire replicated communities. They also permit direct comparisons to be made between the structure of communities under ambient conditions with that of their "future" warmed counterparts. We used this experiment to test the following hypotheses:

(i) Warming will shift the distribution of body size by increasing the prevalence of small sized species, resulting in an overall steepening of the slope of the community size spectrum. We expect this effect to be most pronounced in the phytoplankton assemblages because phytoplankton size structure tends to be strongly related to the prevailing physical and chemical environment (Reynolds 1984) and recent observations in aquatic ecosystems suggest that warming tends to favour smaller phytoplankton

(Finkel *et al.* 2005, Falkowski & Oliver 2007, Daufresne *et al.* 2009, Winder *et al.* 2009, Moran *et al.* 2010).

(ii) Warming will reduce total standing community biomass. Again, we expect this effect to be most pronounced for phytoplankton for two reasons. First, a shift in the community size spectrum towards smaller species should result in an overall reduction in the standing biomass. Second, theoretical expectations from the metabolic theory of ecology (MTE) suggest total standing biomass should decline with increasing temperature (Allen *et al.* 2002), such that the total standing biomass in a community (B_{tot}) is predicted to vary as $B_{\mathrm{tot}} = r_0\, e^{-E/kT} M^{1/4}$ where r_0 is the resource supply rate, $e^{-E/kT}$ is the Boltzmann factor where E is the activation energy of metabolism, k is Boltzmann's constant and T is absolute temperature. Therefore, holding r_0 constant (i.e. if the supply rate of limiting resources does not vary with T), B_{tot} should decline with increases in environmental temperature according to $e^{-E/kT}$.

(iii) Warming will alter the relative distribution of biomass between phytoplankton and zooplankton assemblages. We expect that a shift in phytoplankton size structure and a concomitant reduction in standing biomass will result in elevated zooplankton-to-phytoplankton biomass ratios in the warmed mesocosms. We also predict that relatively high zooplankton biomass will be retained in the warmed mesocosms, because phytoplankton turnover rates should increase in response to metabolic stimulation by warming and by a shift towards smaller species with faster generation times. Comparable shifts in the organisation of plankton communities have been observed in the open ocean (Gasol *et al.* 1997) and in lakes (del Giorgio & Gasol 1995) along large scale spatial gradients of nutrient limitation.

MATERIAL AND METHODS

Experimental Design

The experiment was carried out between December 2005 and April 2008 at the Freshwater Biological Association, River Laboratory (2°10`W, 50°13`N), East Stoke, Dorset, UK. A detailed description of the experimental set-up has been described elsewhere (Yvon-Durocher *et al.* 2010). The experiment consisted of twenty freshwater mesocosms (~1 m^3, 0.5 m water depth): ten replicates remained at ambient temperature, whilst the other ten were warmed and maintained at 3-5°C (mean 4°C) above ambient. The mesocosms were seeded in December 2005 with organic substrates and a suite of organisms to include the main components of organismal composition and physical structure of shallow lake ecosystems. The established communities contained representative species from primary producers (phytoplankton, including: *Botryococcus*, *Chlorella*, *Volvox*, *Scenedesmus*) to top predators (Roach, *Rutilus rutilus*. Linnaeus), and a suite of zooplankton consumers (including cyclopoid and calanoid copepods, caldocerans, and rotifers). The biota was left to establish for ten months prior to experimental warming, which commenced in September 2006, thereby allowing time for further, natural colonisation before the onset of the study in April 2007. Populations of the introduced top predator, *R. rutilus* were maintained at a constant densities [two individuals (age 1+) per mesocosm (~12 g C m^{-3})] in all mesocosoms and monitored via regular eletro-fishing surveys. Because the fish were maintained at predetermined biomass-densities they merely served to “complete” the food webs to mimic natural shallow lakes and were not considered further in the analyses.

Measuring the Size Spectrum

The plankton communities from each of the 20 mescosms were sampled at the beginning and end of the growing season in April and October 2007 respectively (Yvon-Durocher *et al.* 2010). The entire water column (depth 0.5m) from the sediment surface to the water surface was sampled using a 0.8m – long tube sampler (Volume: 2L), which was positioned at random in each mesocosm on each date. Each sample was divided into two size categories for preservation and subsequent analyses, via filtration through a 80µm sieve: organisms that were retained were preserved in 4% Formalin, and of the remaining sample (i.e. organisms <80 µm), a 100ml sub-sample was preserved in 1% Lugol's iodine for microscopy analyses.

Plankton >80 µm were counted, measured and identified by microscopy (using a Nikon SMZ1500 dissection microscope). Zooplankton > 80 µm were typically assigned to taxonomic orders, though in a number of cases rotifers were identified to species level. Planktonic organisms <80µm were counted, measured and identified by inverted microscopy. Phytoplankton <80µm were typically identified to genus level, which is generally considered to be of sufficient taxonomic resolution to detect the effects of a perturbation (Cottingham & Carpenter 1998). Organisms were settled for 24 h in a 10ml Utermöhl sedimentation chamber before viewing under an inverted light microscope (Leica DMIRE2). An initial scan of the sample, viewed under low magnification (150×), of a fixed area (50 mm^2) was used to count and measure large, rare organisms. At higher magnification (630×), *n* fields of view were chosen at random and all organisms were counted, sized and identified until a minimum of 400 individuals were measured from each sample. This was sufficient to estimate 95% of the variance in the distribution of body size (Fig S1 in supplementary material) given that settlement of organisms followed a Poisson distribution within the sedimentation chamber (Fig S2 in supplementary material).

Linear body dimensions were determined with an interactive image analysis system (Hamamatsu C4742-95 camera and Openlab software). Body size of all organisms was expressed in units of carbon (µg C). For organisms >80µm (typically zooplankton), biovolumes were determined by assigning organisms to geometric shapes that closely represented the real shape of the organism (Ruttner-Kolisko 1977, Reiss & Schmid-Araya 2008). Body mass was determined by converting biovolume to freshweight using a factor of 1.1, and carbon content was then estimated from a dry/wet weight ratio of 0.25 and a dry carbon content of 40% (Reiss & Schmid-Araya 2008). For organisms <80µm (typically phytoplankton) biovolumes, were similarly estimated from geometric shapes that were most similar to the shape of the organism (Hillebrand *et al.* 1999). Biovolume was then converted into carbon units assuming a multiplication factor of 0.109 (Montagnes *et al.* 1994). In total 47,699 individual organisms of both phytoplankton and zooplankton were measured.

Phytoplankton turnover

Turnover rates of the phytoplankton assemblages (µg C m^{-3} d^{-1}/µg C m^{-3}) were estimated for each mesocosm on each sampling occasion (n = 40). Phytoplankton turnover was calculated as the quotient of primary production and standing phytoplankton biomass after Gasol *et al*. (1997). This gives an estimate of the biomass specific production, or the rate at which the carbon in the assemblage turns over. Measurements of primary production were made simultaneously using the dissolved oxygen change technique and are presented in detail in Yvon-Durocher *et al*. (2010). Benthic metabolism measured using *in-situ* benthic chambers contributed, on average, 35 % of whole system respiration (see S7 in supplementary material for further details). From this we infer that our measures of primary production predominantly reflect planktonic metabolism and provide reliable estimates of carbon turnover within the phytoplankton assemblages.

Constructing the Size Spectrum

The community size spectrum ($n = 40$), which included phytoplankton and zooplankton, and the phytoplankton assemblage size spectrum ($n = 40$) were constructed for each mesocosm in April and October 2007. The size spectrum of the zooplankton assemblage alone could not be constructed accurately due to the relatively small body mass range and the low number of individuals present in some samples. Size spectra were constructed by logarithmic binning of the body masses (M) of the individuals measured in each mesocosm (either the entire community or just the phytoplankton). The total range of $\log_{10}(M)$ values was divided into 10 bins of equal width and the $\log_{10}$ of the total population abundance of all organisms with $\log_{10}(M)$ in each bin was regressed against the bin centres (Reuman *et al.* 2008, White *et al.* 2008). The slope of the linear model describes how quickly the abundance of individuals declines with increasing size in the size spectrum (see Tables S5 and S6 in supplementary material). We also measured two normalisation constants of the linear model. The intercept at $x = 0$: its variation between warmed and ambient treatments gives information on the relative abundance of large organisms, and the intercept at $x = -8$: its variation provides information on the differences among treatments in the relative abundance of the smallest organisms. For both the community and the phytoplankton size spectrum, non-significant coefficients of the linear models (i.e. at $P>0.05$) were excluded from further analyses ($n = 5$ out of 40 for the phytoplankton size spectrum).

Statistical Analyses

We analysed differences between treatments in the following community properties: size spectrum slopes and intercepts; total community biomass; total phytoplankton biomass; total zooplankton biomass; and mean individual body mass, using ANOVA, with treatment (either warmed or ambient) and sampling occasion (April or October) as fixed factors. The relationships between phytoplankton and zooplankton biomass and the biomass ratio of zooplankton to phytoplankton were determined using ANCOVA, again using treatment and sampling occasion as factors. In all statistical modelling procedures the most parsimonious model was identified using the Akaike Information Criterion (AIC). Statistical analyses were performed using R statistical software (R. Development. Core. 2006).

Multivariate analysis of phytoplankton taxonomic composition was conducted using the vegan package in R. Redundancy analysis (RDA) was used to test for a significant linear trend in community composition. RDA is a constrained form of principal components analysis and assesses the variation in taxonomic composition that can be explained by specific environmental variables defined as the constraints. Here, the first RDA axis quantified the linear component of the between treatment variation in phytoplankton taxonomic composition. Consequently, it was used to assess the strength of the trend and its significance was tested using permutation tests. The *F*-ratio of the first RDA axis was compared with those of 999 permutations, to assess the statistical significance of the linear trend. As well as treatment (warming), NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and total inorganic N:P (see S3 for details on nutrient measurements) were tested as constraining environmental variables. Phytoplankton taxon biomass was transformed prior to the construction of the RDA by taking the proportional contribution of a given taxa as a fraction of the total biomass in a given mesocosm. Furthermore, rare genera defined as those occurring in less than two mesocosms per sampling date were excluded from the RDA analysis to reduce noise in the data.

RESULTS

Effects of Warming on the Size Spectrum

Warming significantly increased the steepness of the slope of the community size spectrum from -0.86 (95% CI -0.83 to -0.89) in the systems at ambient temperature to -0.95 (95% CI -0.92 to -0.98) in the warmed mesocosms (Fig. 1 a, b & c; Table 1), i.e., smaller organisms were relatively more abundant than large organisms in the warmed communities. Furthermore, the intercept of the community size spectrum at $x = 0$ (i.e. at large body masses) was significantly reduced, whilst the intercept at $x = -8$ (i.e. at small body masses) was significantly elevated in the warmed mesocosms (Table 1). Thus, the abundance of larger organisms declined on average, while the abundance of small organisms increased in response to warming.

Comparable patterns were observed for the phytoplankton size spectrum (Fig. 1d, e & f). Warming significantly increased the steepness of the slope of the phytoplankton size spectrum from -0.36 (95% CI -0.32 to -0.40) in the systems at ambient temperature to -0.49 (95% CI -0.43 to -0.55) in the warmed mesocosms (Table 1; Fig. 1d, e & f). Warming also significantly reduced the intercept of the phytoplankton size spectrum (Table 1). Therefore, small organisms were relatively more abundant than large organisms in the warmed mesocosms. Additionally, warming truncated the upper size classes of the phytoplankton size spectrum (Fig. 1 d). The maximum phytoplankton body mass in the ambient mesocosms was 1.36×10^{-2} µg C, while in the heated the maximum body mass was only 3.88×10^{-3} µg C. Furthermore, the average body mass of an individual phytoplankter was almost an order of magnitude smaller in the warmed mesocosms relative to the ambient systems (Fig. 2; Table 1), while the average size of an individual zooplankter was unaffected by warming (Fig. 2; Table 1).

Effects of Warming on Community Composition

Redundancy analysis of the phytoplankton taxa revealed that the composition of the phytoplankton assemblages were significantly different between warmed and ambient treatments in both April (Fig. 3a; *F*-ratio = 5.72; *P* = 0.011; permutation number = 999) and October (Fig. 3b; *F*-ratio = 5.87; *P* = 0.001; permutation number = 999). RDA1 which was constrained by treatment, explained 24.1% and 24.6% of the variation in the taxonomic composition of the phytoplankton assemblages in April and October respectively, which in both cases was greater than the variation explained by PCA1, indicating that treatment effects were the dominant predictor of phytoplankton taxonomic composition. We also tested for significant relationships between phytoplankton taxonomic composition and other environmental variables (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, total inorganic N:P) using permutation tests, though none of these variables significantly predicted taxonomic composition. Certain taxa were strongly associated with either warmed or ambient treatments. For example, in both April and October, the large chlorophyte, *Botryococcus* clustered towards the ambient treatment centroid, while the small cyanophyte *Synechocystis*, and the small chlorophyte *Monoraphidium*, typically clustered towards the heated centroid. The phytoplankton assemblages consisted of many rare, generalist taxa that were present in both treatments; however, in most of the mesocosms the biomass was dominated by a few indicator taxa (named above) that were associated with either the heated or the ambient treatments. Furthermore, figures 3a and 3b show that a large core contingent of the phytoplankton assemblages were present in both April and October and that only a few taxa were present in only one month, suggesting that temporal succession was less important than treatment effects in determining phytoplankton community composition.

In contrast to the phytoplankton assemblages the taxonomic composition of the zooplankton assemblages differed very little between treatments in both April and October (Fig. 4a & b). In heated and ambient treatments calanoid and cyclopoid copepods dominated zooplankton biomass with cladocerans and rotifers forming a smaller secondary contingent of the assemblages. These patterns were consistent between April and October, though ostracods, oligochates and the rotifer *Asplanchna* were absent from the zooplankton assemblage in October.

Effects of Warming on the Distribution of Biomass

Total planktonic community biomass differed between April and October in the ambient but not in the warmed mesocosms (Fig. 5). Overall, warming significantly reduced total community biomass (Fig. 5; Table 1). This was principally driven by a considerable reduction in total phytoplankton biomass in the warmed mescocosms (Fig. 5; Table 1). Overall, warming shifted the distribution of biomass and body size of phytoplankton from assemblages comprised of large individuals with high standing biomass to assemblages with low standing biomass and many small individuals. In contrast, warming appeared to have no effect on the distribution of size and biomass within the zooplankton assemblages (Fig. 5; Table 1).

Zooplankton and phytoplankton biomass were not correlated within the mesocosms (Fig. 6a; Table 2). The former varied by about two orders of magnitude and the latter by three orders of magnitude among mesocosms (Fig. 6a). The ratio of zooplankton to phytoplankton biomass (Z:P) was significantly and negatively correlated with phytoplankton biomass (Fig. 6b; Table 1). Therefore, zooplankton biomass exceeded phytoplankton biomass (i.e. Z:P >1) when phytoplankton biomass was low and vice versa (i.e. Z:P <1) when phytoplankton biomass was high. Warming significantly increased the ratio of Z:P biomass (Table 2). Furthermore, the ratio of Z:P biomass was strongly and positively correlated with the turnover rates of the phytoplankton assemblages, which exhibited distinct variation between warmed and ambient mesocosms (Fig. 6c; Table 2). In summary, the warmed mesocosms were characterised by phytoplankton assemblages comprised of small individuals with low standing stocks of biomass and rapid turnover rates which supported relatively high standing stocks of zooplankton, exemplified by high Z:P biomass ratios.

DISCUSSION

There is ample evidence that ecological responses to recent climate change are already occurring at the species (and therefore the population) level (Walther *et al.* 2002), but scaling from populations to communities and ecosystems is challenging because of the perceived indeterminacy of ecological interactions (Yodzis & Innes 1992). Therefore, there is an increasingly urgent need to explore the effects of the principal components of climate change (e.g., warming) on community structure and ecosystem functioning (Tylianakis *et al.* 2008, Montoya & Raffaelli 2010). Our results broadly supported our experimental hypotheses: i.e., that warming would increase the steepness of the size spectrum slope, reduce total community biomass, and increase the zooplankton to phytoplankton biomass ratio. These findings could provide some novel insights into how future warming might change the distribution of body size and biomass in aquatic ecosystems. The size structure of plankton communities in aquatic ecosystems is a key driver of rates of carbon sequestration and nutrient cycling (Laws *et al.* 2000), and therefore changes in the distribution of planktonic body size and biomass could alter the regulation of biotic feedbacks with warming on a potentially global scale (Falkowski *et al.* 1998).

The general increase in the prevalence of small organisms with increases in environmental temperature that we observed experimentally agrees well with recent studies that have either focused on specific taxa, or subsets of taxa (Atkinson *et al.* 2003, Finkel *et al.* 2005, Daufresne *et al.* 2009, Winder *et al.* 2009), or analysed correlational trends in community structure across latitudinal gradients in temperature (Moran *et al.* 2010). However, here we have developed this understanding further by documenting the effects of warming on the body size, biomass and taxonomic structure of entire planktonic food webs subjected to experimental warming. Experimental mesocosm studies, although inevitably an abstraction of natural ecosystems, afford us the opportunity to isolate the effects of temperature from other potentially confounding variables (e.g. latitudinal and biogeographical effects) whilst studying entire replicated plankton communities.

The increase in the dominance of small phytoplankton and the truncation of the larger size classes in their size spectrum resulted in a general increase in the steepness of the slope of the community size spectrum in the warmed mesocosms. Changes in the distribution of organism size might arise from at least two broad mechanisms, which are not necessarily mutually exclusive. Firstly, organisms might exhibit a degree of phenotypic plasticity to changes in temperature. This hypothesis has been termed the temperature-size rule (Atkinson *et al.* 2003) and posits that reduced organism size at higher temperatures is an adaptive plastic response that results from selection for earlier reproduction as population growth rate increases. The accelerated completion of the life cycle occurs at the expense of maturation size (Atkinson *et al.* 2003). In the second mechanism, changes in the physicochemical environment created by warming select for smaller sized species. In this case, changes in community size structure occur as an indirect effect of warming, mediated for example, by concomitant nutrient limitation, resulting in the competitive exclusion of larger species (Finkel *et al.* 2005, Irwin *et al.*

2006, Falkowski & Oliver 2007, Winder *et al.* 2009, Finkel *et al.* 2010). Here, small cell size increases the efficiency of the acquisition of limiting nutrients because of a higher surface area to volume ratio and is therefore competitively advantageous under conditions of nutrient limitation (Litchman *et al.* 2009).

Our results support the second mechanism. Redundancy analysis revealed that warming dramatically shifted the taxonomic composition of the phytoplankton assemblages. Moreover, warming favoured smaller phytoplankton genera, resulting in a reduction in mean and maximum body size by almost an order of magnitude. For example, the large cholorophyte *Botryococcus* dominated the biomass of the ambient mesocosms in both April and October, but was almost entirely absent from the warmed mesocosms. Similarly the small cyanophyte *Synechocystis* and the small chlorophyte *Monoraphidium* were strongly associated with the warmed mesocosms but were only peripheral members of the assemblages in the ambient mesocosms. Warming therefore resulted in the establishment of phytoplankton assemblages dominated by small species, rather than reducing the body size of the same species composition present in the ambient mesocosoms.

Our experimental design adopted a space-for-time substitution approach to attempt to understand the consequences of warming on the structure of plankton communities. The relatively infrequent but highly replicated sampling regime adopted in our study was a necessary compromise. For example, we documented the size, biomass and taxonomic structure of 20 replicated experimental ecosystems on two separate sampling occasion at the beginning and end of the growing season (identified from measures of primary production; see Yvon-Durocher *et al*., (2010) for details) rather than focusing on the complex temporal dynamics of the plankton assemblages of one or two systems, as would typically be logistically feasible within such a study. As a result, our results come with an associated caveat: we are unable to discern the effects of warming on the temporal succession of the plankton communities. However, analysis of the phytoplankton taxonomic composition suggests that a large, core contingent of these assemblages are present in both April and October but which differ markedly between treatments in both months. These results suggest that temporal succession in the plankton communities was less important than the effect of treatment (i.e. warming) in determining the taxonomic and therefore the body size and biomass structure of these assemblages.

Inorganic nitrogen was limiting in our experiment (N:P ratios were ≈11:1, and were below the 16N:1P expected at Redfield; see S3 & S4 in supplementary material for further details) but to the same extent in both warmed an ambient treatments: i.e., warming did not exacerbate nutrient limitation. Therefore, it is unlikely that a direct effect of nutrient limitation induced by warming caused the observed shifts in phytoplankton size structure that have been frequently documented in the open ocean and in lake ecosystems (Finkel *et al.* 2005, Falkowski & Oliver 2007, Winder *et al.* 2009, Finkel *et al.* 2010). Furthermore, redundancy analysis revealed that inorganic nutrient concentrations (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and total inorganic N:P) were not significantly correlated with phytoplankton taxonomic composition. Therefore, the shift in phytoplankton size and taxonomic structure in the warmed treatments might simply reflect the fact that smaller phytoplankton have lower specific nitrogen requirements than large phytoplankton (Litchman *et al.* 2007). Litchman *et al.* (2007) found that the minimum nitrogen quota required to support growth, $Q_{\min}$, across a wide range of phytoplankton taxa increases allometrically, resulting in a disproportionate increase in cellular nitrogen quota with size. Because metabolic rates and nutrient uptake rates increase with temperature and size (Gillooly *et al.* 2001, Allen & Gillooly 2009), under conditions of nutrient limitation, small cell size should provide a competitive advantage as environmental temperatures rise. This is because species with lower $Q_{\min}$ will be better able to balance the increased demand for limiting nutrients imposed by temperature driven elevated metabolic rates.

An alternative mechanism for the shifts in phytoplankton size and taxonomic structure in the warmed mesocosms is that warming served to increase "top down" control of the phytoplankton community by increasing zooplankton grazing rates. We have previously demonstrated that heterotrophic metabolism increased more rapidly than autotrophic metabolism with increasing temperature in the same experimental system (measurements made simultaneously; see Yvon-Durocher et al., (2010) for details). Therefore, because ingestion rates increase in proportion with metabolic rates (Berlow *et al.* 2009), warming might have increased the strength of top down control of phytoplankton populations by zooplankton grazing. Moreover, zooplankton are often size selective when feeding on phytoplankton, typically consuming the largest size classes possible (Porter 1973, Hall *et al.* 1976, Katechakis *et al.* 2002). Warming might therefore have increased the prevalence of small sized phytoplankton indirectly, by elevating grazing pressure on the larger size classes of the phytoplankton community due to the elevated metabolic demands of zooplankton at higher temperature. Importantly, both the "top down" and "bottom up" hypotheses stated here are not mutually exclusive: both bottom up regulation of phytoplankton competitive ability for limiting nutrients, and top down control of large phytoplankters by zooplankton grazing could occur simultaneously, and combine with the direct effects of warming on metabolism to produce the observed shifts in size, biomass taxonomic structure.

Warming reduced total standing community biomass, largely via a reduction in phytoplankton biomass. These results confirmed our qualitative theoretical predictions. For example, because the potential resource supply rate (i.e. the concentrations of limiting inorganic nutrients) remained constant, we predicted that elevated metabolic demands at higher temperatures should have resulted in a decline in standing community biomass in the warmed mesocosms. Assuming $B_{\mathrm{tot}} = r_0 e^{-E/kT} M^{1/4}$ and that r_0 (i.e. the resource supply rate) and $M^{1/4}$ (i.e. the allometric scaling of biomass with body mass) are constant with temperature we can predict that for ≈4˚C warming (i.e. the average annual temperature increase in our experiment) standing community biomass should decline approximately 1.54 fold according to: $e^{-E/kTh} / e^{-E/kTa}$ where T_h and T_a are the mean annual temperatures of the heated and ambient mesocosms (290.9 and 286.1 K, respectively) and E is the activation energy of metabolism ≈0.65eV (Gillooly *et al.* 2001). In our experiment average total community biomass declined 2.53 fold (i.e. the ratio of mean biomass in the heated and ambient mesocosms), almost double that predicted by metabolic costs alone, suggesting that additional factors may be operating.

The large shift in the distribution of body size from large to small phytoplankton might further reduce standing biomass. For example, the above prediction assumes that the allometric scaling of biomass with body mass (i.e. $B_{\mathrm{tot}} = M^{1/4}$) remains constant with warming. However, we have demonstrated that the slope of the community size spectrum (i.e. the log-log relationship) which is equivalent to the exponent (i.e. -α) of $N = M^{-\alpha}$, where N is abundance (White *et al.* 2007, Reuman *et al.* 2008, White *et al.* 2008), changes from -0.86 to -0.95 in response to warming. Therefore, because $B_{\mathrm{tot}} = N \times M$ the allometric scaling of B_{tot} declined from $B_{\mathrm{tot}} = M^{0.14}$ in the ambient mesocosms to $B_{\mathrm{tot}} = M^{0.05}$ in the warmed mesocosm: i.e., more standing biomass was retained in larger body size classes in the ambient relative to the warmed mesocosoms. We suggest that the effects of increased metabolic costs, associated with warmer temperatures and the shift in the distribution of body size and taxonomic composition of the phytoplankton assemblage, could have acted synergistically to reduce total community biomass in the warmed mesocosms.

The ratio of zooplankton to phytoplankton biomass, Z:P, declined as a function of phytoplankton biomass, in line with our third experimental hypothesis. Our results are qualitatively similar to the findings of Gasol *et al*. (1997) who also demonstrated that the ratio of heterotroph to autotroph biomass (H:A) was a declining function of autotroph biomass in the open ocean and coastal seas, although they attributed the relationship to a nutrient gradient rather than temperature. In our case, the large shifts in community size structure and the distribution of biomass between zooplankton and phytoplankton were independent of the inorganic nutrient status of the mesocosms and appear to have been driven largely by the effects of temperature on metabolism and the relative competitive abilities of large and small phytoplankton.

We found a strong, positive correlation between the Z:P biomass ratio and the turnover rate of the phytoplankton assemblages, which differed profoundly between warmed and ambient treatments. These results offer insight into how these communities might function: the inverted pyramid or squared biomass distributions (i.e. Z>P or Z=P) in the warmed mesocosms contrasted markedly with the pyramidal biomass structure (i.e. Z<P) of the mesocosms at ambient temperature. This suggests that warming of ~4˚C fundamentally altered the structure and functioning (i.e. energy transfer) of our experimental ecosystems. For instance, in the heated mesocosms the high relative biomass of zooplankton may have been supported by a fast turnover rate of the phytoplankton assemblage. For example, for the low standing stocks of phytoplankton biomass in the warmed mesocosms (2.93×10^{5} µg C m^{-3} in heated; 1.12×10^{6} µg C m^{-3} in ambient) to sustain the equivalent biomass of zooplankton as the mesocosms at ambient temperature (1.71×10^{5} µg C m^{-3} in ambient; 1.36×10^{5} µg C m^{-3} heated), the turnover rate of the phytoplankton community would need to be elevated by a factor of ~4. The average turnover rates of the phytoplankton community in the warmed treatments was actually elevated by a factor of ~5 (i.e. 40.9 µg C m^{-3} d^{-1} / µg C m^{-3} in heated; 8.25 µg C m^{-3} d^{-1} / µg C m^{-3} in the ambient) and was therefore sufficient to support the biomass of zooplankton in these systems. Taken together, these results suggest that warming increases the rate of carbon flux between autotrophs and heterotrophs. This effect appears to be driven by the relative increase in small phytoplankton, which have faster turnover times due to the -1/4 allometry of mass specific metabolic rate and generation time (Gillooly *et al.* 2002, Brown *et al.* 2004), and also the direct stimulation of metabolism and generation time by temperature (Gillooly *et al.* 2002).

Conclusion

In general, the results of our experiment reflect patterns in empirical surveys that have analysed phytoplankton communities over macroevolutionary time (Finkel *et al.*

2005, Finkel *et al.* 2007), across latitudinal gradients in temperature (Moran *et al.* 2010), and across gradients of nutrient regime and productivity (del Giorgio & Gasol 1995, Gasol *et al.* 1997). Our results, however, represent the first experimental evidence for a shift in the distribution of body size and biomass of whole plankton communities that can be attributed directly to the effects of warming via a controlled and replicated whole ecosystem manipulation. Although we now have some tantalising hints, the precise mechanism behind the size shifts we observed requires further research. Also, the consequences of such shifts in community size structure for the functioning (e.g. carbon sequestration capacity) of aquatic ecosystems remains an unexplored avenue in ecological research, though with no doubt one that will prove fundamental in addressing the future challenges posed by environmental change.

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Figure Legends

Figure. 1. The size spectrum. (a) The community size spectrum of a heated (red circles) and ambient (black circles) mesocosm, highlighting the increase in the steepness of the slope in the warmed mesocosm. (b) Frequency distribution of the slope of the community size spectrum in the ambient mesocosoms (*n*=20), (c) frequency distribution of the slope of the community size spectrum in the warmed mesocosoms (*n*=20). On average the slope of the community size spectrum in the warmed mesocosms was significantly steeper than the ambient mesocosms (Table 1). (d) The phytoplankton size spectrum of a heated (red circles) and ambient (black circles) mesocosm, highlighting the increase in the steepness of the slope and the truncation of large sized individuals in the warmed mesocosm. (e) Frequency distribution of the slope of the phytoplankton size spectrum in the ambient mesocosoms (*n*=17), (f) frequency distribution of the slope of the community size spectrum in the warmed mesocosoms (*n*=18).

Figure. 2. Effects of warming on mean body mass (±1 s.e.m) of phytoplankton (a) and zooplankton (b) individuals. Data are presented as the overall average of the mean body mass of phytoplankton and zooplankton individuals over 20 mesocosms for each treatment. The mean cell mass of phytoplankton is significantly reduced in response to warming while there is no significant difference in the mean body mass of zooplankton between heated and unheated treatments (table 1).

Figure. 3. Redundancy analysis (RDA) biplot for sites (i.e. mesocosms) and species scores for phytoplankton taxa recorded in the mesocosm experiment in April (a) and October (b). In both cases RDA 1 was constrained by treatment and accounted for 24.1% and 24.6% of the variation in the taxonomic composition of the mesocosms in April and October respectively. In the plot the dotted lines denote the 95% confidence ellipses around the centroids for both treatments. In both April and October these ellipses do not overlap indicating that the community composition was significantly different between warmed and ambient treatments. The solid lines enclose all mesocosms that belong to a particular treatment; in both cases heated treatments (1, 4, 6, 8, 9, 12, 14, 15, 17, 19) cluster to the left, while ambient treatments (2, 3, 5, 7, 10, 11, 13, 16, 18, 20) cluster to the right. Genus abbreviations are as follows: *Aphanothece* (Aph), *Asterococcus* (Ast), *Botryococcus* (Bty), *Bumilleriopsis* (Bum), *C.dinobryonis* (C.d), *Chlorella* (Chl), *Chlorococcum* (Coc), *Chroococcus* (Chr), *Chroomonas* (Cho), *Coencococcus* (Coe), *Cosmarium* (Cos), *Cryptomonas* (Cry), *Goniochloris* (Gon), *Kirchneriella* (Kri), *Monoraphidium* (Mon), *Navicula* (Nav), *Nephrocytium* (Nep), *Rhodomonas* (Rho), *Scenedesmus* (Sce), *Synechococcus* (Syn), *Synechocystis* (Syc), *Spermatozopsis* (Spe).

Figure. 4. Mean biomass of the major zooplankton taxonomic groups documented in the mesocosms in (a) April and (b) October. Note that there is very little difference in the biomass contribution of the different zooplankton taxa between treatments suggesting that the zooplankton community composition was unaffected by warming.

Figure.5. Effects of warming on mean total planktonic biomass (±1 s.e.m). Data are presented as the averages of the total biomass of either phytoplankton and/or zooplankton across the mesocosms for each treatment (n=20 per treatment for the overall mean; n=10 per treatment for each sampling occasion). Total biomass is significantly reduced by warming. This is mainly driven by a reduction in phytoplankton biomass, while there is no significant difference in the biomass of zooplankton in response to warming (table 1).

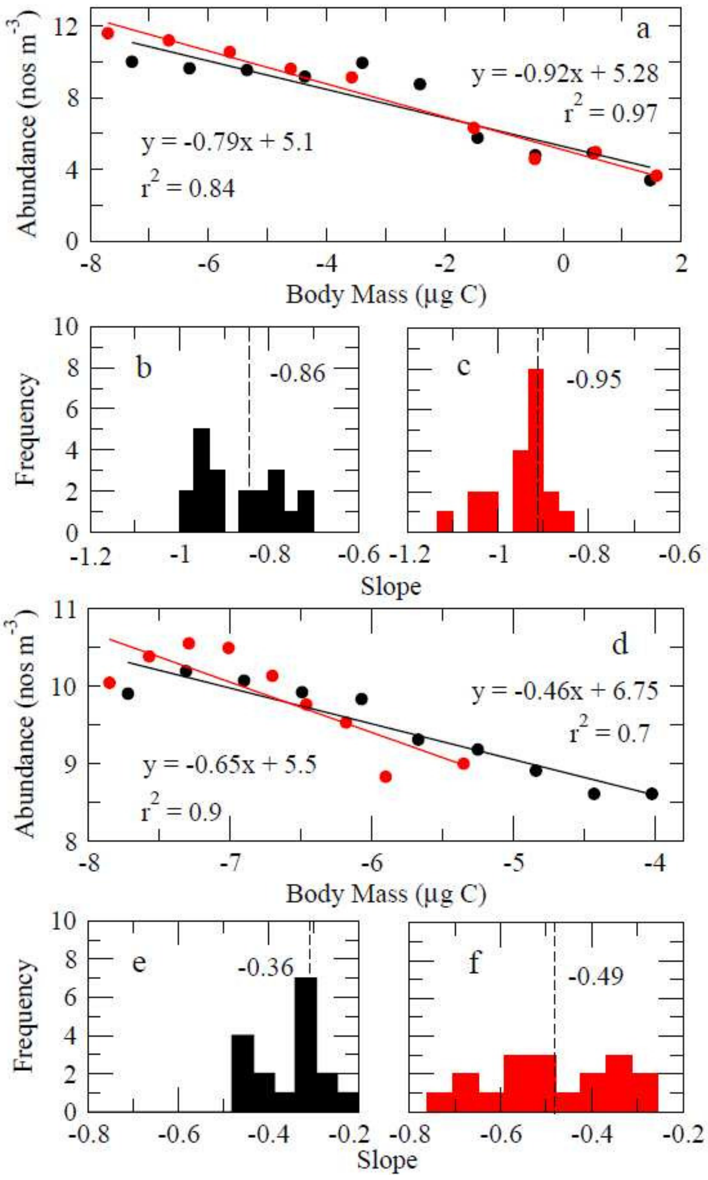
Figure. 6. (a) Relationship between zooplankton and phytoplankton biomass. (b) Relationship between the ratio of zooplankton to phytoplankton biomass (Z:P) and total phytoplankton biomass. (c) The relationship between Z:P and the turnover rate of the phytoplankton communities. Each data point corresponds to either the total zooplankton or phytoplankton biomass or the Z:P in either a heated (red circles) or ambient mesocosm (black circles).

Table 1. The effect of treatment (heated or ambient) on community-level properties. CSS is the community size spectrum and PSS is the phytoplankton size spectrum. ANOVAs were used to isolate treatment effects on individual community-level properties. In each ANOVA month (either April or October) was added as a factor. For each community-level property there was no significant effect of month, which was removed from the model using the AIC score.

Community Property	**DF**	***F***	***P***
CSS slope	1, 38	11.1	**0.002**
CSS intercept (*x* = 0)	1, 38	8.2	**0.007**
CSS intercept (*x* = -8)	1, 38	4.2	**0.047**
PSS slope	1, 33	11.8	**0.002**
PSS intercept	1, 33	8.27	**0.007**
Total community biomass	1, 38	10.8	**0.002**
Total phytoplankton biomass	1, 38	13.1	**<0.001**
Total zooplankton biomass	1, 38	0.47	0.5 (NS)
Mean phytoplankton body mass	1, 38	18.9	**<0.001**
Mean zooplankton body mass	1, 38	1.4	0.2 (NS)
Z:P Biomass ratio	1, 38	4.82	**0.034**

Table 2. Analysis of covariance for the relationships between zooplankton and phytoplankton biomass , the Z:P biomass ratio and phytoplankton biomass, and the Z:P biomass ratio and phytoplankton turnover time.

Relationship	**DF**	***F***	***P***	r^2
Log$_{10}$(Zoo biomass) vs Log$_{10}$ (Phyto biomass)	1, 38	0.062	0.805 (NS)	0.002
Difference in slope	1, 38	3.021	0.073(NS)	N/A
Difference in intercept	1, 38	0.195	0.661 (NS)	N/A
Log$_{10}$(Z:P) vs Log$_{10}$ (Phytoplankton biomass)	1, 38	32.65	**<0.0001**	0.58
Difference in slope	1, 38	1.806	0.187 (NS)	N/A
Difference in intercept	1, 38	0.002	0.956 (NS)	N/A
Log$_{10}$(Z:P) vs Log$_{10}$ (Phytoplankton turnover)	1, 38	52.51	**<0.0001**	0.58
Difference in slope	1, 38	2.171	0.147 (NS)	N/A
Difference in intercept	1, 38	0.538	0.468 (NS)	N/A



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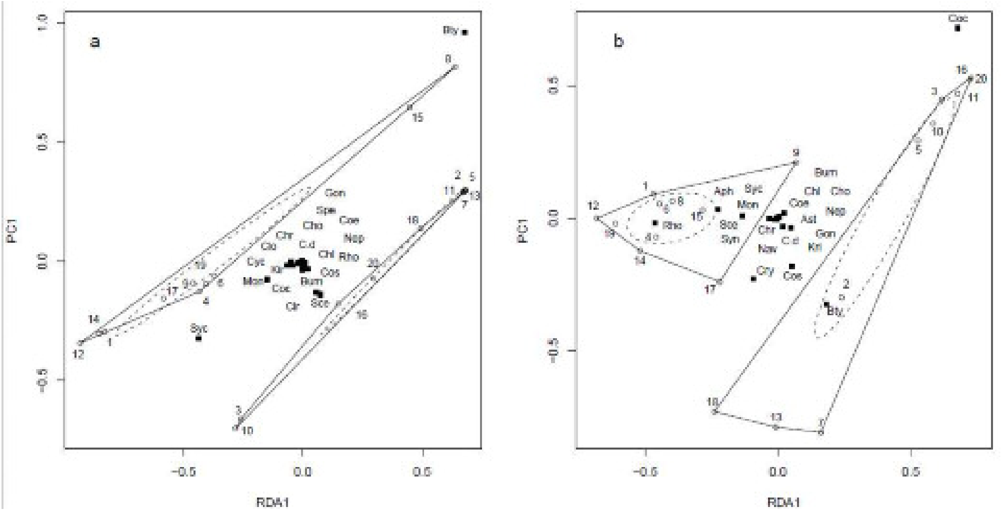

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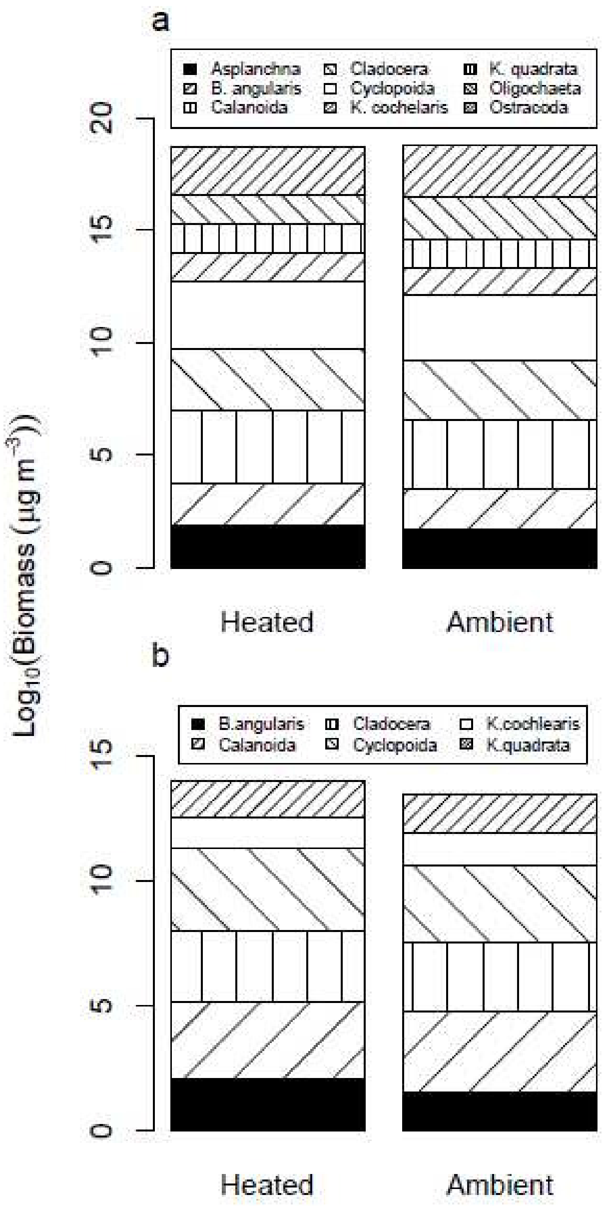

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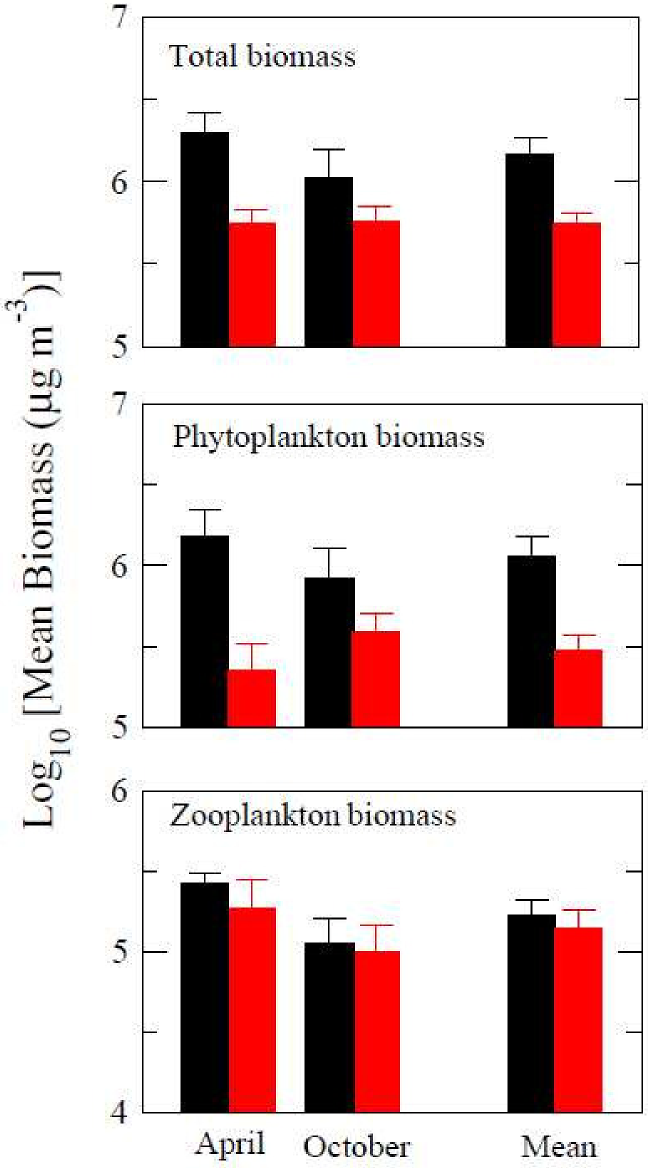

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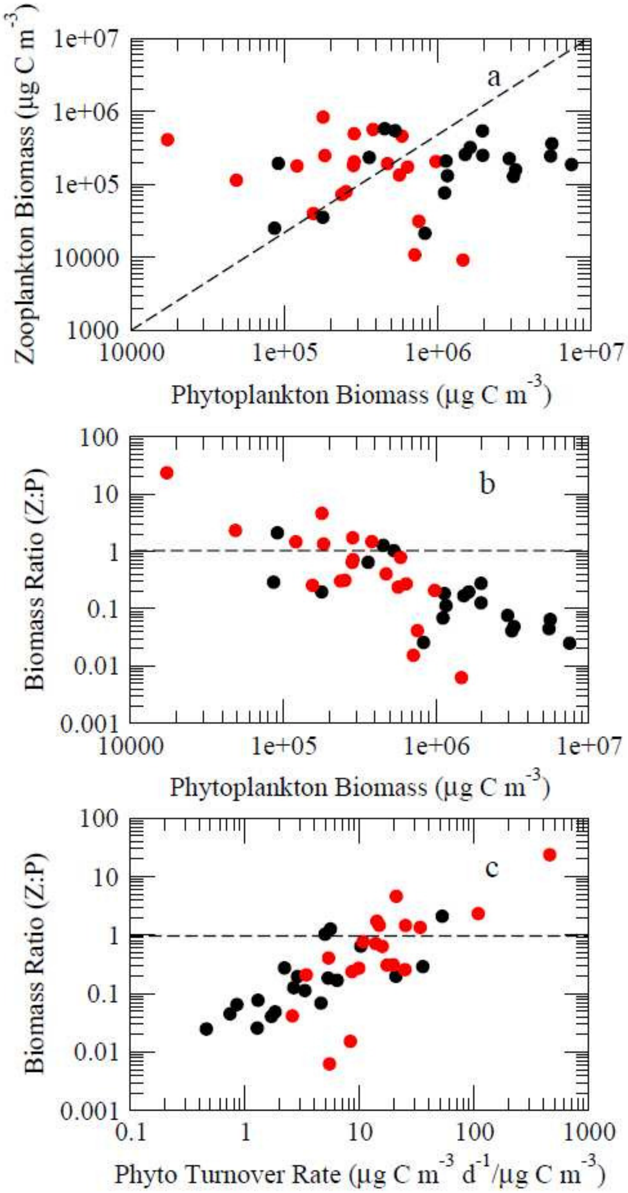

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180x328mm (72 x 72 DPI)

Warming Alters the Size Spectrum and Shifts the Distribution of Biomass in Aquatic Ecosystems

SUPPLEMENTARY MATERIAL

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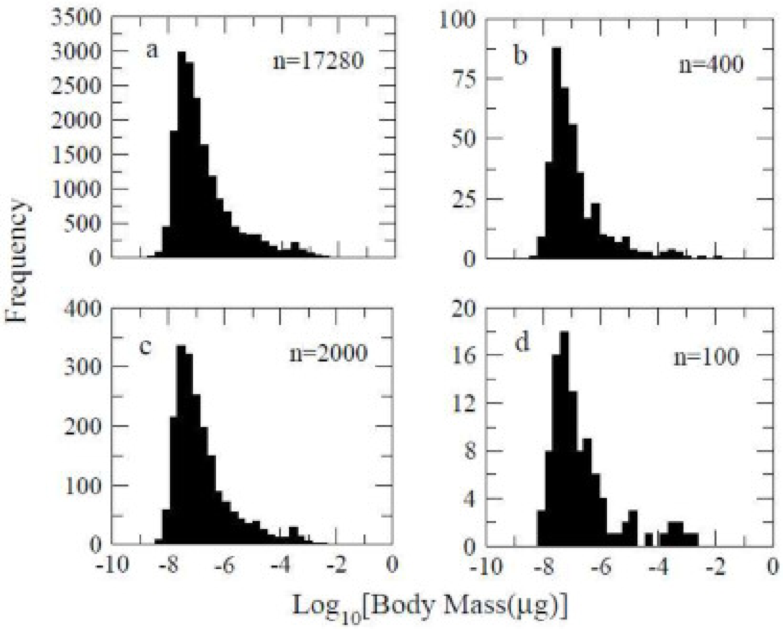

Figure S1. Frequency distributions of individual body mass for (*a*) all individuals measured, (*b*) a random sample of 400 (i.e. the number of individuals actually measured in a sample) from *a*, (*c*) a random sample of 2000 from *a*, (*d*) a random sample of 100 from *a*. Data highlight that a sample of 400 individuals is sufficient to estimate the variance in the distribution of body size comparable to the whole community. When measuring the phytoplankton a minimum of 400 individuals from any given pond were measured over the number of fields of view required to count 400 from the sample in the sedimentation chamber. It is also clear that a sample of 100 is not sufficient to accurately reproduce the variance in the body mass distribution of the whole community. Assuming that organisms of a given body mass are Poisson distributed (figure S2, table S3) on the surface of the sedimentation chamber, the measurement of 400 individuals should be sufficient to attain an error of 5% [if error = 1/sqrt(*n*)].

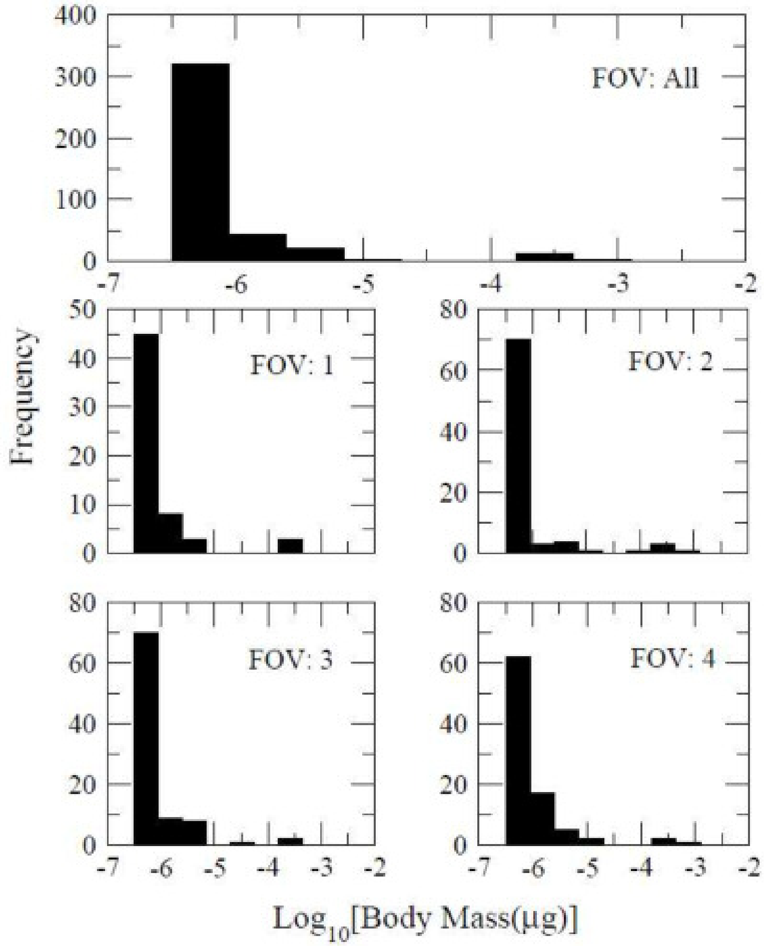

Figure S2. Size-frequency distribution for phytoplankton in pond 14 from April 2007. Panels show the size-frequency distribution after analysing all fields of view (FOV) taken to measure ~400 individuals in the sedimentation chamber, 1 FOV, 2 FOVs, 3 FOVs and 4 FOVs. Data highlight the equitable distribution of body size among fields of view which reflects the random settlement of phytoplankton cells in the sedimentation chamber. Tests for dispersion were carried for all samples and settlement conformed to Poisson statistics in every case (data not shown).

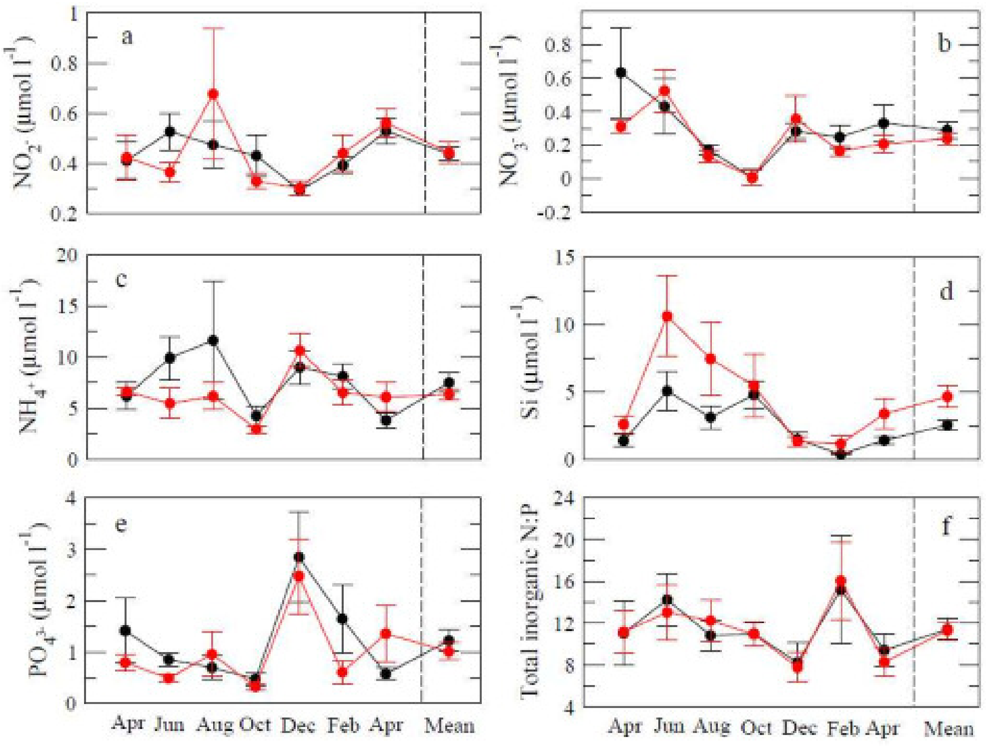

Figure S3. Seasonality of inorganic nutrients in the warmed (red lines) and ambient (black lines) mesocosms. (a) Nitrite, (b) Nitrate, (c) Ammonium, (d) Silicate, (e) Phosphate, (f) the stoichiometry of the inorganic nutrient pool, N:P. Water samples for measuring dissolved inorganic nutrient concentrations were collected from mid depth in the mesocosm at 9am on each sampling occasion. Samples were filtered (Whatmann GF/F) and stored frozen (-20˚C) for subsequent determination of NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and dissolved silica (Si) using a segmented flow auto-analyser (Skalar, San++, Breda, Netherlands), according to (Kirkwood 1996).

Inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} & Si) exhibited strong seasonal trends. For example, NO_3^- concentrations peaked in spring and declined progressively throughout the summer, when rates of primary production were maximal (Yvon-Durocher *et al.* 2010), and were depleted to ≈0.005 µmol l^{-1} by October, before regeneration in the winter. Concentrations of NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} showed identical seasonal patterns in the warmed and ambient treatments, with no significant differences in the overall mean annual concentrations of these nutrients (table S4). Furthermore, the stoichiometry of the inorganic nutrient pool exhibited remarkable similarity between treatments, with a mean annual ratio of total inorganic N to P of ≈11:1 in both heated and ambient mesocosms.

Table S4. Results of the linear mixed effects model testing for differences in the concentration of inorganic nutrients between heated and ambient mesocosms. A linear mixed effects model was conducted with restricted maximum likelihood methods using the *lme* (linear mixed-effects model) function in R, treatment (heated or unheated) was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms over the year was accounted for by including mesocosm identity nested with sampling occasion as random effects.

Inorganic Nutrient	**DF**	***F***	***P***
NO_2^-	1, 120	0.06	0.812 (NS)
NO_3^-	1, 120	0.65	0.420 (NS)
NH_4^+	1, 120	0.23	0.632 (NS)
Si	1, 120	6.08	**0.015**
PO_4^{3-}	1, 120	0.68	0.412 (NS)
Total inorganic N to P	1, 120	0.009	0.922 (NS)

Table S5. Regression statistics for the community size spectrum of each mesocosm for the relationship: log (N_i) = b * log (M_i) + a. Where N_i is the abundance of the size class i and is the mass at the centre of the i^{th} size bin, b and a are the slope and the intercept respectively. These data highlight that the size spectrum was linear for each of the mesocosms and that the individual size distribution was a power law.

Pond	Treatment	Month	Slope	Intercept	r^2	*P*-value
1	Heated	April	-0.92	4.64	0.91	0.00020
2	Ambient	April	-0.94	4.58	0.81	0.00040
3	Ambient	April	-0.93	4.80	0.86	0.00030
4	Heated	April	-0.93	4.50	0.90	0.00003
5	Ambient	April	-0.79	5.28	0.84	0.00020
6	Heated	April	-1.12	3.30	0.78	0.00060
7	Ambient	April	-0.83	5.09	0.84	0.00020
8	Heated	April	-0.90	4.39	0.80	0.00040
9	Heated	April	-1.03	3.94	0.85	0.00040
10	Ambient	April	-0.92	3.89	0.80	0.00100
11	Ambient	April	-0.86	4.71	0.78	0.00200
12	Heated	April	-0.90	4.52	0.97	0.00004
13	Ambient	April	-0.98	4.21	0.74	0.00100
14	Heated	April	-0.88	4.25	0.91	0.00080
15	Heated	April	-0.94	4.64	0.80	0.00100
16	Ambient	April	-0.91	4.90	0.93	0.00001
17	Heated	April	-1.05	4.05	0.88	0.00050
18	Ambient	April	-0.71	5.58	0.81	0.00030
19	Heated	April	-0.92	5.09	0.97	0.00000
20	Ambient	April	-0.75	5.49	0.90	0.00010
1	Heated	October	-0.87	4.58	0.94	0.00001
2	Ambient	October	-0.94	4.15	0.95	0.00001
3	Ambient	October	-0.72	5.46	0.70	0.00200
4	Heated	October	-0.94	4.02	0.90	0.00001
5	Ambient	October	-0.94	4.10	0.81	0.00040
6	Heated	October	-1.06	3.68	0.85	0.00100
7	Ambient	October	-0.97	4.12	0.83	0.00020
8	Heated	October	-0.92	4.50	0.89	0.00040
9	Heated	October	-0.90	4.50	0.84	0.00040
10	Ambient	October	-0.84	5.09	0.87	0.00070
11	Ambient	October	-0.80	5.27	0.83	0.00020
12	Heated	October	-0.93	4.42	0.88	0.00006

13	Ambient	October	-0.78	5.30	0.88	0.00010
14	Heated	October	-0.94	3.75	0.82	0.00080
15	Heated	October	-0.95	4.17	0.93	0.00010
16	Ambient	October	-0.79	5.00	0.87	0.00020
17	Heated	October	-1.01	4.25	0.93	0.00010
18	Ambient	October	-0.95	3.77	0.93	0.00009
19	Heated	October	-0.85	4.70	0.89	0.00010
20	Ambient	October	-0.94	4.24	0.92	0.00004

Table S6. Regression statistics for the phytoplankton size spectrum of each mesocosm for the relationship: log (N_i) = b * log (M_i) + a. Where N_i is the abundance of the size class i and is the mass at the centre of the i^{th} size bin, b and a are the slope and the intercept respectively.

Pond	**Treatment**	**Month**	**Slope**	**Intercept**	r^2	***P*-Value**
1	Heated	April	-0.41	7.50	0.42	0.040000
2	Ambient	April	-0.31	8.20	0.70	0.005000
4	Heated	April	-0.50	6.89	0.90	0.000092
6	Heated	April	-0.35	7.70	0.71	0.002200
7	Ambient	April	-0.22	8.39	0.57	0.012000
8	Heated	April	-0.27	8.03	0.55	0.014000
9	Heated	April	-0.55	6.78	0.88	0.000160
10	Ambient	April	-0.47	6.72	0.91	0.000020
11	Ambient	April	-0.30	7.82	0.53	0.018000
12	Heated	April	-0.76	5.08	0.62	0.011000
13	Ambient	April	-0.34	7.77	0.56	0.013000
14	Heated	April	-0.65	5.52	0.70	0.004600
15	Heated	April	-0.57	6.86	0.87	0.000200
16	Ambient	April	-0.45	7.28	0.75	0.001200
17	Heated	April	-0.52	7.20	0.67	0.004000
18	Ambient	April	-0.27	7.90	0.48	0.030000
19	Heated	April	-0.65	6.36	0.93	0.000006
20	Ambient	April	-0.34	7.60	0.77	0.000900
2	Ambient	October	-0.48	6.65	0.82	0.000300
4	Heated	October	-0.50	6.41	0.78	0.000680
5	Ambient	October	-0.34	7.70	0.85	0.000300
7	Ambient	October	-0.34	7.70	0.62	0.007000
8	Heated	October	-0.30	8.14	0.68	0.004000
9	Heated	October	-0.42	7.16	0.94	0.000005
10	Ambient	October	-0.43	7.50	0.79	0.001400

11	Ambient	October	-0.32	7.90	0.77	0.000880
12	Heated	October	-0.45	7.09	0.87	0.000240
13	Ambient	October	-0.40	7.43	0.90	0.000099
14	Heated	October	-0.35	7.41	0.58	0.010000
15	Heated	October	-0.57	6.25	0.80	0.000400
16	Ambient	October	-0.25	8.20	0.60	0.023000
17	Heated	October	-0.60	6.70	0.91	0.000200
18	Ambient	October	-0.46	6.70	0.82	0.000300
19	Heated	October	-0.36	7.49	0.69	0.003000
20	Ambient	October	-0.38	7.38	0.86	0.000300

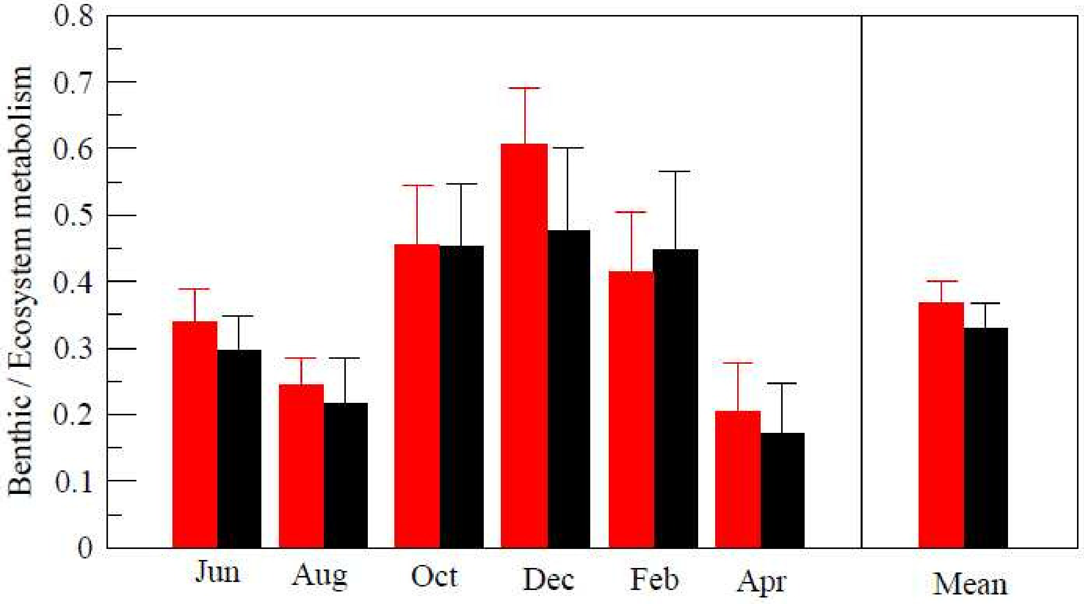

Figure S7. Quotient of benthic to ecosystem metabolism. On average over the course of the year benthic metabolism represented ~35% of whole ecosystem metabolism measured using the dissolved oxygen change technique (see Yvon-Durocher *et al.*, (2010) for details). Benthic metabolism was measured using dark in-situ benthic chambers which enclosed a sample of 500 mL at the sediment-water interface. A magnetic stirrer in the chamber ensured that the sample was evenly mixed. Benthic respiration was measured by the removal of 25mL samples at the beginning and the end of the 6 hour incubations. The samples were gently discharged into gastight vials (12ml, Exetainers, Labco Ltd, High Wycombe, UK) and allowed to overflow twice (to minimize atmospheric gas exchange), and fixed for Winkler analysis. The samples were immediately fixed and stored in a fridge at 5 ^{o}C to minimize light and temperature fluctuations until they could be titrated in the laboratory (< 5 d). To ensure linearity of oxygen uptake a timed series of samples were taken, subsequently only T = 0 and T = final samples were taken to limit sample extraction from the chambers.

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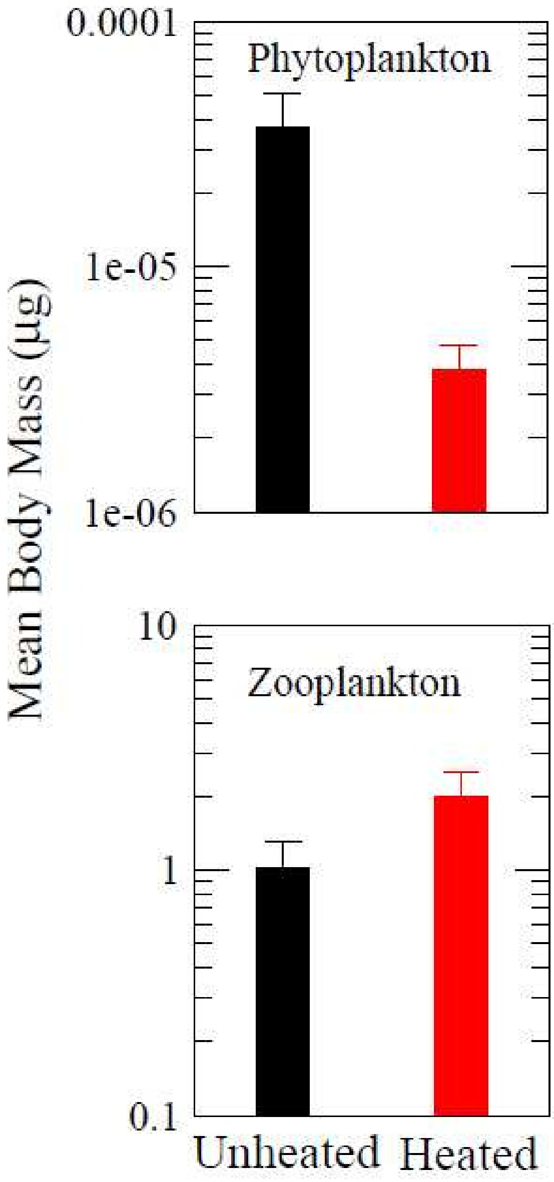

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