Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems

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Abstract

Organism size is one of the key determinants of community structure, and its relationship with abundance can describe how biomass is partitioned among the biota within an ecosystem. An outdoor freshwater mesocosm experiment was used to determine how warming of ∼4 °C would affect the size, biomass and taxonomic structure of planktonic communities. Warming increased the steepness of the community size spectrum by increasing the prevalence of small organisms, primarily within the phytoplankton assemblage and it also reduced the mean and maximum size of phytoplankton by approximately one order of magnitude. The observed shifts in phytoplankton size structure were reflected in changes 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 size towards smaller individuals with rapid turnover and low standing biomass, resulting in a reorganization of the biomass structure of the food webs. These results indicate future environmental warming may have profound effects on the structure and functioning of aquatic communities and ecosystems.

Introduction

Organism size often plays 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 organization, 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 be altered by human impacts is therefore an important contemporary challenge for ecology, especially in the light of growing concern over the accelerating rates of biodiversity loss and climate change (Pimm, 2009; Montoya & Raffaelli, 2010).

The relationship between abundance and organism size 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 a result of size structure at lower levels of organization: for example, 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), whereas the trophic architecture of the community is often important in determining the energy available to a consumer of a given size. The relationship between abundance and organism size therefore integrates size-structure over many levels of organization.

Since the pioneering work of Sheldon et al. (1972) the relationship between abundance and organism size in pelagic food webs has typically been conceptualized as a frequency distribution of individual sizes (Sheldon et al., 1972; Platt & Denman, 1977; Sprules, 1983; Gaedke, 1992; Jennings & Mackinson, 2003; Blanchard et al., 2009). This relationship has been dubbed the 'size spectrum' (Kerr & Dickie, 2001). The negative slopes of size spectra describe how quickly numerical abundance decreases with size, and have often been used to assess the ecological status of ecosystems impacted by

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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 freshwater ecosystems might respond to warming is crucial for predicting the robustness and functioning of these ecosystems in the future warmer climate, especially since 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 microorganisms 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 palaeoecological studies (Finkel *et al.*, 2005) in the open ocean have revealed an increased prevalence of small phytoplankton in warmer regions. These studies suggest that the underlying size structure of aquatic ecosystems might not be robust to global warming (Finkel *et al.*, 2005, 2010; Falkowski & Oliver, 2007; Daufresne *et al.*, 2009; Winder *et al.*, 2009; Moran *et al.*, 2010; Sarmento *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) or documented changes in organism size across latitudinal gradients where other factors (i.e. nutrient limitation) are potentially confounded with temperature (Moran *et al.*, 2010). At present, there is a 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 organization.

The present study attempted 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 outdoor freshwater mesocosms. These experimental systems were maintained at either ambient temperature ($n = 10$) or ~4 °C above ambient ($n = 10$), in line with warming scenarios predicted for temperate latitudes by the end of the 21st century (IPCC, 2007). Mesocosm experiments represent an inevitable compromise between the control and replication of laboratory studies and the realism of descriptive field surveys, therefore caution must be

exercised when extrapolating their findings to natural systems and other ecosystem types (e.g. marine systems). However, despite their limitations, such experiments can provide a useful tool for predicting how global change scenarios might affect ecosystem level processes (Benton *et al.*, 2007). Mesocosm scale experiments 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. This experiment was used to test the following hypotheses:

(i) Warming will shift the distribution of organism size by increasing the prevalence of small species, resulting in an overall steepening of the slope of the community size spectrum. This effect is expected 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, this effect is expected 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 $B_{tot} = r_0 \, e^{-E/kT} M^{1/4}$ 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. B_{tot} should decline with increases in environmental temperature according to $e^{-E/kT}$ because at higher temperatures elevated metabolic rates mean that each organism places a greater demand on their environment, therefore, holding r_0 constant (i.e. if the supply rate of limiting resources does not vary with T) systems at high temperatures should support less biomass.

(iii) Warming will alter the relative distribution of biomass between phytoplankton and zooplankton assemblages. It is predicted that a shift in phyto-

plankton size structure and a concomitant reduction in standing biomass will result in elevated zooplankton-to-phytoplankton biomass ratios in the warmed mesocosms. Furthermore, relatively high zooplankton biomass will be retained in the warmed mesocosms, because phytoplankton turnover rates should increase in response to metabolic stimulation by warming (i.e. because metabolic rates increase with temperature) and by a shift towards smaller species with faster generation times. Comparable shifts in the organization 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.

Materials 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.*, 2010a,b). Briefly, the experiment comprised 20 outdoor freshwater mesocosms (~1 m³, 0.5 m water depth): ten replicates where left at ambient temperature, while the other 10 were warmed to 3–5 °C (mean 4 °C) above ambient. The mesocosms were seeded in December 2005 with organic substrates and organismal inoculums collected from nearby freshwater habitats in an attempt 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, cladocerans and rotifers). The biota was left to establish for 10 months before experimental warming, which commenced in September 2006, thereby allowing time for further, natural colonization and dispersal among mesocosms 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 electro-fishing surveys in April and October. 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.

Characterizing the size spectrum

The plankton communities from each of the 20 mesocosms were sampled at the beginning and end of the growing season in April and October 2007, respectively (Yvon-Durocher *et al.*,

2010a). The entire water column from the sediment surface to the water surface was sampled using a 0.8 m – long tube sampler (volume: 2 L), 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 an 80 μm aperture sieve: organisms that were retained were preserved in 4% formalin, and of the remaining sample (i.e. organisms <80 μm), a 100 mL subsample was preserved in 1% Lugol's iodine for microscopy analyses.

Plankton >80 μm were counted, measured and identified (typically to order level) by microscopy (using a Nikon SMZ1500 dissection microscope, Derby, UK). Zooplankton >80 μm were typically assigned to taxonomic orders, though in a number of cases rotifers were identified to species level. Planktonic organisms <80 μm were counted, measured and identified by inverted microscopy. Phytoplankton <80 μm 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 10 mL Utermöhl sedimentation chamber before viewing under an inverted light microscope (Leica DMIRE2, Milton Keynes, UK). An initial scan of the sample, viewed under low magnification (× 150), of a fixed area (50 mm²) 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 organism size (Fig S1 in Supporting Information) given that settlement of organisms followed a Poisson distribution within the sedimentation chamber (Fig S2 in Supporting Information).

Linear organism dimensions were determined with an interactive image analysis system [Hamamatsu C4742-95 camera (Hamamatsu, Japan) and Openlab software]. The 'size' of all organisms was expressed as mass in units of carbon (μg C). For organisms >80 μm (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). Mass was determined by converting biovolume to fresh weight 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 <80 μm (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 day⁻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.* (2010a). Benthic respiration measured using *in situ* benthic chambers contributed, on average over the year, to 35% of whole system respiration (see Fig. S7 in Supporting Information for further details). It was then inferred that measures of primary production predominantly reflected planktonic metabolism and that the relative contribution of the benthos to whole system respiration and primary production are roughly equivalent. Thus, the measures of primary production 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 size range and the low number of individuals present in some samples. Size spectra were constructed by logarithmic binning of organism masses (M) (either the entire community or just the phytoplankton assemblage of the mesocosm). 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 Supporting Information). Variation between warmed and ambient treatments in two normalization constants of the linear model were also measured. The intercept at $x = 0$ gives information on the relative abundance of large organisms between treatments, and the intercept at $x = -8$ provides information on the differences among treatments in the relative abundance of the smallest organisms. For both the community and the phytoplankton size spectrum, nonsignificant 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

Differences between treatments were analysed in the following community properties: size spectrum slopes and intercepts; total community biomass; total phytoplankton biomass; total zooplankton biomass; and mean organism 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 Team, 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 (PCA) 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, other axes of variation are quantified hierarchically by PCA axes 1 to *n*. If the constraining variable (in this case treatment) is the dominant axis of variation in the response variable (i.e. phytoplankton taxonomic composition) then RDA axis 1 will explain more variability in the data than PCA1. RDA was then used to assess the strength of the trend between treatment and phytoplankton community structure, 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 Table S3 for details on nutrient measurements) were tested as constraining environmental variables in separate analyses. 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 because inclusion of very rare taxa can exert undue influence on the ordination.

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. 1a–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 organism mass) was significantly reduced, whereas the intercept at $x = -8$ (i.e. at small 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–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–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. 1d). The maximum phytoplankton mass in the ambient mesocosms was $1.36 \times 10^{-2} \mu g\,C$, while in the heated the maximum mass was only $3.88 \times 10^{-3} \mu g\,C$. Furthermore, the average 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), whereas the average mass of an individual zooplankter was unaffected by warming (Fig. 2; Table 1).
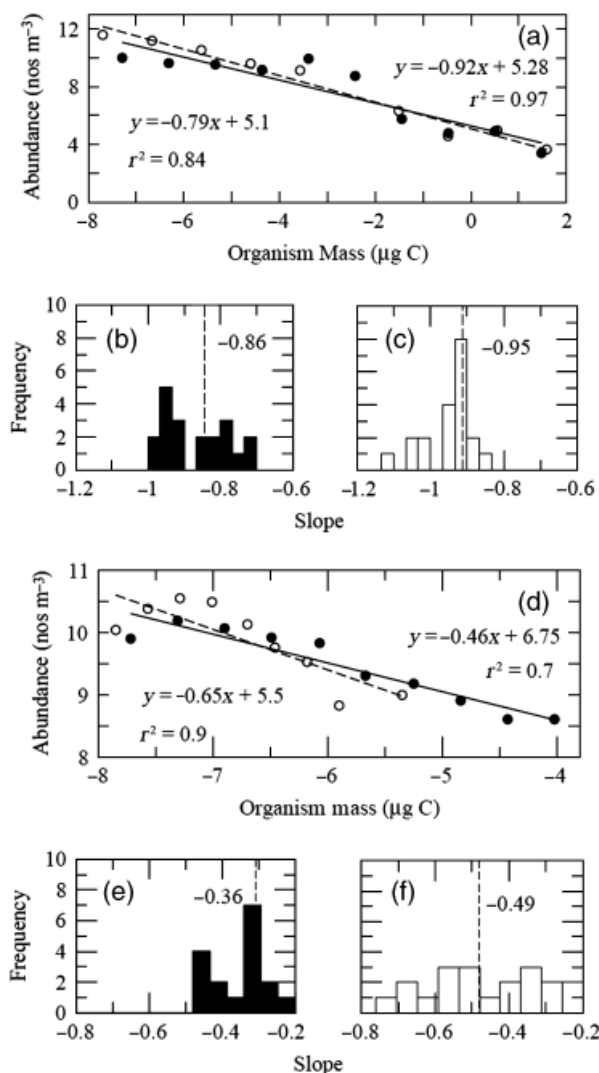
Effects of warming on community composition

RDA 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. Relationships between phytoplankton taxonomic composition and other environmental variables (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, total inorganic N : P) were also tested for in separate analyses using permutation tests, but none of these variables were significantly correlated with taxonomic composition. Certain taxa were strongly associated with either warmed or ambient treatments. For example, in both April and October, the large chlorophyte, *Botryococcus* was located towards the ambient treatment centroid, whereas 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

Fig. 1 The size spectrum. (a) The community size spectrum of a warmed (open circles, dashed line) and ambient (filled circles, solid line) mesocosm, highlighting the increase in the steepness of the slope between a warmed (slope = −0.92, 95% CI −0.9 to −0.94; intercept = 5.09, 95% CI 4.94 to 5.16) and an ambient (slope = −0.79, 95% CI −0.78 to 0.83; intercept = 5.28, 95% CI 5.11 to 5.36) mesocosm. (b) Frequency distribution of the slope of the community size spectrum in the ambient mesocosms ($n = 20$), (c) frequency distribution of the slope of the community size spectrum in the warmed mesocosms ($n = 20$). 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 warmed (open circles, dashed line) and ambient (filled circles, solid line) mesocosm, highlighting the increase in the steepness of the slope and the truncation of large sized individuals between a warmed (slope = −0.65, 95% CI −0.62 to −0.68; intercept = 5.52, 95% CI 5.23 to 5.71) and an ambient (slope = −0.46, 95% CI −0.44 to −0.49, intercept = 6.70, 95% CI 6.61 to 6.78) mesocosm. (e) Frequency distribution of the slope of the phytoplankton size spectrum in the ambient mesocosms ($n = 17$), (f) frequency distribution of the slope of the community size spectrum in the warmed mesocosms ($n = 18$).

Table 1 The effect of treatment (heated or ambient) on community-level properties

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 organism mass	1, 38	18.9	**<0.001**
Mean zooplankton organism mass	1, 38	1.4	0.2 (ns)
Z:P biomass ratio	1, 38	4.82	**0.034**

CSS, community size spectrum; PSS, 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. Bold values correspond to significant *P*-values at 0.05. ns, not significant.

were associated with either the heated or the ambient treatments. Furthermore, Fig. 3a and b 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 1 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 and 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 not found in 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 mesocosms (Fig. 5; Table 1). Warming shifted the distribution of phytoplankton size and biomass from assemblages
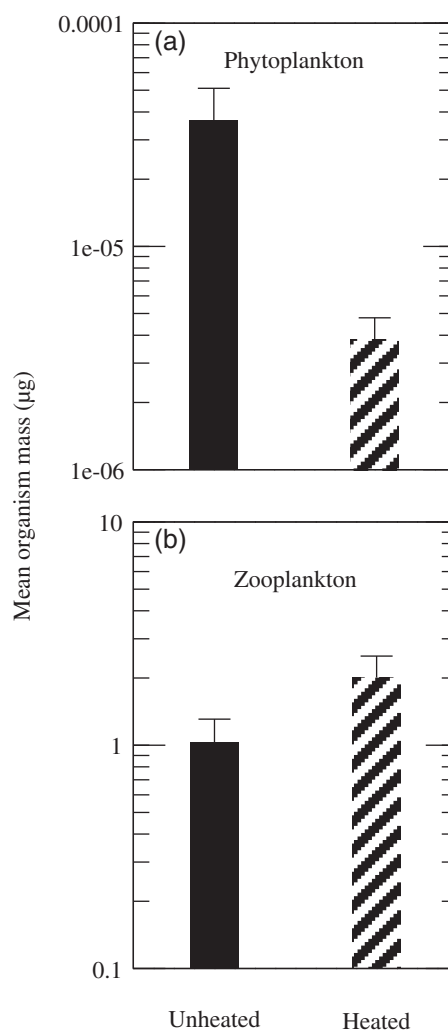

Fig. 2 Effects of warming on mean organism mass (± 1 SEM) of phytoplankton (a) and zooplankton (b) individuals. Ambient treatments are denoted by solid fills, while warmed treatments are given by cross-hatched bars. Data are presented as the overall average of the mean organism mass of phytoplankton 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 organism mass of zooplankton between ambient and warmed treatments (Table 1).

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 (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
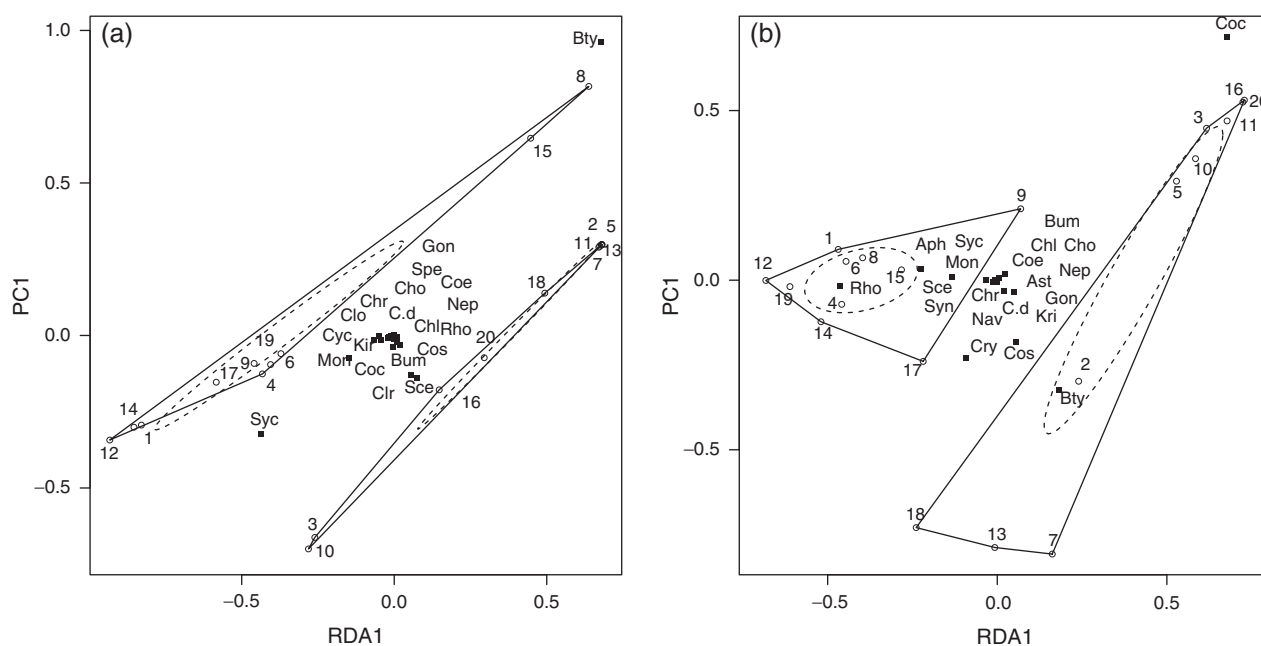
Fig. 3 Redundancy analysis (RDA) biplot for sites (i.e. mesocosms) and species scores for phytoplankton taxa recorded in the mesocosm experiment in April (a) and October (b). In both cases, RDA 1 was constrained by treatment and accounted for 24.1% and 24.6% of the variation in the taxonomic composition 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, whereas 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).

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 characterized 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; Montoya *et al.*, 2006; Woodward *et al.*, 2010). 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). The results presented here broadly supported the 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 organism size and biomass in freshwater ecosystems. The size structure of plankton communities is a key driver of rates of carbon sequestration and nutrient cycling (Laws *et al.*, 2000), and therefore changes in the distribution of planktonic size and biomass could
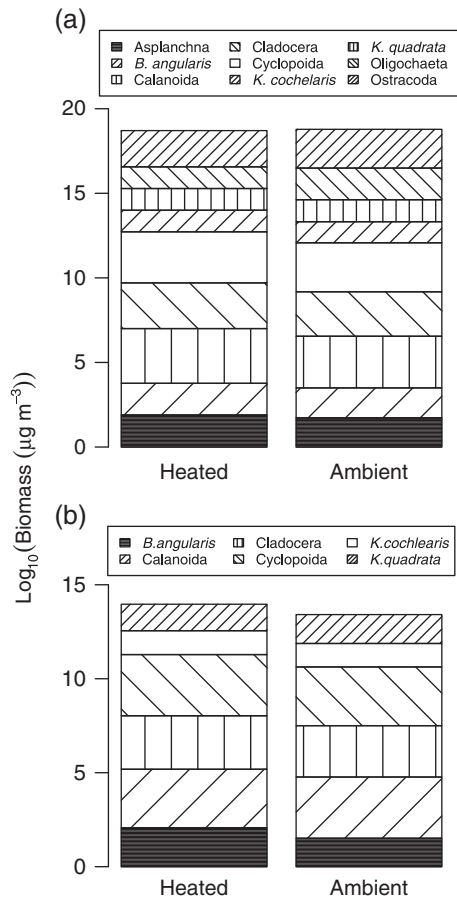
Fig. 4 Mean biomass of the major zooplankton taxonomic groups documented in the mesocosms in (a) April and (b) October. Note that there is very little difference in the biomass contribution of the different zooplankton taxa between treatments suggesting that the zooplankton community composition was unaffected by warming.

Fig. 5 Effects of warming on mean total planktonic biomass (± 1 SEM). 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). Ambient treatments are denoted by solid fills, while warmed treatments are given by cross-hatched bars. Total biomass (a) is significantly reduced by warming. This is mainly driven by a reduction in phytoplankton (b) biomass, while there is no significant difference in the biomass of zooplankton (c) in response to warming (Table 1).

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 were 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, this study has contributed to developing this understanding further by documenting the effects of warming on the 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 the opportunity to isolate the effects of temperature from other potentially confounding vari-
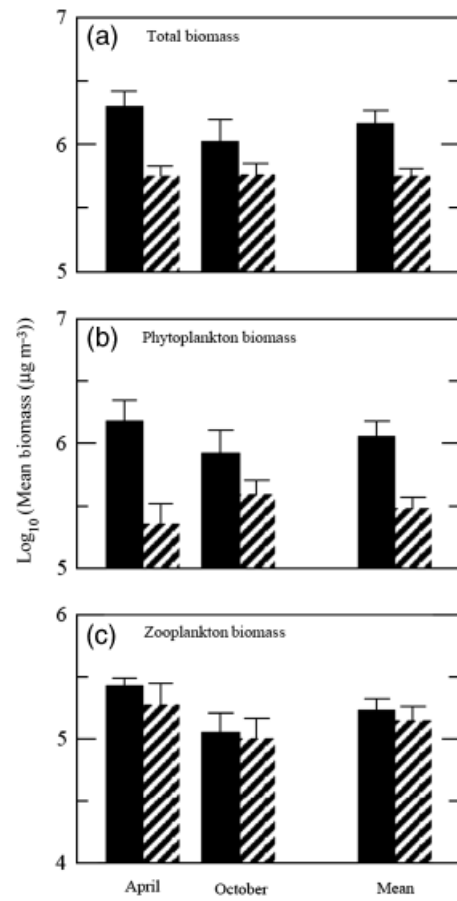
ables (e.g. latitudinal and biogeographical effects) while studying entire replicated plankton communities (Yvon-Durocher *et al.*, 2010a).

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. The first relates to intraspecific changes in organism size, here organisms might exhibit a degree of phenotypic plasticity to changes in temperature. This
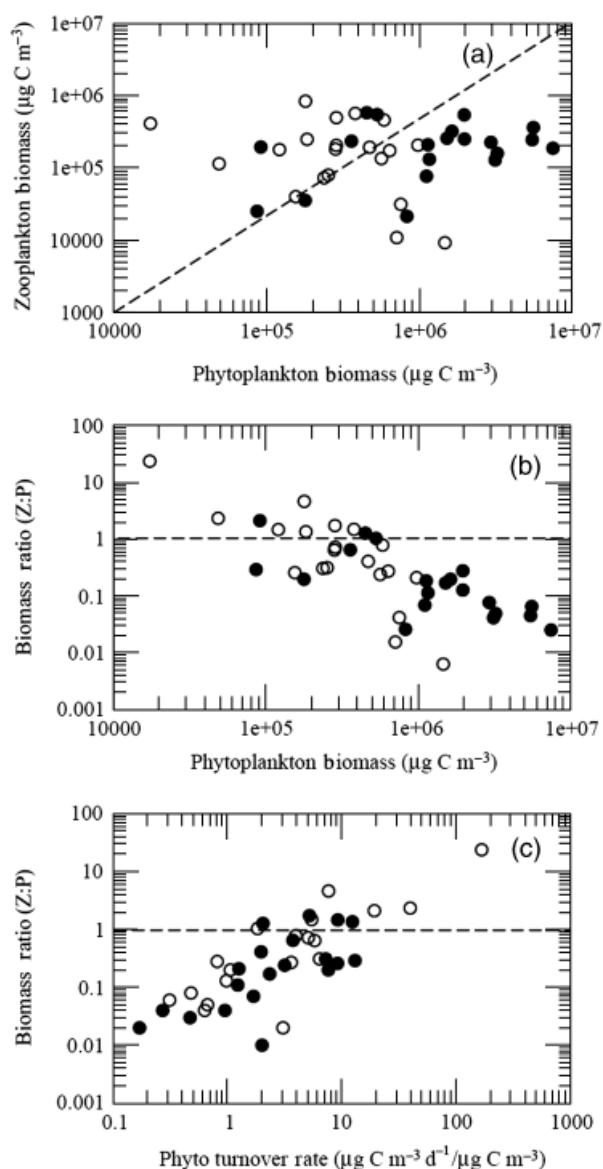
Fig. 6 (a) Relationship between zooplankton and phytoplankton biomass. (b) Relationship between the ratio of zooplankton to phytoplankton biomass (Z : P) and total phytoplankton biomass. (c) The relationship between Z : P and the turnover rate of the phytoplankton communities. Each data point corresponds to either the total zooplankton or phytoplankton biomass or the Z : P in either a warmed (open circles) or ambient mesocosm (filled circles).

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). The second mechanism relates to interspecific changes

in species composition, here changes in the physico-chemical 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, 2010; Irwin *et al.*, 2006; Falkowski & Oliver, 2007; Winder *et al.*, 2009). 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).

The results of this study lend support to the second mechanism. RDA revealed that warming dramatically shifted the taxonomic composition of the phytoplankton assemblages. Moreover, warming favored smaller phytoplankton genera, resulting in a reduction in mean and maximum 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. Conversely, 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 size of the same species composition present in the ambient mesocosms.

The relatively infrequent but highly replicated sampling regime adopted in this study was a necessary compromise. For example, the size, biomass and taxonomic structure of 20 replicated experimental ecosystems was documented on two separate sampling occasions at the beginning and end of the growing season [identified from measures of primary production; see Yvon-Durocher *et al.*, (2010a) 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 such, these results come with an associated caveat: the full effects of warming on the temporal succession of the plankton communities cannot be discerned directly. Nonetheless, analysis of the phytoplankton taxonomic composition suggested that a large, core contingent of these assemblages were present in both April and October but which differed 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 size and biomass structure of these assemblages.

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)	na
Difference in intercept	1, 38	0.195	0.661 (ns)	na
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)	na
Difference in intercept	1, 38	0.002	0.956 (ns)	na
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)	na
Difference in intercept	1, 38	0.538	0.468 (ns)	na

NS, not significant; NA, not applicable. Bold values correspond to significant *P*-values at 0.05.

Inorganic nitrogen was limiting in the experiment (N : P ratios were ≈ 11 : 1, and were below the 16N : 1P expected at Redfield; see Tables S3 and S4 in the Supporting Information 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, 2010; Falkowski & Oliver, 2007; Winder *et al.*, 2009). Furthermore, RDA 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 do large phytoplankton (Shuter, 1978; Litchman *et al.*, 2007). Litchman *et al.* (2007) found that the minimum cellular 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.

Another potential mechanism for the shifts in phytoplankton size and taxonomic structure in the warmed mesocosms is that warming served to increase grazer control of the phytoplankton community by increasing zooplankton grazing rates. It has been 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.* (2010a) for details]. Therefore, because ingestion rates increase in proportion with metabolic rates (Berlow *et al.*, 2009), warming might have increased zooplankton grazing on the phytoplankton assemblages. 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 elevated grazing and 'bottom up' hypotheses are not mutually exclusive: both regulation of phytoplankton competitive ability for limiting nutrients and grazer control of large phytoplankters 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, a pattern that is mirrored by recent trends of phytoplankton biomass and temperature in the modern ocean (Irwin & Finkel, 2008). These results confirmed the qualitative theoretical predictions (i.e. that community biomass would be reduced by warming). For example, because the potential resource supply rate (i.e. the concentrations of limiting inorganic nutrients) remained constant, it was predicted that elevated metabolic demands at higher temperatures should have resulted in a decline in standing community biomass in the warmed mesocosms. Assuming $B_{tot} = r_0 e^{-E/kT} M^{1/4}$ and that r_0 (i.e. the resource supply rate)

and $M^{1/4}$ (i.e. the allometric scaling of biomass with organism mass) are constant with temperature it can be predicted that for $\approx 4\,°C$ warming (i.e. the average annual temperature increase in the experiment) that standing community biomass should decline approximately 1.54-fold according to: $e^{-E/kT_h}/e^{-E/kT_a}$ 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 $\approx 0.65\,eV$ (Gillooly *et al.*, 2001). In this 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 organism size from large to small phytoplankton might further reduce total standing biomass. For example, the above prediction assumes that the allometric scaling of biomass with organism mass (i.e. $B_{tot} = M^{1/4}$) remains constant with warming. However, in this experiment the slope of the community size spectrum (i.e. the log–log relationship), which is equivalent to the exponent (i.e. $-\alpha$) of $N = M^{-\alpha}$, where N is abundance (White *et al.*, 2007, 2008; Reuman *et al.*, 2008), changes from -0.86 to -0.95 in response to warming. Therefore, because $B_{tot} = N \times M$ the allometric scaling of B_{tot} declined from $B_{tot} = M^{0.14}$ in the ambient mesocosms to $B_{tot} = M^{0.05}$ in the warmed mesocosm: i.e., more standing biomass was retained in larger size classes in the ambient relative to the warmed mesocosms. Consequently, the effects of increased metabolic costs, associated with warmer temperatures and the shift in the distribution of organism 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 the third experimental hypothesis. The results presented here 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 the present 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.

This experiment revealed a strong, positive correlation between the $Z:P$ biomass ratio and the turnover

rate of the phytoplankton assemblages. 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 $\sim 4\,°C$ fundamentally altered the structure and functioning (i.e. energy transfer) of the 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 ($2.93 \times 10^5\,\mu g\,C\,m^{-3}$ in heated; $1.12 \times 10^6\,\mu g\,C\,m^{-3}$ in ambient) to sustain the equivalent biomass of zooplankton as the mesocosms at ambient temperature ($1.71 \times 10^5\,\mu g\,C\,m^{-3}$ in ambient; $1.36 \times 10^5\,\mu g\,C\,m^{-3}$ heated), the turnover rate of the phytoplankton community would need to be elevated by a factor of ~ 3.5. The average turnover rates of the phytoplankton community in the warmed treatments were elevated by a factor of ~ 3.5 (i.e. $13.73\,\mu g\,C\,m^{-3}\,day^{-1}/\mu g\,C\,m^{-3}$ in heated; $4.26\,\mu g\,C\,m^{-3}\,day^{-1}/\mu g\,C\,m^{-3}$ in the ambient) which was therefore sufficient to support the biomass of zooplankton in these systems. These results suggest that warming increased the rate of energy flux between autotrophs and heterotrophs in the experiment, a finding which might have important implications for the dynamical stability of aquatic ecosystems in a future warmer climate. This effect appears to be driven by the relative increase in small phytoplankton, which have faster turnover times due to the allometry of mass specific metabolic rate and generation time – i.e. smaller organisms have faster metabolism per unit mass and therefore faster generation times – (Gillooly *et al.*, 2002; Brown *et al.*, 2004; Finkel *et al.*, 2010), and also the direct stimulation of metabolism and generation time by temperature (Gillooly *et al.*, 2002).

Conclusion

In general, the results of this experiment reflect patterns in empirical surveys that have analysed phytoplankton communities over macroevolutionary time (Finkel *et al.*, 2005, 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). However, these findings represent the first experimental evidence for a shift in the distribution of organism 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. Warming is only one (key but not the sole) component of climate change and, for example,

concentrations of dissolved CO_2 due to anthropogenic greenhouse gas emissions are also likely to be significantly enhanced in the coming decades and will no doubt interact with warming to determine changes in plankton community structure (Finkel *et al.*, 2010). The different components of climate change need to be considered together in future projections of changes in plankton community structure, especially as they might act synergistically (Woodward *et al.*, 2010). There are now some tantalizing hints of the potential consequences of future global warming on plankton communities, though the precise mechanisms behind the size shifts that have been observed requires further research. Moreover, the consequences of such shifts in community size structure for the functioning (e.g. carbon sequestration capacity) of freshwater ecosystems remains a still largely unexplored avenue in ecological research, though no doubt one that will prove fundamental in addressing the future challenges posed by climate change.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

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 ($-20\,^{\circ}C$) for subsequent determination of NO_3^-, NO_2^-, NH_4^+, PO_4^{2-} 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 $\approx 0.005\,\mu mol\,L^{-1}$ 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 $\approx 11:1$ 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.

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 ith 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.

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 ith size bin, b and a are the slope and the intercept respectively.

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 25 mL samples at the beginning and the end of the 6 h incubations. The samples were gently discharged into gas-tight vials (12 mL, 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 °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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