





# OIKOS

## Research article

### Direct effect of artificial warming on communities is stronger than its indirect effect through body mass reduction

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Theory predicts that morphological and bioenergetic constraints due to temperature-induced body size reduction can modulate the direct effects of warming on biotic interactions, with consequent effects on trophic cascades and biomass distribution. However, these theoretical predictions have rarely been tested empirically. Our aim was to distinguish the indirect effects of warming-induced body size reductions from the direct effects of warming on community structure. We conducted a mesocosm experiment manipulating factorially 1) body size reduction in the medaka fish *Oryzias latipes* using two populations raised for several generations under contrasted climate conditions and 2) warming (+4°C), to test their independent and interactive effects on the structure of prey and primary resource communities, the predator–prey biomass ratio and the biomass size spectra. We further dissected the effects of seasonal temperature variation from the effects of constant artificial warming. We found that the indirect effects of warming (i.e. fish body size reduction) on composition and structure of communities as well as their biomass size spectra were of marginal amplitude compared to the direct effects of seasonal temperature variation and constant warming. There were no changes in community composition in response to fish body size reduction or constant warming. However, the density of macroinvertebrates and zooplankton were maximal at intermediate seasonal water temperatures and lower in constantly-heated mesocosms. Contrastingly, phytoplankton was not strongly affected by seasonal temperature or warming, but rather responded to grazing effects of zooplankton. Finally, we found a reduction in predator–prey biomass ratio under warming and at the warmest seasonal temperature, inducing a steeper slope of the biomass size spectra under increasing seasonal (but not constant) temperature. We conclude that the direct effects of climate change on freshwater communities are stronger than its indirect effects mediated by body mass reduction.

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## Introduction

Body size reduction has been proposed as the third universal species response to warming (Daufresne et al. 2009, Gardner et al. 2011, Sheridan and Bickford 2011), alongside with shifts in phenology (Visser and Both 2005) and geographical distributions (Parmesan and Yohe 2003). Body size reduction may have important ecological consequences as body size is a fundamental trait influencing many ecological processes at multiple levels of biological organization from metabolism (Brown et al. 2004), intra- and inter-specific interactions (Peters 1983, Emmerson and Raffaelli 2004, Sentis et al. 2017), up to the dynamics and stability of food webs (Woodward et al. 2005, Lindmark et al. 2019, Cuthbert et al. 2020, Vagnon et al. 2021).

Body size reduction is associated with three mutually non-exclusive processes: 1) a selection by climate warming for smaller species (composition shift), 2) a higher proportion of juveniles within populations (population age-structure shift), and 3) an ontogenetic decrease in the size of adult individuals (temperature size rule Atkinson 1994, Daufresne et al. 2009, Martins et al. 2023). Ecological consequences of body size reduction can differ according to the process by which it occurs. Shifts in community composition, population structure and individual size may alter interspecific interactions within food webs (Lindmark et al. 2019, Uszko et al. 2022), intraspecific interactions (e.g. cannibalism and mate-seeking; Ohlberger 2013) and the biological properties and fitness of individuals (Bazin et al. 2023). Recent studies have shown that the decline in body size with warming becomes increasingly negative as the mass of aquatic species increases (Forster et al. 2012). Thus, warming-induced body size reductions are more prevalent and more pronounced at higher trophic levels in aquatic systems, which is likely to affect top–down effects disproportionately compared to bottom–up effects. Because reducing the body size of top predators can alter the strength of trophic interactions by changing predator–prey biomass ratios (PPBRs, Emmerson et al. 2005, Perkins et al. 2022), the temperature size rule can have significant ecological implications on community composition and structure (Post et al. 2008, Renneville et al. 2016, Evangelista et al. 2021), especially in aquatic ecosystems which are often more size-structured than terrestrial ones (Shurin et al. 2002, Sentis et al. 2017, Potapov et al. 2019). Yet, the ecological consequences of temperature-induced body size shifts on communities have so far only been investigated theoretically (Osmond et al. 2017, Sentis et al. 2017, Lindmark et al. 2018, 2019, Bideault et al. 2019, Thunell et al. 2021), and empirical evidence is still lacking, limiting our ability to predict the ecological impacts of climate warming.

Warming-induced body size reduction may also have ecological implications through changes in consumer bioenergetics. The metabolic theory of ecology predicts that

maintenance metabolic rates increase faster than ingestion rate with body size. Thus, the resource density at which the energy gain from ingestion is balanced by the energy loss from maintenance increases with body size, making smaller-bodied individuals dominant in exploitative competition (Persson et al. 1998, Kooijman 2000, De Roos et al. 2003, Persson and De Roos 2006, Edeline et al. 2016). Furthermore, previous studies showed that energetic efficiency (i.e. the ratio of ingestion gains over metabolic losses) decreases with warming (Vucic-Pestic et al. 2011, Lemoine and Burkepile 2012, Sentis et al. 2012) and with increasing body mass (Arim et al. 2007, Rossiter 2017). Smaller organisms are thus more energetically efficient, which can make them more competitive than larger ones (Edeline et al. 2013, Ohlberger 2013). In addition, body size reduction can alter the size-selective predation of a consumer on its prey (Renneville et al. 2016). In the most extreme cases, body size reduction could lead to a rewiring of the food web through loss or gain of trophic links (Polazzo et al. 2022).

Disentangling the direct effects of warming from its indirect effects driven body size reduction is important to better predict future changes in interspecific interactions, size structure and biomass distribution within communities. For instance, by increasing the proportion of small individuals compared to larger ones, warming can change the community size structure and lead to an increase in the steepness of the slope of biomass size spectra (BSS, i.e. the relationship between total biomass and individual biomasses within communities) (Yvon-Durocher et al. 2011). As most of the processes underlying BSS depend on body size (e.g. metabolism and trophic interactions), we can expect body size shifts to affect BSS. However, we lack knowledge about how a reduction in predator size can impact the size structure of the community, and we do not know whether a reduction in predator size can modulate warming effects on community size structure.

The objective of this study was to experimentally test the independent and interactive effects of warming and temperature-induced body size reduction of an aquatic top predator (the medaka fish, *Oryzias latipes*) on community composition (for both prey and basal resource), density, predator–prey biomass ratio and BSS. Warming effects on individual performance depend on the thermal sensitivity of energetic balances in pairwise consumer–resource interactions and on the temperature at which they occur (i.e. below or above the optimum temperature; Angilletta 2006, Kingsolver and Woods 2016, Sinclair et al. 2016, Álvarez-Codesal et al. 2023). Thus, we can expect warming to be beneficial below the average optimal temperature of the species composing the community and unfavourable above this temperature. Therefore, we considered the effects of both artificial warming and water temperature as we expected that artificial warming could have positive effects on the community early in the season when water temperature is low and negative effects when water temperature is high.

We expected that 1) the presence of fish would decrease the density of macroinvertebrates and zooplankton and increase the density of phytoplankton, steepening the slope of BSS compared to fishless mesocosms. This expectation is modulated by the origin of the fish, such that 2) phenotypic variation (i.e. body size differences) of the fish would alter the strength of trophic interactions which in turn affects community structure. We could expect predation rates of small fish reared in warm conditions to be higher than those of large fish reared in cold conditions (as in [Sentis et al. 2015](#)), and therefore the trophic cascades intensity to be higher in mesocosms with warm acclimated fish. Alternatively, warm acclimated fish may focus on smaller prey (such as smaller zooplankton that are less effective grazers of phytoplankton), which should reduce the strength of the cascading effects from fish to phytoplankton. We expected artificial warming 3) to decrease community biomass in response to an increase in individual energy requirements, 4) to steepen the BSS slope in response to a higher prevalence of small individuals (as in [Yvon-Durocher et al. 2010](#)), and 5) to reduce predator-prey biomass ratio (as in [Barneche et al. 2021](#)).

## Material and methods

### Biological model and laboratory-generation of cold- and warm-acclimated lines

Native to East Asia, the medaka *Oryzias latipes* is a small eurythermal fish species (adult size 16–50 mm) living in small ponds and rice paddies at temperatures between 5 and 35°C ([Kirchen and West 1976](#)), with an optimum individual growth temperature of 25°C ([Hirshfield 1980](#), [Dhillon and Fox 2004](#)). Under optimal growth conditions, the medaka reaches sexual maturity in 10–12 weeks, resulting in short generation times. The medaka is omnivorous, feeding mainly on zooplankton but also on diatoms, algae and aquatic insects ([Mano and Tanaka 2012](#), [Edeline et al. 2016](#)).

Four years before the mesocosm experiment, we conducted a laboratory experiment to produce two lines of medaka (cold-acclimated and warm-acclimated), which were used to disentangle the contribution of fish thermal acclimation and body size to the overall effects of global warming in freshwater ecosystems. More precisely, we acclimated the fish over multiple generations at two temperatures (20 versus 30°C), starting with an F<sub>0</sub> generation of 160 male and 160 female fish (approximately 120 days old) belonging to the CAB strain (Carolina Biological Supply Company) and provided by AMAGEN (Gif-sur-Yvette, France). The F<sub>0</sub> generation fish were kept at 25°C in 20 liter tanks (25 × 40 × 20 cm) and then progressively acclimated by reducing and increasing temperature by one degree every two days to 20 or 30°C (160 fish for each temperature). To start a new fish generation, approximately 300 eggs were collected from about 100 females at the fecundity peak (i.e. when most females were laying eggs) and placed in small (2.5 l) temperature-controlled nurseries until hatching. This process was repeated

for each successive generation, leading up to the fifth generation at 30°C and the third generation at 20°C. The generation time of small-bodied fish was shorter because of faster development at 30°C than at 20°C, resulting in asynchronous generations between the two acclimation temperatures.

For each acclimation temperature, eggs were pooled to promote genetic diversity and prevent bottlenecks. Larvae were then evenly distributed among 10 tanks at a final density of about 3 fish per liter. This density does not cause any stress or agonistic behavior in this species ([Denny et al. 1991](#)). Once the fish were sexually mature, they were distributed among the tanks to reach a female:male sex ratio ranging from 1.33:1 to 1.66:1. In each tank, we provided three times a day a portion of TetraMin corresponding to ~ 1.5% of the fish biomass. This quantity is more than the daily need and thus correspond to ad libitum feeding for both rearing temperatures. The water supply of the open water system was controlled by drippers (1 l h<sup>-1</sup>) and the input water quality was maintained by mechanical, biological and UV filtration with a pH of 7.5 at 16°GH. Each tank was equipped with an air filter to prevent high nitrite concentrations and maintain oxygen saturation. The photoperiod was set to 16:8 h (day:night), which is optimal for medaka reproduction ([Hirshfield 1980](#)). For the mesocosm experiment, only males were used to avoid reproduction, since we were interested in their size-dependent effects on communities and not in their population dynamics effects. We used fish reared at 20 and 30°C (hereafter referred to as ‘large-bodied fish’ and ‘small-bodied fish’, respectively). Large-bodied fish of the 3rd generation were 453 days old (= 8190 degree-days) and small-bodied fish of the 5th generation were 273 days old (= 9060 degree-days). Although they had different ages in days, their physiological ages were relatively close to each other. When placed in the mesocosms, the small-bodied fish were on average about 10% smaller (32.9 ± 2.3 mm) and 30% lighter in body mass (409.8 ± 99.9 mg) than the large-bodied fish (which measured on average 36.9 ± 1.9 mm and weighed 589.7 ± 139.3 mg) (Supporting information). Body size is one of the traits that best predicts both individual performance and effects on prey communities ([Raffard et al. 2019](#), [Schmid et al. 2019](#)). We thus use body size as an ‘umbrella’ trait representing a ‘thermal syndrome’. Nevertheless, we acknowledge that body size is not the only trait responding to temperature, and that thermal acclimation can alter biological rates ([Donelson et al. 2011](#), [Gray 2013](#), [Enriquez et al. 2018](#), [Rohr et al. 2018](#), [Alberto-Payet et al. 2022](#)) and interspecific interactions ([Sentis et al. 2015](#), [Sinclair et al. 2016](#), [Rohr et al. 2018](#), [Sohlström et al. 2021](#)). Acclimation could therefore also modulate the effects of size changes on community structure. As a result, distinguishing the effects solely attributable to changes in body size from those resulting from the thermal acclimation of organisms to temperature is challenging.

### Experimental design / mesocosm experiment

The experiment was conducted in 24 outdoor freshwater mesocosms in Aix-en-Provence, France (43°31'25"N,

05°30'40"E). In October 2019, each mesocosm was filled with 1 m<sup>3</sup> filtered freshwater (mechanically and UV) from an irrigation canal originating from a nearby freshwater reservoir (Bimont Lake). They were then inoculated with 10 g of dry white poplar leaves and zooplankton and phytoplankton from a small pond nearby (Aulnes Pond, 43°35'30"N, 04°47'30"E). The mesocosms were left undisturbed and open to natural colonization and dispersal for 18 months to allow the communities to stabilize and diversify, especially through colonization by aquatic insects (diptera, ephemeroptera, odonata or hemiptera). We used a full factorial design with two temperature treatments (unheated and heated by +4°C) and three fish treatments (no fish, large-bodied fish, and small-bodied fish). All the mesocosms followed natural temperature fluctuations. Half of the mesocosms (n=12) were unheated (hereafter NW for no artificial warming) while the other half (n=12) were heated by thermal resistors (hereafter W for artificial warming) compared to the unheated ones. Water temperature was monitored throughout the experiment by HOBO loggers (time step=5 min) to record the temperature difference between the unheated and heated mesocosms. At the beginning of the experiment, water temperature was about 14°C in the unheated mesocosms. Water temperature strongly varied until mid-April, and then increased more or less continuously until it reached 29°C on average at the end of the experiment in June. The heated mesocosms followed the same water temperature fluctuations with an artificial warming of +3.35°C compared to the unheated ones (Fig. 1). One third of the mesocosms (n=8) had four large-bodied fish (LB), another third (n=8) had four small-bodied fish (SB) and the last third (n=8) was fishless (NF for no fish). Fish were acclimated prior to introduction to the mesocosms by decreasing their tank temperatures by 2°C per day to approximately 14°C, which corresponded to the mesocosm temperature at the beginning of the experiment. Fish mortality was monitored weekly. Dead fish were not replaced. Eight large-bodied and two small-bodied fish died toward the end of the experiment. Nevertheless, we found no significant effect of fish origin or artificial warming on the fish survival probability

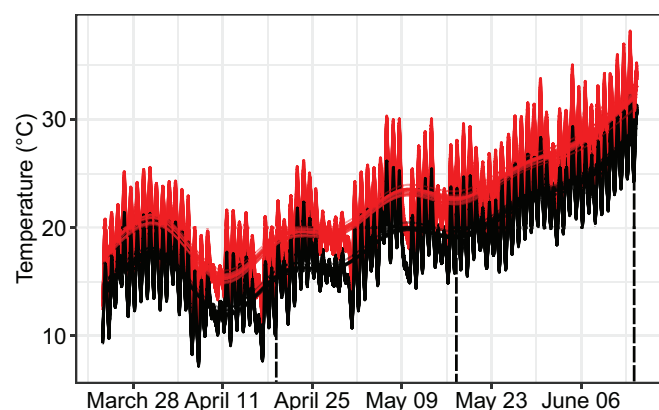


Figure 1. Mesocosms water temperature (°C) during the experiment. Black and red colours represent unheated and heated mesocosms, respectively. Dotted lines represent the sampling dates.

during the experiment (Supporting information). Mortality was thus not considered in the subsequent statistical analyses. Each of the six treatments was randomly assigned to four mesocosms, resulting in 24 experimental communities.

At the start of the experiment, no significant differences were found among mesocosms assigned to our experimental treatments regarding macroinvertebrate community composition, zooplankton density and chlorophyll a concentration (Supporting information). Consequently, we inferred that the initial communities were comparable among mesocosms. The experiment took place in spring and lasted three months, from 23 March 2021 to 15 June 2021, during which we sampled macroinvertebrates, zooplankton and phytoplankton communities monthly (20 April 2021, 18 May 2021, 15 June 2021). Under Mediterranean climate, spring is a season of thermal transition from low to high temperatures up to 35°C, strongly affecting thermal dynamics of aquatic environments (Fig. 1).

## Biological communities sampling and processing

### Macroinvertebrates

Both benthic and pelagic macroinvertebrate communities were sampled. At the beginning of the experiment, 3 benthic traps were placed into each mesocosm. The benthic traps consisted of a net with a 250 µm mesh size and an area of 225 cm<sup>2</sup> (15 × 15 cm). A 50 cm<sup>2</sup> (10 × 5 cm) brush of stiff silicone bristles was inserted into each net to create a habitat and increase the attractiveness of the traps. Traps were randomly placed on the bottom of each mesocosm. They were collected 1, 2 and 3 months after the start of the experiment. At these same dates, pelagic macroinvertebrates were sampled using a 250 µm mesh dip net (surface area 375 cm<sup>2</sup>, 15 × 25 cm) with two net sweeps per mesocosm (filtered volume = 145 l). Macroinvertebrates were preserved in a 70° ethanol solution before being counted and identified to the lowest possible taxonomic level (see the complete taxonomic list in the Supporting information). Each individual was photographed and body length was measured (without appendages such as antennae or cerci) using Image J (Schneider et al. 2012). Published body length – weight relationships were used to estimate individual dry mass (Supporting information; Smock 1980, Benke et al. 1999, Sabo et al. 2002, Baumgärtner and Rothhaupt 2003). The carbon biomass was then determined based on Feller and Warwick (1988), assuming a dry mass: wet mass ratio of 0.25 and that 40% of the dry mass consists of carbon.

### Zooplankton

Sampling tubes of 500 ml were used to randomly collect 10 × 500 ml (= 5 l of water per mesocosm and sampling date) from the entire water column. Each sample was filtered through a 200 µm mesh screen. The upper fraction > 200 µm, consisting of macrozooplankton, was preserved in a 70° ethanol solution. The lower fraction < 200 µm was concentrated in the pellet of a 30 µm mesh net. The pellet,



used for microzooplankton counts, was resuspended in a 50 ml solution of 5% lugol and preserved in the dark at 4°C. Analysis of macrozooplankton samples was performed using the ZooScan (HYDROPTIC, Inc.)/ZooProcess system (Grosjean et al. 2004, Gorsky et al. 2010) and scanning of each microzooplankton sample was performed using the FlowCAM (flow cytometer and microscope; Fluid Imaging Technologies)/ZooProcess system (Sieracki et al. 1998) at the Institut Méditerranéen d'Océanologie (MIO, Marseille, France) (Supporting information).

The automatic classification of thumbnails was performed in Ecotaxa (<http://ecotaxa.obs-vlfr.fr>). Thumbnails from ZooScan and FlowCAM were loaded into the 'EcoTeBo\_macrozooplankton' (<https://ecotaxa.obs-vlfr.fr/prj/4181>) and 'EcoTeBo\_microzooplankton' (<https://ecotaxa.obs-vlfr.fr/prj/4424>) projects, respectively. For each project, a personal 'learning set' was created by identifying between 50 and 100 individuals per taxonomic unit. Based on the variables generated by ZooProcess and our own learning set, all remaining thumbnails were classified by a machine learning algorithm. All thumbnails were checked manually to correct any classification errors. Only the categories corresponding to our lowest taxonomic level 'Cyclopoida', 'Calanoida', 'nauplii < Copepoda' and 'Rotifera' were retained for zooplankton analysis since the other categories corresponded to detritus, insects or phytoplankton.

The individual elliptical biovolume ( $EB_v$ ) was estimated using the ZooProcess variables 'major' and 'minor', representing the principal and secondary axis of the best-fit ellipse for the object, respectively (Eq. 1). 'minor' is the axis orthogonal to the middle of 'major' defining the area of the best-fit ellipse ( $A_e$ ) that is equal to the surface area of the object ( $A_o$ ) (Eq. 2; Vandromme et al. 2012). Elliptical biovolume was preferred over spherical biovolume since many zooplanktonic organisms, including the very abundant copepods in our study, are best represented by an ellipsoid (Herman 1992). Wet biomass was estimated from the individual ellipsoidal biovolume considering a density of 1.1 g ml<sup>-1</sup> and then converted to dry mass assuming a dry mass:wet mass ratio of 0.25 (as in Yvon-Durocher et al. 2015). As with macroinvertebrates, the carbon biomass was determined assuming that 40% of the dry mass consists of carbon (Feller and Warwick 1988).

$$EB_v = \frac{4}{3}\pi \left( \frac{\text{Major}}{2} \right) \left( \frac{\text{Minor}}{2} \right)^2 \quad (1)$$

$$A_e = \pi \left( \frac{\text{Major}}{2} \right) \left( \frac{\text{Minor}}{2} \right) = A_o \quad (2)$$

### Phytoplankton

After zooplankton sampling, 50 ml of water was collected from the remaining fraction < 200 µm. Samples were conserved in a lugol solution (5% final concentration) at 4°C and in the dark. The samples were processed according to

the standard sedimentation method of Utermöhl (1958). After homogenization, 5 ml of each sample was sedimented in a 4.91 cm<sup>2</sup> sedimentation chamber for 24 h. This volume optimized the cell density for counting. Phytoplankton individuals were counted and identified to the lowest possible taxonomic level (see the complete taxonomic list in the Supporting information) using an inverted optical microscope (x40 magnification) and reference books (Bourrelly 1966, 1972, 1981, Lenzenweger 1996, 1997, 1999, 2003).

Depending on the abundance of the taxon, the count was performed on five random fields (0.012 cm<sup>2</sup>; very high abundance), on a single transect (0.1375 cm<sup>2</sup>; high abundance), on a double transect (0.275 cm<sup>2</sup>; medium abundance) or on the entire sedimentation chamber (low abundance) to obtain at least 30 counted cells per taxon. Length and width (or thickness) of each individual were measured using a scale on the eyepiece (1 unit = 1.6 µm), except for the very abundant taxa where a maximum of 30 individuals were measured. For taxa present in colonial form, about ten individual cells were measured in addition to the whole colony. The cell abundance within a colony could therefore be estimated by dividing the colony biovolume by the average biovolume of a single cell constituting the colony.

Individual biovolumes were estimated by assigning a geometric shape to each taxon (following Hillebrand et al. 1999, Supporting information). Individual wet biomasses were estimated considering a density of 1 g ml<sup>-1</sup> (Wetzel and Likens 2000). Carbon biomasses were obtained based on the class-dependent C:wet biomass ratios in Wetzel and Likens (2000) (Supporting information). For each mesocosm and sampling date, the carbon biomass of each counted but unmeasured phytoplankton individual was estimated by a normal distribution with the mean and standard deviation of the observed carbon biomasses as parameters, truncated by the observed minimum and maximum carbon biomasses values of the considered taxon (*rtnorm* function from the 'MCMCglmm' R package (Hadfield 2010)). We also sampled microorganisms (i.e. microalgae, prokaryotes, viruses) collecting 2 ml of the remaining fraction < 200 µm in an Eppendorf tube. Samples were immediately fixed in liquid nitrogen and then conserved at -80°C until flow cytometer analysis (see the Supporting information for more details about methodology and statistical results on density of microorganisms).

### Ecological index

For each mesocosm at each sampling date, we calculated the species richness  $S$  and the Shannon diversity index  $H'$  (Eq. 3) of the macroinvertebrate and phytoplankton communities. For the zooplankton community, we did not calculate these ecological indexes since the taxonomic resolution was too low ( $n = 4$  taxa).

$$H' = - \sum_{i=1}^S p_i \log_2 p_i \quad p_i = \frac{n_i}{N} \quad (3)$$

Where  $S$  represents the species richness,  $i$  is a single species and  $p_i$  is the proportion of the species  $i$  given by its abundance  $n_i$  in relation to the total abundance in the community  $N$ .

### Predator–prey biomass ratio

Log<sub>10</sub>-scale PPBRs were used as an indicator of trophic transfer efficiency between adjacent trophic levels (Yvon-Durocher et al. 2011, García-Comas et al. 2016, Ersoy et al. 2017). For each mesocosm and date, we calculated the PPBR between zooplankton and phytoplankton. We excluded adult cyclopoids from the zooplankton biomass, as they tend to be carnivorous, feeding on copepod nauplii, rotifers and other animals (Fryer 1957). However, we considered all copepod nauplii (calanoid and cyclopoid) in zooplankton biomass, as these larval stages feed on phytoplankton (Brandl 1998). The higher the PPBR, the better the biomass production of the higher trophic level from the lower trophic level.

### Biomass size spectra

BSS allow for the simultaneous integration of size structure across many biological organization levels (White et al. 2008, Yvon-Durocher et al. 2011, Guet et al. 2016, Edwards et al. 2017), and simplify complex biological and ecological processes such as metabolism, trophic interactions and energy transfer (Atkinson et al. 2021). Size spectra can thus be used as an integrative measure of response to perturbations and previous studies have used it to investigate the warming consequences on community size structure and biomass distribution (Yvon-Durocher et al. 2011, Dossena et al. 2012, Pomeranz et al. 2022, Arranz et al. 2023).

We used the individual carbon biomasses (defined as  $X$ ) of all individuals present in the community (i.e. macroinvertebrate, zooplankton and phytoplankton individuals) to construct the BSS. We did not include fish in the BSS because we considered fish as a driving factor whose intensity we controlled, by setting their artificial density to four individuals per mesocosm containing fish. The BSS equation for a community of  $n$  individuals, which describes how the total biomass is distributed as a function of individual carbon biomass, has the following form (Edwards et al. 2017):

$$B(x) = xN(x) = nCx^{b+1}, x_{\min} \leq x \leq x_{\max} \quad (4)$$

Where  $x$  represents the possible values of  $X$ ,  $N(x)$  is the abundance density function,  $b$  is the exponent, and  $C$  is a normalization constant (Supporting information). The slope of the BSS can be estimated by different methods (reviewed by Edwards et al. 2017). According to Sprules and Barth (2016) and Edwards et al. (2017), methods not requiring data binning such as log cumulative distribution LCD or maximum likelihood estimation MLE are more effective for estimating the exponent  $b$  compared to methods requiring binning the data into size classes. As the LCD and MLE methods poorly fitted our experimental data (Supporting information), we used the LBNbiom (log-binning with normalization using

biomass) which is the binning method that most accurately estimates the exponent  $b$  (Edwards et al. 2017).

The LBNbiom method involves binning individual carbon biomasses into classes of equal width on a log<sub>2</sub> scale. The total biomass in each class is normalized by dividing it by the width of the class (in normal scale). We then used a linear regression to fit log<sub>10</sub>(normalized biomass in a class) (= log<sub>10</sub>(BiomNorm)) against log<sub>10</sub>(mid-point of the class) (= log<sub>10</sub>(binMid)). Given the normalized counts, the slope of the regression corresponds to the exponent  $b + 1$  (White et al. 2008). We scaled the x-axis so that its midpoint equals 0 (= log<sub>10</sub>(binMid.scaled)), following the recommendations of Sprules and Barth (2016). Therefore, in the scaled regression, the mean community biomass was reflected by the intercept (representing the midpoint height of BSS) and the size distribution by the slope. The estimate of the slope may depend on the number of classes defined (Edwards et al. 2017). We determined the most appropriate number of classes by constructing BSS using 4–10 body size classes, and then selected the number of classes that maximized the linearity of the regression (i.e. minimized the residuals variance; Supporting information). Based on this criterion, the best number of classes was five. These five equal width classes on a log<sub>2</sub> scale were calculated from the integer value below the smallest observed individual carbon biomass to the integer value above the largest observed individual carbon biomass of our entire experiment (including all mesocosms and all sampling dates). Consequently, the width and the number of size classes were the same for all mesocosms at each sampling date. For each mesocosm at each sampling date, we computed the log<sub>10</sub>(BiomNorm) for each size class (i.e. log<sub>10</sub>(binMid)) which were then used as variables in the statistical analyses.

### Statistical analyses

We performed multivariate analyses on the Hellinger transformed density of macroinvertebrate and phytoplankton taxa to investigate the impact of our experimental treatments on community composition. Hellinger transformation was used to reduce the effect of overrepresented taxa (Legendre and Gallagher 2001). To account for potential temporal autocorrelation between communities sampled at different dates, we used principal response curves. Principal response curves is a special case of redundancy analysis for design with repeated observations (Van den Brink and Ter Braak 1999). This method uses canonical coefficients and species scores for a single axis to calculate coefficients, that represent contrasts with the control condition (here we defined NF\_NW as a control condition). We performed a permutation test ( $n = 999$ ) to investigate the effect of our experimental conditions (i.e. combination of fish and artificial warming) as fixed factor on community composition.

As we expected the effects of artificial warming to depend on seasonal temperature, we calculated the seasonal temperature as the mean temperature of the 15 days prior to the sampling dates ( $T_{15}$ ) for each mesocosm, as it is more integrative than the temperature at the sampling date (we also conducted the statistical analyses using the sampling date as a continuous

variable and presented the results in Supporting information). We have named this variable seasonal temperature, although we recognize that seasonal succession is not only an increase in temperature but also changes in other environmental factors such as photoperiod. In our models, we used a quadratic polynomial for  $T_{15}$  (i.e. to test that low  $T_{15}$  values can have positive effects while high  $T_{15}$  values would have negative effects as mentioned in the Introduction). In addition, we expected interactive effects of artificial warming and fish treatment to depend on biological compartment (i.e. invertebrate, zooplankton and phytoplankton). We thus computed mixed-effects models to investigate the effects of  $T_{15}$ , artificial warming, fish treatments, compartment and their interactions on ecological index ( $S$  and  $H'$ ) and log-transformed community density. We scaled response variables relative to the mean of the response variable per compartment with the goal of analysing the response of ecological indexes of phytoplankton and invertebrate communities in the same analysis. We included sampling date and mesocosm ID as random factors on the intercept to account for repeated measurements and to minimize the potential effects of a correlation between temperature and sampling date. If there was a significant interaction between our fixed factors and the biological compartment, we conducted separate analyses for each compartment. In addition, we estimated the correlation between  $T_{15}$  and the mean temperature of the 3, 7 and 30 days prior to the sampling dates ( $T_3$ ,  $T_7$  and  $T_{30}$ , respectively). We found that  $T_{15}$  was highly correlated with  $T_3$ ,  $T_7$  and  $T_{30}$  (Supporting information), suggesting that our results should be robust to different integration times.

We computed mixed-effects models using a quadratic polynomial for  $T_{15}$  to investigate the effects of  $T_{15}$ , artificial warming, fish treatments and their interactions on PPBR (Eq. 5).

$$\begin{aligned} \log_{10}(\text{PPBR}) = & \beta_0 + \beta_1 T_{15} + \beta_2 T_{15}^2 + \beta_3 \text{fish} \\ & + \beta_4 \text{warming} + \beta_5 T_{15} \text{fish} + \beta_6 T_{15}^2 \text{fish} \\ & + \beta_7 T_{15} \text{warming} + \beta_8 T_{15}^2 \text{warming} \\ & + \beta_9 \text{fish warming} + \beta_{10} T_{15} \text{fish warming} \\ & + \beta_{11} T_{15}^2 \text{fish warming} + b_{0i} + b_{1j} + \epsilon \end{aligned} \quad (5)$$

Where  $\beta_0$  is the intercept term,  $\beta_1$  to  $\beta_4$  are the coefficient for the single terms,  $\beta_5$  to  $\beta_9$  are the coefficient for the double interaction terms and  $\beta_{10}$  and  $\beta_{11}$  are the coefficients for the triple interaction terms.  $b_{0i}$  and  $b_{1j}$  represent the random effect on the intercept for date and mesocosm ID, respectively.  $\epsilon$  is the error term that is assumed to be multivariately normally distributed.

For the BSS, we computed a linear mixed-effects model with the response variable  $\log_{10}(\text{BiomNorm})$  depending on  $\log_{10}(\text{binMid.scaled})$ ,  $T_{15}$ , artificial warming, fish treatment and their interactions as fixed factors and date and mesocosm ID as random factors (Eq. 6).

$$\begin{aligned} \log_{10}(\text{BiomNorm}) = & \beta_0 + \beta_1 \log_{10}(\text{binMid.scaled}) \\ & + \beta_2 T_{15} + \beta_3 \text{fish} + \beta_4 \text{warming} \\ & + \beta_5 \log_{10}(\text{binMid.scaled}) T_{15} \\ & + \beta_6 \log_{10}(\text{binMid.scaled}) \text{fish} \\ & + \beta_7 \log_{10}(\text{binMid.scaled}) \text{warming} \\ & + \beta_8 T_{15} \text{fish} + \beta_9 T_{15} \text{warming} + \beta_{10} \text{fish warming} \\ & + \beta_{11} \log_{10}(\text{binMid.scaled}) T_{15} \text{warming} \\ & + \beta_{12} \log_{10}(\text{binMid.scaled}) T_{15} \text{fish} \\ & + \beta_{13} \log_{10}(\text{binMid.scaled}) \text{fish warming} \\ & + \beta_{14} T_{15} \text{fish warming} \\ & + \beta_{15} \log_{10}(\text{binMid.scaled}) T_{15} \text{fish warming} \\ & + b_{0i} + b_{1j} + \epsilon \end{aligned} \quad (6)$$

Where  $\beta_0$  is the intercept term,  $\beta_1$ – $\beta_4$  are the coefficient for the single terms,  $\beta_5$ – $\beta_{10}$  are the coefficient for the double interaction terms,  $\beta_{11}$ – $\beta_{14}$  are the coefficients for the triple interaction terms and  $\beta_{15}$  is the coefficient for the quadruple interaction term.  $b_{0i}$  and  $b_{1j}$  represent the random effect on the intercept for date and mesocosm ID, respectively.  $\epsilon$  is the error term that is assumed to be multivariately normally distributed.

All the maximum likelihood estimates of the parameters in linear mixed-effects models were determined using the *lmer* function in the R package 'lme4' with the 'Nelder\_Mead' optimizer (Bates et al. 2015). For each complete model, a stepwise backward regression was performed on the fixed factors and their interactions using the *step* function from the 'lmerTest' R package (Kuznetsova et al. 2017) to obtain the most parsimonious model while keeping the same random structure. When the most parsimonious model included the quadratic but not linear coefficient of  $T_{15}$ , we manually added the linear coefficient of  $T_{15}$ . Wald  $\chi^2$  statistic of each fixed effect was calculated in deviance analyses (*Anova* function from 'car' R package, Fox et al. 2012) on the most parsimonious models to determine the significance of the retained fixed effects. Residuals of the most parsimonious models were inspected to verify the assumptions of each model. All statistical analyses were performed with R software (ver. 4.1.3, [www.r-project.org](http://www.r-project.org)).

## Results

### Community composition and ecological index

We did not detect any significant effect of artificial warming on species richness. However, species richness was significantly

affected by linear and quadratic coefficients of  $T_{15}$ , and the effects of  $T_{15}$  depended on fish treatment and compartment (Supporting information). Species richness was not affected by  $T_{15}$  in mesocosms without fish, but increased with the linear coefficient of  $T_{15}$  in mesocosms with large-bodied and small-bodied fish. As the effects of  $T_{15}$  were compartment-dependent (Supporting information), we conducted separate analyses for the macroinvertebrate and phytoplankton compartments. Macroinvertebrate species richness increased with linear coefficient of  $T_{15}$  (Fig. 2a, Table 1), and we found a hump-shaped relationship between phytoplankton species richness and  $T_{15}$ ; it first increased with  $T_{15}$  and then decreased at higher values of  $T_{15}$  (Fig. 2b, Table 1).

There were no significant differences between experimental conditions for macroinvertebrate and phytoplankton community compositions ( $df=1$ ,  $F=5.4$ ,  $p=0.315$  and  $df=1$ ,  $F=15.7$ ,  $p=0.106$ , respectively). The most contributing taxa to the variation in macroinvertebrate density were dipteran (ceratopogonidae, chironominae, tanypodinae and orthocladinae), ephemeroptera (caenidae and *Cloeon* sp.) and odonata (sympetrinae) taxa (Supporting information). Zooplankton community was composed of rotifera and cyclopoids and calanoids copepoda (adults and nauplii). We did not find any cladocerans in the zooplankton community. Cyanobacteria *Microcystis* sp. and *Synechococcus* sp. alone explained most of the variation in phytoplankton density (Supporting information).

We found that the Shannon index  $H'$  was significantly affected by linear and quadratic coefficients of  $T_{15}$ , fish treatment, artificial warming, and that these effects depended on the compartment (Supporting information). To study the response of each compartment to our experimental treatments, we conducted separate analyses for the macroinvertebrate and phytoplankton compartments. Shannon index  $H'$  of the macroinvertebrate community was significantly affected by linear and quadratic coefficients of  $T_{15}$ , and the effects of  $T_{15}$  depended on fish treatment and artificial warming (Table 1). The relationship between  $H'$  and  $T_{15}$  was U-shaped in unheated and heated mesocosms, and the increase in  $H'$  at higher values of  $T_{15}$  was lower in heated

mesocosms. In all fish treatments, the relationship between  $H'$  and  $T_{15}$  was U-shaped (Fig. 3a), it first decreased with  $T_{15}$ , reached a minimum and then increased at higher  $T_{15}$  values. Moreover, the decrease in  $H'$  with  $T_{15}$  and the increase at higher  $T_{15}$  values were lower in mesocosms with small-bodied fish than in mesocosms with large-bodied fish or without fish. The Shannon index  $H'$  of phytoplankton depended on the quadratic coefficient of  $T_{15}$ , and the effect of the linear coefficient of  $T_{15}$  depended on the fish treatment (Table 1). In mesocosms without fish, the relationship between  $H'$  and  $T_{15}$  was U-shaped (Fig. 3b); it first decreased with  $T_{15}$  and then increased at higher  $T_{15}$  values. In mesocosms with large and small-bodied fish, the Shannon index was not affected by the linear coefficient of  $T_{15}$  but increased significantly at higher  $T_{15}$  values.

## Community density

Fish treatments had no significant effect on community density. Community density was significantly affected by the interaction between linear and quadratic coefficients of  $T_{15}$ , artificial warming, and compartment (Supporting information). For macroinvertebrates, we found a hump-shaped relationship between densities and  $T_{15}$  (Fig. 4a, Table 1). Thus, the highest densities were observed at intermediate values of  $T_{15}$  (between 20 and 25°C). In addition, densities were lower in heated mesocosms and the increase in density with the linear coefficient of  $T_{15}$  was higher in heated mesocosms than in unheated ones.

Zooplankton densities followed a similar hump-shaped pattern with  $T_{15}$ , with lower densities in heated mesocosms and a stronger increase in zooplankton density with the linear coefficient of  $T_{15}$  in heated mesocosms compared to unheated ones (Fig. 4b, Table 1).

Phytoplankton density was significantly affected by the interaction between  $T_{15}$  and artificial warming (Table 1). In unheated mesocosms, it tended to increase with  $T_{15}$ , although none of our fixed factors significantly affected it. In heated mesocosms, opposite to our expectations, the relationship between phytoplankton density and  $T_{15}$  was U-shaped, it first

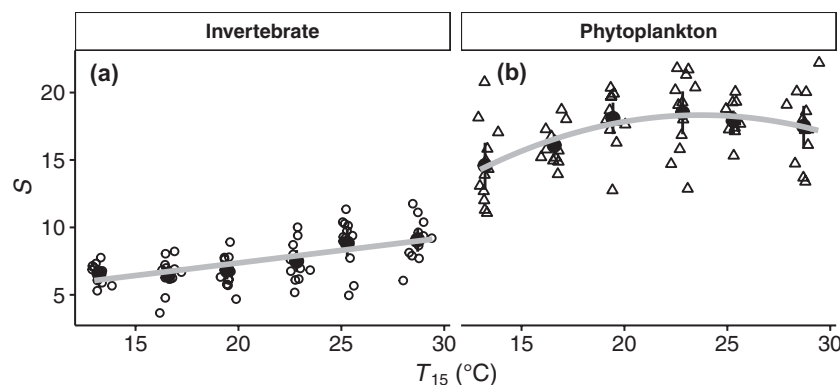


Figure 2.  $T_{15}$  effects on species richness  $S$  for (a) macroinvertebrate and (b) phytoplankton communities. Empty circles and triangles represent experimental raw data for macroinvertebrate and phytoplankton communities, respectively. Filled circles represent the mean values at each sampling date and bars represent the 95 % confidence intervals. Lines represent the fit of the most parsimonious model.



Table 1. Outputs of deviance analysis on the most parsimonious models for the ecological index  $S$  and  $H'$ , community density and biomass, predator–prey biomass ratios and BSS.

Response variable	Compartment	n	Predictors	df	$\chi^2$	p
Ecological index	$S$	72	$T_{15}$	1	29.0	< 0.001 ***
			$T_{15}$	1	14.4	< 0.001 ***
$H'$	Macroinvertebrate Phytoplankton	72	$I(T_{15}^2)$	1	11.4	< 0.001 ***
			$T_{15}$	1	83.7	< 0.001 ***
	Macroinvertebrate	72	$I(T_{15}^2)$	1	88.9	< 0.001 ***
			Artificial warming	1	0.0	0.973
	Phytoplankton	72	Fish	2	1.6	0.442
			$T_{15}$ : Artificial warming	1	2.3	0.128
	Phytoplankton	72	$I(T_{15}^2)$ : Artificial warming	1	5.5	0.019 *
			$T_{15}$ : Fish	2	9.8	0.007 *
	Phytoplankton	72	$I(T_{15}^2)$ : Fish	2	8.9	0.011 *
			$T_{15}$	1	3.6	0.056
Community density	Phytoplankton	72	$I(T_{15}^2)$	1	4.5	0.033 *
			Fish	2	0.6	0.739
	Macroinvertebrate	72	$T_{15}$ : Fish	2	7.8	0.020 *
			$T_{15}$	1	91.4	< 0.001 ***
	Zooplankton	71	$I(T_{15}^2)$	1	85.1	< 0.001 ***
			Artificial warming	1	25.8	< 0.001 ***
	Phytoplankton	72	$T_{15}$ : Artificial warming	1	21.4	< 0.001 ***
			$T_{15}$	1	21.5	< 0.001 ***
	Phytoplankton	72	$I(T_{15}^2)$	1	25.7	< 0.001 ***
			Artificial warming	1	7.3	0.007 **
Community biomass	Macroinvertebrate Zooplankton	72	$T_{15}$ : Artificial warming	1	4.9	0.028 *
			$T_{15}$	1	1.5	0.220
	Phytoplankton	72	$I(T_{15}^2)$	1	2.3	0.131
			Artificial warming	1	0.0	0.992
	Phytoplankton	72	$T_{15}$ : Artificial warming	1	5.6	0.018 *
			$I(T_{15}^2)$ : Artificial warming	1	4.1	0.043 *
	Macroinvertebrate Zooplankton	72	no predictors	1	23.8	< 0.001 ***
			$T_{15}$	1	28.7	< 0.001 ***
	Phytoplankton	72	$I(T_{15}^2)$	1	12.0	< 0.001 ***
			Artificial warming	1	0.3	0.568
Predator–prey biomass ratio	Phytoplankton	72	$T_{15}$	1	1.1	0.291
			$I(T_{15}^2)$	1	0.0	0.995
	Phytoplankton	72	Artificial warming	1	9.7	0.002 **
			$T_{15}$ : Artificial warming	1	7.4	0.006 **
	Zooplankton: Phytoplankton	71	$I(T_{15}^2)$ : Artificial warming	1	2.9	0.090
			$T_{15}$	1	17.2	< 0.001 ***
	Zooplankton: Phytoplankton	71	$I(T_{15}^2)$	1	4.2	0.040 *
			Artificial warming	1	13.2	< 0.001 ***
	All	358	$T_{15}$ : Artificial warming	1	9949.2	< 0.001 ***
			$\text{Log}_{10}(\text{binMid.scaled})$	1	0.2	0.675
Biomass size spectra	All	358	$T_{15}$	1	3.3	0.070
			$\text{Log}_{10}(\text{binMid.scaled})$	1	4.0	0.045 *
	All	358	Artificial warming	1	6.3	0.012 *
			$T_{15}$ : Artificial warming	1	6.3	0.012 *

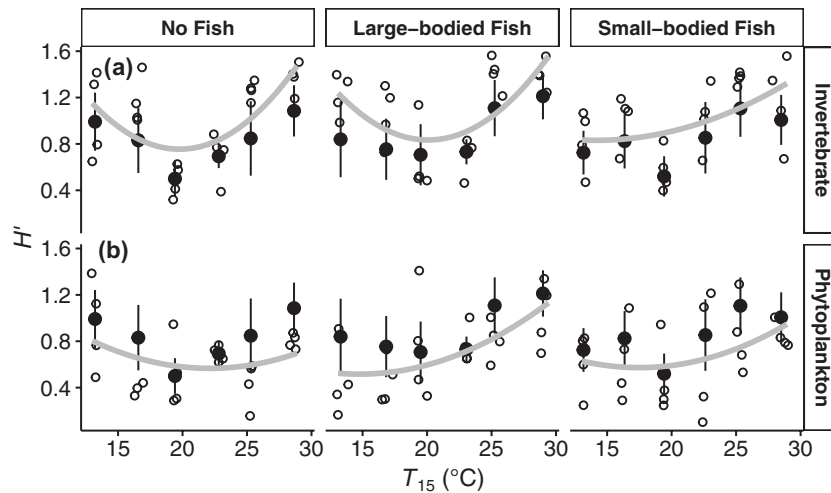


Figure 3. Shannon diversity index  $H'$  depending on  $T_{15}$  and fish treatment for the (a) macroinvertebrate and (b) phytoplankton communities. Empty circles represent raw experimental data. Filled circles represent the mean values at each sampling date and bars represent the 95 % confidence intervals. Lines represent the fit of the most parsimonious model.

decreased with  $T_{15}$ , reached a minimum and then increased slightly at higher  $T_{15}$  values (Fig. 4c).

### Community biomass and predator–prey biomass ratio

Zooplankton and phytoplankton biomass exhibited similar responses to changes in density across seasonal temperature variations and artificial warming. However, invertebrate biomass responded differently compared to density, as invertebrate biomass was not significantly affected by seasonal temperature or artificial warming (Table 1, Supporting information). Fish treatments had no significant effect on the PPBR. PPBR was lower in heated mesocosms, and we found a hump-shaped relationship between the PPBR and  $T_{15}$  in both unheated and heated mesocosms (Table 1), it first increased with  $T_{15}$ , reached a maximum, and then decreased at higher  $T_{15}$  values (Fig. 5). The increase in PPBR with the linear coefficient of  $T_{15}$  was higher in heated mesocosms (Table 1).

### Biomass size spectra

Fish treatments had no significant effect on the BSS slope and intercept (i.e. the height of BSS). We found that BSS slope steepened with increasing  $T_{15}$  in both unheated and heated mesocosms (Table 1). However, we found that  $T_{15}$  effect on the BSS intercept depended on the artificial mesocosm warming (Table 1). In unheated mesocosms, increasing  $T_{15}$  led to an increase on the BSS intercept, whereas  $T_{15}$  had no significant effect on intercept in heated mesocosms. We illustrated this result by plotting the BSS of all the mesocosms at three different  $T_{15}$  values: 10, 20 and 30°C (Fig. 6a). We also illustrated the effect of increasing  $T_{15}$  on BSS intercept of unheated mesocosms, and the intercept of heated mesocosms for a  $T_{15}$  value of 20°C (Fig. 6b). Increasing the  $T_{15}$  by +10°C decreases the slope by 0.04 and increases the intercept by 0.18 in the unheated mesocosms. Finally, BSS intercept is lower in heated mesocosms than in unheated mesocosms when  $T_{15}$  is above 20°C.

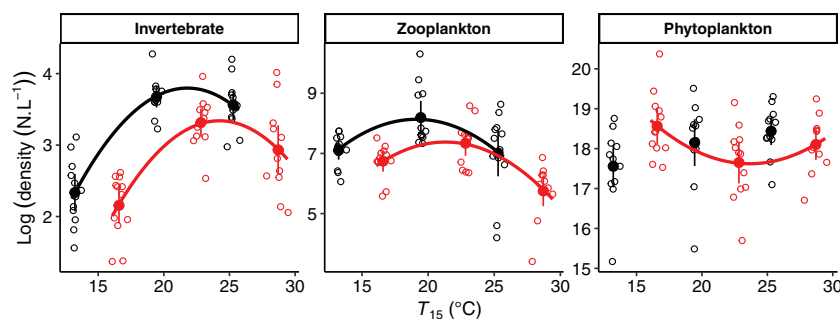


Figure 4.  $T_{15}$  and artificial warming effects on (a) macroinvertebrate, (b) zooplankton and (c) phytoplankton densities in log scale. Black and red colours represent unheated and heated mesocosm, respectively. Empty circles represent experimental data. Filled circles represent the mean values at each sampling date and bars represent the 95 % confident intervals. Lines represent the fit of the most parsimonious model.

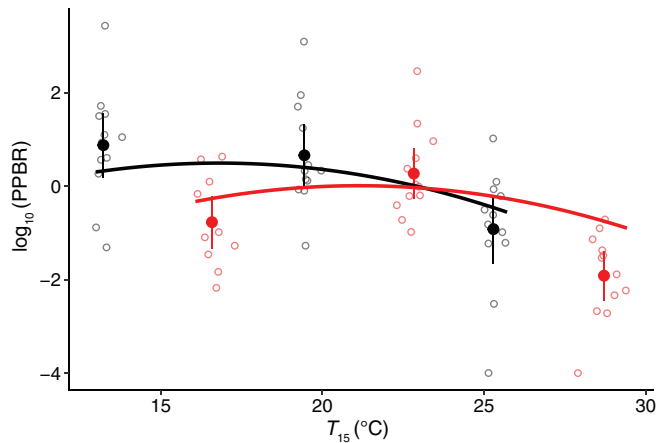


Figure 5.  $T_{15}$  and artificial warming effects on predator–prey biomass ratio on a log scale. Black and red colours represent unheated and heated mesocosms, respectively. Empty circles represent experimental data. Filled circles represent the mean values at each sampling date and bars represent the 95 % confident intervals. Lines represent the fit of the most parsimonious model.

## Discussion

This study aimed to expand our knowledge of the direct effects of warming and the indirect effects via phenotypic response to temperature (body size reduction and acclimation) on community composition and structure, PPBR and biomass distribution. While theoretical approaches have already been proposed to this end (Osmond et al. 2017, Sentis et al. 2017, Bideault et al. 2019), we still lack empirical evidence on the interactive effects of warming and body size reduction on communities to improve our understanding of the ecological impacts of global warming on aquatic ecosystems (Ohlberger 2013). Here, we used a mesocosm experiment to provide a more comprehensive understanding of the diversity of the effects of warming and temperature-induced body size reduction in an aquatic predator. Overall, our hypotheses on the effects of fish and their body size on community properties were not supported. Indeed, our findings suggest that fish

had only small effects on community structure, dynamics and trophic interactions, and that these effects are marginal compared to the stronger direct effects of seasonal temperature and artificial warming. Nevertheless, in line with our expectations, both seasonal and artificial warming had substantial impacts on community biomass, size spectrum and energetic transfer efficiency, providing new experimental evidence for the strong repercussions that global warming could have on aquatic ecosystems.

## Fish treatments and seasonal temperature influenced diversity through effects on species abundances

We observed that in mesocosms with fish, macroinvertebrate and phytoplankton species richness increased with increasing  $T_{15}$ , whereas it was not significantly affected by  $T_{15}$  in mesocosms without fish. In all fish treatments, the relationships between the Shannon index of the macroinvertebrate community and  $T_{15}$  were U-shaped (initially decreasing with  $T_{15}$ , then increasing at higher values of  $T_{15}$ ). Furthermore, this pattern was weaker with small-bodied fish with  $H'$  being less sensitive to  $T_{15}$ . For the phytoplankton community, fish treatments also modulated seasonal temperature effect on the Shannon index  $H'$ , as in mesocosms without fish, the relationship between  $H'$  and  $T_{15}$  was U-shaped, and in mesocosms with large and small-bodied fish Shannon index increased monotonically with  $T_{15}$ . We did not find any significant effect of artificial warming on ecological indices. Nor did we find significant differences in macroinvertebrate and phytoplankton community composition between experimental treatments using multivariate analyses. Taken together, this suggests that the observed differences in diversity were mainly due to variation in relative species abundance rather than species richness. This result is in line with Urrutia-Cordero et al. (2017), who showed that the loss of phytoplankton diversity is driven by the dominance of mixotrophic algae, whereas several other phytoplankton taxa may be displaced from the community. Chironomid density strongly increased in May, making this taxon very dominant in the macroinvertebrate

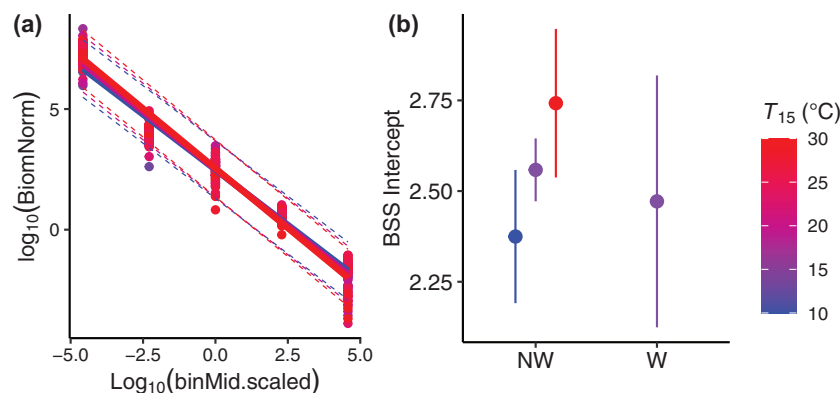


Figure 6. Increasing  $T_{15}$  effect on (a) BSS including all the mesocosms and (b) BSS intercept depending on the artificial warming. Colour gradient from blue to red represent the mean temperature of the 15 days prior the sampling date ( $T_{15}$ ), from 10 to 30 °C. Dotted lines and vertical bars represent the 95 % confidence interval.

community, which may explain why the relationship between the macroinvertebrate community Shannon index and seasonal temperature was U-shaped. In contrast, similar studies in mesocosms found that a +4°C warming increased phytoplankton species richness and diversity due to an increase in productivity (Yvon-Durocher et al. 2015), suggesting that warming can affect ecological indexes through changes in both abundance and community composition.

### **Presence and body size reduction of fish had little effect on community structure and trophic interactions**

Apart from the effects on species richness and the Shannon index, we detected very few independent or interactive effects of the presence or body size of our fish with warming on community composition, structure and BSS. These results were contrary to our expectations and contrast with previous mesocosm studies reporting that the effects of warming on the food chain biomass ratios depend on the presence of fish predators (Kratina et al. 2012, Hansson et al. 2013, Svensson et al. 2017). Our results also contrast with previous studies reporting that phenotypic variation at higher trophic levels and temperature acclimation can alter the trophic interactions strength, impacting community structure (Post et al. 2008, Sentis et al. 2015, 2017, Renneville et al. 2016, Bideault et al. 2019, Sohlström et al. 2021). Theoretically, smaller individuals could have a higher energetic efficiency, especially at higher temperatures, giving them a competitive advantage over larger individuals (Ohlberger 2013). In this scenario, the intensity of trophic cascades induced by smaller predators may have been greater than those induced by larger predators. Almost all the previous studies are either theoretical or laboratory experiments considering simplified systems (e.g. systems with one predator and one prey, but see Edeline et al. 2013).

### **Why fish have such a weak effect on communities?**

The weak effects of fish in our experiment may be explained by three mutually non-exclusive hypotheses. First, the addition of only four small predatory fish was not enough to induce a significant predation pressure on lower trophic levels compared to other invertebrate predators that had colonized the mesocosms, such as odonate larvae and notonecta, which could have dampened the predation effect of our medaka fish. In addition, the abundance of many other invertebrate predators could explain the lack of large zooplankton such as cladocerans, which are preferential prey for medaka fish (Edeline et al. 2013, Renneville et al. 2016). This may have limited our chances to detect any fish-induced trophic cascade. Second, the 10% reduction in body size between small- and large-bodied fish may not have been strong enough to detect impacts on community structure (although in Audzijonyte et al. (2013), 4% body size reduction in fish length-at-age over 50 years had strong impacts on interspecific interactions and community biomass). In addition, body

size differences between small and large-bodied fish decreased during the experiment, and body sizes were no longer significantly different at the end of the experiment (Supporting information). Third, in mesocosm experiments, the effects of differences in fish body size may have potentially be mitigated by uncontrolled environmental factors and compensation phenomena at the community level. This emphasizes the importance of setting complex and realistic experiments to shed light on the direct and indirect impacts of climate change on ecosystems.

### **Zooplankton grazing modifies phytoplankton communities more strongly than the direct effects of warming**

In line with our expectations, we found a unimodal effect of  $T_{15}$  on community density, although this effect differed between biological compartments. Macroinvertebrate density had a hump-shaped relationship with  $T_{15}$ , which can be explained by a strong increase in chironomid density in May (Supporting information). As for macroinvertebrate density, zooplankton density also showed a hump-shaped relationship with  $T_{15}$ . In contrast, we did not detect any effects of  $T_{15}$  on phytoplankton density in unheated mesocosms. In heated mesocosms, we found a U-shaped relationship between phytoplankton density and  $T_{15}$ . This result is surprising as we expected that increasing  $T_{15}$  would stimulate primary productivity, especially at the beginning of the experiment when temperatures were low. It is noteworthy that we observed that increasing  $T_{15}$  increased the density of microalgae in the unheated mesocosms (Supporting information). Although seasonal temperature or artificial warming had little effect on phytoplankton density, we observed architectural changes with an increase in less mobile colonial forms and less developed chloroplasts in heated mesocosms (results not shown). In addition, increased temperature can favour the development of parasitic fungi potentially harming the phytoplankton development (Ibelings et al. 2004). The decrease in phytoplankton density at intermediate  $T_{15}$  values was accompanied by an increase in zooplankton density, especially in the heated mesocosms. These results suggest that the main factor controlling phytoplankton dynamics was not temperature, but rather zooplankton grazing. More structuring effects of trophic cascades than direct temperature effects have already been reported by O'Connor et al. (2009). In addition, previous studies have shown that the capacity of zooplankton to control phytoplankton declines with warming (Petchey et al. 1999, Binzer et al. 2012), which may explain why phytoplankton density increases again at higher seasonal temperatures.

### **Increasing seasonal temperature and warming decreased heterotroph densities and total community biomass by affecting individual energy requirements**

We found that macroinvertebrate and zooplankton density were lower in heated mesocosms than in unheated ones. In



addition, when  $T_{15}$  was above 20°C, BSS intercept was lower in heated mesocosms than in unheated ones, and the decrease of BSS intercept with artificial warming was almost significant, suggesting a decrease in total community biomass with increasing seasonal temperature and artificial warming. These findings support our hypothesis that warming increases the energetic requirements of each individual. Thus, assuming that the resources available in the environment are constant, the environment may supply the needs of fewer individuals when it is heated (Allen et al. 2002). On the other hand, in line with Urrutia-Cordero et al. (2017), we did not detect any significant effects of artificial warming on phytoplankton density. Yvon-Durocher et al. (2015) and Velthuis et al. (2017) also showed that total community biomass was lower in heated mesocosms than in unheated ones, but their results differ from ours since the decrease in total biomass was due to a decrease in phytoplankton biomass rather than a decrease in zooplankton biomass. The difference in compartment responses to artificial warming when the number of trophic levels changes is the result of potential confounding effects of temperature (affecting bottom-up control) and predation (affecting top-down control), adding complexity to our ability to predict the effects of global warming on biological community.

### Warming-induced trophic transfer efficiency reduction as the hypothesized underlying mechanism for steeper slopes of BSS

The seminal study by Raymond Lindeman (1942) showed that the trophic transfer efficiency between adjacent trophic levels is about 10%, i.e. that 10% of the energy produced at one trophic level is returned to the next one (Lindeman 1942, Pauly and Christensen 1995). However, recent studies have suggested that this 10% value may change with warming due to higher metabolic costs at higher temperatures (Barneche and Allen 2018). Barneche et al. (2021) found empirical support for this theory by showing that an artificial warming of +4°C over seven years could reduce trophic transfer efficiency by up to 56% in mesocosm experiments. Consequently, we expected both seasonal and artificial warming to alter the biomass ratio between zooplankton and phytoplankton. Our findings are in line with both our expectations and recent studies, as in our experiment phytoplankton biomass was not strongly affected by either  $T_{15}$  or artificial warming. In contrast, zooplankton biomass decreased at higher  $T_{15}$  values and was lower in heated mesocosms. This suggests that the decrease in PPBR observed at higher  $T_{15}$  values and in heated mesocosms is due to a decrease in trophic transfer efficiency between zooplankton and phytoplankton with warming.

A decrease in trophic transfer efficiency under warming may also be the underlying mechanism explaining the steepening of BSS slopes with increasing  $T_{15}$ . In fact, we found that community composition did not change, that variations in individual carbon biomass were very small (Supporting information) and that primary producers barely responded to our experimental factors, suggesting that the steepening of the

BSS slope with increasing seasonal temperature is rather due to a decrease in the consumers density (i.e. invertebrate and zooplankton) which can be the result of a decrease in trophic transfer efficiency. Most of our results remain similar with the results obtained from analyses conducted using dates (i.e. number of day since the beginning of the experiment) rather than seasonal temperature  $T_{15}$ . However, one divergence is that the dates had no significant effects on the slope of the BSS. This suggests that the observed decline in trophic transfer efficiency was not strong enough to affect the BSS slope. Dossena et al. (2012) also found steeper BSS slopes in heated mesocosms in spring due to a decrease in the proportion of larger organisms, which might be explained by a decrease in trophic transfer efficiency, although the opposite was observed in autumn. Reduced trophic transfer efficiency with warming has important ecological implications, since trophic transfer is a key determinant of biomass distribution in ecosystems (Sheldon et al. 1977, Brown and Gillooly 2003), and reduced trophic transfer efficiency can negatively impact large consumers and affect ecosystem functioning (Barneche et al. 2021).

### Relative importance of direct versus indirect effects through body size changes of global warming on communities

Given the potential impacts of climate warming on communities, which may stem from both the direct effects of temperature and its indirect effects through body size reductions, it is crucial to understand how these direct and indirect effects combine to better predict the impact of global warming on aquatic ecosystems. Our findings indicate that predator body size reduction had negligible effects on community structure and trophic interactions. Conversely, direct seasonal and artificial warming effects significantly influenced community structure and energetic transfer efficiency. It is pertinent to point out that the significant ecological impacts of seasonal warming, emphasized in the study, are highly likely to encompass additional effects from unmeasured factors that vary throughout the season and covary with the rise in seasonal temperature. In the context of global warming, our results suggest that the direct effects of increasing temperature have a dominant effect on communities in comparison to indirect effects driven by changes in body size. While our experimental findings may suggest that accounting for temperature-induced body size reductions is unnecessary when assessing the ecological impacts of global warming, previous studies have suggested otherwise. For instance, Bernhardt et al. (2018) demonstrated that while warming directly reduces the carrying capacity of unicellular phytoplankton, decreasing body size can counteract this effect, mitigating temperature's negative impact. In addition, Réveillon et al. (2022) found that reduced body size weakened direct effects of warming on resource and consumer persistence. Nevertheless, these two studies were conducted under simplified laboratory conditions with one or two species, respectively. We thus need more empirical studies in richer communities under more natural conditions to better determine the relative importance of the

direct and indirect effects of global warming on ecological communities.

## Conclusion

Our results suggest that the effects of fish on community structure, dynamics and biomass distribution are relatively marginal compared to the direct effects of seasonal temperature and artificial warming. At the beginning of the experiment where temperature was low, macroinvertebrates and zooplankton densities increased with rising seasonal temperature but decreased when it reached higher temperature. Macroinvertebrate and zooplankton densities were lower in heated mesocosms, and BSS intercept was also lower in heated mesocosms at high seasonal temperatures (above 20°C), supporting the hypothesis that the environment may supply the needs of fewer individuals when heated (Allen et al. 2002, Yvon-Durocher et al. 2015). In contrast, phytoplankton were not strongly impacted by increasing seasonal temperature or artificial warming, suggesting that top-down effects were more structuring than direct temperature effects on primary production. In line with Barneche et al. (2021), we found empirical support for the theory that warming can alter trophic transfer efficiency since PPBRs were lower in heated mesocosms than in unheated ones and at high seasonal temperatures. The dependence of trophic transfer efficiency on temperature has important ecological implications as it is expected to affect community structure, dynamics and size spectrum and ecosystem functioning (Barneche et al. 2021).

## Speculations

Our study aims at determining which climate warming factor – body size shifts or temperature – has the most significant impacts on natural communities and ecosystems. Although previous study suggested that temperature-induced body size shifts could alter predator-prey interactions (Bideault et al. 2019) and modulate the effects of global warming on communities, our empirical findings suggest that the effects of body size changes are marginal compared to the direct effects of warming. However, we recognize that our result may be context specific (i.e. low-productivity oligotrophic environment), and we argue that further experimental studies in other environmental contexts are needed to broaden our understanding of the impacts of temperature-induced body size shifts on aquatic ecosystems. In addition, Martins et al. (2023) recently showed that changes in community composition contribute more to changes in body size than variations in size within species, but that substantial variations in the magnitude and direction of size changes were observed in response to each mechanism independently. Consequently, we believe that future research should focus on how body size shifts are articulated at different levels of biological organization (i.e. individual, population and community scales), and on their repercussions in natural ecosystems.

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## Author contributions

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## Data availability statement

Data are available from the Figshare Repository: <https://doi.org/10.6084/m9.figshare.23925291.v14> (Bazin et al. 2024).

## Supporting information

The Supporting information associated with this article is available with the online version.

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