RESEARCH ARTICLE

High temperature, predation, nutrient, and food quality drive dominance of small-sized zooplankton in Neotropical lakes

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Abstract

Body size plays a key role in the functioning of communities and ecosystems. However, this ecological trait is commonly under strong selection pressure by environmental drivers, such as temperature, nutrients, predation, and food quality. Understanding how environmental factors interact to shape the body size structure of communities is, therefore, of fundamental and applied interest. Using a unique database from 12 Neotropical lakes, we quantified the community-weighted mean trait (CWM) of zooplankton body size. We investigated how temperature, total phosphorus, abundance of predators (planktivorous fish) and food availability (abundance of edible and inedible algae) affect CWM of zooplankton body size. We also analyzed the interactions among these environmental predictors, and their cascading effects on zooplankton body size. We found that planktivorous fish, inedible algae, and edible algae had strong direct impacts on CWM of zooplankton body size. In particular, planktivorous fish and inedible algae decreased the CWM of body size, whereas edible algae increased it. Temperature and total phosphorus indirectly affected CWM of body size by increasing the abundance of planktivorous fish and inedible algae, and decreasing the abundance of edible algae. Our findings illustrate that environmental factors act in combination and affect zooplankton body size through multiple pathways. Therefore, focusing on the interaction between environmental predictors rather than just their isolated effects may provide a more mechanistic understanding of how environmental changes drive the body size structure of biotic communities.

Keywords Body size · Climate warming · Traits · Zooplankton · Top-down · Bottom-up

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Introduction

Understanding which factors shape the great variety of body sizes observed in nature has been a central challenge in ecology (Hutchinson 1959). Body size plays a key role in processes, such as reproductive fitness, physiology, predator–prey dynamics, and competition (White et al. 2007). Body size is crucial in the transfer of energy along food chains, mainly in aquatic ecosystems, where top-down and bottom-up mechanisms are particularly strong (Shurin et al. 2006; Hildrew et al. 2007). Zooplankton are an important group in aquatic ecosystems due to their central place, mediating energy flow in food webs (Brooks and Dodson 1965; Quirino et al. 2021) and connecting the classic food web with the microbial loop (Segovia et al. 2015). Zooplankton are highly heterogeneous group in terms of body size (Havens and Beaver 2011), being composed of small-sized (less than 100 μm) to relatively large-sized individuals (more than 1000 μm; Bonecker et al. 2011).

Multiple environmental drivers such as temperature (Karpowicz et al. 2020; Brucet et al. 2010), nutrients (Parra et al. 2009), food quality (Sun et al. 2012) and predation (Brooks and Dodson 1965; Iglesias et al. 2011) may directly or indirectly affect zooplankton body size. For instance, rising temperature often decreases the average body size of zooplankton (Havens et al. 2015) due to increase on metabolic demand of the individuals (Brown et al. 2004). This allows the juveniles to reach the adult stage faster and, as a result, adults are smaller (Brown et al. 2004). Higher metabolic demand in warmer temperatures increases the individual costs of growth, potentially leading to oxygen limitation for larger organisms (oxygen limitation hypothesis; Atkinson et al. 2006). Similarly, an increase in the abundance of large inedible algae (e.g., filamentous and colonial cyanobacteria) is directly linked to a decrease in zooplankton body size (Sun et al. 2012; Ger et al. 2014). Inedible algae are a low-quality food resource for zooplankton and affect mainly the larger individuals (e.g., daphniids and copepods), which are more sensitive to changes in food quality (Sun et al. 2012; Sikora et al. 2016). The aggregation of inedible algae into colonies or filaments inhibits feeding by large grazers by clogging the filtration apparatus (Ger et al. 2014). Predation by planktivorous fish also decreases zooplankton body size (Li et al. 2017). Planktivorous fish are size-selective predators and prefer larger individuals, which are more easily detectable and provide the greatest energy return (Brooks and Dodson 1965). Thereby, planktivorous fish are often positively correlated with small-sized individuals, as these zooplankton tend to be below the size spectrum for visual predation and do not represent adequate food quality (Brooks and Dodson 1965; Iglesias et al. 2011).

There are still knowledge gaps on how temperature, nutrients, food quality and predation interact to affect zooplankton body in 'real-world' ecosystems (Parra et al. 2009; Iglesias et al. 2011). Experimental evidence indicates that these environmental factors can act in combination to drive zooplankton body size (Brucet et al. 2010). Furthermore, if environmental factors affect each other, this could have a cascading impact on zooplankton body size. For instance, previous studies in lakes have shown that warming increases the abundance of small planktivorous fishes, which have multiple cohorts per year (i.e., multiple spawning during the year), thereby exerting intense annual predation on large-sized zooplankton (Jeppesen et al. 2007; Teixeira de Mello et al. 2009; Meerhoff et al. 2007, 2012). Warming also increases the abundance of large inedible algae (e.g., cyanobacteria), but decreases the abundance of small edible algae (Paerl and Huisman 2008). The increase in nutrients available (e.g., total phosphorus) is expected to also increase the abundance of planktivorous fish and large inedible algae (Carpenter et al. 2001; Jeppesen et al. 2000; Ger et al.

2014). Consequently, warming and total phosphorus could indirectly affect zooplankton body size by increasing predation (planktivorous fish) and decreasing food quality (increasing inedible algae and decreasing edible algae; Teixeira de Mello et al. 2009; Ger et al. 2014). Perhaps more importantly, however, no study has addressed the interaction of these multiple environmental predictors and their combined impacts on zooplankton body size in real-world ecosystems. This is particularly important in light of current global warming and eutrophication of aquatic ecosystems (Gardner et al. 2011; Moss et al. 2011).

Here we combined 10 years of sampling from 12 Neotropical shallow lakes to investigate the pathways by which temperature, nutrients, predation, and food quality affect the body size of zooplankton communities. In each lake sampled, we quantified the community-weighted mean trait (CWM) of zooplankton body size. We predicted that (i) abundance of planktivorous fish and inedible algae would directly decrease the CWM of zooplankton body size, whereas (ii) abundance of edible algae will increase the CWM of zooplankton body size (Li et al. 2017; Karpowicz et al. 2020; Li and Chen 2020). Moreover, (iii) temperature and total phosphorus would affect CWM of zooplankton body size by altering abundance of planktivorous fish, inedible and edible algae.

Methods

Study site

The study was carried out in 12 shallow lakes (mean depth ± 2.5 m) situated in the upper Paraná River floodplain (Fig. 1). These lakes (22°40'–22°50'S and 53°10'–53°40'W) cover approximately 802,150 km² between the Porto Primavera reservoir and the Itaipu reservoir. The 12 studied lakes are located in three different rivers: four lakes on the Ivinhema River, four lakes on the Paraná River and four lakes on the Baia River (Fig. 1). Importantly, these lakes are isolated and only connect to rivers during intense rainfall periods. Consequently, there is no constant and direct exchange of species between lakes—biotic communities within each lake may be considered independent (Braghin et al. 2018). Three sampling events were carried out during a dry (2000 and 2010) and rainy (2001 and 2011) season, totaling 72 samples. This design was chosen to cover a greater heterogeneity of environments, over space (sub-basins) and time (hydrological period), considering different temperature gradients, resources, productivity and predation. All sampling was standardized and performed during the Long-Term Ecological Research Project (PELD; http://www.peld.uem.br/).
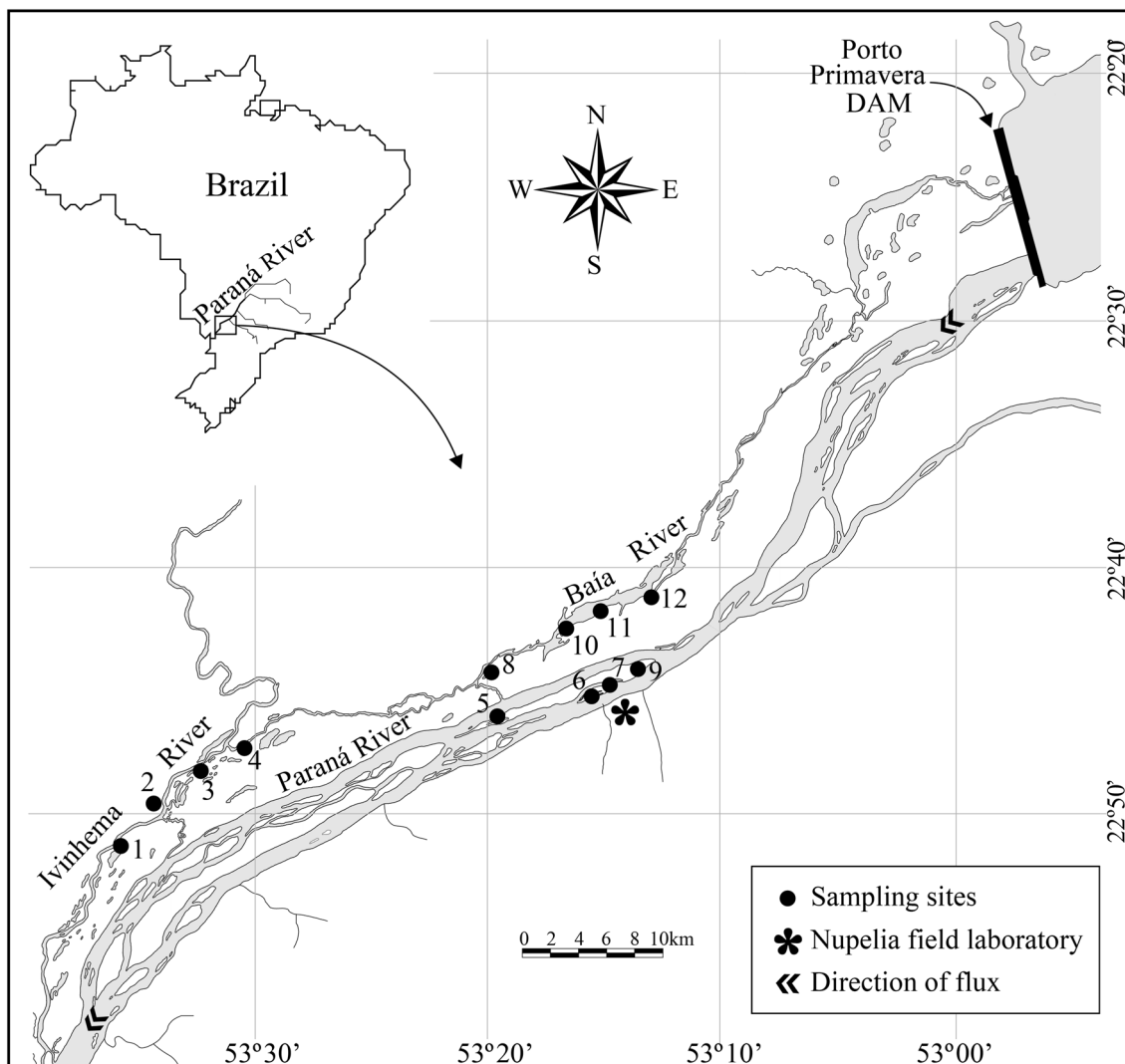
Fig. 1 Map of the upper Parana River floodplain showing the studied Lakes. All Lakers are isolated and indicated by numbers, where 1: Venture Lake; 2: Zé do Paco Lake; 3: Capivara Lake; 4: Jacaré Lake; 5: Osmar Lake; 6: Genipapo Lake; 7: Clara Lake; 8: Traíra Lake; 9: Pousada Lake; 10: Fechada Lake; 11: Pousada Garças Lake; and 12: Aurélio Lake

Sampling of zooplankton

Zooplankton sampling was performed at the subsurface (approximately 0.5 m depth) of the pelagic region of each lake using a motor pump and a plankton net (68 μm mesh size), and filtering 600 l of water. The sampled material was preserved in 4% formaldehyde solution buffered with calcium carbonate. Samples were always obtained using a boat moving at a constant speed to prevent a biased sampling. Large rotifer, cladoceran, and copepod identification and abundance estimation (ind m^{-3}) were carried out according to methodology specified in Lansac-Tôha et al. (2009); see species list in Table S1.

Zooplankton body size

The body size of zooplankton was estimated following specialized literature in which species accounting for 80% of the total abundance to each group are considered (i.e., species that account for 80% of the abundance of large rotifers, cladocerans, and copepods; see Azevedo and Bonecker 2003; Lansac-Tôha et al. 2009). In each lake, the length of each individual was measured through an optical microscope using an objective with a micrometer reticle. Because body shape differs among zooplankton groups (i.e., rotifers, cladocerans, and copepods), the way to measure body size for each group is different. In particular, it is considered: (i) the

distance between the upper and lower edge of the carapace, without spines for the rotifers; (ii) the distance from the head until the end of the carapace, without the helmet and spine for the cladocerans; and (iii) the distance from the head until the genital segment for the copepods. We then calculated the community abundance-weighted mean (CWM; Enquist et al. 2015) for body size trait. CWM is a measure of the trait dispersion within a given community weighted by the abundance of constituent species (Enquist et al. 2015):

$$CWM = \sum_{i=1}^{S} p_i x_i$$

where p_i is the relative abundance of species i ($i = 1, 2, \ldots,$ S), and x_i is the mean body size value for species i.

Environmental predictors

To assess the importance of the environmental predictors in drive body size structure of the zooplankton community, we measured five environmental predictors: (i) temperature, (ii) nutrient concentration using TP as a proxy, (iii) abundance of planktivorous fish, (iv) abundance of large inedible algae and (v) abundance of small edible algae. Collectively, these predictors represent four drivers of zooplankton body size, including warming, nutrients, predators, and food resources.

Temperature

We measured the water temperature (°C) in each lake using an analytical thermometer. The temperature was measured in the subsurface (between 0.5 and 1.0 m depth) of the pelagic region of each lake.

Nutrient available

We obtained in situ measurements of total phosphorus available in the water (mg L^{-1}). Total phosphorus covers all fractions of this nutrients, including particulate phosphate, dissolved organic phosphate, and orthophosphate. To do so, we took water samples in each lake, which were filtered through GF 52-C membranes (<10 h after sampling) and immediately frozen (−20 °C) for further analyses of total phosphorus in the laboratory. The total phosphorus was then measured in a spectrophotometer (Shimadzu, UV-1280), according to Golterman et al. (1978).

Abundance of potential predators

We measure the abundance of fish that could be potential predators of zooplankton (Table S2). These included small omnivorous fish species, which often have zooplankton in their stomachs (e.g., *Serrapinnus notomelas,*

Hyphessobrycon eques, and *Pyrrhulina australis*) (Carniatto et al. 2020) and exclusively planktivorous fish (e.g., *Hypophthalmus oremaculatus*; Santana-Porto and Andrian 2009). Fish were caught using 20 m long seine nets with a mesh size of 0.5 cm in the littoral and middle zones of each lake for a 24 h period (Agostinho et al. 2004). The fish captured were identified to species level (Ota et al. 2018), and abundance (ind m^{-2}) was indexed by Catch Per Unit Effort, using the size of the area dragged by the seine net. Fish sampling and manipulation was approved according to the Ethics Committee on the Use of Animals (CEUA no. 1420221028; ID 001,974).

Abundance of large inedible and edible algae

We measured the abundance of inedible and edible algae and used these two measures as indicators of food quality to zooplankton community (Ger et al. 2014). Inedible algae comprised the large algae species (above 100 μm) that are not easily consumed by Neotropical zooplankton (i.e., filamentous and colonial algae; Moi et al. 2021a). In contrast, edible algae comprised the small algae species, including nanoplankton (2–60 μm) and picoplankton (<2 μm), which are the main resource for Neotropical zooplankton that are small and cannot feed efficiently on large algae (Li and Chen 2020). Both large inedible and small edible algae were sampled at the subsurface (between 0.5 and 1.0 m depth) of the pelagic region of the lakes, using plastic bottles and preserved in 10% acetic acid (Bicudo and Menezes 2006). The analysis and counting of the algae were performed under a microscope. Algae abundance (ind ml^{-1}) was calculated according to the American Public Health Association-APHA (1985) and Utermohl (1958). The greatest axial dimensions (GALD) of phytoplankton cells, colonies and filaments, are used to describe the size structure of whole assemblages of species. In particular, a high abundance of small edible algae indicates higher food quality for zooplankton, whereas a high abundance of large inedible algae indicates poor food quality (Sun et al. 2012; Sikora et al. 2016).

Statistical analysis

In the first step of our analysis, we tested how the water level periods (fixed categorical: rainy and dry) affect (a) CWM of zooplankton body size, (b) temperature, (c) total phosphorus, (d) abundance of planktivorous fish, (e) abundance of inedible algae, and (f) abundance of inedible algae using linear mixed-effects models (LMEs) in the package NLME (Pinheiro et al. 2013). To control for non-independence between sites, we nested the lakes within rivers as a random effect in the model. This allowed the intercept to vary in each lake independently for each river. We ensured that the model assumptions of variance homogeneity, normality and

outliers were met. Thus, we ln-transformed some variables to achieve normality in the residuals.

Second, we investigated the relationship of the five environmental predictors with the CWM of zooplankton body size with a linear mixed effects model. The two seasonal sampling periods were nested within each lake, and lakes were nested within rivers as a random effect in the model. This nested structure allowed us to control the dependency between season periods, as well as control the dependency between lakes within each river. We tested temporal autocorrelation in our data with the function 'acf', but we did not find temporal autocorrelation. Visual analysis of residuals using graphical diagnostics (QQ plots and residual plots) revealed no departures from assumptions of normality or homoscedasticity. We assessed the multicollinearity of each predictor variable within model by calculating the variance inflation factor (VIF), but no VIF > 2 was observed. We ln-transformed the CWM of zooplankton body size to achieve normality in the residuals. To make all slopes in the model amenable for comparison, we standardized all predictors using the z-score (mean-centered and divided by the SD; Schielzeth 2010).

Finally, we fitted a piecewise structural equation modeling (pSEM; Lefcheck 2016) to analyze the relationships among the drivers (temperature, total phosphorus, abundance of planktivorous fish, and abundance of inedible and edible algae) and disentangle the direct and indirect pathways by which each driver influenced CWM of zooplankton body size. Importantly, the pSEM was also constructed using all standardized predictors (z-scored). In this way, standardized effects can be directly compared and thus indicate the relative importance of each path. This allowed us to calculate effect sizes of whole paths that represent cascading environmental predictors effects by summing up all indirect coefficients of each predictor (Grace 2006; Olobatuyi 2006).

Moreover, we applied a multigroup analysis in the pSEM to evaluate whether the relationship among predictors and their cascading effects on zooplankton body size varied across seasons. To do so, we considered the two seasons (dry and rainy) as a grouping variable in the SEM. In the multigroup analysis is implemented a model-wide interaction and tested each path between grouping variable (i.e., dry and rainy periods). In particular, a significant interaction indicates that the path differs among dry and rainy periods. The aim of this analysis was to identify which paths are consistent or differ across seasons when including the key drivers of zooplankton body size. The pSEM was constructed with the 'psem' function from the piecewiseSEM package (Lefcheck 2016). To carry out the piecewiseSEM, we specified an a priori meta-model of causal relationships among all environmental predictors based on our ecological knowledge and previous studies (Fig. S1). We also checked the multicollinearity in pSEM based on the variance inflation

factor (VIF) for each predictor. No predictor showed VIF > 2. We present the standardized coefficient for each path. The indirect effects of environmental predictors on zooplankton body size were calculated through multiplication of direct standardized coefficients. Path significance was obtained by maximum likelihood, and model fit was evaluated using Shipley's test of d-separation through Fisher's C-statistic ($P > 0.05$ indicates no missing path). All analyses were conducted in R version 4.1.1. (R Core Team 2021).

Results

Zooplankton community

Over the 12 sampled lakes, we found 63 species of zooplankton. Rotifers were the most abundant group with 37 species (58.7%), followed by cladocerans with 22 species (34.9%), and copepods with 4 species (6.5; Table S1). Regarding zooplankton body size, the small-size individuals (≤300 μm) accounted for the highest percentage of abundance (51%), followed by medium-size individuals (≥300 ≤600 μm; 35%), and large-size individuals (≥600 μm; 12%; Table S1).

Variation in zooplankton body size and environmental predictors across seasons

We found no significant differences in the CWM of zooplankton body size (difference = −0.056, $t = −0.520$, $P = 0.605$) and in the abundance of small edible algae (difference = 0.429, $t = 1.331$, $P = 0.188$) between the rainy and dry seasons. In contrast, there were significant differences in the temperature (difference = −0.166, $t = −5.641$, $P < 0.001$), total phosphorus (difference = 4.450, $t = 56.670$, $P < 0.001$), abundance of planktivorous fish (difference = 1.941, $t = 4.567$, $P < 0.001$), and abundance of large inedible algae (difference = 1.462, $t = 3.362$, $P = 0.001$) between rainy and dry seasons. Notably, temperature was higher during the rainy season (Fig. 2; Table S3). Conversely, total phosphorus, abundance of planktivorous fish, and abundance of inedible algae were higher during the dry season (Fig. 2b–f; Table S3).

Environmental predictors drive zooplankton body size

Environmental predictors accounted for 60% of the explained variance in CWM of zooplankton body size. There were significant relationships of planktivorous fish, inedible algae, and edible algae with the CWM of zooplankton body size (Fig. 3a; Table 1). Planktivorous fish and large inedible algae were strongly and negatively related with CWM of zooplankton body size (Fig. 3b, c). By contrast, small

Fig. 2 Violin plot showing the differences in **a** CWM of zooplankton body size, **b** temperature, **c** total phosphorus, **d** planktivorous fish, **e** inedible algae, and **f** edible algae between dry and rainy seasons. The blue triangle in the center of each plot denotes mean values. Jitter function was used on the data to prevent overplotting
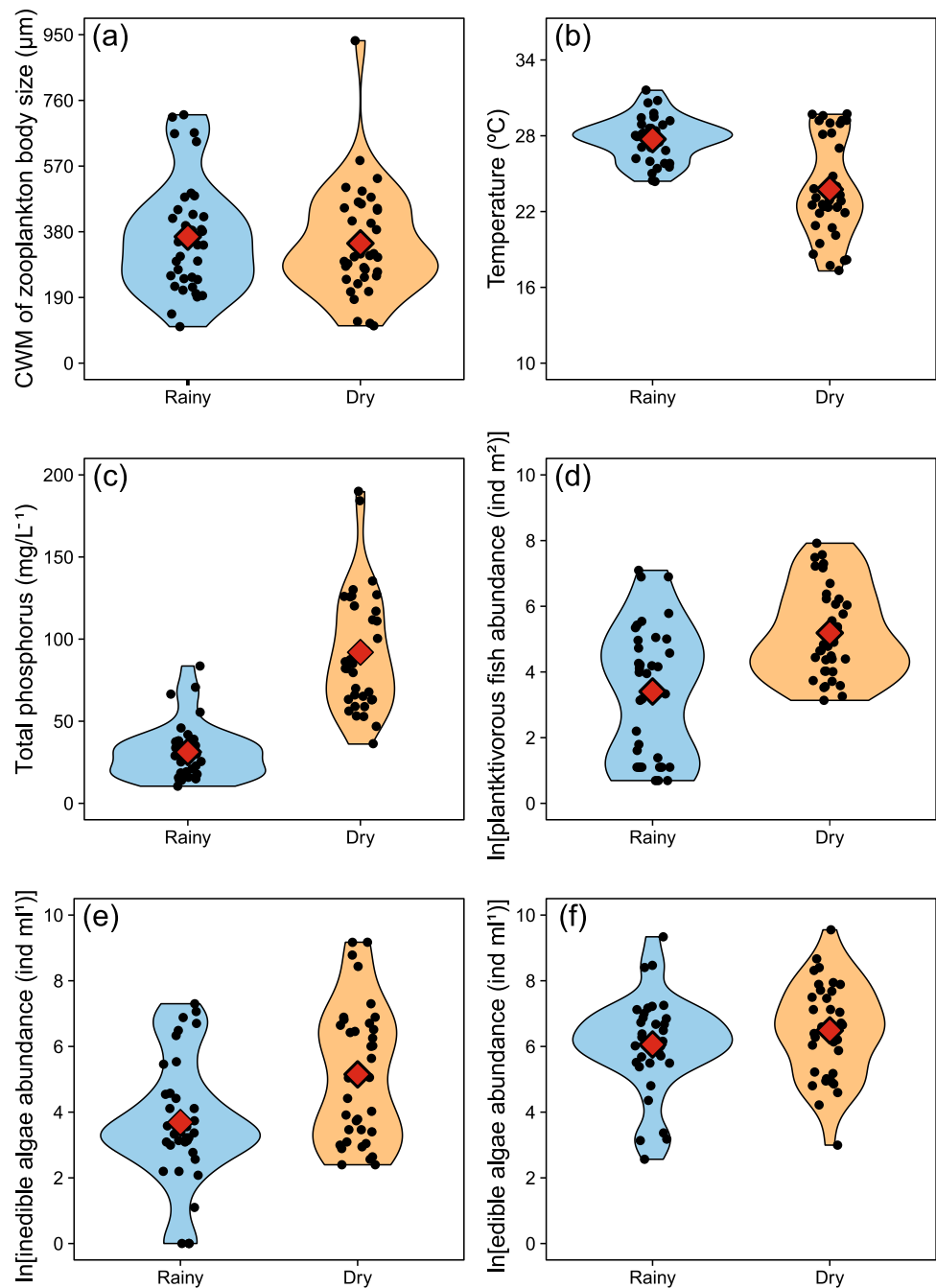
edible algae had a strong positive relationship with CWM of zooplankton body size (Fig. 3d). Temperature and total phosphorus were not significantly related to the CWM of zooplankton body size, although their relationship tended towards the negative (Fig. 3a).

Direct and indirect effects of environmental predictors on zooplankton body size

PiecewiseSEM had a good fit (Fisher'C = 4.454, $P = 0.108$), and revealed consistent interactions between

environmental predictors, with cascading effects on the CWM of zooplankton body size (Table S4). In particular, the temperature had an direct and indirect negative effects on zooplankton body size, mediated by a negative effect on the abundance of small edible algae (Fig. 4a; SEM: temperature—edible algae: –0.390 × edible algae—zooplankton CWM: 0.292 = −0.114). Similarly, total phosphorus had indirect negative effects on zooplankton body size mediated by positive effects on the abundance of planktivorous fish and large inedible algae (Fig. 4a; indirect effect on zooplankton CWM via planktivorous fish
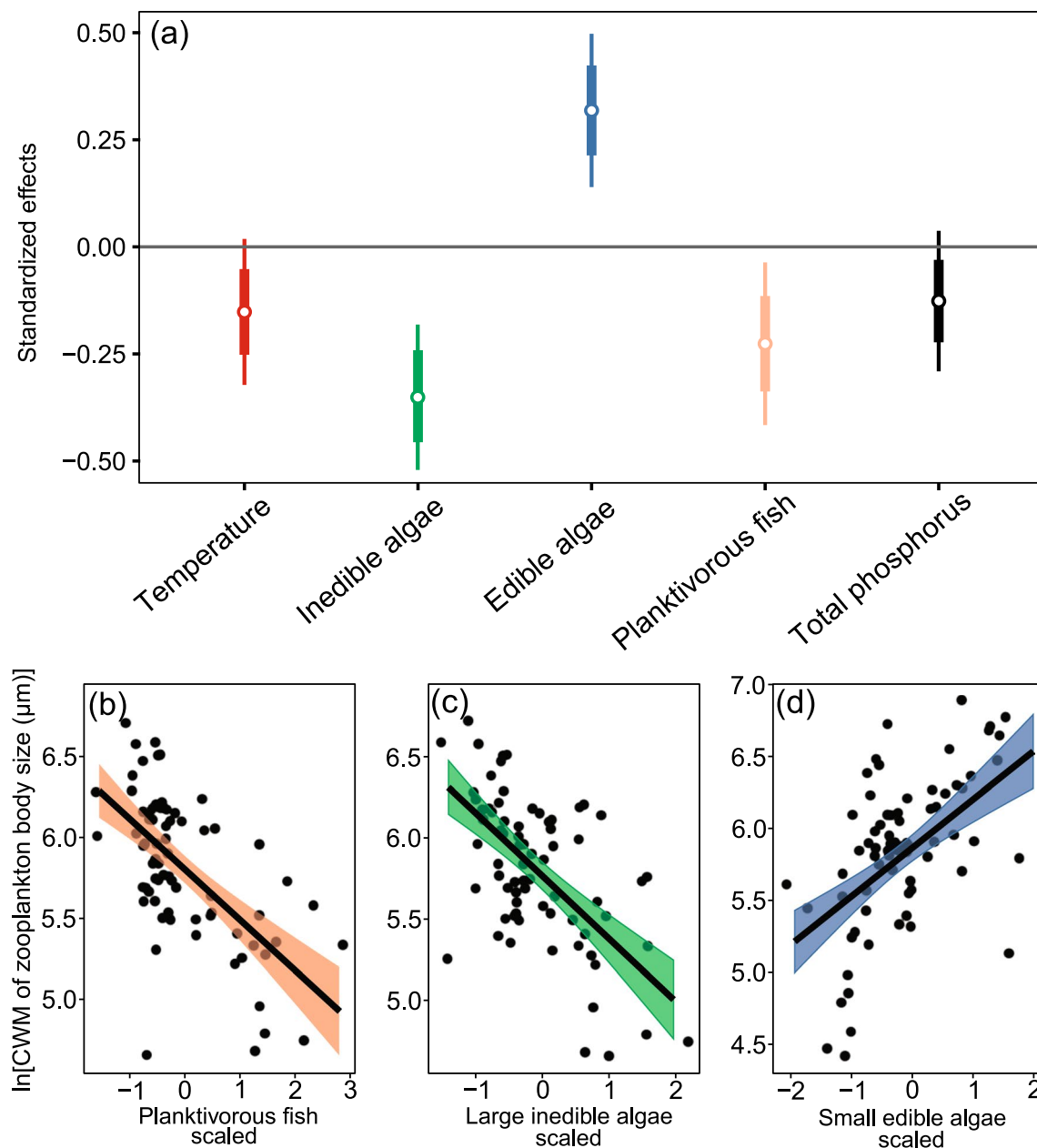
Fig. 3 Linear mixed-effect models showing responses fish body size to environmental predictors. **a** Effects of predictors, including temperature, total phosphorus, abundance of planktivorous fish, abundance of inedible algae, and abundance of edible algae on CWM of fish body size. Effect sizes were adjusted using linear mixed-effects models. Colors represent predictors: red (temperature), green (inedible algae), blue (edible algae), orange (planktivorous fish), and black (total phosphorus). See Table 1 for the full model output. Relationships of the environmental predictors that were significantly related with the CWM of fish body size: **b** abundance of planktivorous fish, **c** abundance of inedible algae, an **d** abundance of edible algae. Lines show the best model fits and colored shaded areas correspond to the 95% confidence interval from linear mixed effect models. Model predictions were calculated using a model averaging procedure (see Methods). Environmental predictors were scaled to interpret parameter estimate on a comparable scale. The CWM of zooplankton body size was ln-transformed to achieve linearity of the residuals

−0.067, indirect effect on zooplankton CWM via inedible algae −0.089). There was also an indirect negative effect of inedible algae on zooplankton body size through a decline in abundance of edible algae (Fig. 4a; indirect effect on zooplankton CWM via edible algae = −0.086).

Decomposing the direct and cascading (indirect) effects of the environmental predictors on CWM of zooplankton body size, we observed that temperature, planktivorous fish, inedible algae and edible algae impacted zooplankton body size through direct effects (Fig. 4b). In addition,

Table 1 Results of the linear mixed-effects models showing effects of the environmental predictors (temperature, total phosphorus, abundance of planktivorous fish, and abundance of inedible and edible algae) on CWM of zooplankton body size

Predictors	CWM of zooplankton boy size						
	Estimate	CI (lower)	CI (upper)	Std.error	df	t-test	P-value
Intercept	0.041	−0.149	0.227	0.097	24.0	0.425	0.674
Temperature	−0.118	−0.329	0.010	0.082	66.1	−2.05	0.054
Total phosphorus	−0.093	−0.254	0.067	0.084	65.9	−1.11	0.272
Planktivorous fish	−0.246	−0.447	−0.049	0.104	63.1	−2.37	0.021*
Inedible algae	−0.456	−0.690	−0.216	0.124	63.3	−3.68	<0.001***
Edible algae	0.359	0.158	0.564	0.106	63.5	3.38	0.001**

Std.error Standard error; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

temperature and total phosphorus impacted zooplankton body size mainly via cascading effects (Fig. 4b).

Finally, comparing the results of PiecewiseSEM between rainy and dry season (multigroup analysis), we observed that the effect of total phosphorus on abundance of large inedible algae significantly differed between the two seasons (Table S5). In particular, there was a strong positive effect of total phosphorus on abundance of large inedible algae during the dry season (Fig. S2 and Table S6). In contrast, the effect of total phosphorus on abundance of large inedible algae was negative and not significant during the rainy season (Fig. S2 and Table S6). Other pathways did not differ between the rainy and dry season.

Discussion

In this study, we analyzed the simultaneous influence of five environmental predictors on the body size of zooplankton communities. By disentangling the direct and cascading effects of these predictors, we found that temperature, planktivorous fish, inedible algae, and edible algae directly affected the zooplankton body size. Moreover, temperature and total phosphorus affected zooplankton body size indirectly via changes in planktivorous fish, inedible algae, and edible algae. Considering that planktivorous fish are major predators of zooplankton (Brooks and Dodson 1965; Iglesias et al. 2011), whereas algae is the major food resource (Onandia et al. 2015), this indicates that changes in predation and food quality are the main drivers of body size of zooplankton communities. Although temperature and nutrients are well known to control the entire dynamics of aquatic ecosystems (Brucet et al. 2010; Bouraï et al. 2020), our results suggest that these drivers affect zooplankton body size by increasing predation and changing food quality. Our findings are consistent with previous analyses from terrestrial realms (Greve et al. 2008; Provost et al. 2020) demonstrating that multiple environmental predictors can work simultaneously to shape the body size structure of biotic communities in different realms. In agreement with our prediction, the effects of environmental predictors on zooplankton body size include direct and indirect pathways. Moreover, some pathways, e.g., effect of total phosphorus on large inedible algae seem to differ between rainy and dry season (Fig. S2), which likely reflect the fact that total phosphorus and large inedible algae also differed between dry and rainy periods (Fig. 2). Collectively, our findings suggest that studies focusing on environmental predictors in isolation or analyzing only their direct effects hinders our ability to understand how biodiversity responds to global environmental changes, such as warming and eutrophication.

Besides weak direct effects, temperature had a strong indirect contribution in dictating zooplankton body size. In particular, temperature indirectly decreased the zooplankton body size by increasing total phosphorus and decreasing the abundance of small edible algae (Fig. 4). During warmer periods, the studied lakes were classified as eutrophic or hypereutrophic (TSI > 60), and were dominated by large inedible algae, whereas those small edible algae were less abundant. Warming may increase total phosphorus in lakes via an increase in the rate of nutrients mineralization in the catchment soil (Brookshire et al. 2011). High temperature also increases water column stratification, which together with lower oxygen solubility, leads to bottom deoxygenation and favors further sediment release of P to the water column (Jeppesen et al. 2009; Moss et al. 2011). Furthermore, warming often favors large inedible algae through an increase in vertical stratification, which results in greater fitness by cyanobacteria that are able to regulate their buoyancy (Visser et al. 2016). Combined with the high total phosphorus, warming may improve the competitive ability of large inedible algae (Paerl and Huisman 2008; but see Lürling et al. 2013). This scenario leads to an impoverishment in the food quality for the zooplankton, which affect mainly the larger individuals (Sun et al. 2012; Sikora et al. 2016). In general, the decrease in zooplankton body size with increasing temperature agree with previous studies (Havens et al. 2015; Evans et al. 2020) suggesting that our results are robust.

Total phosphorus also indirectly affected zooplankton body size mediated by an increased in abundance of planktivorous fish and large inedible algae (Fig. 4). These results are consistent with the idea that increased in nutrient

concentrations favors direct shifts of zooplankton toward small-sized individuals, as small individuals may be better competitors at higher nutrient levels (Vehmaa et al. 2018). This is mainly due to (i) increased abundance of large inedible algae (Ghadouani et al. 2003; Hsieh et al. 2011) and (ii) increased abundance of planktivorous fish (Jeppesen et al. 2000). In eutrophic lakes, large piscivorous fish are often less abundant or absent (Jeppesen et al. 2000; Carpenter et al. 2001), favoring planktivorous fish, which exert strong predation on large-sized zooplankton.

Considering that the effect of total phosphorus on the abundance of large inedible algae was markedly stronger during the dry season (Fig. S2), the indirect effect of total phosphorus on zooplankton body size mediated by large inedible algae was also stronger during the dry season. This can be explained by the fact that during the dry season the concentration of nutrients in the floodplain lakes is markedly higher due to the low water level and the higher resuspension of nutrients from the sediment (Mormul et al. 2012). Similarly, algae abundance is also higher during the dry season, which is mainly due to the high nutrient levels during this period (Moi et al. 2021a). By contrast, during the rainy period, the effect of total phosphorus on abundance of large inedible algae is not significant; consequently, the total phosphorus did not indirectly affect the zooplankton body size. This is likely due to the dilution effect during the rainy season, which decreases the nutrient level and algae abundance (Mormul et al. 2012). These findings suggest that while droughts can potentiate, rainfall can reduce the impact of environmental factors on the body size of biotic communities. Given that our study includes only a dry and rainy period, more studies are needed that focus on how droughts and rains can influence the ability of environmental predictors to shape the body size of biotic communities.

Large inedible algae had a direct negative effect on zooplankton body size. The studied lakes are typically characterized by low water transparency and high nutrient levels (Roberto et al. 2009), which usually favor the development of large inedible algae. These organisms are a poorer food resource for zooplankton, which tends to work against the development of large-sized zooplankton by clogging the filtration apparatus (Gliwicz 1990). In addition, some species of inedible phytoplankton release dissolved organic carbon, favoring the development of bacterioplankton, an important food resource for small-sized zooplankton (Segovia et al. 2015). We found that small edible algae had a positive effect on zooplankton body size. This can be partly explained by fact that the zooplankton in the Neotropics are small and can only feed efficiently on small edible algae (Moi et al. 2021a). As a result, zooplankton are directly dependent on the abundance of these small algae, and as their abundance decreases, the zooplankton body size also decreases. Given that the total phosphorus favored the large inedible

algae, these findings suggest an important bottom-up effect decreasing zooplankton body size (Hiltunen et al. 2021).

Planktivorous fish played a direct role in decreasing the zooplankton body size. These results match to the frequent disappearance of large zooplankton (such as Cyclopidae and Daphniidae), when planktivorous fishes are abundant (Meerhoff et al. 2007; Iglesias et al. 2011). This finding is consistent with the size-specific predation prediction, since planktivorous fish are highly size-selective predators (Ersoy et al. 2019; Karpowicz et al. 2020). While small-sized zooplankton are less visible and vulnerable to fish predation, large-size zooplankton are highly preyed upon by planktivorous fish (Zhang et al. 2017; Sha et al. 2020). Most importantly, the predation by planktivorous fish is often strengthened with increasing nutrient concentrations (Braz et al. 2020). Moreover, by feeding on large-sized zooplankton, fish predation pressure contributes to releasing small-sized zooplankton from exploitative competition and mechanical interference (Gilbert 1988). Collectively, our findings indicate an important top-down effect of planktivorous fish, contributing to decrease zooplankton body size in Neotropical lakes.

Our findings provide support for the occurrence of bottom-up (via food quality depletion) and top-down (via predation) mechanisms operating simultaneously to shape zooplankton body size (Vehmaa et al. 2018; Li and Chen 2020). The results suggest that both bottom-up and top-down mechanisms were intensified by increasing temperature and total phosphorus. This implies that warming and nutrient concentrations may in combination potentiate top-down and bottom-up mechanisms and drive changes in body size structure of biotic communities. These findings are particularly concerning, because warming and eutrophication (by increasing nutrients) have occurred simultaneously in aquatic ecosystems (Bouraï et al. 2020), causing synergistic effects on biotic communities (Moss et al. 2011). Warming and eutrophication are among the major global drivers of biodiversity loss in terrestrial, marine and freshwater systems (Hautier et al. 2009; Butchart et al. 2010). These two global drivers have been strongly linked to reducing average body size of species worldwide, leading even to extirpation of large-sized species (Daufresne et al. 2009; Suikkanen et al. 2013). In turn, body size decline has strong potential to affect the functioning of aquatic ecosystems, as large-sized species often contribute more to ecosystem functioning than small-sized species (Moi et al. 2021b).

Zooplankton underpin multiple functions and services that freshwater ecosystems provide for human well-being. These include primary productivity, biogeochemical cycling, secondary productivity and stabilization of aquatic food webs (Hébert et al. 2017; Sodré and Bozelli 2019). In this study, we show that temperature, total phosphorus, planktivorous fish and large inedible algae work in combination to decrease zooplankton body size. Neotropical shallow lakes

(a)

(b)
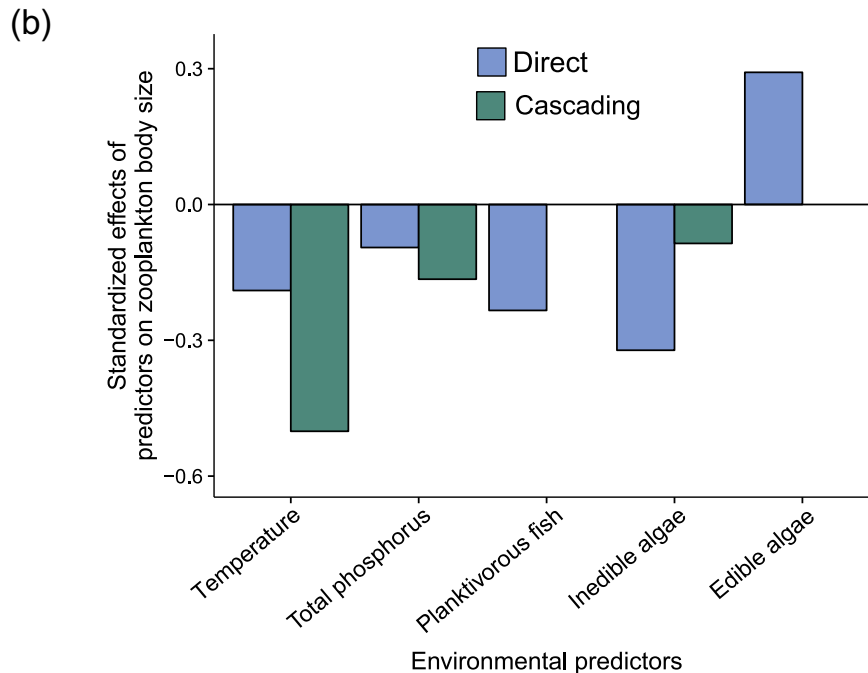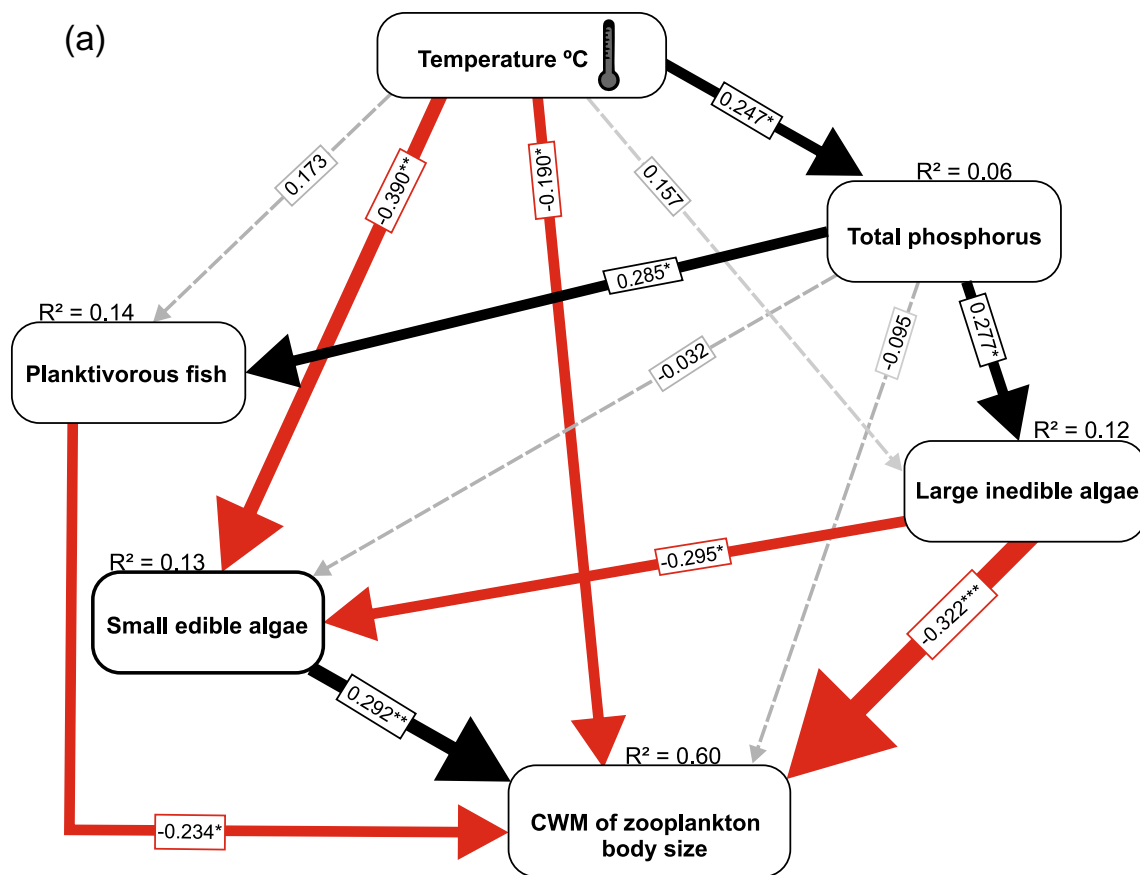
often sustain a high abundance of small planktivorous fish and large inedible phytoplankton. In addition, these lakes have been subject to high temperatures and nutrient concentrations, which have driven the decline in zooplankton body size. These conditions make warm lakes comparatively less resilient than similar cold lakes to changes in external drivers (Meerhoff et al. 2012). In essence, preserving large-sized zooplankton will become increasingly challenging given the

◄**Fig. 4** Structural equation model of **a** relationships among five environmental predictors (i.e., temperature, total phosphorous, planktivorous fish, inedible algae, and edible algae) and their cascading (indirect) effects on zooplankton body size (CWM). Solid black and red arrows represent significant positive and negative paths ($P \leq 0.05$), respectively. Dashed gray arrows represent non-significant paths ($P \geq 0.05$). The thickness of the significant paths represents the magnitude of the standardized regression coefficient or effect sizes, indicated at the arrows. R^2 for component models are given in the boxes of endogenous variables. Significance levels of each predictor are *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Full model results are provided in Table S4. **b** Show the standardized direct and indirect effect of each environmental predictor on zooplankton body size. Importantly, a priori, we standardized all environmental predictors (z-scored: centered to mean and divided by the SD) to interpret slope estimates on a comparable scale. Effects are derived from the SEMs, and standardized indirect coefficient is computed based on multiplication of direct coefficients

projected increases in the temperature and eutrophication over the next few decades in the Neotropics (IPCC 2014).

Declarations

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