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Biodiversity-mediated effects on ecosystem functioning depend on the type and intensity of environmental disturbances

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Environmental disturbances affect ecosystem functioning through changes in organisms' metabolism (direct effect) and biodiversity loss (indirect or biodiversity-mediated effect). It is still a challenge to separate direct and biodiversity-mediated effects of environmental changes on ecosystem functioning due to the difficulties in isolating 'true' biodiversity loss effects. Furthermore, it is still unclear whether biodiversity-mediated effects are as important as direct effects. In this study, we performed an experiment in artificial microcosms to disentangle biodiversity-mediated and direct effects of two major environmental disturbances on the functioning of aquatic ecosystems: increases in temperature and salinity. The ecosystem function analyzed was the microalgae predation by the zooplankton community (zooplankton grazing rates). Temperature and salinity increases affected the zooplankton grazing rates due to changes in community composition and abundance, as well as organism performance. The impact of salinity changes on community structure was higher than that of temperature; however, the importance of biodiversity-mediated and direct effects was similar to regulating the ecosystem functioning, albeit they have presented different directions and magnitude across the treatments. At a moderate level of temperature increase, we observed that the biodiversity-mediated effect was more relevant than the direct effect, with negative effects on the overall grazing rates. Our results suggest that disturbances can affect the functioning of aquatic environments through a set of complex biological mechanisms that balance direct and biodiversity-mediated effects. We concluded that the relative importance of biodiversity-mediated effects depends on the type and intensity of the disturbance.

Keywords: biodiversity-mediated effects, ecosystem functioning, environmental disturbance, grazing rates, zooplankton community

Introduction

Environmental changes, such as climate change, affect all levels of organization in ecology, from the physiology of the organisms to the functioning of entire biomes,

and are major drivers of biodiversity loss (Bellard et al. 2012). Biodiversity loss also decreases and destabilizes ecosystem functioning (Hooper et al. 2012). Therefore, environmental changes can affect ecosystem functioning in, at least, two different ways: directly, through adjustments in the phenology, metabolism and behavior of organisms; and indirectly, by species loss, and changes in the dynamics of different populations and community structure (Hillebrand and Matthiessen 2009). Disentangling the direct and indirect effects of environmental changes on ecosystem functions is still a challenge in ecological studies (De Laender et al. 2016). Furthermore, a significant gap exists in understanding to what extent indirect (or biodiversity-mediated) effects are as important as direct effects.

Climate change particularly threatens aquatic ecosystems, through changes in community composition, trophic interactions and habitat structure (Pires et al. 2016, Woodward et al. 2016, Marino et al. 2017). Changes in the multiple parameters of climate are expected, which include alterations in rainfall distribution, temperature increases and rising sea levels (IPCC 2014). Coastal inland aquatic ecosystems are particularly vulnerable as they are shallow and located near the sea. Shallow aquatic ecosystems are typically characterized by a high diel temperature variability (Esteves et al. 2008) with maximum values reaching beyond 35°C in some tropical coastal lakes (Farjalla et al. 2005). Both temperature variability and maximum temperature is predicted to increase in shallow aquatic ecosystems related to the climate change. Due to the proximity of the sea, rising sea levels is predicted to increase the saltwater intake in coastal inland ecosystems, such as coastal lagoons, river deltas and estuaries. In sum, it is very likely that climate changes will lead to increases in the temperature and salinity of coastal aquatic ecosystems.

Temperature and salinity variation directly and indirectly affect the aquatic ecosystem functioning respectively through changes in the physiology of aquatic organisms and the structure of aquatic communities (Jeppesen et al. 2010, Bellard et al. 2012). At the physiological level, warming accelerates the metabolism (Brown et al. 2004) and salinity increase produces physiological stress especially in oligohaline species due to their limited osmoregulation capability (Achuthankutty et al. 2000). These changes directly affect energy flux in aquatic ecosystems due to changes in the growth and feeding habits of the organisms. At the community level, temperature and salinity increases alter community structure and simplify aquatic food webs, through biodiversity loss (Jeppesen et al. 2010, Loureiro et al. 2013, Castillo et al. 2017, Marino et al. 2018).

Changes in community structure can have strong implications on ecosystem functioning, considering that there is a tradeoff between the ability to function and disturbance tolerance (Gilman et al. 2010). As biodiversity within the community declines, the community's ability to function is also reduced, thus promoting negative biodiversity-mediated effects. As a consequence, the relative importance of biodiversity-mediated effects in the face of the direct effects would be higher in cases of more intense disturbances.

Salinity fluctuations are considered as one of the most important ecological drivers in structuring aquatic communities of coastal lagoons (Esteves et al. 2008). Major changes in salinity occasionally happen and are generally caused by natural or artificial sand barriers opening (Kozlowsky-Suzuki and Bozelli 2004). Most species are physiologically adapted to specific salinity conditions, hence even slight increases can cause abrupt changes in the functioning of these systems (Kozlowsky-Suzuki and Bozelli 2004). On the other hand, we can expect that aquatic organisms are to some extent physiologically adapted to temperature variation in these ecosystems, due to the natural variation of temperature in shallow coastal lagoons. Therefore salinity increase would be a stronger environmental disturbance to aquatic communities of coastal lagoons compared to warming. Consequently, we would expect that the relative importance of biodiversity-mediated effects is higher in disturbances caused by salinity increases than in disturbances caused by warming.

In this study, we used an innovative experimental approach to disentangle biodiversity-mediated and direct effects of temperature and salinity increases on the functioning of aquatic ecosystems. We hypothesized that the relative importance of biodiversity-mediated effects will be higher 1) in disturbances caused by salinity increases than in disturbances caused by warming; 2) at higher than at moderate levels of environmental disturbance. We used artificial microcosms to separate these effects by manipulating zooplankton community structure and environmental disturbance from a freshwater coastal lagoon. We chose the zooplankton community because it plays a key role in aquatic ecosystems, especially in nutrient cycling and energy flow, as it transfers organic matter from primary producers to higher secondary consumers. In addition, most of zooplankton organisms have typical short generation times and their populations can respond quickly to environmental changes (Dam 2013). Previous studies suggested that salinity is one of the main drivers of changes in zooplankton community composition (Schallenberg et al. 2003) and temperature is critical for zooplankton performance (O'Connor et al. 2009). They are also considered 'sentinels' of climate change, since their survival, physiological functions and life history traits are very sensitive to temperature and salinity variations (Chen and Stillman 2012).

We set up an experimental approach that allowed us to measure and compare the relative importance of biodiversity-mediated and direct effects through a set of comparisons between zooplankton grazing rates (GR) under disturbed and undisturbed conditions. First, we started with a community isolated from a natural coastal lagoon. Then we exposed samples of this community to salinity and temperature increases over a sufficient period so that community composition could change in response. Subsequently, we used these communities to test their immediate GR response to specific environmental conditions (physiological, direct effects) and to community structure changes (biodiversity-mediated effects). To do that we measured GR: 1) after removing the disturbance and allowing organismal function to recover (hereafter, 'undisturbed conditions') and; 2) without removing the disturbance ('disturbed

conditions'). In comparison to the GR in a never-disturbed control, the former isolates the biodiversity-mediated effect, while the latter captures the combination of biodiversity-mediated and direct effects.

Material and methods

Study site and experimental set up

Study site
We sampled a zooplankton community from Jurubatiba lagoon, located at the Restinga de Jurubatiba National Park, Rio de Janeiro, Brazil (22°14′S, 41°33′W). Jurubatiba is a shallow freshwater lagoon that is separated from the sea by a 50-m-wide sand barrier; it has a surface area of 0.34 km² and a maximum depth of 3.5 m. Abrupt variations in salinity are rare and they are mainly attributed to storms, high tides or both, causing seawater inflow over the sand barrier (Branco et al. 2008). The annual mean water temperature is about 25°C (Caliman et al. 2010). The high habitat heterogeneity (mostly promoted by macrophytes) allows for the development of a very diverse aquatic fauna, including a large number of zooplankton taxa (Branco et al. 2008).

Zooplankton sampling
We collected the zooplankton community in the central region of the Jurubatiba lagoon using vertical hauls from bottom to surface with a 50 μm plankton mesh. The filtered zooplankton community was concentrated in a 20-l container and brought to the laboratory. At the lab, we immediately diluted the concentrated sample in a 100-l container with Jurubatiba water filtered in a 50 μm mesh (zooplankton-free, but containing nutrients, suspended particles, some microalgae and other organisms smaller than 50 μm) which was considered the 'initial pool'. This procedure allowed us to create a homogenized zooplankton community ensuring similar composition.

Zooplankton assembly
We set up a microcosm experiment to promote disturbance-driven changes in zooplankton community structure. First, we established a control condition treatment according to the temperature and salinity of the Jurubatiba lagoon during sampling (25°C and 0.2 PSU). Then, we established two independent environmental disturbances (temperature and salinity increase), with two levels of intensity, leading to a total of five treatments. Temperature and salinity increase treatments were defined after pilot experiments, in which we tested five potential temperature and salinity levels as drivers to produce changes in the zooplankton community. We defined the two levels of temperature (30°C and 35°C) and salinity (2.0 PSU and 6.0 PSU) based on the differences that these effects had on zooplankton community structure. For the temperature disturbance, we maintained the microcosms in two different BOD chambers regulated at 30°C and 35°C, while salinity was kept at 0.2 PSU. For the salinity increase

treatment, we increased the salinity levels to 2.0 PSU and 6.0 PSU by adding sea salt (phosphate and nitrate free) into the microcosms, while temperature was kept at 25°C. Water bodies with salinity 2.0 PSU are considered oligohaline, while water bodies with a 6.0 PSU salinity are considered mesohaline. This transition can be considered critical for many freshwater organisms, including zooplankton (Santangelo et al. 2014). Each of the five treatments had five replicates, totaling 25 experimental units (Fig. 1a).

We used transparent 2.8-l polyethylene containers as experimental microcosms, which were filled with the 'initial pool' water. The zooplankton community in each experimental microcosm was incubated for five days in a photoperiod of 12:12 h light:dark. According to the pilot experiments, this incubation period was enough to promote changes in the zooplankton community structure. For more details on the effects of the environmental disturbances on zooplankton community during the pilot experiment test see the Supplementary material Apendix 1 Table A7. During this experimental step, zooplankton fed on the resources available in the 'initial pool' water, which includes microalgae and suspended organic matter. We used aquarium air pumps to help circulate water around the microcosms, thus avoiding particle sedimentation and a decline in oxygen. This allowed the zooplankton community to be structured based on the interactions between their composing species and on the specific environmental conditions imposed by each treatment.

Zooplankton grazing rates
After the five-day incubation period, the community in each disturbance treatment was divided into two equivalent parts which later were carefully filtered through a 50-μm plankton mesh. The zooplankters retained in the mesh were transferred to 1-l polyethylene bottles that contained Jurubatiba lagoon water, previously filtered in a fiberglass microfilter (GF-1, 47 mm) – free from suspended particles, microalgae and any organism bigger than 0.7 μm. We then kept one bottle at its respective previous disturbance condition (30°C or 35°C; 2.0 PSU or 6.0 PSU) and returned the other one to the original conditions of Jurubatiba lagoon (25°C and 0.2 PSU). The community in the control treatment was also transferred to 1-l polyethylene bottles and kept at the control environmental conditions. In total, we established one control treatment; two disturbances treatments (salinity and temperature increase), with two levels each (30°C/35°C; 2.0 PSU/6.0 PSU) and two grazing conditions (disturbed and undisturbed) each with five replicates, amounting to 45 experimental units. Before grazing measurement procedures, zooplankters were acclimatised to the environmental conditions established for each microcosm for 10 h. Previous studies used a similar period for the zooplankton community and considered this period enough to promote acclimation without affecting community composition (DeMott 1995, Rhode et al. 2001).

After the 10-h acclimation period, we added to each bottle a mixture of three cultivated microalgae at an
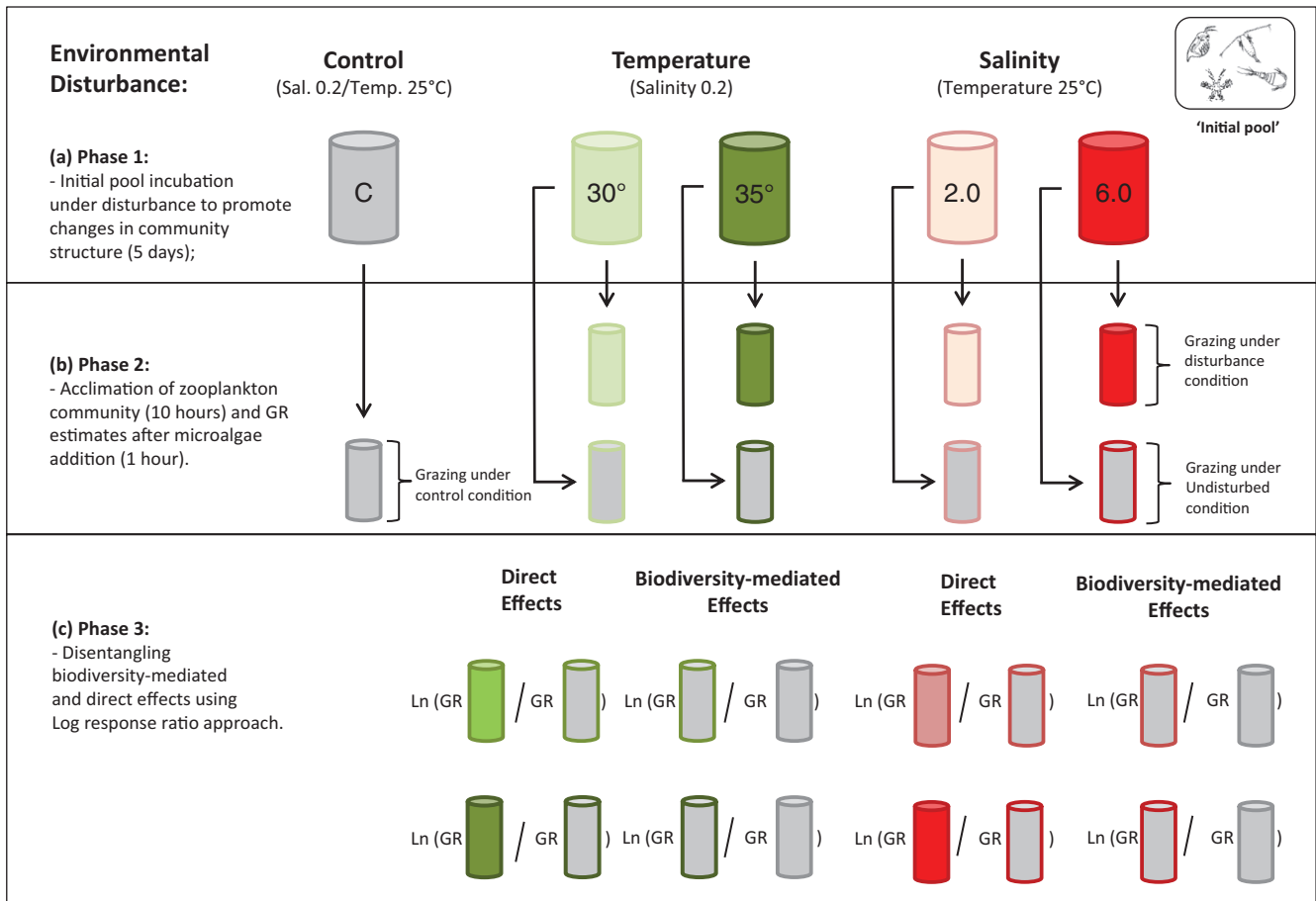
| Environmental Disturbance: | Control (Sal. 0.2/Temp. 25°C) | Temperature (Salinity 0.2) | | Salinity (Temperature 25°C) | | 'Initial pool' |

(a) Phase 1:
- Initial pool incubation under disturbance to promote changes in community structure (5 days);

(b) Phase 2:
- Acclimation of zooplankton community (10 hours) and GR estimates after microalgae addition (1 hour).

(c) Phase 3:
- Disentangling biodiversity-mediated and direct effects using Log response ratio approach.

Direct Effects — Biodiversity-mediated Effects — Direct Effects — Biodiversity-mediated Effects

Ln (GR / GR)   Ln (GR / GR)   Ln (GR / GR)   Ln (GR / GR)

*C: Control; Sal: Salinity; Temp: Temperature; GR: Grazing Rates.

Figure 1. Experimental design outline. The experimental design was carried out in three steps. First, the zooplankton community from Jurubatiba lagoon was exposed to control (25°C and 2.0 PSU, gray) and different disturbance conditions, considering the type (temperature increase, green) and (salinity increase, red) at two different intensities conditions (30°C/35°C; 2.0 PSU/6.0 PSU, respectively) for five days. This allowed the zooplankton community to respond to these five different environmental conditions. Second, the altered zooplankton community was exposed to two different environmental conditions: the disturbed environmental condition that is characterized by the environmental condition in step 1 (colored units) and, the undisturbed condition that is characterized by the return to the control conditions (25°C and 2.0 PSU, gray). After 10 h organisms were acclimated at this environmental condition, each experimental unit received food source to calculate the grazing rates (1 h). Finally, we separated the biodiversity-mediated and direct effects by using the ratio between the grazing rates under disturbed and undisturbed conditions (direct effects) and the ratio between undisturbed and control conditions (biodiversity-mediated effects).

approximate 1.9×10^4 cells ml^{-1} final concentration. We used *Ankistrodesmus gracilis*, *Scenedesmus bijugatu* and *Pseudokirchneriella subcapitata* as food sources. Microalgae species were selected based on differences in shape and/or size to allow food resource diversity. *Ankistrodesmus gracilis* is a fusiform species with an average length of 35 μm; *S. bijugatus* has an oval shape with an average length of 10 μm, and *P. subcapitata* is C-shaped with an average length of 10 μm. Zooplankton in growth and reproduction trials often feed on these three species (Martinez-Jeronimo and Ventura-Lopez 2011), and they are commonly found in tropical regions (Severiano et al. 2012). We obtained the algae strains from the microalgae collection of the Laboratório de Cultivo e Fisiologia do Fitoplâncton at the botanical department of the Federal University of São Carlos, São Paulo, Brazil. We maintained strains in batch monocultures in a WC medium under 12:12 h light:dark cycles (Guillard and Lorenzen 1972).

Then, we allowed zooplankton to graze for one hour in the dark in a saturated food source condition as established by several grazing rates methods protocols (Sanders et al. 1996). During this experimental step, organisms were able to graze considering the ability of the community structured by each environmental disturbance type and intensity. After this period, we collected algae samples in each microcosm and preserved them with Lugol's solution (Fig. 1b). We removed all organisms from each bottle with a plankton mesh and immediately fixed them in a buffered formalin solution (5%, final concentration). We estimated algae densities by the Utermöhl sedimentation method (Utermöhl 1958) using

an inverted microscope. Zooplankters were identified to the lowest possible taxonomic unit and were counted in either a Sedgewick–Rafter chamber under a microscope or in open chambers under a stereomicroscope.

GR were estimated by the reduction of algal density in all microcosms. We estimated the total grazing rates (tGR) and per capita grazing rates (pcGR) using the following equations (Frost 1972):

$$\text{tGR: } V \times \left(\left(\ln Cc - \ln Cf \right) / t \right) \times \left(\left(Ci - Cf \right) / \left(\ln Ci - \ln Cf \right) \right)$$

$$\text{pcGR: } V / N \times \left(\left(\ln Cc - \ln Cf \right) / t \right) \times \left(\left(Ci - Cf \right) / \left(\ln Ci - \ln Cf \right) \right)$$

where V: volume (ml), N: number of individuals, t: time (h), Cc: control phytoplankton concentration (cells ml^{-1}), Cf: final phytoplankton concentration (cells ml^{-1}) and Ci: initial phytoplankton concentration (cells ml^{-1}). We considered the control phytoplankton concentration to be the initial phytoplankton concentration because changes in the phytoplankton community after one hour of incubation are not significant for our goals (Lürling et al. 2013). In this way, tGR variation is a response to environmental changes that include both effects on community composition and abundance, while pcGR variation is related to changes in community composition.

Data analysis

We evaluated the effects of environmental disturbance on zooplankton community structure by using a permutational multivariate analysis of variance (PERMANOVA), based on the Bray–Curtis dissimilarity index, followed by a test for homogeneity of multivariate dispersions (PERMDISP) (Anderson 2001). We compared tGR and pcGR differences among treatments by using a one-way analysis of variance (ANOVA) followed by a Tukey HSD post hoc test. We measured GR: 1) after removing the disturbance and allowing organismal function to recover (hereafter, 'undisturbed conditions') and; 2) without removing the disturbance ('disturbed conditions'). Undisturbed conditions are characterized by the zooplankton community previously exposed to disturbances but under original conditions of salinity and temperature and showed biodiversity-mediated effects. GR responses under disturbed conditions were presented by the zooplankton community kept in environmental disturbances conditions. In comparison to the GR in a never-disturbed control, the former isolates the biodiversity-mediated effect, while the latter captures the combination of biodiversity-mediated and direct effects. We used the pcGR to isolate the biodiversity-mediated effects as pcGR reflects the effects of environmental disturbance on the community functional profile while the tGR also incorporates potential density-mediated effects.

We disentangled and compared biodiversity-mediated and direct effects of environmental disturbances by using the log response ratio approach (ln [treatment response/control

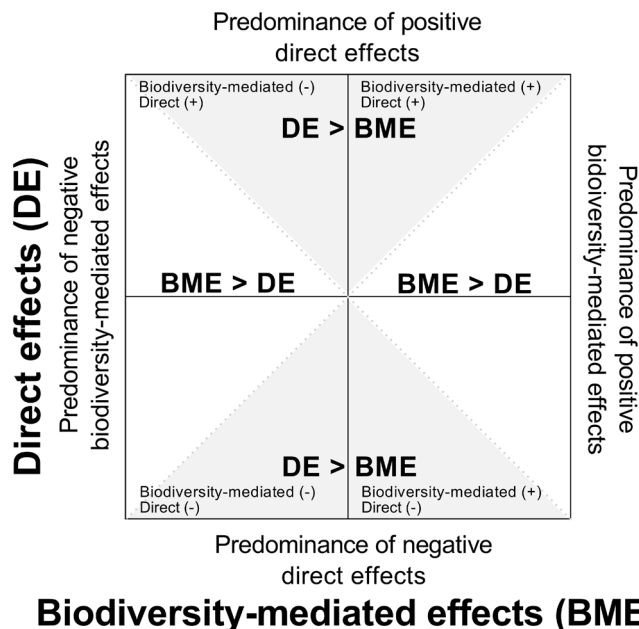## Conceptual model

Predominance of positive
direct effects

Figure 2. Conceptual model proposed to interpret the contributions of biodiversity-mediated (BME) and direct effects (DE) to zooplankton grazing rates. Data in the white region represents predominant biodiversity-mediated effects, and data in the gray region represents predominant direct effects. For both situations, BME and DE can be either positive or negative, considering each portion of the frame.

response]) (Gruner et al. 2008). To calculate the biodiversity-mediated effects, we used the log response ratio between pcGR (under undisturbed conditions) and pcGR from the control for each disturbance level treatment. To calculate the direct effects, we used the log response ratio between the pcGR under disturbed and undisturbed conditions for each disturbance level treatment (Fig. 1c). We established whether biodiversity-mediated and direct effects were significantly different from zero by using a t-test for one sample, considering the hypothetical mean zero. We compared biodiversity-mediated and direct effects of each treatment through a paired t-test. In addition, we compared both effects between disturbance levels through an unpaired t-test. To facilitate interpretation and comparison between the magnitudes of biodiversity-mediated and direct effects, we plotted the values in a scatter plot graph, where the y-axis represents direct effects, the x-axis represents biodiversity-mediated effects and each coordinate pair corresponds to a replicate. Thus, the contribution of biodiversity-mediated and direct effects can be observed following the conceptual model proposed (Fig. 2).

We performed the PERMANOVA analysis by using the function 'adonis' in package 'vegan' in R program, ver. 2.13.0 (<www.r-project.org>). The ANOVAs and t-tests were performed using the statistical software Graph-Pad Prism ver. 7.0. All assumptions relating to the normality and the variance homogeneity of the data were considered.
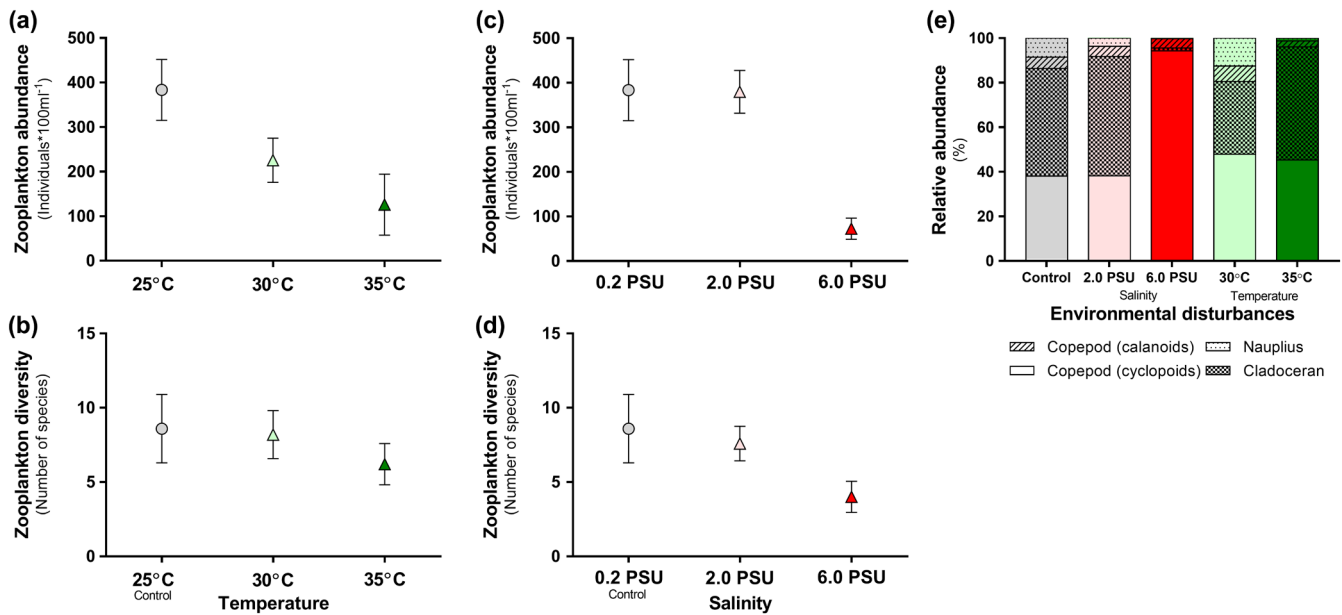
Figure 3. Effects of environmental changes on zooplankton community structure. Effects of temperature increase on zooplankton abundance (a) and diversity (species richness, b), for both disturbance intensities (30°C, light green and 35°C, dark green; mean ± SD). Effects of salinity increase on zooplankton abundance (c) and diversity (species richness, d), for both disturbance intensities (2.0 PSU, light red and 6.0 PSU, dark red; mean ± SD). Note that for both disturbances control values are the same (gray circle; mean ± SD). Effects of environmental disturbance on the relative abundance of the main zooplankton groups: copepod calanoids, copepod cyclopoids, nauplius and cladocerans (e). Relative abundances of rotifers were not representative and they are not described in the graph. For the full description of community composition in each environmental condition, see Supplementary material Appendix 1 Table A7, A8.

Results

We identified 26 zooplankton taxa belonging to the three major zooplanktonic groups: cladocerans, copepods and rotifers (Supplementary material Appendix 1 Table A8). Zooplankton communities from the control treatment were comprised mainly of cladocerans and cyclopoid copepods and presented low densities of nauplii larvae and calanoid copepods and very low densities of rotifers (Fig. 3e).

Temperature increase accounted for 23% of the community structure variation (PERMANOVA, p = 0.0015, R^2 = 0.23). The dispersion of 35°C-driven communities was significantly different from control (PERMDISP, p = 0.027) and from 30°C (PERMDISP, p = 0.006), suggesting higher variability in the former (Supplementary material Appendix 1 Table A1–A2, Fig. A1a). Total zooplankton abundance decreased in both levels of temperature, and zooplankton species richness decreased at 35°C (Fig. 3a–b). The relative abundance of zooplankton groups changed in both disturbance levels. The proportion of cladocerans decreased while cyclopoid copepods and nauplii larvae increased at 30°C. At 35°C, the proportion of cladocerans and cyclopoid copepods increased while calanoid copepods and nauplii larvae declined (Fig. 3e).

In general, tGR decreased significantly with temperature increase (Fig. 4a: ANOVA: $F_{(4,18)}$: 13.09, p < 0.0001; for all comparisons with control Tukey post hoc test: p < 0.007; Supplementary material Appendix 1 Table A3), while pcGR did not differ significantly between control and other

treatments (Fig. 4b: ANOVA: $F_{(4,18)}$: 5.64, p = 0.004; for all comparisons with control Tukey post hoc test: p > 0.178).

At 30°C, tGR and pcGR did not differ significantly under disturbed and undisturbed conditions, despite the upward trend in GR under disturbed conditions in both cases (Fig. 4a and b: for all comparisons Tukey post hoc test: p > 0.115). The direct effects were not significantly different from zero (one-sample t-test p = 0.133; Supplementary material Appendix 1 Table A4), but the biodiversity-mediated effects were significantly negative (Fig. 5, one-sample t-test p = 0.012; Supplementary material Appendix 1 Table A4). The direct and biodiversity-mediated effects were significantly different from each other (BME > DE: Fig. 5, t-test p = 0.041; Supplementary material Appendix 1 Table A5), indicating that biodiversity-mediated effects were stronger than the direct ones at this level of temperature increase (Fig. 5).

At 35°C, tGR and pcGR did not differ significantly under disturbed and undisturbed conditions, despite the downward trend in GR under disturbed conditions in both cases (Fig. 4a–b: Tukey post hoc test: p > 0.339). The biodiversity-mediated and direct effects were not significantly different from zero (Fig. 5; one-sample t-test p = 0.507 and p = 0.583, respectively; Supplementary material Appendix 1 Table A4) or different from each other (BME = DE: Fig. 5, t-test p = 0.549; Supplementary material Appendix 1 Table A5). However, biodiversity-mediated effects were significantly different between temperature levels (Fig. 5; t-test, p = 0.014; Supplementary material Appendix 1 Table A6), while direct
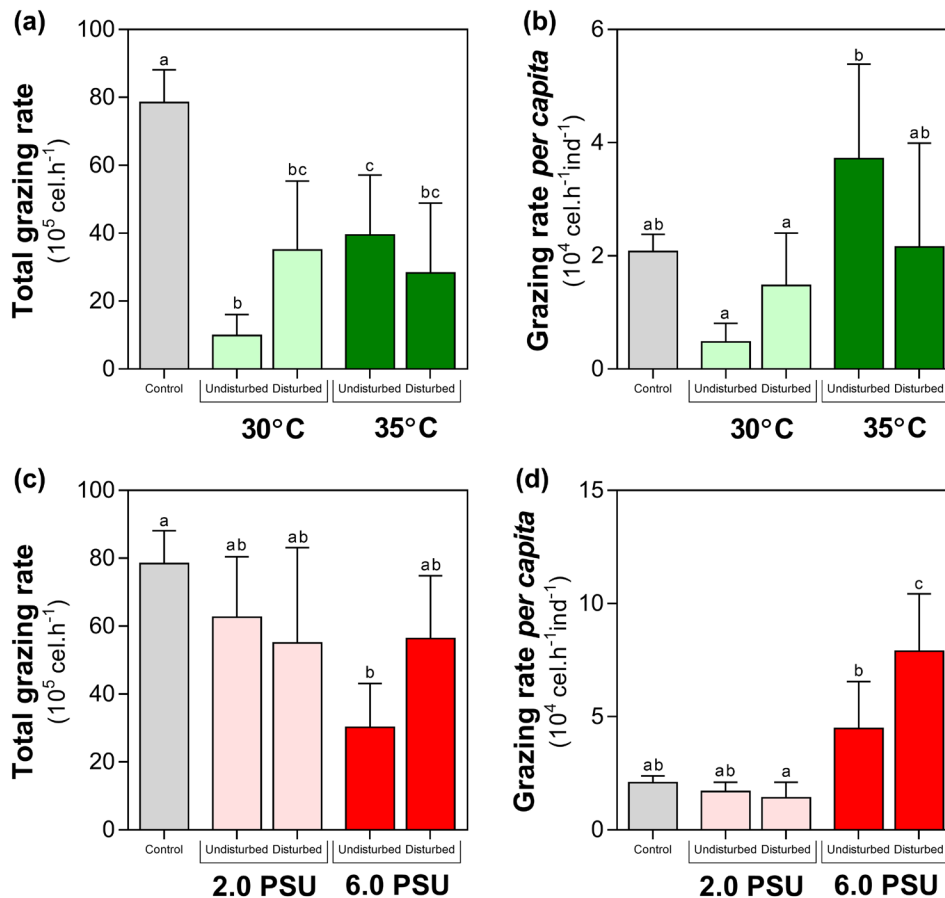
Figure 4. Variation in the grazing rates in the different environmental conditions. Effects of temperature increase on total (a) and per capita (b) grazing rates (mean ± SD). Effects of salinity increase on total (c) and per capita (d) grazing rates (mean ± SD). Note that for both disturbances control values are the same (gray bars; mean ± SD). Different letters indicate significant differences between treatments (Tukey post hoc test). For full statistics see Supplementary material Appendix 1 Table A3.

effects were not (Fig. 5; t-test p = 0.15; Supplementary material Appendix 1 Table A6).

Disturbances caused by salinity increase accounted for 77% of the community structure variation (PERMANOVA, p = 0.0001, R^2 = 0.77). The community dispersion of salinity 6.0 PSU treatment was significantly different from control (PERMDISP, p = 0.042) and from salinity 2.0 PSU treatment (PERMDISP, p = 0.002), indicating higher variability in the former (Supplementary material Appendix 1 Table A1–A2, Fig. A1b). Total zooplankton abundance and diversity decreased at salinity 6.0 PSU (Fig. 3c–d), where cladocerans nearly reached extinction and the cyclopoid copepods became the dominant group (Fig. 3e). At salinity 2.0 PSU the proportion of cladocerans increased while nauplii larvae declined (Fig. 3e).

In general, tGR were not significantly different from control except at salinity 6.0 PSU under undisturbed conditions, which was significantly lower (Fig. 4c; ANOVA: $F_{(4,20)}$: 4.428, p = 0.01; Tukey post hoc test: p = 0.004; Supplementary material Appendix 1 Table A3). Neither were pcGR significantly different from control – except at salinity 6.0 PSU under disturbed conditions, which was significantly higher than

control (Fig. 4d: ANOVA: $F_{(4,20)}$: 16.25, p < 0.001; Tukey post hoc test: p < 0.0001; Supplementary material Appendix 1 Table A3).

At salinity 2.0 PSU, tGR and pcGR under disturbed conditions did not differ significantly from tGR and pcGR under undisturbed conditions(Fig. 4c–d: for all comparisons Tukey post hoc test: p > 0.964; Supplementary material Appendix 1 Table A3). The biodiversity-mediated and direct effects were not significantly different from zero (Fig. 5, one-sample t-test, for all comparisons p > 0.13; Supplementary material Appendix 1 Table A4) or different from each other (BME = DE: Fig. 5, t-test p = 0.86; Supplementary material Appendix 1 Table A5).

At salinity 6.0 PSU, tGR under disturbed conditions did not differ significantly from tGR under undisturbed conditions (Fig. 4c: Tukey post hoc test: p = 0.207; Supplementary material Appendix 1 Table A3), while pcGR under disturbed conditions were significantly higher than pcGR under undisturbed conditions (Fig. 4d: Tukey post hoc test: p = 0.015; Supplementary material Appendix 1 Table A3). The calculated biodiversity-mediated and direct effects were significantly positive (Fig. 5, one-sample t-test, for all comparisons,
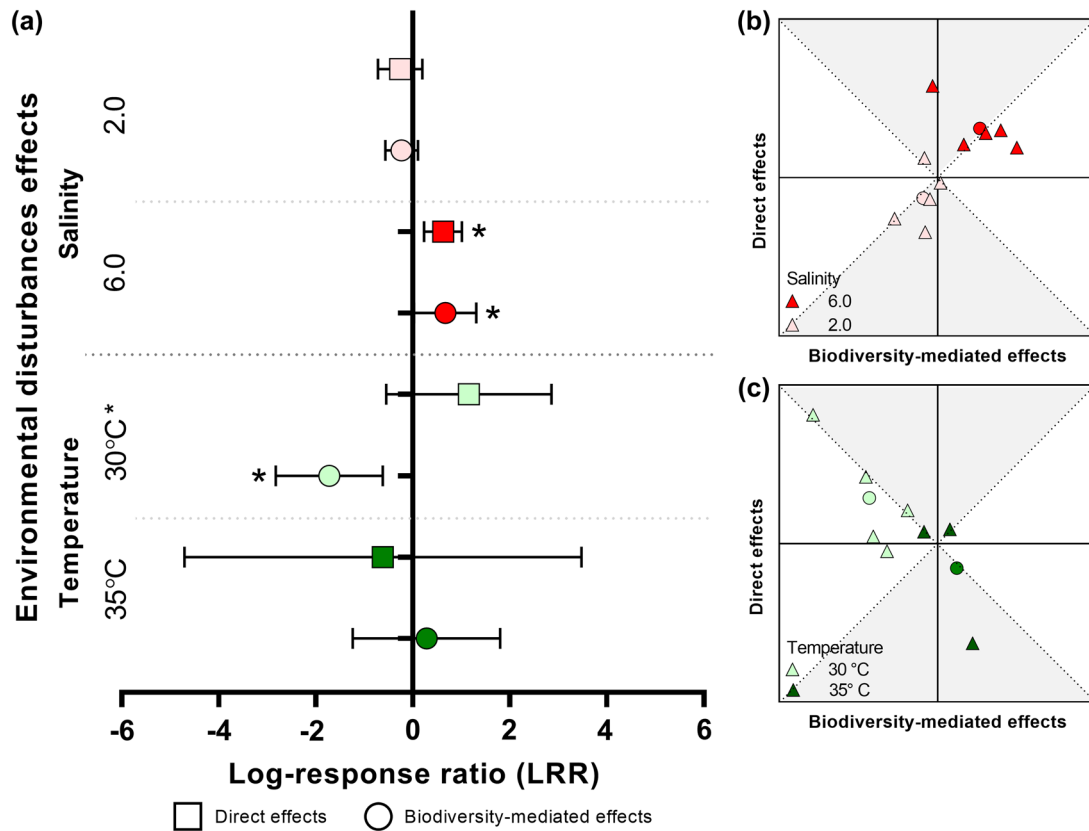
Figure 5. Distribution of the direct and biodiversity-mediated effects among the different types and intensities of disturbances. Direct (squares; mean ± 95% CI) and biodiversity-mediated effects (circles; mean ± 95% CI) were obtained through the log response ratio (LRR) between the disturbed/undisturbed and undisturbed/control treatments, respectively (a). For salinity increase, there were no significant differences between direct and biodiversity-mediated effects for 2.0 PSU (light red) and 6.0 PSU (dark red) treatments. At salinity 6.0 PSU, direct and biodiversity-mediated effects were significantly positive. For temperature increase, the 30°C treatment showed significant differences between direct and biodiversity-mediated effects, where the latter was significantly negative. * means significant differences from zero (symbols next to the bars) and between direct and biodiversity-mediated effects (symbols next to the treatments). For disturbances caused by salinity increase (b) and warming (c), the relationship between direct and biodiversity-mediated effects for each experimental unit (triangle) is described following our conceptual model (Fig. 2). Mean values for each environmental disturbance treatment are described as circles in the graph. Two replicates of 35°C treatment were lost in the last step, so the results for the treatment are based on three replicates.

p < 0.04; Supplementary material Appendix 1 Table A4), but the effects were not significantly different from each other (BME = DE: Fig. 5, t-test p = 0.903; Supplementary material Appendix 1 Table A5). This result indicates that biodiversity-mediated effects were as important as direct ones at salinity 6.0 PSU (Fig. 5). Biodiversity-mediated and direct effects were also significantly higher at salinity 6.0 PSU than at salinity 2.0 PSU (p = 0.009 and p = 0.004, respectively; Supplementary material Appendix 1 Table A6).

Discussion

Disentangling biodiversity-mediated and direct effects of environmental changes on ecosystem functions remains a challenge in ecological studies. We showed that increases in temperature and salinity lead to substantial changes in zooplankton community structure and function. We hypothesized that biodiversity-mediated effects would be more relevant for salinity increase and at higher levels of disturbance based on the expected effects on zooplankton community composition. Biodiversity-mediated and direct effects varied with disturbance type and intensity, but were equivalent in most treatments – except at a moderate temperature increase, where the biodiversity-mediated effects became more relevant. Thus, results were opposite to our hypothesis and suggest that the effects of environmental disturbances on ecosystem functions are significantly more complex than we had expected. Our results were explained by the balance between different mechanisms, including effects on community density, richness and composition and organism performance. We concluded that the relative contribution of biodiversity-mediated effects is dependent on how the disturbance type and intensity affects these underlying mechanisms.

High levels of salinity changed zooplankton community structure, reducing the abundance of organisms and

changing the dominance pattern amongst different groups. Temperature increases caused changes in community structure even at a moderate disturbance level, also affecting the organisms' performance. For nearly all disturbance types and intensities, the zooplankton community declined, except at salinity 2.0 PSU. The density effect has been reported as one of the main mechanisms that explain how environmental disturbances affect ecosystem function by suppressing the number of organisms able to perform a process (McKie et al. 2008). However, the net effect also depends on the balance between changes in community composition and organism performance as shown in our results.

Temperature increase was more important in driving zooplankton community performance at a moderate disturbance level (30°C). Despite a metabolic acceleration – indicated by a positive signal of direct effects – the negative biodiversity-mediated effects were overriding. The most plausible reason for such effect was the reduction of cladocerans' relative abundance, which are considered more efficient grazers than other zooplankton groups (Sommer et al. 2001). These results suggest that the effects of temperature on zooplankton performance will depend on the balance between opposite signals: positive on organism physiology, especially at moderate levels, but negative on community structure. At a high disturbance level (35°C), biodiversity-mediated and direct effects were not relevant. At this level, the zooplankton community responded idiosyncratically, producing a great variation in the overall response of GR (that might also be related to the number of replicates in this treatment). High temperatures can trigger a complex relationship between species interaction and tolerance to disturbance, and the net response is dependent on this balance. We showed that extreme climate events can disrupt important trophic interactions in aquatic food webs by affecting community structure and metabolism. Our results suggest that temperature increase may affect the energy flow in tropical coastal lagoons, compromising multiple biological mechanisms.

Salinity increase represented a stronger driver in shaping zooplankton communities, but only at a high level (salinity 6.0 PSU). Previous studies reported that even a slight salinity increase leads to a decline in the number of organisms and a loss of functional relevant species (Schallenberg et al. 2003, Sarma et al. 2006). However, we observed that a moderate increase in salinity (2.0 PSU) caused no changes in zooplankton community structure or metabolism, thus producing no significant changes in the overall grazing rates. At a high level of salinity (6.0 PSU), we observed the greatest changes in community structure, with an intense reduction in cladocerans' relative abundance. Surprisingly, we observed similar positive biodiversity-mediated and direct effects, where the community structure was dominated by cyclopoid copepods. Unlike cladocerans and rotifers, copepods have a wide range of osmoregulation capacity and can inhabit freshwater and marine ecosystems (Sarma et al. 2006). They may have benefited from the new environmental conditions, thus presenting higher grazing rates. Additionally, major changes in salinity

can cause significant alterations in organism physiology and in the food intake rate as they need extra energy to osmoregulate. These synergic mechanisms show an increase in the overall performance of the surviving species, indicating that salinity increases can affect aquatic environments by its combined effects on community composition and on metabolism rates.

We demonstrated that environmental disturbances can cause changes in ecosystem functioning through direct and biodiversity-mediated effects. However, the prevalence of direct and biodiversity-mediated effects depends on the type and intensity of the disturbance. While higher temperatures can often be experienced by tropical aquatic organisms, salinity increases are occasional, and their effects are more predictable due to the low tolerance of oligohaline species. We showed that the disturbance type and intensity, i.e. the role it plays in determining community structure, is a key factor to explain the prevalence of direct and biodiversity-mediated effects on ecosystem function. In this sense, disturbance intensity becomes more important as it affects species' ability to cope with each disturbance. At moderate disturbance levels, community structure changes might alter the functional groups' dominance. This can be determined by the tradeoff between stress tolerance and the ability to function of its species. In this case, biodiversity-mediated effects can primarily be responsible for changes in ecosystem functioning. At higher intensities, when the performance of most species is severely altered, organisms' metabolic changes escalate and can be as important as the biodiversity-mediated effects, as observed at salinity 6.0 PSU and at 35°C. We suggest that at moderate levels, the type of disturbance determines the importance of biodiversity-mediated effects, while at high levels biodiversity-mediated can be equally important as the direct effects.

It is important to highlight that our experimental approach allowed us to disentangle the biodiversity-mediated and direct effects of environmental disturbances and to show their relative importance. However, in natural conditions other mechanisms might occur and the effects of temperature and salinity increase would interact with other important factors. For example, artificial microcosms are closed systems without recolonization which simplify the complex relationships that occur in nature (Carpenter 1996). In an open natural system, colonization by new species is expected after environmental changes (Leibold et al. 2017), which introduces new functional traits to the community and to its performative processes (Arnoldi et al. 2018). In addition, population growth and organism adjustments are expected in the long term, thus offsetting the initial decrease in abundance (Arnoldi et al. 2018). We adopted the pcGR rather than the tGR when separating biodiversity-mediated and direct effects to minimize this constraint. Also, our study subject (zooplankton community) demands only a short acclimation period to recover functioning after a disturbance event (DeMott 1995). It was essential to measure the biodiversity-mediated effects and to guarantee the consistency of our results, but acclimation time should vary according to the system studied.

Current studies show that effects of diversity loss on ecosystem functioning can be as important as the effects of many other global environmental changes, such as climate warming, acidification and nutrient pollution (Hooper et al. 2012). Other studies showed that biodiversity plays a small role in ecosystem functioning when compared to the direct effects of environmental change (Grace et al. 2007). Our results integrate these conclusions by indicating the existence of a complex balance between species interaction and performance determined by disturbance type and intensity. We suggest that future studies should empirically investigate the multiple ways that environmental disturbances can affect ecosystem functioning considering the contingency of biodiversity-mediated effects.

Speculations

Understanding the tradeoffs between species tolerance and functional habilities is the main component to explore how biodiversity acts in natural disturbed systems. Perhaps, what we haven't attempted (modestly because it wasn't our initial idea) was to use a functional diversity approach to directly observe changes in communities' functional attributes which might be seen in detail in a longer experimental trial. By increasing the exposure time to the disturbances we might possiblysee a robust change in the community functional diversity with the selection of organisms with specific traits. Despite some studies highlighting the role of complementarity mechanisms acting after environmental disturbances, our results reinforce the importance of the selection effects – as seen in 6.0 PPT disturbance which has favored an important grazer, better adapted to the new environmental condition. Furthermore, if we had simulated a continuum gradient of disturbance intensity, we might see a threshold between the disturbance intensity and the relative importance of biodiversity-mediated effects. We would like to support the need for further understanding of species' tolerance and functional responses when organisms are dealing with different disturbances types, intensity and exposure time.

Data availability statement

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.nzs7h44n3> (Dib et al. 2019).

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Supplementary material (available online as Appendix oik-06768 at <www.oikosjournal.org/appendix/oik-06768>). Appendix 1.