Research Article

Interactive effects of warming and eutrophication on zooplankton could reverse the stoichiometric mismatch with phytoplankton

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ABSTRACT

The core ecosystem functioning (e.g. trophic transfer efficiency) is at risk of being disrupted by the growing mismatch between nutrient content of primary producers and nutrient demand of grazing consumers. Ecological stoichiometry provides a conceptual framework that explains this trophic interaction using C, N and P elemental composition across trophic levels. In light of ongoing climate change and eutrophication, previous studies have raised concerns regarding the growing stoichiometric mismatch between phytoplankton and zooplankton, given the stoichiometric plasticity of phytoplankton. However, there is currently little conclusive evidence on the stoichiometric mismatch from a dual perspective of phytoplankton and zooplankton. To address this, we conducted a mesocosm experiment to investigate the separate and combined effects of climate warming (a constant increase of $+3.5\,^{\circ}$C plus heat waves) and eutrophication (nutrient addition) on stoichiometric mismatch between phytoplankton and zooplankton by examining stoichiometric changes in both communities. We observed a growing trend in stoichiometric mismatches when warming or nutrient addition acted individually, which was mediated by the increase in nutrient demand (N, P elements) of zooplankton growth. However, when these stressors acted jointly, the mismatches were reversed. This could be because climate warming and eutrophication combined would lead to changes in species composition, which accordingly reshaped the stoichiometric composition at the community level. These results illustrate the need of stoichiometric mismatches for understanding the implication of global change on trophic interactions and ecosystem functioning, requiring consideration not only of cross-trophic levels but also of compositional changes within communities.

1. Introduction

There is a high degree of variability in trophic transfer efficiency between primary producers and grazing consumers, which constrains ecosystem functioning (Kemp et al., 2005; Karpowicz et al., 2020; Barneche et al., 2021). The classical ecological model proposes that food quantity drives material transfer to higher trophic levels (Lindeman, 1942). More recent evidence has shown that the relationship is modified by the quality of food available to consumers (Thomas et al., 2022). Consumers usually have higher content of essential nutrients such as nitrogen (N) and phosphorus (P) than the foods they eat (van de Waal et al., 2010), resulting in a stoichiometric mismatch between consumer nutrient demands and producer nutrient content. The extent to which the content and ratios of these nutrients match between producers and their consumers determines the potential maximum trophic transfer efficiency, which regulates ecosystem functioning, such as biogeochemical cycling, and carbon sequestration (Sistla et al., 2013).

Ecological stoichiometry provides a conceptual framework that explains this trophic interaction using C, N and P elemental composition of producers and consumers (Sterner and Elser, 2002). In aquatic ecosystems, the stoichiometric plasticity frequently observed in phytoplankton may lead to a stoichiometric mismatch with their consumer's (i.e.,

zooplankton's) demand for essential nutrient elements (Persson et al., 2010). The increase in C:N and C:P ratios of phytoplankton is known to significantly impair zooplankton performance (Sterner and Elser, 2002). So far, less attention has been paid to the consequences of stoichiometric changes in zooplankton (Teurlincx et al., 2017). Zooplankton can regulate the elemental composition (Persson et al., 2010), but this requires trade-offs from other behaviors or processes, such as compensatory grazing (Mandal et al., 2018). Consequently, stoichiometric mismatch can result in additional energy expenditure and nutrient loss (Anderson et al., 2005), which could have wider repercussions for overall ecosystem functioning (van de Waal et al., 2010). In addition to physiological and behavioral responses, recent evidence has shown that changes in species composition at the community level can also contribute to addressing stoichiometric mismatch (Teurlincx et al., 2017).

Within the context of global change, the stoichiometry of phytoplankton and zooplankton may exhibit divergent responses. Climate warming and eutrophication have emerged as prominent drivers of global change, posing threats to aquatic ecosystems as a consequence of anthropogenic activities (Walther, 2010; Smith, 2003; Birk et al., 2020). Research has been conducted on the stoichiometric responses of organisms within this context (van de Waal et al., 2010; De Senerpont Domis et al., 2014; Schulhof et al., 2019; Velthuis et al., 2022). Temperature can affect the stoichiometry of organisms by altering the cellular elemental composition (Sterner and Elser, 2002). For instance, warming-driven increases in growth rates may promote greater P allocation due to RNA (P-rich) enrichment (Elser et al., 2000). On the other hand, it has been proposed that invertebrates (e.g., zooplankton) might require increasingly C-rich diets to meet increased energy demands as temperatures increase (Anderson et al., 2017). This can result in a stoichiometric mismatch between phytoplankton and zooplankton, because nutrient demands of zooplankton may not align with the elemental composition of their phytoplankton diet.

Eutrophication can also alter the stoichiometry of nutrient supply, as well as the elemental and species composition of phytoplankton communities (Prins et al., 2012), and therefore change the quality of phytoplankton as food source for zooplankton (Elser et al., 2001). These changes may impact zooplankton grazing rates and performance (Hillebrand et al., 2009), resulting in a shift in zooplankton community composition towards species that can better adapt to changes in food quality (Elser et al., 2000; Moody and Wilkinson, 2019). While climate warming and eutrophication occur simultaneously in many aquatic ecosystems and either individually or jointly affect the community stoichiometry and ecosystem dynamics (Sardans et al., 2012; Cross et al., 2015; Gerhard et al., 2019; Zhang et al., 2021). Higher temperatures may lead to an increase in nutrient availability through enhanced remineralization (Gudasz et al., 2010), and reduce phytoplankton C:nutrient ratios. Conversely, intensified thermal stratification may limit the nutrient availability and improve light conditions for phytoplankton growth, resulting in higher phytoplankton C:nutrient ratios (van de Waal et al., 2010). There is also evidence that suggests that under increasing environmental stress, communities may undergo species turnover that reshapes community composition and stoichiometry (Teurlincx et al., 2017; Schulhof et al., 2019; Moody and Wilkinson, 2019).

Previous studies have primarily focused on the stoichiometric mismatch caused by unilateral changes in either phytoplankton or zooplankton under climate warming and eutrophication (van de Waal et al., 2010; De Senerpont Domis et al., 2014), rather than considering it from the dual perspective of both phytoplankton and zooplankton. Hence, it remains unclear whether climate warming, eutrophication and/or their combined effects will exacerbate stoichiometric mismatches between phytoplankton and zooplankton communities. To investigate this, we conducted a mesocosm experiment to simulate natural shallow lakes and manipulated both warming and nutrient addition in a full factorial design. Given the diverse range of organisms in the mesocosm, this experiment tested the integrated impact of warming and nutrient addition, on community stoichiometry of both phytoplankton and zooplankton, as well as biotic interactions from a range of higher plants and animals present in the mesocosms.

2. Materials and methods

2.1. Mesocosm and experimental design

The mesocosm system consisted of 24 insulated cylindrical polyethylene containers, each with a total volume of approximately 2,500 L (inner diameter = 1.5 m, depth = 1.45 m) located at Huazhong Agricultural University, Wuhan, China (30°29′N, 113°12′E). Each mesocosm was filled to 100 mm with a layer of lake sediment at half of the bottom and then filled with tap water to a depth of 1 m. Sediments (containing natural inoculum) were collected from Lake Liangzi (30°11′03″N, 114°37′59″E), and were homogenized and sieved through a 5 × 5 mm² metal mesh to remove larger particulates. The mesocosms were left to acclimate at ambient conditions for about four weeks. Phytoplankton, zooplankton, oligochaetes and aquatic insects were introduced via sediment addition from Lake Liangzi, as well as through the addition of 10 L of water from a nearby lake (Lake Nanhu, 30°28′57″N, 114°22′34″E). To simulate a natural shallow lake ecosystem, we also introduced other biological components such as submerged macrophytes (*Potamogeton crispus* and *Hydrilla verticillata*), snails (*Bellamya aeruginosa* and *Radix swinhoei*), shrimp (five individuals of *Macrobrachium nipponense*), and fish (four individuals of *Rhodeus sinensis* and four individuals of *Carassius auratus*). In addition, aquatic insects with incomplete metamorphosis were introduced through the spawning of terrestrial adults.

We used a (two factors × two levels) fully-factorial design and randomly divided the twenty-four mesocosms into four treatments with six replicates each. The control treatment (C) with ambient temperatures and no nutrient addition was used to simulate ambient temperature conditions and nutrient concentrations in many of the lakes in the middle and lower reaches of the Yangtze River. The warming treatment mesocosms (W) were maintained at 3.5 °C above ambient conditions plus simulated heat wave events. Mesocosms with added nutrients (E) were maintained at ambient temperatures, and the fourth treatment (WE) consisted of a combination of the warming and nutrient addition treatments. The amount of warming and the frequency and magnitude of the heat wave were based on model projections from historical meteorological data that are predicted to occur in the middle and lower reaches of the Yangtze River area by the end of this century (IPCC, 2014). Nitrogen (N) and phosphorus (P) were added to the nutrient addition treatments (E and WE) at a mass ratio of 10:1 (a molar ratio of 22:1) following González Sagrario et al. (2005), by dissolving $NaNO_3$ and KH_2PO_4 powder in de-mineralized water. Nutrient addition treatments were applied bi-weekly with the doses changing according to the agricultural activities and precipitation intensities in this area (Xu et al., 2020). This mesocosms were operational from April until November 2021. Evaporative losses from the mesocosms were replenished with distilled water additions, when not supplemented by natural rainfall.

2.2. Sample collection and analyses

Depth-integrated water samples were collected bi-weekly from 200 mm above the sediment to the surface using an acrylic tube (length = 1 m, diameter = 50 mm). Six depth-integrated water samples collected across the diameter of each mesocosm were then pooled and mixed in a bucket. Subsamples were collected for TN, TP and Chl-*a* measurements. TN and TP were first digested with potassium peroxydisulfate, and then measured spectrophotometrically according to standard methods (Chinese National Standards, 2020, GB/T 11894–1989 and GB/T 11893–1989 respectively). Chl-*a* was determined spectrophotometrically by filtering about 1L water through Whatman GF/C filters followed by acetone extraction according to standard methods (Chinese National Standards, 2020, HJ 897–2017).

At the end of the experiment, samples of seston (<30 μm and <130

μm) and crustacean zooplankton were collected for elemental analysis of phytoplankton and zooplankton, . Between 100 and 1,000 mL water was filtered through a 130 μm nylon net, and immediately vacuum filtered through Whatman GF/F filters until the filter was covered with homogeneous material, thus forming samples of seston <130 μm. Samples of seston <30 μm were processed the same way, but through a 30 μm net. More than 20 L water was concentrated with a 64 μm net then aspirated with a pipette until a sufficient amount of crustaceans were visible to the naked eye, thus forming crustacean zooplankton samples. All samples were immediately dried at 60 °C for 24 hours, and then sealed for storage. Each sample was divided into two subsamples for C, N and P analysis. For C and N analysis, 0.2–2 mg dry mass was analyzed on an elemental NC analyzer (Flash EA 1112, CE Instruments, Italy). For P, 1–4 mg dry mass was combusted in a borosillicate glass tube at 550 °C for 120 min. Five millilitres of persulfate (2.5%) was added, and samples were autoclaved for 30 min at 121 °C. Dissolved phosphate concentration was measured spectrophotometrically (Cleverchem380, DeChem-Tech., Germany). One <30 μm seston and six zooplankton samples had insufficient levels of P for analysis. Hereafter, the C, N and P content are presented as the percentage of total dry mass (%), and C:N, C:P and N:P ratios are presented in molar ratios.

2.3. Statistical analysis

We used linear mixed-effects models (LMM) to evaluate the effects of warming, nutrient addition and their interaction on TN, TP and Chl-*a* concentrations by considering time and mesocosms as random factors. We used the "lmer" function from the R-software package "lme4" (Bates et al., 2015). The sequential decomposition of the contributions of fixed-effects was calculated using the "Anova" function with Wald F tests from the R-software package "car" (Fox and Weisberg, 2018).

To test the effects of warming, nutrient addition and seston sizes on phytoplankton stoichiometric ratios and their interactions we also used the LMM method described above, but with warming, nutrient addition and seston sizes as fixed factors and the mesocosm as a random factor. For zooplankton stoichiometric ratios, we examined the effects of warming and nutrient addition and their interaction using a two-way ANOVA. The effects on C, N and P content of phytoplankton and zooplankton were tested using the same method (see results in Fig. S2, Table S3 and Table S4).

Stoichiometric mismatch is defined as the difference between the C:N, C:P and N:P ratios between phytoplankton and zooplankton (Elser and Hassett, 1994). Taking the C:N mismatch as an example, a larger value means that the demand of the latter element (N) by zooplankton is relatively unable to be met by phytoplankton. Similarly, the LMM method was also used to analyse the effects of warming and nutrient addition and their interaction on stoichiometric mismatches between phytoplankton and zooplankton, and to assess whether the stoichiometric mismatch was different for different seston size classes. Multiple comparisons (Tukey, *α* = 0.05) were also performed between treatments for element contents, stoichiometric ratios, and stoichiometric mismatches using the R package "multcomp". All data analyses were performed in R-4.1.3 (R Core Team, 2022).

3. Results

3.1. Environmental factors

During the experiment, water temperatures averaged 3.19 °C higher (Fig. S1; Table S1; Table S2) in the warming treatments (W and WE) than in the ambient controls (C and E). TN and TP concentrations were also significantly higher in treatments with added nutrients (E and WE) than in the ambient controls (C and W, Table S1; Table S2). Warming also significantly increased the TP concentration (Table S2). No significant effect of warming or nutrient addition on Chl-*a* concentration was observed. There was no interaction between warming and nutrient

addition on any of these environmental factors.

3.2. Stoichiometric ratios

No significant effects of warming or nutrient addition on were observed on the C:N, C:P or N:P ratios of phytoplankton (Fig. 1; Table 1). Similarly, no significant differences in the stoichiometric ratios between the <30 μm and <130 μm seston size class samples was observed either, meaning the C:N:P ratios were the same for each group.

Although, neither warming nor nutrient addition showed separate effects on the C:N ratios of zooplankton, there was a significant interactive effect (Fig. 1; Table 2). Warming did not affect the C:P ratios of zooplankton, but nutrient addition significantly decreased it. There was also a significant interaction between warming and nutrient addition on the C:P ratio of zooplankton (Fig. 1; Table 2). Warming and nutrient addition acting individually had a decreasing trend on the C:N and C:P ratios of zooplankton, but this trend was reversed when acting jointly (Fig. 1). The N:P ratio of zooplankton was increased by warming (*P* = 0.08) and was increased significantly by nutrient addition. There was no significant interaction between warming and nutrient addition on the N:P ratio of zooplankton, so the ratio was highest when both factors are combined (Fig. 1; Table 2).

3.3. Stoichiometric mismatches

Neither warming nor nutrient addition showed any significant effects but there was an interactive effect on the C:N mismatch between phytoplankton and zooplankton (Fig. 2; Table 3). Warming did not change the C:P mismatch, while nutrient addition significantly reduced it. There was also a significant interaction between warming and nutrient addition on the C:P mismatch (Fig. 2; Table 3). Warming and nutrient addition individually trended to increase C:N and C:P mismatches, but this trend was reversed when acting jointly (Fig. 2). Warming did not affect the N:P mismatch, while nutrient addition significantly reduced it. An interaction between warming and nutrient addition on the N:P mismatch (*P* = 0.07) was detected (Fig. 2; Table 3). The stoichiometric mismatches of the seston in different size classes with zooplankton were almost identical (Fig. 2; Table 3).

4. Discussion

4.1. Stoichiometric ratios

Temperature typically affects a wide range of cellular mechanisms, including carbon and nutrient assimilation rates (Hancke et al., 2008), and photosynthesis and respiration rates (Cabrerizo et al., 2014). Theoretical and empirical studies suggest that warming increases the growth rate of phytoplankton (Thomas et al., 2012; Brandenburg et al., 2019), and may decrease the phytoplankton C:P ratio (Elser et al., 2000). However, our results showed that warming did not affect C:N:P stoichiometry of phytoplankton. A recent meta-study analyzing 43 experiments showed that warming-induced increases in growth rate do not always coincide with changes in phytoplankton stoichiometry (Velthuis et al., 2022). For instance, prolonged exposure to experimental warming leads to changes in photosynthesis and respiration ratios, which may result in increased rates of carbon fixation (Barton et al. 2020). De Senerpont Domis et al. (2014) also suggested that higher temperatures allow phytoplankton to yield a higher carbon biomass per unit cellular P or N. Besides, phytoplankton community stoichiometry is likely influenced by a range of factors, such as losses from sinking, physiological limits to nutrient storage, physiological adaptation, species composition and species interactions (Hall, 2009). Thus, the interplay of multiple temperature-dependent changes in cellular processes may differ across species and communities and complicates our mechanistic understanding on the effects of climate warming on phytoplankton stoichiometry.

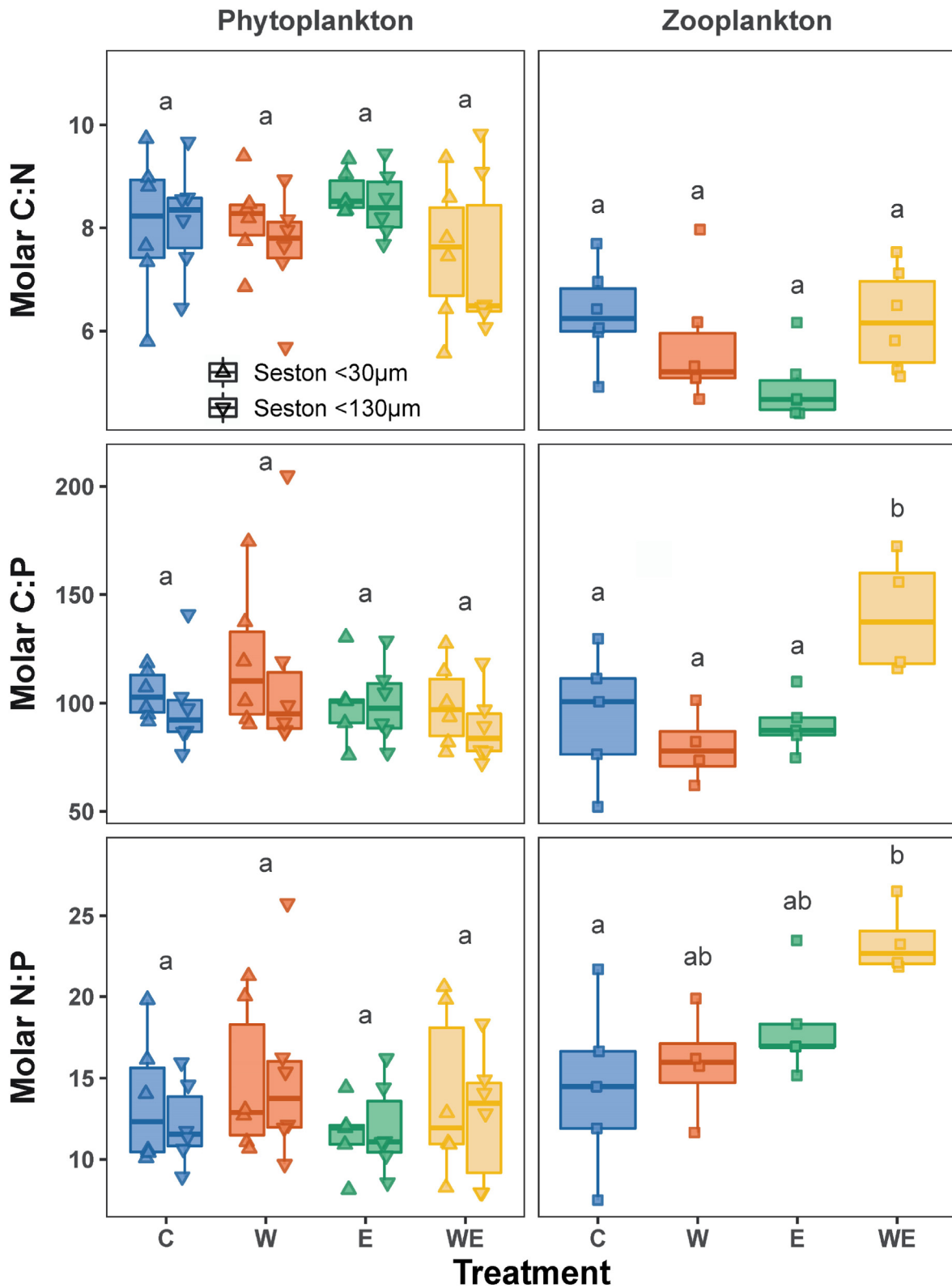Previous studies have shown that phytoplankton have relatively high

Fig. 1. Stoichiometric ratios of phytoplankton and zooplankton for each treatment. Boxplots show the minimum, first quartile, median, third quartile and maximum values. Symbols indicate individual replicate (mesocosm) measurements within each of the treatments. Letters indicate the significance of differences among the treatments ($\alpha = 0.05$). C stands for the control treatment, W for warming, E for nutrient addition, and WE for the combined warming and nutrient addition treatments.

stoichiometric plasticity that varies with environmental nutrient concentrations (Hall, 2009; Galbraith and Martiny, 2015). Therefore, nutrient addition would reduce both the C:N and C:P ratios of phytoplankton communities (Dickman et al., 2006). On the other hand, there is

evidence that grazers can increase the C:N and C:P ratios of phytoplankton communities due to selective foraging for high-quality food (low C:N and C:P ratios) (Mandal et al., 2018; Plum and Hillebrand, 2019). Thus, the C:N and C:P ratios of phytoplankton communities may

Table 1
Results from LMM analyses evaluating effects of warming (W), nutrient addition (E), seston size (F) and their interactions on the C:N:P stoichiometric ratios of phytoplankton.

Stoichiometric ratio	Explanatory	F	Df	Df.res	P
C:N	W	3.23	1.00	20.00	0.09
	E	0.00	1.00	20.00	0.95
	F	0.52	1.00	20.00	0.48
	W*E	1.57	1.00	20.00	0.22
	W*F	0.27	1.00	20.00	0.61
	E*F	0.01	1.00	20.00	0.93
	W*E*F	0.38	1.00	20.00	0.54
C:P	W	0.20	1.00	19.98	0.66
	E	1.44	1.00	19.98	0.24
	F	3.28	1.00	19.12	0.09
	W*E	1.24	1.00	19.99	0.28
	W*F	0.42	1.00	19.14	0.52
	E*F	0.02	1.00	19.14	0.90
	W*E*F	0.76	1.00	19.15	0.39
N:P	W	1.37	1.00	19.98	0.25
	E	0.80	1.00	19.98	0.38
	F	0.75	1.00	19.13	0.40
	W*E	0.03	1.00	19.98	0.86
	W*F	0.00	1.00	19.15	0.98
	E*F	0.00	1.00	19.15	0.96
	W*E*F	2.89	1.00	19.16	0.11

Table 2
Results from ANOVA analyses evaluating effects of warming (W), nutrient addition (E) and their interactions on stoichiometric ratios of zooplankton. Bold numbers indicate significant effects at $\alpha = 0.05$.

Stoichiometric ratio	Explanatory	F	Df	Df.res	P
C:N	W	0.76	1.00	20.00	0.39
	E	1.35	1.00	20.00	0.26
	W*E	5.88	1.00	20.00	**0.02**
C:P	W	2.74	1.00	14.00	0.12
	E	5.22	1.00	14.00	**0.04**
	W*E	8.73	1.00	14.00	**0.01**
N:P	W	3.48	1.00	14.00	0.08
	E	9.27	1.00	14.00	**<0.01**
	W*E	1.14	1.00	14.00	0.30

reflect the combined effect of both these processes.

Our results showed no changes in C:N or C:P ratio of phytoplankton communities, suggesting that phytoplankton community stoichiometry may be driven by both bottom-up and top-down forces (Mandal et al., 2018). Zooplankton graze on phytoplankton and limit phytoplankton growth, while they also effectively recycle nutrients and benefit phytoplankton growth (Schulhof et al., 2019; Vanni, 2002). These forces were likely to be in a dynamic equilibrium over the six-month life of the experiment, in which case the phytoplankton might not be nutrient-limited (Elser and Urabe, 1999; Mandal et al., 2018). Our data showed that all treatments did not significantly alter Chl-*a* concentration, and suggested that there might be no difference in nutrient availability, which laterally verified this conjecture.

Interestingly, warming and nutrient addition interactively affected the C:N and C:P ratios of zooplankton. Our results showed that warming and nutrient addition individually trended to reduce the C:N and C:P ratios of zooplankton, while this trend was relatively reversed when acting jointly. Other studies suggest that zooplankton can increase their grazing rates in response to low nutrient content in food to compensate for lack of essential nutrients required to maintain growth (Mandal et al., 2018; Hillebrand et al., 2009). Our results also support this, with zooplankton having a lower C:N ratio than phytoplankton regardless of what the treatment was. Warming may induce higher growth rates, and nutrient addition may yield more food for zooplankton. Both processes contribute to increase zooplankton grazing rates and lead to lower C:N

and C:P ratios. In addition, the potential decrease in zooplankton C:P ratio may be related to elevated temperature, because temperature alters the lipid (C-rich) and RNA (P-rich) content in zooplankton body through mediated metabolic processes (Prater et al., 2018). However, under combined stress of warming and nutrient addition, the increased C:P ratio in zooplankton was most likely mediated through changes in zooplankton community composition (Hassett et al., 1997). At the same time, the increased N:P ratio of zooplankton suggested that the nutrient demand ratio of zooplankton had changed. In freshwater ecosystems, copepods have higher N:P ratios than cladocerans (Elser and Urabe, 1999; Sterner and Elser, 2002), so changes in zooplankton community composition may be responsible for the higher N:P ratio under the combined stress of warming and nutrient addition.

4.2. Stoichiometric mismatches

The stoichiometric responses of organisms to environmental changes continue to be studied. There are increasing concerns about stoichiometric mismatch across trophic levels that can reduce trophic transfer efficiency and impair ecosystem functioning (van de Waal et al., 2010; De Senerpont Domis et al., 2014).

Currently, many concerns about stoichiometric mismatch are raised from the phytoplankton perspective, because in previous studies a decrease in the quality of phytoplankton as food, i.e., an increase in the C:nutrient ratios, was observed (De Senerpont Domis et al., 2014; Diehl et al., 2022). However, we showed that warming or nutrient addition did not change the stoichiometric ratios of phytoplankton, while each treatment individually trended to decrease the C:N and C:P ratios of zooplankton which resulted in a stoichiometric mismatch between them. Higher temperatures result in increased metabolic rates of zooplankton (Gillooly et al., 2001), a process that requires more C:nutrient ratios than growth (Anderson et al., 2017). Therefore, a lower C:nutrient ratio in the body is expected if metabolism scales faster than growth with temperature (Laspoumaderes et al., 2022). Nutrient addition may also lower C:nutrient ratios in zooplankton through compensatory grazing to acquire more nutrients (Mandal et al., 2018). Notably, stoichiometric mismatches under warming caused by increasing nutrient demands for growth (lower C:nutrient ratios) by zooplankton differ from those caused by poorer food quality (higher C:nutrient ratios) of phytoplankton, because the former is energy-limited (C limited) for zooplankton metabolism while the latter is nutrient-limited (N and/or P limited) for zooplankton growth (Laspoumaderes et al., 2022). Both result in nutrient or energy loss, leading to an impairment in trophic transfer at the phytoplankton-zooplankton interface (Anderson et al., 2005).

The C:N and C:P mismatches between phytoplankton and zooplankton were reversed when warming and nutrient addition acted jointly. At the same time, the N:P mismatch was also unexpectedly reduced. Again, this N:P mismatch was also attributed to the changes in N:P ratio of zooplankton. However, the mechanism by which this interaction occurs is not yet clear. Some current studies suggest that warming under eutrophic conditions reduces the proportion of large cladocerans (*Daphnia*) in the total zooplankton community (He et al., 2018); whereas warming and phosphorus addition have a synergistic contribution to the recruitment and abundance of copepods (Zhang et al., 2021). Therefore, it is likely that the zooplankton community underwent a copepod-dominated compositional change under the combined stress of warming and nutrient addition. Copepods tend to have higher N:P ratios (Elser and Urabe, 1999; Sterner and Elser, 2002), so our N:P ratio results also suggested the possibility of compositional shifts.

5. Conclusions

Our study investigated the separate and combined effects of climate warming and eutrophication on stoichiometric mismatches between phytoplankton and zooplankton by examining stoichiometric responses of both communities. We observed a growing trend in stoichiometric
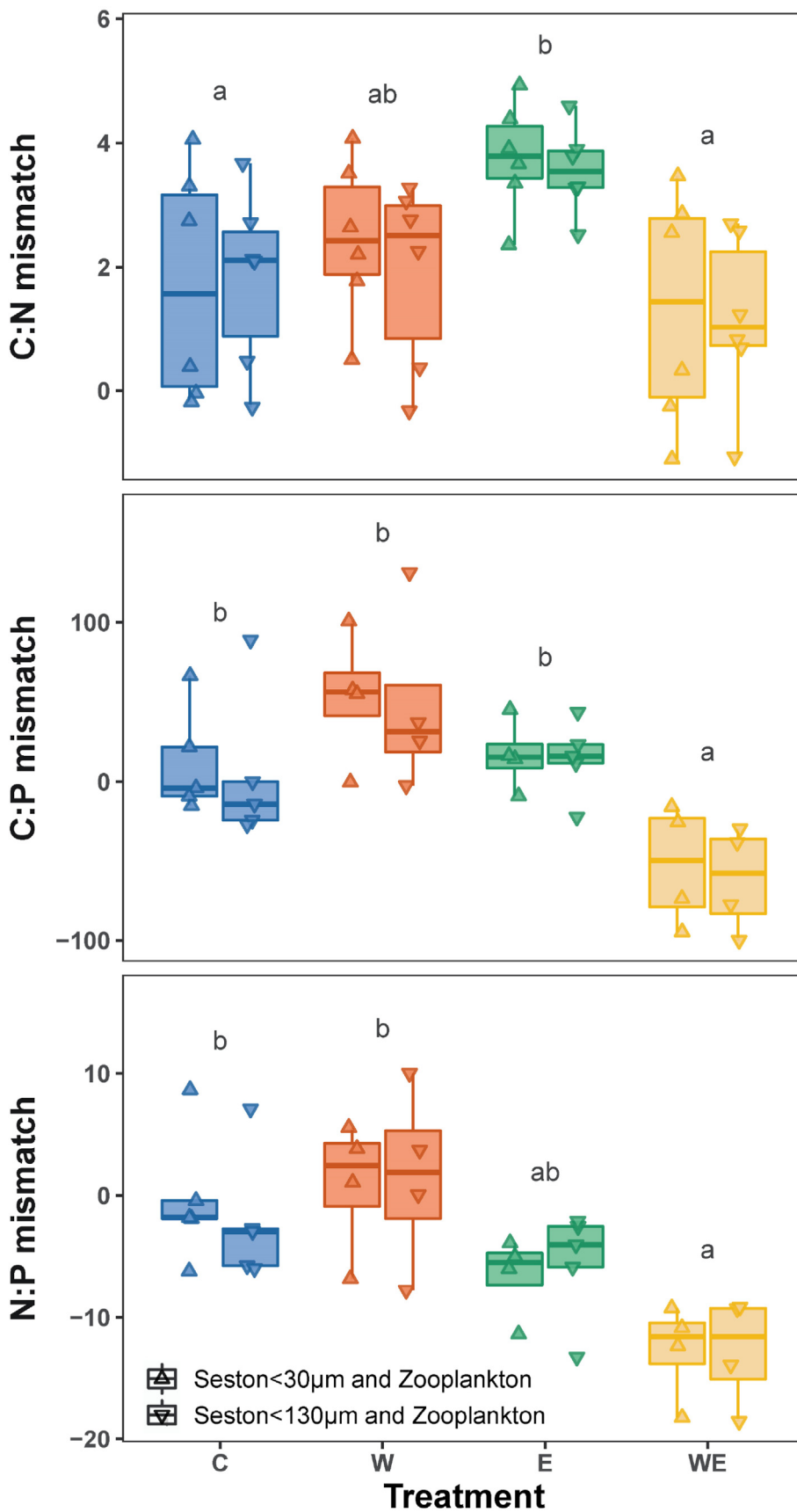
Fig. 2. Stoichiometric mismatches between phytoplankton and zooplankton for each treatment. Boxplots show the minimum, first quartile, median, third quartile and maximum values. Symbols indicate individual replicate (mesocosm) measurements within each of the treatments. Letters indicate the significance of differences among the treatments ($\alpha = 0.05$). C stands for the control treatment, W for warming, E for nutrient addition, and WE for the combined warming and nutrient addition treatment.

Table 3

Results from LMM analyses evaluating effects of warming (W), nutrient addition (E), seston sizes (F) and their interactions on stoichiometric mismatch between phytoplankton and zooplankton. Bold numbers indicate significant effects at $\alpha = 0.05$.

Stoichiometric mismatch	Explanatory	F	Df	Df.res	P
C:N mismatch	W	3.89	1.00	20.00	0.06
	E	0.90	1.00	20.00	0.35
	F	0.52	1.00	20.00	0.48
	W*E	7.80	1.00	20.00	**0.01**
	W*F	0.27	1.00	20.00	0.61
	E*F	0.01	1.00	20.00	0.93
	W*E*F	0.38	1.00	20.00	0.54
C:P mismatch	W	0.72	1.00	13.97	0.41
	E	6.29	1.00	13.98	**0.03**
	F	1.97	1.00	13.06	0.18
	W*E	10.51	1.00	13.97	**<0.01**
	W*F	0.09	1.00	13.06	0.77
	E*F	0.02	1.00	13.08	0.88
	W*E*F	0.30	1.00	13.07	0.60
N:P mismatch	W	0.88	1.00	13.96	0.36
	E	14.16	1.00	13.98	**<0.01**
	F	0.14	1.00	13.07	0.72
	W*E	3.95	1.00	13.97	0.07
	W*F	0.53	1.00	13.07	0.48
	E*F	0.62	1.00	13.09	0.44
	W*E*F	1.61	1.00	13.08	0.23

mismatch when warming or nutrient addition acted individually, which was mediated by an increase in nutrient demand by zooplankton for growth. However, when these stressors acted jointly, the mismatch was reversed. This could be because warming and eutrophication combined drived changes in zooplankton species composition, which accordingly reshaped the stoichiometric composition at the community level.

Overall, ecological stoichiometry integrates multiple life processes and community changes. This provide insights into ecological mechanisms, while cross-trophic level stoichiometry provides a valuable tool to help understand trophic interactions within those ecosystems. Meanwhile, environmental stressors often lead to changes in species composition which can complicate community-level stoichiometry. Therefore, predicting the effects of global change on stoichiometric mismatches requires consideration not only of cross-trophic levels, but of compositional changes within communities as well.

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Author contributions

Konghao Zhu: Conceptualization, Methodology, Software, Writing – original draft. **Huan Zhang:** Conceptualization, Data curation, Funding acquisition, Writing – review & editing. **Peiyu Zhang:** Project administration. **Panpan Wang:** Investigation. **Hailu Li:** Investigation. **Mingjun Feng:** Investigation, Methodology. **Huan Wang:** Investigation. **Hongxia Wang:** Project administration. **Min Zhang:** Writing – review & editing. **Jun Xu:** Conceptualization, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Jun Xu is an editorial board member for *Water Biology and Security* and was not involved in the editorial review or the decision to publish this article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.watbs.2023.100205.

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