Title: Trans-generational plasticity to warming has antagonistic effects on nutrient release by a keystone grazer

Running title: TGP to warming decreases nutrient release

Authors: Sarah S Hasnain[1,2,3,4]*, Valerie McCarthy[5], Zeynep Uyar[1], Lucia Cabrera-Lamanna[1,6], Cihelio Alves Amorim[1,7], Emma Drohan[8], Gülce Yalçın[9,10], Dilvin Yıldız[1,9], Meryem Beklioğlu[1,9], Kemal Ali Ger[11,12]*

Author Contributions CRediT
Sarah S Hasnain: Conceptualization (equal), Data curation (lead), Formal Analysis (lead), Investigation (lead), Methodology (equal), Visualization (lead), Writing-Original Draft Preparation (lead), Writing-Review and Editing (lead). **Valerie McCarthy**: Investigation (supporting), Methodology (equal), Resources (supporting), Funding Acquisition (supporting), Writing-Original Draft Preparation (supporting), Writing-Review and Editing (equal). **Lucia Cabrera-Lamanna**: Investigation (supporting), Data curation (supporting), Writing-Review and Editing (equal). **Zeynep Uyar**: Investigation (supporting), Data curation (supporting), Writing-Review and Editing (supporting). **Emma Drohan:** Investigation (supporting), Data curation (supporting). **Cihelio Alves Amorim:** Investigation (supporting), Writing-Review and Editing (equal), **Gülce Yalçın**: Project administration (supporting). **Dilvin Yıldız:** Project administration (supporting), Investigation (supporting). **Meryem Beklioglu:** Conceptualization (supporting), Methodology (supporting), Resources (lead), Project Administration (lead), Funding Acquisition (lead), Supervision (supporting). **Kemal Ali Ger:** Conceptualization (equal), Data curation (supporting), Formal Analysis (supporting), Investigation (supporting), Methodology (equal), Project Administration (supporting), Resources (supporting), Supervision (lead), Visualization (supporting), Writing-Original Draft Preparation (supporting), Writing-Review and Editing (equal)

*co-corresponding authors

Author affiliations
1 Department of Biological Sciences, Middle East Technical University, Üniversiteler Mahallesi, 06800 Çankaya/Ankara, Türkiye
2 Integrated Sciences and Mathematics, Habib University, Block 18, Gulistan-e-Jauhar, University Avenue Karachi 75290, Sindh, Pakistan
3 Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, LOV, F-06230 Villefranche-sur-Mer, France
4. Institute of Globally Distributed Open Research and Education (IGDORE), Gothenburg, Sweden
5. School of History and Geography, Dublin City University, D09 YT18 9, Dublin, Ireland
6. Department of Ecology, Radboud Institute for Biological and Environmental Sciences, Radboud University, Nijmegen, the Netherlands

7. WasserCluster Lunz – Biologische Station, Dr. Carl Kupelwieser Promenade 5, 3293, Lunz am See, Austria
8. Inland Fisheries Ireland, D24 CK66, Dublin, Ireland
9. Centre for Ecosystem Research (EKOSAM), Middle East Technical University, Üniversiteler Mahallesi, Dumlupınar Bulvarı No: 1, 06800 Çankaya/Ankara, Türkiye
10. Department of Plankton and Microbial Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Stechlin, Germany
11. Departamento de Ecologia, Centro de Biociências Universidade Federal do - R. das Biociências, s/n - Lagoa Nova, Natal - RN, 59078-970, Brazil
12. Institute of Biological Research Cluj, National Institute of Research and Development for Biological Sciences, 48 Republicii Street, 400015, Cluj-Napoca, Cluj County, Romania

Authors' information:

Corresponding authors

SSH – ORCID 0000-0003-4358-5478; Email: sarah.hasnain@queensu.ca

KAG – ORCID 0000-0002-6075-5697; Email: ali.ger@ufrn.br

VMC – ORCID 0000-0002-9169-2043; Email: valerie.mccarthy@dcu.ie

CAA – ORCID 0000-0002-7171-7450; Email: alvescihelio@gmail.com, cihelio.amorim@wcl.ac.at

LCL – ORCID 0000-0002-6144-8429; Email: lucia.cabrera@ru.nl

ZDU – ORCID XXXX; Email: zeynep.uyar@metu.edu.tr

ED – ORCID 0000-0002-3391-3100; Email: emma.drohan@fisheriesireland.ie

GY – ORCID 0000-0002-2921-0197; Email: guelce.yalcin@igb-berlin.de

DY – ORCID 0000-0002-8031-8996; Email:dilvinim@metu.edu.tr

MB – ORCID 0000-0003-2145-3941; Email: meryem@metu.edu.tr

Title: Trans-generational plasticity to warming has antagonistic effects on nutrient release by a keystone grazer

Abstract

Human-driven environmental change can reshape species traits via evolutionary adaptation and plasticity. Yet, how stress-induced plasticity, especially trans-generational responses, cascades to ecosystem processes remains unknown. In particular, warming can lead to marked effects on fitness via trans-generational plasticity (TGP), yet how such changes affect ecosystem functioning is not well understood. Here, we used reciprocal transplant experiments to examine if warming triggers TGP impacts on grazing and fluxes in nitrogen (N) and phosphorus (P) by the keystone freshwater grazer *Daphnia magna*. Individuals were reared for two generations at either 18°C or 24°C. Offspring from the second generation were exposed to either 18°C or 24°C and we quantified algal clearance, and nutrient uptake and release responses. Our results show that warmer maternal temperature exposure did not alter grazing rates. However, warmer maternal temperatures elevated body P content in offspring and altered nutrient release, thereby increasing the ratio of dissolved N:P in the system. These results suggest that TGP can shift body stoichiometry and nutrient release patterns under warming, with potentially strong consequences for N and P cycling in lake ecosystems.

Introduction

Human-induced rapid environmental change is a major global stressor, with serious consequences on ecosystems. Predicting the ecological outcomes requires understanding the multitude of mechanisms by which species can respond (Donelan et al. 2020). These changes can alter species and trait composition in ecological communities by filtering species and traits that can withstand these conditions (Walther 2010, Maclean & Beisinger 2017, Bardgett & Caruso 2020) but also impact key physiological processes such as metabolism (Seebacher et al. 2015), nutrient uptake and excretion (Ganser et al. 2015), with cascading effects on primary production, nutrient cycling and greenhouse gas emissions (Davidson et al. 2015, Hood et al. 2017, Cavicchioli et al. 2019). Yet, we are only in the infancy of understanding how stress-induced trait variation regulates key ecosystem functions.

Organisms respond to environmental challenges through adaptation, plasticity, or range shifts (Seebacher et al. 2015; Donelson et al. 2019; Rodrigues and Beldade 2020). Adaptation is favoured under slow, directional change, while plasticity is favoured in rapidly fluctuating environments without clear directional selection (Kawecki 2000). Through plasticity, organisms can alter their own phenotype; within-generation plasticity (WGP), or the phenotype of their offspring and future generations, often referred to as maternal effects, or trans-generational plasticity (TGP; Donelson et al. 2018; Wadgymar et al. 2018). TGP arises when parental phenotypes influence offspring phenotypes through mechanisms beyond genetic inheritance such as RNA-mediated processes, epigenetic marks, or DNA methylation (Rasanen & Kruuk 2007; Wolf & Wade 2009; Kujiper & Hoyle 2015). While less examined than WGP or adaptation, TGP is a key mechanism regulating how species respond to global stressors (Donelan et al. 2020).

Most studies examining TGP focus on offspring fitness, population dynamics, and species interactions (Donelson & Munday 2015; Quigley et al. 2019; Li et al. 2021). However, TGP has the potential to restructure ecosystem function by altering "effect" traits, regulating ecosystem processes like nutrient cycling and carbon flux (Suding et al. 2008; Hebert et al. 2016). In the dandelion, *Taraxacum brevicorniculatum*, competition-induced TGP via DNA methylation altered offspring leaf decomposition (Puy et al. 2021), highlighting how epigenetic effects can cascade to ecosystem processes. However, little is known about how TGP mediates ecological function under rapid environmental change, such as increasing global average temperatures and increasing frequency of extreme warming events predicted under global change (Collins et al. 2013). Thermal stress in particular, not only induces can alter organismal carbon (C):nutrient ratios via elevated metabolism, with potentially cascading effects on nutrient cycling and energy transfer across trophic levels. While TGP response to warming is known to regulate offspring growth rates, body size, mating success, and thermal performance (Walsh et al. 2014; Cavieres et al. 2019; Diaz et al. 2020; Betini et al. 2020), the impacts on body stoichiometry and nutrient cycling remain largely unexplored.

Daphnia are keystone zooplankton grazers across global freshwater ecosystems, transferring energy from phytoplankton production to higher trophic levels (Miner et al. 2012). They have the highest per-capita grazing rates (Hansen et al. 1997), high phosphorus (P) demand, and the lowest

nitrogen-to-phosphorus (N:P) body ratio among zooplankton (Mackay and Elser 1998; Elser et al. 2000; Paterson et al. 2002), making them influential regulators of C, N and P. Plastic responses to thermal stress are extensively documented in *Daphnia*, including TGP effects on fecundity, size at maturity, growth rates, life span, oxidative stress, and resistance to infectious disease (Mckee and Ebert 1996; Pajk et al. 2012; Garbutt et al. 2014; Toyota et al. 2019; Betini et al. 2020; Im et al. 2020). While these are characterized as "response" traits (Suding et al. 2008, Suding & Goldstein 2008), the prevalence of these responses suggests that warming-induced TGP could also mediate "effect" traits (Suding et al. 2008, Suding and Goldstein 2008, Hebert et al. 2016); grazing, nutrient uptake and excretion, with ecosystem-level consequences.

In this study, we test if warming and subsequent TGP alter the expression of traits linked to ecosystem function, i.e. effect traits, in the keystone grazer *Daphnia magna*. We reared *D. magna* populations at two different temperatures for 2 generations, mimicking natural populations exposed to two different thermal regimes, and subsequently conducted a 12-hour reciprocal transplant grazing assay with their offspring to quantify TGP effects on grazing, and N-P uptake and release rates. We hypothesized that exposure to warmer maternal temperatures would prime offspring to warmer thermal conditions via TGP, leading to increased thermal tolerance of offspring. A match between maternal and offspring thermal conditions was expected to stabilize physiological responses, resulting in stable grazing rates, and nutrient retention. Conversely, we expected that offspring exposure to warmer conditions without warmer maternal conditions would result in compensatory metabolic responses, thereby increasing grazing rates and altering release of N and P.

Materials and Methods

Daphnia magna brood stock and experimental design

Daphnia magna, in particular, is a well-established genomic model organism used across ecological and evolutionary studies, especially those examining eco-evolutionary dynamics (Miner et al. 2012, Walsh et al. 2018, De Meester et al. 2023). We conducted a reciprocal transplant experiment using a brood stock *Daphnia magna* population reared in the laboratory for at least ten years in a temperature- and light-controlled room, with exposure to 18°C at 14:10 h Light:Dark cycle (L:D). This population was periodically stocked over ten years with individuals from Lake Eymir, Turkey in order to maintain genetic diversity. *D. magna* were reared in a modified low-nutrient WC medium (without $NaNO_3$, K_2PO_4, ATE, and Vitamin solution, Guillard & Lorenzen 1972) in 100 mL glass containers and fed with 0.5 mgC L^{-1} of *Chlamydomonas reinhardtii* from an exponentially growing batch culture every three days. A carbon equivalent concentration of *C. reinhardtii* was determined from cell density (via hemocytometer) and biovolume (Duncan and Rocha 1984). Individual daphnids were transferred to clean containers filled with fresh media and algal suspension every 3 days to prevent algal accumulation and differences in food quality. All individuals were kept in temperature-controlled rooms and exposed to a light intensity of 45 µmol photons m^{-2} s^{-1} under a 14:10 h L:D cycle.

We took 100 adult female *D. magna* from the brood stock and arbitrarily assigned 50 each to a to either 18℃ or 24℃ (maternal temperature) for 2 generations (schematic overview in Figure 1) and maintained at

a density of 10 individuals per 100ml glass beaker to standardize any effects of density-dependence. Each container was checked daily for neonate emergence, with neonates removed and placed into a new container, at a maximum density of 10 individuals per container. Neonates with different emergence days were maintained in separate containers allowing us to keep track of age. Twenty neonates from the F_2 generation from both maternal temperature populations were measured using a stereomicroscope to estimate the effect of differences in temperature on size at birth.

Critical thermal maxima assessment

To confirm that maternal exposure to different temperatures over two generations affected thermal tolerance, we assessed the critical thermal maximum (CT_{max}) for adult *D. magna* from the F_2 generation reared at either 18°C or 24°C. A single adult was placed in a 10ml glass test tube in the modified WC medium for each observation, with 15 replicates per maternal exposure temperature population. Test tubes were positioned upright in a rack and placed in a 25L Memmert water bath and temperature was gradually increased from 29.5-30ºC (room temperature) to 50ºC at the rate of 1.6ºC per minute. Individual *D. magna* were observed every 5-10 seconds until the animal stopped swimming and sank to the bottom of the test tube. CT_{max} was defined as the temperature at which individuals lost their motor function and sank to the bottom of the tube. *Daphnia* were transferred to ambient conditions to recover after they stopped moving. Individuals used to assess CT_{max} were not used for subsequent grazing assays.

Grazing and N-P release *experiment*

We conducted a 12-hour grazing trial to assess if differences in maternal temperature exposure altered offspring grazing, dissolved N:P, and body P composition at two different temperatures. F_2 adults and juveniles (at least third instar) from both maternal temperature exposure treatments (18ºC or 24°C) were assigned to two exposure treatments: 18ºC or 24°C for the grazing experiment, resulting in four treatment combinations (Figure 1). There was no acclimation period prior to the application of exposure temperature. Each maternal and exposure treatment combination was replicated four times. To remove any effect of differences in sizes on grazing rates, we standardized the number of adult and juvenile individuals added to each grazing replicate to ensure that all grazer present treatments contained similar biomass (see supplementary information for more details).

For each treatment replicate, we also set up an identical replicate without any *D. magna* to control for any changes in algal density, N, and P concentrations in the absence of *D. magna* grazing and excretion. All *D. magna* individuals were starved for 24 hours prior to the grazing experiment to minimize differences in gut fullness and empty gut contents. Glass jars containing 400ml of N- or P-free WC medium were set up for each treatment and control replicate. We inoculated each jar with 0.375mg C L^{-1} equivalent of *C. reinhardtii* as the sole food source. *C. reinhardtti* provided for feeding was rinsed three times with nutrient-free WC before being added to each replicate. *D. magna* individuals were added to the treatment replicates after the algae addition. All replicates were gently bubbled with air to prevent sedimentation and kept in darkness for the entire 12-hour assay duration to prevent algal growth.

Daphnids are generally phosphorus-limited (Urabe et al. 1997; DeMott & Gulatti 1999; Anderson & Hessen 2005, Xu et al. 2021), therefore, any changes in uptake and excretion of phosphorus due to maternal thermal exposure would likely be detected in body content as well. At the end of the grazing experiment, all *D. magna* individuals were removed and placed in a nutrient-free WC medium for gut content evacuation (24h, standard procedure) to assess differences in phosphorus body content (Symntek et al. 2007). For all replicates, we filtered the entire medium volume through a Whatman GF/C filter, which were subsequently frozen at -20°C for Chl-a analysis. We examined changes in dissolved N and P using the filtrate rather than conducting separate excretion assays as it allowed us to assess the impacts of warming on N and P release in the same organisms that are grazing. The entire volume of the filtrate was also frozen at -20°C for laboratory analysis of dissolved N and P (see below).

Laboratory analyses

The filtrate from each replicate was assessed for ammonium (NH_4), nitrate and nitrite (NO_3+NO_2) using an automated wet chemistry analyser (San++, Skalar Analytical, The Netherlands) using standard protocols (Krom 1980, Kroon 1993). Dissolved inorganic nitrogen (DIN) was calculated by summing NH_4, NO_3 and NO_2 concentrations. We used the molybdenum blue method (Mackerth, 1978) to spectrophotometrically determine soluble reactive phosphate (SRP) concentrations for all replicates. Chlorophyll-a samples (Chl-a) were extracted with ethanol before reading the absorbances in a Perkin Elmer Lambda35 UV–Vis spectrophotometer (limit of detection [LoD]: 0.04 µg Chl-a L^{-1}) (Jespersen & Christoffersen 1987). All daphnids from each replicate were placed on phosphorus-free tin capsules and dried at 60°C for 24 hours. Phosphorus body content was determined spectrophotometrically by the ascorbate-reduced molybdenum-blue method after combustion at 550°C for 2h and digestion with potassium peroxide sulphate ($K_2S_2O_8$) under pressure (Eisenreich, Bannerman & Armstrong, 1975).

Clearance rate, dissolved N:P, and N and P release rate calculations

We calculated clearance rates using the method provided by Frost (1972). Dissolved N:P ratios were determined for the grazer present treatments by calculating molar N from combined NH_4 and NO_2 + NO_3 concentrations and molar P from Soluble Reactive Phosphorus (SRP) concentrations.

We also determined relative release rates for these dissolved nutrients to assess which compounds were driving any observed changes in dissolved N:P. In contrast to traditional excretion rate estimates, which require that the grazer is isolated (i.e., no prey or grazing), relative release rates enable simultaneous quantification of rates of nutrient release and clearance within the same environment. Details for relative release rate calculations and results obtained are provided in supplementary materials.

Statistical analysis

We used model-based methods to assess if maternal and offspring exposure temperature (i.e. independent variables) had a statistically significant effect ($p< 0.05$) on clearance rates, dissolved N:P, N-P release rates, and body P composition (i.e. dependent variables) (Table S1). All dependent variables were visually assessed for normality and homoskedasticity using histograms and boxplots. All data were normally distributed, but heteroskedasticity was observed between different maternal and exposure temperature treatments for changes in dissolved N:P ratios. We used generalized least square

models (GLS) for these variables with maternal temperature, exposure temperature, and the interaction between maternal temperature and offspring exposure temperature as predictor variables (Table S1, see supplementary materials for GLS fitting procedures). Body P composition did not violate any assumptions for normality or heteroskedasticity. Therefore, we assessed the interactive effect of maternal temperature and offspring exposure temperature using linear models. Due to the presence of outliers, we used a gamma-distributed robust regression model with a log link function to assess differences in CT_{max} between F_2 adults reared at 18ºC or 24ºC. Details for model selection procedures are provided in supplementary materials. All analyses were performed using R (version 4.2.2; R Core Team, 2023) with packages *nlme* (version 3.1-153) used for GLS regression, *robustbase* (version 0.93-8) used for robust regression, and *multcomp* (version 1.4-1) for generalized linear hypothesis testing.

Results

Critical thermal maxima

The generation time for *D. magna* populations reared at 18ºC was longer (9-14 days) as compared to those reared at 24ºC (7-8 days). Offspring from the maternal exposure population of 24ºC were larger than offspring from the 18ºC populations (59.120µm, $p < 0.0001$). Maternal exposure to warmer temperatures increased offspring CT_{max}. While the mean CT_{max} of *D. magna* with maternal temperature of 18°C was 0.9 °C lower compared to those reared at 24°C (Mean CT_{max} 18°C = 44.2°C, Mean CT_{max} 24°C = 45.1°C). This difference was not statistically significant (Figure 2, $p = 0.07$, $df = 28$).

Specific Clearance rates

Maternal temperature did not affect *D. magna* clearance rates (Figure 2b, Table S1, $p = 0.622$). Clearance rates were also not impacted by differences in exposure temperature ($p = 0.728$). We observed a large variation in clearance rates for *D. magna* with maternal temperature of and exposed to 18°C (Interquartile Range (IQR) = 140.283 mL mg^{-1} h^{-1}) during the grazing experiment as compared to other treatments (Maternal 18°C – Exposure 24°C: IQR = 31.845 mL mg^{-1} h^{-1}, Maternal 24°C – Exposure 18°C: IQR = 27.802 mL mg^{-1} h^{-1}, Maternal 24°C – Exposure 24°C: IQR = 34.106 mL mg^{-1} h^{-1}).

Dissolved N:P and P body content

We observed an interactive effect of maternal and offspring exposure temperatures on dissolved N:P in the filtrate (Figure 3a, $p = 0.001$, Table S1, compound specific release rates provided in supplementary material). Filtrate N:P ratios were greater in treatments with warmer maternal and offspring exposure temperatures as compared to those with only warmer maternal or warmer offspring exposure temperatures (Maternal 24°C – Exposure 24°C: Mean = 48.825 $\pm$ 10.751, Maternal 18°C – Exposure 24°C: Mean = 6.616 $\pm$ 1.169, Maternal 24°C – Exposure 18°C: Mean = 14.438 $\pm$ 2.505). The lowest N:P ratios were observed in treatments with both maternal and offspring exposure temperatures of 18°C (Mean = 4.045 $\pm$ 0.581).

Warmer maternal and exposure temperature increased *D. magna* body P content, by a factor of up to 60%, depending on offspring exposure temperature (Figure 3b, Table S1). Mean %P dry mass was 10% greater in *D. magna* with maternal temperature of 24°C as compared to those with maternal temperature of 18°C. *D. magna* exposed to 24°C during the grazing assay had an average of 14.7% greater %P dry mass as compared to those exposed to 18°C.

Discussion

Our results provide novel evidence that trans-generational plastic responses to warming impact body P content and the N:P release ratios in *D. magna*. In contrast, both maternal thermal environments and exposure temperatures shaped phosphorus release. Offspring from colder maternal environments (18°C) released more P and retained 10% less P body mass, as compared to offspring from warmer maternal environments. In general, warmer exposure temperatures promoted P retention and reduced P release relative to N. These results provide the first evidence that TGP responses to thermal stress in *D. magna*, a keystone grazer across lakes and pond ecosystems globally (Miner et al., 2012), can impact zooplankton-mediated recycling and consequently have the potential to affect N and P cycling in these systems (Elser et al., 2000; Paterson et al., 2002; Sarnelle, 2007), especially in the context of global climate change (Balseiro et al., 2021; Starke et al., 2021).

We observed an increase in thermal tolerance for *D. magna* reared at warmer (24°C) as compared to colder temperatures (18°C), although this difference was not statistically significant. Previous exposure to different thermal regimes can expand *D. magna* thermal limits, through both plastic and evolutionary processes (Geerts et al., 2015; Vanvelk et al., 2021). Our results suggest that differences in maternal exposure temperatures could have resulted in TGP for CTMax, but this may be more evident with a greater number of replicates.

There were no clear effects of maternal temperature on clearance rates, although offspring from cooler maternal thermal environments (18°C) showed highly variable clearance rates at colder temperatures as compared to those from warmer maternal thermal environments (24°C). At higher exposure temperatures (24°C), this variation was reduced. Our results are contrary to the expectation of increased algal clearance rates with warming (Burns, 1969; Müller et al., 2018). This lack of response likely reflects multiple interacting mechanisms at play. First, *D. magna* filtration rates are impacted by the rate of temperature change (Müller et al., 2018) and rapid increase of six degrees may have imposed an acute thermal stress beyond short-term acclimation capacity. Second, our warmer exposure temperatures may have exceeded the thermal optima for our *D. magna* population, potentially inhibiting grazing in some individuals, thereby leading to the large variation observed. Finally, opposing WGP and TGP responses at warmer exposure temperatures could have masked a consistent grazing signal (Luquet & Tariel, 2016).

Temperature and Nutrient Cycling

Daphnia are keystone grazers that play a central role in cycling nutrients through freshwater ecosystems (Mackay & Elser 1998, Stibor 2010). Mismatches between an organism's elemental

composition and its food resources determine the rates and ratio of nutrient release. Zooplankton, such as *Daphnia*, can actively retain the most limiting element from their diet, while excreting (*i.e.* discharge metabolic wastes) or egesting (*i.e.*, discharge undigested food) others in excess (Frost et al., 2006; Doi et al., 2011). The relative rates at which elements are excreted or egested are largely species-specific, dictated by nutrient imbalances (Urabe, 1993; Sterner & Elser, 2002), and influenced by abiotic conditions, such as increasing temperatures (Halvorson et al. 2019). Our study shows that TGP responses to climate warming have the potential to shift *Daphnia*-mediated nutrient fluxes *via* increasing nitrogen release relative to phosphorus, thereby increasing dissolved N:P ratios in our replicates. These shifts could increase N:P ratios across freshwater systems, favouring the proliferation of certain cyanobacteria taxa (Dolman et al., 2012; Vanderploeg, et al., 2017). These consumer-driven stoichiometric shifts are likely to be significant in low-nutrient systems (McIntyre et al., 2008; Atkinson and Vaughn, 2015), but may be further amplified by warming-driven increases in cyanobacterial biomass (Paerl and Paul, 2012; Bartosiewicz et al., 2019; Urrutia-Cordero et al., 2020). While our results provide a robust, proof-of-concept for TGP effects on nutrient cycling, future research should explicitly measure excretion rates across *Daphnia* populations with different thermal adaptations and algal diets.

Several studies have found that organismal P content declines with increasing temperature (Woods et al., 2003; Balseiro et al., 2021). According to the growth rate hypothesis, organismal RNA content largely determine body P (Elser et al., 2000), with colder temperatures consistently yielding higher RNA content across all poikilothermic (i.e., with variable internal temperature) taxa (Woods et al., 2003). This is hypothesized to result from increased ribosomal translational efficiencies at higher temperatures reducing cellular RNA and P demands (Toseland et al., 2013; Cross et al., 2015). However, decoupling between organismal P content and RNA has been observed across a wide range of taxa, including *Daphnia* (Cross et al., 2015; Prater et al., 2018), suggesting that this link is restricted to P-limited systems. For instance, Prater et al. (2018) show that warmer temperatures can relax %P-RNA coupling, with high P body content observed despite reduced RNA production at increasing temperatures. In our study, warmer maternal and exposure temperatures increased *D. magna* body P content, although we did not measure cellular RNA content or additional pools of organismal P such as phosphosugars or phospholipids, leaving the source of this increase unknown. Comparative warming-driven increases in P concentrations have been reported in damselflies (Janssens et al., 2015) and in planktonic organisms (Mathews et al., 2018). However, other studies found no significant changes in N:P ratios (Ventura et al., 2008; Zhang et al., 2016; Prater et al., 2018). Despite the increasing number of studies investigating temperature and nutrient linkages (*e.g.* Woods et al., 2003; Makino et al., 2011; Cross et al., 2015), a unified framework to mechanistically account for these observations is still lacking. Our results suggest that TGP responses to temperature can impact nutrient cycling, and should be incorporated in future frameworks.

It is possible that the TGP effects we observed for P uptake and N:P release ratios are adaptive for *D. magna*. Both warming and phosphorus limitation alter life history traits including clutch size, mean clutch length, and fecundity, which are indirectly related to fitness (Cavalheri et al. 2019, Harnett et al. 2019). Furthermore, selection for adaptive phenotypic plasticity has been observed in *Daphnia pulicaria* populations from alpine lakes (Cavalheri et al. 2019) and *Daphnia galeata* populations

exposed to thermal effluents (Dzuiba et al. 2021). TGP responses can be locally adapted as well, as shown in *Daphnia ambigua* exposed to fish cues (Walsh et al. 2016). Adaptive associations between our observed TGP responses and warming may arise through selection on P uptake and N:P release ratios under thermal stress, a combination of selection and TGP, or selection on TGP itself (Via et al. 1995; Ghalambor et al. 2007). As our study only assessed TGP in a single *D. magna* laboratory population, we cannot distinguish among these mechanisms. We recommend that future studies should explicitly quantify the relative contributions of these mechanisms to better distinguish the role of selection and plasticity, and to determine their adaptive potential.

Conclusions

To our knowledge, our results are the first to show that TGP responses to warming alter physiological traits governing nutrient cycling in ecosystems. Warmer maternal temperatures increased P content in *Daphnia*, and altered dissolved N:P ratios in our system. These results have potentially significant implications for N:P ratios in lakes where *Daphnia* are key nutrient recyclers (Elser & Urabe, 1999). Shifts in N:P ratios can alter fundamental ecosystem functions, including biogeochemical cycling and nutritional status of species (Penuelas et al. 2020). While our experiment was designed as a proof-of-concept, the clear TGP-driven shifts we observed suggest that this could be an overlooked pathway through which warming alters nutrient cycling. We recommend that future studies of climate change impacts on ecosystem functioning consider the contribution of trans-generational plasticity on potential outcomes.

Acknowledgements

We thank Sinem Yetim, Canberk Kahraman, Öykü Tanrıverdi and Bilge Arıkan for their assistance with *Daphnia* culturing and maintenance. We also thank Luc De Meester, Jeffrey Dudycha and Chao Zhang for their input on our experimental design, analysis, and presentation of results. Travel funding for SSH to perform this experiment and the nutrient analyses were supported by the EU H2020-INFRAIA-project No 731065 “AQUACOSM: Network of Leading European AQUAtic MesoCOSM Facilities Connecting Mountains to Oceans from the Arctic to the Mediterranean”.

References

Anderson, T. R., & Hessen, D. O. (2005). Threshold elemental ratios for carbon versus phosphorus limitation in Daphnia. *Freshwater Biology*, *50*: 2063-2075. doi:10.1111/j.1365-2427.2005.01450.x

Angilletta M.J., Steury T.D., and Sears M.W. 2004. Temperature, growth rate, and body size in ectotherms: fitting pieces of a life-history puzzle. *Integrative and Comparative Biology*, 44: 498–509. doi:10.1093/icb/44.6.498

Atkinson, D. (1994) Temperature and organism size – A biological law for ectotherms. *Advances in Ecological Research*, 25**:**1–58.

Atkinson, C. L., Capps, K. A., Rugenski, A. T., & Vanni, M. J. (2017). Consumer‐driven nutrient dynamics in freshwater ecosystems: From individuals to ecosystems. *Biological Reviews*, *92*: 2003-2023. doi:10.1111/brv.12318

Baird, R., Bridgewater, L. (2017). Standard Methods for the Examination of Water and Wastewater (23rd ed.), American Public Health Association, Washington DC

Balseiro, E., Laspoumaderes, C., Smufer, F. *et al.* (2021) Short term fluctuating temperature alleviates *Daphnia* stoichiometric constraints. *Scientific Reports,* 11. doi: 10.1038/s41598-021-91959-w

Bardgett, R. D., & Caruso, T. (2020). Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states. *Philosophical Transactions of the Royal Society B*, *375*:20190112. doi:10.1098/rstb.2019.0112

Bartosiewicz, M., Przytulska, A., Deshpande, B. N., Antoniades, D., Cortes, A., MacIntyre, S., Lehman, M.F., & Laurion, I. (2019). Effects of climate change and episodic heat events on cyanobacteria in a eutrophic polymictic lake. *Science of the Total Environment*, *693*:133414. doi: 10.1016/j.scitotenv.2019.07.220

Betini, G. S., Wang, X., & Fryxell, J. M. (2020). Transgenerational plasticity mediates temperature effects on fitness in the water flea Daphnia magna. *Canadian Journal of Zoology*, *98*:661-665 doi:10.1139/cjz-2020-0080

Cavalheri, H. B., Symons, C. C., Schulhof, M., Jones, N. T., & Shurin, J. B. (2019). Rapid evolution of thermal plasticity in mountain lake Daphnia populations. *Oikos*, *128*: 692-700. doi: 10.1111/oik.05945

Cavicchioli, R., Ripple, W. J., Timmis, K. N., Azam, F., Bakken, L. R., Baylis, M., Behrenfeld, M. J., Boetius A, Boyd, P. W., Classen, A. T., Crowther, T. W., Danovaro, R., Foreman, C. M., Huisman, J., Hutchins, D. A., Jansson, J. K., Karl, D. M., Koskella, B., Welch, D. B. M, Martiny, J. B. H., Moran, M. A., Orphan, V. J., Reay, D. S., Remais, J. V., Rich, V. I., Singh, B. K., Stein, L. Y., Stewart, F .J., Sullivan, M. B., van Oppen, M. J. H., Weaver, S. C., Webb, E. A., & Webster, N. A. (2019). Scientists' warning to humanity: microorganisms and climate change. *Nature Reviews Microbiology*, *17*: 569-586 doi:10.1002/9781119879534.ch19

Cavieres, G., Rezende, E. L., Clavijo‐Baquet, S., Alruiz, J. M., Rivera‐Rebella, C., Boher, F., & Bozinovic, F. (2020). Rapid within‐and transgenerational changes in thermal tolerance and fitness in variable thermal landscapes. *Ecology and Evolution*, *10*: 8105-8113. doi: 10.1002/ece3.6496

Chevin, L. M., Lande, R., & Mace, G. M. (2010). Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS biology*, *8*: e1000357 doi:10.1371/journal.pbio.1000357

Cross, W.F., Hood, J.M., Benstead, J.P., Huryn, A.D. and Nelson, D. (2015), Interactions between temperature and nutrients across levels of ecological organization. *Global Change Biology*, *21*: 1025-1040. doi: 10.1111/gcb.12809

Davidson, T. A., Audet, J., Svenning, J. C., Lauridsen, T. L., Søndergaard, M., Landkildehus, F., ... & Jeppesen, E. (2015). Eutrophication effects on greenhouse gas fluxes from shallow‐lake mesocosms override those of climate warming. *Global Change Biology*, *21*: 4449-4463. doi:10.1111/gcb.13062

De Meester, L., Declerck, S. A., & Ger, K. A. (2023). Beyond Daphnia: a plea for a more inclusive and unifying approach to freshwater zooplankton ecology. *Hydrobiologia*, *850*: 4693-4703. doi:10.1007/s10750-023-05217-3

DeMott, W. R., & Gulati, R. D. (1999). Phosphorus limitation in Daphnia: evidence from a long term study of three hypereutrophic Dutch lakes. *Limnology and Oceanography*, *44*: 1557-1564. doi:10.4319/lo.1999.44.6.1557

Diaz, F., Kuijper, B., Hoyle, R. B., Talamantes, N., Coleman, J. M., & Matzkin, L. M. (2021). Environmental predictability drives adaptive within‐and transgenerational plasticity of heat tolerance across life stages and climatic regions. *Functional Ecology*, *35*; 153-166. doi:10.1111/1365-2435.13704

Doi, H., Chang, K. H., & Nakano, S. I. (2011). Nitrogen and carbon isotope fractionations of zooplankton consumers in ponds: potential effects of seston C: N stoichiometry. *Marine and Freshwater Research*, *62*: 66-71. doi: 10.1071/MF10090

Dolman, A. M., Rücker, J., Pick, F. R., Fastner, J., Rohrlack, T., Mischke, U., & Wiedner, C. (2012). Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. *PloS one*, *7*: e38757. 10.1371/journal.pone.0038757

Donelan, S. C., Hellmann, J. K., Bell, A. M., Luttbeg, B., Orrock, J. L., Sheriff, M. J., & Sih, A. (2020). Transgenerational plasticity in human-altered environments. *Trends in Ecology & Evolution*, *35*: 115-124. doi:10.1016/j.tree.2019.09.003

Donelson, J. M., & Munday, P. L. (2015). Transgenerational plasticity mitigates the impact of global warming to offspring sex ratios. *Global Change Biology*, *21*:2954-2962. doi:10.1111/gcb.12912

Donelson, J. M., Salinas, S., Munday, P. L., & Shama, L. N. (2018). Transgenerational plasticity and climate change experiments: Where do we go from here? *Global Change Biology*, *24*:13-34. doi:10.1111/gcb.13903

Donelson, J. M., Sunday, J. M., Figueira, W. F., Gaitán-Espitia, J. D., Hobday, A. J., Johnson, C. R., Hobday, A. J., Johnson, C. R., Leiss, J. M., Ling, S. D., Marshall, D., Pandolfi, J. M., Pecl, G., Rodgers, G. G., Booth, D. J. & Munday, P. L. (2019). Understanding interactions between plasticity, adaptation and range shifts in response to marine environmental change. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*: 20180186. doi: 10.1098/rstb.2018.0186

Dziuba, M. K., Kuczyński, L., Wejnerowski, Ł., Cerbin, S., & Wolinska, J. (2021). Countergradient variation concealed adaptive responses to temperature increase in *Daphnia* from heated lakes. *Limnology and Oceanography*, *66*: 1268-1280. doi: 10.1002/lno.11680

Eisenreich, S. J., Bannerman, R. T., & Armstrong, D. E. (1975). A simplified phosphorus analysis technique. *Environmental letters*, *9*: 43-53. doi:10.1080/00139307509437455

Elser, J.J. and Urabe, J. (1999). The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. *Ecology*, *80*: 735-751. doi: 10.1890/0012-9658(1999)080[0735:TSOCDN]2.0.CO;2

Elser, J.J., Sterner, R.W., Gorokhova, E., Fagan, W.F., Markow, T.A., Cotner, J.B., Harrison, J.F., Hobbie, S.E., Odell, G.M. and Weider, L.W. (2000), Biological stoichiometry from genes to ecosystems. *Ecology Letters, 3*:540-550. doi:10.1111/j.1461-0248.2000.00185.x

Fawcett, T.W., Frankenhuis, W.E. (2015) Adaptive explanations for sensitive windows in development. *Frontiers in Zoology 12* (Suppl 1): S3. doi:10.1186/1742-9994-12-S1-S3

Forster, J. and Hirst, A.G. (2012), The temperature-size rule emerges from ontogenetic differences between growth and development rates. *Functional Ecology, 26*: 483-492. doi: 10.1111/j.1365-2435.2011.01958.x

Forster, J., Hirst, A.G. and Atkinson, D. (2011), How do organisms change size with changing temperature? The importance of reproductive method and ontogenetic timing. *Functional Ecology, 25*: 1024-1031. doi:10.1111/j.1365-2435.2011.01852.x

Frost, B. W. (1972). Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod Calanus pacificus 1. *Limnology and Oceanography*, *17*: 805-815. doi:10.4319/lo.1972.17.6.0805

Frost, P. C., Benstead, J. P., Cross, W. F., Hillebrand, H., Larson, J. H., Xenopoulos, M. A., & Yoshida, T. (2006). Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecology Letters*, *9*: 774-779. doi:10.1111/j.1461-0248.2006.00919.x

Ganser, A. M., Newton, T. J., & Haro, R. J. (2015). Effects of elevated water temperature on physiological responses in adult freshwater mussels. *Freshwater Biology*, *60*: 1705-1716. doi:10.1111/fwb.12603

Garbutt, J. S., & Little, T. J. (2014). Maternal food quantity affects offspring feeding rate in Daphnia magna. *Biology Letters*, *10*: 20140356. doi: 10.1098/rsbl.2014.0356

Geerts, A., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feutchmayer, H., Atkinson, D., Moss, B., Davidson, T.A., Sayer, C.D., & De Meester, L. (2015) Rapid evolution of thermal tolerance in the water flea *Daphnia*. *Nature Climate Change* **5**: 665–668. doi: https:10.1038/nclimate2628

Ghalambor, C. K., McKay, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non‐adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional ecology*, *21*: 394-407. doi: 10.1111/j.1365-2435.2007.01283.x

Guillard, R. R., & Lorenzen, C. J. (1972). Yellow-green algae with chlorophyllide c. *Journal of Phycology*, *8*: 10-14. doi: 10.1111/j.0022-3646.1972.00010.x

Halvorson, H. M., Fuller, C. L., Entrekin, S. A., Scott, J. T., & Evans-White, M. A. (2019). Interspecific homeostatic regulation and growth across aquatic invertebrate detritivores: a test of ecological stoichiometry theory. *Oecologia*, *190*: 229-242. doi: 10.1007/s00442-019-04409-w

Hansen, P. J., Bjørnsen, P. K., Hansen, B. W. (1997) Zooplankton grazing and growth: Scaling within the 2-2,-µm body size range. *Limnology and Oceanography*, 42, doi: 10.4319/lo.1997.42.4.0687

Hartnett, R. (2019). Variation in life-history traits among Daphnia and its relationship to species-level responses to phosphorus limitation. *Royal Society Open Science*, *6*: 191024. doi: 10.1098/rsos.191024

Hébert, M. P., Beisner, B. E., & Maranger, R. (2016). A meta‐analysis of zooplankton functional traits influencing ecosystem function. *Ecology*, *97*:1069-1080. doi: 10.1890/15-1084.1

Herman, J. J., & Sultan, S. E. (2011). Adaptive transgenerational plasticity in plants: case studies, mechanisms, and implications for natural populations. *Frontiers in Plant Science*, *2*:. doi: 10.3389/fpls.2011.00102

Hoefnagel KN, de Vries EHJ, Jongejans E, Verberk WCEP. (2018)The temperature-size rule in *Daphnia magna* across different genetic lines and ontogenetic stages: Multiple patterns and mechanisms. *Ecology and Evolution*, *8*: 3828–3841.doi:10.1002/ece3.3933

Hood, J. M., Benstead, J. P., Cross, W. F., Huryn, A. D., Johnson, P. W., Gíslason, G. M., ... & Tran, C. (2018). Increased resource use efficiency amplifies positive response of aquatic primary production to experimental warming. *Global Change Biology*, *24*:1069-1084. doi:10.1111/gcb.13912

Horne, C. R., Hirst, A. G., Atkinson, D., Almeda, R., & Kiørboe, T. (2019). Rapid shifts in the thermal sensitivity of growth but not development rate causes temperature-size response variability during ontogeny in arthropods. *Oikos, 128*: 823-835. doi:10.1111/oik.06016

Im, H., Kang, J., Jacob, M. F., Bae, H., & Oh, J. E. (2023). Transgenerational effects of benzotriazole on the gene expression, growth, and reproduction of Daphnia magna. *Environmental Pollution*, *323*: 121211. doi: 10.1016/j.envpol.2023.121211

Jespersen, A.-M. & K. Christoffersen, 1987. Measurements of chlorophyll *a* from phytoplankton using ethanol as extraction solvent. *Archiv fur Hydrobiologie 109*: 445–454.

Jalal, Marwa, Wojewodzic, Marcin W., Laane, Carl Morten M., & Hessen, Dag O. (2013) Larger *Daphnia* at lower temperature: a role for cell size and genome configuration?. *Genome, 56*: 511-519. doi:10.1139/gen-2013-0004

Janssens, L., Van Dievel, M. & Stoks, R., 2015. Warming reinforces nonconsumptive predator effects on prey growth, physiology, and body stoichiometry. *Ecology*, *96*: 3270-3280. doi:10.1890/15-0030.1

Kariya, T., & Kurata, H. (2004). *Generalized least squares*. John Wiley & Sons. West Sussex, England.

Kawecki, T. J. (2000). The evolution of genetic canalization under fluctuating selection. *Evolution*, *54*:1-12. doi:10.1111/j.0014-3820.2000.tb00001.x

Kuijper, B., & Hoyle, R. B. (2015). When to rely on maternal effects and when on phenotypic plasticity?. *Evolution*, *69*:950-968. doi:10.1111/evo.12635

Kopp, M., & Matuszewski, S. (2014). Rapid evolution of quantitative traits: theoretical perspectives. *Evolutionary Applications*, *7*:169-191 doi: 10.1111/eva.12127

Krom, M.D. (1980). Spectrophotometric determination of ammonia: a study of a modified Berthelot reaction using salicylate and dichloroisocyanurate. *Analyst*, *105*: 305-316. doi: 10.1039/AN9800500305

Kroon, H. (1993). Determination of nitrogen in water: comparison of a continuous-flow method with on-line UV digestion with the original Kjeldahl method. *Analytica Chimica Acta*, *276*: 287-293 doi: 10.1016/0003-2670(93)80396-3

Lampert, W., Fleckner, W., Rai, H., & Taylor, B. E. (1986). Phytoplankton control by grazing zooplankton: A study on the spring clear‐water phase 1. *Limnology and oceanography*, *31*:478-490. doi:10.4319/lo.1986.31.3.0478

Li, Y., Hou, L., Yang, L., & Yue, M. (2021). Transgenerational effect alters the interspecific competition between two dominant species in a temperate steppe. *Ecology and Evolution*, *11*: 1175-118. doi:10.1002/ece3.7066

Luquet, E., & Tariel, J. (2016). Offspring reaction norms shaped by parental environment: interaction between within-and trans-generational plasticity of inducible defenses. *BMC Evolutionary Biology*, *16*: 1-10. doi:10.1186/s12862-016-0795-9

Müller, M. F., Colomer, J., & Serra, T. (2018). Temperature-driven response reversibility and short-term quasi-acclimation of Daphnia magna. *PloS one*, *13*:e0209705. doi:10.1371/journal.pone.0209705

MacKay, N. A., & Elser, J. J. (1998). Factors potentially preventing trophic cascades: Food quality, invertebrate predation, and their interaction. *Limnology and Oceanography*, *43*: 339-347. doi: 10.4319/lo.1998.43.2.0339

Mackerth, F. (1978). Water analysis: some revised methods for limnologists. *Freshwater Biol. Assoc. Sci. Pub.*, *36*, 117.

MacLean, S. A., & Beissinger, S. R. (2017). Species' traits as predictors of range shifts under contemporary climate change: A review and meta-analysis. *Global Change Biology, 23*:4094–4105. doi:10.1111/gcb.13736

Makino, W., Gong, Q. and Urabe, J. 2011. Stoichiometric effects of warming on herbivore growth: Experimental test with plankters. *Ecosphere, 2*:79. doi:10.1890/ES11- 00178.1

Mathews, L., Faithfull, C.L., Lenz, P.H. and Nelson, C.E., 2018. The effects of food stoichiometry and temperature on copepods are mediated by ontogeny. *Oecologia*, *188*: 75-84. doi: 10.1007/s00442-018-4183-6

McIntyre, P. B., Flecker, A. S., Vanni, M. J., Hood, J. M., Taylor, B. W., & Thomas, S. A. (2008). Fish distributions and nutrient cycling in streams: can fish create biogeochemical hotspots. *Ecology*, *89*: 2335-2346. doi: 10.1890/07-1552.1

McKee, D., & Ebert, D. (1996). The interactive effects of temperature, food level and maternal phenotype on offspring size in Daphnia magna. *Oecologia*, *107*:189-196. doi:10.1007/BF00327902

Miner, B. E., De Meester, L., Pfrender, M. E., Lampert, W., & Hairston Jr, N. G. (2012). Linking genes to communities and ecosystems: Daphnia as an ecogenomic model. *Proceedings of the Royal Society B*, *279*: 1873-1882. doi: 10.1098/rspb.2011.2404

Morita, S. H., Morita, K., Hamatsu, T., Chimura, M., Yamashita, Y., Sasaki, K., & Sato, T. (2015). Differential effects of the environment on the growth of arabesque greenling (Pleurogrammus azonus): does rising temperature benefit young but not old fish?. *Environmental Biology of Fishes*, *98*, 801-809. doi:10.1007/s10641-014-0316-x

Olsen, Y., G. Knutsen, & Lien, T. (1983). Characteristics of phosphorus limitation in Chlamydomonas reinhardtii (Chlorophyceae) and its palmelloids. *Journal of Phycology, 19*: 313-319. doi:10.1111/j.0022-3646.1983.00313.x

Paerl, H. W. & Paul. V. J. (2012) "Climate change: links to global expansion of harmful cyanobacteria." *Water research, 46.5*: 1349-1363. doi: 10.1016/j.watres.2011.08.002

Pajk, F., Von Elert, E., & Fink, P. (2012). Interaction of changes in food quality and temperature reveals maternal effects on fitness parameters of a keystone aquatic herbivore. *Limnology and Oceanography*, *57*: 281-292. doi: 10.4319/lo.2012.57.1.0281

Panov, V. E. & McQueen, D. J. (1998) Effects of temperature on individual growth rate and body size of a freshwater amphipod. *Canadian Journal of Zoology* **76**: 1107-1116. doi:10.1139/z98-025

Paterson, M. J., Findlay, D. L., Salki, A. G., Hendzel, L. L., & Hesslein, R. H. (2002). The effects of Daphnia on nutrient stoichiometry and filamentous cyanobacteria: a mesocosm experiment in a eutrophic lake. *Freshwater Biology*, *47*:1217-1233. doi: 10.1046/j.1365-2427.2002.00842.x

Penuelas J, Janssens I. A., Ciais P., Obersteiner M., & Sardans J. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. *Global Change Biol*ogy 2020; 26: 1962–1985. doi: 10.1111/gcb.14981

Prater, C., Wagner, N.D. & Frost, P.C., 2018. Seasonal effects of food quality and temperature on body stoichiometry, biochemistry, and biomass production in Daphnia populations. *Limnology and Oceanography*, *63*(4), pp.1727-1740.doi: 10.1002/lno.10803

Puy, J., de Bello, F., Dvořáková, H., Medina, N. G., Latzel, V., & Carmona, C. P. (2021). Competition‐induced transgenerational plasticity influences competitive interactions and leaf decomposition of offspring. *New Phytologist*, *229*: 3497-3507. doi: 10.1111/nph.17037

Quigley, K. M., Willis, B. L., & Kenkel, C. D. (2019). Transgenerational inheritance of shuffled symbiont communities in the coral Montipora digitata. *Scientific Reports*, *9*:13328. doi: 10.1038/s41598-019-50045-y

Rodrigues, Y. K., & Beldade, P. (2020). Thermal plasticity in insects' response to climate change and to multifactorial environments. *Frontiers in Ecology and Evolution*, 8:271. doi: 10.3389/fevo.2020.00271

Räsänen, K., & Kruuk, L. E. B. (2007). Maternal effects and evolution at ecological time‐scales. *Functional Ecology*, *21*: 408-421. doi: 10.1111/j.1365-2435.2007.01246.x

Schwanz, L. E., Crawford-Ash, J., & Gale, T. (2020). Context dependence of transgenerational plasticity: the influence of parental temperature depends on offspring environment and sex. *Oecologia*, *194*: 391-401.

Seebacher, F., White, C. R., & Franklin, C. E. (2015). Physiological plasticity increases resilience of ectothermic animals to climate change. *Nature Climate Change*, 5:61-66. doi:10.1038/nclimate2457

Sterner, R. W., & Elser, J. J. (2002). Ecological stoichiometry Princeton University Press. *Princeton, NJ*.

Haupt, F., Stockenreiter, M., Reichwaldt, E. S., Baumgartner, M., Lampert, W., Boersma, M., & Stibor, H. (2010). Upward phosphorus transport by *Daphnia* diel vertical migration. *Limnology and Oceanography, 55*: 529-534. doi: 10.4319/lo.2010.55.2.0529

Suding, K. N., & Goldstein, L. J. (2008). Testing the Holy Grail framework: using functional traits to predict ecosystem change. *New Phytologist*, 180: 559-562. doi: 10.1111/j.1469-8137.2008.02650.x

Suding, K.N., Lavorel, S., Chapin, F.S., Iii, Cornelissen, J.H.C., Díaz, S., Garnier, E., Goldberg, D., Hooper, D.U., Jackson, S.T. and Navas, M.-L. (2008), Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology, 14*: 1125-1140. doi:10.1111/j.1365-2486.2008.01557.x

Smyntek P. M. , Teece M. A. , Schulz K. L. and Thackeray S. J. (2007), A standard protocol for stable isotope analysis of zooplankton in aquatic food web research using mass balance correction models, *Limnology and Oceanography*, 52, doi: 10.4319/lo.2007.52.5.2135.

Toseland, A., Daines, S., Clark, J. *et al.*(2013) The impact of temperature on marine phytoplankton resource allocation and metabolism. *Nature Climate Change **3***, 979–984. doi:10.1038/nclimate1989

Toyota, K., Cambronero Cuenca, M., Dhandapani, V., Suppa, A., Rossi, V., Colbourne, J. K., & Orsini, L. (2019). Transgenerational response to early spring warming in Daphnia. *Scientific Reports*, *9*: 4449. doi: 10.1038/s41598-019-40946-3

Urabe, J. (1993). N and P cycling coupled by grazers' activities: food quality and nutrient release by zooplankton. *Ecology*, *74*: 2337-2350. doi:10.2307/1939586

Urabe, J., Togari, J. And Elser, J.J. (2003), Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. *Global Change Biology, 9*: 818-825. doi:10.1046/j.1365-2486.2003.00634.x

Urrutia‐Cordero, P., Zhang, H., Chaguaceda, F., Geng, H., & Hansson, L. A. (2020). Climate warming and heat waves alter harmful cyanobacterial blooms along the benthic–pelagic interface. *Ecology*, *101*: e03025. doi: 10.1002/ecy.3025

Vanderploeg, H. A., Sarnelle, O., Liebig, J. R., Morehead, N. R., Robinson, S. D., Johengen, T. H., & Horst, G. P. (2017). Seston quality drives feeding, stoichiometry and excretion of zebra mussels. *Freshwater Biology*, *62*: 664-680. doi:10.1111/fwb.12892

Ventura, M., Liboriussen, L., Lauridsen, T., Søndergaard, M., Søndergaard, M. and Jeppesen, E., 2008. Effects of increased temperature and nutrient enrichment on the stoichiometry of primary producers and consumers in temperate shallow lakes. *Freshwater Biology*, *53*: 1434-1452. doi:10.1111/j.1365-2427.2008.01975.x

Verberk, W. C. E. P., Atkinson, D., Hoefnagel, K. N., Hirst, A. G., Horne, C. R., & Siepel, H. (2021). Shrinking body sizes in response to warming: explanations for the temperature–size rule with special emphasis on the role of oxygen. *Biological Reviews, 96*: 247-268. doi:10.1111/brv.12653

Vanvelk, H., Govaert, L., Van den Berg, E. M., Brans, K. I., & De Meester, L. (2021). Interspecific differences, plastic, and evolutionary responses to a heat wave in three co‐occurring Daphnia species. *Limnology and Oceanography*, *66*: 1201-1220. doi: 10.1002/lno.11675

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S. M., Schlichting, C. D., & Van Tienderen, P. H. (1995). Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology & Evolution*, *10*:212-217. doi:10.1016/S0169-5347(00)89061-8

Waite, H. R., & Sorte, C. J. (2022). Negative carry‐over effects on larval thermal tolerances across a natural thermal gradient. *Ecology*, *103*: e03565. doi: 10.1002/ecy.3565

Walsh, M. R., Whittington, D., & Funkhouser, C. (2014). Thermal transgenerational plasticity in natural populations of Daphnia. *Integrative and Comparative Biology, 54*: 822-829. doi: 10.1093/icb/icu078

Walther G-R. (2010) Community and ecosystem responses to recent climate change. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*: 2019–2024 doi:10.1098/rstb.2010.0021

Wadgymar, S. M., Ogilvie, J. E., Inouye, D. W., Weis, A. E., & Anderson, J. T. (2018). Phenological responses to multiple environmental drivers under climate change: insights from a long‐term observational study and a manipulative field experiment. *New Phytologist*, *218*: 517-529 doi: 10.1111/nph.15029

Wolf, J. B., & Wade, M. J. (2009). What are maternal effects (and what are they not)?. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*: 1107-1115 doi:10.1098/rstb.2008.0238

Woods, H. A., Makino, W., Cotner, J. B., Hobbie, S. E., Harrison, J. F., Acharya, K., Elser, J.J. (2003). Temperature and the chemical composition of poikilothermic organisms. Functional Ecology 17: 237–245. doi: 10.1046/j.1365-2435.2003.00724.x

Xu, Z., Li, Y., Li, M., & Liu, H. (2021). Transcriptomic response of Daphnia magna to nitrogen‐or phosphorus‐limited diet. *Ecology and Evolution*, *11*: 11009-11019. doi:10.1002/ece3.7889

Yacobi, Y. Z., & Zohary, T. (2010). Carbon: chlorophyll a ratio, assimilation numbers and turnover times of Lake Kinneret phytoplankton. *Hydrobiologia*, *639*: 185-196. doi:10.1007/s10750-009-0023-3

Figures

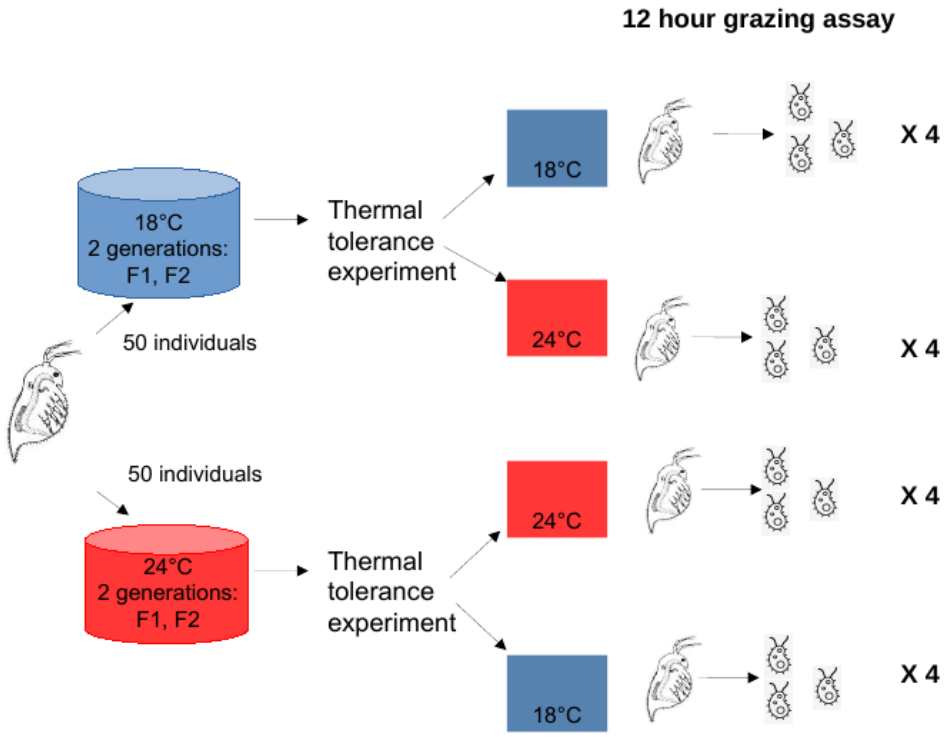

Figure 1: Graphical overview of the experimental design used to assess if transgenerational plasticity in *D. magna* influences grazing, and N-P uptake and release rates. Fifty individuals from a 10-year *D. magna* laboratory population were exposed to either 18℃ or 24℃ for 2 generations. Critical thermal maximum of F2 individuals from both parental exposure treatments was assessed in a thermal tolerance experiment. Individuals from each parental thermal environment were exposed to either 18℃ or 24℃ for a 12-hour grazing assay on *C. reinhardtii*.

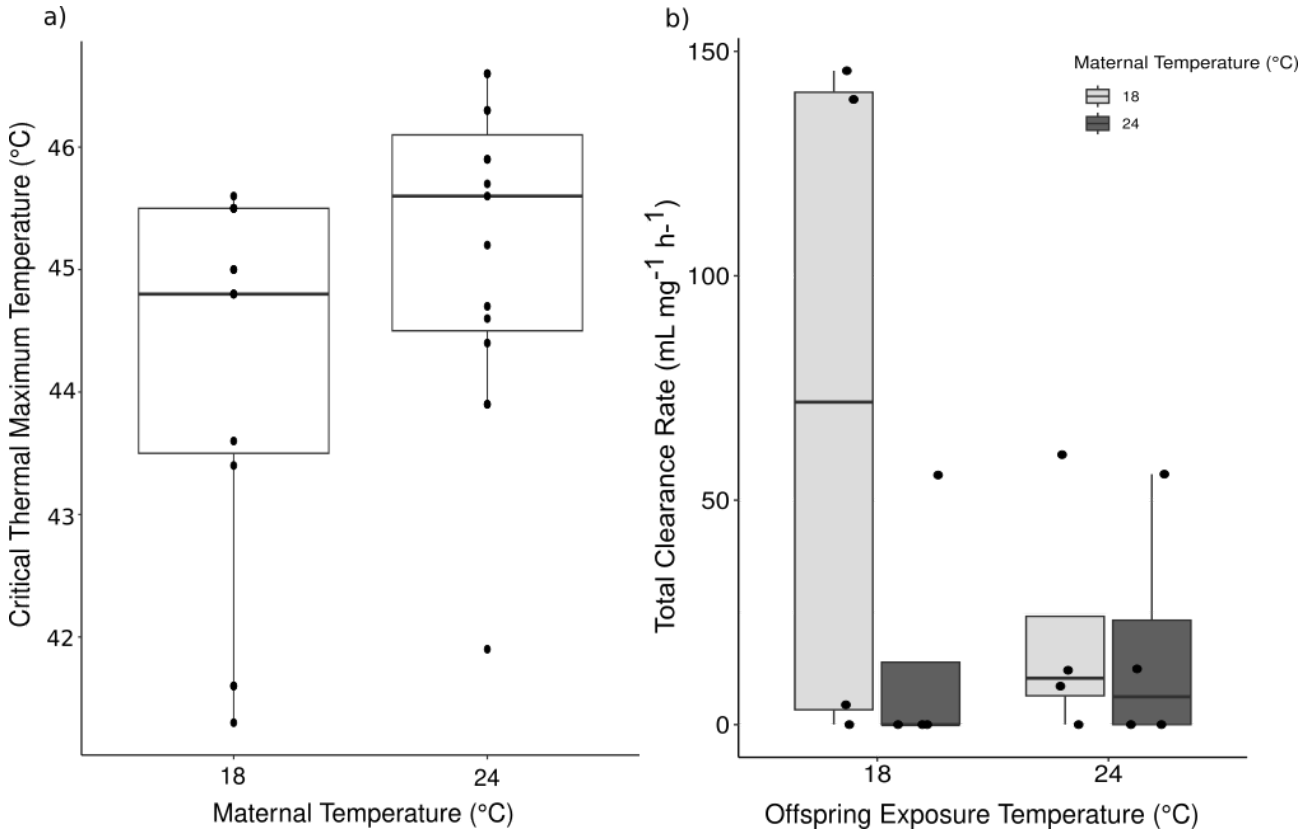

Figure 2**:** a) Critical thermal maximum temperature (°C) for *D. magna* reared at either 18°C or 24°C for two generations; and b) Total clearance rate (mgC ml^{-1} h^{-1}) for *D. magna* F2 generation with maternal temperature of 18°C or 24°C exposed to either 18°C or 24°C during a 12-hour grazing assay. No statistically significant differences were observed between treatments for critical thermal maximum temperature of total clearance rate.

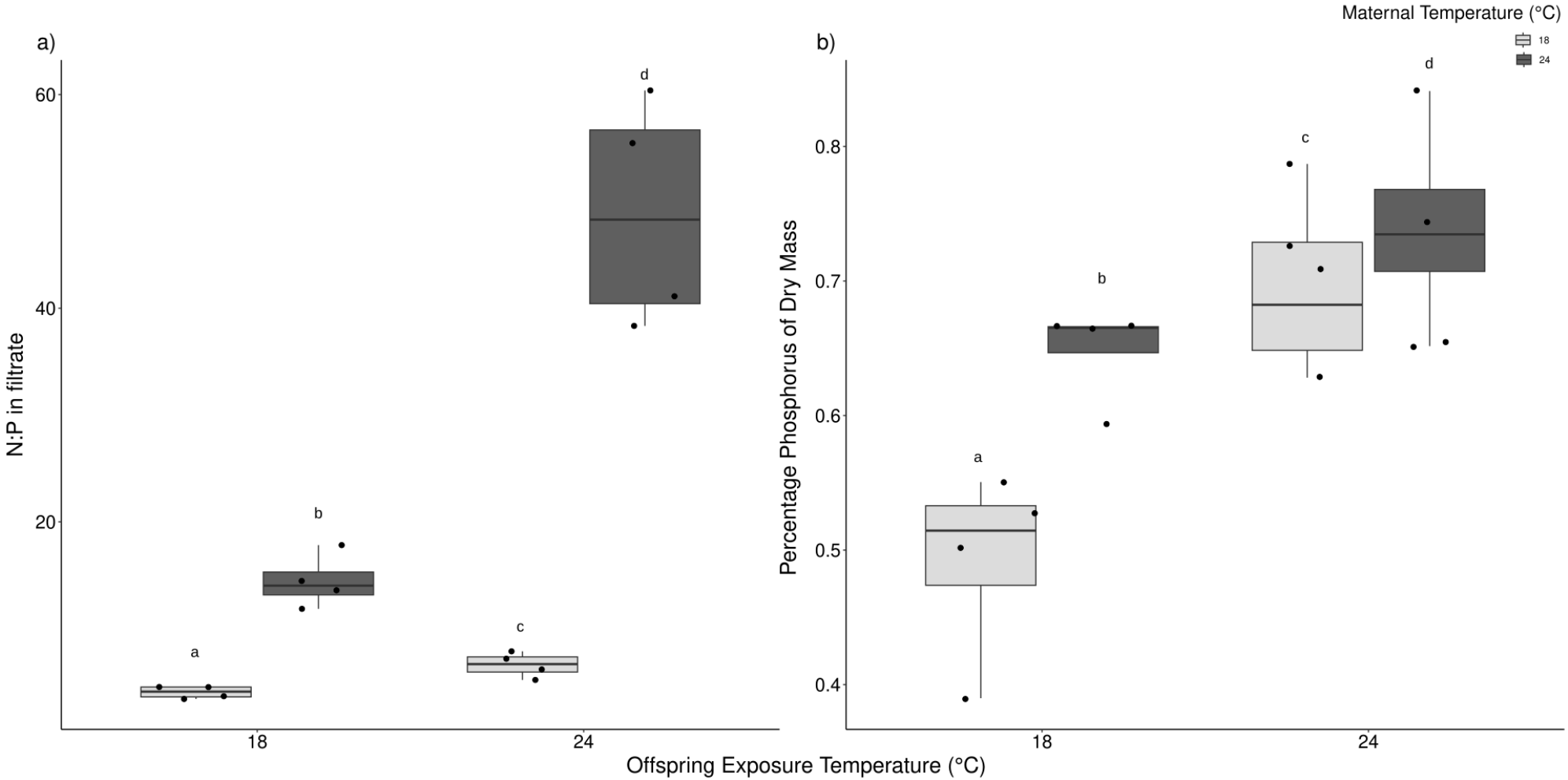

Figure 3: a) The N:P ratio in filtrate and b) Percentage Phosphorus of Dry Mass for *D. magna* F2 generation with maternal temperature of 18°C or 24°C exposed to either 18°C or 24°C during a 12-hour grazing assay. Different letters denote statistically significant differences ($p < 0.05$).

Supplementary Materials

Daphnia biomass standardization

Biomass differences between 18ºC and 24°C were estimated by performing a census of adults and juveniles in each treatment on the same day as the grazing experiment. We used published average lengths for adult and juvenile *D. magna* in our biomass calculations (Ger et al. 2019) to remove any effect of handling on the condition of individuals used in our grazing experiment. A combination of adult and juvenile *D. magna* individuals equivalent to a biomass of 0.5 mg was added to each grazer replicate treatment. This resulted in 4 adults and 30 juveniles reared at 18ºC, and 9 adults and 12 juveniles reared at 24ºC per replicate, densities similar to those used in other studies (Bengtsson et al. 2004; Ger et al. 2011; Park & Post 2018). Due to COVID19 related logistical complications, we were unable to weigh the final biomass of *D. magna* in our replicates.

Physiological rate calculations

Grazing rates

We converted Chl-a to carbon using the 44:1 carbon:Chl-a ratio based on in-lake measurements for Chlorophytes from Yacobi and Zohary (2010). For each grazer-absent control replicate, the rate of change in carbon concentration (k) was determined as:

$$k = \frac{lnC_2 - lnC_1}{t}$$

where C_1 and C_2 represent carbon mass (mg) at the beginning and end of the experiment, and t is the experiment duration (hours).

For each grazer-present replicate, the rate of change in total carbon mass (mg) was determined as:

$$g = \frac{lnT_2 - lnT_1}{t} - k$$

where T_1 and T_2 represent carbon mass at the beginning and end of the experiment.

Clearance rate per jar (F) was calculated as:

$$F = Vg$$

where V is the volume (mL) of each grazer-present replicate. Biomass-specific clearance rate for each jar was determined by dividing F by the *D. magna* biomass in each replicate, which was standardized to 0.5 mg across all replicates. Negative clearance rates were corrected to 0 (Nejstgaard et al. 2001).

N-P Relative release rates

Relative release rates in NH_4, NO_2 + NO_3, DIN, and SRP due to grazing (J) were determined as follows:

$$J = \frac{(T_f - C_f)}{Bt}$$

where T_f is the mass of NH_4, NO_2 +NO_3, DIN, or SRP in the grazer-present treatment (mg), C_f is the mass of these nutrients in the grazer-absent control (mg), B is the *D. magna* biomass. Due to potential increases in NH_4, NO_3, DIN, or SRP as a result of bacterial and algal activity, negative rates of changes in dissolved concentrations were set to 0.

GLS regression

GLS regression fits an ordinary least squares (OLS) model and uses the residual errors to model the heteroskedasticity observed. The variance estimate of a GLS model is determined from the residual error model rather than directly from the OLS regression, making it robust to heteroskedastic and autocorrelated variance (Kariya and Kurata, 2004). All GLS models were fit using maximum likelihood with different variances fit for each maternal temperature and offspring exposure treatment (following Zuur et al. 2009) with fixed variance weights determined by the model variance-covariance structure.

Model selection

Model selection was performed using log-likelihood ratio tests with a Chi-squared distribution for GLS models and Wald test for robust regression models following Crawley's (2008) procedure. We used plots of standardized residual and predicted values to assess the fit of the final GLS and robust regression models chosen. For multiple regression, the final model was visually assessed with plots of residuals, standardized Pearson residuals, and predicted values. If a statistically significant interaction was detected, we assessed differences between treatment combinations using Tukey HSD tests for multiple regression models or generalized linear hypothesis tests with Bonferroni correction for GLS and robust regression.

Results

Individuals from warmer maternal temperatures increased their relative rates of dissolved NO_3+NO_2 (Figure S1b, Table S1) and DIN release (Figure S1c, Table S1). Relative NO_3+NO_2 release was 0.0018 mg NO_3+NO_2 mg DW^{-1} h^{-1} greater in jars with *D. magna* with maternal temperature of 24°C as compared to 18°C. Similarly, relative DIN release was 0.0030 mgDIN mgDW^{-1} h^{-1} greater at maternal temperature of 24°C as compared to 18°C. There was no effect of exposure temperature on relative NO_3+NO_2 and DIN release. We did not observe any effect of maternal temperature on changes in dissolved NH_4 concentrations (Figure S1a, Table S1). There was a decrease in relative NH_4 release in *D. magna* exposed to 24°C as compared to those exposed to 18°C ($p = 0.020$, 0.0012 mgNH_4 mgDW^{-1} h^{-1} less release), regardless of maternal temperature.

Both maternal and offspring exposure temperatures interacted with each other to reduce the relative rate of SRP release. On average, warmer maternal and offspring exposure temperatures reduced relative SRP release (Figure S2, Table S1, $p = 0.028$). An average increase of 0.0004 mg SRP mg DW^{-1} h^{-1} was

observed in *D. magna* with maternal and exposure temperature of 18°C as compared to those with a maternal temperature of 24°C and exposed to 18°C ($p < 0.001$). For *D. magna* with maternal temperature of 24°C, the average release was 0.0003 mgSRP $mgDW^{-1}$ h^{-1} greater for individuals exposed to 18°C as compared to those exposed to 24°C ($p < 0.001$). Average release was 0.0007 mgSRP $mgDW^{-1}$ greater for *D. magna* with maternal and exposure temperature of 18°C as compared to those with maternal temperature of 18°C and exposed to 24°C ($p < 0.001$). We did not observe any differences in relative SRP release for *D. magna* with maternal temperature of 24°C and 18°C with exposure to 24°C during the grazing assay.

References

Bengtsson, G., Hansson, L. A., & Montenegro, K. (2004). Reduced grazing rates in Daphnia pulex caused by contaminants: implications for trophic cascades. *Environmental Toxicology and Chemistry*, *23*: 2641-2648. doi:10.1897/03-432

Crawley, M. J. (2012). *The R book*. John Wiley & Sons. West Sussex, England

Ger, K.A., Naus-Wiezer, S., De Meester, L. and Lürling, M. (2019), Zooplankton grazing selectivity regulates herbivory and dominance of toxic phytoplankton over multiple prey generations. *Limnology and Oceanography, 64*: 1214-1227. doi: 10.1002/lno.11108

Nejstgaard, J. C., Naustvoll, L. J., & Sazhin, A. (2001). Correcting for underestimation of microzooplankton grazing in bottle incubation experiments with mesozooplankton. *Marine Ecology Progress Series*, *221*: 59-75. doi:10.3354/meps221059

Park, J. S., & Post, D. M. (2018). Evolutionary history of Daphnia drives divergence in grazing selectivity and alters temporal community dynamics of producers. *Ecology and Evolution*, *8*: 859-865. doi: 10.1002/ece3.3678

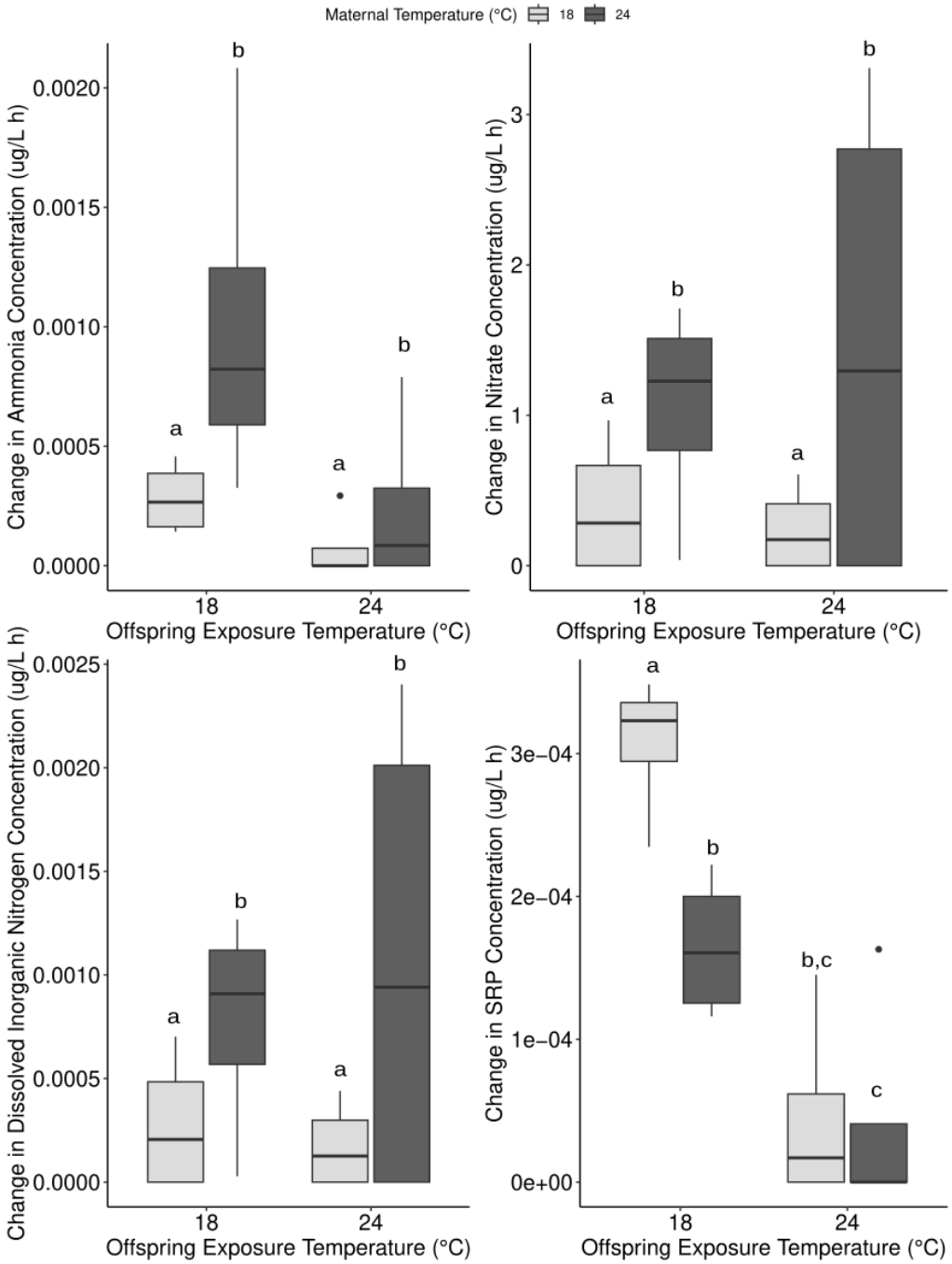

Figure S1: Relative release rates of Ammonia (NH_4, upper left), Nitrite and Nitrate($NO_2 + NO_3$, upper right), Dissolved Inorganic Nitrogen (DIN, lower left), and Soluble Reactive Phosphorus (SRP, lower right) for *D. magna* with maternal temperature of 18°C or 24°C exposed to either 18°C or 24°C during a 12-hour grazing assay. Different letters denote statistically significant differences ($p<0.05$).

Table S1: Degrees of freedom (df) and p-values (p) for generalized least square models (GLS) or multiple regression assessing the interactive effect of maternal temperature (MT, 18°C or 24°C) and offspring exposure temperature (ET, 18°C or 24°C) on total clearance rate, change in NH_4, NO_3+ NO_2, dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), body phosphorus (P) content, and phosphorus release rate (P release). P-values were determined from log-likelihood ratio tests with a Chi-squared distribution for GLS and multiple regression. If a statistically significant interaction was found between maternal temperature and offspring exposure temperature, no further models were evaluated. Statistically significant p-values are provided in bold.

Response Variable	**Explanatory variable**	**df**	**p**	**Analysis**
Total clearance rate	MT:ET	8	0.224	GLS
	MT	7	0.622	GLS
	ET	6	0.728	GLS
N:P	**MT:ET**	8	0.001	GLS
NH_4	MT:ET	8	0.169	GLS
	MT	7	0.146	GLS
	ET	**6**	**0.020**	GLS
$NO_3 + NO_2$	MT:ET	8	0.511	GLS
	MT	**7**	**0.023**	GLS
	ET	6	0.661	GLS
DIN	MT:ET	8	0.816	GLS
	MT	**7**	**0.009**	GLS
	ET	6	0.172	GLS
SRP	**MT:ET**	**8**	**0.018**	GLS
Body P Content	MT:ET	8	0.123	Multiple Regression
	MT	**7**	**0.013**	Multiple Regression
	ET	**6**	**0.001**	Multiple Regression