Transgenerational plasticity to warming decreases nutrient release by a keystone grazer

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Abstract

Increasing temperatures as a result of global climate change can alter the physiology of organisms via selection for tolerant genotypes and individual-level plasticity. Organisms experiencing thermal stress can not only modify their own physiological expression, but also those of future generations; i.e. transgenerational plasticity (TGP). While warming triggered TGP is well documented, its effects on key physiological rates and subsequent ecosystem functioning is poorly understood. In this study, we used reciprocal transplant experiments to examine if warming triggers TGP impacts on grazing, uptake and release of nitrogen (N) and phosphorus (P), in the keystone aquatic herbivore *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 for a period of 12 hours and an assay was conducted to measure rates of algal clearance, N-P uptake and release. Our results show that while differences in maternal temperature exposure did not lead to differences in grazing rates, warmer maternal exposure increased N release rates, increased proportion of body P content, and reduced P release. These results suggest that transgenerational plasticity can alter physiological responses to warming in *Daphnia* with potentially major consequences for N and P cycling in lake ecosystems.

Introduction

Global climate change is a major biological stressor, shifting local and regional temperatures from their long-term averages and increasing the frequency of extreme events (Collins et al. 2013). Changes in thermal regimes 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). These changes can alter key physiological processes such as metabolism (Seebacher et al. 2015) including nutrient uptake and excretion (Ganser et al. 2015), which subsequently affect ecosystem functions including primary production, nutrient cycling and greenhouse gas emissions (Davidson et al. 2015, Hood et al. 2017, Cavicchioli et al. 2019). Yet, we are only beginning to understand how climate-induced trait variation regulates key ecosystem functions.

Organisms can respond to changing thermal conditions through adaptation, plasticity, or range shifts (Seebacher et al. 2015; Donelson et al. 2019; Rodrigues and Beldade 2020). Adaptation is favoured when environmental changes are directional and relatively slow, 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 non-genetic inheritance, anticipatory parental effects, maternal effects, carry-over effects, or transgenerational plasticity (TGP; Donelson et al. 2018; Wadgymar et al. 2018).

Transgenerational plasticity occurs when the phenotype of the parent affects the phenotype of the offspring through processes such as RNA-mediated modifications, epigenetic marks and DNA methylation in addition to the direct effects of the genes contributed by the parent (Rasanen & Kruuk 2007; Wolf & Wade 2009; Kujiper & Hoyle 2015). TGP is a potential source of ecologically and evolutionarily meaningful trait variation (Herman & Sultan 2011) and is predicted to play an important role in the response of organisms to global climate change, as it may buffer immediate effects and allow time for genetic adaptation (Chevin, Lande, & Mace 2010, Kopp & Matuszewski 2014, Donelson et al. 2018). There is increasing evidence that TGP facilitates offspring response to thermal stress by impacting 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), although these responses are not observed consistently (Waite and Sorte 2022) or are mediated by other factors such as offspring sex (Schwanz et al. 2020). As compared with WGP, TGP may play a dominant role in mediating thermal stress as plasticity is often highest during early development (Fawcett & Frankenhuis 2015), and certain plasticity mechanisms such as methylation and histone modification only operate at this life stage (Donelan et al. 2020).

Although most studies primarily evaluate TGP using life history traits associated with offspring fitness, TGP can alter demographic patterns (Donelson & Munday 2015), community composition (Quigley et al. 2019), and species interactions (Li et al. 2021). Moreover, via effects on metabolic rates, TGP can affect ecosystem functions such as grazing and nutrient cycling, for example, differences in DNA methylation have been shown to decrease offspring leaf decomposition rates, with potential

consequences for nutrient cycling (Puy et al. 2020). Yet, relatively little is known about the overall impact of TGP on traits that are linked to ecosystem processes (i.e. “effect” traits, Suding et al. 2008, Hebert et al. 2016).

Daphnia is a common keystone herbivore in freshwater ecosystems globally, occurring in lakes and ponds on every continent and playing a significant role in the transfer of matter and energy (Miner et al. 2012). It is a primary consumer of phytoplankton, and a key food source for secondary consumers such as fish and macroinvertebrates. *Daphnia* populations have a significantly higher per capita grazing rate on phytoplankton as compared to other zooplankton species (Hansen et al. 1997), which drives seasonal patterns in water transparency known as the clear water phase in many lakes (Lampert et al. 1986). *Daphnia* also has high phosphorus (P) demand, and the lowest nitrogen-to-phosphorus (N:P) body ratio as compared to all other zooplankton groups (Elser et al. 2000). The high grazing rates combined with high N-P excretion can alter nutrient availability and N-P cycling in lake ecosystems, especially at high population densities (Mackay and Elser 1998; Paterson et al. 2002).

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). Plastic responses to thermal stress have been documented extensively in this species, including TGP responses such as changes in fecundity, size at maturity, growth rates, life span, oxidative stress, and resistance to infectious disease (e.g. Mckee and Ebert 1996; Pajk et al. 2012; Garbutt et al. 2014; Toyota et al. 2019; Betini et al. 2020; Im et al. 2020), which can be characterized as “response” traits (Suding et al. 2008, Suding & Goldstein 2008). Considering the importance of *D. magna* in regulating primary production and nutrient cycling in freshwater systems and the prevalence of these responses, it is likely that TGP also mediates the expression of “effect” traits (Suding et al. 2008, Suding and Goldstein 2008) in response to thermal stress.

The objective of this study was to examine how thermal stress (i.e., warming) and subsequent TGP impacts the expression of effect traits of the keystone herbivore *D. magna*. We examined the impacts of warming-induced TGP on herbivory, and uptake and release of Nitrogen (N) and Phosphorus (P). Herbivory rates are a direct measure of the top-down control on algal biomass (i.e., primary production), and N-P uptake and release is linked to the cycling of N and P in lakes and ponds. We reared *D. magna* at two different temperatures for 2 generations and subsequently conducted a 12-hour reciprocal transplant grazing assay with their offspring. Our hypotheses were: 1) Warming would result in TGP, improving thermal tolerance for individuals with warmer maternal environments; and 2) TGP would result in differences in grazing, and N-P uptake and release in offspring with different maternal temperatures. For *D. magna* reared for two generations at warmer temperatures, we expected less metabolic stress when exposed to warmer temperatures than those reared for two generations at colder temperatures, resulting in little change in grazing, nutrient uptake or release rates due to a match between parental and offspring temperature exposure. *D. magna* reared for two generations at colder temperatures were expected to exhibit more metabolic stress in warmer environments due to a mismatch between parental and offspring environments. Therefore, these *Daphnia* were expected to

show increased grazing, greater retention of P due to RNA production for TGP response and increased release of N due to increased excretion as a result of increased grazing.

Materials and Methods

Daphnia magna *brood stock and experimental design*

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. The population has been periodically stocked over these ten years with individuals from wild populations from Lake Eymir in order to maintain genetic diversity. *D. magna* were reared in a modified low-nutrient WC medium (without NaNO3, K2PO4, ATE, and Vitamin solution, Guillard & Lorenzen 1972) in 100ml glass containers and fed with 0.5 mgC/L 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 maternal temperature treatment of 18ºC or 24°C for two generations (schematic overview in Figure 1) and maintained at a density of 10 individuals per 100ml glass beaker to standardize any density-dependent maternal effects. Each container was checked daily for neonate emergence, with neonates removed and placed into a new container, at the 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 F2 generation from both maternal temperature treatments were measured using a stereomicroscope.

Critical thermal maxima assessment

To confirm that maternal exposure to different temperatures over two generations affected thermal tolerance, we assessed the critical thermal maximum (CTmax) 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. 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. CTmax 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 maternal temperature exposure influences offspring grazing, and N-P release rates at two different temperatures. F_2 adults and juveniles born five days prior to the grazing trial 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 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. 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. Based on these calculations, 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.

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 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 to assess differences in phosphorus body content. For all replicates, we filtered the entire medium volume through a Whatman GF/C filter, which was 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 nitrogen and phosphorus (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 outlined in Baird and Bridgewater (2017). 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 lg 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, N and P release rate calculations

We calculated clearance rates using the method provided by Frost (1972). 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).

For estimating the effect of maternal thermal acclimation on nutrient recycling, we used relative release rates for main dissolved nutrients. 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. Thus, the relative release rate is useful for measuring nutrient release during grazing. Relative release rates in NH_4, NO_3, DIN, and SRP due to grazing (J) were determined as follows:

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

where T_f is the mass of NH_4, NO_3, DIN, or SRP in the grazer-present treatment (mg), and C_f is the mass of these nutrients in the grazer-absent control (mg) and 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.

To assess the effects of TGP on the direct release of P, we estimated specific P release rates in grazer-present replicates using the zooplankton P recycling model from Olsen and Ostgaard (1985):

$$R = \frac{1}{ZT}\left[P_{dt} - P_{d0} + \frac{P_T N_0 - P_0 N_T}{N_T - N_0} * ln\frac{N_T}{N_0}\right]$$

where P_{d0} is 0, P_{dt} is the SRP concentration at the end of the experiment, N_0 is the algal concentration at the start of the grazing assay, N_t is the algal concentration at the end of the assay, P_0 is the particulate phosphorus at the start of the experiment, P_t is the particulate phosphorus at the end of the experiment, Z is *D. magna* biomass, and T is the duration of the grazing assay (12 hours). P_0 and P_t concentrations were estimated using the median P:C ratio for *Chlamydomonas reinhardtii* from P-enrichment experiments conducted by Olsen (1983).

Statistical analysis

We used model-based methods to assess the impacts of maternal and offspring exposure temperature (i.e. independent variables), on clearance rates, relative release rates in dissolved NH_4, NO_3, DIN, and SRP, body P composition, and P release rates (i.e dependent variables) (Table 1). 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 NH_4, NO_3, DIN, and SRP. We used generalized least squares regressions (GLS) for these variables. This method fits an ordinary least squares (OLS) regression 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).

We fit separate GLS models for each response variable of interest with maternal temperature, exposure temperature, and the interaction between maternal temperature and offspring exposure temperature as predictor variables (Table 1). 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. 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 multiple regression. 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.

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. All analyses were performed using R (version 4.2.2; R Core Team, 2023) with nmle (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* reared at 18ºC was longer (9-14 days) as compared to those reared at 24ºC (7-8 days). 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 less 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, although graphically evident, was not statistically significant (Figure 2, $p = 0.07$, $df = 28$).

Specific Clearance rates

Maternal temperature did not affect *D. magna* clearance rates (Figure 3a, Table 1, $p = 0.622$). 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) = 18.269 mL mg^{-1} h^{-1}) during the grazing experiment as compared to other treatments (Maternal 18°C – Exposure 24°C: IQR = 4.225 mL mg^{-1} h^{-1}, Maternal 24°C – Exposure 18°C: IQR = 4.861 mL mg^{-1} h^{-1}, Maternal 24°C – Exposure 24°C: IQR = 4.895 mL mg^{-1} h^{-1}).

Change in dissolved N and P

Individuals from warmer maternal temperatures increased their relative rates of dissolved NO_3 (Figure 3a, Table 1) and DIN release (Figure 3b, Table 1). Relative NO_3 release was 0.024 mg mL h^{-1} greater in jars with *D. magna* with maternal temperature of 24°C as compared to 18°C. Similarly, relative DIN release was 0.41 mg mL 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 and DIN release. We did not observe any effect of maternal temperature on changes in dissolved NH_4 concentrations (Figure 3a, Table 1). There was an increase in relative NH_4 release in *D. magna* exposed to 24°C as compared to those exposed to 18°C ($p = 0.020$, 0.491 mg mL h^{-1} greater 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 4a, Table 1, $p = 0.028$). An average increase of 0.142 mg mL h^{-1} in SRP 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. For *D. magna* with maternal temperature of 24°C, the average SRP release was 0.117 mg mL h^{-1} greater for individuals exposed to 18°C as compared to those exposed to 24°C. Average SRP release was 0.262 mg mL h^{-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. 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.

Body P composition and P release rate

Warmer maternal and exposure temperature increased *D. magna* body P content, by a factor of up to 60%, depending on offspring exposure temperature (Figure 4b, Table 1). 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. Warmer maternal and exposure temperatures also decreased P release rates (Figure 4c, Table 1). An average of 6.75 µg mL h^{-1} less P was released by all *D. magna* with maternal temperature of 24°C as compared to 18°C.

Discussion

Our results provide novel evidence that transgenerational plastic responses to warming impact body P content and the release of P and N in *D. magna*. Comparisons in dissolved NO_3 and DIN between *D. magna* with different maternal thermal environments show 279.25% greater NO_3 and 531.31% greater DIN release by individuals with higher maternal thermal environments (24°C) regardless of differences in exposure temperatures. Both maternal thermal environments and exposure temperatures influenced P retention and release. We observed a 65.8% greater release in SRP and 10% less percentage of P body mass by individuals with lower maternal temperature as compared to higher maternal temperature. At higher exposure temperatures, greater P was retained and less SRP was released regardless of maternal

exposure. 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 the cycling of N-P 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 also observed an increase in thermal tolerance of *D. magna* reared at 24°C as compared to those reared at 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 resulted in TGP for CTMax, but this result would be more evident with a greater number of replicates.

We observed both within-generation (WGP) and transgenerational plasticity (TGP) effects on nitrogen and phosphorus release in our experiment. While NO_3 and DIN release was moderated by differences in maternal temperature suggesting TGP effects, NH_4 release was only influenced by exposure temperature, regardless of maternal thermal environment, suggesting WGP effects. WGP can often mask any TGP effects (Vu et al., 2015; Groot et al. 2016; Moriuchi et al. 2016). Similarly, TGP can override WGP or operate in an opposing direction (Auge et al. 2017). We observed antagonistic WGP and TGP effects in the release of different N forms, with warmer maternal temperatures increasing NO_3 and DIN release while warmer exposure temperatures reduced NH_4 release. NH_4 release in individuals with warmer maternal temperatures showed a similar, although non-significant pattern, as NO_3 and DIN release. This suggests that WGP effects may be overriding any TGP effects on NH_4 release.

We did not observe any effect of maternal temperature on clearance rates. However, there was a large variation in these rates for *D. magna* with maternal temperature of 18°C when exposed to 18°C as compared to those with maternal temperature of 24°C. At higher exposure temperatures (24°C), this variation was reduced. We expected metabolic rates to increase at higher temperatures, resulting in higher total clearance rates (Burns, 1969; Müller et al., 2018). The lack of change in clearance rates may be an outcome of several mechanisms operating separately or in tandem. First, *D. magna* filtration rates are impacted by the rate of temperature change (Müller et al., 2018) and a sudden increase in six degrees could represent a significant thermal stress to which individuals were unable to acclimate over a short time scale (12 hours). Second, our warmer exposure temperatures exceeded the thermal optima for our *D. magna* population, potentially inhibiting grazing responses in some individuals resulting in the large variation observed. Finally, opposing WGP and TGP responses in total clearance rates to higher temperature exposure could result in the absence of a grazing response (Luquet & Tariel, 2016).

Temperature and Nutrient Cycling

Daphnia are primary consumers that play a central role in cycling nutrients through ecosystems (Mackay & Elser 1998, Stibor 2010). Mismatches between the elemental composition of an organism's body and its food resources can determine the rate and ratio of nutrients processed and released.

Zooplankton, such as *Daphnia*, can actively retain the most limiting element in their diet, while returning other nutrients in excess into the water column (Frost et al., 2006; Doi et al., 2011). The relative rates at which elements are excreted (i.e. discharge metabolic wastes) or egested (i.e. discharge undigested food) are largely species-specific and dictated by nutrient imbalances (Urabe, 1993; Sterner & Elser, 2002), with abiotic conditions, such as increasing temperatures (Halvorson et al. 2019) impacting these rates. Our study shows that climate warming could cause organisms with P-rich tissues such as *Daphnia* to increase the rate of nitrogen release relative to P. This may impact zooplankton-mediated nitrogen recycling at the ecosystem scale, with increased release of N relative to P potentially leading to increased TN:TP ratios, which favour certain cyanobacteria taxa (Dolman et al., 2012; Vanderploeg, et al., 2017). These consumer-driven effects are likely to be particularly significant in low-nutrient systems (McIntyre et al., 2008; Atkinson and Vaughn, 2015), but may also be directly exacerbated by warming, as positive relationships between increasing mean water temperature and cyanobacteria biomass have also been reported (Paerl and Paul, 2012; Bartosiewicz et al., 2019; Urrutia-Cordero et al., 2020).

A number of studies investigating interactions between temperature and nutrients have found that organismal N and P content declines with temperature (Woods et al., 2003; Martiny et al., 2013; Balseiro et al., 2021). Organismal RNA content, considered to be a primary determinant of body P content according to the growth rate hypothesis (Elser et al., 2000), has been observed to be consistently higher at cold temperatures than warmer temperatures for all poikilothermic (i.e., with variable internal temperature) taxa (Woods et al., 2003). These patterns are hypothesized to be a result of increased ribosomal translational efficiencies at higher temperatures reducing cellular RNA and P demands (Toseland et al., 2013; Cross et al., 2015). A decoupling between organismal P content and RNA content 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. Prater et al., (2018) show that warmer temperatures were associated with relaxed %P-RNA coupling as daphnid body RNA content declined but P content remained relatively high. In our study, warmer maternal and exposure temperatures increased *D. magna* body P content. However, we did not measure cellular RNA content or additional pools of organismal P such as phosphosugars or phospholipids and therefore the source of this higher body %P content is unknown. Other studies have also shown increased N and P concentrations in damselfly under warming conditions (Janssens et al., 2015) and in planktonic organisms (Mathews et al., 2018) or no significant changes in N:P ratios under increased temperatures (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), an underlying theoretical framework to mechanistically account for observations is yet to be developed and additional research is, therefore, required to fully investigate these factors. 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 for P uptake and N-P release observed in our study are adaptive for *D. magna*. Both warming and phosphorus limitation alter life history variables including clutch size, mean clutch length, and fecundity, all of which are indirectly related to fitness (Cavalheri et al. 2019, Harnett et al. 2019). Furthermore, selection for adaptive phenotypic plasticity has been observed for

Daphnia pulicaria populations from alpine lakes (Cavalheri et al. 2019) and *Daphnia galeata* populations from lakes heated by thermal effluent (Dzuiba et al. 2021). TGP responses can be locally adapted as well. Walsh et al. (2016) observed that for *Daphnia ambigua* populations exposed to fish predator cues, the magnitude of changes in size at maturation and clutch size were dependent on shared evolutionary history with these predators. Adaptive associations between our observed TGP responses and warming may be achieved through selection on P uptake and N-P release rates under thermal stress, a combination of selection and TGP, or selection of TGP responses (Via et al. 1995; Ghalambor et al. 2007). In our study, we only assessed TGP in a single *D. magna* laboratory population and cannot distinguish between these mechanisms. We recommend future studies examining TGP responses in effect traits explicitly assess 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 among the first to show that TGP responses to warming alter traits linked to nutrient cycling in aquatic ecosystems. The observed increase in N release and reduction in P release by *D. magna* with warmer maternal temperatures has strong implications for the ratio of N:P in lakes where *Daphnia* play a key role in the cycling of these nutrients (Elser and Urabe 1999). Reduced N:P ratios alter fundamental ecosystem functions and services including biogeochemical cycling and food security (Penuelas et al. 2020). More broadly, our results show that maternal warming may moderate the outcomes of climate change on ecosystem functions through transgenerational trait plasticity. We suggest that future research examining climate change impacts on ecosystem functions consider the contribution of transgenerational plasticity on potential outcomes.

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Figures

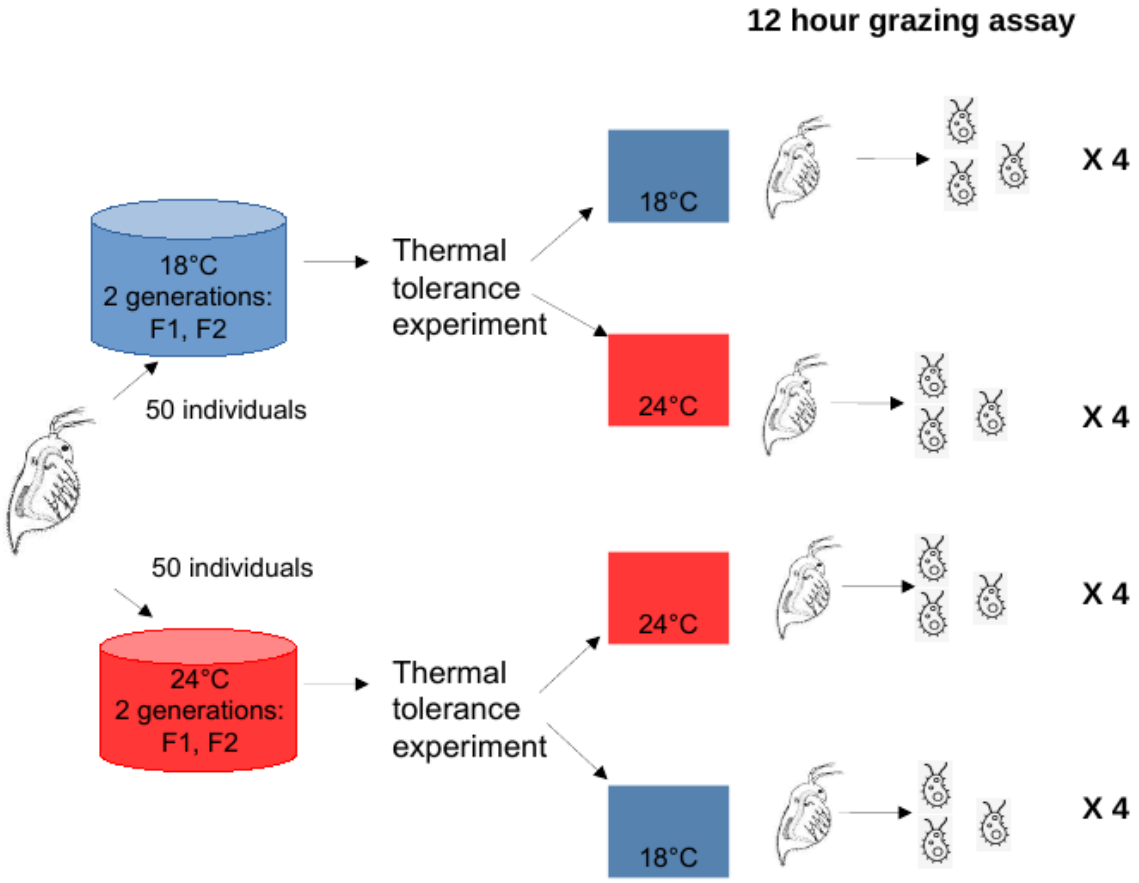

Figure 1: Graphical overview of the experimental design used to assess if transgenerational plasticity in *D. magna* influences grazing, and N-P uptake and release rates. Fifty individuals from a 10-year *D. magna* laboratory population were exposed to either 18℃ or 24℃ for 2 generations. Critical thermal 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*.

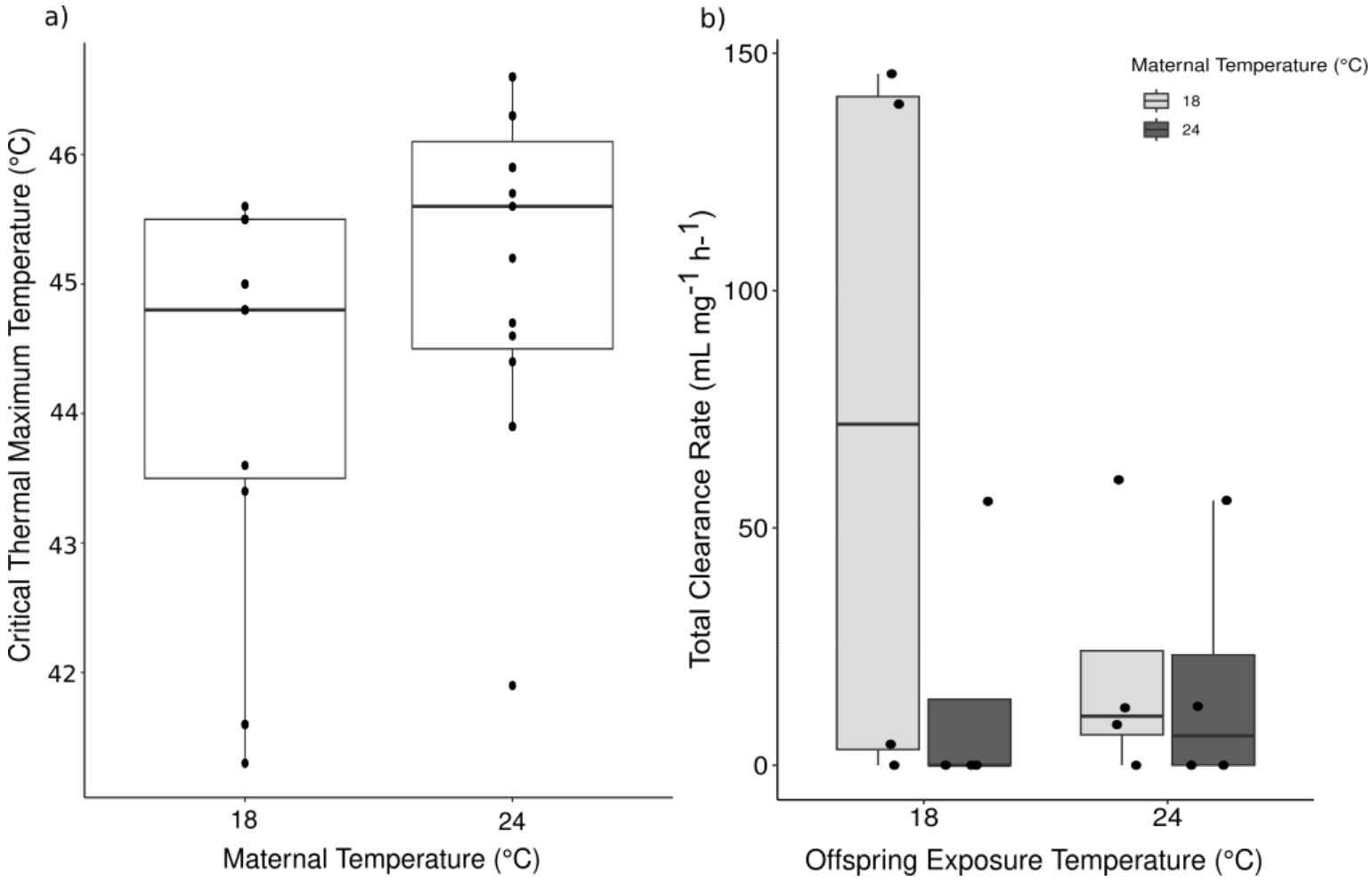

Figure 2**:** a) Critical thermal maximum temperature (°C) for *D. magna* reared at either 18°C or 24°C for two generations; and b) Total clearance rate (mgC ml^{-1} h^{-1}) for *D. magna* F2 generation with maternal temperature of 18°C or 24°C exposed to either 18°C or 24°C during a 12-hour grazing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

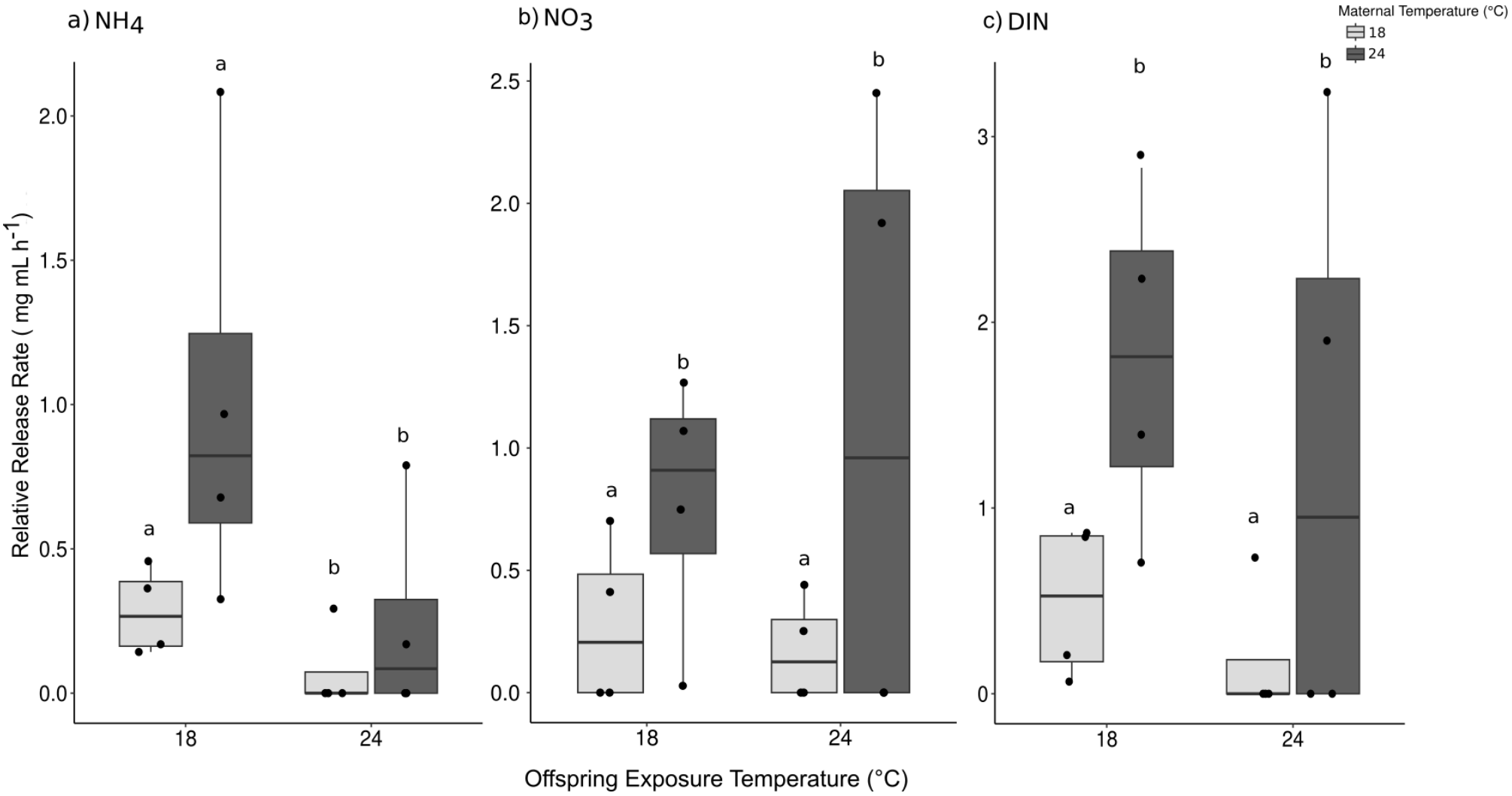

Figure 3: Relative release rates (mL h^{-1}) of a) Ammonia (NH_4), b) Nitrate (NO_3), and c) Dissolved Inorganic Nitrogen (DIN) for *D. magna* with maternal temperature of 18°C or 24°C exposed to either 18°C or 24°C during a 12-hour grazing assay. Different letters denote statistically significant differences ($p<0.05$).

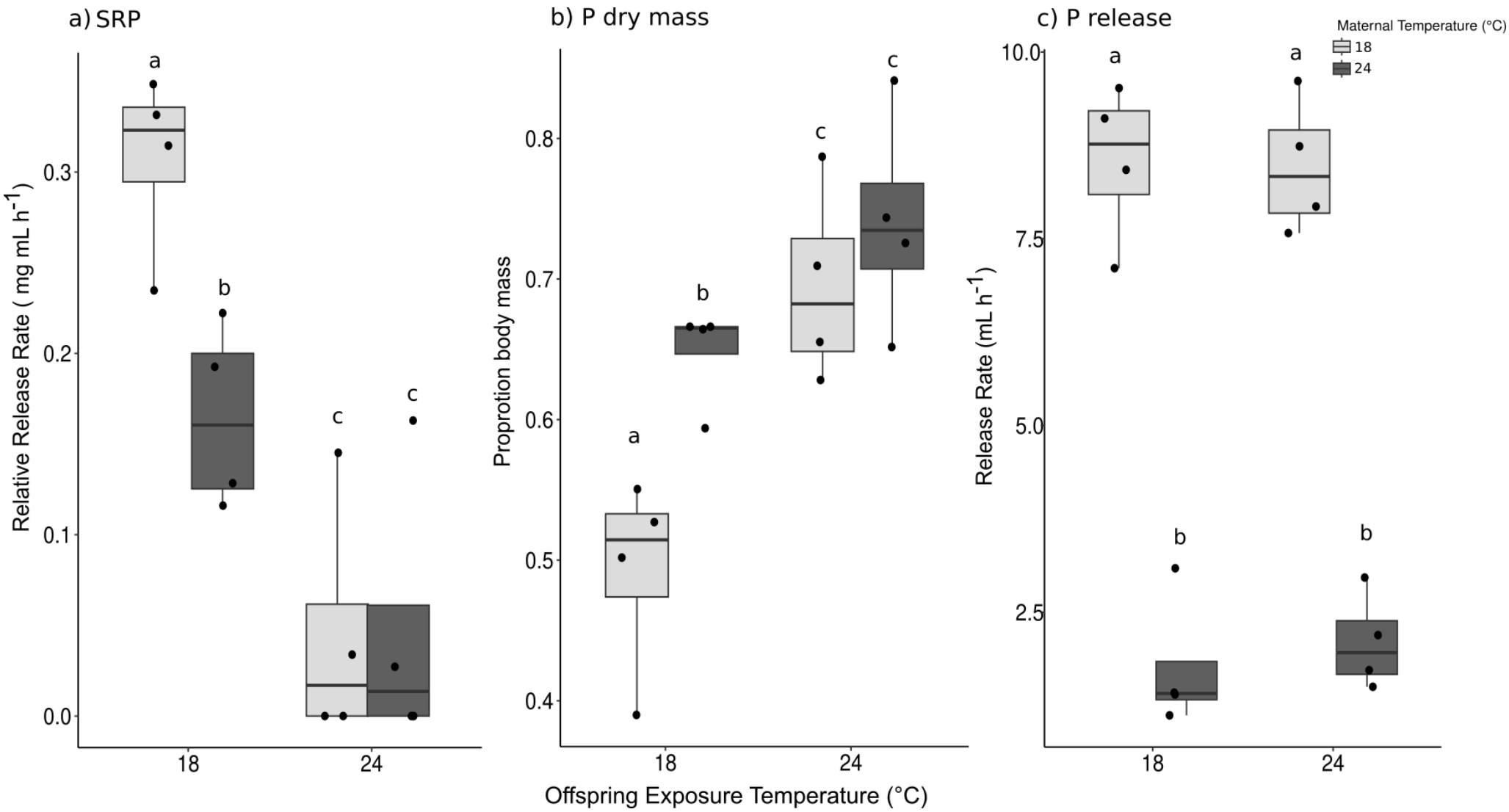

Figure 4: Change in a) relative release rate of Soluble Reactive Phosphorus (SRP mL h^{-1}), b) proportion phosphorus dry mass for *D. magna,* and c) P release rate (mL h^{-1}) with maternal temperature of 18°C or 24°C exposed to either 18°C or 24°C during a 12-hour grazing assay. Different letters denote statistically significant differences ($p<0.05$).

Table 1: 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, 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
NH_4	MT:ET	8	0.169	GLS
	MT	7	0.146	GLS
	ET	**6**	**0.020**	GLS
NO_3	MT:ET	8	0.714	GLS
	MT	**7**	**0.034**	GLS
	ET	6	0.603	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
P release	MT:ET	8	0.594	GLS
	MT	**7**	**<0.0001**	GLS
	ET	6	0.645	GLS