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RESEARCH ARTICLE

Heatwave-mediated decreases in phytoplankton quality negatively affect zooplankton productivity

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Abstract

1. Climate change is expected to increase the frequency of extreme temperature events. The effect of heatwaves on phytoplankton is of particular concern because they are a key source of C, N, P and essential fatty acids to aquatic ecosystems. Laboratory studies have demonstrated that phytoplankton grown at warmer temperatures are a lower quality food source, but how heatwaves affect phytoplankton quality at the community scale is currently unclear.
2. Here we address this knowledge gap by growing natural assemblages of freshwater phytoplankton at "ambient", "constant warming" or "heatwave" conditions. We next fed these phytoplankton communities to natural assemblages of zooplankton to test the prediction that zooplankton that consume heatwave-exposed phytoplankton will exhibit reductions in biomass.
3. Our experiment demonstrated that zooplankton that consumed "heatwave" phytoplankton attained lower community biomass than those fed "constant warming" or "ambient" phytoplankton. Additionally, despite receiving similar total heat input, phytoplankton exposed to "heatwave" conditions contained lower C, N, P and fatty acid concentrations compared to phytoplankton grown in "constant warming" conditions.
4. Correlations between zooplankton biomass and all measured phytoplankton traits revealed that decreases in zooplankton biomass were best explained by low quantities of C, N and monounsaturated fatty acids in "heatwave" phytoplankton.
5. Our study demonstrates that the effects of heatwaves on phytoplankton quality are clearly distinct from those caused by constant warming temperatures and that heatwave-mediated decreases in resource quality have immediate effects on consumer productivity.

KEYWORDS
community, fatty acids, heatwave, nutrients, phytoplankton, stoichiometry, warming, zooplankton

1 | INTRODUCTION

Increased global temperatures have had significant effects on the distribution (Jourdan et al., 2019; Rahel & Olden, 2008), morphology (Mccauley et al., 2015; Riis et al., 2012) and chemical composition (Hixson & Arts, 2016) of aquatic organisms. Of particular concern has been the effects of warming temperatures on phytoplankton. These organisms form the base of aquatic food webs, and they are key sources of C, N, P and fatty acids in aquatic ecosystems. Phytoplankton are a particularly important source of omega-3 polyunsaturated fatty acids (n-3 PUFA), which are products essential to the growth and productivity of higher trophic levels (von Elert & Fink, 2018). The response of phytoplankton to warming temperatures is likely to have ecosystem-wide importance and consequently an increasing number of studies have examined the effects of warming on a range of phytoplankton traits. At the individual scale, warming can favour smaller-celled phytoplankton (Zohary et al., 2021), increase growth rates (Courboulès et al., 2021) and reduce colony formation (Lürling & Van Donk, 1999; Tseng et al., 2021). Warming temperatures can also affect the quality and quantity of phytoplankton. Warming decreased the production of essential fatty acids (Fuschino et al., 2011; Sikora et al., 2014) and total lipids (Tseng et al., 2021) but has been shown to increase (Hessen et al., 2017) or decrease (Bi et al., 2017) C:N and C:P ratios. At the community scale, increases in temperature are expected to shift phytoplankton communities towards smaller-celled (Rasconi et al., 2015; Zohary et al., 2021) or warm-tolerant species (Kosten et al., 2012; Verbeek et al., 2018). Finally, warming temperatures can also alter phytoplankton species richness (Rasconi et al., 2015; Verbeek et al., 2018) and community composition (Machado et al., 2019; Strecker et al., 2004).

Climate change is also resulting in the increased frequency and severity of heatwaves (Woolway et al., 2021). Heatwaves have multiple definitions and are classified by the Intergovernmental Panel on Climate Change (IPCC) as "five consecutive days with maximum temperatures at least 5°C higher than the climatology of the same calendar day" (Pachauri & Reisinger, 2007). Research on how heatwaves affect plankton has thus far focused on changes in phytoplankton traits like biomass and phenology. Several experiments have demonstrated that effects of heatwaves on phytoplankton biomass are affected by nutrient level (Filiz et al., 2020; Weisse et al., 2016; Zhang et al., 2022), and that cyanobacteria phenology and recruitment differ under heatwave versus constant warming conditions (Zhang et al., 2022). At the community scale, heatwaves have been shown to decrease phytoplankton diversity (Bergkemper et al., 2018; Rasconi et al., 2017), and shift communities towards cyanobacteria and Chryptophytes (Hansson et al., 2020). Finally, the response of phytoplankton communities to heatwaves can be strongly affected by the initial temperature and community composition (Striebel et al., 2016). While our knowledge of the effects of heatwaves on phytoplankton traits is increasing, how changes in these traits subsequently affect consumers such as zooplankton is unknown.

Given the importance of phytoplankton-based nutrients for food web productivity (Tseng et al., 2021) and the predicted increased frequency of heatwaves, here we conducted a laboratory mesocosm experiment to investigate how heatwaves directly affect phytoplankton quality and indirectly affect zooplankton productivity. Both the *temperature-dependent physiology hypothesis* and the *RNA-efficiency hypothesis* posit that higher temperatures result in lower phytoplankton N and P (Hessen et al., 2017; Toseland et al., 2013). Similarly, the *homeoviscious adaptation* hypothesis states that saturation level of fatty acids is negatively correlated with temperature and thus at higher temperatures, phytoplankton are comprised of relatively more saturated and mono-unsaturated fatty acids, and fewer polyunsaturated fatty acids (Hazel, 1995; Martin-Creuzburg et al., 2019). Similarly, the ratio of omega-3 to omega-6 (n-3:n-6) fatty acids, which is positively correlated with phytoplankton "quality", has been shown to decrease with warming temperatures (Hixson & Arts, 2016). If the effect of heatwaves is more severe than the effects of an extended constant warming period, we predict that zooplankton that consume "heatwave" phytoplankton will exhibit decreased community-level productivity because their phytoplankton food source should contain lower nutrient content, compared to phytoplankton reared at ambient or constant warming conditions.

2 | METHODS

2.1 | Collection of phytoplankton and zooplankton communities

Natural communities of phytoplankton and zooplankton were collected from the University of British Columbia Research Ponds (49.247770, −123.233236). This research facility contains water bodies ranging in size from 300 L bins to 253,000 L ponds. Some of these containers are used for ongoing experiments while others have been fallow for up to 10 years and have accumulated natural assemblages of phytoplankton, zooplankton, and insects. Phytoplankton, zooplankton, and water for the experiment were collected from the containers that were not being subjected to ongoing experiments using buckets, 64 µm plankton tow nets, and handheld aquarium nets. Collected samples were combined and mixed thoroughly. Phytoplankton were collected approximately 3 weeks prior to the start of the experiment to facilitate propagating sufficient volumes of phytoplankton for the experiment (Figure S1). In the field, zooplankton were isolated using a 64 µm sieve. Larger invertebrates such as adult and larval insects were manually removed. Ethics approval was not required as per the lead author's institutional Animal Care Committee requirements.

2.2 | Zooplankton rearing

Wild-collected zooplankton were transported back to the lab, thoroughly mixed and distributed evenly into 27 20 L plastic food-grade buckets each filled with 6 L of filtered pond water. Pond water was

filtered using BRITA brand (Brita Canada Corporation, Ontario, Canada) filters. These filters are rated as 'NSF 53', have a pore size of approximately 1 μm and are designed to remove substances such as zinc, mercury, lead, asbestos, ibuprofen and chlorine from municipal tap water. Zooplankton were kept in the lab for approximately 11 days before receiving their first experimental phytoplankton feeding (Figure S1). During this time zooplankton were all fed wild-collected phytoplankton assemblages that were being propagated in the lab at ambient temperature. Weekly, zooplankton buckets were haphazardly moved within the lab to minimise location effects. Zooplankton experienced ambient temperature (20–22°C) for the duration of the experiment. See below for the description of how phytoplankton were fed to zooplankton.

2.3 | Phytoplankton rearing and temperature treatments

Each phytoplankton replicate ($n=27$ total) consisted of one 3.8 L glass jar filled with 3 L of wild-caught phytoplankton assemblage. Phytoplankton jars were supplemented with store-bought Schultz brand (Premier Tech Home & Garden Inc., Canada) liquid plant fertiliser (10:15:10 N:P:K). Fertiliser was added throughout the study and a total of four times to each replicate, for a total addition of 2.85 mL per replicate. Jars were topped up with BRITA-filtered pond water as needed. Phytoplankton jars were subjected to one of three temperature treatments (Figure 1, "ambient" 20°C day, 16°C night, $n=9$), "constant warming" (24°C day, 20°C night, $n=9$) and "heatwave" ($n=9$). Lighting within each incubator was provided by SunBlaster brand full-spectrum LED striplights (SunBlaster, British Columbia Canada). For the "heatwave" treatment, the temperature matched the "ambient" treatment for days 1–12. Temperature was increased to match the "constant warming" treatment for days 12–16. Temperature was then ramped up to 34°C/32°C day/night for days 16–27 (peak heatwave), before returning to the "constant warming" treatment for days 27–37, and then to the "ambient" treatment for the remainder of the experiment (days 37–51, Figure 1). We used temperature and light loggers (Onset HOBO® MX2202 Pendant, Massachusetts, USA) to track air and water temperatures inside of each incubator. The maximum, minimum, and average logger temperatures for the "ambient" (20°C/16°C) and "constant warming" (24°C/20°C) water treatments were 21.1°C, 16.6°C, 19.2°C and 24.7°C, 20°C, 22.7°C respectively.

We also calculated total degree-days for each temperature treatment according to standard methods (Solensky & Larkin, 2003). We used temperatures in the ambient treatment as the threshold temperatures and summed degree-days over the total 52 days of the experiment. The total degree-days experienced by the "constant warming" and "heatwave" treatments were 8.17 and 8.58 days, demonstrating that both treatments had similar temperature input. The average summer temperature for this region is 20–22°C. The +4°C implemented in the "constant warming" treatment is in accordance with projected lake temperature increases (Taner et al., 2011). A +4°C warming treatment is also commonly used in other warming studies, for example (Schulhof et al., 2019; Yvon-Durocher et al., 2015). The maximum summer temperature in Vancouver in 2021 was 32.4°C, which coincided with the 2021 Western North America Heat Dome (White et al., 2023). Day length for all treatments was set at 16 h light: 8 h dark. Phytoplankton jars and temperature treatments were haphazardly rotated among incubators at two instances to minimise incubator-specific effects.

2.4 | Feeding of phytoplankton to zooplankton

Wild-collected phytoplankton assemblages were propagated for approximately 20 days in the lab. Phytoplankton replicates were placed into their respective temperature treatments on day 1 of the study (Figure S1). Zooplankton received their first "ambient", "constant warming" or "heatwave" phytoplankton food feeding on Day 6 (Figure S1). This five-day gap between the start of the phytoplankton temperature treatment and the start of the zooplankton feeding schedule was chosen somewhat arbitrarily. Mainly we waited these 5 days before harvesting phytoplankton to feed zooplankton

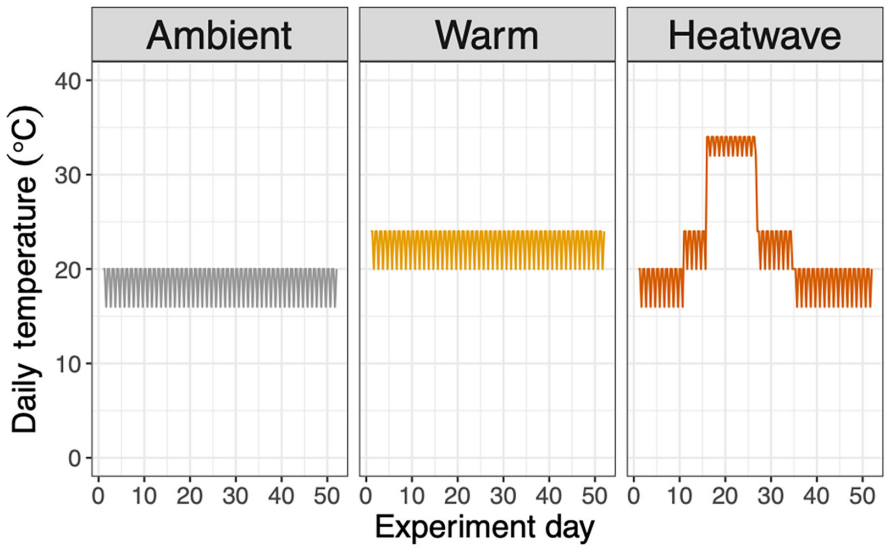

FIGURE 1 Temperature profiles for the three phytoplankton temperature treatments.

to ensure that all of the replicates appeared to be growing normally in the incubators. Throughout the experiment, phytoplankton were simultaneously exposed to their temperature treatments and harvested three times a week to feed to zooplankton (Figure S1).

Each phytoplankton replicate was paired with one corresponding zooplankton community replicate (bucket). For example, throughout the experiment, phytoplankton replicate 11 was used to feed zooplankton replicate. All zooplankton buckets were fed the same density of phytoplankton. The exact density of algae varied slightly week to week because of random stochastic variation among replicates. Each week we quantified phytoplankton density of all replicates, calculated the average density across all replicates, and fed each zooplankton community based on this average value. Phytoplankton density was quantified using an automated cell counter (Corning® Cell Counter with CytoSMART™ App, Version 3, New York, USA). We aimed to feed the zooplankton enough phytoplankton to maintain positive population/community growth, but not enough that there would be considerable uneaten phytoplankton remaining in the zooplankton buckets between treatments. On average over the 7 weeks, zooplankton were fed an estimated $9.84*10^6$ phytoplankton cells/L, three times per week. Additionally, to ensure minimal growth of uneaten phytoplankton within the zooplankton containers, zooplankton were reared in very low light conditions. Our HOBO loggers read 0 Lux for at least 21 h per day indicating that there was insufficient light within the zooplankton buckets for photosynthesis to occur. There was some initial educated guesswork to narrow the range of phytoplankton densities used to feed zooplankton but we used previous experience in the lab plus evidence of continued positive zooplankton population/community growth as an indicator that the density of phytoplankton we were feeding zooplankton was sufficient.

Zooplankton media was not changed during the experiment but approximately 300 mL filtered pond water was added weekly during feeding. Total zooplankton numbers increased throughout the experiment (Figure S2a), suggesting that conditions within the buckets were favourable for zooplankton reproduction. We ran this experiment for 52 to encompass the timing of an environmentally relevant heatwave and to try to account for a potential lag in temperature-mediated shifts in phytoplankton nutrients and observed changes in zooplankton biomass and fatty acids.

2.5 | Data collection

2.5.1 | Community composition and biomass

Zooplankton

For estimation, 1 L from each replicate was collected and filtered for zooplankton at the start, middle and end (days 6, 22, 52) of the study. Water was returned to the replicate bucket and the sample was preserved in 90% ethanol. Zooplankton were counted and identified to genus and life stage using a dissecting microscope (Zeiss Stemi 508, Oberkochen, Germany). Final zooplankton whole-community biomass data were measured at the end of the experiment by collecting all remaining zooplankton using sieves, rinsing, freeze-drying and then weighing the final sample. Freeze-drying occurred during the fatty acid sample preparation.

Phytoplankton

For estimation,100 mL from each replicate jar was collected on at the start, middle and end of the study (Days 1, 22, 51). The "end" sampling day of the experiment was staggered for zooplankton and phytoplankton because we were unable to collect and process both trophic levels on the same day. Phytoplankton was immediately preserved in Lugol's solution. Phytoplankton were counted and classified into five major taxonomic groups: Bacillariophyta (diatoms), Chlorophyta/Charophyta (green algae), Haptophyta/Ochrophyta (golden algae), Cyanophyta (blue-green algae), and Dinophyta (dinoflagellates). Within the five major groups, phytoplankton were further sub-classified as single cells, colonies or filaments. Within colonies and filaments, groups of 2–5 cells were categorised as "small" colonies/filaments, 6–15 cells as "medium" colonies/filaments and over 16 cells (up to 43 cells) as "large". Phytoplankton classification was conducted by Biologica Environmental Services Ltd (Victoria, BC). Phytoplankton morphospecies are listed in Table S1. Phytoplankton biomass (whole-community dry weight) data were obtained during fatty acid processing (see the next section).

2.5.2 | Fatty acids

Zooplankton fatty acids

We quantified zooplankton fatty acids at the end of the experiment to avoid destructively sampling throughout the experiment. All zooplankton per bucket were filtered through a 64 µm sieve, rinsed with distilled water, transferred into a 10 mL Pyrex glass conical centrifuge tube, and frozen at −65°C until analysis. Fatty acids were quantified using the same methods as Tseng et al. (2021). Briefly, the sample was freeze-dried, incubated, chemically separated and fatty acids were analysed using gas chromatography. During fatty acid quantification an "internal standard" is added to the analysis at a set quantity (e.g. 500 µg). The peak of the chromatograph for each fatty acid is then standardised by the peak of the internal standard, and this standardisation allows for the calculation of fatty acid concentrations. Unfortunately, during the analysis of zooplankton fatty acids, the internal standard did not run correctly and thus we are unable to report concentrations of zooplankton fatty acids. Instead, for zooplankton we can only report relative fatty acid percentages. To calculate percent saturated fatty acids (SFA), percent monounsaturated fatty acids (MUFA) and percent polyunsaturated fatty acids (PUFA), we summed across the fatty acid groups listed in Table S2 and divided by the total. We also summed across all n-3 PUFA and n-6 PUFA and calculated the n-3:n-6 ratio. This ratio is commonly reported in studies of fatty acids because products with higher n-3:n-6 are typically thought to be a higher "quality" food source (Hixson & Arts, 2016).

Phytoplankton fatty acids

At the start, middle and end sampling periods (Days 1, 22, and 51) we filtered 25–75 mL of culture from each phytoplankton replicate onto a GF/F borosilicate filter. Filters were placed in individual 10 mL Pyrex glass conical centrifuge tubes and frozen at −65°C until analysis. Fatty acids were quantified using the same methods as described above. Fortunately for phytoplankton, the internal standard did run properly, and thus we are able to report phytoplankton fatty acids concentrations. The fatty acids included in the SFA, MUFA and PUFA groups are listed in Table S2. As we did for zooplankton, we also calculated phytoplankton n-3:n-6 ratio.

2.5.3 | Phytoplankton C, N and P

At the start, middle, and end sampling days (days 1, 22, and 51), we filtered 25-50 mL from each phytoplankton replicate onto a GF/F borosilicate filter (GE Healthcare Life Sciences Whatman, USA) for carbon (C) and nitrogen (N) analysis. At the end of the experiment we filtered an additional 35 mL of culture per replicate for total phosphorus (P) analysis. Phosphorus samples were only obtained at the end due to processing and sample volume limitations. All filters with samples were placed in individual 10 mL Pyrex glass conical centrifuge tubes and frozen at −65°C until analysis. Total C, N and P were analysed at the Marine Geochemistry Laboratory in the Department of Earth, Ocean, and Atmospheric Sciences, University of British Columbia (Vancouver, BC). For total C and N analysis, samples were removed from the freezer and placed overnight in a drying oven at 50°C. Samples were acid fumed to obtain POC, and analysis was conducted using an elemental analyser. For total P analysis, samples were weighed, digested and analysed using ICP-OES (inductively coupled plasma–optical emission spectrometry). Detailed procedures for C and N analysis are available in (Verardo et al., 1990), and for P analysis in (Murray et al., 2000). We did not analyse zooplankton C, N or P because almost all zooplankton biomass at the end of the experiment was required for fatty acid analysis.

2.6 | Data analysis

2.6.1 | Zooplankton biomass, growth rate and Shannon diversity

Zooplankton biomass was measured as the total dry mass of the entire zooplankton community at the end of the experiment. Growth rate was defined as the [(number of zooplankton at the end – number of zooplankton at the beginning)/number of zooplankton at the beginning]. Shannon diversity (H′) was calculated using the "vegan" package in R (Oksanen, 2022). Unlike biomass and growth rate, Shannon diversity was measured at three time periods. We present zooplankton Shannon diversity two ways: (1) at the end of the experiment (to complement the zooplankton biomass and growth rate data) and (2) at the beginning, middle, and end of the study to both confirm that replicate buckets started with the same community of zooplankton, and to examine whether zooplankton Shannon diversity was explained by phytoplankton food type.

We used analysis of variance (ANOVA) to examine whether phytoplankton food type explained variation in zooplankton biomass, growth rate or Shannon diversity at the end of the experiment. We used residual plots and q-q plots to check that the data met assumptions of ANOVA, and we checked for homogeneity of variances using Levene's Test. No data were transformed. When the ANOVA was statistically significant, we used Tukey HSD post-hoc tests to further examine pairwise differences.

We used a linear mixed-effects model (package "lmerTest"; Kuznetsova et al., 2017) to test whether zooplankton Shannon diversity was affected by phytoplankton food type or experiment sampling period (start, middle and end). In this model, the replicate bucket was coded as a random factor and "phytoplankton food type" and "period" were coded as fixed factors. We examined plots of residuals to confirm that the data were appropriate for this model structure. Finally, we used Tukey post-hoc comparisons (via the emmeans package; Lenth et al., 2023) to further examine pairwise differences in zooplankton Shannon diversity.

2.6.2 | Phytoplankton C, N and P; phytoplankton and zooplankton fatty acids

Because the dates chosen for the "middle" and "end" of the study were relatively arbitrary, we averaged C, N, SFA, MUFA, PUFA and n-3:n-6 values across these two sampling points. Averaging across the "middle" and "end" time points provides a more conservative estimate of the overall effects of the "heatwave" or "constant warming" treatments. Phytoplankton P data and zooplankton fatty acid data were only collected at the end of the experiment so those data were not averaged. Trait values from the beginning of the experiment were not included in the averages because phytoplankton would have only experienced the temperature treatments for less than 1 day. We used ANOVA to examine whether phytoplankton temperature treatment explained variation in phytoplankton C, N, P or in phytoplankton and zooplankton SFA, MUFA, PUFA and n-3:n-6. Similar to the zooplankton biomass analyses, we used residual plots and q-q plots to check that all traits met assumptions of ANOVA, and used Levene's Test to check for homogeneity of variances. When the ANOVA was significant, we used Tukey HSD post-hoc tests to test for pairwise differences within each trait. For completeness, we also used the same statistical tests to examine the effects of temperature on phytoplankton C, N, SFA, MUFA, PUFA and n-3:n-6 from just the samples collected at the end of the experiment.

2.6.3 | Phytoplankton biomass and Shannon diversity

To confirm that the phytoplankton treatments started with the same community composition and biomass, we plotted phytoplankton

biomass and Shannon diversity over the three sampling periods (start, middle, end) and used linear mixed effects models to examine the effects of "period" and "temperature". The replicate jar was coded as a "random" factor. Similar to the analysis for zooplankton Shannon diversity over time, we examined plots of residuals to confirm that the data were appropriate for this model and we used Tukey posthoc comparisons (via the emmeans package) to further examine pairwise differences within sampling periods.

2.6.4 | Relationships between phytoplankton traits and zooplankton biomass

Finally, our paired phytoplankton–zooplankton replicate design allowed us to investigate whether phytoplankton C, N, P, SFA, MUFA, PUFA or n-3:n-6 directly predicted zooplankton biomass. Phytoplankton traits for this analysis were averaged across the "middle" and "end" time points. We conducted individual linear regressions with each phytoplankton trait as the predictor, and zooplankton biomass as the response variable. All model assumptions were checked using residual and q-q plots and no data were transformed. For completeness, we also conducted the same analyses using just phytoplankton data collected at the "end" sampling period.

All figures were created using the package "ggplot2" (Wickham, 2016). We considered $p < 0.05$ as statistically significant and all statistical analyses were conducted in R Version 4.2.0 (R Core Team, 2023). All raw data are available from the Dryad Digital Repository (Kim et al., 2024).

3 | RESULTS

3.1 | Zooplankton biomass, growth rate and diversity

The temperature at which phytoplankton were reared significantly affected zooplankton biomass at the end of the experiment (Figure 2a; $F_{2,21} = 4.82$, $p = 0.02$; Table S3). Post-hoc pairwise comparisons demonstrated that zooplankton biomass was higher when they were fed "constant warming" versus "heatwave" phytoplankton (Figure 2a; Tukey HSD: $p = 0.014$). There was no effect of phytoplankton food type on overall zooplankton growth rate (Figure 2b, $F_{2,23} = 0.29$, $p = 0.75$). Phytoplankton food type also did not affect zooplankton Shannon diversity at the end of the study (Figure 2c; $F_{2,23} = 0.54$, $p = 0.59$) nor at other time points (Figure S2a,b: treatment: $F_{2,24} = 0.99$, $p = 0.38$; period $F_{2,48} = 5.1$, $p = 0.01$; treatment: period $F_{4,48} = 0.36$, $p = 0.84$).

3.2 | Phytoplankton C, N, P, fatty acids, biomass and diversity

Temperature treatment had consistent effects on phytoplankton C, N and P. Phytoplankton that experienced the heatwave exhibited

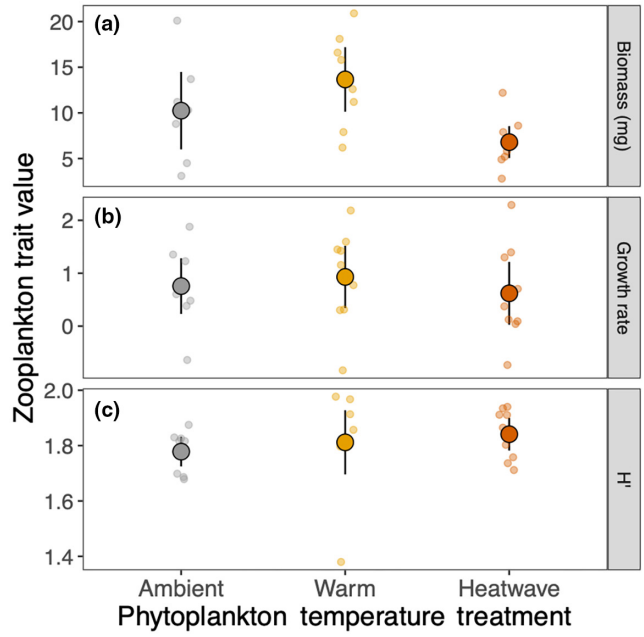

FIGURE 2 The effects of phytoplankton temperature treatment on zooplankton (a) biomass, (b) growth rate and (c) Shannon diversity (H'). Large dots and error bars denote the mean ± 95% confidence intervals. Smaller dots denote individual replicates. The three temperature treatments are coloured grey (ambient), dark yellow (constant warming), and dark orange (heatwave). See Results for ANOVA and posthoc test results.

the lowest concentrations of these elements (Figure 3, Table 1, Table S3). Tukey post-hoc tests demonstrated that C, N and P were significantly lower in "heatwave" versus "constant warming" phytoplankton, and in "heatwave" versus "ambient" phytoplankton (Table 1). These patterns remained when we examined only data from the final sampling point (Figure S4, Table S4). Temperature also had clear effects on phytoplankton fatty acids (Figure 4). Concentrations of SFA, MUFA and PUFA were all lower in "heatwave" versus "constant warming" phytoplankton (Figure 4a–c; Table 1, Table S3). SFA and MUFA concentrations were similar between "heatwave" and "mbient" phytoplankton (Figure 4a,b) but PUFA concentrations were lowest in phytoplankton from the "heatwave" treatment (Figure 3c, Table 1). Finally, phytoplankton from the "ambient" treatment exhibited higher n-3:n-6 ratios compared to either "constant warming" or "heatwave" phytoplankton (Figure 4d, Table 1). The effect of temperature on phytoplankton SFA and MUFA remained the same when we examined only data from the final sampling point (Figure S5, Table S4). However, PUFA and n-3:n-6 of "heatwave" phytoplankton resembled that of the "ambient" treatment (Figure S5, Table S4).

There were no differences in phytoplankton biomass among the temperature treatments at the start or middle of the experiment but by the end, phytoplankton exposed to "constant warming" temperatures exhibited lower biomass than either "ambient" or "heatwave" phytoplankton (Figure 5a, treatment: $F_{2,24.3} = 2.54$, $p = 0.1$; period: $F_{2,48} = 601$, $p < 0.0001$; treatment: period $F_{4,48} = 5.8$, $p = 0.0001$; "end" period contrasts: "constant warming" vs. "ambient" $p = 0.006$,

"constant warming" vs. "heatwave" $p < 0.0001$; Table S3). We examined Shannon diversity (H′) of phytoplankton morphospecies and although diversity decreased over the course of the experiment, there was no effect of temperature treatment on diversity at any point in the experiment (Figure 4b, Figure S3, treatment: $F_{2,24.3} = 1.9$, $p = 0.2$; period: $F_{2,47} = 209$, $p < 0.0001$; treatment: period: $F_{4,47} = 2.16$, $p = 0.09$).

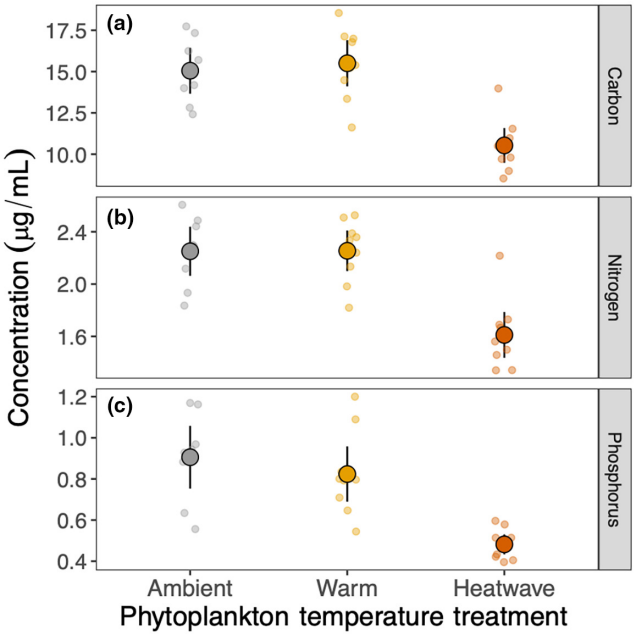

FIGURE 3 Effect of temperature treatment on concentrations of phytoplankton (a) carbon, (b) nitrogen, and (c) phosphorus. Large dots and error bars denote the mean ± 95% confidence intervals. Smaller dots denote individual replicates. The three temperature treatments are coloured grey (ambient), dark yellow (constant warming), and dark orange (heatwave). See Table 1 for ANOVA and posthoc test results.

3.3 | Relationship between phytoplankton traits and zooplankton biomass

To investigate which phytoplankton traits were potentially responsible for the low biomass of zooplankton in the "heatwave phytoplankton" treatment, we examined the relationship between individual phytoplankton traits (C, N, P, fatty acids) and zooplankton biomass (Figures 6 and 7). We found statistically significant positive relationships between zooplankton biomass and phytoplankton C, N, and MUFA (Figure 6a,b and 7b; Table 1). The correlation between zooplankton biomass and phytoplankton PUFA was also positive but not statistically significant (Figure 6c; $p = 0.08$; Table 1). When we examined the same set of correlations except with just phytoplankton fatty acid data sampled at the "end" sampling period, phytoplankton C, N and MUFA were still strong predictors of total zooplankton biomass (Figures S6 and S7, Table S4). Zooplankton biomass was also predicted by phytoplankton n-3:n-6 ratios (Figure S7, Table S4).

3.4 | Effect of phytoplankton food type on zooplankton fatty acids

Zooplankton fatty acid composition was significantly affected by phytoplankton food type (Figure 8, Table 2, Table S3). Zooplankton fed "ambient" phytoplankton exhibited the lowest percentages of SFA, highest percentages of PUFA and highest n-3:n-6 ratio. MUFA

TABLE 1 Summary results for ANOVA investigating the effect of rearing temperature on phytoplankton traits (columns 1–2), and for linear regression analyses examining the relationship between each phytoplankton trait and zooplankton biomass (column 4). Column 3 displays Tukey HSD posthoc tests for each corresponding ANOVA.

1. Phytoplankton trait	2. Effect of temperature	3. Tukey HSD posthoc tests	4. Correlation with zooplankton biomass
Carbon	$F_{2,23} = 18.1$; $p < 0.0001$	HW < Warm: $p < 0.0001$ HW < Ambient: $p = 0.0002$	$F_{1,22} = 9.6$, $p = 0.005$; slope = 0.29, $r^2 = 0.27$
Nitrogen	$F_{2,23} = 18.1$; $p < 0.0001$	HW < Warm: $p = 0.0001$ HW < Ambient: $p = 0.001$	$F_{1,22} = 5.6$, $p = 0.027$; slope = 0.03, $r^2 = 0.17$
Phosphorus	$F_{2,23} = 14.1$; $p = 0.0001$	HW < Warm: $p = 0.0012$ HW < Ambient: $p = 0.0002$	$F_{1,22} = 0.86$, $p = 0.36$
SFA	$F_{2,22} = 12.8$; $p = 0.0002$	Warm > Ambient: $p = 0.0002$ Warm > HW: $p = 0.0012$	$F_{1,21} = 2.2$, $p = 0.16$
MUFA	$F_{2,22} = 27.7$; $p < 0.0001$	Warm > Ambient: $p < 0.0001$ Warm > HW: $p < 0.0001$	$F_{1,21} = 9.4$, $p = 0.0006$; slope = 1.4, $r^2 = 0.28$
PUFA	$F_{2,22} = 7.8$; $p = 0.003$	HW < Ambient: $p = 0.05$ HW < Warm: $p = 0.002$	$F_{1,21} = 3.3$, $p = 0.08$; slope = 0.4, $r^2 = 0.1$
n-3:n-6	$F_{2,22} = 14.1$; $p = 0.0001$	Warm < Ambient: $p = 0.0003$ HW < Ambient: $p = 0.0005$	$F_{1,21} = 2.9$, $p = 0.10$

Abbreviations: Ambient, "ambient" temperature treatment; HW, "heatwave" treatment; MUFA, monounsaturated fatty acids; n-3:n-6, the ratio of omega-3 PUFA to omega-6 PUFA; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Warm, "constant warming" treatment.

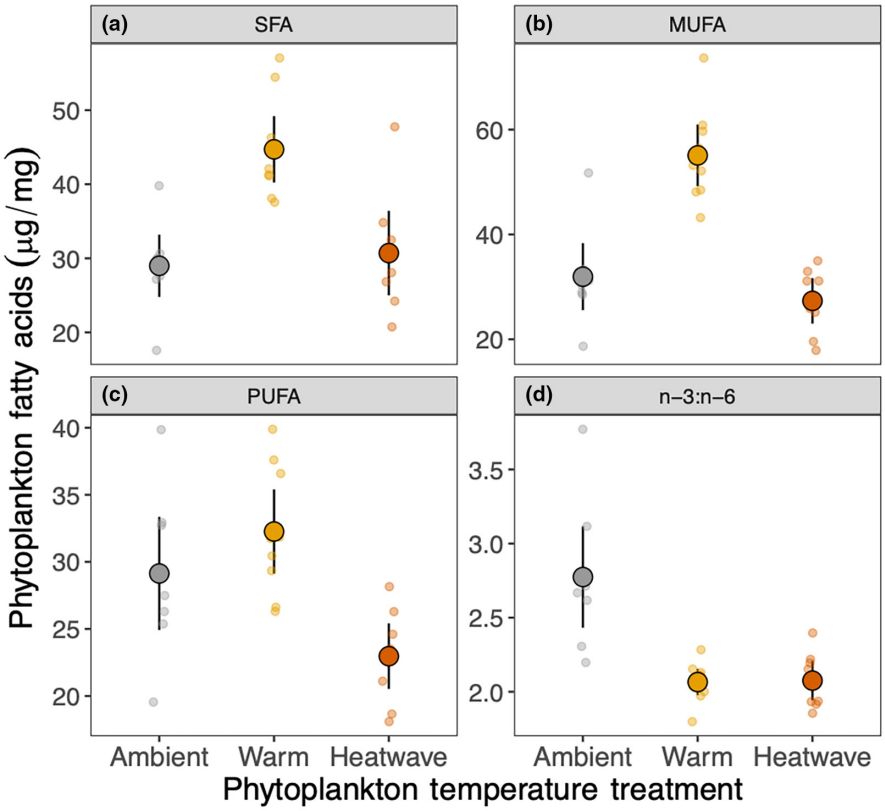

FIGURE 4 Effect of temperature treatment on phytoplankton fatty acid concentrations. Abbreviations are (a) SFA, saturated fatty acids; (b) MUFA, monounsaturated fatty acids; (c) PUFA, polyunsaturated fatty acids; and (d) n-3:n-6 ratio for omega-3 PUFA to omega-6 PUFA. Large dots and error bars denote the mean ± 95% confidence intervals. Smaller dots denote individual replicates. The three temperature treatments are coloured grey (ambient), dark yellow (constant warming), and dark orange (heatwave). See Table 1 for ANOVA and posthoc test results.

and PUFA fatty acid percentages were also significantly different between zooplankton fed "constant warming" versus "heatwave" phytoplankton (Figure 7b,c, Table 2, Table S3).

4 | DISCUSSION

The overall goal of this experiment was to examine the indirect effects of heatwaves on zooplankton communities via shifts in the nutritional quality of heatwave-exposed phytoplankton. We predicted that zooplankton that consumed "heatwave" phytoplankton would exhibit lower productivity than those that were fed phytoplankton that were reared "ambient" or "constant-warming" temperatures. We found that zooplankton communities fed "heatwave" phytoplankton grew at the same rate and were comprised of the same taxonomic diversity as zooplankton fed other types of phytoplankton, and as predicted, those fed "heatwave" phytoplankton achieved the lowest overall biomass. Phytoplankton C, N, P and fatty acid data revealed that the decline in zooplankton biomass was mostly due to decreased concentrations of C, N and MUFA in "heatwave" exposed phytoplankton. Phytoplankton in the "constant warming" and "heatwave" treatments were exposed to very similar total amounts of heat but while warming was mostly concentrated between days 15 and 25 in the "heatwave" treatment, it was spread out over the duration of the study in the "constant warming" treatment. The clear effects of phytoplankton temperature treatment on all measured phytoplankton "quality" traits, as well as on zooplankton biomass demonstrate that how heat is delivered into an aquatic system has important effects on both phytoplankton and zooplankton productivity.

Low levels of zooplankton biomass could also arise if, compared to zooplankton fed "ambient" or "constant warming" phytoplankton, zooplankton in the "heatwave phytoplankton" treatment received less food, or a different community of phytoplankton. Phytoplankton biomass was not affected by temperature treatment at the start or middle of the experiment, but by the end of the study, biomass was lowest in the "constant warming" treatment. Because phytoplankton biomass was never lowest in the "heatwave" treatment, we infer that the depressed biomass of zooplankton fed "heatwave" phytoplankton was not due to low food availability. There was also no effect of temperature treatment on phytoplankton Shannon diversity and thus decreases in zooplankton biomass in the "heatwave" treatment were likely not due to temperature-specific shifts in phytoplankton community composition. However, phytoplankton diversity was calculated using coarse taxonomic groupings of phytoplankton and it is possible that fine-scale shifts in species composition were not captured in the Shannon diversity metric. Still, freshwater phytoplankton communities are most often delineated by the relative contributions of the coarse taxonomic groupings included in this study rather than by individual species (De Senerpont Domis et al., 2014; Galloway & Winder, 2015). Thus, we believe the results presented here are broadly applicable. Finally, among-treatment differences in zooplankton biomass could also be driven by changes in zooplankton community composition. We did not observe any effects of phytoplankton food type on zooplankton Shannon diversity or community composition. Overall, the data support the conclusion

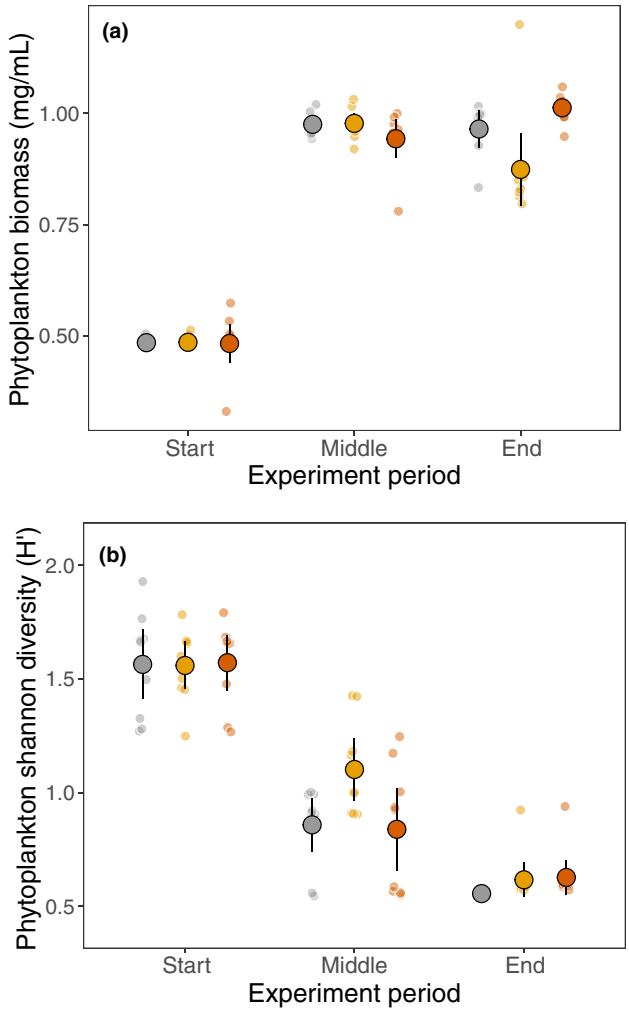

FIGURE 5 Effect of temperature treatment and experiment period on phytoplankton (a) biomass and (b) Shannon diversity (H′). Large dots and error bars denote the mean ± 95% confidence intervals. (95% CI are masked by the larger dot in some cases.) Smaller dots denote individual replicates. The three temperature treatments are coloured grey (ambient), dark yellow (constant warming) and dark orange (heatwave). For biomass there was a statistically significant effect of period and a significant period by treatment interaction. For Shannon diversity, there was a significant effect of period. See Results for full analyses.

that depressed biomass in zooplankton fed "heatwave" phytoplankton was primarily explained by shifts in phytoplankton nutritional quality.

The community-wide responses of phytoplankton fatty acids to constant warming observed in this study are similar to those reported in single species studies. *Scenedesmus obliquus*, *Isochrysis galbana* and *Chroomonas salina* produced more SFA, MUFA, and exhibited lower n-3 to n-6 ratios when reared at warmer temperatures (Fuschino et al., 2011; Henderson & Mackinlay, 1989; Zhu et al., 1997). In outdoor flow-through mesocosms or whole lake studies, the effects of warming on community-wide phytoplankton fatty acids also depended on nutrient levels and phytoplankton community composition (Calderini et al., 2023; Keva et al., 2021; Strandberg et al., 2022). In our experiment, heatwave-exposed phytoplankton contained the lowest total concentrations of fatty acids and although the mechanism underlying this overall decrease in fatty acid production is unclear, total fatty acid production was also lowest in *Scenedesmus obliquus* grown at 32°C (vs. 16°C or 24°C; Sikora et al., 2014). We are currently conducting follow-up studies to investigate why phytoplankton responses to temperature increases are clearly different between the "constant warming" versus "heatwave" treatments. We do not have clear mechanisms at the moment but we are exploring whether decreases in C, N and MUFA could be linked to the reshuffling of proteins or other molecules in response to temporary heat stress.

Our study is unique in that in addition to shifts in fatty acid content and composition, we document concomitant temperature-mediated reductions in phytoplankton C, N and P. Additionally, we demonstrate immediate knock-on effects of decreases in phytoplankton quality on zooplankton community biomass and on zooplankton fatty acid composition. Zooplankton fatty acid composition often closely reflects that of their food (Brett et al., 2006; Meyers et al., 2022). Here we see some parallels between zooplankton and phytoplankton MUFA and n-3:n-6 ratios but because we were limited to presenting zooplankton fatty acid data as percentages (see Section 2), we refrain from drawing concrete conclusions regarding similarities between zooplankton and phytoplankton fatty acid profiles. We know of no other experiments that have also examined the effects of phytoplankton food quality on community-wide zooplankton biomass but two previous studies have reported that *Daphnia* zooplankton produced fewer offspring and had lower population sizes when they were fed *Scenedesmus* phytoplankton grown at warmer temperatures (Sikora et al., 2014; Tseng et al., 2021). Here we have extended these studies to show that temperature directly affects resource quality and indirectly affects consumer productivity at the whole-community level.

The dates we chose at the middle and end of the study to sample phytoplankton were somewhat arbitrary and thus for the main analyses presented here we report the values of phytoplankton C, N, and fatty acids averaged across the two sampling periods. (Phytoplankton P was only sampled at the end). Although averaging the data prevents us from examining temporal variation in phytoplankton responses, our results are a more conservative estimate of the effects of "constant warming" versus "heatwave" conditions on phytoplankton traits. For completeness we have also included phytoplankton trait data for just the "end" sampling point, as well as correlations between zooplankton biomass and each of the phytoplankton traits for this sampling point (Figures S4–S7, Table S3). The results from the "end only" analyses are qualitatively very similar to the "middle and end averaged" analyses for all data except for phytoplankton n-3:n-6. At the "end" sampling point, n-3:n-6 values for "heatwave" phytoplankton resemble values for "ambient" phytoplankton (rather than resembling values for "constant warming"). Because the PUFA do not change very much when comparing the "middle+end averaged" data versus the "end" only data (Figure 4c

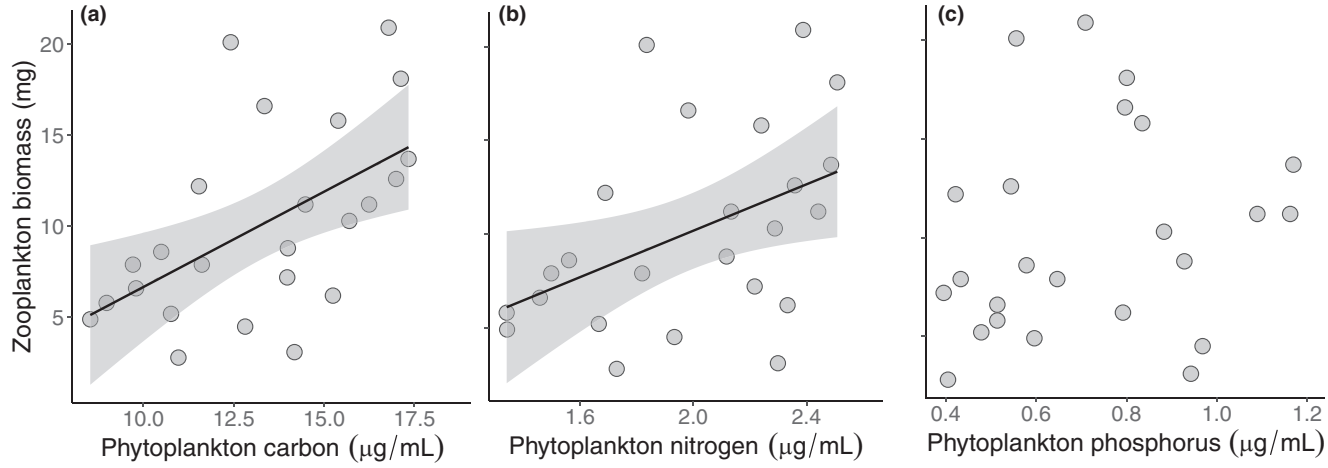

FIGURE 6 Relationships between concentrations of phytoplankton (a) carbon, (b) nitrogen, and (c) phosphorus and total zooplankton community biomass. See Table 1 (column 4) for statistical analyses. The solid line is the line of best fit estimated from linear regression and the grey shading represents the 95% confidence interval. (No line = no statistically significant relationship).

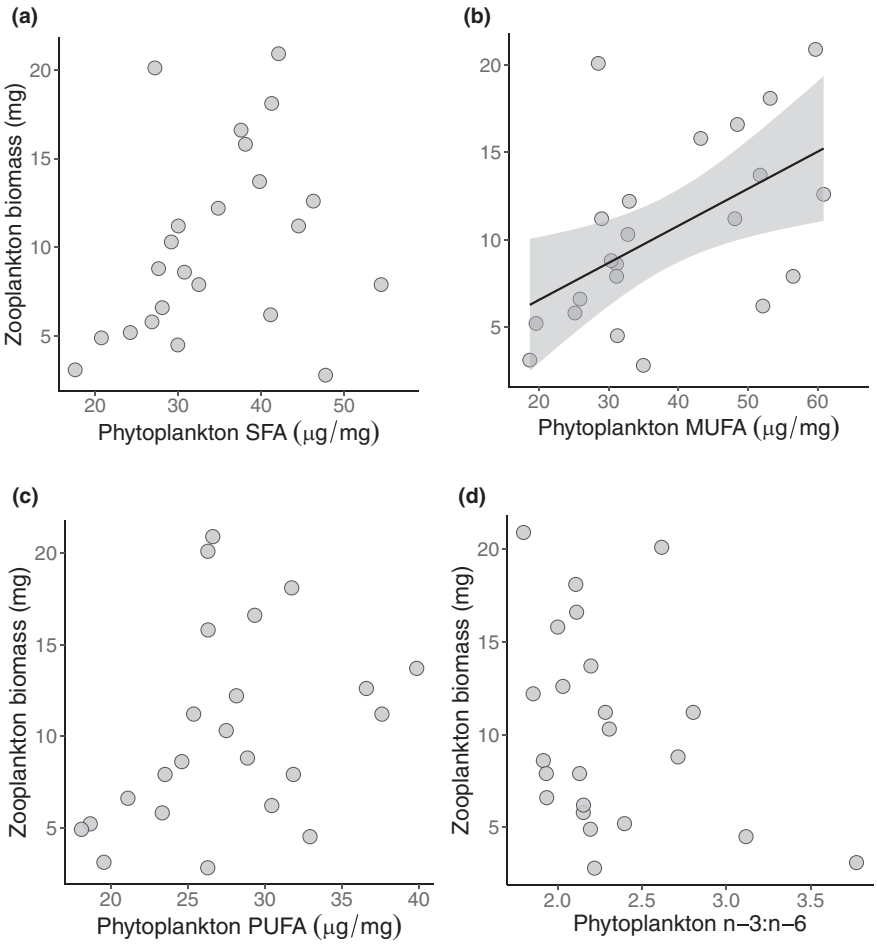

FIGURE 7 Relationships between phytoplankton (a) SFA, (b) MUFA, (c) PUFA and (d) n-3:n-6 ratio on total zooplankton community biomass. See Table 1 (column 4) for statistical analyses. The solid line is the line of best fit estimated from linear regression and the grey shading represents the 95% confidence interval. (No line = no statistically significant relationship).

vs. Figure S5c), the clear shift in n-3:n-6 in these two data sets (Figure 4d vs. Figure S5d) suggests that the types of PUFAs phytoplankton make are highly responsive to temperature.

Overall, we have documented that exposure to a heatwave had clear negative effects on phytoplankton-based nutrients. Reductions in key phytoplankton "quality" traits contributed to

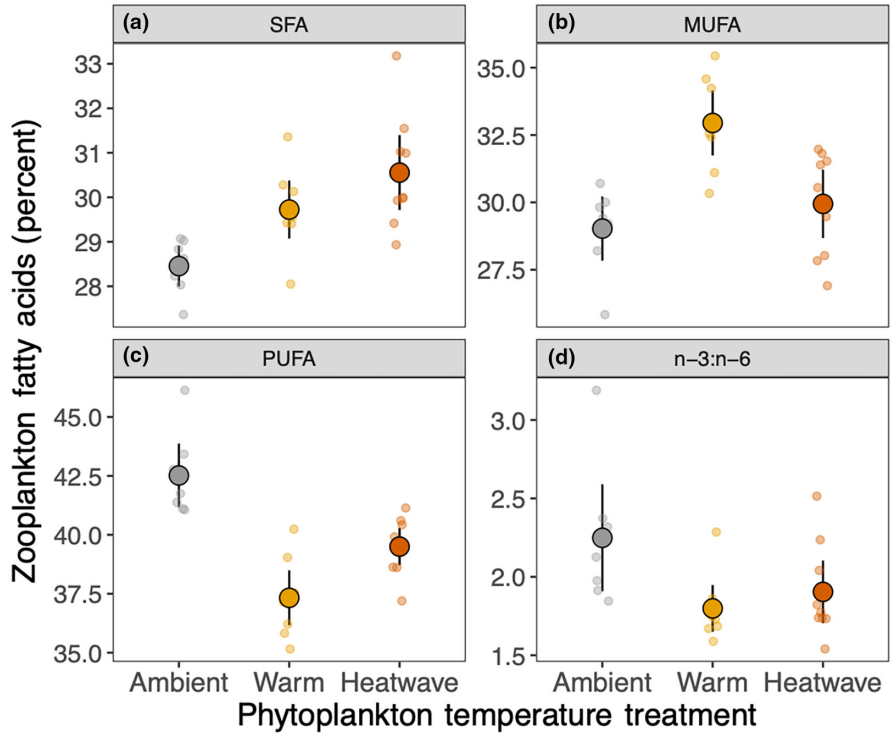

FIGURE 8 Effect of phytoplankton temperature treatment on zooplankton (a) SFA, (b) MUFA, and (c) PUFA percentages, and on (d) n-3:n-6 ratio. Large dots and error bars denote the mean ± 95% confidence intervals. (95% CI are masked by the larger dot in some cases.) Smaller dots denote individual replicates. The three temperature treatments are coloured grey (ambient), dark yellow (constant warming) and dark orange (heatwave). See Table 2 for ANOVA and posthoc test results.

TABLE 2 Summary results for ANOVA investigating the effect of phytoplankton food type on zooplankton fatty acid percentages (columns 1–2). Column 3 displays the Tukey HSD results for each corresponding ANOVA.

1. Zooplankton fatty acid	2. Effect of phytoplankton food type	3. Tukey HSD posthoc tests
SFA	$F_{2,21}=8.4$; $p=0.002$	HW > Ambient: $p=0.001$
MUFA	$F_{2,21}=10.24$; $p=0.0008$	Warm > Ambient: $p=0.001$ Warm > HW: $p=0.006$
PUFA	$F_{2,21}=20.6$; $p<0.0001$	HW < Ambient: $p=0.003$ Warm < Ambient: $p<0.0001$ Warm < HW: $p=0.02$
n-3:n-6	$F_{2,21}=3.67$; $p=0.04$	Warm < Ambient: $p=0.04$

Abbreviations: Ambient, "ambient" temperature treatment; HW, "heatwave" treatment; MUFA, monounsaturated fatty acids; n-3:n-6, the ratio of omega-3 PUFA to omega-6 PUFA; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; Warm, "constant warming" treatment.

overall decreases in zooplankton community biomass and shifts in zooplankton fatty acid profiles. Our study is innovative in that we have examined shifts in community-scale phytoplankton and zooplankton nutrients, but is limited by the fact that we did not also assay zooplankton communities across multiple temperature treatments, nor did we allow for natural dispersal into zooplankton replicates (as would occur in nature). We acknowledge that the existing design does not provide a complete picture of the effects of heatwaves at the zooplankton-phytoplankton interface, and we suggest that future experiments carefully examine both direct and indirect effects of heatwaves on plankton dynamics. Additionally, studies have shown that nutrient levels can significantly affect the responses of phytoplankton-based nutrients to temperature (Kosten et al., 2012; Schulhof et al., 2019; Verbeek et al., 2018). Given that rates of freshwater eutrophication are expected to increase concomitantly with climate warming (De Senerpont Domis et al., 2014; Hsieh et al., 2010), it would be informative for future studies to examine the combined effects of heatwaves and nutrient shifts on phytoplankton nutrients.

AUTHOR CONTRIBUTIONS

JOK: Idea conception, experimental design, data acquisition and analyses, and manuscript writing and editing. AD: data acquisition and manuscript editing. IF: data acquisition and manuscript editing. MT: Idea conception, experimental design, data analyses, manuscript writing and editing, and acquisition of funding.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Raw data are available in Dryad: https://doi.org/10.5061/dryad.q83bk3jqc.

STATEMENT ON INCLUSION

This is a laboratory experiment that was conducted on campus at the University of British Columbia, Vancouver, Canada. Fatty acid analysis was conducted in collaboration with the Department of Oceans and Fisheries in West Vancouver, British Columbia, Canada.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. List of phytoplankton morphospecies.

Table S2. List of fatty acids included in zooplankton and phytoplankton fatty acid analyses.

Table S3. Parameter estimates for linear models investigating the effects of phytoplankton food type on zooplankton traits, and for linear models investigating the effects of temperature treatment on Phytoplankton traits.

Table S4. Summary results for ANOVA investigating the effect of rearing temperature on phytoplankton traits (columns 1-2), and for linear regression analyses examining the relationship between each phytoplankton trait and zooplankton biomass (column 4).

Figure S1. Schematic diagram of the experiment timeline.

Figure S2. Effect of phytoplankton food type and experiment period on (a) total zooplankton, (b) zooplankton Shannon diversity (H') and (c) proportion of zooplankton taxa in each treatment group.

Figure S3. The effect of phytoplankton temperature treatment and experiment period on the proportion of phytoplankton morphospecies.

Figure S4. Effect of temperature treatment on concentrations of phytoplankton carbon, nitrogen, and phosphorus.

Figure S5. Effect of temperature treatment on phytoplankton fatty acid concentrations.

Figure S6. Relationships between concentrations of phytoplankton (a) carbon, (b) nitrogen, and (c) phosphorus and total zooplankton community biomass.

Figure S7. Relationships between phytoplankton (a) SFA, (b) MUFA, and (c) PUFA, and (d) n-3:n-6 ratio on total zooplankton community biomass.

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