Running title: Diet and temperature effects on *Daphnia*

Seasonal effects of food quality and temperature on body stoichiometry, biochemistry, and biomass production in *Daphnia* populations

Clay Prater[1†], Nicole D. Wagner[1], and Paul C. Frost[2]

[1] Environmental and Life Science Graduate Program, Trent University, Peterborough Ontario

[2] Department of Biology, Trent University, Peterborough Ontario

[†]contact information of corresponding author, mail to: prater.clay@gmail.com 705-748-1011 ext. 7676

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Abstract

Food quality and temperature can affect zooplankton production in lakes by altering organismal metabolism. However, the influence of these factors on consumer nutritional physiology and population biomass remains relatively understudied in natural populations. Here, we examined seasonal changes in body stoichiometry, biochemistry, and population biomass in two *Daphnia* species collected from two separate lakes differing in dietary phosphorus (P) supply. Food quality, measured as seston carbon:P (C:P) ratios, varied throughout the study in each lake, and water temperatures generally increased across the growing season. Daphnid elemental composition was correlated with food quality in both populations, but relationships between daphnid body stoichiometry and temperature were consistently stronger as *Daphnia* body C:P ratios and content of major biochemical pools declined simultaneously throughout the summer, which largely coincided with increased water temperatures. Warmer temperatures were associated with relaxed %P-RNA coupling as daphnid body RNA content declined and P content remained relatively high. These responses combined with temperature related decreases in *Daphnia* body %lipids and %C appeared to explain declines in daphnid body C:P ratios in both lakes over the growing season. Seasonal changes in population biomass were related to both food quality and water temperature in the lower nutrient lake. Biomass production under more eutrophic conditions however was unrelated to food quality and was instead associated with seasonal temperature changes in the higher nutrient lake. Overall, our study shows that seasonal changes in temperature and resource quality may differentially affect consumer stoichiometry and biomass production in lake ecosystems by altering consumer elemental metabolism.

Introduction

Freshwater zooplankton assemblages can show considerable phenological changes in biomass production in temperate lake ecosystems (Sommer et al. 1986; Pantel et al. 2014). These seasonal dynamics have traditionally been considered to be predominantly regulated by biological constraints such as predation and food quantity (McCauley & Kalff 1981; Carpenter et al. 1985; Sommer et al. 1986). In addition, recent studies have demonstrated the importance of elemental food *quality* in controlling zooplankton production through its effects on consumer nutritional physiology and community biomass (Elser et al. 1998, 2003; Hessen et al. 2005; Sommer et al. 2012). Experimental work has also found that food quality can interact with other temporally dynamic variables such as temperature to alter consumer growth and metabolic rates in laboratory environments (Makino et al. 2011; McFeeters and Frost 2011). Despite considerable seasonal differences in dietary elemental composition and temperature in temperate lakes (Kreeger et al. 1997; Hessen et al. 2005), their relative influence on consumer metabolism and population dynamics in natural assemblages remains poorly understood. Here, we examine how zooplankton consumer (*Daphnia spp.*) body stoichiometry, biochemistry, and population biomass relate to temporal changes in food quality and temperature in two different lake ecosystems.

Nutrient availability differs widely within and among aquatic habitats (Elser et al. 2000a; Sterner et al. 2008), and food elemental content is a well-known factor influencing consumer nutritional physiology and life-history (Frost et al. 2005; Wagner et al. 2013). Imbalances between the proportional supplies of key dietary elements like carbon (C), nitrogen (N), and phosphorus (P) and consumer metabolic demands can alter the synthesis of major macromolecules such as lipids, proteins, and nucleic acids, respectively (Elser et al. 1996;

Wagner et al. 2015). As these biochemical pools are tied to consumer elemental composition (Elser et al. 1996), poor food quality could indirectly affect consumer body stoichiometry by altering their elemental metabolism. Specifically, the growth hypothesis states that dietary P-limitation can slow the production of P-rich ribosomal RNA in animals and increase their body N:P ratios (Elser et al. 2003; Loladze and Elser 2011). These metabolic changes are further known to reduce individual growth, reproduction, and survivorship rates (Sterner et al. 1993; Frost et al. 2005), which suggests that poor food quality could ultimately lead to decreased biomass production in consumer populations (Loladze et al. 2000). Thus, elemental imbalances between producers and consumers represent potentially strong controls on aquatic food webs by regulating the proportion and amount of elements found within the consumer trophic level (Andersen 1997; Cebrian et al. 2009).

In addition to food quality, temperature also affects consumer nutrient metabolism. Within biologically relevant ranges, temperature drives exponential changes in organismal metabolic rates (Gillooly et al. 2001; Brown et al. 2004), which in turn influence animal biochemical composition and elemental content (Woods et al. 2003; Bullejos et al. 2014). For example, higher temperatures can reduce cellular RNA and P demands due to increased ribosomal translational efficiencies (Sievers et al. 2004; Toseland et al. 2013) and decrease body lipid stores by increasing C respiration (Evjemo et al. 2001; McFeeters and Frost 2011; Alcaraz et al. 2013) leading to proportional changes in consumer body C:P ratios. Further, by influencing consumer life-history trait expression and elemental composition, temperature can also affect population growth rates and regulate elemental flows through ecosystems (Petchey et al. 1999; Savage et al. 2004). In all, temperature and food quality play key roles in shaping consumer metabolism, and changes in these variables may have cascading effects on organismal life-

history, body stoichiometry, and population dynamics in aquatic ecosystems (Hessen et al. 2005; Cross et al. 2015).

In this study, we documented weekly variation in daphnid body elemental composition, gross biochemistry, and biomass production of two daphnid species (*D. pulicaria* and *D. mendotae*) collected from two separate lakes across a summer growing season. As these species show little overlap in our study region and are predominantly found in low and high P environments, respectively (Prater et al. 2017), we examined changes in each species independently. Food quality and temperature changed seasonally in each lake allowing us to compare the compare their relative effects on 1) daphnid elemental-biochemical relationships and 2) biomass production within each population. By focusing on two elements and their major molecular pools with well-known connections to daphnid nutritional physiology (P-RNA) and that account for the majority of consumer biomass (C-lipids), we provide *in situ* observations to better understand the effects of temperature and dietary nutrient supply on consumer elemental metabolism within the context of stoichiometric theory.

Methods

Study Sites. We sampled *Daphnia* populations from two lakes that are geographically close (~40 km apart) but are found in two distinct ecoregions in south central Ontario. Wolf Lake is located in the Kawartha Highland Provincial Park on the southern edge of the Canadian Shield where landuse is mostly forested with little to moderate shoreline development (Hicks and Frost 2011). Pigeon Lake is in the Kawartha Lakes region, which is located just south of the Canadian Shield. This area is characterized by significant agricultural landuse (~50%) and high lake shore residential development (Crins et al. 2009). These lakes were chosen due to their differences P supply and trophic state (Suppl. Table 1) as Wolf Lake is considered to be an oligo-mesotrophic

lake and Pigeon Lake is meso-eutrophic (sensu, Carlson 1977). In addition, each site is also inhabited by a different species of *Daphnia* (Pigeon: *D. mendotae* and Wolf: *D. pulicaria*, Prater et al. 2017).

Field Sampling. Lake sampling began immediately after ice off, which occurred 2 weeks earlier in Pigeon Lake than Wolf Lake. Lakes were sampled weekly from May through September for a total of 22 and 20 weeks, respectively. This time span roughly represents a normal growing season in many temperate regions of the northern hemisphere. Water samples for total phosphorus (TP) and seston analyses (stoichiometry and biomass) were collected at the surface of the water column and 1 m from bottom (8-10 m) using a Van Dorn sampler. These samples were poured into acid-washed 4 L carboys and transported back to the lab on ice. Quantitative *Daphnia* biomass samples were collected by taking fixed-depth vertical tows at these sites. These samples were rinsed into 500 ml plastic bottles and kept cool at ~4°C during transport. Temperature depth profiles were measured during each collection period (YSI Pro20, Yellow Springs, OH), and lakes were sampled at roughly the same time of the day (1000-1200h) to minimize the influence of diurnal temperature fluctuations.

Sample Processing and Preservation: In the laboratory, we saved whole water samples for TP analysis at 4°C until processing. We pre-filtered water samples for seston analysis with 80 µm mesh to remove inedible particles and then filtered the remaining suspended materials onto pre-ashed 0.7 µm GF/F glass fiber filters. Samples for stoichiometric analysis of surface and bottom samples (n=2 CN and n=2 P for each) were dried at 60°C and stored at 20°C, and chlorophyll a (Chl *a*, n=2 for each) was frozen and stored in the dark at -20°C until analysis. Between 5-10 daphnids were pooled into 5 separate samples for elemental analysis (n=5 CN and n=5 P analytical replicates). Animals were rinsed twice with deionized water, placed into pre-

140 weighed tins, dried at 60°C, and desiccated prior to weighing on a microbalance (±1 µg; Mettler-
141 Toledo, Markham, ON). For gross biochemical analysis, daphnids were also rinsed and saved in
142 separate 1.5 ml vials for each analysis. Lipid samples (10-20 pooled individuals, n=5 samples)
143 were immediately flash-frozen using liquid nitrogen, stored at minus -80°C, and lyophilized. For
144 RNA samples, we measured lengths of 10 individuals (from the top of the eyespot to the base of
145 the tail), placed each animal into a numbered vial, added 100 µl RNA-later (ThermoFisher,
146 Burlington, ON) to each vial, flash-froze all samples, and stored them at -80°C. Only live
147 animals were preserved for stoichiometric and biochemical analysis to prevent elemental
148 leaching and molecular degradation. Samples used to estimate daphnid biomass (n= 3 tows) were
149 divided using a zooplankton splitting wheel (n=2 analytical replicates for each tow) and were
150 preserved using a 4% sugar buffered formalin solution (Haney and Hall 1973).
151 *Elemental and biochemical analyses.* Seston and *Daphnia* C and N content were
152 measured on an elemental analyzer (Vario EL III, Elementar Inc. Mt. Laurel, NJ). Seston P,
153 water TP, and daphnid P content were measured after persulfate digestion through molybdate-
154 blue ascorbic acid colorimetry (APHA 1992) and absorbance spectroscopy (Cary-50, Varian,
155 Palo Alto, CA). We then used daphnid masses to calculate %C, N, and P for each animal and
156 converted all elemental ratios to molar ratios.
157 Prior to biochemical analyses we first weighed lyophilized *Daphnia* (lipids) or used
158 length/mass regressions (RNA, see below for details) to estimate total animal dry mass. All
159 biochemical analyses were then conducted using procedures from Wagner et al. (2015), and to
160 ensure proper extraction and analysis for all fractions, we included the same *D. magna* clone
161 used in that study as an internal control in each run. We analyzed total lipid content by first
162 homogenizing *Daphnia* tissues using a motorized pestle in 2:1 chloroform:methanol (*v/v*). Then,

we followed a sulfophosphovanillan (SPV) heat block procedure to extract the lipid fraction (Gardner et al. 1985). Standards were prepared by dissolving cholesterol in 2:1 chloroform: methanol (*v/v*), and samples and standards were analyzed with a spectrophotometer. Total nucleic acid content (DNA/RNA) was analyzed as described by Gorokhova et al. (2002). *Daphnia* were rinsed to remove residual RNA-later and homogenized in 200 µl of TE buffer. Then, we pippetted 50 µl of daphnid homogenate two 2 separate tubes, added 50 µl of 5 µg L^{-1} DNAse and RNase to separate tubes, and incubated them at 37°C for 15 min. We ran samples and RNA/DNA standards using a RiboGreen fluorometric analysis on a microplate reader (Synergy HT, Biotech, Winooski, VT). We divided total biochemical concentrations by total animal mass to calculate %RNA and %Lipids. We also estimated the proportion of daphnid body %P in the RNA pool (%P-RNA) by assuming a fixed P content (9%) for RNA (Elser et al. 2003; Acharya et al. 2004) and dividing RNA bound P by total body %P.

Daphnia biomass estimates. Daphnid biomass estimates were made with methods described in McCauley (1984). Briefly, we divided each tow replicate (n=3) into analytical subsamples (n=2), and for each subsample we counted individuals in 5 separate 1 ml samples on a Sedgewick-Rafter slide using a compound microscope. While counting, we also measured the body lengths of at least 25 individuals using digital photo software (iSolution, iMTechnology, Coquitlam, BC). Length-mass relationships for individual species from each lake were determined by growing field-caught *Daphnia* to different 0.1 mm size classes (n=10-20 per class) in the lab while feeding them lab cultured algae (*Scenedesmus Obliquus* Canadian Physiological Culture Centre strain 10). Pooled individuals for each size class were then dried at 60°C, desiccated, and weighed using a microbalance. We then used power functions to estimate the mass of each daphnid from length measurements (R^2= 0.96-0.98) and multiplied the mean

186 *Daphnia* mass by the total number of individuals found in each 1 ml sample to obtain a biomass
187 estimate for each subsample. Finally, we scaled these mass estimates up from the 1 ml samples
188 to the volume of water sampled in each tow ($\mu g\ L^{-1}$).

189 *Statistical Analyses.* Before conducting temporal analyses, we plotted temperature depth
190 profiles and top and bottom seston stoichiometry values for each lake. Pigeon Lake was well
191 mixed for most of the year, and seston C:P values were similar in top and bottom waters.
192 Therefore, we used integrated seston stoichiometry values and water column temperature
193 measurements in our subsequent data analyses. In contrast, Wolf Lake showed seasonal
194 stratification and had systematically higher C:P ratios in the top waters (Fig. 1A). As we could
195 not track daphnid diel migration patterns and thus could not determine their precise daily food
196 quality regimes, we analyzed relationships between daphnid response variables and top, bottom,
197 and integrated food C:P and measurements separately.

198 All other data were also visualized using scatter plots. Temperature and seston
199 stoichiometry were highly skewed due to our sampling regime, and we also detected non-linear
200 and non-monotonic trends in the data. As traditional parametric time-series analysis methods
201 were inappropriate, we estimated the strength of relationships between variables through a
202 distance correlation (dcor) approach using the 'energy' package in R (Rizzo and Szekely 2008;
203 Székely and Rizzo 2009). This technique is similar to other traditional non-parametric
204 correlational statistics such as Spearman's (ρ) or Kendall's (τ). However, distance correlation
205 does not assume monotonic relationships between variables, and the test statistic (D) is reported
206 from 0-1 with a value of 0 indicating a complete independence of two variables and values
207 approaching 1 indicating stronger correlations.

In general, daphnid correlations in Wolf Lake were more strongly related with top seston C:P values than with bottom values (Suppl. Table 2). Although correlation strength differed slightly between top and integrated measurements, relationships between these values and daphnid responses were qualitatively similar. Therefore, our inferences do not change using either measurement, and we report our Wolf Lake results using top seston C:P and temperature values to minimize the influence of bottom waters on our analyses.

Results

Seasonal changes in food quality, temperature, and Daphnia elemental content. Seston C:P ratios changed over the growing season and differed for most of the year between the two lakes (Fig. 1A&B). In general, seston stoichiometry in Wolf Lake was P poor and varied considerably over the summer (c.v.= 40%), whereas Pigeon Lake seston was P rich throughout the study and varied less (c.v.= 19%). Temperature regimes were similar in these lakes with a peak in temperature occurring in mid-July (Fig. 1 C&D). In both Wolf and Pigeon Lake, daphnid body C:P ratios were relatively more constrained than their food resources (c.v.= 10-12%) and declined steadily across the growing season (Fig. 1 E&F).

Irrespective of lake and species, *Daphnia* body elemental composition was more strongly correlated with temperature than food quality (Figs. 2&3). In Wolf Lake, *D. pulicaria* body %C and C:P ratios were negatively related to seston C:P ratios and temperature. In contrast, daphnid body %P was positively correlated with seston C:P and increased with higher seasonal temperatures. In Pigeon Lake, *D. mendotae* body %C was positively correlated to seston C:P ratios but declined precipitously at higher temperatures (Fig. 3 A&B). Daphnid body P content was not significantly related to seston C:P ratios and instead increased non-linearly with temperature (Fig. 3 C&D). Similar to body %C, *Daphnia* body C:P ratios were differentially

related to food quality and temperature with temperature effects showing relatively stronger correlations.

Correlations between temperature, Daphnia biochemistry, and body stoichiometry. Temperature effects on daphnid body stoichiometry seemed to be mediated by changes in their biochemical and elemental metabolism. In both species, body %RNA declined with higher seasonal temperatures (Fig. 4 A&B), but body %P remained relatively high (~1.2-1.6%) resulting in weak correlations between daphnid body %P and RNA in *Daphnia* from both Wolf and Pigeon Lakes (Fig. 4C&D). These temperature related metabolic changes appeared to alter organismal P investment into RNA production (Fig. 4E&F) as reduced %P-RNA ratios corresponded with lower body C:P ratios in each study population. Similar to %RNA, body lipid content also decreased with higher seasonal temperatures in both daphnid species (Fig. 5A&B). However, unlike %P-RNA relationships, daphnid body %lipid was more strongly related to body C content (Fig. 5C&D), and reduced lipid stores corresponded with lower *Daphnia* body C:P ratios in each population (Fig. 5E&F).

Relationships between Daphnia biomass, food quality, and temperature. In Wolf Lake, daphnid biomass displayed a large population increase soon after ice-off, which was quickly followed by a rapid population decline (Fig. 6A). Population biomass remained near zero during the middle of the growing season but was reestablished to moderate levels in the later summer months. *Daphnia* biomass in this low P lake was related to both food quality and temperature with the highest biomass occurring at low seston C:P ratios and moderate temperatures (Fig. 6 C&E). In the more eutrophic Pigeon Lake, daphnid biomass also showed a large early season spike, but biomass quickly decreased and remained low afterwards for the remainder of the study (Fig. 6B). Biomass production was not significantly related to seston C:P ratios in this lake

(Figure 6D) where daphnid biomass was instead correlated with temperature and peaked at moderate temperatures (Figure 6F).

Discussion

In each study lake, *Daphnia* elemental composition was related to seasonal changes in both food quality and temperature. However, we found negative correlations between seston C:P and daphnid C:P in Wolf Lake and weak relationships between these variables in Pigeon Lake suggesting that daphnid stoichiometry was poorly related to food quality overall. Instead, temperature appeared to more strongly alter *Daphnia* elemental composition as body C:P declined with higher summer temperatures. These changes were consistent with temperature effects on daphnid biochemical pools as higher temperatures were associated with relaxed coupling between body P and RNA content and reduced C-rich lipid stores. Although temperature seemed to be mostly responsible for driving seasonal variation in *Daphnia* stoichiometry, both temperature and food quality were related to total biomass production in study lakes.

Seston and *Daphnia* stoichiometry varied seasonally within each lake. As in other studies, we observed phenological changes in seston C:P ratios (Kreeger et al. 1997; Hessen et al. 2005), which fell within previously documented measurements (C:P 100-800; Elser et al. 2000a; Sterner et al. 2008). Fine-scale (weekly) variation was also high in the low P Wolf Lake due to differences in seston stoichiometry between the epi- and hypolimnion. While *Daphnia pulicaria* body stoichiometry also changed temporally in Wolf Lake, their body stoichiometry seemed to decouple from seston C:P as daphnid and seston C:P ratios were negatively correlated across the growing season. Stoichiometric food quality was high for the entire study period in Pigeon Lake (C:P <200) where *Daphnia mendotae* body C:P ratios were positively correlated

with seston C:P, similar to patterns observed in other temperate lakes (DeMott et al. 2004). However, this relationship was not as strong in our study due to extensive stoichiometric variation in this taxon. Together, the decoupling of seston C:P and daphnid C:P in Wolf Lake and weak relationships in Pigeon Lake suggest that food quality likely played a minor role in shaping *Daphnia* body stoichiometry in both populations. Instead, seasonal declines in daphnid C:P appeared to be more connected to temperature effects on daphnid elemental composition and biochemistry.

Temperature was strongly related to daphnid P and RNA content in field-caught animals. Seasonal temperature increases were associated with linear increases in *D. pulicaria* body %P in Wolf Lake and non-linear responses in *D. mendotae* from Pigeon Lake. Our results resemble those from a previous laboratory experiment showing species differences in body %P across temperature gradients (McFeeters and Frost 2011). But unlike this study, daphnid responses in our lakes appeared to be mostly independent from food quality effects, suggesting that organismal responses to temperature in natural populations are likely to be both context and species dependent (Bullejos et al. 2014; Moody et al. 2017). Body RNA content declined in both of our study species with increased temperatures, which is consistent with adaptive physiological thermal responses commonly observed across many taxa in the wild (Woods et al., 2003). However, relationships between daphnid body %P and %RNA were weak for *D. mendotae* and were even negative in *D. pulicaria* suggesting that temperature unexpectedly modified consumer nutrient metabolism in our lakes.

Investment of P into *Daphnia* RNA pools declined substantially with higher seasonal temperatures. This observation contrasts with many studies that have found consistent positive relationships between organismal body %P and %RNA (Elser et al., 2000; Bullejos et al., 2014;

300 Zhang et al., 2016). Our results could thus at first glance seem to contradict the central premise
301 of the growth rate hypothesis. However, this hypothesis as currently formulated is most
302 applicable to consumers experiencing P-limitation and growing at the same temperature (Elser et
303 al. 2003; Moody et al. 2017). Since relaxed coupling of daphnid body %P and %RNA has been
304 documented outside of these narrow set of conditions (Elser et al. 2003; Acharya et al. 2004;
305 Wagner et al. 2015), temperature mediated changes in elemental-biochemical coupling may
306 explain the weak relationships between *Daphnia* C:P and seston C:P in our study. As a majority
307 of consumer body %P is thought to be associated with ribosomal RNA (Elser et al. 1996), it
308 remains unclear how *Daphnia* in our study maintained a high body %P despite exhibiting
309 reduced body RNA content. We can eliminate the possibility of increased investment into DNA
310 since it was a relatively small component of daphnid biomass (<0.4%; Suppl. Fig. 1A&B). As
311 we did not measure additional P pools (e.g., phosphosugars, phospholipids), more work is
312 required to identify the molecular form of the remaining unaccounted-for body %P. These
313 studies should include other important elemental-biochemical relationships, such as body %N-
314 protein content, which also seemed to be temperature dependent in our populations (Suppl. Fig.
315 1C&D). Understanding how consumers regulate their nutrient metabolism across temperature
316 gradients is clearly an important step towards the further integration of temperature effects into
317 stoichiometric theory.

318 Seasonal changes in water temperature were also related to daphnid body C and lipid
319 content. In both populations, we saw sharp declines in daphnid body %C, which corresponded to
320 a reduction of ~10-15% of their total body dry mass at higher temperatures. These changes were
321 likely due to elevated metabolic rates (Darchambeau et al. 2003; McFeeters and Frost 2011),
322 which have been shown to decrease *Daphnia* body lipid and C content (Zhang et al. 2016). We

provide further support for this mechanism as we observed synchronous declines in C-rich *Daphnia* body lipid stores and C:P ratios with higher seasonal temperatures in these two ecologically distinct species. These changes along with altered P metabolism provide a likely explanation for declines in *Daphnia* body C:P ratios across the growing season and highlight the important role of temperature in shaping consumer elemental composition in field assemblages (Bullejos et al. 2014).

Although temperature was strongly related to *Daphnia* stoichiometry in our study, it is necessary to consider temperature effects within the hierarchy of other factors potentially affecting animal C:P ratios in nature. Consumer body stoichiometry reflects the influence of a number of environmental and biological factors that operate simultaneously across spatial and temporal scales (Cherif et al. 2017). Within individuals (level-1; L1), consumer body stoichiometry is proximately controlled by the biochemical/elemental content of its subcellular components and body tissues (Elser et al. 1996). For instance, differences in the elemental content of somatic vs. reproductive tissues such as eggs can alter daphnid body stoichiometry and account for size-specific differences across developmental stages (Ventura and Catalan 2005; Frost et al. 2008). At an environmental-level (L2), variables that affect consumer physiology, life-history, or behavior can alter the intake and investment of dietary elements at L1 (Frost et al. 2005). In addition to food quality, this list includes a suite of abiotic variables (e.g., light and CO_2,), biotic factors such as food quantity and algal taxonomic composition, and food web dependent factors such as predation and parasitism (Dickman et al. 2008; Yamamichi et al. 2015). Finally, organismal stoichiometry is shaped by the evolutionary history (L3) of a given taxon, which can influence both immediate responses of organisms to environmental conditions (i.e., elemental plasticity) and shape species and population differences through space and time

(Elser et al. 2000b; Frisch et al. 2014; Prater et al. 2017). As our study examined the seasonal effects of temperature and food quality (L2) on organismal stoichiometry (L1) of two separate species (L3) in complex natural environments, we are unable to fully differentiate among the effects of all of these factors and their interactions. Nevertheless, our results suggest that temperature is likely to be an important variable controlling organismal elemental content in field populations, despite the possible roles of other factors, as it accounted for a substantial amount of variation in daphnid stoichiometry in both study lakes.

Both temperature and stoichiometric food quality appeared to influence *Daphnia* biomass production in our study lakes. Biomass in the lower nutrient Wolf Lake was correlated with both food quality and temperature and was highest at low food C:P ratios and moderate temperatures, which occurred in the early spring and fall. Thus, although temperature seemed to predominantly control daphnid stoichiometry in this lake, nutrient availability represents an important factor determining zooplankton production and likely interacts with temperature to influence seasonal patterns in *Daphnia* biomass in oligo- and mesotrophic systems (Elser et al. 1998; Makino et al. 2002). *Daphnia* biomass was not related to food quality in Pigeon Lake where seston was P-rich year-round. Instead, biomass peaked at moderate temperatures early in the year and remained low for the remainder of the growing season. We were unable to quantify predation pressure in our study, which could have influenced seasonal variation in Pigeon Lake biomass. Similarly, we did not measure differences in algal taxonomic composition, but daphnid biomass remained low despite high food quantities and was negatively related to algal biomass (Suppl. Fig 2). As cyanobacteria blooms can develop in the mid-summer and persist throughout the growing season in Pigeon Lake, it is possible that either feeding inhibition (Abrams and Walters 1996; DeMott et al. 2001) or reduced growth and reproductive rates due to fatty acid-limitation (Ravet et al.,

2012; Ger et al., 2016) could explain failed *Daphnia* recruitment following the spring die off. If true, our results suggest that food quality effects on daphnid nutritional physiology and biomass production may act along a continuum controlled by dietary elemental stoichiometry in oligotrophic systems and switching to physical and/or biochemical regulation under more eutrophic conditions.

In this study, we documented complex relationships between seston food C:P ratios and temperature and consumer elemental metabolism and biomass production over a summer growing season in two separate lake ecosystems. While the correlational nature of our study necessarily limits the strength and breadth of our conclusions, we provide observational evidence that seasonal temperature changes were likely responsible for decoupling producer-consumer stoichiometry and altering consumer elemental-biochemical investment in natural populations. These observations provide important insights for stoichiometric theory as they might partially explain contrasting responses to elemental limitation among species adapted to different habitats (Bullejos et al., 2014; Zhang et al., 2016) and could account for the weak relationships sometimes found between consumer biochemistry and elemental composition (Wilder and Jeyasingh 2016). However, metabolic changes in our populations did not translate into straight-forward predictable biomass responses in either lake highlighting current theoretical limitations in linking organismal-level physiology and life-history to higher-order ecological processes (Cherif et al. 2017). Moving ahead, careful laboratory studies in conjunction with manipulative field-based experiments are needed to better understand these cross-scale dynamics while controlling for and estimating the relative influence of other important ecological factors. These studies will allow for temperature effects on consumer metabolic physiology to be more fully integrated into existing stoichiometric models (e.g., Cross et al. 2015) to better predict how

consumer population dynamics and ecosystem functions may change under increasingly variable climatic conditions occurring across the planet.

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Figure Captions

Fig 1. Seasonal variation in seston carbon:phosphorus (C:P) ratios, lake temperature, and *Daphnia* body C:P ratios. Weekly means ± standard error are plotted for C:P ratios. Top (white) and bottom (light grey) seston C:P values and top water column temperature values are shown for Wolf Lake. Water column integrated seston C:P and temperature values are displayed for Pigeon Lake (dark grey).

Fig 2. Changes in *Daphnia* body elemental composition across seston stoichiometry and temperature gradients in Wolf Lake. Distance correlations are reported for: A) seston carbon:phosphorus (C:P) ratios and daphnid body %C, B) temperature and daphnid body %C, C) seston C:P ratios and daphnid body %P, D) temperature and daphnid body %P, E) seston C:P ratios and daphnid body C:P ratios, and F) temperature and daphnid body C:P ratios. *P*-values and correlation coefficients (D) are reported for each correlation.

Fig 3. Changes in *Daphnia* body elemental composition across seston stoichiometry and temperature gradients in Pigeon Lake. Distance correlations are reported for: A) seston carbon:phosphorus (C:P) ratios and daphnid body %C, B) temperature and daphnid body %C, C) seston C:P ratios and daphnid body %P, D) temperature and daphnid body %P, E) seston C:P ratios and daphnid body C:P ratios, and F) temperature and daphnid body C:P ratios. *P*-values and correlation coefficients (D) are reported for each correlation.

Fig 4. Correlations between temperature, *Daphnia* body RNA content, and body elemental composition. Distance correlations are shown for: A&B) temperature and daphnid body RNA content, C&D) daphnid body phosphorus (%P) content and body %RNA, and E&F) daphnid body C:P ratios and the proportion of body P bound in RNA (%P-RNA). *P*-values and

correlation coefficients (D) are reported separately for each lake. Wolf Lake values are shown in white, and Pigeon Lake values are displayed in grey.

Fig 5. Correlations between temperature, *Daphnia* body lipid content, and body elemental composition. Distance correlations are shown for: A&B) temperature and daphnid body %lipid, C&D) daphnid body carbon (%C) content and body %lipid, and E&F) daphnid body C:phosphorus (P) ratios and body %lipid. *P*-values and correlation coefficients (D) are reported separately for each lake. Wolf Lake values are shown in white, and Pigeon Lake values are displayed in grey.

Fig 6. Changes in *Daphnia* biomass across the growing season and correlations between seston nutrient content, temperature, and daphnid biomass production. Weekly means ± standard error are plotted for seasonal changes (A&B). Scatterplots and distance correlations are shown for: C&D) seston carbon:phosphorus (C:P) ratios and daphnid biomass and between E&F) temperature and daphnid biomass. *P*-values and correlation coefficients (D) are reported separately for each lake. Wolf Lake values are shown in white, and Pigeon Lake values are displayed in grey.

Figure 1.

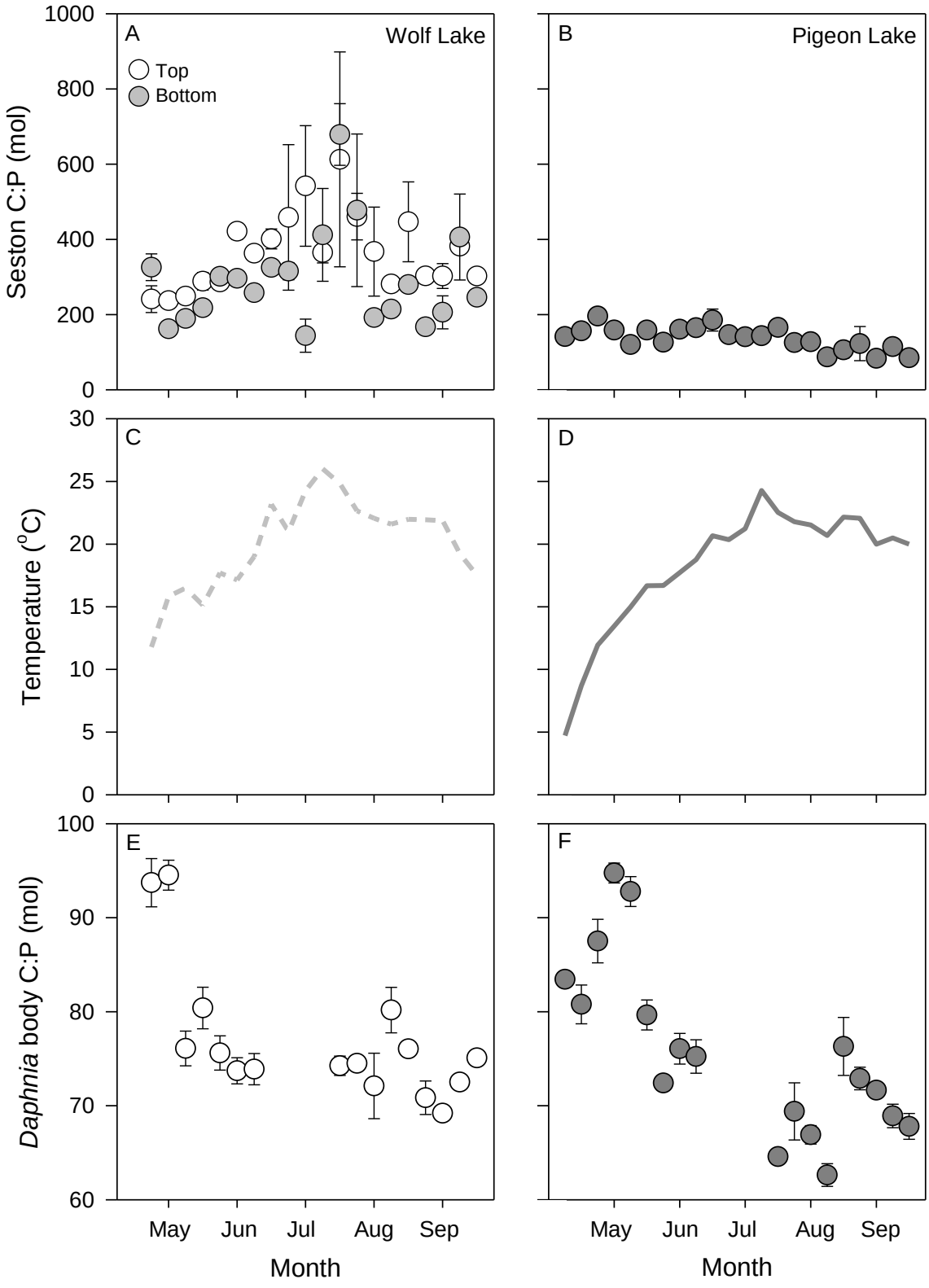

A
Wolf Lake
Top
Bottom
B
Pigeon Lake
C
D
E
F
Seston C:P (mol)
Temperature (°C)
Daphnia body C:P (mol)
1000
800
600
400
200
0
30
25
20
15
10
5
0
100
90
80
70
60
May
Jun
Jul
Aug
Sep
Month

Figure 2.

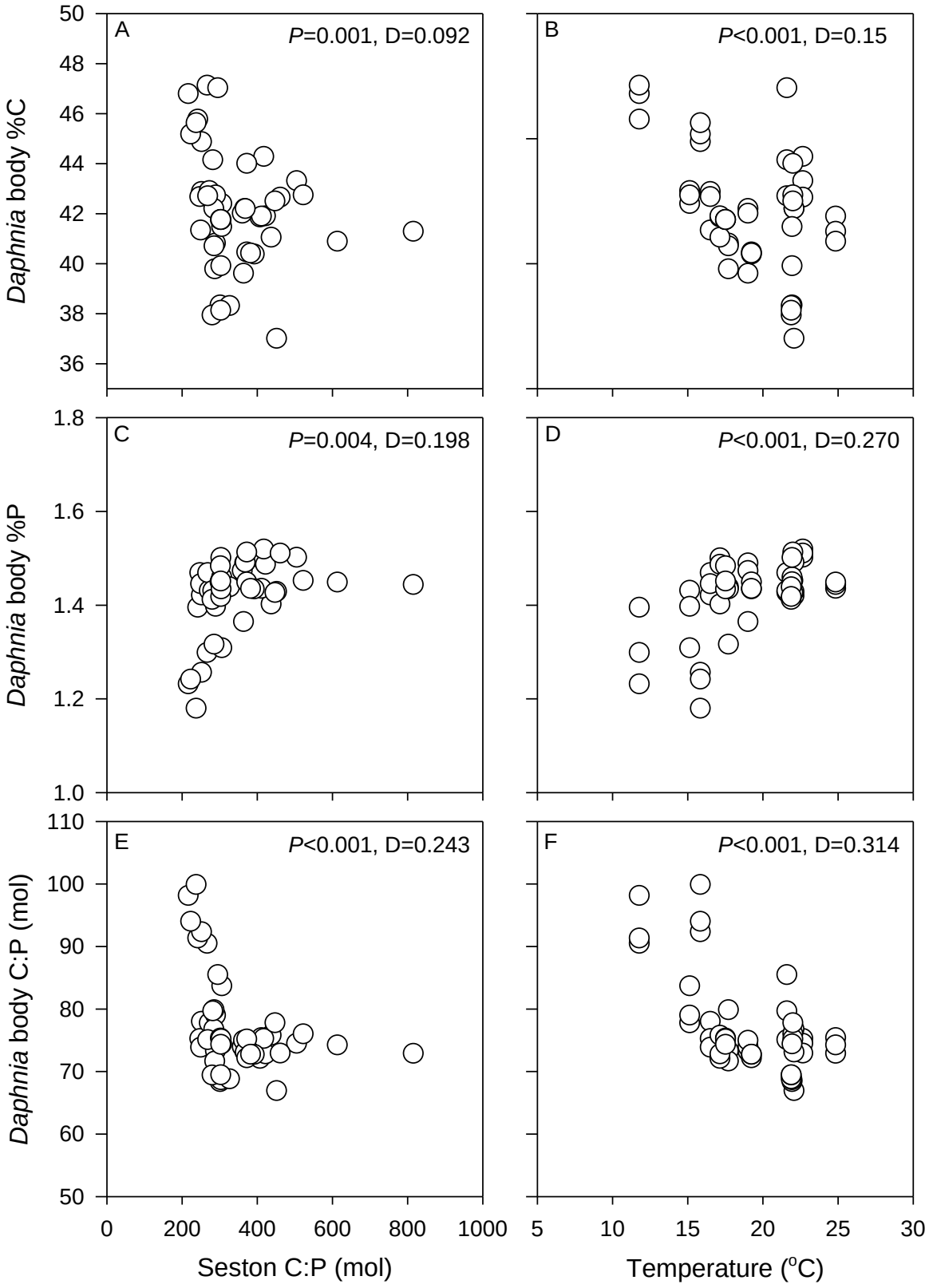

A
P=0.001, D=0.092
B
P<0.001, D=0.15
C
P=0.004, D=0.198
D
P<0.001, D=0.270
E
P<0.001, D=0.243
F
P<0.001, D=0.314
Daphnia body %C
Daphnia body %P
Daphnia body C:P (mol)
Seston C:P (mol)
Temperature (°C)

Figure 3.

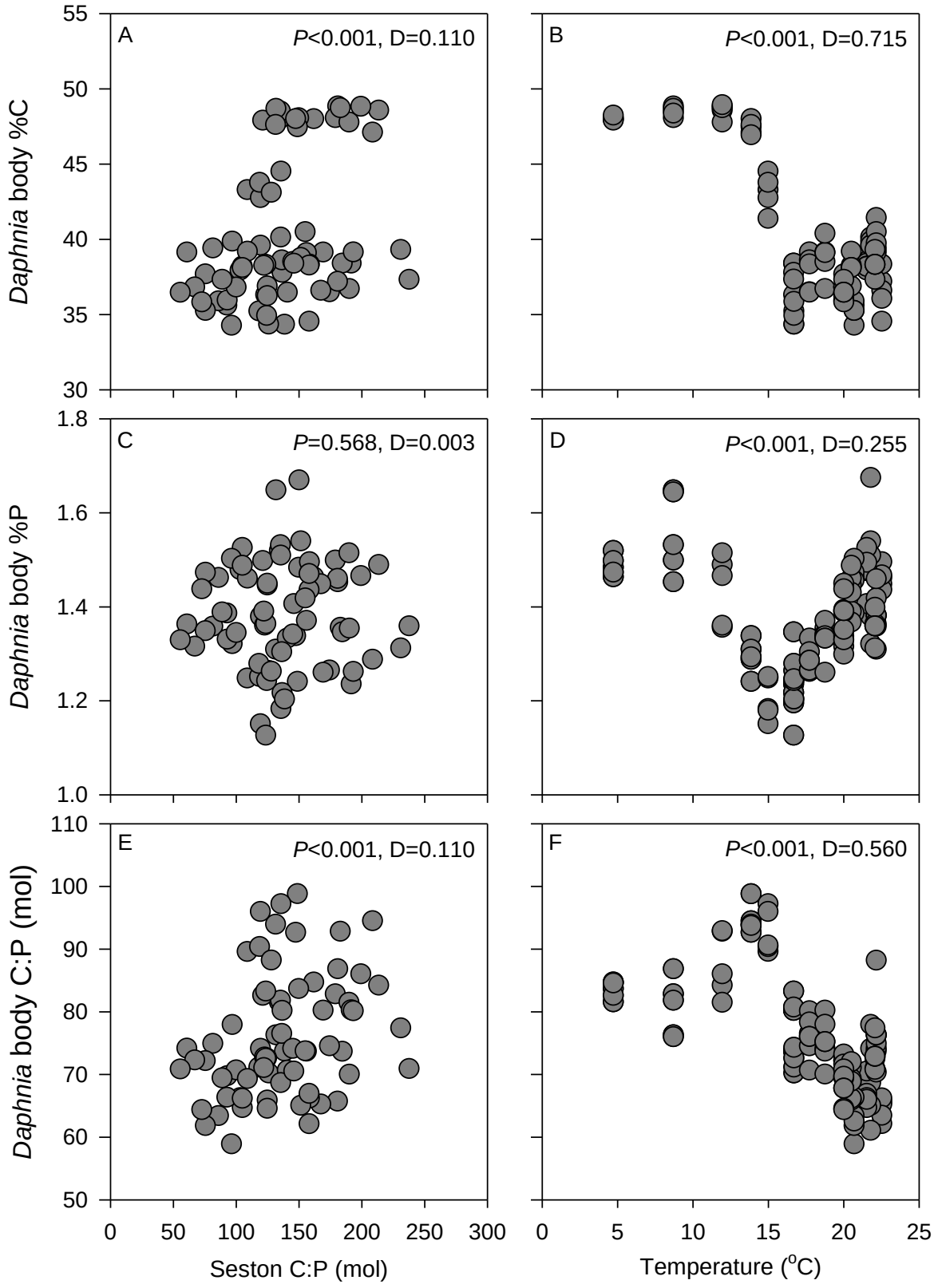

A
P<0.001, D=0.110
B
P<0.001, D=0.715
C
P=0.568, D=0.003
D
P<0.001, D=0.255
E
P<0.001, D=0.110
F
P<0.001, D=0.560
Daphnia body %C
Daphnia body %P
Daphnia body C:P (mol)
Seston C:P (mol)
Temperature (°C)

Figure 4.

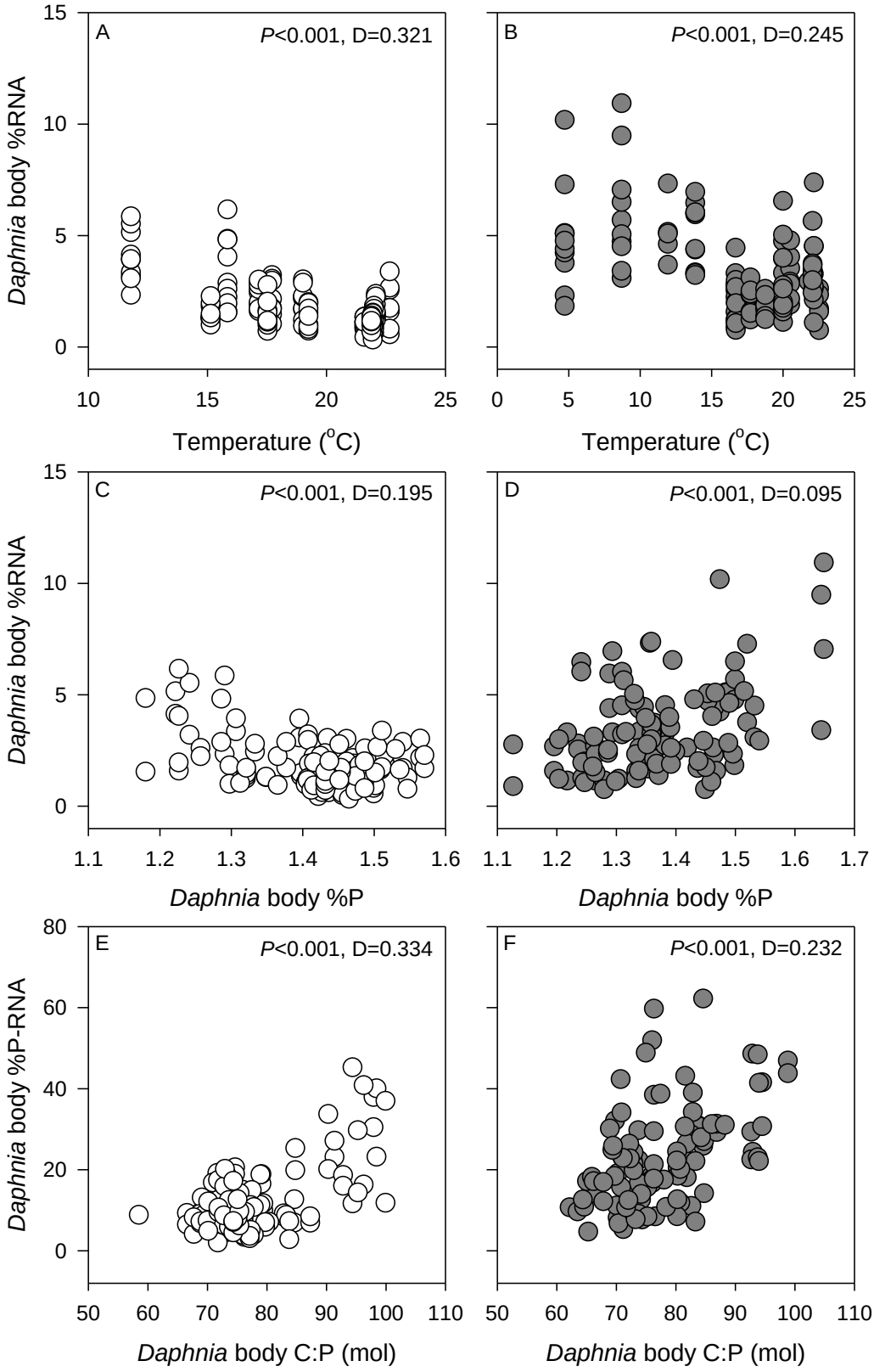

A
P<0.001, D=0.321
B
P<0.001, D=0.245
C
P<0.001, D=0.195
D
P<0.001, D=0.095
E
P<0.001, D=0.334
F
P<0.001, D=0.232
Daphnia body %RNA
Daphnia body %P-RNA
Temperature (°C)
Daphnia body %P
Daphnia body C:P (mol)

Figure 5

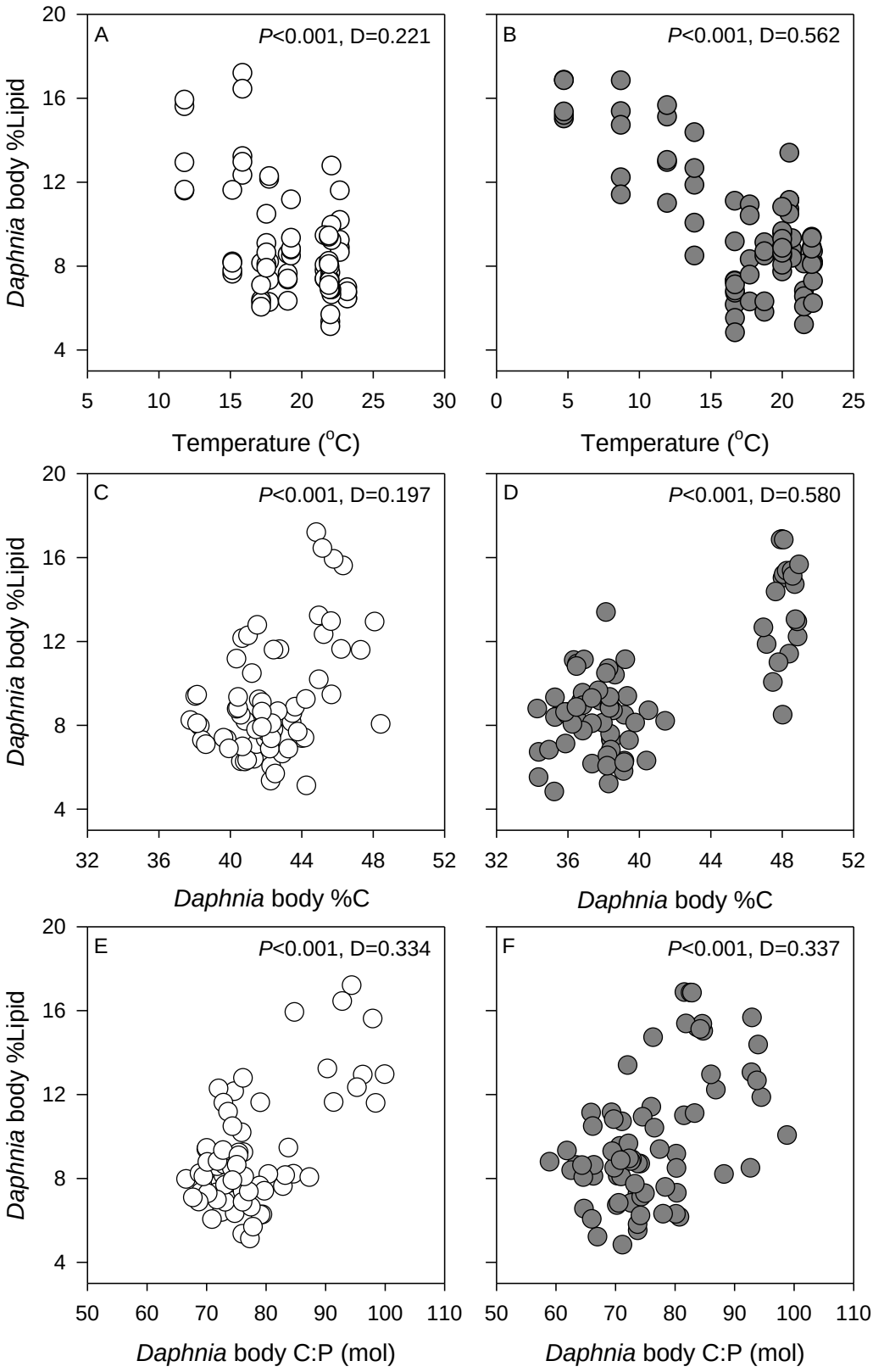
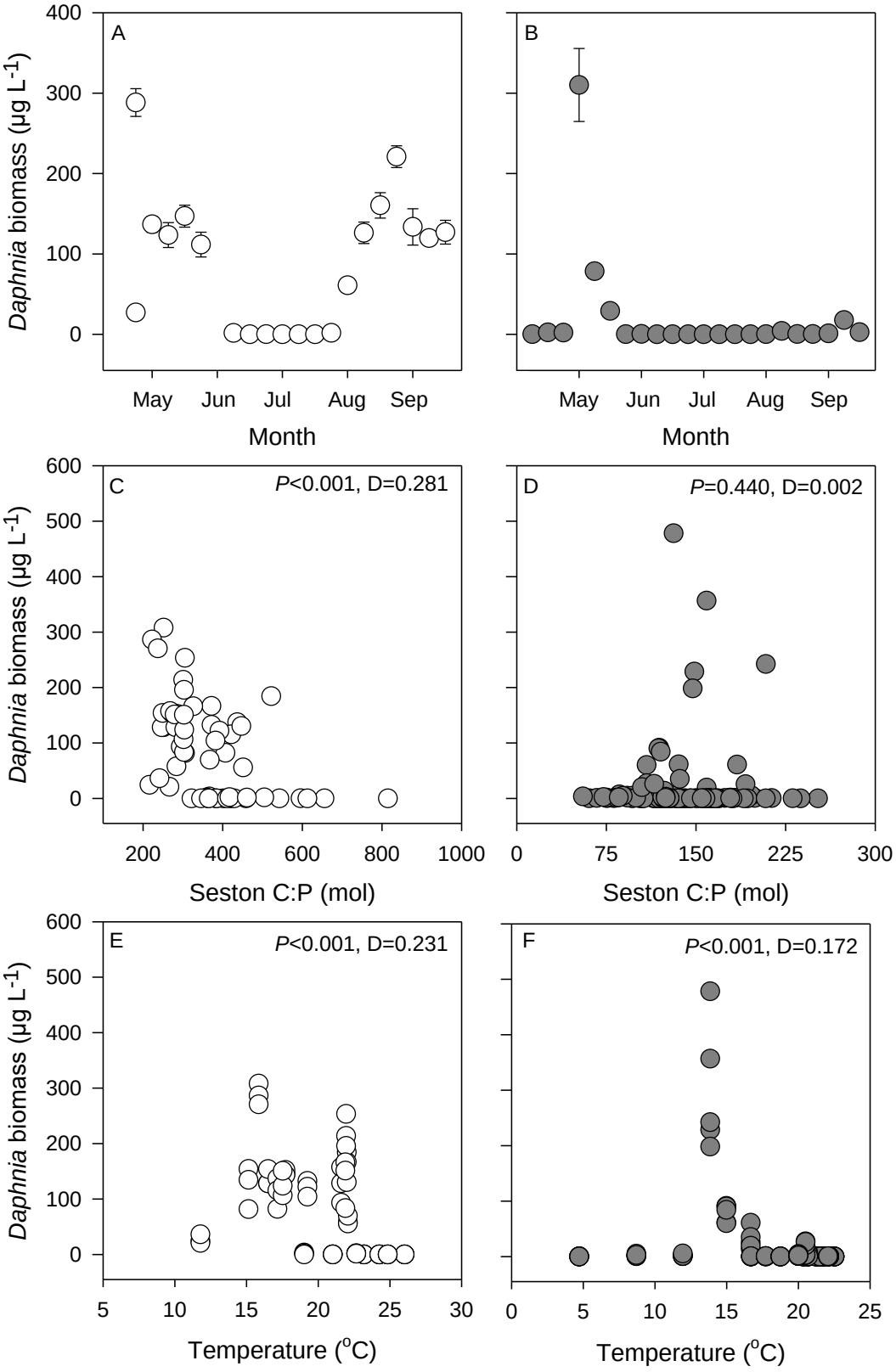

A
P<0.001, D=0.221
B
P<0.001, D=0.562
C
P<0.001, D=0.197
D
P<0.001, D=0.580
E
P<0.001, D=0.334
F
P<0.001, D=0.337
Daphnia body %Lipid
Temperature (°C)
Daphnia body %C
Daphnia body C:P (mol)

Figure 6.


A
B
C
D
E
F
Daphnia biomass (µg L-1)
Month
May
Jun
Jul
Aug
Sep
Seston C:P (mol)
P<0.001, D=0.281
P=0.440, D=0.002
P<0.001, D=0.231
P<0.001, D=0.172
Temperature (°C)