

1 Running title: Diet and temperature effects on *Daphnia*

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

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8 Clay Prater^{1†}, Nicole D. Wagner¹, and Paul C. Frost²

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

12 ² Department of Biology, Trent University, Peterborough Ontario

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

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17 Author Contributions: CP, NDW, and PCF conceived and designed the experiments. NDW and

18 CP collected and processed samples. CP and PCF analyzed the data and wrote the manuscript.

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25 **Abstract**

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

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48 **Introduction**

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

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

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

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

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

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

108 **Methods**

109 *Study Sites.* We sampled *Daphnia* populations from two lakes that are geographically
110 close (~40 km apart) but are found in two distinct ecoregions in south central Ontario. Wolf Lake
111 is located in the Kawartha Highland Provincial Park on the southern edge of the Canadian Shield
112 where landuse is mostly forested with little to moderate shoreline development (Hicks and Frost
113 2011). Pigeon Lake is in the Kawartha Lakes region, which is located just south of the Canadian
114 Shield. This area is characterized by significant agricultural landuse (~50%) and high lake shore
115 residential development (Crins et al. 2009). These lakes were chosen due to their differences P
116 supply and trophic state (Suppl. Table 1) as Wolf Lake is considered to be an oligo-mesotrophic

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

120 *Field Sampling.* Lake sampling began immediately after ice off, which occurred 2 weeks
121 earlier in Pigeon Lake than Wolf Lake. Lakes were sampled weekly from May through
122 September for a total of 22 and 20 weeks, respectively. This time span roughly represents a
123 normal growing season in many temperate regions of the northern hemisphere. Water samples
124 for total phosphorus (TP) and seston analyses (stoichiometry and biomass) were collected at the
125 surface of the water column and 1 m from bottom (8-10 m) using a Van Dorn sampler. These
126 samples were poured into acid-washed 4 L carboys and transported back to the lab on ice.

127 Quantitative *Daphnia* biomass samples were collected by taking fixed-depth vertical tows at
128 these sites. These samples were rinsed into 500 ml plastic bottles and kept cool at ~4°C during
129 transport. Temperature depth profiles were measured during each collection period (YSI Pro20,
130 Yellow Springs, OH), and lakes were sampled at roughly the same time of the day (1000-1200h)
131 to minimize the influence of diurnal temperature fluctuations.

132 *Sample Processing and Preservation:* In the laboratory, we saved whole water samples
133 for TP analysis at 4°C until processing. We pre-filtered water samples for seston analysis with 80
134 µm mesh to remove inedible particles and then filtered the remaining suspended materials onto
135 pre-ashed 0.7 µm GF/F glass fiber filters. Samples for stoichiometric analysis of surface and
136 bottom samples (n=2 CN and n=2 P for each) were dried at 60°C and stored at 20°C, and
137 chlorophyll a (Chl *a*, n=2 for each) was frozen and stored in the dark at -20°C until analysis.
138 Between 5-10 daphnids were pooled into 5 separate samples for elemental analysis (n=5 CN and
139 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 ($\pm 1 \mu\text{g}$; 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,

163 we followed a sulfophosphovanillan (SPV) heat block procedure to extract the lipid fraction
164 (Gardner et al. 1985). Standards were prepared by dissolving cholesterol in 2:1 chloroform:
165 methanol (v/v), and samples and standards were analyzed with a spectrophotometer. Total
166 nucleic acid content (DNA/RNA) was analyzed as described by Gorokhova et al. (2002).
167 *Daphnia* were rinsed to remove residual RNA-later and homogenized in 200 μ l of TE buffer.
168 Then, we pipetted 50 μ l of daphnid homogenate into two separate tubes, added 50 μ l of 5 μ g L⁻¹
169 DNase and RNase to separate tubes, and incubated them at 37°C for 15 min. We ran samples
170 and RNA/DNA standards using a RiboGreen fluorometric analysis on a microplate reader
171 (Synergy HT, Biotech, Winooski, VT). We divided total biochemical concentrations by total
172 animal mass to calculate %RNA and %Lipids. We also estimated the proportion of daphnid body
173 %P in the RNA pool (%P-RNA) by assuming a fixed P content (9%) for RNA (Elser et al. 2003;
174 Acharya et al. 2004) and dividing RNA bound P by total body %P.

175 *Daphnia biomass estimates.* Daphnid biomass estimates were made with methods
176 described in McCauley (1984). Briefly, we divided each tow replicate (n=3) into analytical
177 subsamples (n=2), and for each subsample we counted individuals in 5 separate 1 ml samples on
178 a Sedgewick-Rafter slide using a compound microscope. While counting, we also measured the
179 body lengths of at least 25 individuals using digital photo software (iSolution, iMTechnology,
180 Coquitlam, BC). Length-mass relationships for individual species from each lake were
181 determined by growing field-caught *Daphnia* to different 0.1 mm size classes (n=10-20 per
182 class) in the lab while feeding them lab cultured algae (*Scenedesmus Obliquus* Canadian
183 Physiological Culture Centre strain 10). Pooled individuals for each size class were then dried at
184 60°C, desiccated, and weighed using a microbalance. We then used power functions to estimate
185 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 ($\mu\text{g L}^{-1}$).

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 Székely 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.

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

214 **Results**

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

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

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

233 *Correlations between temperature, Daphnia biochemistry, and body stoichiometry.*

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

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

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

256 **Discussion**

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

268 Seston and *Daphnia* stoichiometry varied seasonally within each lake. As in other
269 studies, we observed phenological changes in seston C:P ratios (Kreeger et al. 1997; Hessen et
270 al. 2005), which fell within previously documented measurements (C:P 100-800; Elser et al.
271 2000a; Sterner et al. 2008). Fine-scale (weekly) variation was also high in the low P Wolf Lake
272 due to differences in seston stoichiometry between the epi- and hypolimnion. While *Daphnia*
273 *pulicaria* body stoichiometry also changed temporally in Wolf Lake, their body stoichiometry
274 seemed to decouple from seston C:P as daphnid and seston C:P ratios were negatively correlated
275 across the growing season. Stoichiometric food quality was high for the entire study period in
276 Pigeon Lake (C:P <200) where *Daphnia mendotae* body C:P ratios were positively correlated

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

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

297 Investment of P into *Daphnia* RNA pools declined substantially with higher seasonal
298 temperatures. This observation contrasts with many studies that have found consistent positive
299 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

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

329 Although temperature was strongly related to *Daphnia* stoichiometry in our study, it is
330 necessary to consider temperature effects within the hierarchy of other factors potentially
331 affecting animal C:P ratios in nature. Consumer body stoichiometry reflects the influence of a
332 number of environmental and biological factors that operate simultaneously across spatial and
333 temporal scales (Cherif et al. 2017). Within individuals (level-1; L1), consumer body
334 stoichiometry is proximately controlled by the biochemical/elemental content of its subcellular
335 components and body tissues (Elser et al. 1996). For instance, differences in the elemental
336 content of somatic vs. reproductive tissues such as eggs can alter daphnid body stoichiometry
337 and account for size-specific differences across developmental stages (Ventura and Catalan
338 2005; Frost et al. 2008). At an environmental-level (L2), variables that affect consumer
339 physiology, life-history, or behavior can alter the intake and investment of dietary elements at L1
340 (Frost et al. 2005). In addition to food quality, this list includes a suite of abiotic variables (e.g.,
341 light and CO₂), biotic factors such as food quantity and algal taxonomic composition, and food
342 web dependent factors such as predation and parasitism (Dickman et al. 2008; Yamamichi et al.
343 2015). Finally, organismal stoichiometry is shaped by the evolutionary history (L3) of a given
344 taxon, which can influence both immediate responses of organisms to environmental conditions
345 (i.e., elemental plasticity) and shape species and population differences through space and time

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

353 Both temperature and stoichiometric food quality appeared to influence *Daphnia* biomass
354 production in our study lakes. Biomass in the lower nutrient Wolf Lake was correlated with both
355 food quality and temperature and was highest at low food C:P ratios and moderate temperatures,
356 which occurred in the early spring and fall. Thus, although temperature seemed to predominantly
357 control daphnid stoichiometry in this lake, nutrient availability represents an important factor
358 determining zooplankton production and likely interacts with temperature to influence seasonal
359 patterns in *Daphnia* biomass in oligo- and mesotrophic systems (Elser et al. 1998; Makino et al.
360 2002). *Daphnia* biomass was not related to food quality in Pigeon Lake where seston was P-rich
361 year-round. Instead, biomass peaked at moderate temperatures early in the year and remained
362 low for the remainder of the growing season. We were unable to quantify predation pressure in
363 our study, which could have influenced seasonal variation in Pigeon Lake biomass. Similarly, we
364 did not measure differences in algal taxonomic composition, but daphnid biomass remained low
365 despite high food quantities and was negatively related to algal biomass (Suppl. Fig 2). As
366 cyanobacteria blooms can develop in the mid-summer and persist throughout the growing season
367 in Pigeon Lake, it is possible that either feeding inhibition (Abrams and Walters 1996; DeMott et
368 al. 2001) or reduced growth and reproductive rates due to fatty acid-limitation (Ravet et al.,

369 2012; Ger et al., 2016) could explain failed *Daphnia* recruitment following the spring die off. If
370 true, our results suggest that food quality effects on daphnid nutritional physiology and biomass
371 production may act along a continuum controlled by dietary elemental stoichiometry in
372 oligotrophic systems and switching to physical and/or biochemical regulation under more
373 eutrophic conditions.

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

392 consumer population dynamics and ecosystem functions may change under increasingly variable
393 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 \pm 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 \pm 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.

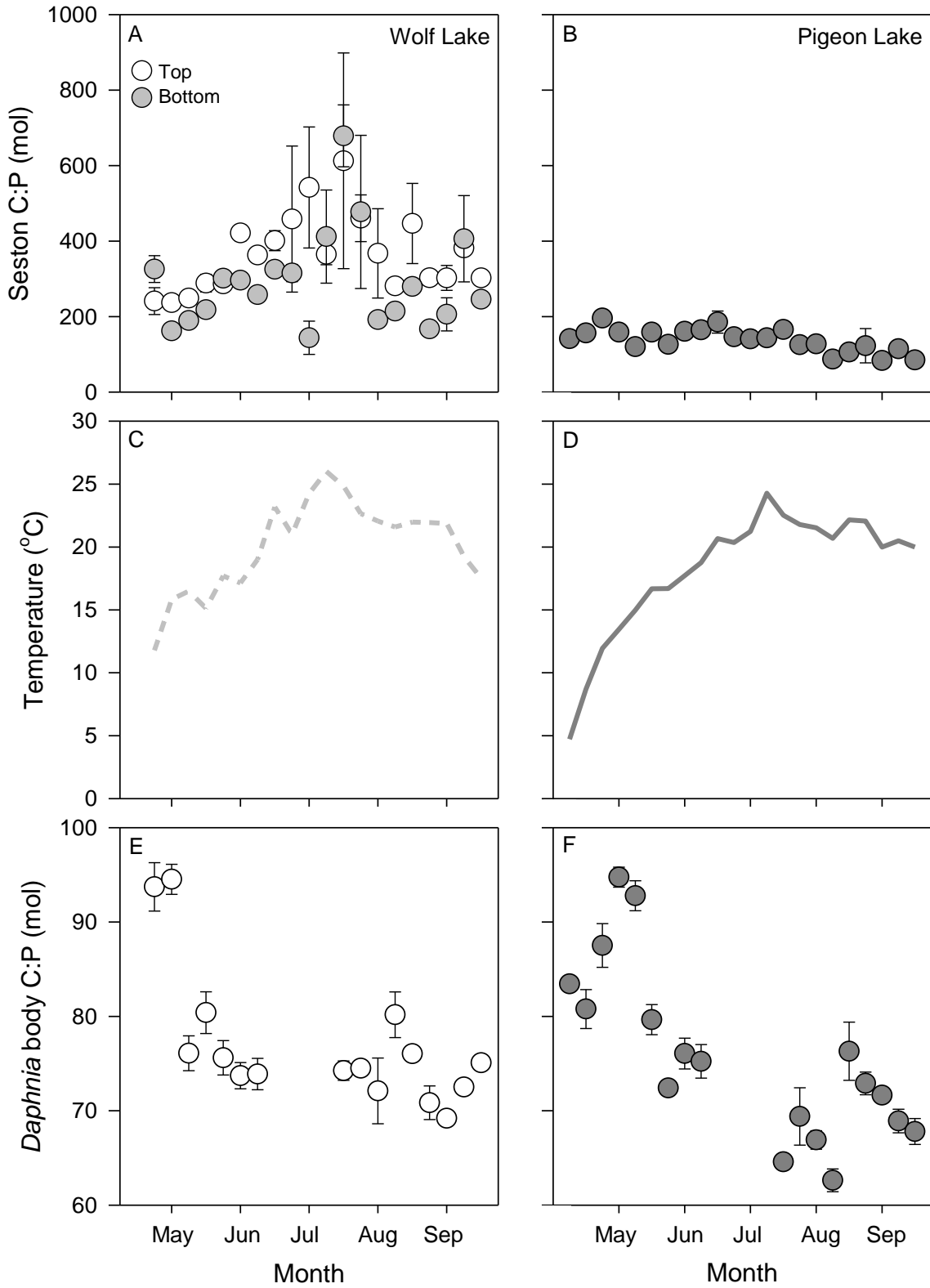


Figure 2.

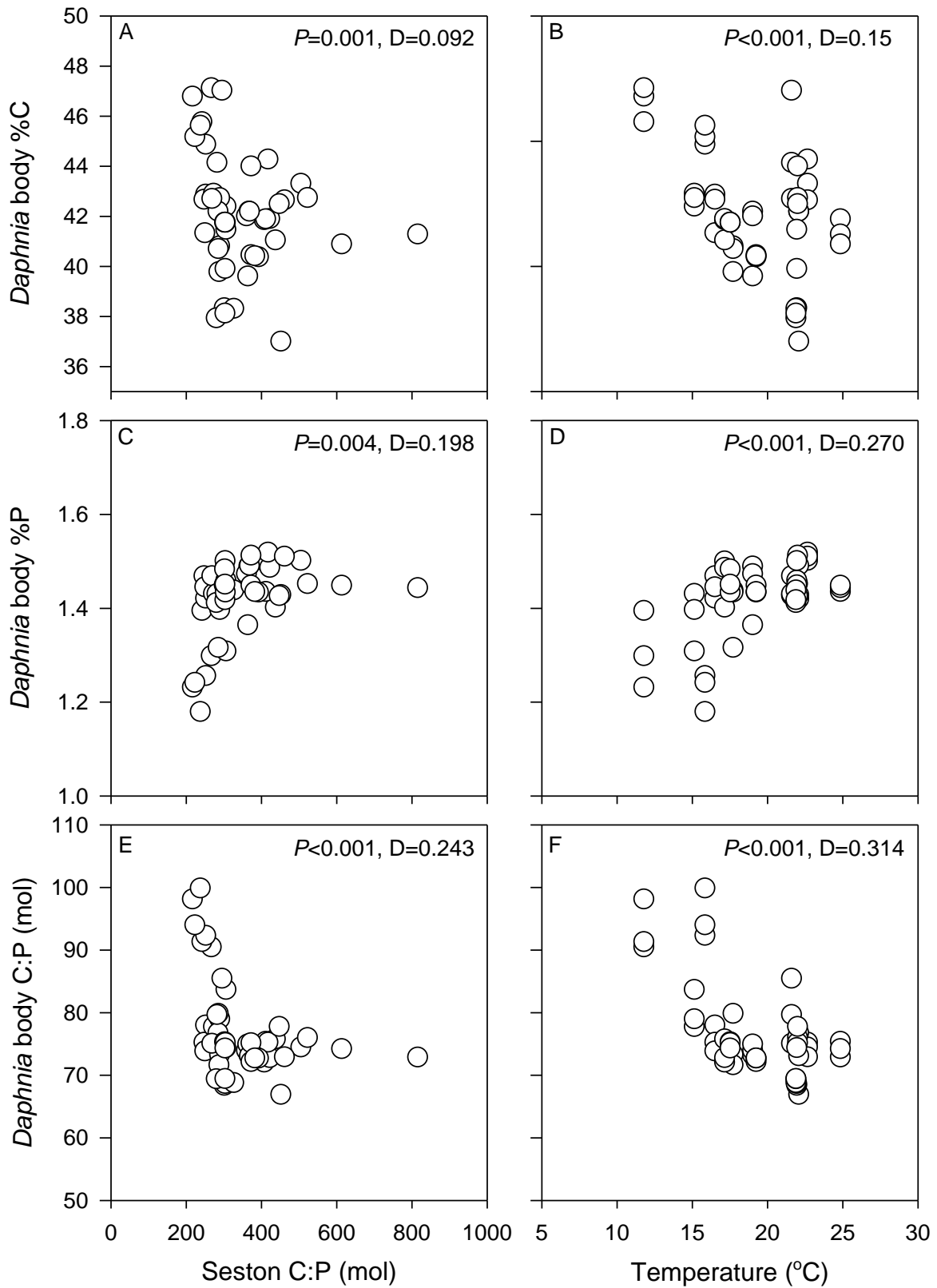


Figure 3.

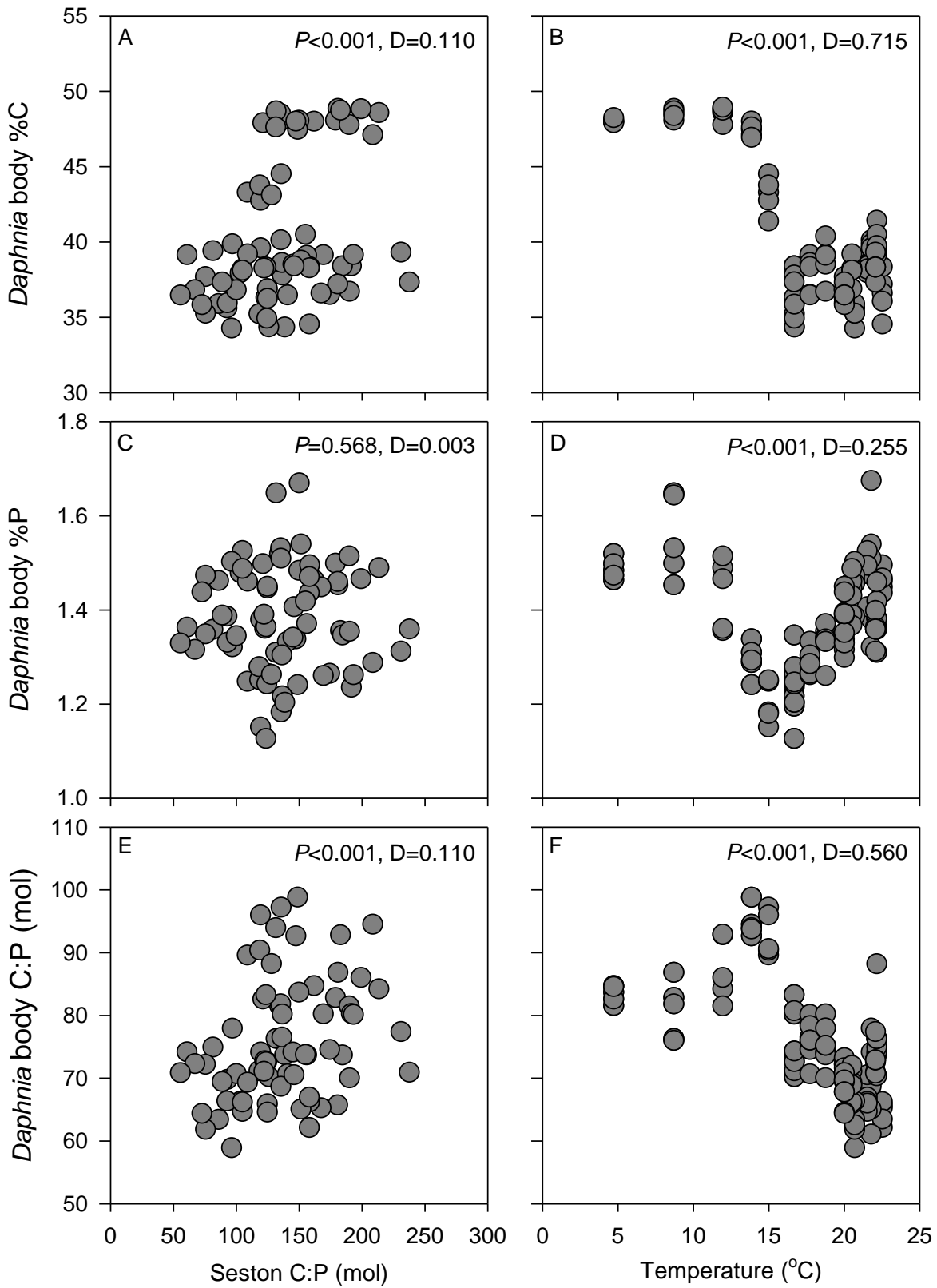


Figure 4.

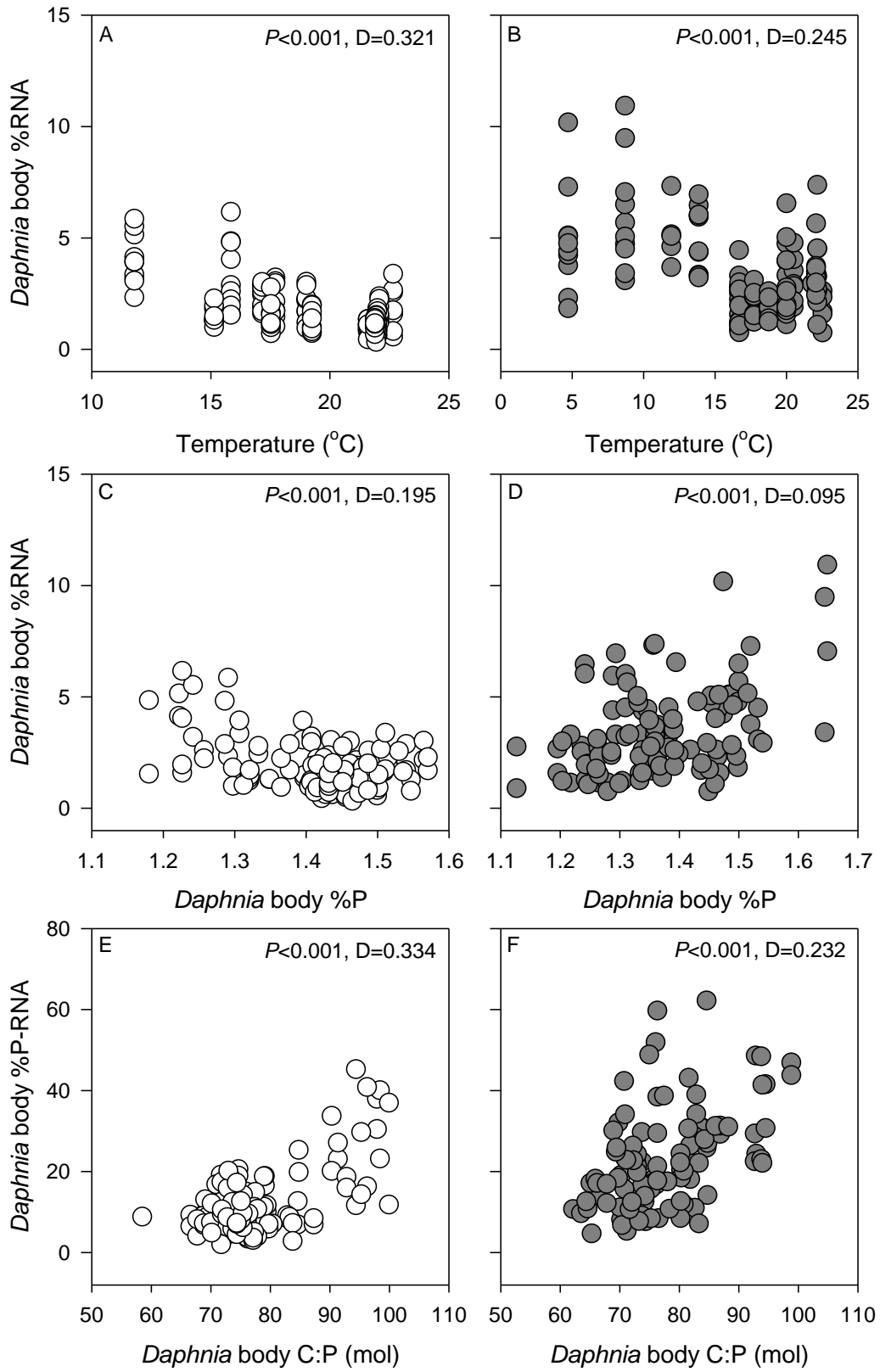


Figure 5

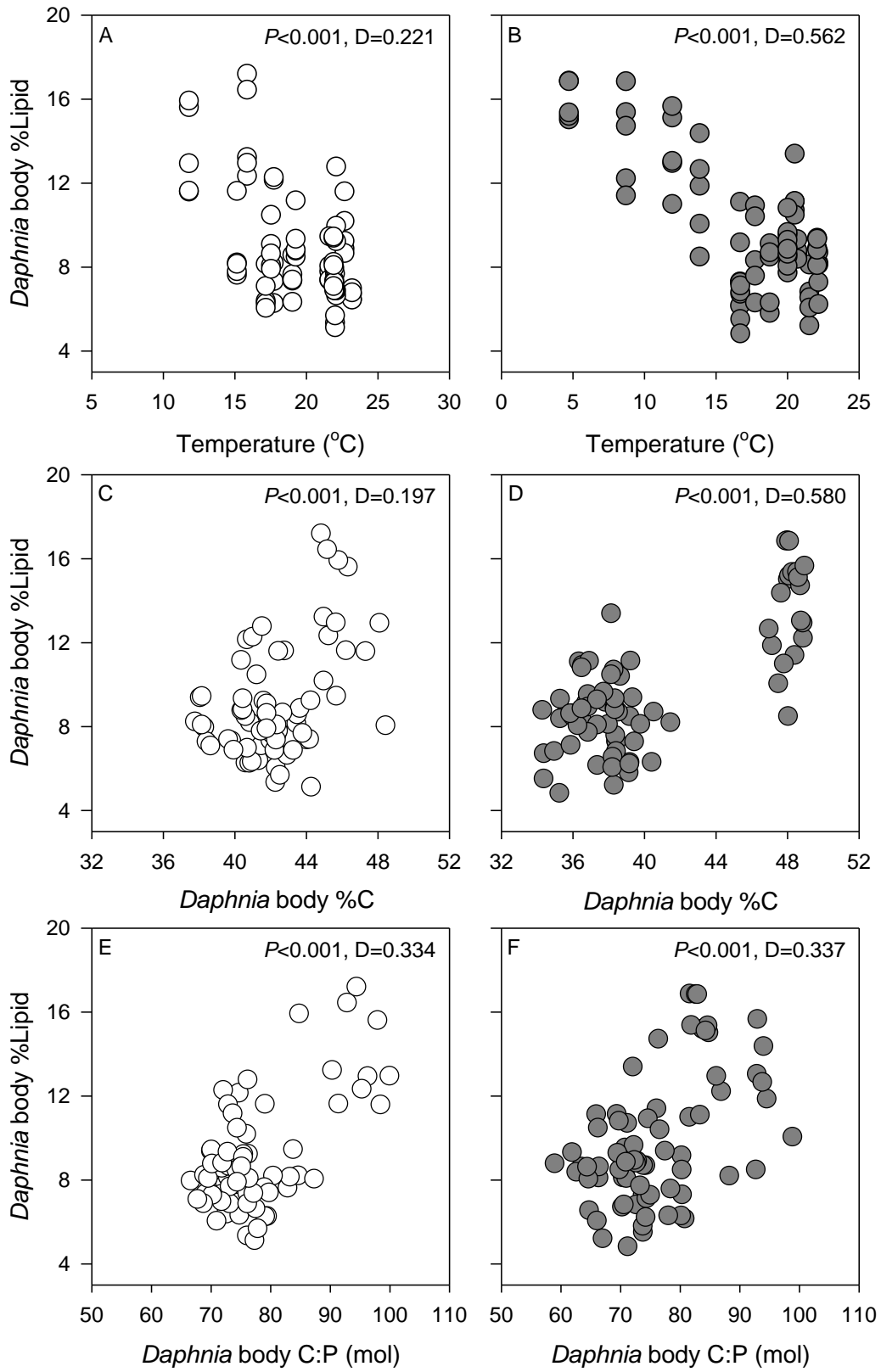


Figure 6.

