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The Jellification of North Temperate Lakes

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24 Calcium (Ca) concentrations are decreasing in softwater lakes across eastern North
25 America and western Europe. Using long-term contemporary and palaeo-environmental field
26 data, we show that this is precipitating a dramatic change in Canadian lakes: the replacement of
27 previously dominant pelagic herbivores (Ca-rich *Daphnia* species) by *Holopedium glacialis*, a
28 jelly-clad, Ca-poor competitor. In some lakes, this transformation is being facilitated by
29 increases in macro-invertebrate predation, both from native (*Chaoborus* spp.) and introduced
30 (*Bythotrephes longimanus*) zooplanktivores, to which *Holopedium*, with its jelly coat, is
31 relatively invulnerable. Greater representation by *Holopedium* within cladoceran zooplankton
32 communities will reduce nutrient transfer through food webs, given their lower phosphorus
33 content relative to daphniids, and greater absolute abundances may pose long-term problems to
34 water users. The dominance of jelly-clad zooplankton will likely persist while lakewater Ca
35 levels remain low.

36 **Keywords** *Holopedium*, *Daphnia*, lakes, calcium decline, jelly, jellification, nutrients

37 1. INTRODUCTION

38 The ecological impacts associated with calcium (Ca) decline are a growing concern in
39 aquatic ecosystems of eastern North America and northern Europe [1,2,3]. This is especially true
40 in soft-water lakes where abundances of Ca-rich *Daphnia* (Crustacea, Cladocera), often the
41 dominant and keystone herbivores in the pelagic zone, are declining [3,4,5]. However, not all
42 taxa suffer when Ca declines. Daphniids compete for food resources with another large
43 Cladoceran, *Holopedium glacialis* [6,7,8] (previously *H. gibberum* [9] and hereafter called
44 *Holopedium*). Because *Daphnia* has a heavily-calcified carapace supported by a dense network
45 of phosphorus-rich, polyploid cells [10], it has high Ca and phosphorus (P) needs. *Holopedium*
46 lacks such a carapace, giving it about one-tenth the Ca [11] and one-half the P [12,13]

47 requirements of daphniids. This likely explains why *Holopedium* commonly outcompetes
48 *Daphnia* in softwater, oligotrophic lakes [14]. *Holopedium* also sports a mucopolysaccharide
49 jelly-capsule that largely protects it from macro-invertebrate predators (figure 1), including the
50 larval phantom midge, *Chaoborus*, and the recent Eurasian invader, *Bythotrephes longimanus*
51 (the spiny water flea), both of which can be major energy conduits to fish [15], and to which
52 most daphniid species are vulnerable [8,16].

53 Here we report the replacement of previously dominant Ca-rich daphniids by
54 *Holopedium* in two major lake regions of Canada separated by ~1500 km, i.e. south-central
55 Ontario and Nova Scotia. In recent decades, these Ontario lakes have changed in many ways: Ca
56 [3] and P concentrations [17] have declined in response to changes in climate and acid
57 deposition, coloured dissolved organic carbon (DOC) has increased [16, 18] partly because
58 increasingly base-poor catchments are retaining less organic matter [19], and *Bythotrephes* has
59 been introduced and is spreading [20]. *Bythotrephes* reduces populations of most daphniids more
60 than it does that of *Holopedium* [21]. The Nova Scotia lakes have experienced similar acid
61 deposition and Ca trends [22,23], but they have not, as yet, been invaded by *Bythotrephes*.

62 Our goal was to determine whether declining lakewater Ca concentrations are increasing
63 the contribution of *Holopedium* to crustacean zooplankton abundance at the expense of *Daphnia*.
64 We tested two hypotheses:

- 65 **1.** *Holopedium* abundances have increased since pre-industrial times. We tested this hypothesis
66 using sedimentary cladoceran assemblages from 84 Ontario and Nova Scotia lakes (see
67 electronic supplementary material, table S1).
- 68 **2.** Relative and/or absolute abundances of *Holopedium* have increased during the period of
69 recent lakewater Ca decline. We tested this hypothesis by surveying pelagic zooplankton in

70 31 Ontario lakes between 1981-1990 and resampling from 2004-05, and with a longitudinal
71 study of 8 other Ontario lakes that were sampled monthly for 30 years (see electronic
72 supplementary material, tables S2-S3).

73 We expected that Ca decline would be the ultimate driver of increases in relative and/or
74 absolute *Holopedium* abundance, both of which we term “jellification”, but it might not be the
75 proximate cause. The proximate causes could be either bottom-up, i.e. changes in competition
76 for food, or top-down, i.e. changes in predation. To tease these apart, we used the 30-year
77 longitudinal study to test four potentially co-occurring mechanisms:

78 *Declining populations mechanism: Holopedium* increased only in relative abundance
79 within the planktonic cladoceran assemblage because non-*Holopedium* Cladocera declined with
80 falling lake Ca.

81 *Less competition mechanism: Holopedium* increased in absolute abundance because there
82 was more phytoplankton available to support its growth, as *Daphnia*, the dominant cladoceran
83 herbivore, declined with falling lake water Ca, releasing *Holopedium* from their primary
84 cladoceran competitor [14].

85 *More food mechanism: Holopedium* increased in absolute abundance in response to
86 greater phytoplankton abundance in lakes where P inputs have increased over time.

87 *Increasing predation mechanism: Holopedium* increased in absolute and/or relative
88 abundance because they suffered less than daphniids from increasing predator densities.

89 *Chaoborus* spp., the primary native invertebrate predator in our lakes [15], should benefit from
90 temporal increases in the volume of their refuge from fish predation [24]. We predicted greater
91 refuge volume would accompany increased hypolimnetic oxygen depletion associated with

92 climate warming [25] and/or greater coloured DOC concentrations [24], both of which might
93 reduce predation from fish. As *Chaoborus* preferentially select *Daphnia* over *Holopedium* [26],
94 greater *Chaoborus* densities should thus increase the relative abundance of *Holopedium*.
95 Predation could also promote greater absolute abundances of *Holopedium* if reductions in
96 *Daphnia* reduce competition for food.

97 By using structural equation modelling (SEM), we were explicitly able to test the strength
98 and direction of causal associations underlying these four mechanisms [27]. SEM works by
99 optimizing the fit between predicted and observed patterns of covariance, while respecting the
100 constraints of a hypothesized network of interactions [28]. Thus, it is more powerful for
101 disentangling the associations between the many interacting chemical and ecological factors that
102 co-vary with zooplankton abundance than simple linear regression [29]. An additional advantage
103 of SEM is its incorporation of latent (i.e. unmeasured) variables, which can be inferred from
104 measured data [see 27,29 for details].

105 2. METHODS

106 *Have Holopedium abundances increased since pre-industrial times?*

107 We examined long-term changes in the relative abundance of *Holopedium* within the
108 pelagic cladoceran assemblages in lake sediments from 36 south-central Ontario lakes in 2007
109 [4], and 48 Nova Scotia lakes in 2002-04 [5] (see electronic supplementary material, table S1).
110 Sediment cores were collected from the deepest basin of each lake using a gravity corer, and
111 sectioned at 0.25 cm intervals. We then employed the well-established “top/bottom”
112 palaeolimnological sampling design [30], wherein the chitinous cladoceran remains were
113 isolated, identified and compared between a “top” interval (surface sediments) representing
114 present-day assemblages and a “bottom” interval (sediment depth >15.0 cm) representing

115 assemblages present prior to the onset of lake acidification. Although this protocol cannot
116 document the timing of changes, it clearly separates modern from historical assemblages, which
117 is what the test of our first hypothesis required.

118 We counted and identified remains from a minimum of 90 individual cladocerans per
119 sample [31,32], of which a mean of 78% and 61% were pelagic taxa in the Ontario and Nova
120 Scotia lakes, respectively. This count is sufficient to accurately characterize the taxonomic
121 assemblage [33]. We tested whether the contribution of *Holopedium* to planktonic cladoceran
122 assemblages had changed since pre-industrial times using paired t-tests comparing *Holopedium*
123 relative abundance in the pre-industrial vs. recent sediment datasets. We excluded lakes where
124 *Holopedium* was absent from both intervals.

125 *Are Holopedium increases consistent with recent lakewater Ca decline?*

126 We tested our second hypothesis using two datasets: a longitudinal study of 8 Ontario
127 lakes sampled over a 30 year period [34], and a regional survey of 31 other Ontario lakes
128 sampled in the 1980s and 2004 or 2005 [35] (see table S2). Zooplankton were sampled during
129 the ice-free season near the point of maximum depth by using a metered, 76 µm mesh, tow net.
130 The net was deployed in 4 to 7 vertical hauls and its contents were combined to produce a
131 composite that corrected for the diminution of lake stratum volume with depth. A minimum of
132 250 crustacean zooplankton were enumerated in each composite, subsampling to ensure that no
133 taxon comprised more than 10% of the total count. All Cladocera were identified to the species
134 level [34].

135 To test whether the relative and absolute abundance of *Holopedium* had increased in the
136 31 lake regional dataset, we compared the 1980s vs. 2004-05 ice-free means using, respectively,
137 a paired t-test and a Wilcoxon signed-rank test. The five lakes that were invaded by *Bythotrephes*

138 between the two sampling periods were analysed separately. For the 8 lake longitudinal dataset,
139 we used Mann-Kendall trend tests to identify whether the absolute and relative abundances of
140 *Holopedium*, daphniids and lakewater Ca concentrations had changed in a monotonic fashion
141 over time when averaged across lakes in each year of the dataset. Prior to averaging absolute
142 abundances, we standardized observations to a mean of 0 and SD of 1 across years in each lake.

143 *Discriminating among alternative mechanisms underlying increases in Holopedium abundance*

144 We attempted to distinguish the causal processes underlying the long-term changes in
145 daphniid and *Holopedium* abundances using ice-free season means from the eight longitudinal
146 study lakes [34,36] (see table S3). These lakes vary in acid-sensitivity [34], and have patterns of
147 environmental change reflecting those of the broader region [17]. We needed composition and
148 abundance data for crustacean zooplankton and phytoplankton, water chemistry, oxygen profiles,
149 and abundances of *Chaoborus* spp. to choose among our four hypothesized causal mechanisms.
150 Briefly, zooplankton were enumerated from the volume-weighted composites as described
151 above. Phytoplankton were sampled through the euphotic zone, and water chemistry samples
152 were volume-weighted composites of all depths during isothermal periods, and of the mixed
153 layers during periods of vernal and autumnal stratification. Dissolved oxygen profiles were
154 generated at 1-2 m intervals from all depths. Finally, abundances of the zooplanktivore
155 *Chaoborus* spp. were quantified in samples from night-time vertical hauls at 10 stations visited
156 during the autumn in all lakes in 1986 and in one lake from 1987-1994 [37]. In the laboratory, a
157 minimum of 300 cells, colonies, or filaments of phytoplankton were counted within the
158 Bacillariophyceae, Chlorophyceae, Cryptophyceae, Dinophyceae, and Euglenophyceae.
159 Dimensions of all counted algae were measured, and standing stocks were expressed as
160 biovolumes [38]. We derived an index of “edible phytoplankton” by summing phytoplankton

161 biovolumes across these five families, which are preferentially consumed by zooplankton (see
162 electronic supplementary material).

163 Using a SEM approach, we then estimated a series of equations (2.1-2.6) describing a
164 causal network that simultaneously tested our four hypothesised mechanisms.

165 *Declining populations mechanism:* We estimated the probability of observing that a
166 random cladoceran individual in each lake i in year j was a *Holopedium* [$p_{ij}^{(1)}$], independent of
167 the other species in the community. We did so assuming that the number of *Holopedium*
168 individuals $y_{ij}^{(1)}$ annually counted in each lake could be described by a binomial distribution with
169 N_{ij} total number of planktonic cladoceran individuals:

$$170 \quad y_{ij}^{(1)} \sim B(p_{ij}^{(1)}, N_{ij}).$$

171 Thus, the relative abundance of *Holopedium* within the planktonic cladoceran assemblage
172 could increase simply because the absolute number of non-*Holopedium* Cladocera declined (i.e.
173 N_{ij} becomes smaller over time). Relative increases over time could also arise because both $p_{ij}^{(1)}$
174 and N_{ij} increased. Alternatively, there could be an increase in the absolute abundance of
175 *Holopedium*, calculated independently of any other taxa in the assemblage. The probability $p_{ij}^{(1)}$
176 would correspondingly have increased over time.

177 *Less competition mechanism:* To test whether the number of *Holopedium* $y_{ij}^{(1)}$ increased
178 with food availability (ξ_{ij}), and whether ξ_{ij} increased as the dominant Cladocera in our lakes
179 (*Daphnia* spp.) declined with falling lake Ca, we first modelled $p_{ij}^{(1)}$ as an inverse-logit of ξ_{ij} :

$$180 \quad \text{logit}(p_{ij}^{(1)}) = \alpha^{(1)} + \gamma_1 \xi_{ij} + \gamma_2 \text{Chaob}_{ij} + \nu_i^{(1)} + \nu_j^{(1)}, \quad (2.1)$$

181 where $\alpha^{(1)}$ is the estimated mean probability of observing *Holopedium* across all lakes and years,
182 γ_1 is the effect of food availability, γ_2 is an estimated effect accounting for predation by

183 *Chaoborus* spp. (Chaob_{ij} , square-root transformed, individuals m^{-3}), and $v_i^{(1)}$ and $v_j^{(1)}$ account for
184 random variation among the i lakes and j years and are each drawn from a normal distribution
185 with a mean of 0 and separately estimated standard deviation (SD). Although we did not observe
186 values directly for ζ_{ij} , a latent variable, we parameterized it using observed data.

187 We assumed that ζ_{ij} was $\sim N(\eta_{ij}, \sigma_\zeta)$, and modelled mean food availability η_{ij} in each lake i
188 in year j given abundances of potential *Daphnia* competitors. Exploitative competition for
189 limiting resources, namely food, is a major factor structuring zooplankton communities [14].
190 Although larger zooplankton species can exclude smaller species because their greater body
191 reserves survive low food periods, this competitive hierarchy depends on how the abundances of
192 species vary with levels of predation and abiotic conditions [39]. For *Daphnia*, the abundances of
193 species, and thus their competitive effects, depend on species-specific Ca requirements [14]. We
194 therefore classified *Daphnia* species into either relatively Ca-rich (*D. dubia*, *D. longiremis*, *D.*
195 *mendotae*, *D. pulicaria*, and *D. retrocurva*) or Ca-poor (*D. ambigua* and *D. catawba*) groups
196 using inter-specific differences in body Ca content [11,40] and prevalence thresholds in field
197 surveys [20,40] (figure S1). However, one single metric cannot summarize the responses of all
198 Ca-rich daphniids because body-size and Ca sensitivity still vary within this group (figure S2).
199 We additionally allowed for different responses within this group by summarizing community
200 composition with an index D_{ij} that reflects both the relative abundances of species and their
201 differences in Ca sensitivity [41]. The resulting D_{ij} is an “effective” diversity measure; e.g. a
202 community with 3 species and $D_{ij} = 2.5$ is slightly less diverse than a community of 3 equally-
203 abundant taxa with totally dissimilar Ca requirements (see electronic supplementary material).
204 This combination of approaches essentially allows both linear and non-linear interactions
205 between *Holopedium* and *Daphnia* in our model, without assuming any specific effect. Finally,

206 we could not ignore herbivorous copepod species (Cop_{ij}), as their biomass ($\mu\text{g dry weight m}^{-3}$)
 207 can account for the majority of zooplankton biomass in our study lakes, and temporal changes in
 208 their densities will influence food available for Cladocera [6,42]. η_{ij} was then a function of the
 209 probability of observing Ca-poor [$p_{ij}^{(2)}$] and Ca-rich daphniids [$p_{ij}^{(3)}$], the composition of Ca-rich
 210 daphniid communities (D_{ij}), and Cop_{ij} :

$$211 \quad \eta_{ij} = \gamma_3 p_{ij}^{(2)} + \gamma_4 p_{ij}^{(3)} + \gamma_{5[i]} D_{ij} + \gamma_6 Cop_{ij}, \quad (2.2)$$

212 where γ_3 - γ_6 are estimated effects and σ_ξ is the estimated SD.

213 Mean food availability η_{ij} is directly proportional to $p_{ij}^{(2)}$ and $p_{ij}^{(3)}$. As these probabilities
 214 will vary with lakewater Ca, we modelled them using binomial distributions based on the
 215 number of individuals of Ca-poor [$y_{ij}^{(2)}$] and Ca-rich [$y_{ij}^{(3)}$] daphniids, respectively, counted in
 216 lake i in each year j :

$$217 \quad y_{ij}^{(2)} \sim B(p_{ij}^{(2)}, N_{ij}),$$

$$218 \quad \text{logit}(p_{ij}^{(2)}) = \alpha^{(2)} + \gamma_7 Ca_{ij} + \gamma_8 Chaob_{ij} + v_i^{(2)} + v_j^{(2)}, \quad (2.3)$$

$$219 \quad y_{ij}^{(3)} \sim B(p_{ij}^{(3)}, N_{ij}),$$

$$220 \quad \text{logit}(p_{ij}^{(3)}) = \alpha^{(3)} + \gamma_9 Ca_{ij} + \gamma_{10} Ca_{ij}^2 + \gamma_{11} Chaob_{ij} + v_i^{(3)} + v_j^{(3)}. \quad (2.4)$$

221 $\alpha^{(k)}$ is the estimated mean probability of observing individuals of life group k across all
 222 lakes and years, γ_7 , γ_9 , γ_{10} are estimated effects of lake Ca, γ_8 and γ_{11} are estimated effects
 223 accounting for the fact that predation by *Chaoborus* influences daphniid abundances, and $v_i^{(k)}$
 224 and $v_j^{(k)}$ account for random variation among the i lakes and j years and are drawn from zero-
 225 mean normal distributions with separately estimated SD for each source of variation associated

226 with each life group k . We allowed the response of Ca-rich daphniids to lakewater Ca to be non-
 227 linear, as observed in mid-summer surveys of 304 regional lakes [40], by including the quadratic
 228 effect $\gamma_9 \text{Ca}_{ij}^2$. There was no reason to do so for Ca-poor taxa as these are not positively
 229 associated with lake water Ca [40].

230 *More food mechanism:* We then defined food availability ξ_{ij} as increasing with edible
 231 phytoplankton biovolume (P_{ij} , $\text{mm}^3 \cdot \text{m}^{-3}$), and drew P_{ij} from a Poisson distribution modelled as:

$$232 \quad P_{ij} \sim \text{Pois}(\lambda_{ij}),$$

$$233 \quad \log(\lambda_{ij}) = \alpha^{(4)} + \xi_{ij} + \gamma_{12} \text{Nsamp}_{ij} + \gamma_{13} \text{TP}_{ij} + \nu_i^{(4)} + \nu_j^{(4)}, \quad (2.5)$$

234 where $\alpha^{(4)}$ is mean biovolume across all years and lakes, γ_{12} - γ_{13} are estimated effects of the
 235 number of sampling events and total phosphorus (TP), and ν_i and ν_j account for variation among
 236 the i lakes and j years, respectively, and are drawn from zero-mean, normal distributions with
 237 separately estimated SD. We expected P_{ij} to increase primarily with TP, so we did not include
 238 other chemistry variables in equation 2.5 because they co-varied with TP (e.g. Pearson's
 239 correlation with DOC = 0.68).

240 *Increasing predation mechanism:* Finally, we considered whether the relative abundances
 241 of *Holopedium* and their competition for food could be changed by *Chaoborus*, a principal
 242 predator of *Daphnia*. We modelled the densities of *Chaoborus* spp. (Chaob_{ij}) as a function of the
 243 thickness of the hypolimnetic water layer with $<3 \text{ mg} \cdot \text{L}^{-1} \text{ O}_2$ (O_{ij}) and concentration of DOC in
 244 lakewater (DOC_{ij}), which both reduce predation from fish [24]:

$$245 \quad \text{Chaob}_{ij} \sim N(\mu_{ij}, \sigma_{\text{Chaob}}),$$

$$246 \quad \mu_{ij} = \alpha^{(5)} + \gamma_{14} \text{O}_{ij} + \gamma_{15} \text{DOC}_{ij} + \nu_i^{(5)}, \quad (2.6)$$

247 where $\alpha^{(5)}$ is the estimated mean density of *Chaoborus*, γ_{14} - γ_{15} are estimated effects of O₂ and
248 DOC, and $v_i^{(5)}$ accounts for random variation among the *i* lakes and is drawn from a zero-mean
249 normal distribution with estimated SD. *Chaoborus* spp. were measured only in one lake outside
250 of 1986, and so we could not adequately estimate both variation among lakes and years (total $n =$
251 16). Thus, we only accounted for variation among lakes. For all the other year×lake
252 combinations in equations (2.1, 2.3-2.4), we let Chaob_{ij} take on mean observed values.

253 We did not directly consider how variation in predation on *Chaoborus* spp. themselves
254 influenced daphniid abundances. We expect *Chaoborus* populations are likely to experience
255 similar top-down control among lakes and whole-lake manipulative work on nearby lakes has
256 shown that changes in piscivorous fish communities do not influence the overall importance of
257 *Chaoborus* as zooplanktivores [43]. We did not model copepod biomass as a function of
258 *Chaoborus* densities as we were not interested in estimating the associated effects. Any changes
259 in copepod biomass associated with *Chaoborus* was reflected in the raw data and allowed us to
260 determine the influence of copepods on food availability.

261 *Model estimation:* The SEM was estimated within a hierarchical Bayesian framework
262 using Markov chain Monte Carlo (MCMC) sampling by calling Stan v.2.0 [44] from R v.3.0
263 [45]. Four MCMC chains of 1000 iterations were simulated, with a burn-in period of 4000 runs
264 (see electronic supplementary material for details). We standardized all estimated coefficients to
265 a common scale with a mean of 0 and SD of 1, so that their effects were directly comparable.
266 This allowed us to test the relative importance of different causal linkages. To infer effects, we
267 calculated posterior means and 95% credible intervals (CIs) for each parameter by drawing a
268 subset of 800 simulations. We did not reject our predictions about specific causal linkages if 95%
269 CIs excluded zero. To summarize overall model fit, we calculated a Bayesian R^2 at the level of

270 our measured data, analogous to the proportion of variance explained by a model in classical
271 linear regression [46]. We used three approaches to verify convergence of our model and the
272 structure of the SEM was assessed using a graphical modelling approach [47] (see electronic
273 supplementary material).

274 3. RESULTS

275 *Have Holopedium abundances increased since pre-industrial times?*

276 Across both study regions, the relative contribution of *Holopedium* to planktonic
277 cladoceran assemblages clearly increased since pre-industrial times (i.e. pre-1850 or prior to the
278 onset of acid deposition). These increases were evident in both of our palaeolimnological surveys
279 as the relative abundance of *Holopedium* increased in 25 of the 35 Ontario lakes and 17 of the 23
280 Nova Scotia lakes in which it was present (paired *t*-test, $t_{34} = 2.7$, $p = 0.012$ and $t_{22} = 2.7$, $p =$
281 0.013 , respectively; figures 2*a* and 2*b*). Furthermore, *Holopedium* appeared (i.e. was present in
282 the “top” samples, but was absent from the pre-industrial samples) in 15 Ontario lakes and 11
283 Nova Scotia lakes, while disappearing from only 2 and 1 lakes, respectively, suggesting that
284 increases in absolute abundances have also occurred.

285 *Have Holopedium abundances increased during the recent period of lakewater Ca decline?*

286 *Holopedium* has become more abundant in the 31 south-central Ontario lakes between the
287 1980s and 2004-05 as Ca declined by a median of 25% [17]. *Holopedium* relative abundances
288 increased in 17 of the 26 lakes that have not been invaded by *Bythotrephes*, these increases
289 ranged from 3-37% (paired *t*-test, $t_{25} = 2.8$, $p = 0.009$, figure 2*c*). In each of the five invaded
290 lakes *Holopedium* was more abundant (by 5-30%) in 2004-2005 than in the 1980s (paired *t*-test,
291 $t_4 = 3.5$, $p = 0.024$, figure 2*c*). The absolute abundance of *Holopedium* also increased; median

292 abundance in the lakes doubled, from 304 animals·m⁻³ in the 1980s to 607 animals·m⁻³ in 2004-
293 2005 ($W_{30} = 123$, $p = 0.013$, figure 2d).

294 Increasing absolute abundances of *Holopedium* in the eight longitudinal study lakes
295 mirror the trends in the regional dataset. The relative abundance of *Holopedium* increased in
296 these lakes as lakewater Ca declined (figure 3a; Mann-Kendall test: $\tau = 0.41$, $p = 0.002$ and $\tau = -$
297 0.71 , $p < 0.001$, respectively). This arose partly because there were lower absolute abundances of
298 both Ca-rich daphniids and all other Cladocera as Ca declined ($\tau = -0.48$ and -0.55 , respectively;
299 $p < 0.001$ for both). Greater absolute abundances of the two Ca-poor daphniid species over this
300 period ($\tau = 0.50$, $p < 0.001$; figure 3b) could not offset these declines, i.e. the trend in absolute
301 abundance of all non-*Holopedium* Cladocera was negative ($\tau = -0.48$, $p < 0.001$). The increased
302 relative abundance of *Holopedium* in the longitudinal study lakes was also attributable to an
303 increase in its absolute abundance in Harp, Heney and Red Chalk lakes from 1981 to 2009 ($\tau >$
304 0.37 , $p < 0.010$), and in Dickie Lake prior to recent dust suppressant (CaCl₂) additions [48],
305 which raised lakewater Ca levels (one-tailed test for increasing trend: $\tau = 0.33$, $p = 0.029$; see
306 table S3).

307 *Discriminating among alternative mechanisms underlying increases in Holopedium abundance*

308 Our SEM revealed that the relative rise of *Holopedium* in the eight lakes of the
309 longitudinal study could be explained by both declines in non-*Holopedium* Cladocera (“declining
310 populations” mechanism) and increases in absolute numbers of *Holopedium*. The latter changes
311 were specifically associated with reduced inter-specific competition for food and declines in
312 Cladocera predicted by the “less competition” and “increasing predation” mechanisms (figure 4).

313 Falling lakewater Ca was the primary explanation for declines in dominant *Daphnia* spp.
314 that have favoured *Holopedium*. The probability of observing *Daphnia* increased with lakewater

315 Ca in the SEM, with the effect much stronger for Ca-rich as opposed to Ca-poor species (95%
316 CIs: 1.09 – 1.14 and 0.13 – 0.25, respectively). Thus, as Ca has fallen over time, so too have Ca-
317 rich daphniids (trends reported above), thereby favouring a greater proportional representation of
318 *Holopedium* within communities (i.e. lower N_{ij} predicted by “declining populations”
319 mechanism).

320 Falling abundances of Ca-rich *Daphnia* have also reduced competition for food,
321 supporting the “less competition” mechanism. The SEM revealed that Ca-rich but not Ca-poor
322 *Daphnia* spp. reduced food availability (95% CIs: -0.06 – <-0.01 and -0.32 – 0.17, respectively),
323 and the probability of observing *Holopedium* increased with more food (95% CI: 1.01 – 1.17).
324 Thus, as Ca-rich species declined, there was more food available for *Holopedium*. For example, a
325 decline in lakewater Ca from 3 to 1 mg·L⁻¹ corresponded with an approximately 34% increase in
326 the absolute probability of observing *Holopedium* at mean levels of all other covariates (e.g. TP
327 and *Chaoborus* spp. densities) when the pathways influenced by Ca were followed from start to
328 finish in our SEM (figure 4). Copepods have similarly declined over time ($\tau = -0.40$, $p = 0.002$),
329 increasing food available for *Holopedium* (95% CI for their effect on food: -0.15 – -0.12).
330 However, food availability did not increase in all lakes despite declines in non-*Holopedium*
331 zooplankton. This was because the total food supply, determined by the abundance of edible
332 phytoplankton, depended on TP concentrations (95% CI: 0.02 – 0.08), which have declined over
333 time across lakes ($\tau = -0.33$, $p < 0.014$). These declining trends lend no support to the “more
334 food” mechanism.

335 Although Ca-rich *Daphnia* have declined, the net effect on food availability could be
336 offset by changes associated with their species composition (95% CIs for composition were
337 negative in five lakes and positive in three; table S4). Thus, overall food availability increased

338 only in two lakes (Honey and Red Chalk), explaining why the absolute number of *Holopedium*
339 has increased in these two lakes but not the others. There was no increase in food availability in
340 Harp Lake despite increases in *Holopedium* reported earlier, because we only included years
341 prior to *Bythotrephes* invasion in the SEM to avoid this complication (1980 to 1992; table S3).
342 Absolute abundances of *Holopedium* only increased when we also considered the years after the
343 invasion.

344 Finally, the SEM supported our “increasing predation” mechanism, which predicted that
345 *Holopedium* increased in relative abundance because they suffered less from predation by
346 *Chaoborus* than did *Daphnia* (95% CI for effect on Ca-rich *Daphnia*, Ca-poor *Daphnia*,
347 *Holopedium*: -0.21 – -0.11, -0.20 – -0.09, -0.26 – 1.01, respectively). Declines in *Daphnia* from
348 predation will have also increased food availability, promoting greater absolute abundances of
349 *Holopedium* (figure 4). These changes may continue into the future as *Chaoborus* densities
350 increased with the thickness of hypoxic water (95% CI: 0.23 – 13.6), which has increased over
351 time ($\tau = 0.30$, $p = 0.023$), and most strongly in recent years.

352 4. DISCUSSION

353 Collectively, our analyses reveal that: 1) the contribution of *Holopedium* to pelagic
354 cladoceran abundance has increased in central and eastern softwater Canadian lakes since pre-
355 industrial times; 2) relative and absolute abundances of *Holopedium* have increased over the last
356 3 decades, a time of recent lakewater Ca decline; and 3) declining lakewater Ca concentration is
357 the ultimate driver explaining the *Holopedium* rise, though changes in TP, hypolimnetic anoxia
358 and invading *Bythotrephes* may be contributing causes in some lakes.

359 *Drivers of increased relative and absolute abundances*

360 *Holopedium* abundance increased principally because Ca-rich daphniid populations fell
361 with declining lakewater Ca. The survival, growth, development and reproduction of daphniids is
362 known to be reduced at Ca levels below 1.5-2 mg·L⁻¹ [49,50]. Delayed maturation at low Ca
363 consequently limits the population growth of daphniids consistent with our “declining
364 populations” mechanism. Daphniids are also more vulnerable to at least one key predator
365 (*Chaoborus*) at low Ca because their ability to produce anti-*Chaoborus* defences (larger bodies,
366 more rigid carapaces and projecting neck teeth) is compromised [50]. Thus, greater predation on
367 daphniids at low Ca, predicted by our “increasing predation” mechanism, likely further increased
368 the relative abundance of *Holopedium*.

369 Our SEM also suggests that “less competition” for resources with other herbivorous
370 zooplankton, principally daphniids, is linked to increasing absolute abundances of *Holopedium*
371 as Ca levels decline. Competition between *Holopedium* and *Daphnia* is well documented
372 [6,7,8,14], and a comparison of recent daphniid and *Holopedium* fecundity supports the
373 competition mechanism. In the regional-scale monitoring survey, the average clutch size of
374 *Holopedium* was twice that of its most common daphniid competitors (figure S2), suggesting it
375 was better able to secure the limiting food resources that reproduction requires. Among
376 daphniids, only the relatively rare *D. dentifera* and *D. pulicaria*, which are much larger and thus
377 more likely controlled by fish predation equalled *Holopedium*'s fecundity.

378 Declining TP levels did not support our "more food" mechanism, but they could be
379 promoting greater absolute abundances of *Holopedium* by reducing the competitive effects of
380 *Daphnia*. First, daphniids likely have to eat more than *Holopedium* to get the P they need,
381 because their P content is twice as high [12,13]. This should make them more vulnerable than
382 *Holopedium* to falling food levels, particularly if they are poorer competitors. Thus, the

383 competitive effects of daphniids may further depend on whether food supplies exceed the levels
384 required to meet basic metabolic needs. *Holopedium*'s advantage could also be enhanced if algal
385 P content declines with falling TP levels [51]. Additional data on nutritional thresholds and
386 phytoplankton community composition would help to test these explanations.

387 Many environmental changes other than Ca decline influence zooplankton, though they
388 are unlikely to be the dominant factor explaining the rise of *Holopedium*. For example, the SEM
389 suggests that recent declines in hypolimnetic oxygen levels due to climate warming [25] can
390 promote abundances of *Chaoborus*, which prey more heavily on daphniids than *Holopedium*
391 ("increasing predation" mechanism). However, predation risk is not increasing universally, as
392 factors such as the spread of the piscivorous bass across Ontario [52] may lower the abundance
393 of some planktivores, in turn reducing pressure on both *Daphnia* and *Holopedium*. Levels of
394 coloured DOC [17,18] have also been rising in many Ontario lakes, though we found no
395 evidence in our SEM that this has been large enough to benefit *Holopedium*. *Holopedium*
396 abundances have increased over a broad range of DOC concentrations in both Ontario and Nova
397 Scotia (1.4-12.7 mg·L⁻¹) [4,5], so high DOC is clearly not a requirement of *Holopedium*
398 increases. While our survey data also suggest the *Bythotrephes* invasion is promoting
399 *Holopedium* (figure 2), *Holopedium* have risen in the majority of our study lakes in the absence
400 of this invasion.

401 One factor we excluded from our SEM was pH, because lake acidity was an unlikely
402 factor in the observed increases in *Holopedium*. Although *Holopedium* is much more acid-
403 tolerant than *Daphnia* [14,53], and its relative abundance increased in Nova Scotia lakes that
404 declined in pH [22], there have also been widespread reductions in lake acidity in south-central

405 Ontario [34]. We might have expected increases in *Daphnia* abundance at the expense of
406 *Holopedium* in Ontario, if lake acidity was the key driver, but the reverse was observed.
407 *Consequences of an increasingly jellied future*

408 Our results signal a shift in the ecological state of temperate softwater lakes of eastern
409 North America. *Holopedium* is widespread in these lakes [54], and we have shown both its
410 relative and absolute abundances are increasing with widespread Ca decline. This will likely
411 reduce vertical energy and nutrient transport in lake food webs [55]. Both the declining
412 availability of daphniid prey, and the relative ease with which planktivorous fish consume
413 *Holopedium* [56], suggest that this taxon will increasingly dominate the diet of zooplanktivorous
414 fish. Although *Holopedium* and daphniids have similar fatty acid content and composition [57],
415 *Holopedium* has much lower P and Ca content than its daphniid competitors [11,12,13]. Thus,
416 with its increasing dominance, fewer essential nutrients will be transferred to planktivorous fish
417 [58]. The co-occurring copepods have low P content matching *Holopedium* [12,59]. These
418 changes may also be quite long-lasting if acid deposition rates continue to exceed mineralogical
419 weathering rates [60,61], reducing exchangeable Ca levels in soils, and ultimately in downstream
420 waters.

421 As Ca declines, the rise in the absolute abundance of *Holopedium* may also have
422 socioeconomic consequences, because increased concentrations of pelagic jelly may impede the
423 withdrawal of lake water for residential, municipal and industrial uses. The costs of operating
424 infrastructure for water users will therefore rise as Ca continues to decline and densities of filter-
425 clogging jelly-capsules increase [62]. In Ontario, 20% of government-monitored drinking water
426 systems draw only from surface waters within landscapes containing lakes with Ca
427 concentrations $\leq 3.5 \text{ mg} \cdot \text{L}^{-1}$ (figure S4), levels that favour *Holopedium* (figure S3). Our results

428 show that the continued jellification of pelagic food webs, due to ongoing declines in lakewater
429 Ca, and exacerbated by other environmental stressors, has the potential to negatively impact the
430 functioning of, and services provided by, temperate lakes in eastern North America.

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435 REFERENCES

- 436 1. Stoddard JL *et al.* 1999 Regional trends in aquatic recovery from lake acidification in North
437 America and Europe. *Nature* **401**: 575-578.
- 438 2. Skjelkvåle BL *et al.* 2005 Regional scale evidence for improvements in surface water
439 chemistry 1990-2001. *Environ. Pollut.* **137**, 165-176.
- 440 3. Jeziorski A *et al.* 2008 The widespread threat of calcium decline in fresh waters. *Science* **322**,
441 1374-1377.
- 442 4. Jeziorski A, Paterson AM, Smol JP. 2012 Changes since the onset of acid deposition among
443 calcium-sensitive cladoceran taxa within softwater lakes of Ontario, Canada. *J Paleolimnol.*
444 **48**, 323-337.
- 445 5. Korosi JB, Smol JP. 2012 A comparison of present-day and pre-industrial cladoceran
446 assemblages from softwater Nova Scotia (Canada) lakes with different regional acidification
447 histories. *J. Paleolimnol.* **47**, 43-54.
- 448 6. Hessen DO. 1985 Filtering structures and particle size selection in coexisting Cladocera.
449 *Oecologia* **66**, 368-372.

- 450 7. Persaud AD, Dillon PJ, Lasenby D, Yan ND. 2009 Stable isotope variability of meso-
451 zooplankton along a gradient of dissolved organic carbon. *Freshw. Biol.* **54**, 1705-1719.
- 452 8. Allan JD. 1973 Competition and the relative abundances of two cladocerans. *Ecology* **54**,
453 484-498.
- 454 9. Rowe CL, Adamowicz SJ, Hebert PDN. 2007 Three new cryptic species of the freshwater
455 zooplankton genus *Holopedium* (Crustacea: Branchiopoda: Ctenopoda), revealed by genetic
456 methods. *Zootaxa* **1656**, 1-49.
- 457 10. Beaton MJ, Hebert PDN. 1989 Miniature genomes and endopolypoidy in cladoceran
458 crustaceans. *Genome* **32**, 1048-1053.
- 459 11. Jeziorski A, Yan ND. 2006 Species identity and aqueous calcium concentrations as
460 determinants of calcium concentrations of freshwater crustacean zooplankton. *Can. J. Fish.*
461 *Aquat. Sci.* **63**, 1007-1013.
- 462 12. Andersen T, Hessen DO. 1991 Carbon, nitrogen, and phosphorus content of freshwater
463 zooplankton. *Limnol. Oceanogr.* **36**, 807-814.
- 464 13. McCarthy V, Irvine K. 2010 A test of stoichiometry across six Irish lakes of low-moderate
465 nutrient status and contrasting hardness. *J. Plankton Res.* **32**, 15-29.
- 466 14. Hessen DO, Faafeng BA, Andersen T. 1995 Competition or niche segregation between
467 *Holopedium* and *Daphnia*; empirical light on abiotic key parameters. *Hydrobiol.* **307**, 253-
468 261.
- 469 15. McQueen DJ, Ramcharan CW, Yan ND. 2001 Summary and emergent properties - Part 12 of
470 the Dorset food web piscivore manipulation project. *Arch. Hydrobiol. Spec. Issues Advanc.*
471 *Limnol.* **56**, 257-288.

- 472 16. Wissel B, Boeing WJ, Ramcharan CW. 2003 Effects of water color on predation regimes and
473 zooplankton assemblages in freshwater lakes. *Limnol. Oceanogr.* **46**, 1965-1976.
- 474 17. Palmer ME, Yan ND, Paterson AM, Girard RE. 2011 Water quality changes in south-central
475 Ontario lakes and the role of local factors in regulating lake response to regional stressors.
476 *Can. J. Fish. Aquat. Sci.* **68**, 1038-1050.
- 477 18. Monteith DT *et al.* 2007 Dissolved organic carbon trends resulting from changes in
478 atmospheric deposition chemistry. *Nature* **450**, 537-541.
- 479 19. Kerr JG, Eimers MC. 2012 Decreasing soil water Ca²⁺ reduces DOC adsorption in mineral
480 soils: Implications for long-term DOC trends in an upland forested catchment in southern
481 Ontario, Canada. *Sci. Total Environ.* **427-428**, 298-307.
- 482 20. Kim N, Walseng B, Yan ND. 2012 Will environmental calcium declines in Canadian Shield
483 lakes help or hinder *Bythotrephes* establishment success? *Can. J. Fish. Aquat. Sci.* **69**, 810-
484 820.
- 485 21. Kim N, Yan ND. 2010 Methods for rearing the invasive zooplankter *Bythotrephes* in the
486 laboratory. *Limnol. Oceanogr. Methods* **8**, 552-561.
- 487 22. Clair TA, Dennis IF, Vet R. 2011 Water chemistry and dissolved organic carbon trends in
488 lakes from Canada's Atlantic Provinces: no recovery from acidification measured after 25
489 years of lake monitoring. *Can. J. Fish. Aquat. Sci.* **68**, 663-674.
- 490 23. Korosi JB, Ginn BK, Cumming BF, Smol JP. 2013 Establishing past environmental
491 conditions and tracking long-term environmental change in the Canadian Maritime provinces
492 using lake sediments. *Environ. Rev.* **21**, 15-27.

- 493 24. Wissel B, Yan N, Ramcharan C. 2003 Predation and refugia: implications for *Chaoborus*
494 abundance and species composition. *Freshw. Biol.* **48**, 1421–1431.
- 495 25. Stefan HG, Hondzo M, Fang X, Eaton JG, McCormick JH. 1996 Simulated long-term
496 temperature and dissolved oxygen characteristics of lakes in the north-central United States
497 and associated fish habitat limits. *Limnol. Oceanogr.* **41**, 1124-1135.
- 498 26. Vinyard GL, Menger RA. 1980 *Chaoborus americanus* predation on various zooplankters;
499 functional response and behavioral observations. *Oecologia* **45**, 90-93.
- 500 27. Shipley B. 2000 *Cause and Correlation in Biology*. Cambridge: Cambridge University Press.
- 501 28. Shipley B. 1999 Testing causal explanations in organismal biology: causation, correlation
502 and structural equation modelling. *Oikos* **86**, 374-382.
- 503 29. Tanentzap AJ *et al.* 2014 Identifying pathways for managing multiple disturbances to limit
504 plant invasions. *J. Appl. Ecol.* **51**, 1015-1023.
- 505 30. Smol JP. 2008 *Pollution of lakes and rivers: A paleoenvironmental perspective*. 2nd edn.
506 Oxford: Wiley-Blackwell Publishing.
- 507 31. Korosi JB, Smol JP. 2012 An illustrated guide to the identification of cladoceran subfossils
508 from lake sediments in northeastern North America: part 1—the Daphniidae, Leptodoridae,
509 Bosminidae, Polyphemidae, Holopedidae, Sididae, and Macrothricidae. *J. Paleolimnol.* **48**,
510 571-586.
- 511 32. Korosi JB, Smol JP. 2012 An illustrated guide to the identification of cladoceran subfossils
512 from lake sediments in northeastern North America: part 2—the Chydoridae. *J. Paleolimnol.*
513 **48**, 587-622.

- 514 33. Kurek J, Korosi JB, Jeziorski A, Smol JP. 2010 Establishing reliable minimum count sizes
515 for cladoceran subfossils sampled from lake sediments. *J. Paleolimnol.* **44**, 603-612.
- 516 34. Yan ND *et al.* 2008 Long-term trends in zooplankton of Dorset, Ontario, lakes: the probable
517 interactive effects of changes in pH, total phosphorus, dissolved organic carbon, and
518 predators. *Can. J. Fish. Aquat. Sci.* **65**, 862-877.
- 519 35. Palmer ME, Yan ND. 2013 Decadal-scale regional changes in Canadian freshwater
520 zooplankton: the likely consequence of complex interactions among multiple anthropogenic
521 stressors. *Freshw. Biol.* **58**, 1366-1378
- 522 36. Paterson AM *et al.* 2008 Long-term changes in phytoplankton composition in seven
523 Canadian Shield lakes in response to multiple anthropogenic stressors. *Can. J. Fish. Aquat.*
524 *Sci.* **65**, 846–861.
- 525 37. Yan N, Nero R, Keller W, Lasenby D. 1985 Are *Chaoborus* larvae more abundant in
526 acidified than in non-acidified lakes in central Canada. *Holarct. Ecol.* **8**, 93–99.
- 527 38. Hopkins GE, Standke SJ. 1992 Phytoplankton methods manual: with special emphasis on
528 waterworks operation internal methods manual. Queen's Printer for Ontario, Toronto, ON.
529 ISBN 0-7729-8923-0.
- 530 39. Lynch M. 1979 Predation, competition, and zooplankton community structure: An
531 experimental study. *Limnol. Oceanogr.* **24**, 253-272.
- 532 40. Cairns A. 2010 Field assessments and evidence of impact of calcium decline on *Daphnia*
533 (Crustacea, Anomopoda) in Canadian Shield lakes M.Sc. Thesis. York University, Canada.
- 534 41. Leinster T, Cobbold CA. 2012 Measuring diversity: the importance of species similarity.
535 *Ecology* **93**, 477-489.

- 536 42. Geller W, Müller H. 1981 The filtration apparatus of Cladocera: Filter mesh-sizes and their
537 implications on food selectivity. *Oecologia*. **49**, 316-321.
- 538 43. Ramcharan C *et al.* 2001 Complex responses of *Chaoborus* to changes in fish populations.
539 *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* **56**, 81–100.
- 540 44. Stan Development Team. 2012 *Stan Modeling Language: User's Guide and Reference*
541 *Manual. Version 2.0.* See <http://mc-stan.org/>.
- 542 45. R Development Core Team. 2013 *R: A Language and Environment for Statistical*
543 *Computing.* Vienna: R Foundation for Statistical Computing. See <http://www.R-project.org/>.
- 544 46. Gelman A, Pardoe I. 2006 Bayesian measures of explained variance and pooling in
545 multilevel (hierarchical) models. *Technometrics* **48**, 241–251.
- 546 47. Grace JB *et al.* 2012 Guidelines for a graph-theoretic implementation of structural equation
547 modeling. *Ecosphere*. **3**, art73.
- 548 48. Yao H, McConnell C, Somers KM, Yan ND, Watmough S, Scheider W. 2011 Nearshore
549 human interventions reverse patterns of decline in lake calcium budgets in central Ontario as
550 demonstrated by mass-balance analyses. *Water Resour. Res.* 47, W06521,
551 doi:10.1029/2010WR010159
- 552 49. Ashforth D. Yan ND. 2008 The interactive effects of calcium concentration and temperature
553 on the survival and reproduction of *Daphnia pulex* at high and low food concentrations.
554 *Limnol. Oceanogr.* **53**, 420-432
- 555 50. Riessen HP *et al.* 2012 Changes in water chemistry can disable plankton prey defenses. *Proc.*
556 *Natl. Acad. Sci. U. S. A.* **109**, 15377–15382.

- 557 51. Painter DS, Kamaitis G. 1987 Reduction of Cladophora biomass and tissue phosphorus in
558 Lake Ontario, 1972-1983. *Can. J. Fish. Aquat. Sci.* **44**, 2212-2215.
- 559 52. Vander Zanden MJ, Olden JD, Thorne JH, Mandrak NE. 2004 Predicting occurrences and
560 impacts of smallmouth bass introductions in north temperate lakes. *Ecol. Appl.* **14**, 132-148.
- 561 53. Keller W, Pitblado JR. 1984 Crustacean plankton in Northeastern Ontario lakes subjected to
562 acidic deposition. *Water Air Soil Pollut.* **23**, 271-291.
- 563 54. Merrix-Jones FL, Thackeray SJ, Ormerod SJ. 2013 A global analysis of zooplankton in
564 natural and artificial fresh waters. *J. Limnol.* **72**, 140-153
- 565 55. Ramcharan CW *et al.* 2001 Analyses of lake food webs using individual-based models to
566 estimate *Chaoborus* production and consumption. *Arch. Hydrobiol. Spec. Issues Advanc.*
567 *Limnol.* **56**, 101–126.
- 568 56. Arts MT, Sprules WG. 1989 Use of enclosures to detect the contribution of particular
569 zooplankton to growth of young-of-the-year yellow perch (*Perca flavescens* Mitchell).
570 *Oecologia* **81**, 21-27.
- 571 57. Persson J, Vrede T. 2006 Polyunsaturated fatty acids in zooplankton: variation due to
572 taxonomy and trophic position. *Freshw. Biol.* **51**, 887-900.
- 573 58. Malzahn A, Aberle N, Clemmesen C, Boersma M. 2007 Nutrient limitation of primary
574 producers affects planktivorous fish condition. *Limnol. Oceanogr.* **52**, 2062-2071.
- 575 59. Sterner RW, Elser JJ, Hessen DO. 1992 Stoichiometric relationships among producers,
576 consumers and nutrient cycling in pelagic ecosystems. *Biogeochemistry.* **17**, 49-67.

- 577 60. Whitfield CJ, Watmough SA, Aherne J, Dillon PJ. 2006 A comparison of weathering rates
578 for acid-sensitive catchments in Nova Scotia, Canada and their impact on critical load
579 calculations. *Geoderma* **136**, 899-911.
- 580 61. Watmough SA, Aherne J. 2008 Estimating calcium weathering rates and future lake calcium
581 concentrations in the Muskoka-Haliburton region of Ontario. *Can. J. Fish. Aquat. Sci.* **65**,
582 821-833.
- 583 62. Thelen AC. 2012 Population dynamics of *Holopedium gibberum* in a Pacific Northwest
584 drinking water reservoir: effects of temperature, food, and competition. M.Sc. Thesis.
585 University of Washington, USA.
586

587 **FIGURE CAPTIONS**

588 **Figure 1.** A handful of *Holopedium* collected in a <10 m horizontal net haul from Plastic Lake,
589 ON, Canada. Photo by Ron Ingram, Aug 2011. (Inset) *Holopedium* painting by G.O. Sars
590 (Property of the National Library of Norway), with arrows demarcating the margins of the jelly
591 capsule.

592 **Figure 2.** Temporal increases in *Holopedium* across two lake districts. Change in relative
593 abundance of *Holopedium* since pre-industrial times in the sedimentary pelagic cladoceran
594 assemblages of (a) 36 lakes in south-central Ontario and (b) 48 lakes in Nova Scotia, Canada
595 (*Holopedium* remains were absent from 1 and 25 of the lakes, respectively). Change in (c)
596 relative abundance of *Holopedium* in the planktonic cladoceran assemblage and (d) absolute
597 abundance of *Holopedium* from the 1980s to 2004/5 in 31 south-central Ontario lakes, 5 of
598 which were invaded by *Bythotrephes* between the two surveys.

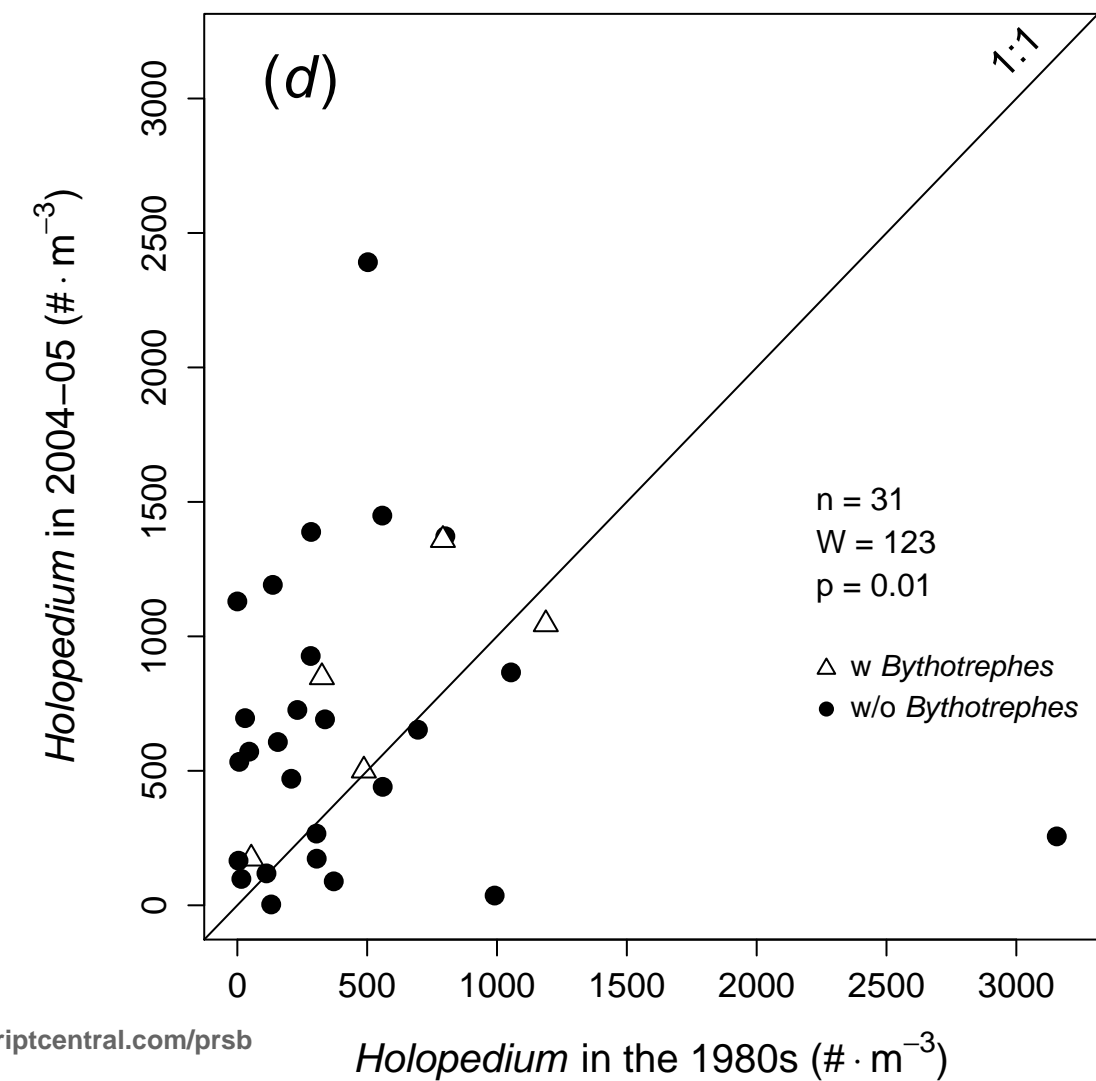
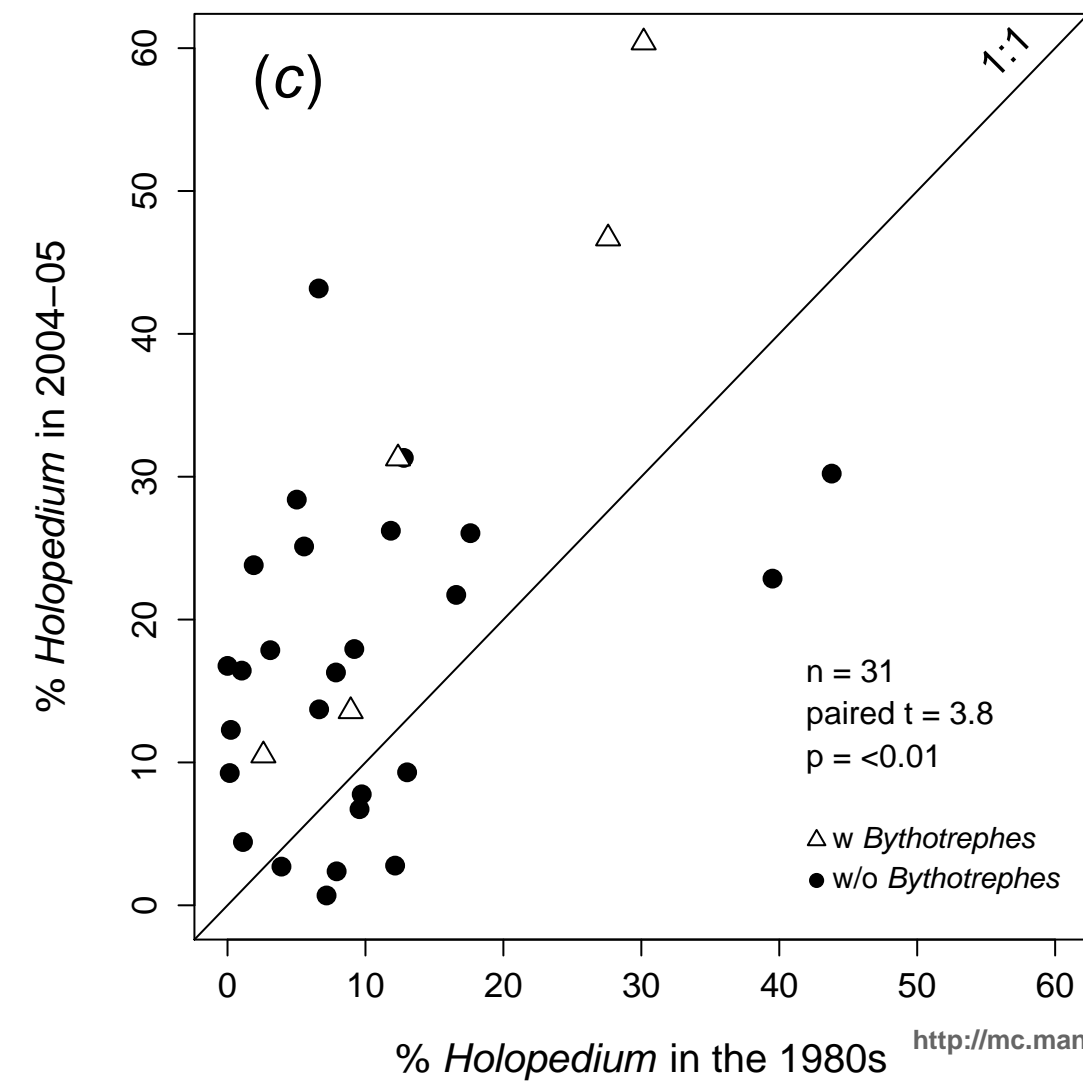
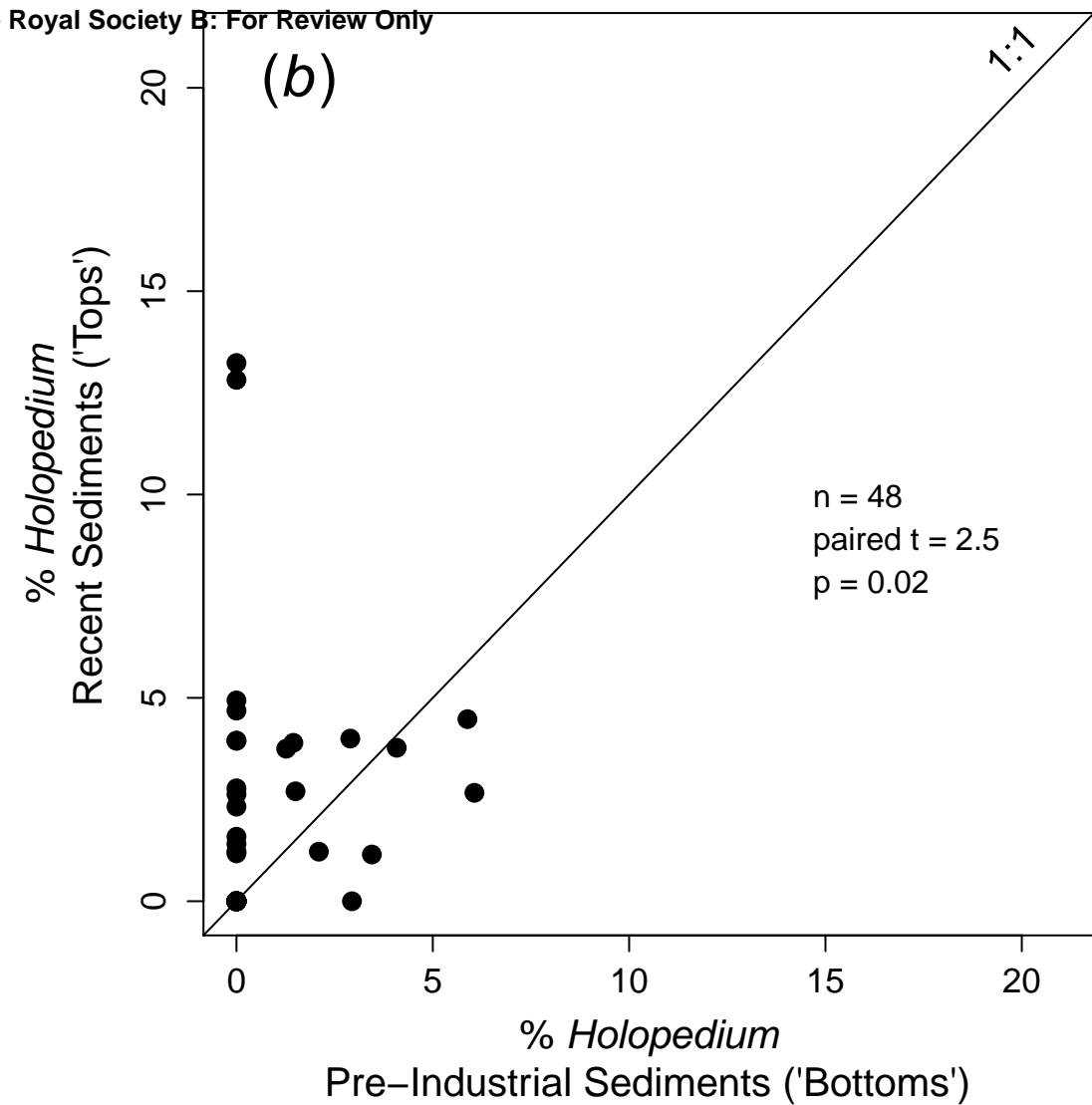
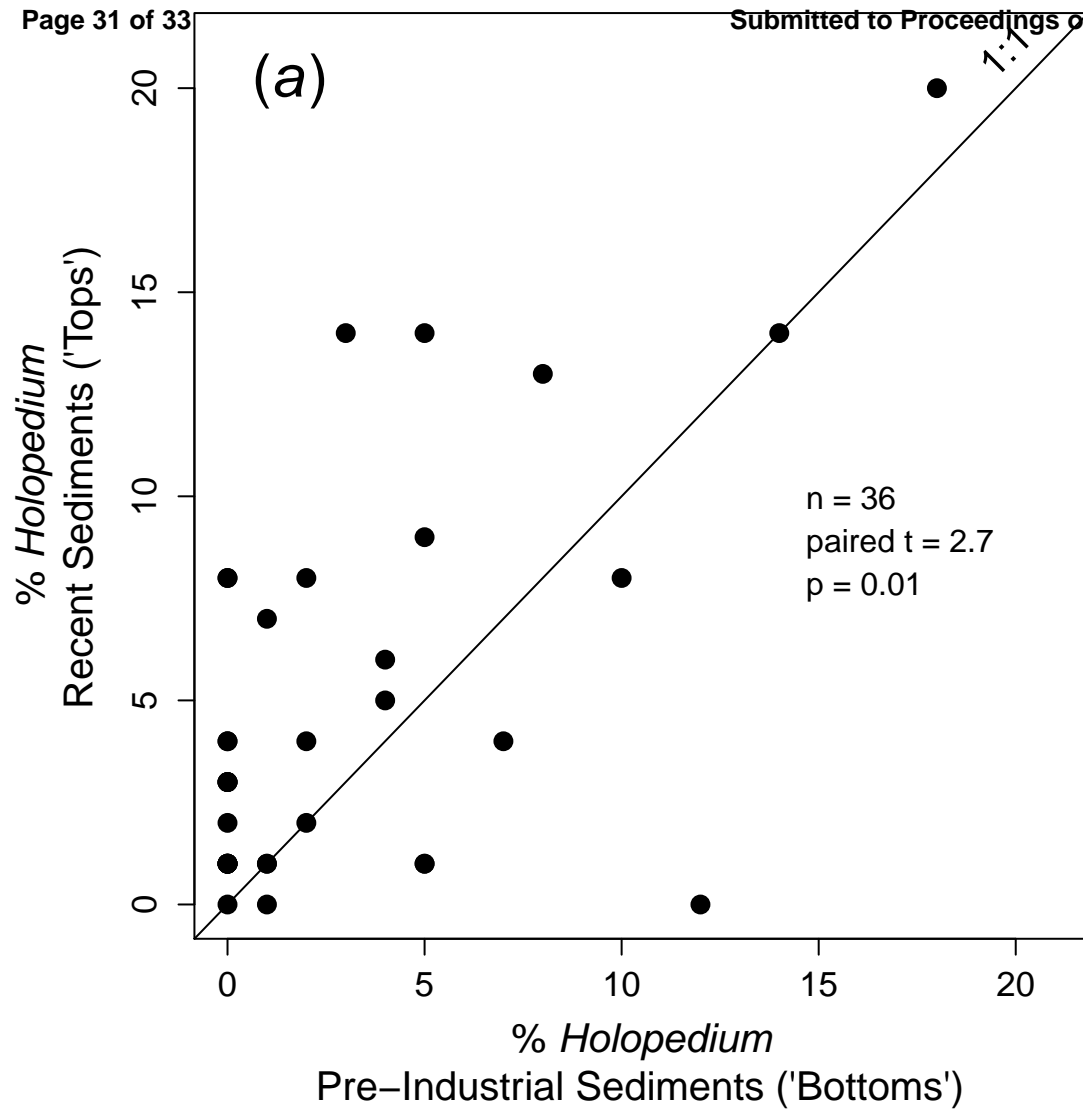
599 **Figure 3.** Effects of Ca decline on abundances of large Cladocera in eight lakes in south-central
600 Ontario, Canada, from 1980-2009. (a) Declines in mean Ca \pm SE (gray) across the study lakes
601 and increases in mean relative abundances of *Holopedium* \pm 95% CIs summed across the eight
602 lakes (black). (b) Temporal changes (mean \pm 95% CIs) in relative abundances of Ca-rich (black)
603 and Ca-poor daphniids (gray).

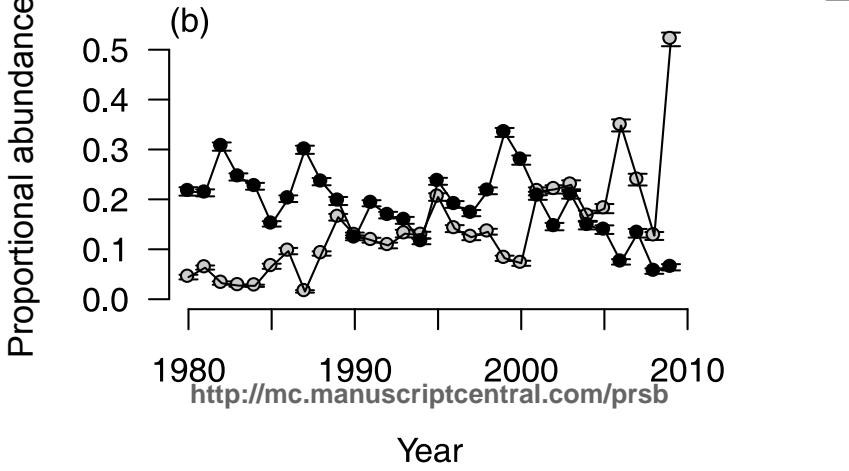
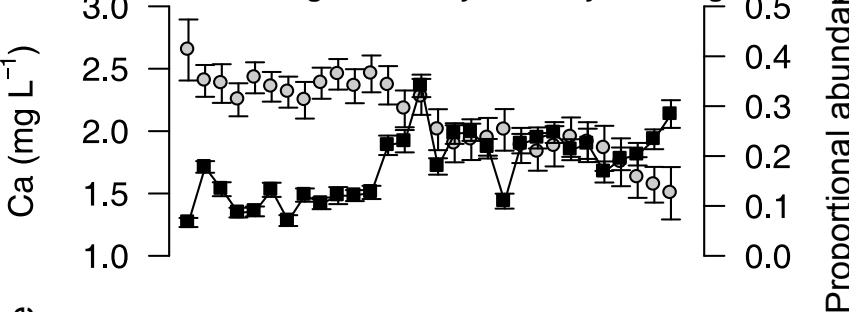
604 **Figure 4.** Structural equation model predicting causal linkages among lakewater Ca and
605 Cladocera groups. Directional arrows denote direct effects of one variable on another and are
606 proportional to effect size (see legend). Four alternate and mutually-inclusive mechanisms
607 explaining increased abundances of *Holopedium* are associated with broken boxes. These test

608 whether *Holopedium* are more abundant because they have more access to food, associated with:
609 (i) increased predation by *Chaoborus* on *Daphnia* due to larger refuge from fish predation; (ii)
610 declines in competition from lower abundances of Ca-rich and Ca-poor *Daphnia* due to
611 lakewater Ca, accounting for compositional changes in *Daphnia* (Ca-rich diversity) and copepod
612 biomass; (iii) eutrophication; and/or (iv) because other cladocera have become rarer. Bayesian R^2
613 measuring model fit to *Holopedium*, Ca-rich and Ca-poor daphniids, phytoplankton, and
614 *Chaoborus* measurements = 0.99, 0.65, 0.60, 0.50, and 0.75, respectively (see electronic
615 supplementary material, figure S3).



Figure 1. A handful of *Holopedium* collected in a <10 m horizontal net haul from Plastic Lake, ON, Canada. Photo by Ron Ingram, Aug 2011. (Inset) *Holopedium* painting by G.O. Sars (Property of the National Library of Norway), with arrows demarcating the margins of the jelly capsule.
254x190mm (150 x 150 DPI)





(i) Increasing predation



Lakewater Ca

Chaoborus

(ii) Less competition



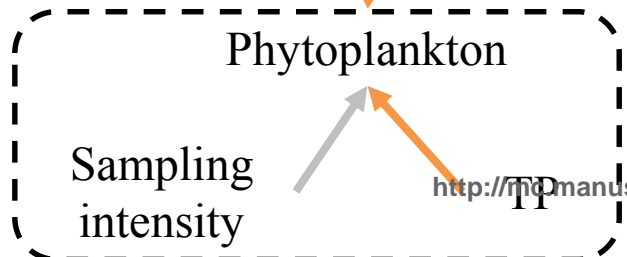
(iv) Declining populations



Probability of *Holopedium*

Holopedium

(iii) More food



Effect direction
negative
 neutral
 positive

Absolute effect size

—	0.0 – 1.0
—	1.0 – 4.0
—	4.0 – 8.0