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THE ROYAL SOCIETY
PUBLISHING

The jellification of north temperate lakes

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

1. Introduction

The ecological impacts associated with calcium (Ca) decline are a growing
concern in aquatic ecosystems of eastern North America and northern Europe
[1–3]. This is especially true in softwater lakes where abundances of Ca-rich
Daphnia (Crustacea, Cladocera), often the dominant and keystone herbivores in
the pelagic zone, are declining [3–5]. However, not all taxa suffer when Ca
declines. Daphniids compete for food resources with another large cladoceran,
Holopedium glacialis [6–8] (previously *H. gibberum* [9] and hereafter called *Holope-
dium*). Because *Daphnia* has a heavily calcified carapace supported by a dense
network of phosphorus-rich, polyploid cells [10], it has high Ca and phosphorus
(P) needs. *Holopedium* lacks such a carapace, giving it about one-tenth the Ca [11]
and one-half the P [12,13] requirements of daphniids. This likely explains why
Holopedium commonly outcompetes *Daphnia* in softwater, oligotrophic lakes
[14]. *Holopedium* also sports a mucopolysaccharide jelly capsule that largely pro-
tects it from macro-invertebrate predators (figure 1), including the larval
phantom midge, *Chaoborus*, and the recent Eurasian invader, *Bythotrephes longi-
manus* (the spiny water flea), both of which can be major energy conduits to
fish [15], and to which most daphniid species are vulnerable [8,16].

Here we report the replacement of previously dominant Ca-rich daphniids by
Holopedium in two major lake regions of Canada separated by approximately

Figure 1. A handful of *Holopedium* collected in a less than 10 m horizontal net haul from Plastic Lake, Ontario, Canada. Photo by Ron Ingram, August 2011. (Inset) *Holopedium* painting by G.O. Sars (property of the National Library of Norway), with arrows demarcating the margins of the jelly capsule. (Online version in colour.)

1500 km, i.e. south-central Ontario and Nova Scotia. In recent decades, these Ontario lakes have changed in many ways: Ca [3] and P concentrations [17] have declined in response to changes in climate and acid deposition, coloured dissolved organic carbon (DOC) has increased [16,18] partly because increasingly base-poor catchments are retaining less organic matter [19], and *Bythotrephes* has been introduced and is spreading [20]. *Bythotrephes* reduces populations of most daphniids more than it does that of *Holopedium* [21]. The Nova Scotia lakes have experienced similar acid deposition and Ca trends [22,23], but they have not, as yet, been invaded by *Bythotrephes*.

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

(1) *Holopedium* abundances have increased since pre-industrial times. We tested this hypothesis using sedimentary cladoceran assemblages from 84 Ontario and Nova Scotia lakes (see electronic supplementary material, table S1).
(2) Relative and/or absolute abundances of *Holopedium* have increased during the period of recent lakewater Ca decline. We tested this hypothesis by surveying pelagic zooplankton in 31 Ontario lakes between 1981 and 1990, and resampling from 2004 to 2005, and with a longitudinal study of eight other Ontario lakes that were sampled monthly for 30 years (see electronic supplementary material, tables S2 and S3).

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

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

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

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

Increasing predation mechanism: *Holopedium* increased in absolute and/or relative abundance because they suffered less than daphniids from increasing predator densities. *Chaoborus* spp., the primary native invertebrate predator in our lakes [15], should benefit from temporal increases in the volume of their refuge from fish predation [24]. We predicted greater refuge volume would accompany increased hypolimnetic oxygen depletion associated with climate warming [25] and/or greater coloured DOC concentrations [24], both of which might reduce predation from fish. As *Chaoborus* preferentially select *Daphnia* over *Holopedium* [26], greater *Chaoborus* densities should thus increase the relative abundance of *Holopedium*. Predation could also promote greater absolute abundances of *Holopedium* if reductions in *Daphnia* reduce competition for food.

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

2. Material and methods

(a) Have *Holopedium* abundances increased since pre-industrial times?

We examined long-term changes in the relative abundance of *Holopedium* within the pelagic cladoceran assemblages in lake sediments from 36 south-central Ontario lakes in 2007 [4], and 48 Nova Scotia lakes in 2002–2004 [5] (see electronic supplementary material, table S1). Sediment cores were collected from the deepest basin of each lake using a gravity corer, and sectioned at 0.25 cm intervals. We then employed the well-established 'top/bottom' palaeolimnological sampling design [30], wherein the chitinous cladoceran remains were isolated, identified and compared between a 'top' interval (surface sediments) representing present-day assemblages and a 'bottom' interval (sediment depth > 15.0 cm) representing assemblages present prior to the onset of lake acidification. Although this protocol cannot document the timing of changes, it clearly separates modern from historical assemblages, which is what the test of our first hypothesis required.

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

sediment datasets. We excluded lakes where *Holopedium* was absent from both intervals.

(b) Are *Holopedium* increases consistent with recent lakewater Ca decline?

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

To test whether the relative and absolute abundance of *Holopedium* had increased in the 31 lake regional dataset, we compared the 1980s versus 2004–2005 ice-free means using, respectively, a paired *t*-test and a Wilcoxon signed-rank test. The five lakes that were invaded by *Bythotrephes* between the two sampling periods were analysed separately. For the eight lake longitudinal dataset, we used Mann–Kendall trend tests to identify whether the absolute and relative abundances of *Holopedium*, daphniids and lakewater Ca concentrations had changed in a monotonic fashion over time when averaged across lakes in each year of the dataset. Prior to averaging absolute abundances, we standardized observations to a mean of 0 and s.d. of 1 across years in each lake.

(c) Discriminating among alternative mechanisms underlying increases in *Holopedium* abundance

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

Using an SEM approach, we then estimated a series of equations (2.1)–(2.6) describing a causal network that simultaneously tested our four hypothesized mechanisms.

(i) Declining populations mechanism

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

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

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

(ii) Less competition mechanism

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

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

where $\alpha^{(1)}$ is the estimated mean probability of observing *Holopedium* across all lakes and years, γ_1 is the effect of food availability, γ_2 is an estimated effect accounting for predation by *Chaoborus* spp. (Chaob$_{ij}$, square-root transformed, individuals m^{-3}) and $\nu_i^{(1)}$ and $\nu_j^{(1)}$ account for random variation among the i lakes and j years and are each drawn from a normal distribution with a mean of 0 and separately estimated standard deviation. Although we did not observe values directly for ξ_{ij}, a latent variable, we parametrized it using observed data.

We assumed that ξ_{ij} was approximately $N(\eta_{ij}, \sigma_\xi)$, and modelled mean food availability η_{ij} in each lake i in year j given abundances of potential *Daphnia* competitors. Exploitative competition for limiting resources, namely food, is a major factor structuring zooplankton communities [14]. Although larger zooplankton species can exclude smaller species because their greater body reserves survive low food periods, this competitive hierarchy depends on how the abundances of species vary with levels of predation and abiotic conditions [39]. For *Daphnia*, the abundances of species, and thus their competitive effects, depend on species-specific Ca requirements [14]. We therefore classified *Daphnia* species into either relatively Ca-rich (*D. dubia*, *D. longiremis*, *D. mendotae*, *D. pulicaria* and *D. retrocurva*) or Ca-poor (*D. ambigua* and *D. catawba*) groups using interspecific differences in body Ca content [11,40] and prevalence thresholds in field surveys [20,40] (electronic supplementary material, figure S1). However, one single metric cannot summarize the responses of all Ca-rich daphniids because body-size and Ca sensitivity still vary within this group (electronic supplementary material, figure S2). We additionally allowed for different responses within this group by summarizing community composition with an index D_{ij} that reflects both the relative abundances of species and their differences in Ca sensitivity [41]. The resulting D_{ij} is an 'effective' diversity measure; e.g. a community with three species and $D_{ij} =$ 2.5 is slightly less diverse than a community of three equally abundant taxa with totally dissimilar Ca requirements (see the electronic supplementary material). This combination of approaches essentially allows both linear and nonlinear interactions between *Holopedium* and *Daphnia* in our model, without assuming any specific effect. Finally, we could not ignore herbivorous copepod species (Cop$_{ij}$), as their biomass (μg dry weight m^{-3})

can account for the majority of zooplankton biomass in our study lakes, and temporal changes in their densities will influence food available for Cladocera [6,42]. η_{ij} was then a function of the probability of observing Ca-poor [$p_{ij}^{(2)}$] and Ca-rich daphniids [$p_{ij}^{(3)}$], the composition of Ca-rich daphniid communities (D_{ij}) and Cop_{ij}

$$\eta_{ij} = \gamma_3 p_{ij}^{(2)} + \gamma_4 p_{ij}^{(3)} + \gamma_{5[i]} D_{ij} + \gamma_6 \mathrm{Cop}_{ij}, \qquad (2.2)$$

where $\gamma_3 - \gamma_6$ are estimated effects and σ_ξ is the estimated s.d.

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

$$\left.\begin{array}{l} y_{ij}^{(2)} \sim B(p_{ij}^{(2)}, N_{ij}) \\[4pt] \text{and} \quad \mathrm{logit}(p_{ij}^{(2)}) = \alpha^{(2)} + \gamma_7 \mathrm{Ca}_{ij} + \gamma_8 \mathrm{Chaob}_{ij} + v_i^{(2)} + v_j^{(2)}, \end{array}\right\}$$
$$(2.3)$$

$$\left.\begin{array}{l} y_{ij}^{(3)} \sim B(p_{ij}^{(3)}, N_{ij}) \\[4pt] \text{and} \quad \mathrm{logit}(p_{ij}^{(3)}) = \alpha^{(3)} + \gamma_9 \mathrm{Ca}_{ij} + \gamma_{10}\mathrm{Ca}_{ij}^2 + \gamma_{11}\mathrm{Chaob}_{ij} + v_i^{(3)} + v_j^{(3)}, \end{array}\right\}$$
$$(2.4)$$

where $\alpha^{(k)}$ is the estimated mean probability of observing individuals of life group k across all lakes and years, γ_7, γ_9, γ_{10} are estimated effects of lake Ca, γ_8 and γ_{11} are estimated effects accounting for the fact that predation by *Chaoborus* influences daphniid abundances, and $v_i^{(k)}$ and $v_j^{(k)}$ account for random variation among the i lakes and j years and are drawn from zero-mean normal distributions with separately estimated s.d. for each source of variation associated with each life group k. We allowed the response of Ca-rich daphniids to lakewater Ca to be nonlinear, as observed in mid-summer surveys of 304 regional lakes [40], by including the quadratic effect $\gamma_9 \mathrm{Ca}_{ij}^2$. There was no reason to do so for Ca-poor taxa as these are not positively associated with lakewater Ca [40].

(iii) More food mechanism

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

$$\left.\begin{array}{l} P_{ij} \sim \mathrm{Pois}(\lambda_{ij}) \\[4pt] \text{and} \quad \log(\lambda_{ij}) = \alpha^{(4)} + \xi_{ij} + \gamma_{12}\mathrm{Nsam\,p}_{ij} + \gamma_{13}\mathrm{TP}_{ij} + v_i^{(4)} + v_j^{(4)}, \end{array}\right\}$$
$$(2.5)$$

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

(iv) Increasing predation mechanism

Finally, we considered whether the relative abundances of *Holopedium* and their competition for food could be changed by *Chaoborus*, a principal predator of *Daphnia*. We modelled the densities of *Chaoborus* spp. (Chaob_{ij}) as a function of the thickness of the hypolimnetic water layer with less than $3\,\mathrm{mg\,l}^{-1}$ O_2 (O_{ij}) and concentration of DOC in lakewater (DOC_{ij}), which both reduce predation from fish [24]

$$\left.\begin{array}{l} \mathrm{Chaob}_{ij} \sim N(\mu_{ij}, \sigma_{\mathrm{Chaob}}) \\[4pt] \text{and} \quad \mu_{ij} = \alpha^{(5)} + \gamma_{14}O_{ij} + \gamma_{15}\mathrm{DOC}_{ij} + v_i^{(5)}, \end{array}\right\}$$
$$(2.6)$$

where $\alpha^{(5)}$ is the estimated mean density of *Chaoborus*, $\gamma_{14}-\gamma_{15}$ are estimated effects of O_2 and DOC and $v_i^{(5)}$ accounts for random variation among the i lakes and is drawn from a zero-mean normal distribution with estimated s.d. *Chaoborus* spp. were measured only in one lake outside of 1986, and so we could not adequately estimate both variation among lakes and years (total $n = 16$). Thus, we only accounted for variation among lakes. For all the other year × lake combinations in equations (2.1), (2.3) and (2.4), we let Chaob_{ij} take on mean observed values.

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

(v) Model estimation

The SEM was estimated within a hierarchical Bayesian framework using Markov chain Monte Carlo (MCMC) sampling by calling Stan v. 2.0 [44] from R v. 3.0 [45]. Four MCMC chains of 1000 iterations were simulated, with a burn-in period of 4000 runs (see the electronic supplementary material for details). We standardized all estimated coefficients to a common scale with a mean of 0 and s.d. of 1, so that their effects were directly comparable. This allowed us to test the relative importance of different causal linkages. To infer effects, we calculated posterior means and 95% credible intervals (CIs) for each parameter by drawing a subset of 800 simulations. We did not reject our predictions about specific causal linkages if 95% CIs excluded zero. To summarize overall model fit, we calculated a Bayesian R^2 at the level of our measured data, analogous to the proportion of variance explained by a model in classical linear regression [46]. We used three approaches to verify convergence of our model and the structure of the SEM was assessed using a graphical modelling approach [47] (see the electronic supplementary material).

3. Results

(a) Have *Holopedium* abundances increased since pre-industrial times?

Across both study regions, the relative contribution of *Holopedium* to planktonic cladoceran assemblages clearly increased since pre-industrial times (i.e. pre-1850 or prior to the onset of acid deposition). These increases were evident in both of our palaeolimnological surveys as the relative abundance of *Holopedium* increased in 25 of the 35 Ontario lakes and 17 of the 23 Nova Scotia lakes in which it was present (paired t-test, $t_{34} = 2.7$, $p = 0.012$ and $t_{22} = 2.7$, $p = 0.013$, respectively; figure 2a,b). Furthermore, *Holopedium* appeared (i.e. was present in the 'top' samples, but was absent from the pre-industrial samples) in 15 Ontario lakes and 11 Nova Scotia lakes, while disappearing from only two and one lakes, respectively, suggesting that increases in absolute abundances have also occurred.

(b) Have *Holopedium* abundances increased during the recent period of lakewater Ca decline?

Holopedium has become more abundant in the 31 south-central Ontario lakes between the 1980s and 2004–2005 as Ca declined
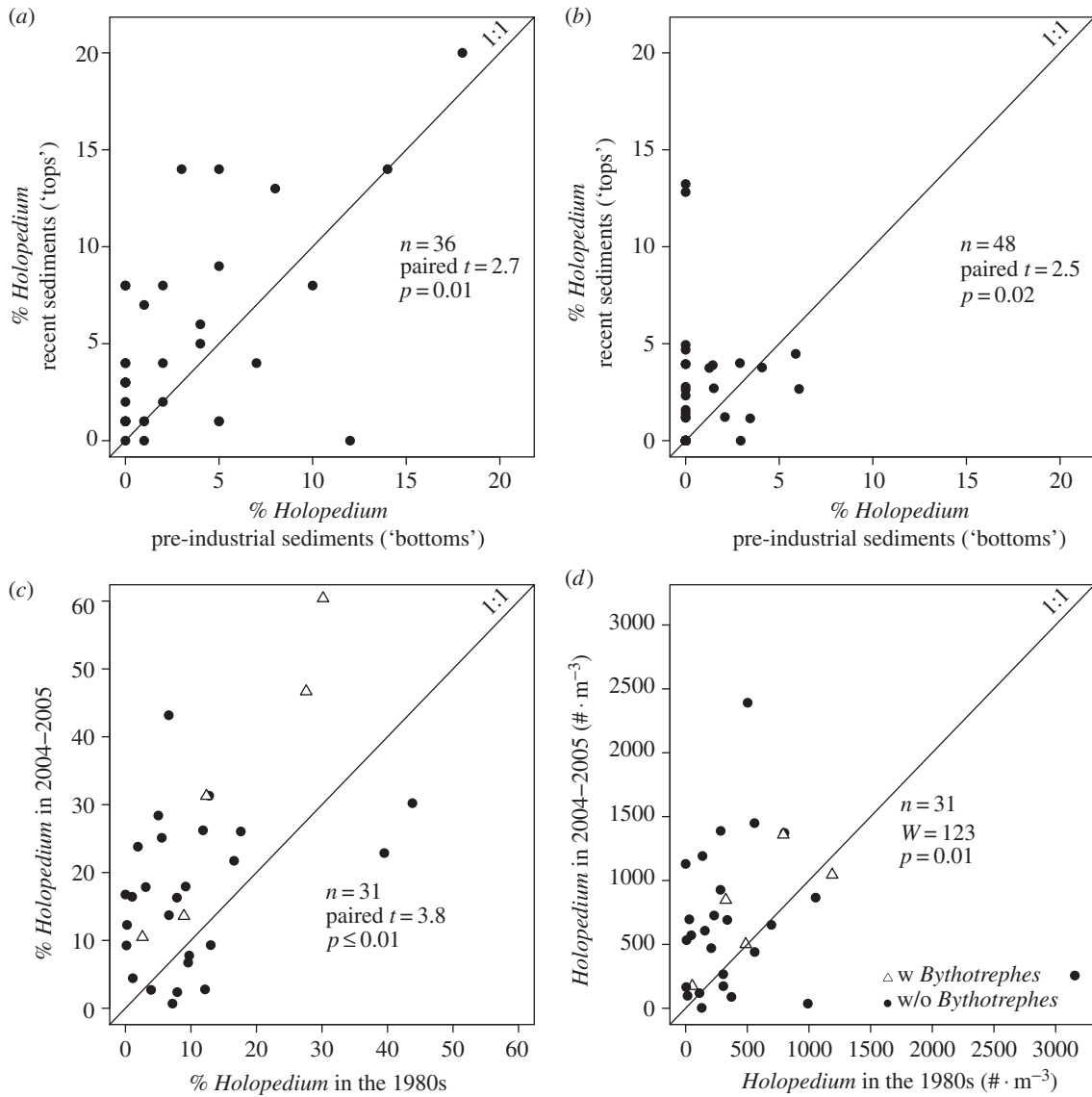
Figure 2. Temporal increases in *Holopedium* in two lake districts. Change in relative abundance of *Holopedium* since pre-industrial times in the sedimentary pelagic cladoceran assemblages of (*a*) 36 lakes in south-central Ontario and (*b*) 48 lakes in Nova Scotia, Canada (*Holopedium* remains were absent from 1 and 25 of the lakes, respectively). Change in (*c*) relative abundance of *Holopedium* in the planktonic cladoceran assemblage and (*d*) absolute abundance of *Holopedium* from the 1980s to 2004/2005 in 31 south-central Ontario lakes, five of which were invaded by *Bythotrephes* between the two surveys.

by a median of 25% [17]. *Holopedium* relative abundances increased in 17 of the 26 lakes that have not been invaded by *Bythotrephes*; these increases ranged from 3 to 37% (paired *t*-test, $t_{25} = 2.8$, $p = 0.009$, figure 2*c*). In each of the five invaded lakes, *Holopedium* was more abundant (by 5–30%) in 2004–2005 than in the 1980s (paired *t*-test, $t_4 = 3.5$, $p = 0.024$, figure 2*c*). The absolute abundance of *Holopedium* also increased; median abundance in the lakes doubled, from 304 animals m^{-3} in the 1980s to 607 animals m^{-3} in 2004–2005 ($W_{30} = 123$, $p = 0.013$, figure 2*d*).

Increasing absolute abundances of *Holopedium* in the eight longitudinal study lakes mirror the trends in the regional dataset. The relative abundance of *Holopedium* increased in these lakes as lakewater Ca declined (figure 3*a*; Mann–Kendall test: $\tau = 0.41$, $p = 0.002$ and $\tau = -0.71$, $p < 0.001$, respectively). This arose partly because there were lower absolute abundances of both Ca-rich daphniids and all other Cladocera as Ca declined ($\tau = -0.48$ and -0.55, respectively; $p < 0.001$ for both). Greater absolute abundances of the two Ca-poor daphniid species over this period ($\tau = 0.50$, $p < 0.001$; figure 3*b*) could not offset these

declines, i.e. the trend in absolute abundance of all non-*Holopedium* Cladocera was negative ($\tau = -0.48$, $p < 0.001$). The increased relative abundance of *Holopedium* in the longitudinal study lakes was also attributable to an increase in its absolute abundance in Harp, Heney and Red Chalk Lakes from 1981 to 2009 ($\tau > 0.37$, $p < 0.010$), and in Dickie Lake prior to recent dust suppressant ($CaCl_2$) additions [48], which raised lakewater Ca levels (one-tailed test for increasing trend: $\tau = 0.33$, $p = 0.029$; see electronic supplementary material, table S3).

(c) Discriminating among alternative mechanisms underlying increases in *Holopedium* abundance

Our SEM revealed that the relative rise of *Holopedium* in the eight lakes of the longitudinal study could be explained by both declines in non-*Holopedium* Cladocera ('declining populations' mechanism) and increases in absolute numbers of *Holopedium*. The latter changes were specifically associated with reduced interspecific competition for food and declines
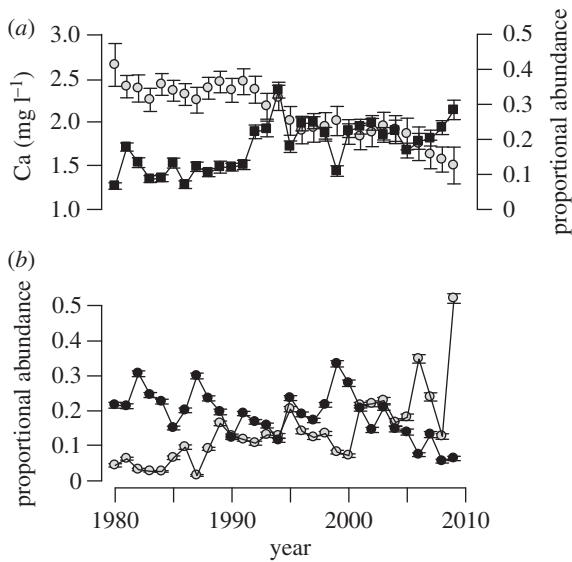
Figure 3. Effects of Ca decline on abundances of large Cladocera in eight lakes in south-central Ontario, Canada, from 1980 to 2009. (*a*) Declines in mean Ca $\pm$ s.e. (grey) across the study lakes and increases in mean relative abundances of *Holopedium* $\pm$ 95% CIs summed across the eight lakes (black). (*b*) Temporal changes (mean $\pm$ 95% CIs) in relative abundances of Ca-rich (black) and Ca-poor daphniids (grey).

in Cladocera predicted by the 'less competition' and 'increasing predation' mechanisms (figure 4).

Falling lakewater Ca was the primary explanation for declines in dominant *Daphnia* spp. that have favoured *Holopedium*. The probability of observing *Daphnia* increased with lakewater Ca in the SEM, with the effect much stronger for Ca-rich as opposed to Ca-poor species (95% CIs: 1.09–1.14 and 0.13–0.25, respectively). Thus, as Ca has fallen over time, so too have Ca-rich daphniids (trends reported above), thereby favouring a greater proportional representation of *Holopedium* within communities (i.e. lower N_{ij} predicted by 'declining populations' mechanism).

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

Although Ca-rich *Daphnia* have declined, the net effect on food availability could be offset by changes associated with their species composition (95% CIs for composition were

negative in five lakes and positive in three; electronic supplementary material, table S4). Thus, overall food availability increased only in two lakes (Heney and Red Chalk), explaining why the absolute number of *Holopedium* has increased in these two lakes but not the others. There was no increase in food availability in Harp Lake despite increases in *Holopedium* reported earlier, because we only included years prior to *Bythotrephes* invasion in the SEM (1980–1992; electronic supplementary material, table S3). Absolute abundances of *Holopedium* only increased when we also considered the years after the invasion.

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

4. Discussion

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

(a) Drivers of increased relative and absolute abundances

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

Our SEM also suggests that 'less competition' for resources with other herbivorous zooplankton, principally daphniids, is linked to increasing absolute abundances of *Holopedium* as Ca levels decline. Competition between *Holopedium* and *Daphnia* is well documented [6–8,14], and a comparison of recent daphniid and *Holopedium* fecundity supports the competition mechanism. In the regional-scale monitoring survey, the average clutch size of *Holopedium* was twice that of its most common daphniid competitors (electronic supplementary material, figure S2), suggesting it
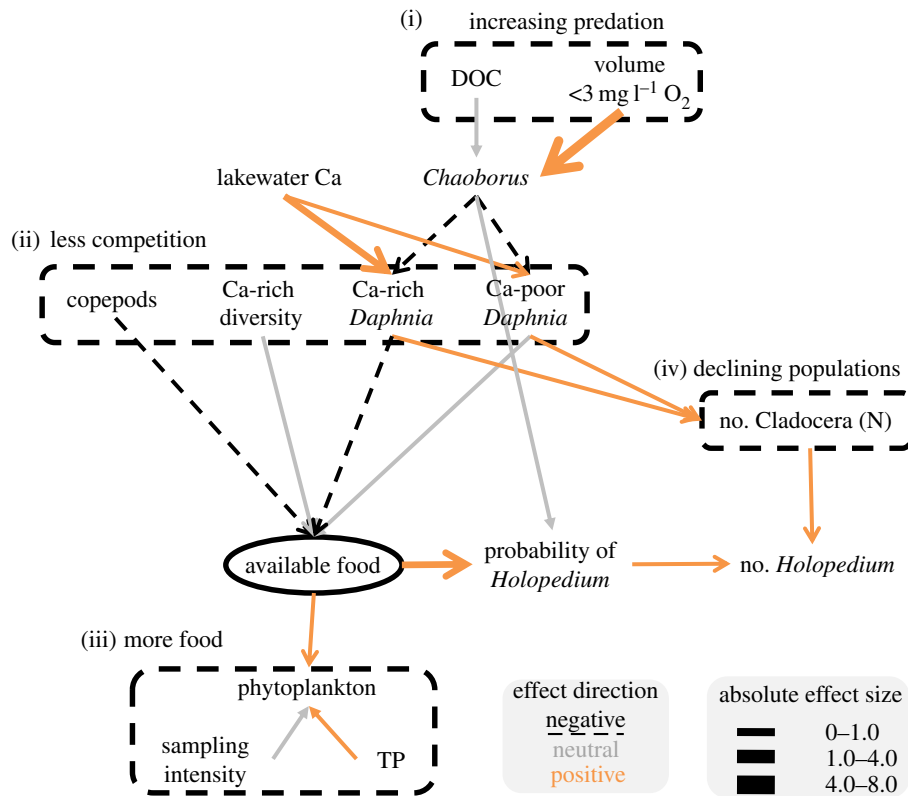
Figure 4. Structural equation model predicting causal linkages among lakewater Ca and Cladocera groups. Directional arrows denote direct effects of one variable on another and are proportional to effect size (see legend). Four alternate and mutually inclusive mechanisms explaining increased abundances of *Holopedium* are associated with broken boxes. These test whether *Holopedium* are more abundant because they have more access to food, associated with (i) increased predation by *Chaoborus* on *Daphnia* due to larger refuge from fish predation; (ii) declines in competition from lower abundances of Ca-rich and Ca-poor *Daphnia* due to lakewater Ca, accounting for compositional changes in *Daphnia* (Ca-rich diversity) and copepod biomass; (iii) eutrophication and/or (iv) because other Cladocera have become rarer. Bayesian R^2 measuring model fit to *Holopedium*, Ca-rich and Ca-poor daphniids, phytoplankton and *Chaoborus* measurements = 0.99, 0.65, 0.60, 0.50 and 0.75, respectively (see electronic supplementary material, figure S3). (Online version in colour.)

was better able to secure the limiting food resources that reproduction requires. Among daphniids, only the relatively rare *D. dentifera* and *D. pulicaria*, which are much larger and thus more likely controlled by fish predation, equalled *Holopedium*'s fecundity.

Declining TP levels did not support our 'more food' mechanism, but they could be promoting greater absolute abundances of *Holopedium* by reducing the competitive effects of *Daphnia*. First, daphniids likely have to eat more than *Holopedium* to get the P they need, because their P content is twice as high [12,13]. This should make them more vulnerable than *Holopedium* to falling food levels, particularly if they are poorer competitors. Thus, the competitive effects of daphniids may further depend on whether food supplies exceed the levels required to meet basic metabolic needs. *Holopedium*'s advantage could also be enhanced if algal P content declines with falling TP levels [51]. Additional data on nutritional thresholds and phytoplankton community composition would help to test these explanations.

Many environmental changes other than Ca decline influence zooplankton, though they are unlikely to be the dominant factor explaining the rise of *Holopedium*. For example, the SEM suggests that recent declines in hypolimnetic oxygen levels due to climate warming [25] can promote abundances of *Chaoborus*, which prey more heavily on daphniids than *Holopedium* ('increasing predation' mechanism). However, predation risk is not increasing universally, as factors such as the spread of the piscivorous bass across Ontario [52] may lower the abundance of some planktivores, in turn reducing pressure

on both *Daphnia* and *Holopedium*. Levels of coloured DOC [17,18] have also been rising in many Ontario lakes, though we found no evidence in our SEM that this has been large enough to benefit *Holopedium*. *Holopedium* abundances have increased over a broad range of DOC concentrations in both Ontario and Nova Scotia (1.4–12.7 mg l^{-1}) [4,5], so high DOC is clearly not a requirement of *Holopedium* increases. While our survey data also suggest the *Bythotrephes* invasion is promoting *Holopedium* (figure 2), *Holopedium* have increased in the majority of our study lakes in the absence of this invasion.

One factor we excluded from our SEM was pH, because lake acidity was an unlikely factor in the observed increases in *Holopedium*. Although *Holopedium* is much more acid-tolerant than *Daphnia* [14,53], and its relative abundance increased in Nova Scotia lakes that declined in pH [22], there have also been widespread reductions in lake acidity in south-central Ontario [34]. We might have expected increases in *Daphnia* abundance at the expense of *Holopedium* in Ontario, if lake acidity was the key driver, but the reverse was observed.

(b) Consequences of an increasingly jellied future

Our results signal a shift in the ecological state of temperate softwater lakes of eastern North America. *Holopedium* is widespread in these lakes [54], and we have shown both its relative and absolute abundances are increasing with widespread Ca decline. This will likely reduce vertical energy and nutrient transport in lake food webs [55]. Both the

declining availability of daphniid prey and the relative ease with which planktivorous fish consume *Holopedium* [56], suggest that this taxon will increasingly dominate the diet of zooplanktivorous fish. Although *Holopedium* and daphniids have similar fatty acid content and composition [57], *Holopedium* has much lower P and Ca content than its daphniid competitors [11–13]. Thus, with its increasing dominance, fewer essential nutrients will be transferred to planktivorous fish [58]. The co-occurring copepods have low P content matching *Holopedium* [12,59]. These changes may also be quite long-lasting if acid deposition rates continue to exceed mineralogical weathering rates [60,61], reducing exchangeable Ca levels in soils, and ultimately in downstream waters.

As Ca declines, the rise in the absolute abundance of *Holopedium* may also have socioeconomic consequences, because increased concentrations of pelagic jelly may impede the withdrawal of lakewater for residential, municipal and industrial uses. The costs of operating infrastructure for water users will therefore rise as Ca continues to decline and densities

of filter-clogging jelly capsules increase [62]. In Ontario, 20% of government-monitored drinking water systems draw only from surface waters within landscapes containing lakes with Ca concentrations less than or equal to 3.5 mg l^{-1} (electronic supplementary material, figure S4), levels that favour *Holopedium* (electronic supplementary material, figure S3). Our results show that the continued jellification of pelagic food webs, due to ongoing declines in lakewater Ca, and exacerbated by other environmental stressors, has the potential to negatively impact the functioning of, and services provided by, temperate lakes in eastern North America.

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