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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 dramatic 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 phosphorus 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.

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

1. INTRODUCTION

The ecological impacts associated with calcium (Ca) decline are a growing concern in aquatic ecosystems of eastern North America and northern Europe [1,2,3]. This is especially true in soft-water lakes where abundances of Ca-rich *Daphnia* (Crustacea, Cladocera), often the dominant and keystone herbivores in the pelagic zone, are declining [3,4,5]. However, not all taxa suffer when Ca declines. Daphniids compete for food resources with another large Cladoceran, *Holopedium glacialis* [6,7,8] (previously *H. gibberum* [9] and hereafter called *Holopedium*). 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 protects it from macro-invertebrate predators (figure 1), including the larval phantom midge, *Chaoborus,* and the recent Eurasian invader, *Bythotrephes longimanus* (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 ~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 1981-1990 and resampling from 2004-05, and with a longitudinal study of 8 other Ontario lakes that were sampled monthly for 30 years (see electronic supplementary material, tables S2-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 lake water 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 co-vary 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. METHODS

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 2002-04 [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 accurately characterize the taxonomic assemblage [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 vs. recent sediment datasets. We excluded lakes where *Holopedium* was absent from both intervals.

Are Holopedium increases consistent with recent lakewater Ca decline?

We tested our second hypothesis using two datasets: a longitudinal study of 8 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 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 4 to 7 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 vs. 2004-05 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 8 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 SD of 1 across years in each lake.

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 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 1987-1994 [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 electronic supplementary material).

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

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:

$$y_{ij}{}^{(1)} \sim B(p_{ij}{}^{(1)}, \mathrm{N}_{ij}).$$

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.

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}:

$$\mathrm{logit}\left(p_{ij}{}^{(1)}\right) = \alpha^{(1)} + \gamma_1 \xi_{ij} + \gamma_2 \mathrm{Chaob}_{ij} + \nu_i{}^{(1)} + \nu_j{}^{(1)}, \tag{2.1}$$

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 $v_i^{(1)}$ and $v_j^{(1)}$ 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 (SD). Although we did not observe values directly for ξ_{ij}, a latent variable, we parameterized it using observed data.

We assumed that ξ_{ij} was $\sim N(\eta_{ij},\sigma_{\xi})$, 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 inter-specific differences in body Ca content [11,40] and prevalence thresholds in field surveys [20,40] (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 (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 3 species and $D_{ij} = 2.5$ is slightly less diverse than a community of 3 equally-abundant taxa with totally dissimilar Ca requirements (see electronic supplementary material). This combination of approaches essentially allows both linear and non-linear 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}:

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

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

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:

$$y_{ij}^{(2)} \sim B\left(p_{ij}^{(2)}, N_{ij}\right),$$

$$\mathrm{logit}\left(p_{ij}^{(2)}\right) = \alpha^{(2)} + \gamma_7 \mathrm{Ca}_{ij} + \gamma_8 \mathrm{Chaob}_{ij} + \nu_i^{(2)} + \nu_j^{(2)}, \tag{2.3}$$

$$y_{ij}^{(3)} \sim B\left(p_{ij}^{(3)}, N_{ij}\right),$$

$$\mathrm{logit}\left(p_{ij}^{(3)}\right) = \alpha^{(3)} + \gamma_9 \mathrm{Ca}_{ij} + \gamma_{10} \mathrm{Ca}_{ij}^{2} + \gamma_{11} \mathrm{Chaob}_{ij} + \nu_i^{(3)} + \nu_j^{(3)}. \tag{2.4}$$

$\alpha^{(k)}$ 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 $\nu_i^{(k)}$ and $\nu_j^{(k)}$ account for random variation among the i lakes and j years and are drawn from zero-mean normal distributions with separately estimated SD for each source of variation associated with each life group *k*. We allowed the response of Ca-rich daphniids to lakewater Ca to be non-linear, 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 lake water Ca [40].

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:

$$\mathrm{P}_{ij} \sim \mathrm{Pois}(\lambda_{ij}),$$

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

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

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 <3 $\mathrm{mg \cdot L^{-1}}$ $\mathrm{O_2}$ (O_{ij}) and concentration of DOC in lakewater (DOC_{ij}), which both reduce predation from fish [24]:

$$\mathrm{Chaob}_{ij} \sim N(\mu_{ij}, \sigma_{Chaob}),$$

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

where $\alpha^{(5)}$ is the estimated mean density of *Chaoborus*, γ_{14}-γ_{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 SD. *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, 2.3-2.4), 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.

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 electronic supplementary material for details). We standardized all estimated coefficients to a common scale with a mean of 0 and SD 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 electronic supplementary material).

3. RESULTS

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; figures 2*a* and 2*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 2 and 1 lakes, respectively, suggesting that increases in absolute abundances have also occurred.

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 2004-05 as Ca declined 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 3-37% (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 table S3).

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 inter-specific competition for food and declines 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 – <-0.01 and -0.32 – 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 $\mathrm{mg \cdot L^{-1}}$ 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 (τ = -0.40, p = 0.002), increasing food available for *Holopedium* (95% CI for their effect on food: -0.15 – -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 (τ = -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; 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 to avoid this complication (1980 to 1992; 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% CI for effect on Ca-rich *Daphnia*, Ca-poor *Daphnia*, *Holopedium*: -0.21 – -0.11, -0.20 – -0.09, -0.26 – 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: 1) the contribution of *Holopedium* to pelagic cladoceran abundance has increased in central and eastern softwater Canadian lakes since pre-industrial times; 2) relative and absolute abundances of *Holopedium* have increased over the last 3 decades, a time of recent lakewater Ca decline; and 3) 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.

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 $mg \cdot L^{-1}$ [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 [6,7,8,14], 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 (figure S2), suggesting it 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 $mg \cdot L^{-1}$) [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 risen 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.

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 [11,12,13]. 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 lake water 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 $\leq 3.5\ \mathrm{mg \cdot L^{-1}}$ (figure S4), levels that favour *Holopedium* (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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REFERENCES

1. Stoddard JL *et al*. 1999 Regional trends in aquatic recovery from lake acidification in North America and Europe. *Nature* **401**: 575-578.

2. Skjelkvåle BL *et al.* 2005 Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environ. Pollut.* **137**, 165-176.

3. Jeziorski A *et al.* 2008 The widespread threat of calcium decline in fresh waters. *Science* **322**, 1374-1377.

4. Jeziorski A, Paterson AM, Smol JP. 2012 Changes since the onset of acid deposition among calcium-sensitive cladoceran taxa within softwater lakes of Ontario, Canada. *J Paleolimnol.* **48**, 323-337.

5. Korosi JB, Smol JP. 2012 A comparison of present-day and pre-industrial cladoceran assemblages from softwater Nova Scotia (Canada) lakes with different regional acidification histories. *J. Paleolimnol.* **47**, 43-54.

6. Hessen DO. 1985 Filtering structures and particle size selection in coexisting Cladocera. *Oecologia* **66**, 368-372.

7. Persaud AD, Dillon PJ, Lasenby D, Yan ND. 2009 Stable isotope variability of meso-zooplankton along a gradient of dissolved organic carbon. *Freshw. Biol.* **54**, 1705-1719.

8. Allan JD. 1973 Competition and the relative abundances of two cladocerans. *Ecology* **54**, 484-498.

9. Rowe CL, Adamowicz SJ, Hebert PDN. 2007 Three new cryptic species of the freshwater zooplankton genus *Holopedium* (Crustacea: Branchiopoda: Ctenopoda), revealed by genetic methods. *Zootaxa* **1656**, 1-49.

10. Beaton MJ, Hebert PDN. 1989 Miniature genomes and endopolypoidy in cladoceran crustaceans. *Genome* **32**, 1048-1053.

11. Jeziorski A, Yan ND. 2006 Species identity and aqueous calcium concentrations as determinants of calcium concentrations of freshwater crustacean zooplankton. *Can. J. Fish. Aquat. Sci.* **63**, 1007-1013.

12. Andersen T, Hessen DO. 1991 Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnol. Oceanogr.* **36**, 807-814.

13. McCarthy V, Irvine K. 2010 A test of stoichiometry across six Irish lakes of low-moderate nutrient status and contrasting hardness. *J. Plankton Res.* **32**, 15-29.

14. Hessen DO, Faafeng BA, Andersen T. 1995 Competition or niche segregation between *Holopedium* and *Daphnia*; empirical light on abiotic key parameters. *Hydrobiol.* **307**, 253-261.

15. McQueen DJ, Ramcharan CW, Yan ND. 2001 Summary and emergent properties - Part 12 of the Dorset food web piscivore manipulation project. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* **56**, 257-288.

16. Wissel B, Boeing WJ, Ramcharan CW. 2003 Effects of water color on predation regimes and zooplankton assemblages in freshwater lakes. *Limnol. Oceanogr.* **46**, 1965-1976.

17. Palmer ME, Yan ND, Paterson AM, Girard RE. 2011 Water quality changes in south-central Ontario lakes and the role of local factors in regulating lake response to regional stressors. *Can. J. Fish. Aquat. Sci.* **68**, 1038-1050.

18. Monteith DT *et al.* 2007 Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* **450**, 537-541.

19. Kerr JG, Eimers MC. 2012 Decreasing soil water Ca2+ reduces DOC adsorption in mineral soils: Implications for long-term DOC trends in an upland forested catchment in southern Ontario, Canada. *Sci. Total Environ.* **427-428**, 298-307.

20. Kim N, Walseng B, Yan ND. 2012 Will environmental calcium declines in Canadian Shield lakes help or hinder *Bythotrephes* establishment success? *Can. J. Fish. Aquat. Sci.* **69**, 810-820.

21. Kim N, Yan ND. 2010 Methods for rearing the invasive zooplankter *Bythotrephes* in the laboratory. *Limnol. Oceanogr. Methods* **8**, 552-561.

22. Clair TA, Dennis IF, Vet R. 2011 Water chemistry and dissolved organic carbon trends in lakes from Canada's Atlantic Provinces: no recovery from acidification measured after 25 years of lake monitoring. *Can. J. Fish. Aquat. Sci.* **68**, 663-674.

23. Korosi JB, Ginn BK, Cumming BF, Smol JP. 2013 Establishing past environmental conditions and tracking long-term environmental change in the Canadian Maritime provinces using lake sediments. *Environ. Rev.* **21**, 15-27.

24. Wissel B, Yan N, Ramcharan C. 2003 Predation and refugia: implications for *Chaoborus* abundance and species composition. *Freshw. Biol*. **48**, 1421–1431.

25. Stefan HG, Hondzo M, Fang X, Eaton JG, McCormick JH. 1996 Simulated long-term temperature and dissolved oxygen characteristics of lakes in the north-central United States and associated fish habitat limits. *Limnol. Oceanogr.* **41**, 1124-1135.

26. Vinyard GL, Menger RA. 1980 *Chaoborus americanus* predation on various zooplankters; functional response and behavioral observations. *Oecologia* **45**, 90-93.

27. Shipley B. 2000 *Cause and Correlation in Biology*. Cambridge: Cambridge University Press.

28. Shipley B. 1999 Testing causal explanations in organismal biology: causation, correlation and structural equation modelling. *Oikos* **86**, 374-382.

29. Tanentzap AJ *et al.* 2014 Identifying pathways for managing multiple disturbances to limit plant invasions. *J. Appl. Ecol.* **51**, 1015-1023.

30. Smol JP. 2008 *Pollution of lakes and rivers: A paleoenvironmental perspective.* 2nd edn. Oxford: Wiley-Blackwell Publishing.

31. Korosi JB, Smol JP. 2012 An illustrated guide to the identification of cladoceran subfossils from lake sediments in northeastern North America: part 1—the Daphniidae, Leptodoridae, Bosminidae, Polyphemidae, Holopedidae, Sididae, and Macrothricidae. *J. Paleolimnol.* **48**, 571-586.

32. Korosi JB, Smol JP. 2012 An illustrated guide to the identification of cladoceran subfossils from lake sediments in northeastern North America: part 2—the Chydoridae. *J. Paleolimnol.* **48**, 587-622.

33. Kurek J, Korosi JB, Jeziorski A, Smol JP. 2010 Establishing reliable minimum count sizes for cladoceran subfossils sampled from lake sediments. *J. Paleolimnol.* **44**, 603-612.

34. Yan ND *et al.* 2008 Long-term trends in zooplankton of Dorset, Ontario, lakes: the probable interactive effects of changes in pH, total phosphorus, dissolved organic carbon, and predators. *Can. J. Fish. Aquat. Sci.* **65**, 862-877.

35. Palmer ME, Yan ND. 2013 Decadal-scale regional changes in Canadian freshwater zooplankton: the likely consequence of complex interactions among multiple anthropogenic stressors. *Freshw. Biol.* **58**, 1366-1378

36. Paterson AM *et al.* 2008 Long-term changes in phytoplankton composition in seven Canadian Shield lakes in response to multiple anthropogenic stressors. *Can. J. Fish. Aquat. Sci.* **65**, 846–861.

37. Yan N, Nero R, Keller W, Lasenby D. 1985 Are *Chaoborus* larvae more abundant in acidified than in non-acidified lakes in central Canada. *Holarct. Ecol.* **8,** 93–99.

38. Hopkins GE, Standke SJ. 1992 Phytoplankton methods manual: with special emphasis on waterworks operation internal methods manual. Queen's Printer for Ontario, Toronto, ON. ISBN 0-7729-8923-0.

39. Lynch M. 1979 Predation, competition, and zooplankton community structure: An experimental study. *Limnol. Oceanogr.* **24**, 253-272.

40. Cairns A. 2010 Field assessments and evidence of impact of calcium decline on *Daphnia* (Crustacea, Anomopoda) in Canadian Shield lakes M.Sc. Thesis. York University, Canada.

41. Leinster T, Cobbold CA. 2012 Measuring diversity: the importance of species similarity. *Ecology* **93**, 477-489.

42. Geller W, Müiller H. 1981 The filtration apparatus of Cladocera: Filter mesh-sizes and their implications on food selectivity. *Oecologia.* **49**, 316-321.

43. Ramcharan C *et al.* 2001 Complex responses of *Chaoborus* to changes in fish populations. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* **56**, 81–100.

44. Stan Development Team. 2012 *Stan Modeling Language: User's Guide and Reference Manual. Version 2.0.* See http://mc-stan.org/.

45. R Development Core Team. 2013 *R: A Language and Environment for Statistical Computing.* Vienna: R Foundation for Statistical Computing. See http://www.R-project.org/.

46. Gelman A, Pardoe I. 2006 Bayesian measures of explained variance and pooling in multilevel (hierarchical) models. *Technometrics* **48**, 241–251.

47. Grace JB *et al.* 2012 Guidelines for a graph-theoretic implementation of structural equation modeling. *Ecosphere.* **3**, art73.

48. Yao H, McConnell C, Somers KM, Yan ND, Watmough S, Scheider W. 2011 Nearshore human interventions reverse patterns of decline in lake calcium budgets in central Ontario as demonstrated by mass-balance analyses. *Water Resour. Res.* 47, W06521, doi:10.1029/2010WR010159

49. Ashforth D. Yan ND. 2008 The interactive effects of calcium concentration and temperature on the survival and reproduction of *Daphnia pulex* at high and low food concentrations. *Limnol*. *Oceanogr*. **53**, 420-432

50. Riessen HP *et al*. 2012 Changes in water chemistry can disable plankton prey defenses. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 15377–15382.

51. Painter DS, Kamaitis G. 1987 Reduction of Cladophora biomass and tissue phosphorus in Lake Ontario, 1972-1983. *Can. J. Fish. Aquat. Sci.* **44**, 2212-2215.

52. Vander Zanden MJ, Olden JD, Thorne JH, Mandrak NE. 2004 Predicting occurrences and impacts of smallmouth bass introductions in north temperate lakes. Ecol. Appl. **14**, 132-148.

53. Keller W, Pitblado JR. 1984 Crustacean plankton in Northeastern Ontario lakes subjected to acidic deposition. *Water Air Soil Pollut.* **23**, 271-291.

54. Merrix-Jones FL, Thackeray SJ, Ormerod SJ. 2013 A global analysis of zooplankton in natural and artifical fresh waters. *J. Limnol.* **72**, 140-153

55. Ramcharan CW *et al.* 2001 Analyses of lake food webs using individual-based models to estimate *Chaoborus* production and consumption. *Arch. Hydrobiol. Spec. Issues Advanc. Limnol.* **56**, 101–126.

56. Arts MT, Sprules WG. 1989 Use of enclosures to detect the contribution of particular zooplankton to growth of young-of-the-year yellow perch (*Perca flavescens* Mitchell). *Oecologia* **81**, 21-27.

57. Persson J, Vrede T. 2006 Polyunsaturated fatty acids in zooplankton: variation due to taxonomy and trophic position. *Freshw. Biol.* **51**, 887-900.

58. Malzahn A, Aberle N, Clemmesen C, Boersma M. 2007 Nutrient limitation of primary producers affects planktivorous fish condition. *Limnol. Oceanogr.* **52,** 2062-2071.

59. Sterner RW, Elser JJ, Hessen DO. 1992 Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. *Biogeochemistry.* **17**, 49-67.

60. Whitfield CJ, Watmough SA, Aherne J, Dillon PJ. 2006 A comparison of weathering rates for acid-sensitive catchments in Nova Scotia, Canada and their impact on critical load calculations. *Geoderma* **136**, 899-911.

61. Watmough SA, Aherne J. 2008 Estimating calcium weathering rates and future lake calcium concentrations in the Muskoka-Haliburton region of Ontario. *Can. J. Fish. Aquat. Sci.* **65**, 821-833.

62. Thelen AC. 2012 Population dynamics of *Holopedium gibberum* in a Pacific Northwest drinking water reservoir: effects of temperature, food, and competition. M.Sc. Thesis. University of Washington, USA.

FIGURE CAPTIONS

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.

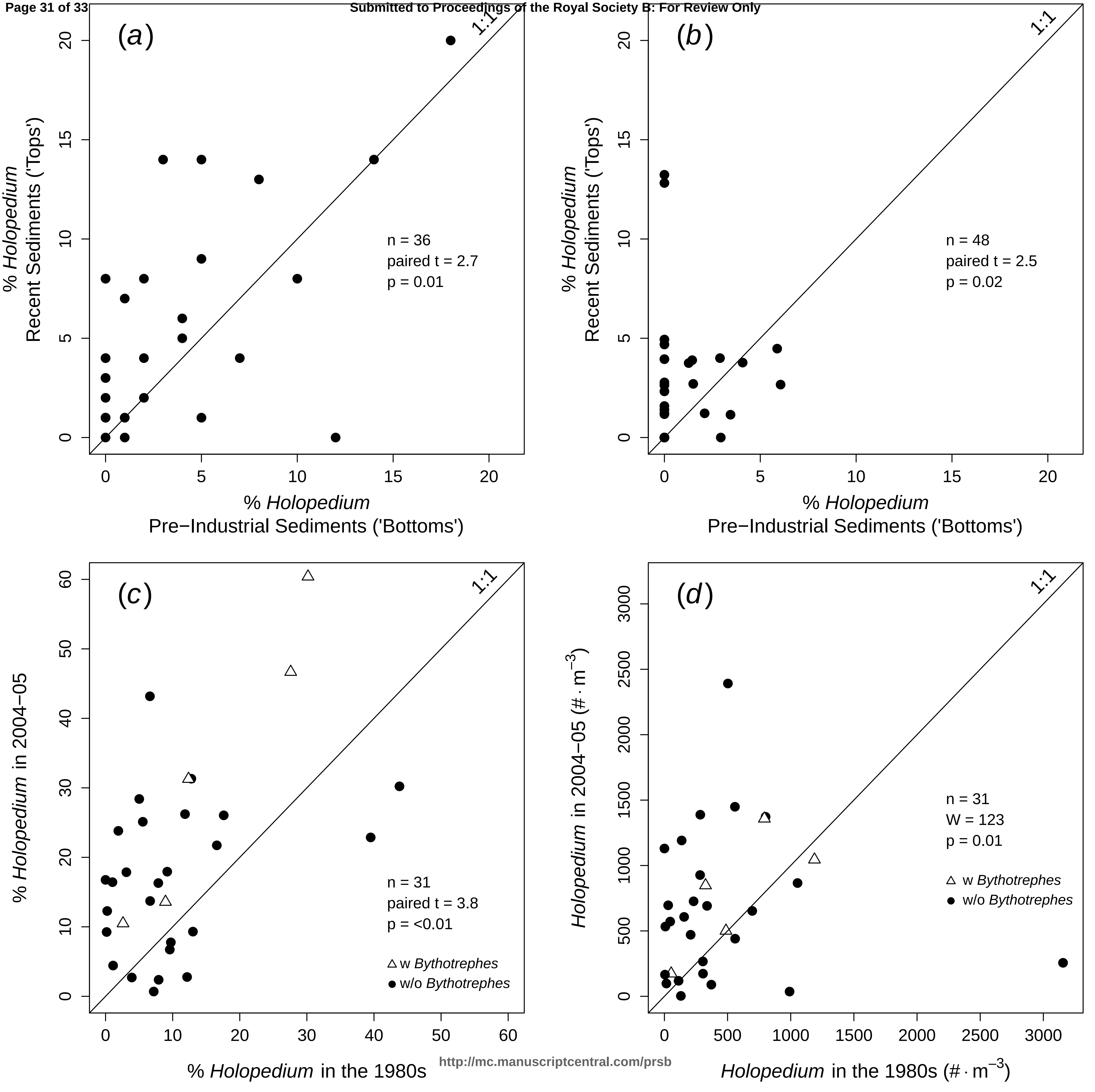
Figure 2. Temporal increases in *Holopedium* across two lake districts. Change in relative abundance of *Holopedium* since pre-industrial times in the sedimentary pelagic cladoceran assemblages of (*a*) 36 lakes in south-central Ontario and (*b*) 48 lakes in Nova Scotia, Canada (*Holopedium* remains 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 2004/5 in 31 south-central Ontario lakes, 5 of which were invaded by *Bythotrephes* between the two surveys.

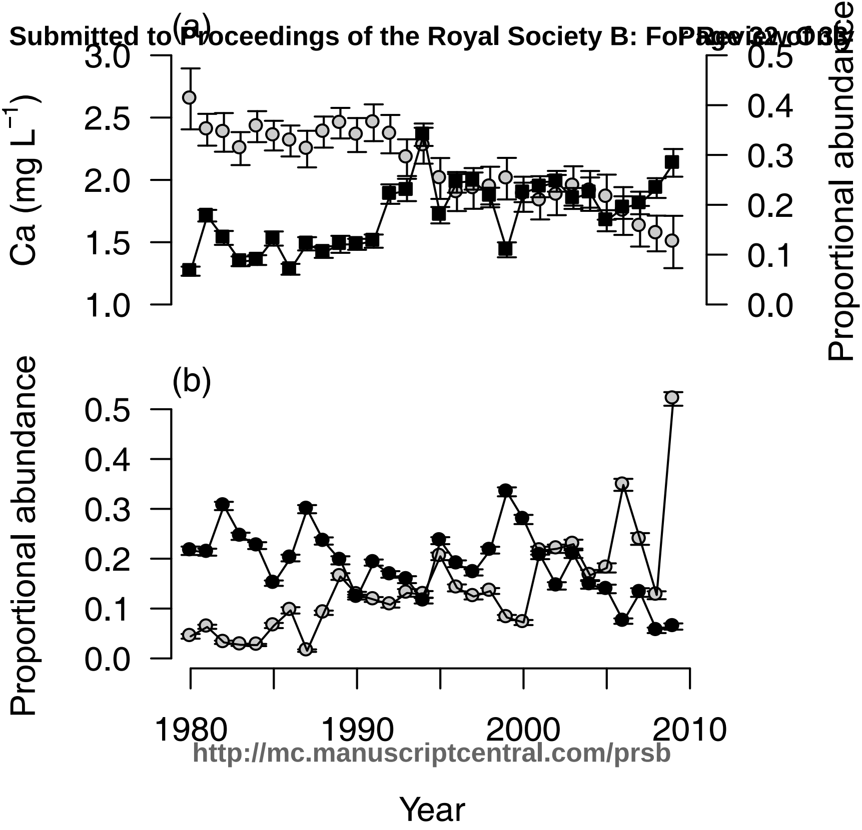
Figure 3. Effects of Ca decline on abundances of large Cladocera in eight lakes in south-central Ontario, Canada, from 1980-2009. (*a*) Declines in mean Ca ± SE (gray) across the study lakes and increases in mean relative abundances of *Holopedium* ± 95% CIs summed across the eight lakes (black). (*b*)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

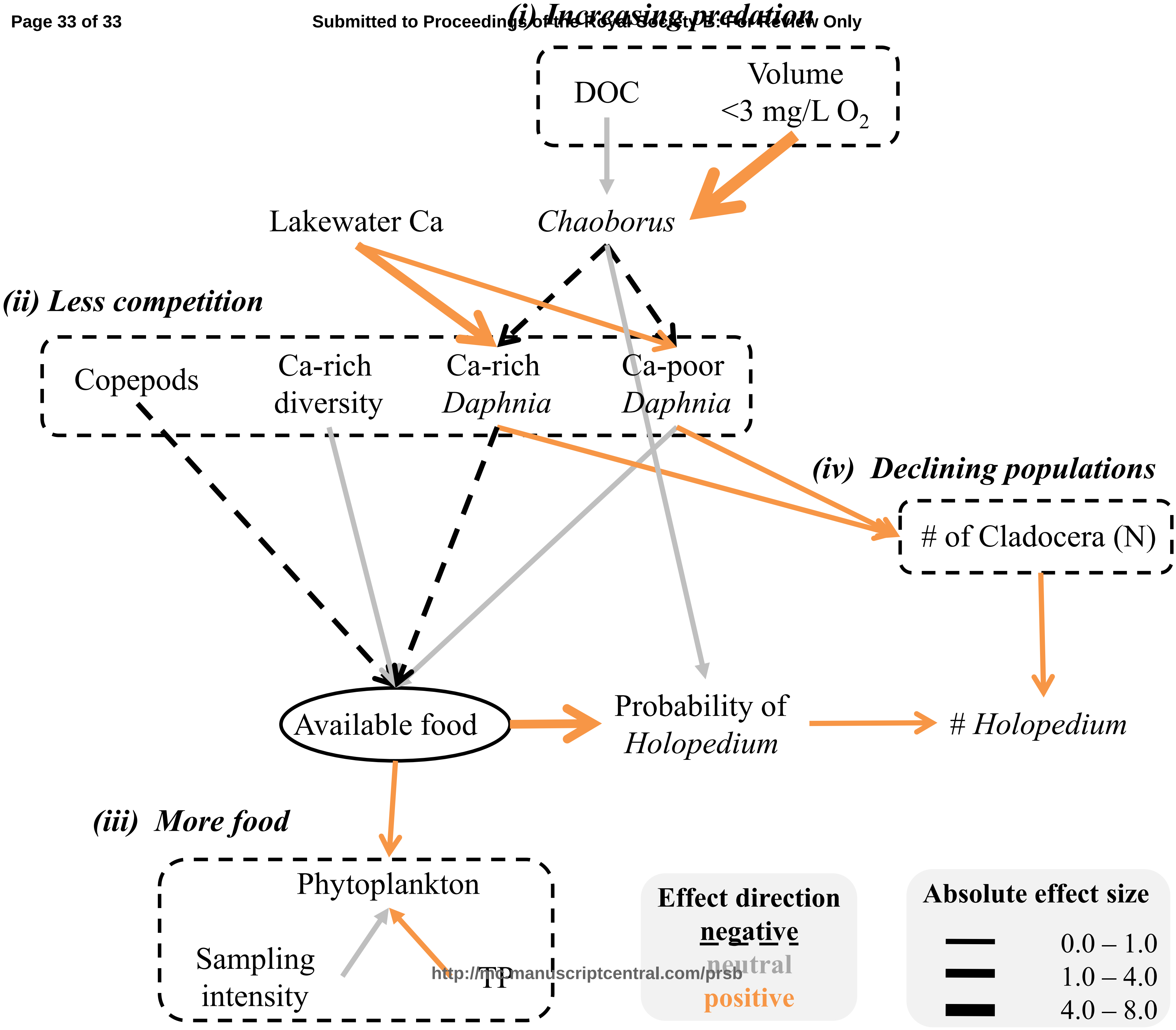
Figure 4. Structural equation model predicting causal linkages among lakewater Ca and Cladocera groups. Directional arrows denote direct effects of one variable on another and are proportional to effect size (see legend). Four alternate and mutually-inclusive mechanisms explaining increased abundances 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).

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)


(a)
1:1
n = 36
paired t = 2.7
p = 0.01
% Holopedium
Recent Sediments ('Tops')
% Holopedium
Pre−Industrial Sediments ('Bottoms')
0
5
10
15
20
(b)
1:1
n = 48
paired t = 2.5
p = 0.02
(c)
1:1
n = 31
paired t = 3.8
p = <0.01
△ w Bythotrephes
● w/o Bythotrephes
% Holopedium in 2004−05
% Holopedium in the 1980s
0
10
20
30
40
50
60
(d)
1:1
n = 31
W = 123
p = 0.01
Holopedium in 2004−05 (# · m−3)
Holopedium in the 1980s (# · m−3)
0
500
1000
1500
2000
2500
3000


(a)
Ca (mg L^{-1})
3.0
2.5
2.0
1.5
1.0
Proportional abundance
0.5
0.4
0.3
0.2
0.1
0.0
(b)
Proportional abundance
0.5
0.4
0.3
0.2
0.1
0.0
1980
1990
2000
2010
Year


(i) Increasing predation
DOC
Volume
<3 mg/L O2
Lakewater Ca
Chaoborus
(ii) Less competition
Copepods
Ca-rich diversity
Ca-rich Daphnia
Ca-poor Daphnia
(iv) Declining populations
# of Cladocera (N)
Available food
Probability of Holopedium
Holopedium
(iii) More food
Phytoplankton
Sampling intensity
TP
Effect direction
negative
neutral
positive
Absolute effect size
0.0 – 1.0
1.0 – 4.0
4.0 – 8.0