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A review of the effects of *Bythotrephes longimanus* and calcium decline on zooplankton communities – can interactive effects be predicted?

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A review of the effects of *Bythotrephes longimanus* and calcium decline on zooplankton communities – can interactive effects be predicted?

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Abstract

Anthropogenic stressors including acid deposition, invasive species, and calcium decline, have produced widespread damage to Canadian Shield lakes, especially to their zooplankton communities. Here, we review current knowledge on the individual effects on zooplankton by the non-indigenous predator, *Bythotrephes longimanus*, and calcium decline; we identify knowledge gaps in this literature; and we examine the likely interactive impacts of *Bythotrephes* invasions and Ca decline on zooplankton. The negative impacts of *Bythotrephes longimanus* on zooplankton communities are well known, whereas current understanding of the effects of declining calcium on zooplankton is restricted to *Daphnia* spp.; hence, there is a large knowledge gap on how declining calcium may affect zooplankton communities in general. The co-occurring impacts of *Bythotrephes* and declining Ca have rarely been studied at the species level, and we expect daphniids, particularly *Daphnia retrocurva* and *Daphnia pulicaria* to be the most sensitive to both stressors. We also expect a synergistic negative interaction on cladocerans in lakes with both stressors leaving a community dominated by *Holopedium glacialis* and/or copepods. Our predictions form testable hypotheses but since species and ecosystem response to multiple stressors are difficult to predict, we may actually see ecological surprises in Canadian Shield lakes as *Bythotrephes* continues to spread, and calcium levels continue to fall.

Key words: Cladocera, zooplankton, daphniids, *Bythotrephes*, multiple stressors, calcium decline

Introduction

Freshwater ecosystems cover only ~0.8% of the Earth’s surface (Dudgeon et al. 2006), but they are hotspots of biodiversity, and provide vital resources for humans (Strayer and Dudgeon 2010). Human needs for the essential services that freshwater ecosystems provide have increased over time, but our activities have often resulted in their degradation (e.g. increased nutrient loading, pollution, and introduction of alien invasive species), threatening the provision of these services. While there has been considerable effort to understand how these environmental stressors individually influence biodiversity and ecosystem function, the stressors commonly occur in combination (e.g. Schindler 2001). In freshwater conservation and management, therefore, a major challenge is to understand the effects of multiple stressors on species, populations, communities, and ecosystems (Altshuler et al. 2011; Schindler et al. 1996; Yan et al. 1996), given that multiple stressors are now so pervasive (Halpern et al. 2008; Christensen et al. 2006; Breitburg et al. 1998).

The Canadian Shield biome contains 80-90% of Canada’s (Schindler and Lee 2010) and 60% of the Earth’s available surface freshwater (Schindler 2001). The Canadian Shield region is underlain by predominantly silicate bedrock, capped with thin glacial tills. In consequence, Shield lakes and streams have soft waters that are low in nutrients and ions (Watmough et al. 2003). Over the last several decades, eastern Canadian Shield lakes have experienced several widespread stressors, two of which are the introduction of invasive species and a decline in lake water Ca concentrations.

Bythotrephes longimanus (the spiny water flea, hereafter *Bythotrephes*) is a generalist cladoceran predator (Schulz and Yurista 1999) that invaded the Laurentian Great Lakes during the 1980s from Europe (Sprules et al. 1990). First detected in Lake Ontario in 1982 (Johannsson et al. 1991), *Bythotrephes* has subsequently spread into 179 inland lakes in Ontario (EDDMaps Ontario). In its native range, *Bythotrephes* can survive under a wide range of conditions, tolerating broad temperature (4-30°C), and salinity gradients, but preferring temperatures between 10 and 24°C and low salinity between 0.04 and 0.06‰ (Grigorovich et al. 1998). *Bythotrephes* can tolerate waters with pH between 4 and 8 (Hessen et al. 2011; Grigorovich et al. 1998). It prefers large, deep, circumneutral, oligotrophic lakes although it has been observed in shallow lakes and ponds and small tundra pools (Grigorovich et al. 1998). In North America, *Bythotrephes* occupies lakes similar to those in its native range, but its distribution in the early days of the North American invasion was linked to greater lake depth and lake area (MacIsaac et al. 2000).

Human assistance appears to be key for the spread of *Bythotrephes* into many small to mid-size lakes on the Canadian Shield. Weisz and Yan (2010) demonstrated a strong correlation between *Bythotrephes* occurrence and human activity on lakes. Invaded lakes were more accessible to humans through public or private boat launches, had more heavily developed shorelines, and had more motorized boats than uninvaded lakes. Gravity models have also shown that invaded lakes are closer to roads and experience higher boater traffic from other invaded lakes in contrast to uninvaded lakes (Muirhead and MacIsaac 2005; MacIsaac et al. 2004). These models also indicate that larger lakes closer to highly populated areas are more frequently invaded (Gertzen and Leung 2011). *Bythotrephes* propagules are most likely dispersed by recreational anglers

moving among lakes, moving animals or resting eggs attached to anchor ropes, fishing lines, or minnow seines, or in bait buckets and live wells. Propagules may also be spread among lakes by migrating fish that can carry live *Bythotrephes* resting eggs in their stomachs (Kerfoot et al. 2011).

Bythotrephes is a zooplanktivore that detects potential prey within the water column by vision, using its enormous compound eye (Pangle and Peacor 2009; Muirhead and Sprules 2003). *Bythotrephes* captures its prey with its large, first thoracic legs, dismembers the prey using its mandibles, and imbibes the prey's liquid content (Burkhardt and Lehman 1994; Monakov 1972). *Bythotrephes* is a voracious predator, consuming about 75% of its body weight in prey each day (Lehman et al. 1997). It prefers slow moving and visible prey (e.g. *Bosmina*, *Daphnia*) (Grigorovich et al. 1998; Vanderploeg et al. 1993; Monakov 1972). Thus, *Bythotrephes* has impacted pelagic biodiversity in larger (MacIsaac et al. 2000) and smaller lakes (Weisz and Yan 2010), reducing cladoceran abundance and species richness (e.g. Strecker et al. 2006; Yan et al. 2002). *Bythotrephes* occupies epilimnetic waters during the day (Young and Yan 2008), and it induces vertical migration of its prey to cooler hypolimnetic waters, lowering their growth rates (Pangle et al. 2007; Pangle and Peacor 2006; Lehman and Cáceres 1993). These impacts of *Bythotrephes* on the abundance, composition, and vertical migration of its prey can also cascade down the food chain to lower trophic groups such as rotifers that appear to benefit from competitive release and/or predatory release as native invertebrate populations decline (Hovius et al. 2007, 2006).

Ca decline is an emerging stressor for soft water lakes on the Canadian Shield and similar soft water lakes in Europe (e.g. Skjelkvåle et al. 2005; Evans et al. 2001; Kirchner and Lydersen, 1995; Hedin et al. 1994). In these lakes, base cation concentrations have declined in response to long-term exposure to atmospheric acid deposition. Of 770 lakes within Ontario that were monitored in the 1980s, and again in the early 2000s, 35% have <1.5 mg of Ca/L and 62% have <2 mg of Ca/L (Jeziorski et al. 2008). Steady state Ca concentrations are projected to decline between 10-40% in comparison to 1980 to 1990 levels (Watmough and Aherne 2008), which may result in Ca concentrations in a majority of Canadian Shield lakes that are low enough to cause ecological damage.

The local causes of Ca decline may vary, but must be related to changes in the soil Ca pool, i.e., reductions in the rates of input to, or export from, the exchangeable Ca pool in watershed soils. Inputs come from the atmosphere (wet and dry deposition), from chemical weathering of calcium-containing minerals within the soil and bedrock, and perhaps from human activities (e.g. liming of forest soils). Export rates are influenced by unit water runoff and Ca concentrations in the runoff waters, which themselves will be a function of soil base saturation and thickness, and human activities, such as additions of dust suppressants (Yao et al. 2011). Ca in its ionic form runs off into aquatic systems within the watershed, and the runoff rate, coupled with direct atmospheric inputs, regulate Ca levels in lakes. If the watershed or atmospheric supply of Ca decline, or the hydrological input itself decline, Ca levels in lakes may fall, for several specific reasons, including: 1) reduced atmospheric Ca deposition (Watmough et al. 2005; Keller et al. 2001; Likens et al. 1998; Driscoll et al. 1995); 2) historic depletion of exchangeable Ca ions in the soil caused by decades of elevated rates of Ca leaching from the soil, during recent decades

when rates of acid deposition exceeded mineral weathering rates (Watmough and Dillon 2003a, 2003b; Lawrence et al. 1999); and 3) logging in watersheds and the ensuing uptake of Ca by trees as the forest re-grows (Piiraninen et al. 2004; Watmough et al. 2003; Likens et al. 1998).

Calcium is an essential macro-element for organisms and declines in aqueous Ca will likely have implications for aquatic biodiversity. Crustaceans (e.g. *Daphnia* and crayfish) have high Ca requirements akin to other Ca-rich aquatic organisms, i.e. those with calcareous shells or heavily-calcified carapaces (Table 1), as they shed their exoskeleton (carapace) regularly as they grow (Greenaway 1985). Water and food are the main sources of Ca for crustaceans, but dissolved ionic Ca is the most important source for zooplankton (Cowgill et al. 1986). The carapace of daphniids contains the majority of their Ca, and 90% of body Ca is either trapped in the shed exuviae or lost to the surrounding medium during moulting (Alstad et al. 1999). After moulting, hardening of the carapace follows rapid uptake of Ca; however, this uptake is dependent on the Ca concentration in the water, which is the major source of Ca for post-moult calcification (Greenaway 1985). In lakes with low Ca concentrations, remineralisation of the carapace is compromised (Greenaway 1985) and the survival, growth, and reproduction of daphniids are threatened (Ashforth and Yan 2008). A poorly calcified carapace increases the vulnerability of daphniids to predation (Riessen et al. 2012); thus there may be both direct and indirect effects of Ca decline on crustaceans. Reduced crayfish abundances (Edwards et al. 2009) and loss of daphniids in pelagic communities (Cairns 2010; Hessen et al. 1995) have been attributed to Ca decline. Low Ca can also affect the distribution and size of zooplankton communities as species with high Ca demands are mainly found in lakes with high ambient Ca (Wærvågen et al. 2002). Furthermore, small-bodied cladocerans such as *Daphnia retrocurva*, *Daphnia parvula*, *Daphnia*

160 *ambigua*, and *Daphnia catawba* occur in softer lakes with Ca levels <2 mg/L compared to their
161 larger counterparts (e.g. *Daphnia pulex*, *Daphnia pulicaria* and *Daphnia mendotae*) that are
162 largely restricted to high Ca lakes (Tessier and Horwitz 1990).

163
164 *Bythotrephes* and Ca decline are large-scale threats to the Canadian Shield as they are impacting
165 a large number of lakes that cover a broad area within North America. Their individual impacts
166 on different Cladoceran species have the potential to decimate many herbivorous cladocerans,
167 important animals within the aquatic food web. *Bythotrephes* and Ca decline also have the
168 potential to co-occur in Canadian Shield lakes (Jeziorski et al. 2015; Palmer and Yan 2013) that
169 may also be experiencing other stressors to produce ecological surprises (Paine et al. 1998) that
170 could exceed (synergistic), or fall below (antagonistic) their expected additive effects (Folt et al.
171 1999). Here we: 1) review current knowledge on the effects of *Bythotrephes* and Ca decline on
172 zooplankton; 2) identify gaps in the literature; and 3) examine the likely impacts of a co-
173 occurrence of *Bythotrephes* invasions and Ca decline on zooplankton. We hypothesized that in
174 combination, the impacts of declining aqueous Ca and *Bythotrephes* would: 1) be synergistic (the
175 most severe non-additive effect) on daphniids as Ca decline would impair reproduction, as well
176 as increase their vulnerability to *Bythotrephes* predation due to the reduction or absence of anti-
177 predator defences. Declining daphniid abundances would result in the concomitant increase of
178 more resistant species that are co-tolerant of both stressors such as *Holopedium glacialis* and
179 copepods (antagonistic interaction); 2) be antagonistic on small cladocerans (e.g. *Bosmina*) that
180 would be consumed by *Bythotrephes* but may increase with Ca decline as they would benefit
181 from reduced competition with daphniids; 3) have no effect on copepods, either through
182 predation or impaired reproduction.

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Methods

Literature on *Bythotrephes* and Ca decline were identified using the search engines Web of Science and Google Scholar. Key words used during the search were: *Bythotrephes*, *Bythotrephes longimanus*, spiny water flea, calcium, calcium decline, base cations, calcium content, acid deposition, multiple stressor, Boreal/Canadian Shield lakes, *Daphnia*, freshwater, and crustacean zooplankton. Identified papers were reviewed and subsequent searches based on author or cited literature were also performed. A paper was included if it provided information relevant to our stated objectives.

Two over-arching categories were identified in the *Bythotrephes* retrievals: field surveys and experiments. Field surveys were subdivided into lake survey, long-term study of an inland lake (Harp Lake), and long-term study in the Great Lakes. Experiments were also subdivided into field mesocosms and laboratory work. Lake surveys were conducted mainly during the ice-free season, from May to September. Lakes were either sampled fortnightly at the deepest spot in the lake, or once or twice during the ice-free season using zooplankton conical nets that varied between 65 and 285 μm mesh size (diameter range 0.3 to 0.75 m). The number of lakes sampled ranged from eight to 193 lakes in the Muskoka-Haliburton-Parry Sound regions of south-central Ontario, Canada. Only one survey compared 212 Canadian Shield lakes to 342 Norwegian lakes. The Norwegian data on zooplankton was not included except as a comparison to North American results. Canadian Shield lakes surveyed ranged from 0.01 to 122.56 km^2 . Long-term analysis of Harp Lake was achieved primarily through weekly, fortnightly or monthly sampling since 1978 at a single mid-lake station, with the exception of one study that collected fortnightly samples and diel samples over a 24 hour period. An 80 μm mesh size conical net (0.12 m diameter) was

mainly used. Great Lakes research, for the most part, focused on Lake Michigan using time-series data collected from a 100 m deep reference station, multiple stations, or data from cruises, all of which were conducted, at some point, between May and September. Zooplankton were collected using a Puget Sound 130 μm (1 m diameter), 62 μm (0.5 m diameter), or 153 μm mesh size conical net. Lakes Erie, Huron, Superior and Ontario were rarely included.

The mesocosm experiments we included were conducted in Canada and Sweden. Both studies used clear, plastic cylinders (1 to 1.6 m in diameter) suspended on floating wooden frames in a lake, and elevated 0.3 m above the surface water level to prevent immigration of new species. Mesocosms ranged in depth from 8 (Sweden) to 8.7 m (Canada). Sampling frequency also varied with location. In Canada, samples were collected once a week using an 80 μm (0.15 m diameter) mesh size conical net, whereas in Sweden, sampling was done twice a week using a 100 μm (0.25 m diameter) net. Experimental manipulations included *Bythotrephes* addition, or non-added control, fully crossed with stages of recovery from acidification (recovered versus acid-damaged) for 28 days in Canada and zooplankton communities inoculated with *Bythotrephes* densities (0, 100, 600, 1000 individuals/ m^3) for 16 days in Sweden. The majority of the laboratory experiments included were conducted in Canada and used clear acrylic cylinders, 60-80 cm tall (18-19 mm diameter) and diffuse light (50 W halogen bulbs) to investigate the response of *Daphnia mendotae* and copepods to *Bythotrephes* water-borne cues. Wide-mouth Nalgenes have also been used to examine the prey preference of *Bythotrephes* over a short time frame (24 to 96 hours).

Within the above five study sub-categories, we scored impacts on zooplankton abundance and species richness, first at the whole crustacean community level, and for Cladocera and Copepoda, and finally, for individual Cladocerans and Copepod species.

To further address our first objective, we employed a different approach to assess the impact of Ca decline on zooplankton. Calcium thresholds were tallied only for daphniids, the cladoceran genus reported to be most sensitive to Ca decline. All Ca thresholds were reported in mg/L, and all ambient Ca was assumed to be present as free Ca since the majority of the studies were conducted in soft waters. Unless otherwise indicated, Ca thresholds were reported as nominal concentrations if they were presented as such in the literature. Laboratory-defined Ca thresholds for experiments using various Ca concentrations and other stressors such as high/low and good/poor food, temperature, and ultra-violet (UV) radiation, were also recorded. The laboratory-defined thresholds were for the section of the experiment that was conducted at or above incipient food levels, and at standard, control experimental temperatures. Ca thresholds were also provided for daphniids derived from field studies such as lake surveys and a microcosm study. Ca content was recorded for all zooplankton species found in the literature. Paleolimnological studies were also included to increase our understanding of the subsequent changes in cladoceran remains associated with Ca decline. The majority of these studies used the “top/bottom” approach (Smol 2008). Some studies focused on changes between modern and pre-industrial times, whereas others had a secondary objective that examined changes in the sedimentary remains of lakes above and below 1.5 mg Ca/L, the laboratory-defined threshold for *Daphnia pulex* (Ashforth and Yan 2008). To capture the objectives of all studies reviewed, we divided the data into two groups: those that examined the impact on cladocerans below and

above 1.5 mg Ca/L; and those that examined changes in the relative abundance of cladocerans between modern and pre-industrial times.

Cairns and Yan (2009) identified three gaps in the literature on the effects of Ca decline on crustacea. These gaps were: 1) no verification of experimental thresholds of daphniids in situ; 2) use of species that originate from or are adapted to Ca-poor waters to fully understand impacts of Ca decline; and 3) lack of knowledge of Ca saturation points and lower lethal thresholds for aquatic species other than daphniids. During our review, we determined if these gaps have been filled.

Finally, our concern was with the co-occurrence of these two stressors, a factor little examined in the literature. To examine the likely joint impacts of a *Bythotrephes* invasion and Ca decline, we used a population demographics conceptual model to ascertain how both stressors would impact birth and death rates and thus overall population size. We consider factors such as growth rates, predation, time to maturation and primiparity. For this model and based on the details of our review, we assessed the likely sensitivity of differing zooplankton species to the co-occurrence of the stressors, identifying those species that would most likely suffer synergistic impacts.

How does *Bythotrephes* impact zooplankton communities?

Bythotrephes is by far, the world's most studied invasive zooplankter (Strecker 2011; Bollens et al. 2002). In its native range in Europe, *Bythotrephes* inhabits lakes that are larger and deeper, with higher transparency, lower maximum bottom temperatures during the summer, lower maximum surface temperature, and lower total chlorophyll concentrations in comparison to uninvaded lakes (MacIsaac et al. 2000) and it has a relatively minor impact on zooplankton communities compared to changes observed in Canadian Shield lakes (Kelly et al. 2012).

At the overall community level in North America, crustacean zooplankton abundance declined in response to *Bythotrephes* invasion. Abundance declined in three out of six studies, increased in one study, and no effects were observed in the remaining studies (Table 2). These studies ranged from broad scale lake surveys including long-term monitoring in the Great Lakes to controlled field mesocosm experiments. There were, however, a few contrasting studies. Increased crustacean zooplankton abundance in Lake Huron was likely due to higher densities of copepods and their nauplii post-invasion, which was attributed to *Bythotrephes*' higher clearance rates on cladocerans than fast-swimming copepods (Fernandez et al. 2009). Boudreau and Yan (2003) detected no differences in overall crustacean zooplankton abundance between invaded and uninvaded lakes, because they sampled only once and may not have captured seasonal zooplankton changes. It would appear that studies that examine zooplankton over the entire ice-free season, irrespective of study design, are better able to capture changes in crustacean zooplankton abundance in response to *Bythotrephes* than synoptic surveys (Table 2; Table S1).

Species richness, like crustacean zooplankton abundance, declined post-invasion. This decline occurred in seven out of nine studies (Table 2). In Harp Lake, ice-free species richness declined by 17-18%, from 9.92-9.98 species/count pre-invasion to 8.1-8.25 species/count post-invasion (Yan et al. 2002, 2001). Strecker et al. (2006) observed similar reductions from a mean of 15 species in uninvaded lakes to 11.6 species in invaded lakes during the ice-free season from May to September. Similar trends were observed in 26 invaded Canadian lakes (a reduction from 10.9 species pre-invasion to 9.65 species post-invasion) (Kelly et al. (2012) and in the Great Lakes (Table 2). Like crustacean zooplankton abundance, Fernandez et al. (2009) observed an overall increase in species richness post-invasion in Lake Huron, despite a reduction in cladoceran abundance. Increased species richness was attributed to the increased number of copepods sampled, and the large number of samples collected post-invasion. Another theory postulated was the presence of offshore species in ecological niches that became available due to the disruption of nearshore communities by *Bythotrephes*. In addition, predation on the visible, slow-moving, more dominant prey, could have resulted in the increased abundances of faster-moving species (e.g. copepods) or small cladocerans that are able to escape *Bythotrephes*, which in turn become dominant ("size efficiency hypothesis"; Brooks and Dodson 1965). Strecker and Arnott (2005) detected no effect on species richness using a four week enclosure experiment. The short length of this experiment, however, may be the reason why no effect on species richness was observed when compared to North American lake surveys that consistently observed declines over time. The absence of an effect on species richness suggests that short-term studies (e.g. mesocosms) may not be ideal to identify the potential long-term impacts of *Bythotrephes*. Over time, studies conducted in inland lakes may reflect increased species richness post-invasion as

documented in the Great Lakes (e.g. Fernandez et al. 2009) and in Norwegian lakes (cf. Hessen et al. 2011) that have been invaded for centuries (Kelly et al. 2012).

A reduction in crustacean zooplankton abundance and richness was primarily associated with the loss of cladoceran zooplankton taxa. Cladoceran richness declined by 36% post-invasion in Harp Lake (Boudreau and Yan 2003) with average cladoceran species/count declining from 5.8 to 2.44 species/count (Yan et al. 2001). This decline was not restricted to Harp Lake, however, and was documented in a survey of 26 invaded Canadian lakes (Kelly et al. 2012) and 10 invaded lakes in the Muskoka-Haliburton-Parry Sound regions (Strecker et al. 2006). Cladoceran abundance like cladoceran richness also declined post-invasion in Harp Lake (Yan et al. 2001), other invaded lakes in the Canadian Shield lakes (Strecker et al. 2006), and in the Great Lakes (Barbiero and Tuchman 2004). Changes in cladoceran abundance were attributed to the loss of small cladocerans in the Great Lakes (Fernandez et al. 2009) and Harp Lake (Yan et al. 2002). *Bythotrephes* impact on small cladoceran abundance was also documented in field mesocosm experiments (Strecker and Arnott 2005).

The pervasive loss of cladoceran taxa observed by different studies in response to *Bythotrephes* indicates that we can expect a similar outcome in lakes that may become invaded in the future. But are we sure that small cladocerans are more sensitive to *Bythotrephes* than other species? And of this group, can we identify the most sensitive species to *Bythotrephes*? Based on the frequency of negative impacts observed per study, *Bosmina* is the most sensitive Cladoceran to *Bythotrephes*, declining in 12 out of 15 studies (Fig. 1). The macro-invertebrate predator, *Leptodora kindtii* and *Daphnia retrocurva* are the second and third most sensitive species

respectively (Fig. 1). Only one copepod, the carnivore *Mesocyclops edax* appears sensitive to *Bythotrephes*. *Bythotrephes* may interact competitively with the native macro-invertebrate predators, *Leptodora kindtii* and the glacial relict *Mysis diluviana* (opossum shrimp). In long-term monitoring studies in Harp Lake (e.g. Yan and Pawson 1997), the Great Lakes (e.g. Lehman and Cáceres 1993), and lake surveys (e.g. Weisz and Yan 2011; Foster and Sprules 2009; Strecker et al. 2006), *Bythotrephes* has severely reduced *Leptodora* abundances. *Bythotrephes* is also responsible for changes in the diet of *Mysis diluviana* in Canadian Shield lakes (Nordin et al. 2008); no effect of *Mysis diluviana* has been detected on *Bythotrephes* abundance (Jokela et al. 2011; Foster and Sprules 2009).

Negative impacts on copepods (e.g. *Mesocyclops edax*, *Leptodiaptomus minutus*) are surprising since the majority of North American studies have not detected effects on overall copepod abundance or richness (Table 2). This suggests that *Bythotrephes* may have greater impacts at the species level for copepods, in which more sensitive species are replaced by more tolerant ones that may perform similar ecological functions (“functional complementarity”; Frost et al. 1995). *Bythotrephes* can eat copepods (Grigorovich et al. 1998), but they have faster escape responses than do *Daphnia retrocurva*, and *Daphnia pulicaria* under light and dark conditions (Pichlová-Ptáčníková and Vanderploeg 2011). When prey escape responses are greater than the swimming speed of their predator, the prey can move out of the sensory range of the predator. Copepods also migrate with increasing *Bythotrephes* abundance (Bourdeau et al. 2011) to darker, cooler hypolimnetic waters, which can act as a refuge from predation. The negative impacts documented on copepods documented may be attributed to *Bythotrephes*’ ability to catch slow-swimming species that do not reproduce as quickly as cladocerans.

If we disregard the sensitivity level of *Leptodora kindtii* and *Mesocyclops edax* to *Bythotrephes* in Fig. 1, we see that small cladocerans such as *Bosmina*, *Daphnia retrocurva*, *Diaphanosoma birgei*, *Eubosmina tubicen*, and *Chydorus sphaericus* are more sensitive to *Bythotrephes* than large cladocerans such as *Daphnia mendotae*, *Holopedium glacialis*, and *Daphnia pulicaria* and most copepods (Fig. 1). The lack of sensitivity of *Daphnia mendotae* to *Bythotrephes* is interesting as eight out of 15 studies observed increased abundance post-invasion in contrast to seven out of 15 studies that observed the converse. Increased abundance of *Daphnia mendotae* in invaded lakes is probably due to: 1) its ability to migrate to cooler waters during the day to escape predation and to upper, warmer waters at nights in response to *Bythotrephes* (Pangle and Peacor 2006); 2) faster swimming speeds, comparable to diaptomid copepods, to escape predation (Pichlová-Ptáčníková and Vanderploeg 2011); and 3) its ability to escape predation in low light conditions (Jokela et al. 2013). High predation on *Daphnia mendotae* occurred with increasing light intensities ($>100\mu\text{mol}/\text{m}^2\text{s}$) in the laboratory (Pangle and Peacor 2009).

The impact of *Bythotrephes* on *Holopedium glacialis* is site-specific since there is conflicting evidence that the abundance of this species may increase or decrease in experiments. The relative abundance of *Holopedium glacialis* has increased between 5 and 30% in invaded lakes in south-central Ontario possibly due to: 1) increased predation pressure on daphniids by *Bythotrephes* but also 2) reduced interspecific competition for food with larger Ca-rich daphniids (*Daphnia dubia*, *Daphnia longiremis*, *Daphnia mendotae*, *Daphnia pulicaria*, and *Daphnia retrocurva*) that declined with falling Ca levels (Jeziorski et al. 2015). Increased *Holopedium glacialis* abundances in invaded lakes that were acid-damaged or recovering from acidification is also likely since this species is acid-tolerant, and is found across a broad pH range (e.g. Strecker and

Arnett 2008). Daphniids are vulnerable, not only to *Bythotrephes* but also to *Chaoborus* larvae (phantom midge) that occurs in 100% of invaded lakes surveyed (Jokela et al. 2011). In invaded, low Ca lakes, daphniids are probably more susceptible to predation by both *Bythotrephes* and *Chaoborus* due to their inability to produce anti-predator defences (e.g. in low Ca they have a less calcified carapace, reduced body size, and lack neck teeth) (Riessen et al. 2012), thereby facilitating increased abundances of *Holopedium glacialis* that are superior competitors to daphniids in low Ca environments (Hessen et al. 1995). Increased *Holopedium glacialis* abundance may also occur due to its large gelatinous mantle, which provides protection from invertebrate planktivores (e.g. Vanni 1988) and may prove difficult for *Bythotrephes* to grasp with its thoracic legs. In contrast, neonates and moulted individuals have a thin gelatinous mantle (Hamilton 1958), which suggests that these individuals or life stages would be easier to capture. Increased predation by *Bythotrephes* would affect their recruitment to adults and may account for the reduced abundances observed in five out of 13 studies (Table 2). Reduced abundances may also occur due to size-selection predation, in which *Holopedium glacialis* densities are reduced in lakes with fish (e.g. Stenson 1973). Where these lakes are invaded by *Bythotrephes*, we may see an example of “emergent facilitation” (i.e., stage-specific biomass overcompensation of prey), in which the presence of one predator may help the persistence of another predator, by size-selective foraging on a shared prey, especially when resources are limited (de Roos et al. 2008, 2007). Using mesocosms, Huss and Nilsson (2011) detected an increase in juvenile *Holopedium glacialis* due to increased per capita reproduction, as a result of competitive release, as adults were harvested from treatments (proxy for fish predation). *Bythotrephes* subsequently consumed juveniles, as favoured prey.

Our review allows us to confidently predict that *Bythotrephes* invasion will be followed by a decline in the abundance of small Cladocera and some larger daphniids that are efficient grazers. These daphniids and smaller Cladocera are directly vulnerable to *Bythotrephes* predation (Schulz and Yurista 1999; Grigorovich et al. 1998; Vanderploeg et al. 1993). The reduction in abundance of these herbivorous cladocerans, which are key components of pelagic ecosystems, could result in cascading effects through the food web. For example, algal standing stocks could increase, although the evidence for this is mixed. No such increase was observed in Lake Michigan, despite a decline in herbivory following invasion (Lehman, 1988), in several Shield lakes (Strecker and Arnott 2008) and in experimental mesocosms (Wahlström and Westman 1999). In contrast, algal biomass increased with *Bythotrephes* introduction in mesocosms with zooplankton communities from lakes recovering from acidification (Strecker and Arnott 2005) and in Harp Lake (Paterson et al. 2008). *Bythotrephes* also had an indirect effect on algal composition in Harp Lake and other lakes (Strecker et al. 2011) on the Canadian Shield. Based on the literature, it would appear that we have more to learn about indirect effects of *Bythotrephes* on algae. Any effects may well be site specific, and where they do occur, the effects may be small, given that zooplankton grazing does not have much of an influence on average algal standing stocks in oligotrophic lakes on the Shield (Paterson et al. 2008).

Overall, cladocerans suffer more than copepods from *Bythotrephes* invasions, but sensitivity varies among Cladocera species, with *Bosmina* being most vulnerable. But is there a similar trend at different levels of taxonomic resolution? *Bythotrephes* has greater impacts at higher levels of taxonomic resolution, i.e. at the species, rather than at the order level (Fig. 2). We can confirm that cladocerans are more sensitive to *Bythotrephes* than copepods. In freshwater

ecosystems, loss of cladocerans may result in the concomitant increase in more tolerant species (e.g. copepods), which may alter lake food web interactions. For example, loss of herbivorous zooplankton could result in bottom-up cascades through the food web as copepods are less efficient grazers of algae than herbivorous cladocerans. In addition, increased copepod abundances may alter nutrient cycling within lakes as they sequester less phosphorus than *Daphnia* and require more nitrogen (Williamson and Reid 2009).

The impacts of *Bythotrephes* in North American lakes are quite consistent across lake regions, but we cannot as yet assume that the impacts will be permanent. Community, ordinal, and species response to *Bythotrephes* were quite similar in the Laurentian Great Lakes (e.g. Barbiero and Tuchman 2004), Harp Lake (e.g. Yan and Pawson 1997), and other inland lakes on the Canadian Shield. Across these quite different landscapes species richness declined, especially among cladocerans. However, some site-specific impacts have occurred. In some invaded lakes, species richness (e.g. Bernard Lake in Ontario) and abundance (e.g. Peninsula and Vernon lakes in Ontario) were higher in comparison to uninvaded lakes. Also, longer persistence times of *Bythotrephes* resulted in increased species richness possibly due to competitive interactions with other invertebrate predators (Strecker et al. 2006). Lakes in Norway, which *Bythotrephes* invaded centuries ago have greater zooplankton diversity, especially amongst the copepods (Kelly et al. 2012). There were, however, some differences in species response across lakes. In the Great Lakes, variation in species response was likely due to multiple stressors, which may confound the issue of identifying single-stressor impacts of *Bythotrephes*. Differences between inland lakes and the Great Lakes may be a result of factors such as species naiveté to invasion,

species behaviour, lake morphometry and chemistry, the number of lakes used in the study, and sampling design.

While there is no evidence across the landscape of rescue effects by local or regional dispersers on zooplankton communities in invaded lakes, there is evidence of community resilience to *Bythotrephes* with the addition of native dispersers in mesocosm experiments (Strecker and Arnott 2010). Although the majority of Canadian Shield lakes have not recovered in the ~22 years since it was first detected in inland lakes, we can expect that over time regional dispersers may assist in the colonisation of empty niches created by *Bythotrephes* by providing new propagules that are resistant to or can tolerate predation. Through behavioural and morphological adaptations, we may also see communities dominated by copepods with a few cladocerans (e.g. *Daphnia mendotae*) that are able to increase their population over time.

Since multiple environmental stressors are acting on Shield lakes (e.g. increased dissolved organic carbon (DOC), falling total phosphorus (TP); Yan et al. 2008), we may see complementary responses with resistant species dominating. In invaded lakes, we would expect *Bythotrephes* to primarily reduce the abundance of small Cladocera, while abundances of larger taxa such as *Daphnia mendotae* and *Holopedium glacialis* might increase. Declining TP levels would facilitate increased abundances of these species, as they should outcompete smaller cladocerans for food, as this resource decreases (Gliwicz 1990). Increasing DOC would likely reduce the impact of *Bythotrephes* because decreased water clarity would lower the risk of all taxa to visual predators. For example, high predation rates on daphniids and small cladocerans were detected in small plastic enclosures (~24 L) under ambient light than those under dark light conditions (Jokela et al. 2013).

479
480 In summary, quite broad agreement between studies indicates that we do know a lot about the
481 relatively short-term (a few decades) effects of *Bythotrephes* on zooplankton communities.
482 Following invasion, community abundance and richness fall mainly because cladoceran
483 abundance and richness fall, with small Cladocera being particularly vulnerable. Should long-
484 term dynamics of Canadian Shield lakes match Norwegian lakes, zooplankton communities may
485 eventually recover from *Bythotrephes* invasion.

486
487
488 **How does Ca decline affect zooplankton?**

489 Our knowledge on the impacts of declining Ca on zooplankton is poor despite the comprehensive
490 review by Cairns and Yan (2009). In that review, the authors determined that crayfish would
491 likely be the most sensitive crustacean group to ongoing Ca decline. A review of the then-current
492 literature, revealed a lower lethal threshold (the point of 50% mortality and no reproduction
493 possible) of 0.5 mg/L for daphniids. Life history or physiological changes such as diminished
494 longevity, reduced calcification leading to smaller animal size, and delayed maturity and
495 primiparity were theorised to occur in the suboptimal range (point between lower lethal threshold
496 and saturation). However, the authors cautioned that the Ca thresholds for daphniids identified in
497 the laboratory might not reflect actual thresholds for survival and reproduction in the field.

498
499 Cairns and Yan (2009) identified three main knowledge gaps: Ca saturation points and lower
500 lethal thresholds for aquatic species other than daphniids, further research using species that
501 originate from or are adapted to Ca-poor soft waters, and no verification of experimental

daphniid thresholds in situ. Of the three, only one has been subsequently addressed. Thresholds for five daphniid species were estimated by Cairns (2010) using data from a survey of 304 lakes. The critical lower threshold ranged from 1.26 to 1.69 mg/L whereas the optimum Ca varied between 2.76 and 16.1 mg/L (Table 3). The larger daphniids (*Daphnia retrocurva*, *Daphnia mendotae*, and *Daphnia dubia*) were the most sensitive to low Ca with *Daphnia longiremis* being the least sensitive. Distribution of daphniids along a Ca gradient in 346 Norwegian lakes also indicate that the sensitivity of daphniids to low Ca varies among species, for example, *Daphnia cucullata* is more sensitive than *Daphnia hyalina* (Table 3). Related species also vary in their response to low Ca; for example, *Daphnia galeata* has a lower Ca threshold in Norway (0.7 mg/L) than its North American counterpart, *Daphnia mendotae* (1.63 mg/L). Interestingly, these two related taxa are both particularly tolerant to *Bythotrephes* (Hessen et al. 2011). Key knowledge gaps remain. For example, clones of daphniids isolated from Ca-poor waters have rarely been used for ecotoxicological research (but see Ashforth and Yan 2008), and Ca saturation points and lower lethal thresholds have yet to be identified for non-daphniid species.

Daphniids are key herbivores in pelagic food webs that are filter feeders of phytoplankton (Cyr and Curtis 1999), and food for planktivorous invertebrates and fish. Due to their short moult cycles, sensitivity to environmental perturbations, regular parthenogenic reproduction, and ease of culture, daphniids are model organisms to investigate the effects of declining aqueous Ca (Table 3). Survival thresholds for *Daphnia magna* varied between 0.1 and 5 mg Ca/L irrespective of the [Ca] and the secondary stressor used (e.g. high food and UV radiation; Table 3). Survival thresholds for *Daphnia pulex* varied under different experimental conditions with field microcosm experiments providing a higher threshold (1.3 mg/L) compared to the

laboratory-derived threshold (0.1-0.5 mg/L). We would expect laboratory thresholds to be low as *Daphnia pulex* neonates cultured in the laboratory are well fed compared to individuals in the field, where food is a limiting factor since the majority of Shield lakes are oligotrophic. Reproduction and the development of anti-predator defences were impaired at the laboratory-derived threshold of 1.5 mg Ca/L for *Daphnia pulex*. In contrast, reduced reproduction in *Daphnia magna* occurred at 0.5, 1, and 5 mg Ca/L. Saturation levels for *Daphnia magna* ranged from 5 to 10 mg/L and with a Ca content of 7.7% Ca DW (Cowgill 1976), this species clearly requires high Ca waters. The saturation levels for *Daphnia galeata*, and *Daphnia tenebrosa* were similar with complete calcification occurring between 1 and 5 mg/L, suggesting these species are more tolerant of low Ca or soft waters, and require less Ca for complete calcification than *Daphnia magna*. In addition, these results suggest species-specific variation in carapace calcification among daphniids of similar body size.

The lowest lethal Ca level for larger daphniids, such as *Daphnia magna*, *Daphnia mendotae*, and *Daphnia pulex*, is 0.5 mg/L in various experiments. Unfortunately, this threshold, while applicable to controlled environments in the laboratory, may not reflect thresholds in nature. Thresholds observed in the field may be up to three times higher than those in the laboratory (Table 3). The differences observed between laboratory and field thresholds are most likely attributed to the influence of food sufficiency on animal responses to Ca. In the laboratory, test organisms are usually fed to a point of saturation, whereas in soft water, oligotrophic lakes, algal densities are usually well below satiating concentrations, and they are frequently falling of late, as levels of TP, the main determinant of algal densities, are commonly declining (Palmer and

Yan 2013). Low food increases the sensitivity of daphniid survival to falling Ca levels [Ca] (Ashforth and Yan 2008), as it does for other stressors such as road salt (Brown and Yan 2015).

While much research has focused on survival, it is not the key metric needed to assess if populations can persist as aqueous Ca declines. Persistence requires survival, maturation, and reproduction, and these appear to have quite different sensitivities to falling Ca, i.e., daphniid can survive at Ca levels that slow maturation and reduce reproduction. The laboratory-derived threshold for reproduction of *Daphnia pulex* is 1.5 mg/L (Ashforth and Yan 2008), very similar to the field (microcosm)-derived threshold of 1.3 mg/L (Cairns 2010), albeit for population persistence. Field survival thresholds (the [Ca] where the greatest reduction in daphniid presence along a Ca gradient occurs) for other North American daphniids such as *Daphnia longiremis* (1.26 mg/L), *Daphnia dubia* (1.58 mg/L), *Daphnia mendotae* (1.63 mg/L), and *Daphnia retrocurva* (1.69 mg/L) (Cairns, 2010) were also relatively close to the 1.5 mg/L laboratory derived reproduction threshold for *Daphnia pulex* (Ashforth and Yan 2008).

Since Cairns and Yan's review, paleolimnological studies have documented changes in daphniid assemblages associated with declining aqueous Ca. Coincident with declining Ca levels, the relative abundances of daphniids from the *Daphnia longispina* species complex (*Daphnia ambigua*, *Daphnia dubia*, *Daphnia mendotae*, *Daphnia longiremis*, and *Daphnia retrocurva*) declined in post-industrial (modern) sediments in all but one of 37 lakes (Jeziorski et al. 2012a). Dickie Lake, the single lake where declines in the *Daphnia longispina* species complex was not detected, had Ca levels that increased from 2.1 mg/L to 3.2 mg/L in the late 1990s, following additions of calcium chloride (CaCl₂) as a dust suppressant to gravel roads (Shapiera et al. 2012).

As the relative abundances of the *Daphnia longispina* species complex declined in the modern sediments of 36 lakes (Jeziorski et al. 2012a), there was a concomitant increase in the relative abundances of the *Daphnia pulex* species complex (*Daphnia catawba*, *Daphnia pulex*, and *Daphnia pulicaria*), *Bosmina* and *Holopedium glacialis* (Table 4). In contrast, as the *Daphnia longispina* species complex increased in the sediment profile of Dickie Lake, the relative abundances of the *Daphnia pulex* species complex and *Holopedium glacialis* also increased, but bosminid relative abundances declined (Shapiera et al. 2012) (Table 4). When low- and high-Ca lakes were considered separately, contrasting responses were observed in the two categories. Daphniid relative abundances declined in lakes with current $\text{Ca} < 1.5 \text{ mg/L}$, the laboratory-derived reproduction threshold, while bosminid relative abundances increased. Conversely, in lakes with $\text{Ca} > 2 \text{ mg/L}$ daphniid relative abundances increased as bosminid relative abundance declined (Table 5). Where aqueous Ca has declined, Ca-rich daphniids may be at a competitive disadvantage against species with much lower Ca content such as bosminids and *Holopedium glacialis* (Yan et al. 1989).

Although most paleolimnological studies observed reductions in daphniid relative abundances in lakes with $\text{Ca} < 1.5 \text{ mg/L}$, there is varied response to low Ca within daphniid species complexes. Although species within the *Daphnia pulex* species complex cannot be distinguished in the paleolimnological record, plankton samples taken from the study lakes revealed that *Daphnia catawba* was the dominant daphniid present in lakes with $\text{Ca} < 1.5 \text{ mg/L}$ (Jeziorski et al. 2012b). Higher abundances suggest that *Daphnia catawba* has greater tolerance to low Ca than other members in the same species complex and would also explain why relative abundances of *Daphnia pulex* species complex increased in modern sediments. Cairns (2010) similarly

observed no change in the occurrence of *Daphnia catawba* with falling Ca in her survey of 304 Ontario lakes confirming Cairns and Yan's (2009) prediction that smaller daphniids including *Daphnia catawba* have lower Ca lethal thresholds and Ca saturation points allowing them to persist in lake with lower Ca levels than their larger counterparts. This prediction requires additional experimental examination.

The Ca content of zooplankton varies among and within species, depending on body size, life stage, and ambient Ca in the water. The Ca content of daphniids is certainly high (1-7% DW) compared to other non-daphniid cladocerans (0.04-2.32% DW) and copepods (0.05-0.4% DW) (Table 6). Surface area to volume ratio decreases with increased body size and the majority of post-moult Ca extracted from the water is deposited in the carapace to make a calcified exoskeleton. Therefore, increased body size should result in decreased the carapace Ca-content (Alstad et al. 1999), assuming that carapace calcification does not vary with body size. This prediction, however, does not apply to most daphniids. Large daphniids have higher Ca content (e.g. 5.2 % DW for *Daphnia mendotae*) compared to smaller daphniids (e.g. 0.8% DW *Daphnia cristata*) (Table 6). Juveniles, however, on average, have higher Ca content than adults (e.g. 3.35% DW versus 2.79% DW) cultured under similar laboratory conditions (Table 6). Differences in Ca content between life stages is not surprising since juveniles have a larger surface to body volume ratio, and therefore has a greater Ca demand than adults in order to overcome "juvenile bottleneck" that is associated with Ca deficiency (Hessen et al. 2000).

Smaller daphniids generally have low Ca content, suggesting that these animals may require less Ca to make a calcified, albeit thinner carapace compared to their larger counterparts. This may

have implications for predator-prey relationships as small daphniids with thinner carapaces could be easily crushed and without a defence mechanism, there would be more successful predator attacks. To date, our knowledge on daphniid response to predators in low Ca is limited to *Daphnia pulex* and *Chaoborus americanus* larvae. In this predator-prey relationship, *Daphnia pulex* defences were impaired resulting in weaker carapaces, and a smaller body size in low Ca environments in the presence of *Chaoborus* (Riessen et al. 2012); as such, further research is required for other daphniids found on the Canadian Shield.

Calcium concentration of the ambient water can also influence the Ca content of daphniids. The Ca content of *Daphnia pulex*, *Daphnia catawba*, and *Daphnia ambigua* cultured in the laboratory at 2.5 mg Ca/L was four to seven times lower (Table 6) than from populations collected from the wild from higher Ca lakes (Jeziorski et al. 2015). Jeziorski and Yan (2006) similarly observed significant differences between the Ca content of *Daphnia catawba* and *Daphnia pulex* collected from lakes ranging widely in [Ca]. The influence of the [Ca] of the water on the Ca content of an individual is, however, not limited to daphniids. The Ca content of *Bosmina freyi* cultured in FLAMES medium at 2.5 mg Ca/L was three times lower than related taxa collected in the field (Table 6); however, unlike *Daphnia pulex* and *Daphnia catawba*, there were no significant differences in Ca content for *Bosmina* collected from lakes of varying [Ca] (Wærvågen et al. 2002).

Non-daphniid cladocerans, for the most part, have lower Ca content than daphniids (0.04-0.8% Ca DW). The low Ca content of *Holopedium glacialis* is not surprising, since this species is covered by a transparent, gelatinous, polysaccharide mantle, lacks a calcified carapace, and is

often a dominant species in low Ca lakes in Norway (Wærvågen et al. 2002), and Canada (Jeziorski et al. 2015). However, there are some non-daphniid cladocerans that have higher Ca content compared to other species in the non-daphniid cladoceran group. The littoral taxa *Disparalona* sp., *Pleuroxus truncatus*, *Scapholebris rammneri*, and *Alona rectangula* (1.46-2.32% Ca DW) were on par with other daphniids (Table 6). The Ca content of these littoral species would suggest that Ca-rich daphniids may not be the only cladocerans to be impacted by declining Ca; however, this possibility has not been tested to date. Since the majority of non-daphniid cladocerans are small, we would expect them to have large surface area to volume ratios and therefore, higher Ca content; however, this is not the case as they have low Ca content that ranged from 0.04 to 1.2%Ca DW. It is possible that these small cladocerans, like daphniids, are able to regulate their Ca through influx and efflux rates (Tan and Wang 2010; 2009); as such, these species may have high efflux and low influx rates, which could explain their low Ca content. Like smaller daphniids, we are unaware how the Ca-content of these small cladocerans may impact their response to predators in low Ca environments.

Copepods have lower Ca content than daphniids and non-daphniid cladocerans (0.05-0.4% Ca DW) (Table 6). The low Ca content of copepods suggests a weakly calcified carapace and tolerance to declining Ca; however, there are no studies that have examined the effects of declining Ca on copepods and there is no available information on calcium storage within copepods (Greenway 1985). Since specific Ca content can vary with body size (e.g. Alstad et al. 1999), it is possible that copepod juveniles have greater Ca demands to overcome “juvenile bottleneck” associated with Ca deficiency; however, there is no evidence to support our hypothesis. Limited information on the Ca content of copepods suggest that we have more to

learn; for example, we are unsure: 1) how copepod life stages would respond to Ca deficiency; 2) how food supply may impact copepods in declining Ca environments; and 3) how copepods respond to predators in Ca deficient environments.

We have shown that Ca content varies between crustacean zooplankton taxa, with 71% of the daphniids having higher Ca concentration (2.4-7.7% Ca DW) than non-daphniid cladocerans and copepods. There is also a positive relationship between body Ca content and the [Ca] in the water (Alstad et al. 1999; Cowgill et al. 1986; Jeziorski and Yan 2006); as such, species with high Ca content should be more sensitive to declining Ca than their counterparts. We tested this hypothesis on the daphniids, since they are the only group for which we have Ca thresholds for survival and reproduction. Although survival is not a key metric for population persistence in response to declining Ca, it was the only metric for which we had a Ca threshold for all daphniids (Table 3). We use survival as a proxy for sensitivity to declining Ca. A measure of sensitivity was achieved by averaging the [Ca] threshold listed for each species, where applicable. A similar approach was taken for Ca content (Table 6). Sensitivity to declining Ca appears not to be dependent on the Ca content of daphniids (Fig. 3).

Species with high Ca content (>2.4% Ca DW) such as *Daphnia magna*, *Daphnia pulex*, *Daphnia mendotae*, and *Daphnia hyalina* had low sensitivities to declining Ca. A similar trend was observed in daphniids with low Ca content, such as *Daphnia cristata*, *Daphnia longiremis*, *Daphnia longispina*, and *Daphnia galeata* (Fig. 3). *Daphnia tenebrosa* and *Daphnia cucullata* were the only daphniids with low Ca content and high sensitivity to declining Ca. As expected, *Daphnia ambigua* and *Daphnia catawba* have low sensitivities to declining Ca as well as

Daphnia longiremis, which agrees with the sensitivities described by Cairns (2010). We were unable to determine the relationship between sensitivity and Ca content for *Daphnia retrocurva*, nor for *Daphnia pulicaria*, and *Daphnia dubia* due to missing data on their Ca content. The majority of the Norwegian daphniids have low sensitivities to declining Ca. Our findings are not surprising, since soft water lakes in this region have lower [Ca] than lakes in North America, and the daphniids from Norwegian lakes have lower Ca content than their North American counterparts (Fig. 3; Table 6). Similarly, Ca content for *Holopedium glacialis* in Norway is lower compared to North America, while the Ca content for *Diaphanosoma birgei* is the same, irrespective of origin.

Our findings indicate that Ca content is not a key indicator of the sensitivities of daphniids to low Ca. These results also suggest that haemolymph Ca content or some other source of Ca within the body might be a better indicator of species response to declining Ca. Ca content as the imperfect metric for daphniid sensitivity was also documented by Tan and Wang (2010) using life table experiments and four cladocerans. The daphniids, *Daphnia carinata* and *Daphnia mendotae*, had higher Ca content but mortality was 100% after 21 and seven days respectively at 0.5 mg Ca/L. In contrast to daphniids, the Ca-poor species, *Ceriodaphnia dubia* and *Moina macrocopa*, had lower influx and higher efflux rates than their Ca-rich counterparts. Survival for *Ceriodaphnia dubia* was better at 0.5 mg Ca/L in contrast to *Moina macrocopa* that had 100% mortality within 15 days of birth. The authors theorised that a species response to declining Ca may be dependent on the supply of Ca in the environment and the ability of the individual to extract and retain Ca from the environment. Water is the major source of Ca for post-moult calcification. The length of time required for post-moult calcification is dependent on the [Ca] of

the water and the availability of food (Greenaway 1985). Unfortunately, regardless of the [Ca] of the water, daphniid moulting rate is fixed and therefore, they cannot increase the post-moult time period for Ca influx (Hessen et al. 2000). Although food is a minor contributor to the Ca requirements of aquatic organisms, it is also necessary for the synthesis of an organic matrix in the endoskeleton in which the Ca will be deposited (Greenaway 1985).

In summary, we found strong evidence that populations of Ca-rich daphniids are damaged by environmental Ca decline. However, most laboratory studies have employed Ca-rich daphniids such as *Daphnia mendotae*, *Daphnia pulex*, and *Daphnia magna* as test animals, and there is much more limited evidence on how declining Ca will affect other daphniids (e.g. *Daphnia longiremis*, *Daphnia dubia*, and *Daphnia retrocurva*), other cladocerans and copepods. Specifically, lethal and optimum Ca thresholds have not been identified for non-daphniid Cladocerans and copepods. There is also limited research on the particular effects of declining Ca levels on entire zooplankton communities.

To date, possible clonal, or population differences of species to Ca decline have received little if any attention. Most studies have employed single clones from single lakes or ponds. Rukke (2002b) proves that this research gap might be serious. She compared the sensitivity to declining Ca of *Daphnia galeata* isolated from two lakes, and found the neonates from a low Ca lake (2-3 mg/L) had higher mortality at 0 mg Ca/L compared to the neonates from a high Ca lake (>10 mg/L). Clearly, inter-population variation may exist; thus determining how zooplankton communities from lakes of varying [Ca] respond to falling Ca is an important knowledge gap. Examining how individual taxa and populations from different soft water lakes respond to declining Ca is important as there could be local adaptation in which some taxa and populations

731 have lower Ca thresholds. We may also see the impact of maternal effects, which would impact
732 an offspring's fitness and survival to environmental variation. Controlled field mesocosm and
733 laboratory experiments using daphniids and zooplankton communities from low Ca soft water
734 lakes should be conducted to aid our understanding of species response in areas where Ca
735 decline is projected to continue. Further research is also required to determine if populations
736 from lakes with high and low Ca have different survival thresholds to declining Ca.

737
738 We have also shown that body Ca content is not a good indicator of daphniid sensitivity to
739 declining Ca, and as such, cannot be used to predict how different species will respond to
740 declining Ca. Their response may depend on their Ca uptake mechanisms; but there is limited
741 knowledge on how the uptake mechanism changes for daphniids, other cladocerans, and
742 copepods in low Ca environments. In addition, we are unaware how haemolymph Ca content or
743 other sources of Ca in the body may impact a species' response to declining Ca.

744
745 Paleolimnological field studies have documented large changes in the relative abundances of
746 taxa over recent decades during a time when lake water Ca levels have fallen. However, these
747 studies have drawbacks in taxonomic resolution (Smol 2010), i.e., they cannot currently
748 distinguish the daphniid remains of species that have high (e.g. *Daphnia catawba* and *Daphnia*
749 *ambigua*) or low (e.g. *Daphnia pulicaria* and *Daphnia mendotae*) tolerance to declining aqueous
750 Ca, as these species are present in the two species complexes that can be distinguished.
751 Paleolimnology could resolve these taxonomic drawbacks by resurrecting or conducting genetic
752 analysis on *Daphnia ephippia* present in the sediments of low Ca lakes to identify those taxa that
753 are sensitive or tolerant to Ca decline. The other drawback of paleolimnological studies is it

provides mainly correlational information during a time when many limnological variables have been changing. Nonetheless, we have few, if any, other sources of information that provide such long monitoring records.

Given that multiple stressors are impacting lakes on the Canadian Shield, we need more studies that investigate Ca decline as one among many stressors. Although some laboratory experiments have included secondary stressors, there are few studies that have adopted a multiple stressor approach in the field (e.g. Palmer and Yan 2013; Cairns 2010). As such, further field research is required to increase our knowledge on multiple stressor impacts on zooplankton communities. One such combination of stressors is the possible joint impacts of predation from the invading zooplanktivore, *Bythotrephes*, in Shield lakes that are also suffering widespread, and long-term Ca decline.

How does the presence of both *Bythotrephes* and declining Ca impact zooplankton?

Across Canadian Shield lakes, *Bythotrephes* has colonized both large and small lakes that are experiencing Ca decline. Only one study has considered how zooplankton might respond to both stressors. Using data from a large-scale field survey of 34 lakes that were sampled repeatedly in the 1980s and then again in 2004 to 2005, Palmer and Yan (2013) examined the effects of changes in physicochemical parameters and *Bythotrephes* on crustacean zooplankton assemblages. Total zooplankton abundance decreased whereas species richness and diversity increased over time across all lakes; however in invaded lakes, both metrics decreased. Changes in community composition of crustacean zooplankton were detected in response to *Bythotrephes*

and water quality changes. *Bythotrephes* had a negative effect on species richness and diversity. Interactions between declining TP and *Bythotrephes* and between Ca and mean autumn lake temperature negatively impacted small cladoceran abundance; however, a direct interaction between *Bythotrephes* and Ca on zooplankton communities or on individual species was not detected. As the survey was designed to investigate multiple stressor impacts and their interactions at a regional scale, and as it was conducted not too long after the *Bythotrephes* invasion of local lakes, the study had only five invaded lakes and the number of lakes was not balanced around a putative Ca threshold. Thus the study was not ideally designed to detect any combined effect of *Bythotrephes* and low Ca, and indeed this was not the study's intent.

Bythotrephes is quite tolerant of low environmental Ca levels. It has very low body Ca levels and can produce many offspring at 1.5 mg Ca/L in the laboratory (Kim et al. 2012). As such, it is unlikely that the spread of *Bythotrephes* will be affected by Ca decline. *Bythotrephes* are found in Norwegian lakes with Ca levels as low as 0.4 mg/L (Wærvågen *et al.*, 2002), suggesting that Ca levels in Canadian Shield lakes are certainly not now, nor will they ever be low enough to be lethal to *Bythotrephes*. Therefore, it is likely that crustacean zooplankton in Canadian Shield lakes will experience both stressors simultaneously, i.e., falling Ca will not itself harm *Bythotrephes* directly. To understand how low Ca and *Bythotrephes* could impact zooplankton, we built a conceptual model (i.e. a hypothesis of potential biotic and abiotic interactions) of how *Bythotrephes* and declining Ca might influence the birth and death rates, and thus the population sizes of zooplankton. Due to its tolerance to low Ca, we did not include any direct effects of Ca decline on *Bythotrephes* in the model.

800 Conceptual model

801 *Bythotrephes* remain in the epilimnion or upper metalimnion in most lakes, where there is
802 enough light for them to hunt, thus we did not include a *Bythotrephes* migration component in
803 the conceptual model. On the other hand, in the presence of *Bythotrephes*, some of their daphniid
804 prey do vertically migrate into hypolimnetic waters (Pangle et al. 2007; Lehman and Cáceres
805 1993), whose cold waters will slow growth rates. We included this proven possibility in the
806 conceptual model. With such predator-induced migrations, reduced growth rates would lower
807 daphniid birth rates while also reducing death rates by predation, both a cost and a benefit.
808 *Bythotrephes* also increase prey death rates directly through predation, thus reducing population
809 size (Fig. 4). From laboratory-derived thresholds we know that Ca levels at or around 0.5 mg/L
810 are lethal to daphniids and 1.5 mg/L is a threshold at which reproduction can fall. Ca levels in
811 Canadian Shield lakes are not low enough, as yet, to be directly lethal to daphniids, but many
812 lakes have $\text{Ca} < 1.5$ mg/L (Jeziorski et al. 2015, 2008), and at such levels, several daphniid species
813 may experience reduced growth that delays maturation and primiparity (Fig. 4).

814
815 With our conceptual model, we are able to assess the sensitivities of different crustacean
816 zooplankton to both stressors at an aggregated level. Presently, daphniids appear to be sensitive
817 to both stressors since *Bythotrephes* directly impacts them through predation and declining Ca
818 can reduce their birth rates and thus, their population size. The model can also be expanded to
819 examine the effect of both stressors on small cladocerans (Fig. 4) that are negatively impacted by
820 *Bythotrephes* (Table 2; Fig. 1), and for which we have limited information on their impacts with
821 declining Ca (Table 3). A similar exercise was pursued for copepods (Fig. 4). *Bythotrephes*
822 negatively impacts small cladocerans through predation, reducing their abundances. Most small

cladocerans, with the exception of *Daphnia retrocurva*, can be considered Ca-poor species based on their Ca content or the Ca content of related species (range 0.09-0.6% DW). Copepods are also Ca-poor species with low Ca content (body Ca ranges from 0.05-0.4% DW) but not effected by *Bythotrephes* in general. Although the conceptual model allows us to explore sensitivities to both stressors on zooplankton taxonomic groups, our findings cannot extend to higher levels of taxonomic resolution, as it does not include species interaction in terms of competition or different responses to both stressors. In addition, our findings may not reflect how daphniids, non-daphniid cladocerans, and copepods would respond to *Bythotrephes* predation in high or low Ca in lakes with multiple stressors. Daphniids may use vertical migration, armaments (e.g. neck spines), swimming speeds, or morphological changes in helmets and tail spines (e.g. Bungartz and Branstrator 2003), as an avoidance response to predation; however, there is no existing data, on how migration responses and anti-predator defences may change in the presence of both stressors.

To determine the sensitivity of daphniids, small cladocerans, and copepods (for which we have data) to both stressors, we examined the additive joint sensitivity of each species using the following criteria. Where negative impacts on a species by *Bythotrephes* was documented >10 times (Table 2) a six was assigned; six to eight negative impacts, a four; three to five negative impacts, a two; and one to two negative impacts, a one. With regards to declining Ca, high sensitivity was a six; and low sensitivity, a one. The scores for *Bythotrephes* and low Ca were subsequently added to get the additive sensitivity value. We determined synergism and antagonism for each species based on the likely combined effects of *Bythotrephes* and low Ca (Table 7). Based on the additive values, the order of daphniid sensitivity to both stressors was

846 *Daphnia mendotae* and *Daphnia retrocurva* > *Daphnia pulicaria* > *Daphnia catawba* > *Daphnia*
847 *dubia*, *Daphnia longiremis* and *Daphnia ambigua*. Small cladocerans were ordered as *Bosmina*
848 sp., *Diaphanosoma birgei*, *Eubosmina tubicen*, *Chydorus sphaericus* > *Ceriodaphnia dubia*, and
849 copepods, *Mesocyclops edax* > *Leptodiptomus minutus* and *Cyclops scutifer* (Table 7).

850

851 *Hypothesis 1: synergism on daphniids*

852 Based on our additive sensitivity values, *Daphnia retrocurva* appear to be one of the most
853 sensitive daphniids to both stressors. *Daphnia retrocurva* is heavily preyed upon by
854 *Bythotrephes* (nine out of nine studies: Table 2), which would increase its death rate and
855 consequently, reduce its population size. This species also has a low field prevalence threshold of
856 1.69 mg/L ($\pm 1.15\text{SE}$) (Cairns 2010), making it vulnerable to declining Ca. *Daphnia retrocurva*
857 may experience an additive response from both stressors if *Bythotrephes* directly impacts it
858 through predation, and declining Ca reduces its birth rates relative to death rates. A synergistic
859 response to both stressors is likely, since exposure to declining Ca could also reduce *Daphnia*
860 *retrocurva*'s anti-predator defences (e.g. a less calcified carapace), thus increasing its
861 vulnerability to predation.

862

863 *Daphnia mendotae* was the other daphniid with a high additive sensitivity value (Table 7). As
864 one of the larger daphniids with a Ca content of 5.2% DW (Jeziorski and Yan 2006) and a low
865 field prevalence threshold of 1.63 mg/L ($\pm 0.59\text{SE}$) (Cairns 2010), *Daphnia mendotae* would be
866 extremely vulnerable to declining Ca. Mixed responses to *Bythotrephes* have been documented
867 (Table 2); but coexistence tends to occur in long-term lake studies (e.g. Harp, Great Lakes). To
868 date, *Daphnia mendotae* is the only species with a documented diurnal vertical migration that

leads to increased population abundances post-invasion. Migration to cooler hypolimnetic waters to escape predation would result in reduced growth (e.g. Pangle et al. 2007), but would likely lower death rates more than birth rates; thus, the population would be maintained despite the presence of both stressors. In addition, *Daphnia mendotae* has fast swimming speeds, so its death rate, regardless of migration, may be lower compared to other daphniids, since population increases have been observed post-invasion (e.g. Yan et al. 2001). In addition, we are unaware how behavioural responses (e.g. migration and fast escape rates) to predation are influenced by declining Ca. We therefore conclude that although *Daphnia mendotae* had a high additive sensitivity value, it may not be one of the most sensitive species to both stressors because its effective anti-predator strategies are unlikely influenced by Ca

Daphnia pulicaria, *Daphnia catawba*, and *Daphnia ambigua* populations have been lost in invaded lakes in five out of six, three out of three, and two out of two studies respectively (Table 2). Through direct predation, the death rates of these daphniids would increase and ultimately decrease their population size. Since *Daphnia pulicaria* has a higher frequency of negative impacts than the others, we would expect its death rate to be higher thereby resulting in a smaller population size compared to *Daphnia catawba* and *Daphnia ambigua*. *Daphnia pulicaria* has a high optimum Ca threshold of 16.1 mg/L in the field, which suggests that this species has high Ca requirements and would also be highly susceptible to declining Ca. Like *Daphnia retrocurva*, we may see an additive response of both stressors on *Daphnia pulicaria*, since it is a preferred prey of *Bythotrephes* (Schulz and Yurista 1999), and has high Ca demands. However, a synergistic response is expected since low Ca could impair anti-predator defences for *Daphnia pulicaria* (e.g. a less calcified carapace, and reduced body size), which would increase its

vulnerability to *Bythotrephes*. In addition, in the presence of *Bythotrephes*, *Daphnia pulicaria* migrates to hypolimnetic waters, whose cold waters would slow their growth rates and therefore lower birth rates (Pangle et al. 2007). Death rates would increase and population size would decrease for *Daphnia catawba* and *Daphnia ambigua* in the presence of *Bythotrephes* but the high Ca content of *Daphnia catawba* (4.25% Ca DW) and *Daphnia ambigua* (3.03% Ca DW) would suggest that these species are vulnerable to declining aqueous Ca; however, lower prevalence field thresholds for these daphniids could not be determined using Gaussian logistic regression models, which suggests that both may have high tolerances for declining aqueous Ca (Cairns 2010). Jeziorski et al. (2015) also observed increased abundances for these species in Ontario lakes as Ca levels have declined. We therefore expect an antagonistic response on both *Daphnia catawba* and *Daphnia ambigua*. As larger daphniids such as *Daphnia pulicaria* decline, these species would likely increase in low Ca lakes due to competitive release, which would reduce the magnitude of predation and Ca combined. In addition, we expect some predation by *Bythotrephes* but low Ca would not likely impact body size, since smaller daphniids would require less Ca for calcification.

Last, negative impacts on *Daphnia dubia* and *Daphnia longiremis* associated with *Bythotrephes* invasion have been documented in two out of two and two out of four studies respectively; as such, death rates would likely increase and population sizes would decline over time in invaded lakes. Both *Daphnia dubia* and *Daphnia longiremis* have low prevalence field thresholds of 1.58 mg/L (± 0.96 SE) and 1.26 mg/L (± 0.69) (Cairns 2010) respectively, making them less susceptible to declining aqueous Ca than *Daphnia retrocurva*, *Daphnia mendotae*, and *Daphnia pulicaria*. Like *Daphnia catawba* and *Daphnia ambigua*, we would expect an antagonistic response on

these daphniids, since they would likely increase in low Ca environments due to competitive release, and be consumed by *Bythotrephes*.

Our hypothesis may change in unstratified lakes of various sizes that have no hypolimnion or a dark layer for refuge against predation. Cairns (2010) observed reduced frequency of occurrence for all daphniids in unstratified lakes with low Ca. We therefore predict that the effects of both stressors may be greater in shallow or unstratified lakes. We may lose *Daphnia mendotae*, a common pelagic species that is found in 56-63% of Canadian Shield lakes (Keller and Pitblado 1984; Locke et al. 1994). *Daphnia pulicaria*, a key herbivore in the aquatic food web would also be lost in unstratified lakes with both stressors. Loss of these daphniids along with *Daphnia retrocurva*, and *Daphnia dubia*, could result in the concomitant rise of *Daphnia catawba* and *Daphnia ambigua* through competitive release; however, these species are also prey for *Bythotrephes*. Over time, we may also see the loss of these daphniids in unstratified lakes with both stressors, which may result in the increase of *Holopedium glacialis*, a species that exhibits both a neutral to positive response to *Bythotrephes* but has a competitive advantage over daphniids in low Ca environments (Hessen et al. 1995; Jeziorski et al. 2015) and/or copepods that are Ca-poor and relatively unaffected by *Bythotrephes*.

Hypothesis 2: antagonism on small cladocerans

As one of the preferred prey of *Bythotrephes*, direct predation would increase small cladoceran death rates, ultimately leading to a reduced population. Based on their low Ca content, declining aqueous Ca would likely increase their birth rates and population size through release from competition as the negative impacts of declining Ca would be greater on daphniids; as such, the

likely impacts of *Bythotrephes* and declining aqueous Ca presented supports our hypothesis of an antagonistic response on small cladocerans. The additive sensitivity values calculated also suggests that small cladocerans are similar to daphniids in their expected response to both stressors. Additive effects were similar for most of the small cladocerans but were higher than *Daphnia catawba*, *Daphnia longiremis*, *Daphnia dubia*, and *Daphnia ambigua* (Table 7). Higher additive effects for small cladocerans compared to some small (e.g. *Daphnia ambigua*) and medium-sized (e.g. *Daphnia dubia*) daphniids suggest that body size does not play a key role in species response to both stressors.

Hypothesis 3: no effect on copepods

Unlike small cladocerans and daphniids, we expected no effect of *Bythotrephes*, declining Ca or both on copepods since: 1) copepods are relatively unaffected by *Bythotrephes*; and 2) their Ca content suggests tolerance to declining Ca. However, we saw some response at the species level. *Mesocyclops edax* was the only copepod with an additivity value comparable to small cladocerans (mainly driven by its susceptibility to *Bythotrephes*), and the additivity values for *Leptodiaptomus minutus*, and *Cyclops scutifer* were low and similar to *Daphnia ambigua*, *Daphnia catawba*, *Daphnia dubia*, and *Daphnia longiremis* (Table 7).

As we pointed out earlier, Ca content represents the Ca demand of a species but does not indicate how the species will cope with Ca deficiency. In addition, there are no existing experimental data about the effects of declining Ca for non-daphniids and copepods. Nevertheless, based on our joint effects assessment, zooplankton susceptibility to both stressors may be more pronounced at the species level. If we combine what is known and projected for species response in lakes with

both stressors, declining Ca will negatively impact the larger daphniids such as *Daphnia pulicaria* that would allow the increase of small cladocerans (including small daphniids) and copepods through competitive release (e.g. Jeziorski et al. 2015). *Bythotrephes*, in turn, would reduce the abundance of small cladocerans and daphniids, leaving a community dominated by copepods and *Holopedium glacialis*. This double impact on daphniids is an example of negative species co-tolerance (Vinebrooke et al. 2004), a form of synergistic interaction in which the presence of one stressor increases the effect of the other stressor.

Our hypotheses on how a particular group or individual species may respond to *Bythotrephes* and declining aqueous Ca may not reflect what happens in reality as invaders can modulate the impact of other stressors, making it harder to distinguish their individual effects (Strayer 2010). Ecological surprises (Paine et al. 1998) that result in synergistic, antagonistic, or additive effects (Folt et al. 1999) are also possible. Nevertheless, we can test our hypotheses using controlled field experiments such as mesocosms and/or data from large-scale field surveys. Mesocosms are advantageous in providing a semi-natural and reasonably controlled environment (Townsend et al. 2008). Use of zooplankton communities in field experiments would allow for the identification of those species within the community that are most sensitive to either *Bythotrephes* or declining Ca, as well as the integration of ecological processes (e.g. food web dynamics) that naturally occur in lake ecosystems (Culp et al. 2000). Field surveys of a large number of invaded lakes could provide information on how a community with varying functional and life-history attributes may respond to *Bythotrephes* and declining Ca, and show patterns that may arise in the community because of both stressors (Thrush et al. 2008).

Summary

The literature is clear that *Bythotrephes* exerts a negative effect on crustacean zooplankton at least for a few decades after invasion, but as Ca decline has been studied mainly on daphniids, we do not yet know the overall effects of Ca decline on zooplankton communities. However, given their Ca requirements and their general sensitivity to *Bythotrephes* we do expect daphniids to be the most sensitive species to both *Bythotrephes* and declining Ca. It is unclear, however, how other zooplankton, cladocerans as a taxonomic group, and the zooplankton community will respond in lakes with both stressors.

Bythotrephes are found in lakes with $\text{Ca} < 0.5 \text{ mg/L}$ in Norway (Kim et al. 2012); however, in south-central Ontario, we are at an early stage of the *Bythotrephes* invasion. Of the 179 invaded inland lakes in Ontario (EDDMaps Ontario) for which we have Ca data (i.e., 147 lakes), 22 lakes currently have Ca levels $< 2 \text{ mg/L}$, and 5 have $\text{Ca} < 1.5 \text{ mg/L}$ (Dorset Environmental Science Centre, unpublished data). Such a small representation of invaded, low Ca lakes suggests that the prevalence of both stressors in combination is not yet widespread. In addition, although 27 Ontario lakes with both stressors is minor relative to the number of lakes on the Canadian Shield, as *Bythotrephes* spreads through regions where Ca levels are declining, we are likely to see an increase in the co-occurrence of these stressors. Nonetheless, we have to consider that: 1) we have likely underestimated the number of invaded lakes; 2) the presence of more than one stressor in an ecosystem could result in indistinguishable direct and indirect causal linkages among multiple drivers (Didham et al. 2005); 3) communities within ecosystems may vary in response to multiple stressors because different interactions may occur between the component species (Crain et al. 2008); and 4) multiple stressors effects may be unpredictable - biotic and

abiotic factors and the magnitude of either stressor could change, thereby dictating how individual species may respond to the stressors present (Breitburg et al. 1998). The complexity of these factors suggest that we may not be able to predict the possible impacts of the co-occurrence of *Bythotrephes* and declining Ca on zooplankton in any particular Canadian Shield lake with confidence. Nevertheless, on average, and in lakes with both stressors, we expect to see the loss of large daphniids such as *Daphnia pulicaria*, a key herbivore in the aquatic food web. Over time, we expect the loss of other daphniids (e.g. *Daphnia retrocurva* and *Daphnia dubia*) and small cladocerans, with lake communities dominated by *Holopedium glacialis* and/or copepods. Indeed, we have some field evidence that these changes are indeed occurring (e.g. Jeziorski et al. 2015).

The conservation and management of Shield lakes may depend on our ability to understand the simultaneous influence of the stressors present. The unpredictability of multiple stressors and the varied response that can be seen in lake ecosystems supports further research on multiple stressors on zooplankton within Canadian Shield lakes. Research on the likely impacts of *Bythotrephes* and Ca decline on zooplankton communities is crucial since *Bythotrephes* is spreading rapidly despite declining Ca, and daphniids appear to be group most likely to be extirpated. We suggest that controlled field experiments and large-scale lake surveys will be useful to predict the interacting effects on zooplankton of *Bythotrephes* and declining Ca.

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REFERENCES

- Alstad, N. E. W., Skardal, L., and Hessen, D. O. 1999. The effect of calcium concentration on the calcification of *Daphnia magna*. *Limnology and Oceanography* **44**(8):2011-2017.
- Altshuler, I. et al. 2011. An integrated multi-disciplinary approach for studying multiple stressors in freshwater ecosystems: *Daphnia* as a model organism. *Integrative and Comparative Biology* **51**(4):623-633.
- Ashforth, D. and Yan, N. D. 2008. The interactive effects of calcium concentration and temperature on the survival of *Daphnia pulex* at high and low food concentrations reproduction. *Limnology and Oceanography* **53**(2):420-432.
- Baby, R. L. et al. 2010. Nutrient analysis of some commercially important molluscs of Bangladesh. *Journal of Scientific Research* **2**(2):390-396.
- Barbiero, R. P. and Tuchman, M. L. 2004. Changes in the crustacean communities of Lakes Michigan, Huron, and Erie following the invasion of the predatory cladoceran *Bythotrephes longimanus*. *Canadian Journal of Fisheries and Aquatic Sciences* **61**(11):2111–2125.
- Baudouin, M. F., and Rivera, O. 1972. Weight, size, and chemical composition of some freshwater zooplankters: *Daphnia hyalina* (Leydig). *Limnology and Oceanography* **17**(4):645-9.
- Bollens, S. M. et al. 2002. Zooplankton invasions: a brief review, plus two case studies from the northeast Pacific. *Hydrobiologia* **480**(1-3):87–110.
- Boudreau, S., and Yan, N. D. 2003. The differing crustacean zooplankton communities of Canadian Shield lakes with and without the nonindigenous zooplanktivore *Bythotrephes longimanus*. *Canadian Journal of Fisheries and Aquatic Sciences* **60**(11):1307-1313.

- 1056 Bourdeau, P. E., Pangle, K. L., and Peacor, S. D. 2011. The invasive predator *Bythotrephes*
1057 induces vertical migration in native copepods of Lake Michigan. *Biological Invasions*
1058 **13**(11):2533-2545.
- 1059 Bourdeau, P. E. et al. 2013. Finely tuned response of native prey to an invasive predator in a
1060 freshwater system. *Ecology* **94**(7):1449-1455.
- 1061 Brown, A. H., and Yan, N. D. 2015. Food quantity affects the sensitivity of *Daphnia* to road salt.
1062 *Environmental Science and Technology* **49**(7):4673-4680.
- 1063 Breitburg, D. L. et al. 1998. Understanding effects of multiple stressors: ideas and challenges. *In*
1064 Successes, limitations, and frontiers in ecosystem science. *Edited by* M. L. Pace and P. M.
1065 Groffman. Springer Science+Business Media, LLC, New York, USA, pp. 416-431.
- 1066 Brooks, J. L., and Dodson, S. I. 1965. Predation, body size, and composition of plankton. *Science*
1067 **150**(3692):28-35.
- 1068 Bungartz, B., and Branstrator, D. K. 2003. Morphological changes in *Daphnia mendotae* in the
1069 chemical presence of *Bythotrephes longimanus*. *Archiv für Hydrobiologie* **158**(1):97–108.
- 1070 Burkhardt, S., and Lehman, J. T. 1994. Prey consumption and predatory effects of an
1071 invertebrate predator (*Bythotrephes*: Cladocera, Cercopagidae) based on phosphorus
1072 budgets. *Limnology and Oceanography* **39**(5):1007-1019.
- 1073 Cairns, A., and Yan, N. D. 2009. A review of the influence of low ambient calcium
1074 concentrations on freshwater daphniids, gammarids, and crayfish. *Environmental Reviews*
1075 **17**(NA):67-79.
- 1076 Cairns, A. 2010. Field assessments and evidence of impact of calcium decline on *Daphnia*
1077 (Crustacea, Anomopoda) in Canadian Shield lakes. MSc. Thesis, Department of Biology,
1078 York University, Toronto, ON.

- Christensen, M. R. et al. 2006. Multiple anthropogenic stressors cause ecological surprises in boreal lakes. *Global Change Biology* **12**(12):2316-2322.
- Cowgill, U. M. 1976. The chemical composition of two species of *Daphnia*, their algal food and their environment. *The Science of the Total Environment* **6**(1):79-102.
- Cowgill, U. et al. 1986. Influence of water on reproductive success and chemical composition of laboratory reared populations of *Daphnia magna*. *Water Research* **20**(3):317–323.
- Crain, C. M., Kroeker, K., and Halpern, S. 2008. Interactive and cumulative effects of multiple human stressors in marine systems. *Ecology Letters* **11**(12):1304-1315.
- Culp, J. et al. 2000. A research strategy for using stream microcosms in ecotoxicology: integrating experiments at different levels of biological organization with field data. *Journal of Aquatic Ecosystem Stress and Recovery* **7**(2):167-176.
- Cyr, H., and Curtis, J. M. 1999. Zooplankton community size structure and taxonomic composition affects size-selective grazing in natural communities. *Oecologia* **118**(3):306-315.
- de Roos, A. M. et al. 2007. Food-dependent growth leads to overcompensation in stage-specific biomass when mortality increases the influence of maturation versus reproduction regulation. *American Naturalist* **170**(3):E59-76.
- de Roos, A. M. et al. 2008. Stage specific predator species help each other to persist while competing for a single prey. *Proceedings of the National Academic of Science of the United States of America* **105**(37):13930-13935.
- DeSellas, A. M. et al. 2008. Cladocera assemblages from the surface sediments of southcentral Ontario (Canada) lakes and their relationships to measured environmental variables. *Hydrobiologia* **600**(1):105–119.

- 1102 DeSellas, A. M. et al. 2011. Assessing the effects of multiple environmental stressors on
1103 zooplankton assemblages in Boreal Shield lakes since pre-industrial times. *Journal of*
1104 *Limnology* **70**(1):41–56.
- 1105 Didham, R. K. et al. 2005. Are invasive species the drivers of ecological change? *Trends in*
1106 *Ecology and Evolution* **20**(9):470-474.
- 1107 Driscoll, C. T. et al. 1995. Long-term trends in the chemistry of precipitation and lake water in
1108 the Adirondack region of New York, USA. *Water, Air, Soil Pollution* **85**(2):583-588.
- 1109 Dudgeon, D. et al. 2006. Freshwater biodiversity: importance, threats, status, and conservation
1110 challenges. *Biological Reviews* **81**(2):163-182.
- 1111 Dumitru, C., Sprules, W. G., and Yan, N. D. 2001. Impact of *Bythotrephes longimanus* on
1112 zooplankton assemblages of Harp Lake, Canada: an assessment based on predator
1113 consumption and prey production. *Freshwater Biology* **46**(2):241–251.
- 1114 Early Detection and Distribution Mapping System (EDDMaps) Ontario. n.d. *Bythotrephes*
1115 *longimanus*, Leydig, 1860, distribution map. Available at [http://www.eddmaps.org/](http://www.eddmaps.org/ontario/distribution/viewmap.cfm?sub=59823)
1116 [ontario/distribution/viewmap.cfm?sub=59823](http://www.eddmaps.org/ontario/distribution/viewmap.cfm?sub=59823) (accessed May 19, 2015).
- 1117 Edwards, B., Jackson, D., and Somers, K. M. 2009. Multispecies crayfish declines in lakes:
1118 Implications for species distributions and richness. *Journal of the North American*
1119 *Benthological Society* **28**(3):719-732.
- 1120 Edwards, B., Jackson, D., and Somers, K. M. 2015. Evaluating the effect of lake calcium
1121 concentration on the acquisition of carapace calcium by freshwater crayfish. *Hydrobiologia*
1122 **744**(1):91-100.
- 1123 Evans, C. D., Monteith, D. T., and Harriman, R. 2001. Long-term variability in the deposition of
1124 marine ions at west coast sites in the UK Acid Waters Monitoring Network: impacts on

surface water chemistry and significance for trend determination. *Science of the Total Environment* **265**(1-3):115-129.

Fernandez, R. J., Rennie, M. D., and Sprules, W. G. 2009. Changes in nearshore zooplankton associated with species invasions and potential effects on larval lake whitefish (*Coregonus clupeaformis*). *International Review of Hydrobiology* **94**(2):226–243.

Folt, C. L. et al. 1999. Synergism and antagonism among multiple stressors. *Limnology and Oceanography* **44**(3 part 2):864-877.

Foster, S. E., and Sprules, W. G. 2009. Effects of the *Bythotrephes* invasion on native predatory invertebrates. *Limnology and Oceanography* **54**(3):757–769.

Frost, T. M. et al. 1995. Species compensation and complementarity in ecosystem function. In *Linking species and ecosystems. Edited by C. G. Jones and J. H. Lawton*. Chapman & Hall, New York, USA, pp. 224-239.

Gertzen, E., and Leung, B. 2011. Predicting the spread of invasive species in an uncertain world: accommodating multiple vectors and gaps in temporal and spatial data for *Bythotrephes longimanus*. *Biological Invasions* **13**(11):2433-2444.

Gliwicz, Z. M. 1990. Food thresholds and body size in Cladocera. *Nature* **343**(6259):638-640.

Greenaway, P. 1985. Calcium balance and moulting in the Crustacea. *Biological Reviews* **60**(3):425-454.

Grigorovich, I. A. et al. 1998. *Bythotrephes longimanus* in the Commonwealth of Independent States: variability, distribution and ecology. *Hydrobiologia* **379**(1):183-198.

Halpern, B. S. et al. 2008. A global map of human impact on marine ecosystems. *Science* **319**(5865):948-952.

- 1147 Hamilton, J. D. 1958. On the biology of *Holopedium gibberum* Zaddach (Crustacea: Cladocera).
1148 *Verhandlungen des Internationalen Verein Limnologie* **13**:785-788.
- 1149 Havas, M. 1985. Aluminium bioaccumulation and toxicity to *Daphnia magna* in soft water at
1150 low pH. *Canadian Journal of Fisheries and Aquatic Sciences* **42**(11):1741-1748.
- 1151 Hedin, L. O. et al. 1994. Steep declines in atmospheric base cations in regions of Europe and
1152 North America. *Nature* **367**(6461):351-354.
- 1153 Hessen, D. O., Faafeng, B. A., and Andersen, T. 1995. Competition or niche segregation
1154 between *Holopedium* and *Daphnia*: empirical light on abiotic key parameters.
1155 *Hydrobiologia* **307**(1-3):253- 261.
- 1156 Hessen, D. O., and Alstad Rukke, N. 2000. UV radiation and low calcium as mutual stressors for
1157 *Daphnia*. *Limnology and Oceanography* **45**(8):1834-1838.
- 1158 Hessen, D. O., Alstad, N. E., and Skardal, L. 2000. Calcium limitation in *Daphnia magna*.
1159 *Journal of Plankton Research* **22**(3):553-568.
- 1160 Hessen, D., Bakkestuen, V., and Walseng, B. 2011. Ecological niches of *Bythotrephes* and
1161 *Leptodora*: lessons for predicting long-term effects of invasion. *Biological Invasions*
1162 **13**(11):2561-2572.
- 1163 Hovius, J., Beisner, B., and McCann, K. S. 2006. Epilimnetic rotifer community responses to
1164 *Bythotrephes longimanus* invasion in Canadian shield lakes. *Limnology and Oceanography*
1165 **51**(2):1004–1012.
- 1166 Hovius, J. T. et al. 2007. Indirect food web effects of *Bythotrephes* invasion: responses by the
1167 rotifer *Conochilus* in Harp Lake, Canada. *Biological Invasions* **9**(3):233–243.

- 1168 Huner, J. V., and Lindqvist, O. V. 1985. Exoskeleton mineralization in Astacid and Cambarid
1169 crayfishes (Decapoda, Crustacea). *Comparative Biochemistry and Physiology Part A: Physiology* **80A**(4):515-521.
- 1171 Huss, M., and Nilsson, K. A. 2011. Experimental evidence for emergent facilitation: promoting
1172 the existence of an invertebrate predator by killing its prey. *Journal of Animal Ecology*
1173 **80**(3): 615-621.
- 1174 Jeziorski, A., and Yan, N. D. 2006. Species identity and aqueous calcium concentrations as
1175 determinants of calcium concentrations of freshwater crustacean zooplankton. *Canadian*
1176 *Journal of Fisheries and Aquatic Sciences* **63**(5):1007-1013.
- 1177 Jeziorski, A. et al. 2008. The widespread threat of calcium decline in fresh waters. *Science*
1178 **322**(5906):1374-1377.
- 1179 Jeziorski, A., Paterson, A., and Smol, J. 2012a. Changes since the onset of acid deposition
1180 among calcium-sensitive cladoceran taxa within soft water lakes of Ontario, Canada.
1181 *Journal of Paleolimnology* **48**(2):323-327.
- 1182 Jeziorski, A., Paterson, A., and Smol, J. 2012b. Crustacean zooplankton sedimentary remains
1183 from calcium-poor lakes: complex responses to threshold concentrations. *Aquatic Sciences*
1184 **74**(1):121-131.
- 1185 Jeziorski, A. et al. 2015. The jellification of north temperate lakes. *Proceedings of the Royal*
1186 *Society B.* **282**(1798): 20142449. Doi: 10.1098/rspb.2014.2449.
- 1187 Jiang, X. et al. 2014. Combined effects of calcium deficiency, cyanobacteria, and warming on
1188 the population fitness of three cladocerans. *Fundamentals of Applied Limnology* **185**(3-
1189 4):321-328.

- 1190 Johannsson, O. E., Mills, E. L., and O’Gorman, R. 1991. Changes in the nearshore and offshore
1191 zooplankton communities in Lake Ontario: 1981-88. *Canadian Journal of Fisheries and*
1192 *Aquatic Sciences* **48**(8):1546-1557.
- 1193 Jokela, A., Arnott, S., and Beisner, B. 2011. Patterns of *Bythotrephes longimanus* distribution
1194 relative to native macroinvertebrates and zooplankton prey. *Biological Invasions*
1195 **13**(11):2573-2594.
- 1196 Jokela, A., Arnott, S. E., and Beisner, B. E. 2013. Influence of light on the foraging impact of an
1197 introduced predatory cladoceran, *Bythotrephes longimanus*. *Freshwater Biology*
1198 **58**(9):1946-1957.
- 1199 Jurkiewicz-Karnkowska, E. 2005. Some aspects of nitrogen, carbon and calcium accumulation in
1200 molluscs from the Zegrzyński Reservoir Ecosystem. *Polish Journal of Environmental*
1201 *Studies* **14**(2):173-177.
- 1202 Keller, W., and Pitblado, J. R. 1984. Crustacean plankton in Northeastern Ontario lakes
1203 subjected to acid deposition. *Water, Air and Soil Pollution* **23**(3):271-291.
- 1204 Keller, W., Dixit, S. S., and Heneberry, J. 2001. Calcium declines in northeastern lakes.
1205 *Canadian Journal of Fisheries and Aquatic Sciences* **58**(10):2011-2020.
- 1206 Kelly, N. E. et al. 2012. Differential short- and long-term effects of an invertebrate predator on
1207 zooplankton communities in invaded and native lakes. *Diversity and Distributions*
1208 **19**(4):396-410.
- 1209 Kerfoot, W. C. et al. 2011. Temperature, recreational fishing and diapause egg connections:
1210 dispersal of spiny water fleas (*Bythotrephes longimanus*). *Biological Invasions*
1211 **13**(11):2513-2531.

- 1212 Kim, N., Walseng, B., and Yan, N. D. 2012. Will environmental calcium declines hinder
1213 *Bythotrephes* establishment success in Canadian Shield lakes? *Canadian Journal of*
1214 *Fisheries and Aquatic Sciences* **69**(5):810-820.
- 1215 Kirchner, J. and Lydersen, E. 1995. Base cation depletion and potential long-term acidification
1216 of Norwegian catchments. *Environmental Science and Technology* **29**(8):1953-1960.
- 1217 Korosi, J. B., and Smol, J. P. 2012a. A comparison of present-day and pre-industrial cladoceran
1218 assemblages from soft water Nova Scotia (Canada) lakes with different regional
1219 acidification histories. *Journal of Paleolimnology* **47**(1):43-54.
- 1220 Korosi, J. B., and Smol, J. P. 2012b. Examining the effects of climate change, acidic deposition,
1221 and copper sulphate poisoning on long-term changes in cladoceran assemblages. *Aquatic*
1222 *Sciences* **74**(4):781-792.
- 1223 Lawrence, G. B. et al. 1999. Soil calcium status and the response of stream chemistry to
1224 changing acidic deposition rates. *Ecological Applications* **9**(3):1059-1072.
- 1225 Lehman, J. T. 1988. Algal biomass unaltered by food web changes in Lake Michigan. *Nature*
1226 **32**(6164): 537–538.
- 1227 Lehman, J. T. 1991. Causes and consequences of cladoceran dynamics in Lake Michigan:
1228 implications of species invasion by *Bythotrephes*. *Journal of Great Lakes Research*
1229 **17**(91):437–445.
- 1230 Lehman, J. T., and Cáceres, C. E. 1993. Food-web responses to species invasion by a predatory
1231 invertebrate: *Bythotrephes* in Lake Michigan. *Limnology and Oceanography* **38**(4):879–
1232 891.

- 1233 Lehman J. T., Bilkovic, D. M., and Sullivan, C. 1997. Predicting development, metabolism and
1234 secondary production for the invertebrate predator *Bythotrephes*. *Freshwater Biology*
1235 **38**:343–352.
- 1236 Likens, G. E. et al. 1998. The biogeochemistry of calcium at Hubbard Brook. *Biogeochemistry*
1237 **41**(2):89-173.
- 1238 Locke, A. et al. 1994. Zooplankton communities and water chemistry of Sudbury area lakes:
1239 changes related to pH recovery. *Canadian Journal of Fisheries and Aquatic Sciences*
1240 **51**(1):151-160.
- 1241 MacIsaac, H. J. et al. 2000. Modelling *Bythotrephes longimanus* invasions in the great lakes
1242 basin based on its European distribution. *Archiv für Hydrobiologie* **149**(1):1–21.
- 1243 MacIsaac, H. J. et al. 2004. Backcasting and forecasting biological invasions of inland lakes.
1244 *Ecological Applications* **14**(3):773–783.
- 1245 Makarewicz, J. C. et al. 1995. A decade of predatory control of zooplankton species composition
1246 in Lake Michigan. *Journal of Great Lakes Research* **21**(4):620-640.
- 1247 Muirhead, J. R., and Sprules, W. G., 2003. Reaction distance *Bythotrephes longimanus*,
1248 encounter rate and index of prey risk for Harp Lake, Ontario. *Freshwater Biology*
1249 **48**(1):135-146.
- 1250 Muirhead, J. R., and MacIsaac, H. J. 2005. Development of inland lakes as hubs in an invasion
1251 network. *Journal of Applied Ecology* **42**(1):80-90.
- 1252 Monakov, A. V. 1972. Review of studies on feeding of aquatic invertebrates conducted at the
1253 Institute of Biology of Inland Waters, Academy of Science USSR. *Journal of the Fisheries*
1254 *Research Board of Canada* **29**(4):363-383.

- 1255 Nordin, L. J. et al. 2008. An evaluation of the diet of *Mysis relicta* using gut contents and fatty
1256 acid profiles in lakes with and without the invader, *Bythotrephes longimanus*
1257 (Onychopoda, Cercopagidae). *Aquatic Ecology* **42**(3):421-436.
- 1258 Paine, R., Tegner, M. J., and Johnson, E. A. 1998. Compounded perturbations yield ecological
1259 surprises. *Ecosystems* **1**(6):535–545.
- 1260 Palmer, M., and Yan, N. D. 2013. Decadal-scale regional changes in Canadian freshwater
1261 zooplankton: the likely consequence of complex interactions among multiple
1262 anthropogenic stressors. *Freshwater Biology* **58**(7):1366-1378.
- 1263 Pangle K. L., and Peacor S. D. 2006. Non-lethal effect of the invasive predator *Bythotrephes*
1264 *longimanus* on *Daphnia mendotae*. *Freshwater Biology* **51**(6):1070-1078.
- 1265 Pangle K. L., Peacor S. D. and Johannsson, O. E. 2007. Large nonlethal effects of an invasive
1266 invertebrate predator on zooplankton population growth rate. *Ecology* **88**(2):402-412.
- 1267 Pangle K. L., and Peacor S. D. 2009. Light-dependent predation by the invertebrate planktivore,
1268 *Bythotrephes longimanus*. *Canadian Journal of Fisheries and Aquatic Sciences*
1269 **66**(10):1748-1757.
- 1270 Paterson, A. M. et al. 2008. Long-term changes in phytoplankton composition in seven Canadian
1271 Shield lakes in response to multiple anthropogenic stressors. *Canadian Journal of Fisheries*
1272 *and Aquatic Sciences* **65**(5):846-861.
- 1273 Pichlová-Ptáčníková, R., and Vanderploeg, H. A. 2011. The quick and the dead: might
1274 differences in escape rates explain the changes in the zooplankton community composition
1275 of Lake Michigan after invasion by *Bythotrephes*? *Biological Invasions* **13**(11):2595-2604.
- 1276 Piirainen, S. et al. 2004. Effects of forest clear-cutting on the sulphur, phosphorous and base
1277 cation fluxes through podzolic soil horizons. *Biogeochemistry* **69**(3):405-424.

- 1278 Riessen, H. P. et al. 2012. Changes in water chemistry can disable plankton prey defences.
1279 *Proceedings of the National Academy of Sciences of the United States of America*
1280 **109**(38):15377-15382.
- 1281 Rukke, N. A. 2002a. Effects of low calcium concentrations on two common freshwater
1282 crustaceans, *Gammarus lacustris* and *Astacus astacus*. *Functional Ecology* **16**(3):357-
1283 366.
- 1284 Rukke, N. A. 2002b. Tolerance to low ambient calcium shows inter-population differences in
1285 *Daphnia galeata*. *Journal of Plankton Research* **24**(5):527-531.
- 1286 Scheuhammer, A. M. et al. 1997. Relationships between lake chemistry and calcium and trace
1287 metal concentrations of aquatic invertebrates eaten by breeding insectivorous waterfowl.
1288 *Environmental Pollution* **96**(2):235-247.
- 1289 Schindler, D. et al. 1996. Consequences of climate warming and lake acidification for UV-B
1290 penetration in North American boreal lakes. *Nature* **379**(6567):705-708.
- 1291 Schindler, D. W. 2001. The cumulative effects of climate warming and other human stresses on
1292 Canadian freshwaters in the new millennium. *Canadian Journal of Fisheries and Aquatic*
1293 *Sciences* **58**(1):18-29.
- 1294 Schindler, D. W., and Lee, P. G. 2010. Comprehensive conservation planning to protect
1295 biodiversity and ecosystem services in Canadian boreal regions under a warming climate
1296 and increasing exploitation. *Biological Conservation* **143**(7):1571-1586.
- 1297 Schulz, K. L., and Yurista, P. M. 1999. Implications of an invertebrate predator's (*Bythotrephes*
1298 *cederstroemi*) atypical effects on a pelagic zooplankton community. *Hydrobiologia* **380**(1-
1299 3):179–193.

- 1300 Shapiera, M. et al. 2011. Calcium content of littoral Cladocera in three soft water lakes of the
1301 Canadian Shield. *Hydrobiologia* **678**(1):77-83.
- 1302 Shapiera, M. et al. 2012. Cladoceran response to calcium decline and the subsequent inadvertent
1303 liming of a soft water Canadian Lake. *Water, Air, & Soil Pollution* **223**(5):2437-2446.
- 1304 Skjelkvåle, B. L. et al. 2005. Regional scale evidence for improvements in surface water
1305 chemistry 1990-2001. *Environmental Pollution* **137**(1):165-176.
- 1306 Smol, J. 2010. Paleolimnology. In *Lake Ecosystem ecology: a global perspective*. Edited by G.
1307 E. Likens. Academic Press, San Diego, C.A., pp. 56-64.
- 1308 Smol, J. 2008. *Pollution of lakes and rivers: a paleoenvironmental perspective*, 2nd ed. Blackwell,
1309 Oxford.
- 1310 Sprules, W. G., Riessen, H. P., and Jin, E. H. 1990. Dynamics of the *Bythotrephes* invasion of
1311 the St. Lawrence Great Lakes. *Journal of Great Lakes Research* **16**(3):346-351.
- 1312 Stenson, J. A. E. 1973. On predation and *Holopedium gibberum* (Zaddach) distribution.
1313 *Limnology and Oceanography* **18**(6):1005-1010.
- 1314 Strayer, D. L. 2010. Alien species in fresh waters: ecological effects, interactions with other
1315 stressors, and prospects for the future. *Freshwater Biology* **55**(Suppl. 1):152-174.
- 1316 Strayer, D. L., and Dudgeon, D. 2010. Freshwater biodiversity conservation: recent progress and
1317 future challenges. *Journal of the North American Benthological Society* **29**(1):344-358.
- 1318 Strecker, A. and Arnott, S. E. 2005. Impact of *Bythotrephes* invasion on zooplankton
1319 communities in acid-damaged and recovered on the Boreal Shield. *Canadian Journal of*
1320 *Fisheries and Aquatic Sciences* **62**(11):2450-2462.

- 1321 Strecker, A. L. et al. 2006. Variation in the response of crustacean zooplankton species richness
1322 and composition to the invasive predator *Bythotrephes*. *Canadian Journal of Fisheries and*
1323 *Aquatic Sciences* **63**(9):2126–2136.
- 1324 Strecker, A. L., and Arnott, S. E. 2008. Invasive predator, *Bythotrephes*, has varied effects on
1325 ecosystem function in freshwater lakes. *Ecosystems* **11**(3):490–503.
- 1326 Strecker, A. L., and Arnott, S. E. 2010. Complex interactions between regional dispersal of
1327 native taxa and an invasive species. *Ecology* **91**(4):1035-1047.
- 1328 Strecker, A. L. 2011. An overview of invasive freshwater cladocerans: *Bythotrephes longimanus*
1329 as a case study. *In Handbook of global freshwater invasive species. Edited by R. Francis.*
1330 *Earthscan, London, UK, pp. 149-156.*
- 1331 Strecker, A. L., Beisner, B. E., Arnott, S. E., Paterson, A. M., Winter, J. G., Johannsson, O. E.,
1332 and Yan, N. D. 2011. Direct and indirect effects of an invasive planktonic predator on
1333 pelagic food webs. *Limnology and Oceanography* **56**(1):179-192.
- 1334 Tan, Q., and Wang, W. 2009. The regulation of calcium in *Daphnia magna* reared in different
1335 calcium environments. *Limnology and Oceanography* **54**(3):746-756.
- 1336 Tan, Q., and Wang, W. 2010. Interspecies differences in calcium content and requirement in four
1337 freshwater cladocerans explained by biokinetic parameters. *Limnology and Oceanography*
1338 **55**(3):1426-1434.
- 1339 Tessier, A. J., and Horwitz, R. J. 1990. Influence of water chemistry on size structure of
1340 zooplankton assemblages. *Canadian Journal of Fisheries and Aquatic Sciences*
1341 **47**(10):1937-1943.

- Thrush, S. F. et al. 2008. Multiple stressor effects identified from species abundance distributions: Interactions between urban contaminants and species habitat relationships. *Journal of Experimental Marine Biology and Ecology* **366**(1-2):160-168.
- Townsend, C. R., Uhlmann, S. S., and Matthaei, C. D. 2008. Individual and combined responses of stream ecosystems to multiple stressors. *Journal of Applied Ecology* **45**(6):1810-1819.
- Vanderploeg, H. A., Liebig, J. R., and Omair, M. 1993. *Bythotrephes* predation on Great Lakes zooplankton measured by an in-situ method implication for zooplankton community structure. *Archiv für Hydrobiologie* **127**:1–8.
- Vanni, M. J. 1988. Freshwater zooplankton community structure: introduction of large invertebrate predators and large herbivores to a small species community. *Canadian Journal of Fisheries and Aquatic Sciences* **45**(10):1758-1770
- Vinebrooke, R. D. et al. 2004. Impacts of multiple stressors on biodiversity and ecosystem functioning: the role of species co-tolerance. *Oikos* **104**(3):451–457.
- Wærvågen, S. B., Rukke, N. A., and Hessen, D. O. 2002. Calcium content of crustacean zooplankton and its potential role in species distribution. *Freshwater Biology* **47**(10):1866–1878.
- Wahlström, E., and Westman, E. 1999. Planktivory by the predacious cladoceran *Bythotrephes longimanus*: effects on zooplankton size structure and abundance. *Canadian Journal of Fisheries and Aquatic Sciences* **56**(10):1865-1872.
- Watmough, S. A., Aherne, J. and Dillon, P. J. 2003. Potential impact of forest harvesting on lake chemistry in south-central Ontario at current levels of acid deposition. *Canadian Journal of Fisheries and Aquatic Sciences* **60**(9):1095-1103.

- 1364 Watmough, S. A., and Dillon, P. J. 2003a. Base cation and nitrogen budgets for seven forested
1365 catchments in central Ontario, 1993-1999. *Forest Ecology and Management* **177**(1-3):155-
1366 177.
- 1367 Watmough, S. A., and Dillon, P. J. 2003b. Calcium losses from a forested catchment in South-
1368 Central Ontario. *Environmental Science and Technology* **37**(14):3085-3089.
- 1369 Watmough, S. A. et al. 2005. Sulphate, nitrogen and base cation budgets at 21 forested
1370 catchments in Canada, the United States and Europe. *Environmental Monitoring*
1371 *Assessment* **109**(1-3):1-36.
- 1372 Watmough, S. A., and Aherne, J. 2008. Estimating calcium weathering rates and future lake
1373 calcium concentrations in the Muskoka-Haliburton region of Ontario. *Canadian Journal of*
1374 *Fisheries and Aquatic Sciences* **65**(5):821-833.
- 1375 Weisz, E. J., and Yan, N. D. 2010. Relative value of limnological, geographic and human use
1376 variables as predictors of the presence of *Bythotrephes longimanus* in Canadian Shield
1377 lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **67**(3):462-472.
- 1378 Weisz, E. J., and Yan, N. D. 2011. Shifting invertebrate zooplanktivores: watershed-level
1379 replacement of the native *Leptodora* by the non-indigenous *Bythotrephes* in Canadian
1380 Shield lakes. *Biological Invasions* **13**(11):115-123.
- 1381 Williamson, C. E., and Reid, J. W. 2009. Copepoda. *In* Encyclopaedia of inland waters, volume
1382 3. Edited by G. E. Likens. Academic Press, San Diego, C.A., pp. 633-642.
- 1383 Yao, H. et al. 2011. Nearshore human interventions reverse patterns of decline in lake calcium
1384 budgets in central Ontario as demonstrated by mass-balance analyses. *Water Resources*
1385 *Research* **47**(6), W06521, doi:10.1029/2010WR010159.

- Yan, N. D., Mackie, G. L., and Boomer, D. 1989. Seasonal patterns in metal levels of the net plankton of three Canadian Shield lakes. *The Science of the Total Environment* **87/88**:439-461.
- Yan, N. D. et al. 1996. Increased UV-B penetration in a lake owing to drought-induced acidification. *Nature* **381**(6578):141-143.
- Yan, N. D., and Pawson, T. W. 1997. Changes in the crustacean zooplankton community of Harp Lake, Canada, following the invasion by *Bythotrephes cederstroemi*. *Freshwater Biology* **37**(2):409–425.
- Yan, N. D. et al. 2001. Changes in the zooplankton and the phenology of the spiny water flea, *Bythotrephes*, following its invasion of Harp Lake, Ontario, Canada. *Canadian Journal of Fisheries and Aquatic Sciences* **58**(12):2341–2350.
- Yan, N. D., Girard, R., and Boudreau, S. 2002. An introduced predator (*Bythotrephes*) reduces zooplankton species richness. *Ecology Letters* **5**(4):481–485.
- Yan, N. D. 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. *Canadian Journal of Fisheries and Aquatic Sciences* **65**(5):862-877.
- Young, J. D., and Yan, N. D. 2008. Modification of the diel vertical migration of *Bythotrephes longimanus* by the cold-water planktivore, *Coregonus artedii*. *Freshwater Biology* **53**(5):981-995.

Supporting information

Additional supporting information may be found in the online version of this article:

1409

1410 **Table S1.** Study design detail of published studies on *Bythotrephes* effect on zooplankton

1411 communities (e.g. richness, abundance, and composition). The number of stations sampled in

1412 each lakes is one, unless indicated otherwise. “NA” is presented where information was not

1413 provided.

Draft

1414 **Table 1.** Comparison of Ca content of different groups of heavily calcified organisms

Organism	Ca content (%DW)
Daphniids	2-8 ^a
Molluscs	8-30 ^b , 20-30 ^c , 37-39 ^d
Decapods	13.5-33.4 ^e , 15.3-28 ^f
Amphipods	~4.4-12.7 ^g

1415 ^aJeziorski and Yan (2006); ^bBaby *et al.* (2010); ^cScheuhammer *et al.* (1997); ^dJurkiewicz-

1416 Karnkowska (2005); ^eEdwards *et al.* (2015); ^fHuner and Lindqvist (1985); ^gRukke (2002a)

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Table 2. *Bythotrephes longimanus* impacts on zooplankton community attributes in different studies and study design categories. Results are classed as: -, negative effect ($p < 0.05$ and $p < 0.1$); +, positive effect ($p < 0.05$); 0, no effect. Letters in the table legend provide the study identity.

Variable	Field surveys			Experiments	
	Lake survey	Long-term study Harp Lake	Long-term study Great Lakes	Field mesocosms	Laboratory
Crustacean zooplankton					
Abundance	-(a), 0(f)	-(c)	+(n), 0(j)	-(g), -(h)	NA
Richness	-(a, o), -(f)	-(b, c, d)	-(j), +(n)	0(g)	NA
Diversity	-(o)	NA	NA	0(g)	NA
Biomass	-(f)	NA	NA	-(g), -(h)	NA
Productivity	-(m)	NA	NA	NA	NA
Cladocera					
Abundance	-(a, f)	-(c)	-(j, n)	-(g), 0(g)	NA
Richness	-(a, f, o)	-(b)	NA	NA	NA
Diversity	-(a)	NA	NA	NA	NA
Biomass	NA	NA	NA	NA	NA

Variable	Field surveys			Experiments	
	Lake survey	Long-term study Harp Lake	Long-term study Great Lakes	Field mesocosms	Laboratory
Copepod					
Productivity	-(m), 0(m)	NA	NA	NA	NA
Abundance	-(a), 0(f)	0(c)	0(j), +(n)	-(g), 0(g)	NA
Richness	0(a, f)	NA	NA	NA	NA
Diversity	0(a)	NA	NA	NA	NA
Biomass	NA	0(d)	NA	-(g)	NA
Productivity	-(m)	NA	NA	NA	NA
Species					
Cladocera					
<i>Bosmina</i>	-(a, f, o), 0(u)	-(b, c, d, e)	+(i, j), -(l, n, q)	-(h)	-(t)
<i>Holopedium glacialis</i>	-(a), 0(o, u), +(x)	+(c, d, e)	-(j, l), 0(n)	+(g), -(h)	-(t)
<i>Eubosmina tubicen</i>	-(a, o)	-(b, c, d)	NA	NA	NA
<i>Eubosmina longispina</i>	+(o)	NA	NA	NA	NA
<i>Leptodora kindtii</i>	-(a, o, s, v)	-(b, d)	-(i, j, l, n)	NA	NA
<i>Daphnia longispina</i>	NA	NA	NA	-(h)	NA

Variable	Field surveys			Experiments	
	Lake survey	Long-term study Harp Lake	Long-term study Great Lakes	Field mesocosms	Laboratory
<i>Daphnia pulicaria</i>	+(o)	NA	-(i, j, k, l)	NA	-(t)
<i>Daphnia mendotae</i>	-(a), +(o, u)	+(c, d, e)	-(j, q, n), +(i, k, l)	NA	-(p, r, t)
<i>Daphnia catawba</i>	-(a, f, o)	NA	NA	NA	NA
<i>Daphnia longiremis</i>	-(a), 0(o)	NA	+(j) ,-(n)	NA	NA
<i>Daphnia ambigua</i>	-(a, o)	NA	NA	NA	NA
<i>Daphnia retrocurva</i>	-(a), 0(o)	-(b, c, d)	-(i, j, k, l, n, q)	NA	NA
<i>Daphnia dubia</i>	-(f, o)	NA	NA	NA	NA
<i>Sida crystallina</i>	-(a, f, o)	NA	-(n)	NA	NA
<i>Diaphanosoma birgei</i>	-(a, f, o)	-(b, c, d, e)	-(j, n)	NA	NA
<i>Chydorus sphaericus</i>	-(a, f), 0(o)	-(b, c, d, e)	-(n)	NA	NA
<i>Polyphemus pediculus</i>	-(a, o)	NA	NA	NA	NA
<i>Acroperus harpae</i>	-(a)	NA	NA	NA	NA
<i>Alona</i> sp.	-(o)	NA	NA	NA	NA
<i>Ceriodaphnia lacustris</i>	-(o)	NA	-(n)	-(h)	NA
Copepods					

Variable	Field surveys			Experiments	
	Lake survey	Long-term study Harp Lake	Long-term study Great Lakes	Field mesocosms	Laboratory
<i>Acanthocyclops</i> sp.	-(a)	NA	NA	NA	NA
<i>Mesocyclops edax</i>	-(a, f, o)	-(b, c, d)	-(j, l)	NA	NA
<i>Leptodiaptomus minutus</i>	-(a), 0(o)	NA	-(w)	-(g)	-(w)
<i>L. sicilis</i>	NA	+(d)	+(n), -(w)	NA	0(w)
<i>Diacyclops thomasi</i>	-(a), +(o)	NA	NA	NA	-(w)
<i>Epischura lacustris</i>	-(a), +(o)	NA	NA	NA	-(t)
<i>Tropocyclops extensus</i>	0(o)	-(d, e)	NA	NA	NA
<i>Eucyclops agilis</i>	-(o)	NA	NA	NA	NA
<i>E. elegans</i>	+(o)	NA	NA	NA	NA
<i>Senecella calanoides</i>	+(o)	NA	NA	NA	NA
<i>Cyclops scutifer</i>	-(o), 0(u)	NA	NA	NA	NA
<i>Skistodiaptomus oregonensis</i>	-(o)	NA	NA	NA	NA
<i>Skistodiaptomus reighardii</i>	-(o)	NA	NA	NA	NA

1421 ^aStrecker et al. (2006); survey of 20 lakes

1423 ^cYan et al.. (2001); survey of Harp L.

1422 ^bYan et al. (2002); survey of Harp L.

1424 ^dYan and Pawson (1997); survey of Harp L.

1425 ^eDumitru et al. (2001); diel samples over a 24 hr period
1426 ^fBoudreau and Yan (2003); single mid-summer sampling
1427 ^gStrecker and Arnott (2005); 30 day mesocosm experiment
1428 in Canada
1429 ^hWahlström and Westman (1999); 21 day mesocosm
1430 experiment in Sweden
1431 ⁱLehman and Cáceres (1993); time series data in L. Michigan
1432 ^jBarbiero and Tuchman (2004); multiple stations in L.
1433 Michigan, Erie, and Huron
1434 ^kLehman (1991); time series data in L. Michigan
1435 ^lMakarewicz et al. (1995); multiple stations in L. Michigan
1436 ^mStrecker and Arnott (2008); survey of 8 lakes
1437 ⁿFernandez et al. (2009); L. Huron, 10 sample sites in South
1438 Bay
1439 ^oKelly et al.. (2012); comparison of 212 lakes in Canada and
1440 342 lakes in Norway
1441 ^pBourdeau et al. (2013); *D. mendotae* and water-borne cues
1442 ({P/A})
1443 ^qPangle et al. (2007); surveys in L. Erie and Michigan at
1444 multiple stations
1445 ^rPangle and Peacor (2006); *D. mendotae* and *Bythotrephes*
1446 karimones
1447 ^sFoster and Sprules (2009); survey of 8 lakes
1448 ^tSchulz and Yurista (1999); prey preference of *Bythotrephes*
1449 in L. Michigan
1450 ^uHessen et al. (2011); survey data of 1541 lakes (sampled
1451 1970-2000) and 400 lakes (sampled 1998-1992) in Norway
1452 ^vWeisz and Yan (2011); survey of 193 lakes: single stations
1453 ^wBourdeau et al. (2011); field and lab experiment in L.
1454 Michigan
1455 ^xJeziorski et al. (2015); paleolimnological analysis and
1456 survey of south-central Ontario and Nova Scotia lakes

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1462 **Table 3.** Ca threshold and threshold responses on daphniids observed in laboratory and field studies

Species	[Ca] used (mg/L) &/or 2° stressor	[Ca] threshold (mg/L)	Ca] threshold response	Reference	Notes
Laboratory experiments					
<i>D. magna</i>	0.5, 1.0, 5.0, 10.0, 20.0	5.2 - 10.0	Saturation ^a	Alstad et al. (1999)	10 d experiment using neonates. Ca %DW measured in adults. Incomplete calcification below 5 mg/L with significant differences in Ca content between 5 and 10 mg/L. No significant difference between 10 and 20 mg/L.
<i>D. magna</i>	0.1, 0.5, 1.0, 5.0, 10.0 and High (0.4 - 0.8 mg C/L) and Low (0.1 - 0.4 mg C/L) food	0.1 - 0.5 (high food) 0.5 - 5.0 (high food) 5.0 - 10.0 (low food)	Survival Reduced reproduction	Hessen et al. (2000)	30 d experiment using neonates. Neonates survived at 0.5 mg/L for 10 d but 100% mortality by 30 d. One individual reproduced at 0.1 mg/L but all animals died within 48 hours. 30 day experiment using neonates. <i>D. magna</i> reared at high food and different ambient Ca did not produce 3rd and 4th clutches between 0.5 and 5 mg/L. Second reproduction occurred

					between 1 and 10 mg/L.
<i>D. pulex</i>	0.5, 1.5, 2.5, 5.0 and <i>Chaoborus</i> <i>americanus</i> karimones (P/A)	0.5 - 1.5	Reduced development of anti- predator defences	Riessen et al. (2012)	6 d experiment using juvenile instars, in which the anti-predator defences normally develop.
<i>D. pulex</i>	0.1, 0.5, 1.5, 2.0, 5.0, 10.0, High (30µg/L) and Low (3µg/L) food, and four temperatures (20°, 24°, 28°, 32°C)	0.1 - 0.5 (<i>no influence of food or temperature</i>)	Survival	Ashforth and Yan (2008)	15 d experiment using neonates. 0% survival at 0.1 mg/L within 10 d at 20°C; 60% survival and reproduction at 0.5 mg/L; 0% reproduction at 32°C irrespective of food source.
		0.5 - 1.5 (<i>high temperature, low food</i>)	Reduced reproduction		

Species	[Ca] used (mg/L) &/or 2° stressor	[Ca] threshold (mg/L)	Ca] threshold response	Reference	Notes
<i>D. pulex</i>	5.0 and 20.0, cyanobacteria (good and poor food), and two temperatures (25°, 29°C)	5.0	Survival	Jiang <i>et al.</i> (2014)	Life table experiments using 20 neonates within 24 hours of birth. A decline in aqueous Ca from 20mg/L to 5 mg/L, decreased <i>D. pulex</i> fitness regardless of food type and temperature.
<i>D. galeata</i>	0.0, 1.0, 2.0, 5.0, 10.0 and populations from Lake Erken (>10 mg/L) and Lake Ånnsjön (2-3 mg/L)	>10.0 (<i>Lake Erken population</i>)	Saturation	Rukke (2002b)	Mean specific Ca %DW measured in adults reared for 7 d. Significant differences observed between 2 and 5 mg/L and 5 and 10 mg/L in L. Erken populations.
		2.0 - 5.0 (<i>Lake Ånnsjön population</i>)	Saturation		Mean specific Ca %DW measured in adults reared for 7 d. Significant difference observed between 1 and 2 mg/L and 2 and 5 mg/L in L. Ånnsjön populations.
		0.5 - 2.2	Survival		7 d experiment using neonates and adults. At 0.5

					mg/L: L. Ånnsjön (neonates, 0% and adults 60% survival) and L. Erken (neonates 80% survival and adults 100% survival).
<i>D. magna</i>	0.5, 1.0, 5.0, 10.0 and UV radiation (35.95 W/m ² over 300-400nm)	5.0 - 10.0 <i>(D. magna)</i>	Saturation	Hessen and Alstad Rukke (2000)	Mean specific Ca %DW measured in adults reared for 10 d. Significant difference observed between 5 and 10 mg/L.
<i>D. tenebrosa</i>		0.5 - 5.0 <i>(D. magna)</i>	Survival		20% mortality at 0.5 mg/L at 6 hours of UV exposure for <i>D. tenebrosa</i> and 100% mortality within 24 hours for <i>D. magna</i> .
		0.5 - 10.0 <i>(D. tenebrosa)</i>			
		1.0 - 5.0 <i>(D. tenebrosa)</i>	Saturation		Mean specific Ca %DW measured in adults reared for 10 d. Significant difference observed between 1 and 5 mg/L.
Field studies					
<i>D. cristata</i>	NA	~1.1	P/A ^b		
<i>D. cucullata</i>	NA	~4.3	P/A		

<i>D. hyalina</i>	NA	~0.5	P/A	Hessen et al. (1995)	Lake survey (n=346) in Norway with a Ca gradient of 0.4 to 44 mg/L
<i>D. galeata</i>	NA	~0.7	P/A		
<i>D. longispina</i>	NA	~0.6	P/A		
<i>D. longiremis</i>	NA	1.26 ^c - 2.76 ^d	P/A	Cairns (2010)	Lake survey (n=304) of stratified and unstratified lakes in the Muskoka-Haliburton region. P/A analysed using Gaussian or logistic regression, with threshold identified from the probability of 50% presence.
<i>D. dubia</i>	NA	1.58 - 2.82	P/A		
<i>D. mendotae</i>	NA	1.63 - 4.87	P/A		
<i>D. retrocurva</i>	NA	1.69 - 3.28	P/A		
<i>D. pulicaria</i>	NA	16.1 ^d	P/A		
<i>D. ambigua</i>	NA	NTD ^e	P/A		
<i>D. catawba</i>	NA	NTD	P/A		
<i>D. pulex</i>	1.1, 1.3, 1.4, 2.3 and natural vs artificial food levels	1.3 (<i>natural food levels</i>)	Survival	Cairns (2010)	Microcosm study (n=4) in the Muskoka-Haliburton region

^aExposure concentration where there is no significant difference in the rate of calcification in comparison to other exposure concentrations along an increasing Ca gradient (Cairns and Yan 2009). Calcification is generally measured as Ca percentage of total dry weight (Ca %DW)

1466 ^bP/A indicates presence or absence as defined by field experiments

1467 ^cCritical lower threshold (ip) - the [Ca] where the greatest reduction in daphniid presence along a Ca gradient occurs (Cairns 2010)

1468 ^dProbability of 50% presence (u) - maximum Ca at which a species was observed (Cairns 2010)

1469 ^eNTD indicates that no threshold was detected and that these species are not sensitive to low Ca (Cairns 2010)

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Table 4. Effects of calcium decline on daphniid complexes, *Bosmina*, and *Holopedium glacialis*. Changes in the relative abundance of cladoceran remains from pre-industrial (“bottom”) to modern (“top”) sediments are classed as: D-decreased, and I-increased. A similar classification is used for the only full sediment profile study included. Superscript labels in the table legend provide the study identity.

Species	Change in modern sediments	Sediment profile changes
<i>Daphnia longispina</i> species complex (<i>D. ambigua</i> , <i>D. dubia</i> , <i>D. mendotae</i> , <i>D. longiremis</i> , <i>D. retrocurva</i>)	D ^b	I ^a
<i>Daphnia pulex</i> species complex (<i>D. catawba</i> , <i>D. pulex</i> , <i>D. pulicaria</i>)	I ^b	I ^a
<i>Bosmina</i>	I ^{b,c,d}	D ^a
<i>Holopedium glacialis</i>	I ^{b,c,e}	I ^a

^aShapiera et al. (2012); Dickie Lake, Muskoka. Dust suppression programme in the late 1990s raised [Ca] to >2.5 mg/L.

^bJeziorski et al. (2012a); 36 Muskoka-Haliburton lakes along a Ca gradient of 1-3 mg/L

^cKorosi and Smol (2012a); 48 lakes in Nova Scotia

^dKorosi and Smol (2012b); 4 lakes in Nova Scotia

^eJeziorski et al. (2015); paleolimnological analysis and survey of south-central Ontario and Nova Scotia lakes

Table 5. Effects of calcium decline on daphniids and bosminids in Canadian Shield lakes below and above 1.5 mg/L, the laboratory derived reproduction threshold for *Daphnia pulex* below which, survival, reproduction, and fecundity are reduced (Ashforth and Yan 2008). Changes in the relative abundance of cladoceran remains from pre-industrial (“bottom”) to modern (“top”) sediments are classed as: D-decreased, and I-increased. Superscript labels in the table legend provide the study identity.

Species	Ca levels (mg/L)			
	<1.5	1.5-2.0	2.0-2.5	>2.5
Daphniids	D ^{a,b,c}	D ^{a,b}	I ^{a,b}	I ^{a,b,d}
Bosminids	I ^{a,b,c}	I ^{a,b}	D ^b	D ^b

^aJeziorski et al. (2008); dated lake cores, 43 Muskoka-Haliburton lakes

^bdeSellas et al. (2011); 42 Muskoka-Haliburton lakes

^cdeSellas et al. (2008); 42 Muskoka-Haliburton lakes

^dShapiera et al. (2012); Dickie Lake, Muskoka

Table 6. Measured Ca content of crustacean zooplankton species

Species	Ca content (%DW)
Daphniids	
<i>Daphnia ambigua</i>	3.03 ^a , 0.64 ^f
<i>Daphnia carinata</i>	1.7-2.8 ^d
<i>Daphnia catawba</i>	4.25 ^a , 0.62 ^f
<i>Daphnia cristata</i>	0.8 ^b
<i>Daphnia cucullata</i>	1.2 ^b
<i>Daphnia galeata</i>	0.8 ^b , 1.3-2.4 ^d
<i>Daphnia hyalina</i>	5-7 ^j
<i>Daphnia longiremis</i>	1.0 ^b
<i>Daphnia longispina</i>	1.5 ^b
<i>Daphnia magna</i>	4.4 ^b , 1.99-4.71 ^e , 1.77-3.82 ^e , 1.78 ^f , 7.7 ^h , 2.8-4.8 ⁱ , 4.2 ^k
<i>Daphnia mendotae</i>	5.2 ^a
<i>Daphnia pulex</i>	4.59 ^a , 1.23 ^f , 2.1-4.8 ^g , 3.7 ^h
<i>Daphnia pulicaria</i>	1.31 ^f
<i>Daphnia tenebrosa</i>	2.5 ^b , 2.3 ^l
Other Cladocerans	
<i>Acroperus harpae</i>	0.36 ^c
<i>Alona rectangula</i>	1.46 ^c
<i>Alonella excisa</i>	0.38 ^c
<i>Bosmina freyi</i>	0.09 ^f

<i>Bosmina longispina</i>	0.3 ^b
<i>Bosmina</i> sp.	0.32 ^a
<i>Ceriodaphnia dubia</i>	0.8-1.2 ^d
<i>Ceriodaphnia quadrangula</i>	0.6 ^b
<i>Chydorus brevilebris</i>	0.49 ^c
<i>Chydorus piger</i>	0.43 ^c
<i>Daphniopsis ephemeralis</i>	0.56 ^c
<i>Diaphanosoma brachyurum</i> (syn. <i>birgei</i>)	0.2 ^b , 0.18 ^g
<i>Disparalona</i> sp.	2.32 ^c
<i>Holopedium glacialis</i> (syn. <i>gibberum</i>)	0.24 ^a , 0.1 ^b , 0.2-0.5 ^g
<i>Limnospira frontosa</i>	0.2 ^b
<i>Moina macrocopa</i>	0.04-0.07 ^d
<i>Pleuroxus truncatus</i>	2.15 ^c
<i>Scapholebris rammneri</i>	1.7 ^c
Copepods	
<i>Acantodiaptomus denticornis</i>	0.4 ^b
<i>Cyclops scutifer</i>	0.2 ^b
<i>Eudiaptomus gracilis</i>	0.2 ^b
<i>Heterocope appendiculata</i>	0.2 ^b
<i>Leptodiaptomus minutus</i>	0.28 ^a , 0.062-0.063 ^g
<i>Mesocyclops edax</i>	0.17 ^a , 0.048-0.071 ^g

^aJeziorski and Yan (2006); survey of 9 Muskoka-Haliburton lakes with [Ca] 0.4 to 35 mg/L

^bWærvågen et al. (2002); survey of 59 Norway lakes

1503 ^cShapiera et al. (2011); survey of 3 Muskoka-Haliburton lake with [Ca] 1.4 and 9.5mg/L

1504 ^dTan and Wang (2010); 3 to 6 d neonates and [Ca] 2, 20, and 200 mg/L

1505 ^eTan and Wang (2009); 4 d juveniles and 10 d adults of *Daphnia magna* cultured in 0.5 and 50

1506 mg Ca/L. First set of numbers are for juveniles.

1507 ^fJeziorski et al. (2015); Supplementary data

1508 ^gYan et al. (1989); survey of Plastic and Red Chalk L. in Muskoka region

1509 ^hCowgill (1976); chemical composition of laboratory cultured *Daphnia magna* and *Daphnia*

1510 *pulex*

1511 ⁱHavas (1985); laboratory experiment on *Daphnia magna* to assess impact of aluminium at low

1512 pH

1513 ^jBaudouin and Ravera (1972); chemical composition of *Daphnia hyalina* collected from Lago di

1514 Monate, Italy

1515 ^kAlstad et al. (1999); laboratory experiment on the calcification of *Daphnia magna*

1516 ^lHessen and Rukke (2000); 10 d adults cultured in 0.5, 1, 5, and 10 mg Ca/L to assess impact of

1517 UV radiation

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Table 7. Sensitivity assessment of *Bythotrephes* and declining Ca on some zooplankton species. No response (N) or a dash (-) is assigned to a species that is not negatively impacted by both *Bythotrephes* and low Ca or where no synergistic or antagonistic response is expected respectively.

Species	<i>Bythotrephes</i> assigned score	Sensitivity to Ca decline assigned score	Additive sensitivity value	Synergistic/ Antagonistic/ No response
Daphniids				
<i>Daphnia ambigua</i>	1	1	2	A
<i>Daphnia catawba</i>	2	1	3	A
<i>Daphnia dubia</i>	1	1	2	A
<i>Daphnia longiremis</i>	1	1	2	A
<i>Daphnia mendotae</i>	4	6	10	-
<i>Daphnia pulicaria</i>	2	6	8	S
<i>Daphnia retrocurva</i>	4	6	10	S
Small cladocerans				
<i>Bosmina</i> sp.	4	1	5	A
<i>Ceriodaphnia dubia</i>	2	1	3	A
<i>Chydorus sphaericus</i>	4	1	5	A
<i>Diaphanosoma birgei</i>	4	1	5	A
<i>Eubosmina tubicen</i>	4	1	5	A
Copepods				
<i>Cyclops scutifer</i>	1	1	2	N
<i>Leptodiaptomus</i>	2	1	3	N

<i>minutus</i>				
<i>Mesocyclops edax</i>	4	1	5	N

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Figure legends

Fig. 1. Frequency of impacts observed per species from different study categories. In some cases, a single point represents frequency of impacts for two or more species. Abbreviations for species are: *Bm*: *Bosmina*; *Lk*: *Leptodora kindtii*, *Dr*: *Daphnia retrocurva*, *Db*: *Diaphanosoma birgei*, *Mx*: *Mesocyclops edax*, *Cs*: *Chydorus sphaericus*, *Et*: *Eubosmina tubicen*, *Dp*: *Daphnia pulicaria*, *Ho*: *Holopedium glacialis*, *Dm*: *Daphnia mendotae*, *Scr*: *Sida crystallina*, *Cl*: *Ceriodaphnia lacustris*, *Lm*: *Leptodiptomus minutus*, *Dc*: *Daphnia catawba*, *Da*: *Daphnia ambigua*, *Dl*: *Daphnia longiremis*, *El*: *Epischura lacustris*, *Dt*: *Diacyclops thomasi*, *Te*: *Tropocyclops extensus*, *Dd*: *Daphnia dubia*, *Pp*: *Polyphemus pediculus*, *Csc*: *Cyclops scutifer*, *Ah*: *Acroperus harpae*, *Ac*: *Acanthocyclops* sp., *Al*: *Alona* sp., *Ea*: *Eucyclops agilis*, *Dlg*: *Daphnia longispina*, *So*: *Skistodiptomus oregonensis*, *Sr*: *Skistodiptomus reighardii*, *Sca*: *Senecella calanoides*, *Ee*: *Eucyclops elegans*, and *Ls*: *Leptodiptomus sicilis*.

Fig. 2. *Bythotrephes longimanus* impacts on zooplankton taxonomic resolution

Fig. 3. Sensitivity of daphniids derived from their sensitivity thresholds (mg/L) for survival to declining Ca and based on their mean Ca content (%DW) estimated from field collections. Abbreviations for species are: *Dh*: *Daphnia hyalina*, *Dme*: *Daphnia mendotae*, *Dma*: *Daphnia magna*, *Dpx*: *Daphnia pulex*, *Dc*: *Daphnia catawba*, *Da*: *Daphnia ambigua*, *Dlpa*: *Daphnia longispina*, *Dg*: *Daphnia galeata*, *Dlms*: *Daphnia longiremis*, *Dcr*: *Daphnia cristata*, *Dcu*: *Daphnia cucullata*, and *Dt*: *Daphnia tenebrosa*.

1553 **Fig. 4.** Conceptual diagram of the likely effects of *Bythotrephes* and declining Ca on daphniids,
1554 small cladocerans and copepod demographics
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Frequency of impacts observed per species from different study categories. In some cases, a single point represents frequency of impacts for two or more species. Abbreviations for species are: Bm: Bosmina; Lk: Leptodora kindtii; Dr: Daphnia retrocurva; Db: Diaphanosoma birgei; Mx: Mesocyclops edax; Cs: Chydorus sphaericus; Et: Eubosmina tubicen; Dp: Daphnia pulicaria; Ho: Holopedium glacialis; Dm: Daphnia mendotae; Scr: Sida crystallina; Cl: Ceriodaphnia lacustris; Lm: Leptodiptomus minutus; Dc: Daphnia catawba; Da: Daphnia ambigua; Dl: Daphnia longiremis; El: Epischura lacustris; Dt: Diacyclops thomasi; Te: Tropocyclops extensus; Dd: Daphnia dubia; Pp: Polyphemus pediculus; Csc: Cyclops scutifer; Ah: Acroperus harpae; Ac: Acanthocyclops sp.; Al: Alona sp.; Ea: Eucyclops agilis; Dlg: Daphnia longispina; So: Skistodiptomus oregonensis; Sr: Skistodiptomus reighardii; Sca: Senecella calanoides; Ee: Eucyclops elegans, and Ls: Leptodiptomus sicilis

249x240mm (300 x 300 DPI)

Bythotrephes longimanus impacts on zooplankton taxonomic resolution
199x166mm (300 x 300 DPI)

Sensitivity of daphniids derived from their sensitivity thresholds (mg/L) for survival to declining Ca and based on their mean Ca content (%DW) estimated from field collections. Abbreviations for species are: Dh: *Daphnia hyalina*, Dme: *Daphnia mendotae*, Dma: *Daphnia magna*, Dpx: *Daphnia pulex*, Dc: *Daphnia catawba*, Da: *Daphnia ambigua*, Dlpa: *Daphnia longispina*, Dg: *Daphnia galeata*, Dlms: *Daphnia longiremis*, Dcr: *Daphnia cristata*, Dcu: *Daphnia cucullata*, and Dt: *Daphnia tenebrosa*
 179x134mm (300 x 300 DPI)