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The ecological impacts of lakewater calcium decline on softwater boreal ecosystems

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Abstract:

In recent decades, marked declines in calcium (Ca) concentrations have been noted in many softwater boreal lakes, and are believed to be a long-term consequence of acid deposition as well as other stressors (such as timber harvesting). Reduced Ca availability may act as a potent environmental stressor. Investigations of the direct ecological impacts of lower Ca concentrations in freshwater systems have largely focused on Ca-rich members of the Cladocera; however, a growing body of work, spanning several scientific fields, suggests Ca decline will have profound direct and indirect consequences for aquatic ecosystems. Here, we synthesize recent laboratory analyses and field surveys to provide an overview of these consequences, while highlighting paleolimnological investigations that provide some long-term perspective on the phenomenon. However, considerable questions remain regarding 'baseline' or pre-impact conditions, due to the accelerated leaching of Ca from watershed soils during the period of anthropogenic influence. Furthermore, catchment-specific differences in both leaching rates and the initial size of the Ca pool in watershed soils complicate determination of the eventual endpoints of the declines. Despite these uncertainties, persistent low Ca concentrations are anticipated to impede biological recovery from lake acidification, and that ongoing declines will have cascading effects throughout aquatic ecosystems due to the loss of vulnerable taxa. To better understand how reduced Ca availability will continue to change affected surface waters and how these changes will interact with other environmental stressors will require continued investigation of the declines from multiple scientific perspectives.

Key words: calcium decline, Cladocera, lakes, acidification, timber harvesting

Introduction

Calcium (Ca) is an essential nutrient critical for many aspects of cellular function (e.g. signal transduction and gene expression; Clapham 2007), the vertebrate endoskeleton, and Ca minerals are often a fundamental component of invertebrate exoskeletal structure (Greenaway 1985). A widespread decline in the aqueous Ca concentration of many softwater lakes has been documented across eastern North America and northern Europe (Stoddard et al. 1999; Alewell et al. 2000; Skjelkvåle et al. 2005), and the

potential impact of these declines has been recognized as an emerging environmental stressor (Jeziorski et al. 2008a). Since the mid-20th century, emission reduction programs have allowed many formerly acidified lakes to approach or return to pre-impact pH levels (Stoddard et al. 1999; Keller et al. 2003; Futter et al. 2014; Garmo et al. 2014). However, biological recovery has often been slower than anticipated (Monteith et al. 2005; Quinlan et al. 2008; Gray and Arnott 2009), and this slow pace may in part be due to lower aqueous Ca availability (Keller 2009). Declining Ca concentrations are attributed to decades of acid deposition depleting Ca from the catchment soils of softwater lakes and have been observed principally in regions where reductions in acid deposition have lowered the rate of removal of residual Ca (Kirchner and Lydersen 1995; Stoddard et al. 1999; Alewell et al. 2000; Wright et al. 2005; Hessen et al. 2016). Concern regarding the ecological impact of reduced Ca in aquatic environments, and questions regarding how modern Ca concentrations compare with pre-industrial values, have prompted investigations from a variety of perspectives, including the identification of organisms vulnerable to low Ca, effects on the competition among organisms differing in Ca sensitivity, and the interaction of low Ca with other environmental stressors (e.g. Jeziorski and Yan 2006; Hessen et al. 1995; Azan et al. 2015; Jeziorski et al. 2015).

Here we present a synthesis of research on Ca decline including laboratory and field experiments, and regional limnological and paleolimnological surveys, to better understand the impacts on surface waters. Our focus is on eastern Canada, however we have also attempted to provide a global perspective. We conclude that the impacts of Ca decline are expected to grow in severity and geographic extent, with site-specific responses dependent on continued levels of acid deposition and local geology, which may be exacerbated by timber harvesting.

Underlying mechanisms for declining lakewater Ca concentrations

Declining lakewater Ca concentrations (most notably in softwater regions) have been documented over the past ~40 years by numerous long-term monitoring programs across eastern North America (Likens et al. 1998; Keller et al. 2001; Molot and Dillon 2008) and northern Europe (Kirchner and

Lydersen 1995; Stoddard et al. 1999; Skjelkvåle et al. 2005). The Ca declines are closely related to acid deposition and the emission control programs that have successfully lowered levels of SO₄ deposition (Stoddard et al. 1999; Garmo et al. 2014). Lower levels of acid deposition have also reduced acid anion leaching from watershed soils; thus decreases in the SO₄ concentration of runoff have been matched by similar decreases in base cation concentrations (principally Ca; Kirchner and Lydersen 1995; Tomlinson 2003). At local scales the pool of base cations in watershed soils can also be influenced by: timber harvesting through the removal of Ca sequestered in the long-lived stemwood and the increased Ca uptake associated with forest regrowth (Allen et al. 1997; Watmough et al. 2003), wildfires and periods of drought reducing the Acid Neutralizing Capacity (ANC) of run-off (Bayley et al. 1992), and reduced particulate deposition (i.e. dust) of Ca from industrial emissions, unpaved roads, and soil erosion (Hedin et al. 1994). In regions such as the western United States, however, increases in atmospheric dust related to increasing aridity may be counteracting lower Ca particulate emissions (Brahney et al. 2013). Nonetheless, despite the number of local factors with the potential to lower lakewater Ca concentrations, the acidification of lakes and watershed soils by acid precipitation is the principal mechanism acting on a regional or continental scale.

Acid deposition and lakewater Ca decline

The influence of industrial emissions on the acidity of precipitation was first recognized in the 19th century (Smith 1872), and ecological changes to surface waters due to acid deposition have long concerned environmental scientists, managers and policy-makers (Schindler 1988). Reduced lakewater pH has been detrimental to a wide range of aquatic biota, reducing growth and survival rates, and lowering reproductive success, leading to a wide variety of local population extirpations (Beamish and Harvey 1972; Schindler et al. 1985; Havens et al. 1993). In many jurisdictions, the consequences of acid deposition have prompted legislation placing strict controls on the emission of acid precursors (SO₂ and NO_x), allowing widespread improvements in water quality (Stoddard et al. 1999; Garmo et al. 2014). Following reductions in acid precipitation and the subsequent (albeit slow) recovery of many acidified

surface waters (Keller et al. 1992; Stoddard et al. 1999; Jeffries et al. 2003; Vet et al. 2005; Garmo et al. 2014), research interests shifted from identifying the impacts of reduced pH, to the evaluation of emission controls (Kirchner and Lydersen 1995; Alewell et al. 2000; Chestnut and Mills 2005), to determine whether regulations are sufficient to allow for some biological recovery despite continued deposition of acidifying compounds at levels with harmful effects on ecosystem structure and function (Yan et al. 1996; Keller et al. 1999; Jeffries et al. 2003; Vet et al. 2005). More recently, interest has grown regarding whether a return to pre-disturbance conditions can be expected given the continued influence of other ongoing environmental stressors such as dispersal limitation, altered predation dynamics and climate warming (Gray and Arnott 2009; Labaj et al. 2015; Futter et al. 2014).

The slow recovery from acidification is most notable in poorly-buffered lakes where the large reductions in SO_4 deposition have been accompanied by a smaller compensating increase in pH and/or alkalinity (Stoddard et al. 1999; Jeffries et al. 2003; Vinebrooke et al. 2003). This muted response to emission reductions has largely been attributed to a depletion of base cations (principally Ca) from watershed soils, which has counteracted reductions of acid inputs (Kirchner and Lydersen 1995; Likens et al. 1996). The H^+ ions introduced through acid deposition are able to displace base cations from soil particles facilitating their export to surface waters (Johnson and Cole 1980; Christ et al. 1999; Tomlinson 2003). Lakewater Ca concentrations are dependent on the Ca reservoirs in watershed soils (Houle et al. 2006), thus the accelerated export of base cations due to acid deposition provided short-term increases in lakewater Ca, that have been followed by the current declines as the soil Ca reservoirs were depleted reducing the amount available for export to surface waters. Lakewater Ca declines are now prevalent in regions underlain by Ca-poor granitic bedrock (such as the North American Precambrian Shield and much of Scandinavia; Jeziorski et al. 2008a; Skjervåle et al. 2001; 2007; Hessen et al. 2016), where mineral weathering is the primary source of Ca replenishment for catchment soils.

Lakewater Ca concentrations that are potentially limiting to some biota are currently widespread both on the Precambrian Shield in Canada and the USA (Jeziorski et al. 2008a) and across Scandinavia (Skjervåle et al. 2001; 2007). In the relatively young soils overlying Precambrian Shield bedrock, modern

removal rates of base cations (and other chemical constituents) are higher than the long-term removal rates (Kirkwood and Nesbitt 1991), and given continued rates of acid deposition the Ca declines are anticipated to continue (Skeffington and Brown 1992; Watmough et al. 2005). The declines are anticipated to be most pronounced in catchments with active timber harvesting, where lakewater Ca may fall 10-40% lower than present-day values (Federer et al. 1989; Watmough and Aherne 2008). Declines of this magnitude will make Ca concentrations of $<1.0 \text{ mg}\cdot\text{L}^{-1}$ much more common in lakes on the Precambrian Shield (Watmough et al. 2003; Jeziorski et al. 2008a; Reid and Watmough 2016), values potentially lower than historically experienced by many of the native aquatic organisms, presenting a variety of physiological and ecological problems for those taxa with relatively high Ca demands.

Baseline Ca conditions

Despite a general understanding of the mechanisms driving recent Ca declines in softwater lakes, there is little information regarding the pre-impact or baseline Ca levels of lakes currently experiencing falling Ca concentrations. This knowledge gap is due to the putative principal cause of the Ca declines (i.e. acid deposition) pre-dating even the longest environmental monitoring records in regions affected by acid deposition such as eastern North America (Likens et al., 1996). Therefore, indirect methods are necessary to determine the onset and extent of the Ca declines. Reasonable alternatives to direct monitoring data include geochemical models and paleolimnological approaches (both already used extensively in the study of a wide variety of lake management issues; Smol 2008).

Biogeochemical models indicate that Ca concentrations in surface waters were elevated by the accelerated release of Ca from watershed soils induced by acid deposition (Skeffington and Brown 1992; Likens et al. 1996; Watmough and Aherne 2008), thus the earliest available direct observations may not reflect 'natural' Ca conditions and the recent declines may be a return from an elevated state. Although there has been considerable interest in modeling the future availability of base cations in watershed soils and surface waters due to the implications for both forestry and recovery from acid deposition (e.g. Watmough et al. 2003; Akselsson et al. 2007; Clair et al. 2011; Strock et al. 2014), analyses comparing

current Ca concentrations to modeled hindcasts have been relatively rare. In south-central Ontario, the steady state water chemistry model (Henriksen et al. 1992) indicates that even with the substantial declines over the past ~40 years, current lakewater Ca concentrations still exceed pre-industrial values (Watmough et al. 2003; Watmough and Aherne 2008; Reid and Watmough 2016), although there may have been some overestimation of historical Ca concentrations in the earlier analyses (Reid and Watmough, 2016). Due to site-specific weathering rates and variation in forest composition, density, growth rates, as well as timber harvesting frequency and type, inferring historical Ca concentrations on a broad scale using biogeochemical approaches may prove difficult.

Similarly, the use of paleolimnological indicators preserved in lake sediments to produce inference models for historical lakewater Ca has also proven challenging. Although sedimentary algal (i.e. diatom and chrysophyte) assemblages are well-established as superb indicators of lakewater pH (e.g. Charles et al. 1986; Cumming et al. 1994; Dixit et al. 1992), diatoms are poor indicators of Ca and other major cations (Flower et al. 1997) presumably due to relatively low Ca demands. Attempts to infer historical lakewater Ca using the remains of siliceous algae (Keller et al. 2001; Dixit et al. 2002) have been limited by their insensitivity to the short-term variations in Ca availability captured by direct monitoring records.

Concurrent with the quantitative paleolimnological efforts, long-term changes in lakewater Ca have also been examined using qualitative approaches, especially regarding the responses of vulnerable biota. An early focus of these analyses was the identification of organisms with high Ca demands relative to their competitors, and now a growing body of field, lab and paleoecological analyses has identified members of the crustacean order Cladocera to be important indicator taxa for tracking historical lakewater Ca concentrations.

Ca decline and the Cladocera

Ca minerals (e.g. calcite, apatite, and aragonite) are critical components of structural materials such as bones, carapaces, scales and shells. Therefore, the acquisition of Ca either directly from the

surrounding environment or through the consumption of prey is essential for the growth and survival of many organisms. Freshwater biota with high Ca demands relative to their competitors (e.g. crustaceans and mollusks) will be particularly vulnerable to declining Ca availability, especially those that shed large amounts of Ca with each moult (Greenaway 1985). Although many larger invertebrates are able to store Ca in their hemolymph between molts, smaller taxa, such as the crustacean zooplankton, must replenish the Ca lost with the exuviae via the uptake of aqueous Ca (Greenaway 1985; Tan and Wang 2009). Large differences in the sensitivity to low aqueous Ca concentrations among crustacean zooplankton taxa prompted interest in the impact of Ca decline on this group, as they are often the principal prey items of small fish and invertebrate predators, while also the dominant algal grazers in many surface waters (Hessen et al. 1995).

The vulnerability of crustacean zooplankton to lake acidification has been studied extensively (Havas and Rosseland 1995; Yan et al. 1996; Yan et al. 2008; Gray and Arnott 2009), and elements of their physiology and life-cycle (i.e. the extensive use of Ca minerals as a structural component within their carapace; Stevenson 1985) suggest similar sensitivities to environmental Ca decline. There are stark differences in the Ca content (i.e. the total body Ca content as a fraction of dry weight) among both planktonic and littoral crustacean zooplankton taxa (Wærvågen et al. 2002; Jeziorski and Yan 2006; Shapiera et al. 2011). For example, the cladoceran *Daphnia* spp. (the dominant planktonic grazer in many softwater lakes) has a much higher Ca content than either their main cladoceran (e.g. *Bosmina* spp., *Holopedium glacialis*) or copepod (e.g. *Leptodiptomus minutus*) competitors (Wærvågen et al. 2002; Jeziorski and Yan 2006). The Ca-rich daphniid exoskeleton (>90% of their body Ca; Alstad et al. 1999) must be regenerated after each molt, suggesting a relationship between Ca content and Ca requirements. However, Ca content alone is not a proxy for Ca sensitivity, as Ca uptake efficiencies vary significantly among daphniid taxa (Tan and Wang 2010; Prater et al. 2016).

To date, experimental examinations of daphniid Ca demand have largely focused on the hardwater taxon *D. magna*, demonstrating that the direct absorption of Ca from the surrounding water is their principal Ca source (Cowgill et al. 1986), and that elevated environmental Ca concentrations (both

waterborne and dietary) can increase their Ca content (Tan and Wang 2008; Tan and Wang 2009). Adequate Ca is essential for proper carapace development; however, as the moulting rate is independent of both Ca and food concentrations, the impacts of low Ca are greatest during the juvenile stages of development due to their rapid growth (Hessen et al. 2000; Rukke 2002a), and in low Ca environments mothers must provide Ca to developing young at the expense of their own growth (Giardini et al. 2015; Pérez-Fuentetaja and Goodberry 2016). At low Ca concentrations, the Ca uptake mechanisms of *D. pulicaria* become less efficient at discriminating between Ca and elemental analogues such as strontium (Peters et al. 2008), while Ca concentrations $<1.5 \text{ mg L}^{-1}$ negatively impact the growth, reproduction and survival of *D. pulex* (a daphniid common in ponds on the Precambrian Shield) under laboratory conditions (Ashforth and Yan 2008). Outside of the laboratory, the negative impacts of low Ca availability are likely to be more pronounced due to the impacts on anti-predator defences, reducing body size and carapace strength, while also impeding the production of neck spines (Riessen et al. 2012). As the Ca concentrations of many lakes in softwater regions are already approaching or are below 1.5 mg L^{-1} (Keller et al. 2001; Waervagen et al. 2002; Skjelkvåle et al. 2007; Jeziorski et al. 2008a; Arseneau et al. 2011; Clair et al. 2011; Hessen et al. 2016), the effects of lakewater Ca declines on crustacean zooplankton communities are likely already widespread.

Lakewater Ca concentration has been identified as a factor regulating crustacean zooplankton community composition in both North American and Europe (Tessier and Horwitz 1990; Hessen et al. 1995; Wærvågen et al. 2002; Sweetman and Smol 2006). Ca-rich taxa such as large daphniids are most prevalent in high-Ca lakes, and are less abundant in softer waters relative to taxa with lower Ca content, such as *Bosmina* spp. and *Holopedium glacialis* (Jeziorski and Yan 2006). Differences in daphniid Ca uptake efficiency and the weakness of Ca content as an indicator of Ca sensitivity (Tan and Wang 2010) identified in laboratory analyses are also apparent in the differences in the Ca optima of daphniids common in lakes on the Precambrian Shield, with many daphniid populations persisting at Ca concentrations far below their species-specific optima, namely: *D. longiremis* (2.76 mg L^{-1}), *D. dubia* (2.82 mg L^{-1}), *D. retrocurva* (3.28 mg L^{-1}), *D. mendotae* (4.87 mg L^{-1}), *D. pulicaria* (16.1 mg L^{-1} ; Cairns

2010). Meanwhile, taxa such as *D. ambigua* and *D. catawba* appear relatively indifferent to current field Ca concentration, despite a similar Ca content to *D. mendotae* and *D. pulex* (Jeziorski and Yan 2006; Cairns 2010). Despite the complex relationships between daphniids and lakewater Ca concentrations, the sensitivity of some members of the genus to falling Ca availability has prompted their use in paleolimnological investigations of Ca decline.

Investigations of Ca decline using paleolimnological techniques

Initial investigations of Ca decline using paleolimnological techniques (i.e. the analysis of indicators preserved in lake sediments; Smol 2008) focused on differences in the Ca content of daphniid resting eggs (ephippia: Wyn et al. 2007; Jeziorski et al. 2008b). However, the large sediment volumes required for such analyses prompted more conventional paleolimnological approaches (i.e. using sedimentary assemblages of cladoceran remains) to provide historical perspectives on the long-term responses of cladocerans to regional Ca declines as well as insight regarding pre-industrial Ca concentrations.

In south-central Ontario (Canada), a series of paleolimnological analyses have investigated whether evidence supporting the laboratory threshold of 1.5 mg L^{-1} Ca for *D. pulex* can be detected in the cladoceran sediment record, and also whether this threshold can be distinguished from the influence of low pH. An initial “top/bottom” paleolimnological analysis (Smol 2008) across a broad Ca gradient compared cladoceran assemblages from surface sediments (i.e. modern-day “tops”) with those from depths corresponding to times pre-dating industrial development in eastern North America (i.e. ~1850 “bottoms”). Multiple instances of daphniid increase since pre-industrial times were identified, but only in lakes with present-day Ca concentrations $>2.0 \text{ mg L}^{-1}$ (Jeziorski et al. 2008a; DeSellas et al. 2011), prompting the analysis of cladoceran subfossils from the surface (i.e. recent) sediments of 36 lakes selected to be evenly distributed about both Ca (1.5 mg L^{-1}) and pH (6) thresholds, previously identified as important to cladoceran community composition (Holt et al. 2003; Ashforth and Yan 2008). Significant differences among daphniid relative abundances were observed across the Ca threshold, but

the differences did not extend to the broader cladoceran assemblage, and considerable variability in Ca tolerance was observed within the *D. pulex* species complex (Jeziorski et al. 2012a). When the surface sediment assemblages from both investigations were combined (extending the Ca gradient from 1-3 mg L⁻¹ to 1-24 mg L⁻¹), Ca replaced pH as a significant explanatory variable for the cladoceran assemblages (Jeziorski et al. 2012a). The stronger relationship over the much wider gradient suggests either that community changes related to Ca availability had already occurred at the low Ca levels (i.e. 1-3 mg L⁻¹), or that perhaps the analyses were insensitive to some community changes that occur at lower Ca concentrations.

To better characterize the cladoceran changes through time, the “top/bottom” approach was used to compare modern and pre-industrial sedimentary assemblages in the 36 low Ca lakes (Jeziorski et al. 2012b). Substantial declines in the relative abundance of Ca-rich pelagic cladoceran taxa (particularly the *Daphnia longispina* species complex) since pre-industrial times were accompanied by increases in Ca-poor taxa (such as *Bosmina* spp. and *Holopedium glacialis*) irrespective of modern-day pH (Jeziorski et al. 2012b). To determine whether these changes in the cladoceran sedimentary assemblages could be confidently attributed to lakewater Ca decline (rather than the influence of pH; Holt et al. 2003), diatom assemblages from the same sediment intervals were also examined. The diatom analysis revealed that, although the lakes experienced regional acid deposition and may have acidified/recovered, the current pH differs little from pre-industrial times (Barrow et al. 2014). Similar replacement of Ca-rich cladoceran taxa by Ca-poor competitors has also been observed in “top/bottom” analyses of lakes affected by both Ca decline and acidification in Nova Scotia, Canada (Korosi and Smol 2012b).

Long-term impacts on cladoceran communities as lakewater Ca concentrations fall near or below 1.5 mg L⁻¹ are evident in paleolimnological analyses from multiple sites in eastern North America. Declines in daphniid abundance relative to Ca-poor taxa coincident with falling Ca concentrations have been identified in sediment cores collected from lakes that have experienced a variety of acidification trends since pre-industrial times, including stable circumneutral pH, naturally acidic conditions, as well as acidification and subsequent recovery (Jeziorski et al. 2008a). The substantial contributions of daphniids

to the cladoceran sedimentary assemblages prior to their replacement by Ca-poor competitors as lakewater Ca concentrations declined, suggest that current Ca availability is lower than it was prior to acid deposition (Jeziorski et al. 2008a), contradicting biogeochemical models that indicate current lakewater Ca concentrations still exceed pre-industrial values (Reid and Watmough 2016).

More detailed interpretation of paleolimnological investigations of Ca decline using daphniid subfossils have been confounded by the limited taxonomic resolution available in the cladoceran sediment record (Korosi et al., 2011). Although the genus *Daphnia* likely contains many of the cladoceran taxa most sensitive to reduced Ca availability, the most abundant daphniid subfossil is typically the postabdominal claw that can only be attributed to one of two species complexes: the *D. longispina* complex (represented in south-central Ontario by *D. ambigua*, *D. dubia*, *D. longiremis*, *D. mendotae*, *D. parvula*, and *D. retrocurva*), or the *D. pulex* complex (represented in south-central Ontario by *D. catawba*, *D. minehaha*, *D. pulex*, *D. pulicaria*; Hebert 1995; Korosi et al. 2011), and at least one taxon identified as relatively tolerant of low Ca concentrations is present in each complex (*D. ambigua* and *D. catawba*; Cairns 2010).

Differences in Ca tolerances among daphniids are clearly demonstrated in a paleolimnological analysis of Dickie Lake (Ontario, Canada) where, in response to acidification and Ca decline, members of the *D. longispina* complex were virtually eliminated from the cladoceran assemblages only to be replaced by members of the *D. pulex* complex (Shapiera et al. 2012). However, following inadvertent Ca additions to the watershed (through the use of a Ca-rich dust suppressant on local roads; Yao et al. 2011), the relative abundance of the *D. longispina* complex recovered to pre-impact levels (Shapiera et al. 2012). Direct limnological monitoring records indicate that the *D. pulex* complex within Dickie Lake was primarily represented by *D. catawba*, illustrating the limitations imposed by the inability to distinguish between the Ca-sensitive *D. pulex* and the Ca-indifferent *D. catawba*. Furthermore, despite straightforward responses, such as the appearance of the *Daphnia longispina* complex in the sediment record of Middle Lake (near Sudbury, Ontario) following its liming and fertilization (Labaj et al. 2014),

there are difficulties in distinguishing the effects of low pH on Ca-rich daphniids from those of low Ca availability due to their frequent co-occurrence outside of the laboratory.

Examinations of the littoral cladocerans from low Ca shallow lakes have also encountered difficulties in distinguishing Ca-related changes, suggesting that perhaps littoral cladoceran taxa are less sensitive to low Ca, or that ecological thresholds for these taxa have not yet been crossed (Moss crop et al. 2015). Further research on shallow lakes is required, as small shallow lakes that are common on the Precambrian Shield are particularly vulnerable to Ca decline (Jeziorski et al. 2008a). Ambiguities encountered when using the sediment record to identify taxa negatively impacted by Ca decline have prompted examination of the issue from different perspectives, including the identification of organisms that may benefit from decreasing lakewater Ca concentrations.

Identifying taxa favoured by lower lakewater Ca concentrations

Ongoing lakewater Ca declines will increasingly present a disadvantage for organisms with high Ca demands (e.g. some Ca-rich daphniids) with dramatic possibilities for ecosystem composition as daphniids are often the dominant algal grazers in softwater lakes (Hessen et al. 1995). For example, following the replacement of *Daphnia* spp. by the less efficient grazer *Bosmina* spp. associated with lakewater Ca concentrations falling to >1.5 mg/L in Lake George, Nova Scotia (Canada), the reduced grazing pressure was associated with increased algal production, raising concerns of increased algal bloom frequency (Korosi et al. 2012). However, prior to falling to levels that directly reduce the survival of Ca-demanding taxa (Ashforth and Yan 2008), lower Ca availability can increase their vulnerability to predation (Riessen et al. 2012), providing an indirect advantage to both their prey and competitors. Multiple investigations of softwater lakes have identified decreases in the abundance of large daphniids at lower Ca concentrations to be coincident with increases in their competitor, the larger gelatinous cladoceran *Holopedium glacialis* (Hessen et al. 1995; Waervagen et al. 2002; Korosi and Smol 2012a,b; Jeziorski et al. 2014). *Holopedium* is a filter feeding planktonic taxon with a much lower Ca content than its principal daphniid competitors (Hessen et al. 1995; Jeziorski and Yan 2006) because its protection

from predators stems from a protective jelly-capsule composed principally of a mucopolysaccharide (Montvilo et al. 1987; Rowe et al. 2007) rather than a heavily calcified carapace. *Holopedium* is tolerant of low pH and common in acidified lakes (Havas and Likens 1985; Wærvågen et al. 2002). However, recent analyses of direct monitoring data and paleolimnological records have identified increases in the relative abundance of *Holopedium* in lakes recovering from acidification (Jeziorski et al. 2015; Redmond et al. 2016). By combining structural equation modeling, paleolimnological analyses and long-term direct limnological records spanning three decades for a set of lakes on the Precambrian Shield, it was demonstrated that recent *Holopedium* increases are indirectly attributable to reduced Ca availability, as lower Ca concentrations reduced the populations of large Ca-rich daphniids, freeing up food resources for *Holopedium* (Jeziorski et al. 2015). The widespread replacement of *Daphnia* by *Holopedium* would have varied ecological (and economic) consequences, as in addition to a ten-fold lower Ca content, *Holopedium* have only half the phosphorus content of daphniids (Andersen and Hessen 1991), and are less vulnerable to invertebrate predators such as *Chaoborus* larvae and small planktivorous fish (Allan 1973; Campbell 1991). Thus, predation rates may be lower, with implications for vertical energy transport, although it remains uncertain how the broader aquatic community will react to ongoing Ca declines.

Ca decline and other organisms

Impacts of lakewater Ca decline will extend beyond members of the crustacean zooplankton, and there is growing evidence indicating the effect of reduced aqueous Ca concentrations on other biota including many other freshwater crustaceans (Cairns and Yan 2009). For example, among crayfish (Family: Astacidae), changes in pH and Ca concentration have been linked to the near extirpation of the native crayfish, *Cambarus bartonii*, from some wilderness lakes in Algonquin Park, Ontario (Hadley et al. 2015). At the same time the northward expansion of the rusty crayfish (*Oronectes rusticus*) may be limited by the low Ca concentrations on the Precambrian Shield (Edwards et al. 2013). Low Ca concentrations can limit the distribution of gammarids (Rukke 2002b), and also raises the metabolic

demand of Ca acquisition in some gastropods, increasing respiration rates and slowing locomotion (Dalesman and Lukowiak 2010). The direct effects of Ca declines will be greatest on organisms with relatively high Ca demands (e.g. Ca-rich invertebrates); however, these taxa are also a major source of Ca for larger organisms, therefore reduced dietary Ca can allow lakewater Ca-decline to transcend the aquatic-terrestrial boundary with potential cascading effects (e.g. reducing eggshell integrity and growth in birds; Scheuhammer 1991; St. Louis and Breebaart 1991). As our focus is on the influence of Ca decline on aquatic ecosystems, the effects of declining Ca levels in soil are beyond our scope; however such impacts have been intensively studied elsewhere, particularly with respect to forest health (e.g. Tomlinson 2003; Watmough et al. 2003; Lovett et al. 2016). In all environments experiencing Ca decline, interactions of falling Ca concentrations with other environmental stressors are of considerable interest.

Interaction of Ca decline and other environmental stressors

In addition to the direct and indirect effects of Ca decline on the ecology of softwater lakes, there are many potential interactions between reduced Ca availability and the variety of other environmental stressors influencing the landscape (Figure 1). The complicated relationship between pH and Ca has already been discussed, and is an ongoing concern as in many formerly acidified lakes despite rising pH. Decreases in Ca concentrations are already impeding biological recovery from acidification (Keller et al. 2001), and without additional reductions in acid deposition many lakes may re-acidify (Reid and Watmough 2016). Therefore, the recovery of many zooplankton communities that are limited by the ability of populations to cope with current conditions, rather than the arrival of new colonists (Piscia et al. 2016), may slow down or perhaps reverse.

Reduced Ca availability will have physiological consequences for much of the biota in affected water bodies. Disruptive effects of acidic conditions on Ca metabolism have been observed in a variety of organisms, both through the inhibition of calcification and the deformation of carbonate body parts. For example, crayfish living in acidic conditions have reduced exoskeleton rigidity relative to those in circumneutral waters, contributing to increased population mortality from predation (France 1987). Ca

deficiencies are also known to enhance the negative effects of metal toxicity for both fish (Brown 1983; Deleebeeck et al. 2007) and zooplankton taxa (De Schamphelaere and Janssen ,2002; Celis-Selgado et al. 2016). The increased toxicity is related to competition between Ca and divalent metals (e.g. copper) for membrane binding sites, allowing greater metal uptake in low Ca conditions (De Schamphelaere and Janssen ,2002; Kozlova et al. 2009). Therefore, even in regions where metal concentrations are stable and not a current management concern, Ca decline may induce toxicity issues. Other physiological concerns associated with falling Ca include the increased sensitivity of some biota to UV-radiation at low Ca levels (Hessen and Rukke 2000) and the potential for enhanced bacterial uptake of Hg^{II} at both reduced pH levels and lower base cation concentrations (Daguené et al. 2012).

Regional climate warming may also exacerbate the effects of Ca decline. Ca demands may increase with the higher metabolic rates that accompany higher temperatures (Ashforth and Yan 2008), while movement and behavior may also be influenced by low Ca availability at higher temperatures (Betini et al. 2016). Furthermore, specific Ca requirements will likely influence changes in species ranges associated with warming temperatures, as well as the spread of invasive species such as the he predatory cladoceran *Bythotrephes longimanus* (commonly known as the spiny water flea). *Bythotrephes* is spreading to many softwater lakes across the Precambrian Shield (Weisz and Yan 2010), and is tolerant of low Ca conditions (Kim et al. 2012). By preferentially feeding on Ca-rich daphniids over Ca-poor taxa such as *Holopedium* (Kim and Yan 2010), *Bythotrephes* can have significant effects on cladoceran community composition (Yan et al. 2001).

Broader consequences and future research directions

Despite considerable success in the worldwide efforts to reduce acid deposition, acidic emissions have not been eliminated and critical loads continue to be exceeded in some regions (Jeffries et al. 2003; Vet et al. 2005). With increasing evidence demonstrating that low Ca concentrations are impeding recovery from acidification (Keller et al. 2001, 2003; Arseneau et al. 2011), while also acting as an independent environmental stressor (Jeziorski et al. 2008a), the ability to identify the potential ecological

and economic impacts of Ca decline is of great importance. However, evaluating the consequences of Ca concentrations falling below critical thresholds will require detailed information regarding historical conditions. Therefore, future Ca decline analyses should focus on reconciling the current disagreement between paleolimnological and biogeochemical modeling efforts regarding the relationship between current Ca concentrations and pre-industrial levels, while remaining mindful of the possibility that declines in Ca availability in recent decades may be occurring on top of Ca depletion processes operating on much longer time-scales (Leys et al. 2016). Limitations inherent to the paleolimnological analyses of cladoceran remains (i.e. taxonomic resolution, threshold responses of Ca-sensitive daphniids to insufficient Ca, and the small magnitude of the declines in absolute terms) make development of a cladoceran-inferred Ca model unlikely. Future paleolimnological efforts can address some of the taxonomic limitations by searching for sub-lethal effects among other sensitive taxa and increasing the number of detailed investigations outside of south-central Ontario. The accuracy of modeling analyses will continue to improve as more direct monitoring data become available; however, to better understand discrepancies between the two approaches will require studies that combine modeling and paleolimnological approaches on the same lake. Given that Ca concentrations in many softwater lakes are already below critical thresholds for some organisms, and that number of affected lakes is anticipated to grow, the importance of understanding the impacts of continued lakewater Ca declines will only increase.

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764 **Figure Captions:**

765 Figure 1: Summary diagram of the relationships between lakewater Ca declines and the other
766 environmental stressors discussed in the text. Directional arrows indicate the effect of one stressor
767 upon another.

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Draft

220x171mm (300 x 300 DPI)