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Response of *Bosmina* size structure to the acidification and recovery of lakes near Sudbury, Canada

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ABSTRACT

In response to biotic and abiotic cues, the cladoceran genus Bosmina *can undergo changes in body size and appendage length and shape over successive generations. To improve our understanding of the environmental controls on* Bosmina *size structure, we used paleolimnological techniques to examine* Bosmina *size responses to the extreme acidification and metal contamination, and then subsequent chemical recovery, of lakes in the vicinity of mining and smelting operations near Sudbury, Canada. During the acidification period,* Bosmina *antennule and carapace length significantly increased in three of the five study lakes, while mucro length significantly decreased in four of the five lakes. However, despite the recent return to pre-impact pH levels, the size structure of the present-day* Bosmina *community still differs from the pre-impact size distributions. We suggest that the continued dominance of the food webs by small invertebrate predators (*e.g.*, cyclopoid copepods) is responsible for the persistent changes to* Bosmina *size structure.*

Key words: Cladocera; Bosmina*; paleolimnology; multiple-stressors; industrial contamination.*

INTRODUCTION

The Cladocera are a widespread order of crustacean zooplankton that occupy an intermediate trophic position in aquatic food webs. Many cladoceran taxa undergo cyclomorphosis, altering body size and morphology in response to environmental stimuli over successive generations. The influence of invertebrate predation on cladoceran cyclomorphosis has been well-studied, with invertebrate predators inducing an increase in body size and the development of defensive structures such as neck teeth in *Daphnia* (Hebert and Grewe, 1985; Black, 1993) and elongated mucros and antennules in *Bosmina* (Kerfoot, 1975; Black, 1980). However, other environmental factors also affect cladoceran size structure, including temperature (Coker, 1939; Brooks, 1946; Hebert, 1978; Kappes and Sinsch, 2002), food availability (Hebert, 1978), and pH (Locke and Sprules, 2000; Korosi *et al.*, 2008). Size structure is an important attribute of zooplankton ecology, influencing algal grazing rates (larger-sized animals tend to be more efficient grazers; DeMott, 1982; Tóth and Kato, 1997), fitness, competitive abilities, and energy transfer up the food web (Lynch, 1977; Hart and Bychek, 2011). As cladocerans are often both the primary algal grazers and the principal prey items for planktivorous fish and invertebrate predators in many aquatic food webs, changes to cladoceran size structure can also impact trophic dynamics.

Bosmina, a herbivorous filter-feeding cladoceran genus, is one of the most common pelagic invertebrates in temperate lakes of North America. In response to predation and environmental conditions, cyclomorphosis in *Bosmina* manifests as changes in carapace size and the length and shape of the antennules and mucros (Kerfoot, 1975; Black, 1980). These shifts are most often linked to predation by invertebrate predators such as *Chaoborus* and copepods (Kerfoot, 1975; Black, 1980; Post *et al.*, 1995; Korosi *et al.*, 2013), rather than population age structure, as antennules and mucros change little throughout the life of an individual animal (Deevey and Deevey, 1971). When the risk of injury by a predator outweighs the risk of mortality (*e.g.*, attacks from small invertebrates such as copepods), longer antennule lengths may confer protection from damage by curving over the vulnerable swimming appendages, shielding them from damage in an attack (Kerfoot, 1975; Post *et al.*, 1995). When mortality risk is high (*e.g.*, predation by larger invertebrates such as *Chaoborus*), longer mucros and increased carapace size increase prey-handling time and decrease the success rate of attacks (Black, 1980; Sprules *et al.*, 1984; Post *et al.*, 1995; Korosi *et al.*, 2013). In the presence of visual predators such as planktivorous fish, smaller size attributes may be beneficial to reduce visibility to these predators, which often selectively prey upon larger individuals (Kerfoot, 1975; Nilssen and Sandøy, 1990).

Abiotic factors may also impact *Bosmina* size structure; notably, acidification has been associated with shifts in antennule and carapace length (Locke and Sprules, 2000; Korosi *et al.*, 2008). However, reported changes in size structure associated with lake acidification have not been consistent. In some cases, acidification was associated with decreases in *Bosmina* body size (Locke and Sprules, 1993, 2000; Korosi *et al.*, 2008), while others observed increases in body size and appendage length (Nilssen and Sandøy, 1990). Acidification not only impacts organisms through direct physiological mechanisms, but can also alter food webs and predation regimes, which may account for these discrepancies. For example, as lakes acidify and fish are extirpated, *Chaoborus* often becomes the dominant predator of zooplankton (Yan *et al.*, 1991) and these changes in the predation regime may alter zooplankton community composition and size structure. Therefore, the mechanisms affecting size structure, whether direct physiological stress or changes in predation, remain unclear.

Long-term perspectives are required to relate changes in size structure with known environmental stressors at both local and regional scales. *Bosmina* leave abundant, identifiable remains in lake sediments, allowing changes in size structure through time to be tracked using paleolimnological techniques (Korhola and Rautio, 2001; Korosi *et al*., 2013; Labaj *et al.*, 2013). Furthermore, paleolimnological methods are ideal for assessing the response of *Bosmina* to acidification, allowing long-term (*i.e.*, from pre-impact times to the present-day) trends in size structure to be examined. As lake sediments integrate organisms across whole-lake environments, incorporating seasonal and inter-annual variability, they can provide a holistic view of biotic shifts through time. In a *top-bottom* (*i.e.*, pre-impact to present-day comparison) paleolimnological survey of 44 softwater Ontario lakes located around Dorset, Ontario, Canada (~200 km SE of Sudbury), Korosi *et al*. (2010) noted decreased antennule and mucro lengths in *Bosmina*, and decreased postabdominal claw lengths in *Daphnia* spp. in modern times, compared to specimens collected from pre-industrial sediments. The differences in size were attributed to decreased copepod predation, as well as moderate acidification that occurred in the region (Korosi *et al*., 2010). However, since only two sediment samples are analysed when using a *top-bottom* paleolimnological approach, this procedure does not allow for the timing of size changes to be linked with the onset of individual stressors.

We use a paleolimnological approach to assess the response of *Bosmina* size structure to severe acidification, metal contamination, and the subsequent chemical recovery of five intensively studied Boreal Shield lakes through time in the vicinity of Sudbury, Ontario. Previously, Labaj *et al*. (2015) documented the cladoceran species assemblage changes that had occurred in these lakes as a response to anthropogenic stressors. Here, we compare *Bosmina* size attributes from the pre-impact, acidification/metal contamination, and recovery periods to address the hypothesis that *Bosmina* size structure changed in response to acidification and/or metal contamination. We predicted that: i) If the *Bosmina* size structure changes are primarily a response to metal contamination, size differences will be more pronounced in lakes that were acidified and contaminated with heavy metals, relative to those that only acidified; ii) If *Bosmina* size responds to acidification through direct physiological impacts, body size should decrease during the period of acidification, and increase again following recovery of pH; and iii) If *Bosmina* size is responding indirectly to acidification through changes in predator assemblages, size changes between lakes will reflect a shift in the dominant predators.

METHODS

Site description and field methods

Lakes near Sudbury (Ontario, Canada) experienced severe acidification and contamination with copper and nickel due to emissions from nearby smelting operations that began in the late-1800s (Gunn, 1996). At the peak of industrial emissions in the 1960s, many lakes within a ~17,000 km^2 region acidified, with the pH of circumneutral lakes declining to as low 4 (Neary *et al.*, 1990; Gunn, 1996).

Three of the study lakes (Daisy, Middle, Clearwater) are located within the city limits of Sudbury. The lakes were heavily acidified by the 1970s (to pH <5; Keller *et al.*, 2004) and have aqueous Cu and Ni concentrations that continue to exceed provincial water quality guidelines (Cooperative Freshwater Ecology Unit, Laurentian University, 2013). Each of the three Sudbury study lakes has undergone prior diatom-based reconstructions of pH (Dixit *et al*., 1987, 1990, 1996), analyses of sedimentary metals (Snetsinger, 1993; Tropea, 2008; Tropea *et al*., 2010), cladoceran assemblages and sedimentary chlorophyll-*a* (Labaj *et al*., 2015), and regular water chemistry monitoring since 1973 (Middle, Clearwater) or 1991 (Daisy; Cooperative Freshwater Ecology Unit, Laurentian University, 2013).

Lumsden and George lakes are situated in Killarney Provincial Park, ~60 km southwest of Sudbury. Although lakes in Killarney generally acidified to a similar level as lakes in the immediate vicinity of Sudbury, they experienced lower inputs of Cu and Ni due to their greater distance from the smelters (Yan and Strus, 1980; Labaj *et al*., 2015). Both Killarney lakes have also been examined in paleolimnological reconstructions of pH (Dixit *et al.*, 1992), cladocerans and chlorophyll-*a* (Labaj *et al*., 2015), sedimentary metals (Snetsinger, 1993), and water chem-

istry monitoring since 1981 (George) and 1998 (Lumsden; Cooperative Freshwater Ecology Unit, Laurentian University, 2013).

Sediment cores were collected from each lake in July 2012 using a Glew (1989) gravity corer, and sectioned on shore using a Glew (1988) extruder. Sediments were sectioned at 0.25-cm resolution from 0 to 15 cm, and at 0.5-cm resolution for the remainder of the core. Sediments were stored in a cooler with cold packs until transportation to the laboratory. Only the surface (0-0.25 cm) sediments of the 2012 Daisy Lake core were analysed (representing the year 2012), with the remainder of the sediments sourced from a dated core previously analysed for sedimentary metals (Tropea, 2008; Tropea *et al.*, 2010). The 2012 surface sediments allowed the most recent cladoceran assemblage of Daisy Lake to be assessed (Labaj *et al.*, 2015), without the need to establish a ^{210}Pb-based chronology from a second core from the lake. The cores were collected from locations as close as possible to each other within Daisy Lake. Due to the widespread (~17,000 km^2 area) acidification surrounding Sudbury, locating an unimpacted control lake in the immediate region is not feasible. Nonetheless, our use of the paleolimnological approach allows pre-impact conditions to be assessed and compared with impacted and recovery periods.

Laboratory methods

Sediment dates were established with gamma spectroscopy using ^{210}Pb chronologies, and the constant rate of supply (CRS) method described by Appleby (2001). Sediment ages beyond background ^{210}Pb levels were extrapolated using a second-order polynomial function fit to the established dating curve (Labaj *et al.*, 2015). Sample

processing largely followed methods outlined in Korhola and Rautio (2001). Briefly, sediment samples were deflocculated in 10% KOH solution on a hotplate for 20 minutes, then collected on a 38 μm mesh sieve, and mounted on glass slides with glycerine jelly. *Bosmina* sizes were measured at 200x magnification on a Leica DMR light microscope. All identifiable and intact *Bosmina* remains were digitally photographed, and measured using Northern Eclipse imaging software (Empix Imaging, Inc.). A minimum of 40 headshields, mucros, and carapaces were measured to ensure variation was captured in the assemblage (Brahney *et al.*, 2010). *Bosmina* were classified as either *Bosmina* spp. or *Eubosmina* spp., based on the arrangement of pores on the headshields.

Data analysis methods

To assess the impacts of acidification on *Bosmina* size attributes, the history of each study lake was divided into three periods: pre-impact (from ~1800 to the beginning of pH decline; Tab. 1), acidification (period of lowest pH; Tab. 1), and recovery (time that pH ≥ background levels to present), based on previously published diatom-inferred pH profiles and monitoring data for the study lakes (Dixit *et al.*, 1987, 1990, 1992, 1996; Cooperative Freshwater Ecology Unit, Laurentian University, 2013; Tab. 1). Due to differences in acidification trends in each lake, the number of samples from each period differed between lakes (Tab. 1). A nonparametric Kruskal-Wallis test was used to assess whether distribution of each size attribute differed across the time periods. Pairwise Mann-Whitney U-tests (with Holm corrections applied to P-values) were used to assess significance ($P<0.05$) of shifts in distribution between pre-impact, acidification, and recovery periods.

Tab. 1. Time periods in each of the 5 study lakes, based on diatom-inferred pH (Dixit *et al.*, 1987, 1990, 1992, 1996) and pH monitoring (Cooperative Freshwater Ecology Unit, Laurentian University, 2013).

Lake	Period	pH during period	Number of intervals	Sediment interval midpoints (cm)	Approximate dates of period
Daisy	Pre-impact	>6	6	9.13-4.63	1800-1952
	Acidification	<5	1	2.13	1987-1992
	Recovery	>6	2	0.13-Surface	1999-2012
Middle	Pre-impact	>5.8	7	16.25-7.63	1800-1940
	Acidification	<5.5	1	4.63	1966-1972
	Recovery	>5.8	11	4.13-0.13	1975-2012
Clearwater	Pre-impact	>5.8	5	9.63-5.63	1800-1930
	Acidification	<5	3	3.63-2.13	1964-1992
	Recovery	>5.8	4	1.63-0.13	1997-2012
George	Pre-impact	>5.8	5	16.25-9.13	1800-1919
	Acidification	<5.5	3	6.13-4.63	1958-1986
	Recovery	>5.8	7	3.13-0.13	1993-2012
Lumsden	Pre-impact	>5.3	9	20.25-7.63	1800-1958
	Acidification	<5	1	4.63	1973-1983
	Recovery	>5.3	2	1.13-0.13	2004-2012

RESULTS

Bosmina spp. was the dominant bosminid taxon present in Middle, Clearwater, Daisy, and Lumsden lakes (with only one *Eubosmina* spp. headshield found in Middle Lake). Within George Lake, *Eubosmina* spp. appeared in the 1930s, and increased to ~50% of the identifiable species by 2012. Pre-impact *Bosmina* size attributes were variable between the study lakes, with median mucro sizes ranging from 44-65.5 µm (Fig. 1), antennules from 88.5-118.5 µm (Fig. 2), and carapaces from 193-222.5 µm (Fig. 3).

Mean shifts in distribution as identified by Kruskal-Wallis tests occurred between time periods within all lakes and size attributes (P<0.05), with the exception of Lumsden Lake antennule and carapace length (P=0.90 and 0.10, respectively). Between the background and acidification periods, mucro length declined significantly in Daisy, Middle, Clearwater, and George lakes (Fig. 1), while antennule and carapace lengths increased significantly in Middle, Clearwater, and George lakes (Figs. 2 and 3).

From the acidification to recovery period, mucro length increased significantly in George Lake (a return towards
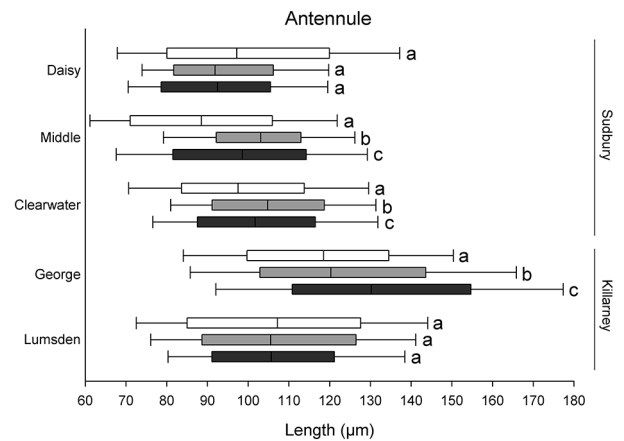

Fig. 2. Antennule lengths during pre-impact (white boxes), acidification (light gray boxes), and recovery (dark gray boxes) periods in the lakes' histories. Whiskers encompass the 10th and 90th percentile, and the box encompasses the 25th and 75th percentile. The line indicates the median. Boxes with different letters indicate periods that are significantly different from each other (within each lake), as assessed by pairwise Mann-Whitney U-tests (significance of P<0.05).
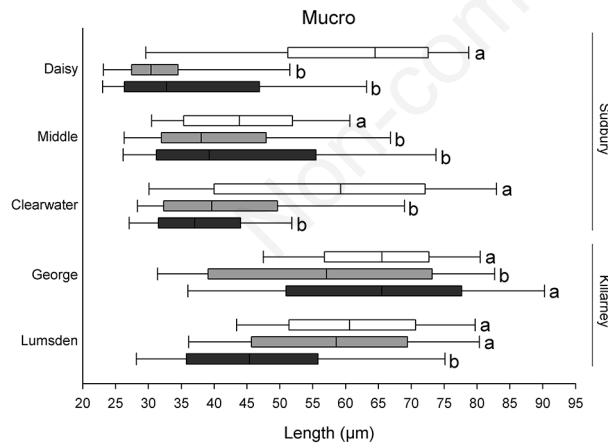

Fig. 1. Mucro lengths during pre-impact (white boxes), acidification (light gray boxes), and recovery (dark gray boxes) periods in the lakes' histories. Whiskers encompass the 10th and 90th percentile, and the box encompasses the 25th and 75th percentile. The line indicates the median. Boxes with different letters indicate periods that are significantly different from each other (within each lake), as assessed by pairwise Mann-Whitney U-tests (significance of P<0.05).
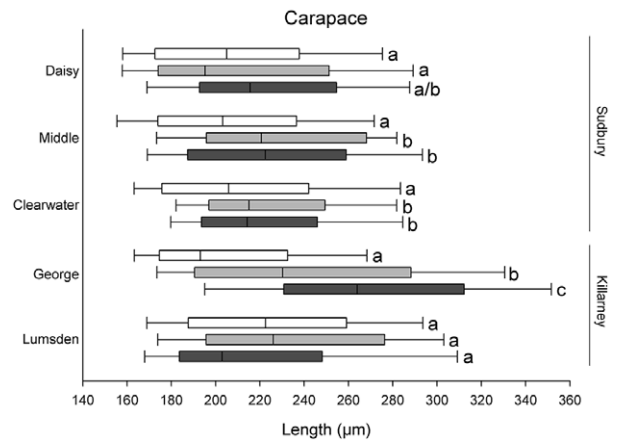

Fig. 3. Carapace lengths during pre-impact (white boxes), acidification (light gray boxes), and recovery (dark gray boxes) periods in the lakes' histories. Whiskers encompass the 10th and 90th percentile, and the box encompasses the 25th and 75th percentile. The line indicates the median. Boxes with different letters indicate periods that are significantly different from each other (within each lake), as assessed by pairwise Mann-Whitney U-tests (significance of P<0.05). Daisy Lake carapace length differed significantly between background and recovery periods (P=0.04), but not between background and acidification (P=0.93) or acidification and recovery periods (P=0.25).

pre-impact size distributions), and decreased significantly in Lumsden Lake (moving further from their pre-impact distributions; Fig. 1). Antennule length decreased significantly in Middle and Clearwater lakes (towards pre-impact size distributions), and increased significantly in George Lake (moving further from pre-impact distributions; Fig. 2). Carapace length increased significantly in George Lake from the acidification to recovery period (moving further from pre-impact distributions; Fig 3).

DISCUSSION

We hypothesized that acidification and metal contamination in the Sudbury and Killarney study lakes would alter *Bosmina* size structure. Prior to the onset of anthropogenic disturbances, *Bosmina* size attributes were variable, reflecting the diversity present in food web composition (*e.g.*, competitor species, planktivorous fish, invertebrate predators; Labaj *et al.*, 2015; Figs. 1-3). During the period of acidification and metal contamination, we noted significant directional shifts in *Bosmina* size structure that were consistent among many of the study lakes (Figs. 1-3), suggesting that acidification and/or metal contamination influenced size structure either through direct physiological effects, or indirectly by transforming food webs. The bosminid assemblage in most of our lakes was dominated by *Bosmina* spp., however in George Lake, *Eubosmina* spp. began increasing in relative abundance during the acidification period, and comprised ~50% of the bosminids by 2012 (Labaj *et al.*, 2015). Replacement of *Bosmina* spp. by *Eubosmina* spp. may confound identification of the effects of acidification on bosminid size structure, as *Eubosmina* tend to be larger than *Bosmina* (Post *et al.*, 1995). Therefore the increased abundance of *Eubosmina* spp. in George Lake during the acidification and recovery periods may be responsible for the larger *Bosmina* size attributes observed in George Lake, especially in the recovery period.

Our lakes differed in severity of contamination, and we predicted that, if *Bosmina* size structure was impacted by metal contamination, shifts in size structure would be most pronounced in the highly metal-contaminated Sudbury lakes (Daisy, Middle, and Clearwater) compared to those in Killarney (Lumsden and George). Long-term exposure to elevated Cu levels can reduce growth rate in *Bosmina* (Koivisto *et al.*, 1992), and similarly, Cu and Ni exposure can reduce growth rate in the cladoceran genus *Daphnia* (Ingersoll and Winner, 1982; Münzinger, 1990), leading to smaller individuals at maturity. Contamination with Cu and Ni has previously been identified as an important factor structuring the cladoceran species assemblages in these lakes, with assemblages experiencing more pronounced shifts in Sudbury lakes, relative to those in Killarney (Labaj *et al.*, 2015). However, many of the

Bosmina size attributes in Sudbury and Killarney showed shifts of similar magnitude and direction, despite different levels of metal contamination. Furthermore, increased carapace size (Fig. 3) in response to acidification and metal contamination of the study lakes contrasts with reports of reduced cladoceran body size with metal contamination elsewhere (Ingersoll and Winner, 1982; Münzinger, 1990; Koivisto *et al.*, 1992). We further predicted that, if *Bosmina* was responding directly to pH, sizes would decrease with acidification, and return to pre-impact levels following recovery of pH. However, despite recovery of pH, size structure did not return to pre-impact levels. Moreover, the increases in size of antennules and carapaces (Figs. 2 and 3) with acidification differ with results of studies that report smaller *Bosmina* size attributes in low-pH lakes (Locke and Sprules, 2000; Korosi *et al.*, 2008). We therefore suggest that direct physiological effects of metal contamination and low pH were not the main drivers of *Bosmina* size structure in these systems.

Acidification often precipitates major shifts in the structure and function of aquatic food webs (Appelberg *et al.*, 1993). In the Sudbury region, acidification caused widespread loss of fish (Beamish and Harvey, 1972), as well as a shift toward acid-tolerant invertebrate predators (Yan *et al.*, 1991). Changes to food web structure and predation regime are likely dominant factors shifting cladoceran (Locke and Sprules, 1993), and specifically *Bosmina*, size structure in acidified lakes (Nilssen and Sandøy, 1990; Korosi *et al.*, 2008). We predicted that if *Bosmina* size structure was responding indirectly to acidification (*i.e.*, through changes in predator assemblages), then size attributes would reflect a shift in the dominant predation regime. The increased antennule and carapace length (Figs. 2 and 3) in many of the lakes during the acidification period suggests that invertebrate predation was more intense during this time (Kerfoot, 1975; Post *et al.*, 1995). However, mucro length, often considered the size attribute most sensitive to invertebrate predation (Sprules *et al.*, 1984; Post *et al.*, 1995; Labaj *et al.*, 2014, 2015), declined in our lakes as they acidified (Fig. 1). Shifts in mucro length often occur in response to predation regime; for example, increasing in response to predation from larger invertebrate predators (*e.g.*, *Chaoborus*, large copepods), as this increases prey-handling time (Kerfoot, 1975; Black, 1980; Post *et al.*, 1995). However, elongated features carry a physiological cost, namely increasing drag, and reducing feeding rate (Lagergren *et al.*, 1997), favouring smaller features when elongated features are not beneficial. Our results may indicate a shift from large invertebrate predators (capable of causing outright mortality) to smaller invertebrate predators, capable of damaging swimming appendages (*e.g.*, cyclopoid copepods), as longer antennules may protect swimming appendages under attack (Post *et al.*, 1995). Similarly, increased cara-

pace length likely reduces overall risk of mortality from small gape-limited invertebrate predators (Korosi *et al.*, 2013). Small, acid-tolerant cyclopoid copepods can become dominant invertebrate predators under acidified conditions (Locke and Sprules, 1993), however their abundance may also increase with increasing pH (Valois *et al*., 2010, 2011). Moreover, cyclopoid abundance has increased in Middle Lake (Yan *et al*., 2004) and Daisy and Clearwater lakes (Cooperative Freshwater Ecology Unit, unpublished) in the past 40 years, concurrent with pH improvements. The decrease in mucro length (Fig. 1) and increases in antennule and carapace length (Figs. 2 and 3) in many of our lakes may reflect a predation regime increasingly dominated by cyclopoid copepods.

Dominance of cyclopoid copepods and other small invertebrate predators in the lakes may be influenced by predation from acid-tolerant yellow perch (*Perca flavescens*), which consume cladocerans and invertebrate predators such as copepods and *Chaoborus* as part of their diet (Falk, 1971; Graeb *et al*., 2004). Due to its preference for larger prey items (*e.g.*, size-selective predation), yellow perch predation can induce a shift towards smaller body size in its prey (Post and Cucin, 1984). Yellow perch is currently present in each of our lakes (with the exception of Lumsden Lake; Snucins and Gunn, 1998; City of Greater Sudbury, 2014), and its predation may have been responsible for the shift to smaller invertebrate predators (*e.g.*, cyclopoid copepods) that was inferred from *Bosmina* size attributes. The absence of yellow perch in Lumsden Lake may explain why only minimal *Bosmina* size changes were observed. With the exception of Lumsden Lake (which remains fishless), the study lakes now contain between 4 - 11 fish species, including piscivorous smallmouth bass and northern pike (Snucins and Gunn, 1998; City of Greater Sudbury, 2014), suggesting that food web recovery is underway. However, despite a return of many fish species to the lakes, *Bosmina* size attributes have shown little evidence of recovery, indicating the persistence of altered food webs (Figs. 1-3).

In addition to shifts in predation regime, the alteration of food webs within the lakes may have impacted competition for food and resources. *Bosmina* was the dominant cladoceran species in each of our study lakes; however, the Sudbury study lakes experienced declines in cladoceran species richness (Yan *et al*., 2004; Labaj *et al*., 2015) and shifts in species composition as the lakes were contaminated. Notably, *Chydorus brevilabris* reached relative abundances of 15-70% in the Sudbury lakes during the period of contamination, and remained at relative abundances of ~15 and 30% in Clearwater and Daisy lakes, respectively (Labaj *et al*., 2015). In comparison, the Killarney lakes have experienced subtle increases in species richness, and minor (<10%) increases in *C. brevilabris* through time (Labaj *et al*., 2015). Nonetheless,

Bosmina size attributes in George Lake (Killarney) show shifts of similar direction as the Sudbury lakes, suggesting that competition with other cladoceran species was not a prominent driver of *Bosmina* size structure.

During the recovery period, following a return of pre-impact pH levels, size attributes largely continued to differ significantly from their pre-impact distributions in both Sudbury and Killarney lakes, and in many cases continued to shift further from their pre-impact distributions (Figs. 1-3). Climate change is expected to become increasingly important in structuring the biotic recovery of lakes (Keller, 2007; Labaj *et al.*, 2015; Smol, 2010), further altering food webs that have not yet recovered from acidification. Within Sudbury, warming air temperatures in recent decades have been accompanied by increased surface mixed layer water temperature (Keller, 2007). Smaller organisms are predicted to be favoured under warmer conditions (Daufresne *et al.*, 2009; Forster *et al.*, 2012), and this may result in *Bosmina* with smaller size attributes in the future. Primary production in our study lakes has increased over the past ~50 years, likely linked to longer growing season and warmer water temperatures (Labaj *et al.*, 2015). With increased primary production, *Bosmina* increase reproductive and growth rates, resulting in larger individuals (Goulden *et al.*, 1982; Vanni, 1987). However, this relationship is unpredictable, and may be complicated by shifts in the timing of phytoplankton blooms, leading to an overall reduction of available food resources for zooplankton (Winder and Schindler, 2004), and consequently smaller individuals. Warmer water temperatures may also result in shifts to predation patterns, ultimately impacting *Bosmina* size structure. Specifically, predation by *Chaoborus* may intensify in warm isothermal waters, while decreasing in thermally stratified systems, due to the influences of temperature on *Chaoborus* metabolism (MacPhee *et al.*, 2011; Labaj *et al.*, 2013). Due to the unpredictable nature of thermal stratification under future climate scenarios (Keller, 2007), the impact of changing *Chaoborus* predation on *Bosmina* size structure is difficult to predict.

CONCLUSIONS

Our results suggest that the size structure of *Bosmina* communities changed significantly following the acidification of lakes in Sudbury and Killarney, with significant decreases in mucro length in four lakes and significant increases in carapace and antennule length in three lakes during the period of acidification. Despite the pH of each study lake having returned to pre-impact levels, bosminid size attributes remain significantly altered from their pre-impact distributions in many of the lakes. Similar changes in bosminid size structure occurred in both the Sudbury and Killarney regions, despite the vastly lower Cu and Ni levels in Killarney, suggesting that the size changes were not attributable to metal contamination. Instead the shifts in size

structure were likely mediated by changes to food webs and predation regimes in response to decreasing pH. The lack of a return to pre-impact *Bosmina* size distributions in many of the lakes suggests persistence of altered food webs. As these systems continue to recover, modern stressors such as climate change will become increasingly important in structuring biotic assemblages and size structure.

Acidification has led to the restructuring of aquatic food webs, which persists despite chemical recovery that has occurred in this region. We show that *Bosmina* size attributes preserved in lake sediments are responsive to changes in predator-prey dynamics that occur as a result of anthropogenic stressors. Furthermore, our results suggest that acidification and metal contamination impacted *Bosmina* sizes indirectly, through food web shifts, rather than through direct physiological effects. However, our results also show that the paradigm of larger appendages under invertebrate predation and smaller appendages under fish predation (Korosi *et al.*, 2013) may be overly simplistic, as the size structure of invertebrate predators can lead to differences in bosminid morphology. Overall, the study of size attributes of *Bosmina* subfossils has the potential to provide important insights into the effects of acidification and other anthropogenic disturbances on aquatic food web structure in a long-term context.

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