Assessing the potential environmental factors affecting cladoceran assemblage composition in arsenic-contaminated lakes near abandoned silver mines

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ABSTRACT

Silver mining in Cobalt (Ontario, Canada) has a long history that has left a complex environmental legacy where many lakes are contaminated with arsenic-rich mine tailings. In this exploratory survey, we examined subfossil Cladocera remains in the surface sediments of 22 lakes in the abandoned mining region to assess which environmental variables may be influencing the recent assemblage structure. Further, using a "top-bottom" paleolimnological approach, we compared the recent (top) and older (bottom) assemblages from a subset of 16 lakes to determine how cladoceran composition has changed in these lakes. Our regional survey suggests that the cladoceran assemblages in the Cobalt area are primarily structured by differences in lake depth, while site-specific limnological characteristics, including those related to past mining activities, may have limited roles in shaping the recent cladoceran compositions. The top-bottom paleolimnological analysis suggests that the cladoceran assemblages have changed in most lakes around Cobalt, however the magnitude and nature of changes varied across the study sites. As with most regional biological surveys, the responses to historical mining activities were not uniform across all sites, which further emphasizes the importance of considering site-specific limnological characteristics and multiple environmental stressors when assessing the impacts of mining pollution.

INTRODUCTION

Cladocera (Branchiopoda) are widely distributed in freshwaters around the world and occupy key intermediate positions in aquatic food webs. These mid-trophic zooplankters are excellent biological indicators of limnological change as they are sensitive to changes in chemical (*e.g*., metals), physical (*e.g*., depth, habitat) and biological (*e.g*., fish predation) conditions (Amsinck *et al.* 2006; Davidson *et al.* 2010; Leppänen 2018). In paleolimnological investigations, the well-preserved chitinous remains of Cladocera are often used to infer past ecological conditions and to assess the effects of various environmental stressors (Korhola and Rautio, 2001). Many cladoceran taxa (*e.g*., *Daphnia* spp., *Ceriodaphnia* spp.) have also been used as model organisms in laboratory-based *in-vitro* experiments to determine the toxicity of environmental contaminants to aquatic biota (Sarma and Nandini, 2006), and therefore Cladocera are often included as biological indicators in environmental monitoring programs of mining operations (Environment Canada, 2012). In addition to the *in vitro* toxicity studies, *in situ* examinations of Cladocera from mine-impacted lakes have reported changes in assemblage structure (Leppänen *et al.,* 2017a; Pociecha *et al.,* 2020), with decreases in diversity and richness (Labaj *et al.,* 2015; Winegardner *et al,*. 2017), and, in extreme cases, functional loss (Thienpont *et al.,* 2016).

In this study, we analyzed subfossil cladoceran remains from temperate lakes in the vicinity of abandoned silver mines near Cobalt (Ontario) where arsenic (As) levels in some lakes have exceeded the guidelines for the protection of aquatic life (5 μg L^{-1}; Canadian Council of Ministers of the Environment, 2001) and drinking water (10 μg L^{-1}; Heath Canada, 2006) by orders of magnitude (Sprague and Vermaire, 2018a). Silver was discovered around Cobalt in 1903 and by 1905 there were 16 mines in the area that continued production well into the 1930s and some small operations persisted until the 1980s (Dumaresq, 1993). The hard-rock mining at the Cobalt camps produced a substantial amount of waste rock and tailings that were rich in arsenic, cobalt, nickel and mercury (Dumaresq, 1993). However, due to the lack of environmental regulations for the disposal of mine waste at the time, waste rock was piled around the local landscape, while the tailings were discharged into surrounding waterbodies (Dumaresq, 2007). Although mine waste has not been

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produced for many decades in the Cobalt area, recent limnological surveys have reported significantly higher levels of As in the water column and sediments of lakes impacted by historical mine tailings (Dumaresq, 1993; Sprague and Vermaire, 2018a; Little *et al.,* 2020). These surveys and As mobility studies suggest that the tailings in the local waterbodies are sources of As in the Cobalt area (Percival *et al.,* 2004; Kwong *et al.,* 2007). The deposition of tailings into some lakes have also led to the reduction of the lakes' surface areas (Dumaresq, 1993; Patterson *et al.,* 1996). Furthermore, recent investigations have indicated that small connecting streams in the local landscape are bringing mine tailings into previously uncontaminated lakes (Sprague *et al.,* 2016).

In this exploratory regional survey, we used two different paleolimnological approaches to assess if metal(loid) contamination from past silver mining activities have impacted cladoceran assemblages in the lakes around Cobalt. First, we examined the cladoceran remains from the surface sediments of 22 lakes (10 impacted and 12 unimpacted by mining activities) and compared assemblage composition to a suite of measured environmental variables to determine the influence of multiple limnological variables in structuring the recent cladoceran assemblages. Second, we used a "top-bottom paleolimnological approach" (Smol, 2008) to determine if the cladoceran assemblages from the surface sediments (top) were different compared to those observed in deeper sediments (bottom, which represents older time periods) in 16 lakes (6 impacted and 10 unimpacted by mining activities). The top-bottom approach provides a snapshot of changes in biological composition between recent and older periods; however, this method has limitations such as the timing of change cannot be determined and the bottom sediment from all lakes may not be from the same time period (Smol, 2008). Despite these limitations, this approach has been an effective paleolimnological tool to track regional environmental change in Arctic and temperate lakes, especially in areas where historical biological data are not available (Jeziorski *et al.,* 2015; Leppänen *et al.,* 2017b; Armstrong and Kurek, 2019; Persaud *et al.,* 2021). Since the top-bottom approach provides a regional snapshot of biological change, it also helps to identify lakes where substantial biotic changes have occurred and select sites for future detailed paleolimnological analyses.

Study area

The Town of Cobalt (47°23'47.8"N 79°40'55.3"W) is in northeastern Ontario near Lake Timiskaming, which forms part of the border between the Canadian provinces of Ontario and Québec (Fig. 1). The local vegetation is characterized by a mixture of coniferous and deciduous species within the Lake Temagami Ecoregion of the Ontario Shield (Crins *et al,.* 2009). This region experiences a humid and cool climate (Crins *et al.,* 2009) where mean annual air temperature and total annual precipitation ranged between 0.8°C and 4.6°C, and 685 mm and 1229 mm, respectively (data collected at Earlton Climate Station - 6072230 between 1939 and 2004 and retrieved from Adjusted and Homogenized Canadian Climate Data https://climate.weather.gc.ca/). Detailed descriptions of the local geology are provided in Dumaresq (1993), Percival *et al.* (2004), and Sprague and Vermaire (2018 a, 2018b). Briefly, the study area is located within the Southern Structural Province of the Canadian Shield and is overlain by Proterozoic sediments of the Cobalt Group, which are intruded by the Proterozoic Nipissing diabase that contain veins rich in silver, arsenic, cobalt, and nickel. Most of the study lakes are around the Town of Cobalt, however a few lakes were sampled near the mines in Silver Center, ~20 km southwest of Cobalt (Fig. 1). The study lakes are underlain by Nipissing diabase, granite, basalt/rhyolite, Gowganda, or Lorraine formations (Sprague and Vermaire, 2018b) and alkaline (median pH 8.2) except for Ice Chisel Lake that had a pH of 4.7. Unfortunately, fish presence/absence data are not available for all lakes. Detailed limnological characteristics are provided in Supplementary Material 1 and discussed in more detail in Sprague and Vermaire (2018a). During the silver mining years, only a few study lakes received As-rich tailings directly from the nearby mining camps. However, the migration of tailings through the local landscape has resulted in the contamination of more lakes over the last century (Sprague *et al.,* 2016; Sprague and Vermaire, 2018a). The 22 study lakes have been categorized into the three groups based on the contamination history (Sprague and Vermaire, 2018a). The first group consists of six study lakes that received direct mine wastes (Brady, Cart, Cobalt, Crosswise, Fourclaim, and Tooth), while the second group includes four lakes that received migrated tailings (Ibsen, Kirk, New Lake, and Maidens), and the third group is composed of 12 uncontaminated lakes (Clear, Frog, Goodwin, Green, Ice Chisel, Mary Ann, Nicole, North Pickerel, Oxbow, Pine, Silver, South Pickerel) (Sprague and Vermaire, 2018a).

METHODS

Sample collection

The study lakes were sampled during the summers of 2015 and 2017 as part of a regional limnological survey; the details of site selection are provided in Sprague and Vermaire (2018a) and Little *et al.* (2020). Water samples for chemical analyses and sediment cores were collected from the middle of the study lakes. Specifically, water samples were obtained from a depth of ~20 cm below the surface and the samples collected for metal analyses were preserved with 0.5% HNO_3. Then water samples were refrigerated and transported to Caduceon Environmental Laboratories in Ottawa (Ontario), a Canadian Association for Laboratory Accreditation certified facility, for chemical analyses. A Glew (1989) gravity corer was used to retrieve sediment cores and extruded using a Glew (1988) extruder. Sediment samples were refrigerated and transported to Carleton University, Ottawa, Ontario, and were stored at 4°C.

Laboratory analyses

We analyzed the surface sediments (0-1 cm) for Cladocera remains following standard protocols described by Korhola and Rautio (2001), with the exception of Cobalt and Green lakes where sediments from the 1-2 cm interval were processed as the surface sediments were used up completely for other analyses. The sediments from Green Lake were radiometrically dated and the ^{210}Pb date associated with the 1-2 cm interval was 2011.7 (±0.8 years). Hence, we are confident that the usage of sediments from the 1-2 cm interval, while not preferred usually, was a suitable alternative to examine recent cladoceran assemblages because surface sediments (0-1 cm) were not available from the two lakes. Briefly, the sediments were deflocculated in 10% potassium hydroxide (KOH) for ~20 mins on a hotplate at ~70°C. The samples were then rinsed through a 38-µm sieve with deionized water to remove fine particles and the resulting residue was retained in glass vials. A few drops of safranin-glycerol and ethanol were added to stain the Cladocera remains and preserve the samples, respectively. Aliquots of the samples were pipetted on to microscope slides and covered with glass cover slips using glycerin jelly.

In addition to the surface sediments, we processed bottom (>20 cm) sediments of 16 lakes (3 impacted by direct deposition of tailings, and 3 impacted by migrated tail-

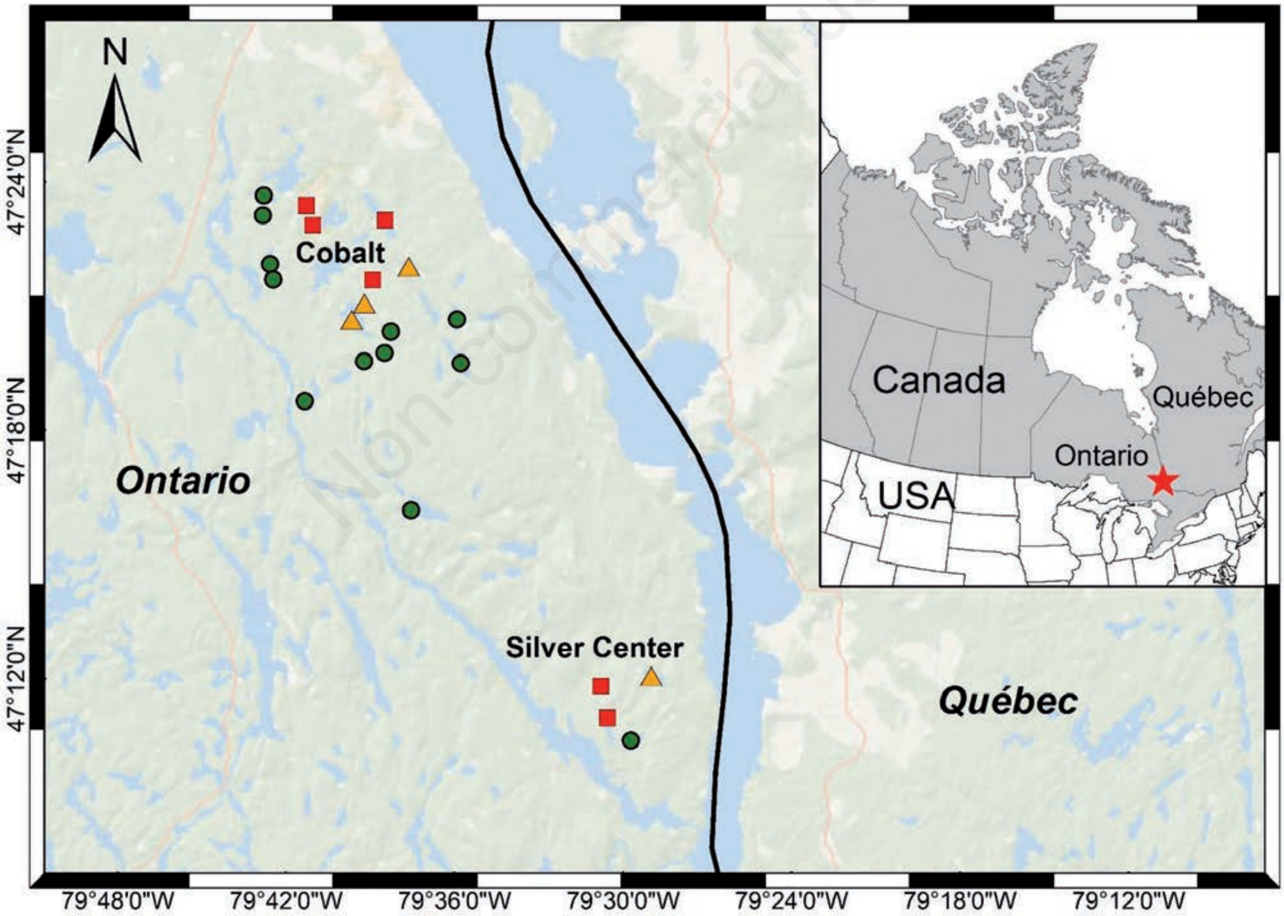

Fig. 1. Map of the study area of lakes around Cobalt, Ontario, Canada. The black line delineates the border between the provinces of Ontario and Québec. The lakes that received direct tailings are represented by red squares (n=6) and the lakes impacted by migrated tailings are indicated by yellow triangles (n=4). The unimpacted sites are represented by green circles (n=12). The red star in the inset map indicates the location of Cobalt within Canada.

ings, and 10 unimpacted lakes) for our top-bottom paleolimnological analysis following the same laboratory methods as described above. The bottom samples used for all study lakes are provided in Supplementary Material 2. Although we did not radiometrically date all the sediment cores for this study, the ^{210}Pb analysis of cores from two unimpacted lakes (Green, Oxbow) and a lake that received migrated tailings (Ibsen) suggest that sediments below ~20 cm represented the pre-mining time periods in some lakes in this region (Supplementary Material 2). The dating profiles for the three lakes are provided in Supplementary Material 2. However, as is common when using the top-bottom paleolimnological approach, due to differences in sedimentation rates among lakes, the time represented by the deeper samples may vary among lakes. Specifically, in lakes that received mine wastes, sediments representing pre-mining conditions may occur deeper in the sediment core. We used the deepest sediment samples available for the six impacted lakes and the bottom sediment sample depth ranged between 33 and 56 cm for these sites. Despite our best efforts to capture pre-mining assemblages, it may be possible that the bottom sediments from the impacted lakes may be from post-mining era. Hence, the results from these impacted sites should be interpreted with caution.

Cladoceran remains were identified from each sample at 200× – 400× magnification using a Leica DMR light microscope under bright-field optics and the counting effort exceeded the recommended minimum of 70 individuals (range: 95 to 206; mean: 126; median: 114; Kurek *et al.* 2010). The photomicrographs presented in Szeroczyńska and Sarmaja-Korjonen (2007) and Korosi and Smol (2012a, 2012b) were used to identify the sub-fossil cladoceran remains. Since Cladocera are disarticulated in the sediments, we tabulated the different body parts (*e.g.*, carapaces, headshields, post-abdominal claws) separately and the most common remains from each taxon were used to quantify the number of individuals for each species (Korhola and Rautio, 2001). Bosminid (*i.e.*, *Bosmina* species or *Eubosmina* species) headshields were not differentiated to the species level because there were challenges in locating the lateral head pores due to the high amounts of clastic materials from the contaminated lakes. All attempts were made to differentiate between the remains of *Alona circumfimbriata* and *Alona guttata* when enumerating the samples; however, due to challenges in differentiating the headshields of these taxa (the most common remain from these taxa), we grouped them as the *Alona circumfimbriata-guttata* complex (*sensu* Labaj *et al.,* 2018). Cladocera assemblage composition is presented as relative abundances (*i.e.*, percent abundance of each species relative to the total number of individuals from a sample) and all taxa were included in subsequent numerical analyses.

Numerical analyses

We used ordination approaches to determine what environmental variable(s) influenced the recent cladoceran assemblage composition in the lakes around Cobalt. Prior to conducting ordination analyses, we prescreened the data and removed chemical variables where measurements were below the detection limits in more than 10% of the lakes. During the pre-screening procedure, we noted that Ice Chisel Lake was an acidic lake as it had anomalously low pH (4.7), while all other study lakes were neutral to alkaline. Hence, Ice Chisel Lake was deemed an outlier in this dataset and removed from ordination analyses. A principal component analysis (PCA) with environmental variables from the 21 lakes was performed to visualize the distribution of our study lakes along measured environmental gradients in R statistical software. A Pearson correlation matrix was generated to assess the correlation among the environmental variables using the Hmisc package (Harrell, 2019) in R (Supplementary Material 3). A detrended correspondence analysis (DCA) of the square-root-transformed cladoceran data revealed a gradient length of 1.76, therefore a linear direct ordination method (redundancy analysis - RDA) was deemed appropriate to assess the relationships between the environmental variables and biological data (Birks, 2010). An RDA with forward selection of normalized explanatory variables (Blanchet *et al.,* 2008) was performed to determine which measured environmental variable(s) explained the most amount of variation in the cladoceran assemblage. The DCAs and RDA were performed in R using the vegan package (Oksanen *et al.,* 2019).

Analysis of similarities (ANOSIM; Clarke, 1993) was used to assess if the cladoceran assemblage composition were different among the three a priori defined groups based on contamination history [*i.e.*, direct tailing inputs (n=6), migrated tailings (n=4), uncontaminated (n=12)]. The cladoceran data were square-root transformed to equalize the variance and the ANOSIM based on Bray-Curtis similarity matrix was conducted in R (R Core Team, 2019) using the vegan statistical package (Oksanen *et al.,* 2019). Cladoceran species richness and Hill's N2 diversity were calculated in the R software environment using the vegan (Oksanen *et al.,* 2019) and rioja (Juggins, 2017) statistical packages, and the data were rarefied to 95 individuals per sample, as that was the lowest count. The non-parametric Kruskal-Wallis test was used to determine if Hill's N2 and species richness were varied significantly ($P<0.05$) among the three a priori defined groups. Since there were notable differences in the number of sites within the three a priori defined categories, we grouped the lakes that received direct (n=6) and migrated (n=4) tailings together and reran the ANOSIM and Kruskal-Wallis tests between two groups [*i.e.*, impacted (n=10) *vs* unimpacted (n=12)].

The change in the percent relative abundance of the most common cladoceran taxa between the top and bottom sediments were calculated (% top - % bottom) and plotted along with the changes in Hill's N2 diversity and species richness. The changes in cladoceran assemblage composition between the top and bottom samples from all 16 lakes were explored separately using Bray-Curtis (B-C) dissimilarity coefficients in R using the vegan statistical package (Oksanen *et al.,* 2019). Similar to the ANOSIM, the B-C dissimilarity coefficients were calculated on square-root transformed data. A B-C dissimilarity coefficient value of 0 indicates identical assemblage composition between two samples while a value of 100 indicates a complete compositional turnover. Hence, larger B-C dissimilarity coefficient values usually indicate a greater degree of difference between two samples. ANOSIM was used to assess if the cladoceran assemblages in the top and bottom samples were significantly ($P<0.05$) different across all 16 lakes. However, we have not explored if the changes in the assemblage composition were significantly different within each a priori defined group as the sample size of the lakes impacted by direct ($n=3$) and migrated ($n=3$) tailings were very small, which could potentially lead to spurious results. Similarly, the nonparametric Wilcoxon signed rank test were conducted on the full 16-lake dataset to assess if the changes in diversity indices were significantly different between the top and bottom samples.

RESULTS

Limnology of lakes around Cobalt, Ontario

The study lakes are generally small (range: 2.74-102.18 ha; median: 18.35 ha) and shallow (range: 1.2-19.2 m; median: 6.4 m; Fig. 2; Supplementary Material 1). The concentrations of As, nickel (Ni), and sulphate (SO_4^{2}) varied between 0.4-972 µg L^{-1}, 0.5-10.2 µg L^{-1}, and 1-10 mg L^{-1}, respectively (Fig. 2; Supplementary Material 1). Concentrations of As, Ni, potassium (K), and SO_4^{2} were significantly (Kruskal-Wallis test: $P<0.05$) different among the three a priori groups of lakes as they were generally higher in lakes that received direct and migrated mine tailings (Fig. 2). Most study lakes were circumneutral to alkaline (range: 6.9-9.2; median: 8.2) and calcium (Ca^{2+}) concentrations (range: 3.5- 49 mg L^{-1}; median: 20.7 mg L^{-1}) were generally high, with the exception of Ice Chisel Lake that had a pH of 4.7 and [Ca^{2+}] of 0.36 mg L^{-1} (Fig. 2; Supplementary Material 1). Interestingly, Ice Chisel Lake also had the lowest values for alkalinity, magnesium (Mg^{2+}), sodium (Na^{+}), TDS, and specific conductance in this data set (Supplementary Material 1). The concentrations for Na^{+} and chloride (Cl^{-1}) were higher at Green, Clear, Cobalt, and Maidens lakes relative to other study sites (Supplementary Material 1).

The PCA and Pearson correlation of the water chemistry variables highlighted the high correlation among the variables associated with the ionic strength of the water in our study lakes. Specifically, alkalinity, Ca^{2+}, Mg^{2+}, specific conductance, and TDS were highly correlated ($r > 0.8$) and plotted along axis 1 of the PCA biplot (Supplementary Material 3; Fig. 3). Lakes with varying physical characteristics (*i.e.,* depth and surface area) and metal concentrations (As and Al) spread along axis 2 of the PCA (Fig. 3). The axes 1 and 2 of the PCA explained 64% of the variation in the environmental variables and some separation was apparent among the contaminated and uncontaminated lakes (Fig. 3). Generally, deep lakes impacted by mining activities plotted in the bottom quadrant while unimpacted shallower lakes with lower As concentrations plotted in the upper quadrant (Fig. 3).

Cladocera assemblages from contaminated and uncontaminated lakes

The recent Cladocera assemblages from the lakes around Cobalt were dominated by bosminids (range: 7-94%; median: 58%) except for a severely contaminated lake (Cart) and an uncontaminated site (Nicol) where *Chydorus brevilabris* was dominant (Fig. 4). Pelagic *Daphnia pulex* complex was only observed in two impacted lakes (Cobalt and Maidens; Fig. 4). The *Daphnia longispina* complex was observed in both impacted and unimpacted lakes, however it was present in higher abundances (>15%) at two unimpacted lakes (Goodwin and Pine; Fig. 4). Several littoral taxa, such as *C. brevilabris*, *Alona circumfimbriata-guttata* complex, and *Alonella nana*, were present in most lakes and in notable abundances (Fig. 4).

The ANOSIM did not identify significant differences in the cladoceran assemblage composition among the three a priori defined groups. The median values for species richness and diversity were generally higher in uncontaminated lakes and sites that received migrated tailings (Tab. 1). However, there were substantial variation in the range (*i.e.*, minimum and maximum) within each group (Tab. 1) and Kruskal-Wallis test indicates that the differences in the diversity indices were not statistically significant ($P>0.05$) among the three a priori defined groups. Furthermore, the reruns of the ANOSIM and Kruskal-Wallis tests with two groups (impacted *vs* unimpacted) also yielded similar results. The RDA with forward selection identified lake depth as the only variable explaining a significant portion of the variation in the cladoceran assemblages from Cobalt. The total variation in the cladoceran dataset was 20.2 (inertia) and depth explained 14% of this variation (2.8).

Changes in Cladocera assemblages between and top and bottom sediments

Bosminids have increased in relative abundance between the two time periods in ten lakes (Fig. 5). At <1% abundance, *Alona intermedia* and *Leptodora kindtii* were only observed in the top sediments of five lakes. Generally, the relative abundances of the *A. circumfimbriata-guttata* complex, *A. nana*, *Alona quadrangularis*, and the *Daphnia longispina* complex have decreased

Tab. 1. Summary of the cladoceran species richness, and diversity (Hill's N2) for lakes that received direct and migrated tailings, and unimpacted sites.

	Species richness				Species diversity			
	Min	Max	Mean	Median	Min	Max	Mean	Median
Direct tailings (n=6)	6.25	15.40	10.26	10.44	1.22	3.85	2.12	2.03
Migrated tailings (n=4)	10.70	17.49	13.03	11.97	1.71	4.32	2.97	2.92
Unimpacted (n=12)	7.62	16.00	12.11	12.01	1.27	3.70	2.78	2.78

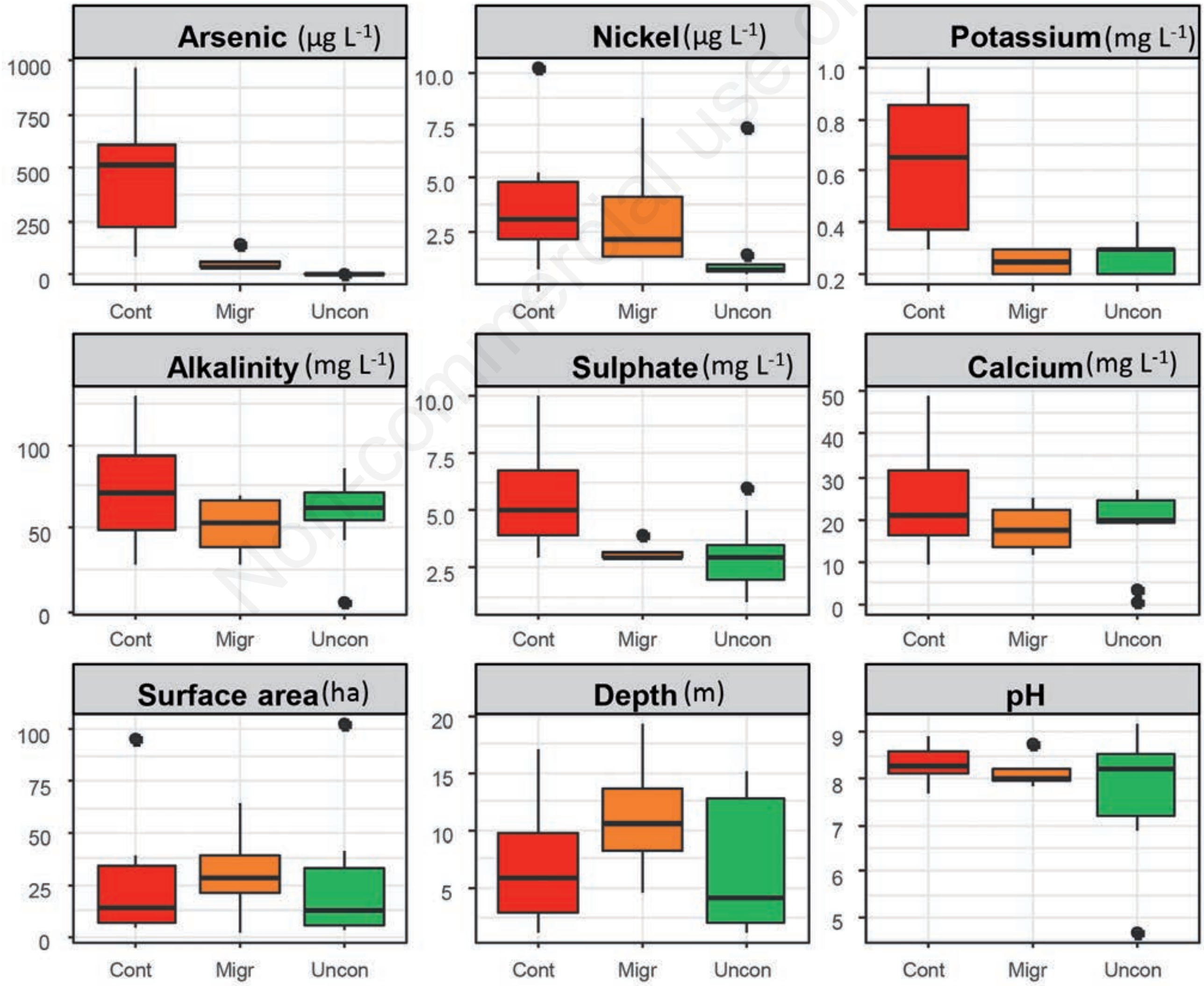

Fig. 2. Boxplots summarizing and comparing measured water chemistry and physical variables among lakes that received direct ("cont", n=4) and migrated ("migr", n=6) tailings, and unimpacted sites ("uncon", n=12). The median for each category is indicated with a thick black line. Concentrations of arsenic and nickel are reported in µg L^{-1}, and alkalinity, potassium, calcium and sulphate are reported in mg L^{-1}. Depth and surface area are reported in ha and m, respectively.

between the two periods (Fig. 5). The relative abundance of *C. brevilabris* remained stable across most lakes with the exception of Nicol Lake (unimpacted site) where it increased by 37% (Fig. 5). The B-C dissimilarity coefficient ranged between 19% (North Pickerel Lake) and 50% (Clear Lake), with a median of 30% across all 16 lakes (Fig. 5). The B-C dissimilarity values were classified into 4 categories and plotted on a regional map to visualize the differences in the magnitude of changes between the top and bottom samples (Fig. 6). Although notable changes in assemblages have occurred around Cobalt (based on the B-C dissimilarity coefficients), the ANOSIM of the full dataset did not detect significant differences between the top and bottom samples. Overall, cladoceran species diversity and richness have decreased between the two periods (n=16; median diversity: recent = 2.8, older = 3.4; median richness: recent = 11.6, older = 12.5) (Fig. 5). Specifically, species diversity and richness have decreased in 10 and 9 lakes, respectively; however, the magnitude of change between the top and bottom samples varied notably among lakes (Fig. 5; Tab. 2) and were not statistically significant (P>0.05) as indicated by the Wilcoxon signed rank test.

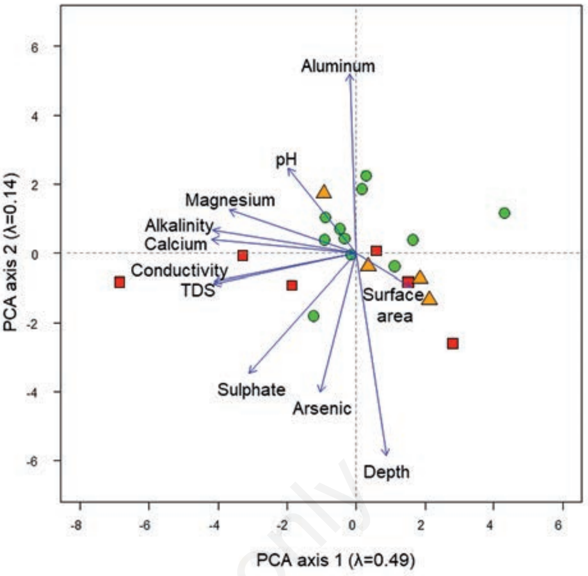

Fig. 3. The distribution of the 21 lakes along the gradients of the measured environmental variables using principal component analysis (PCA). Lakes that received direct tailings are represented by red squares while the lakes impacted by migrated tailings are indicated by orange triangles. The unimpacted sites are represented by green circles.

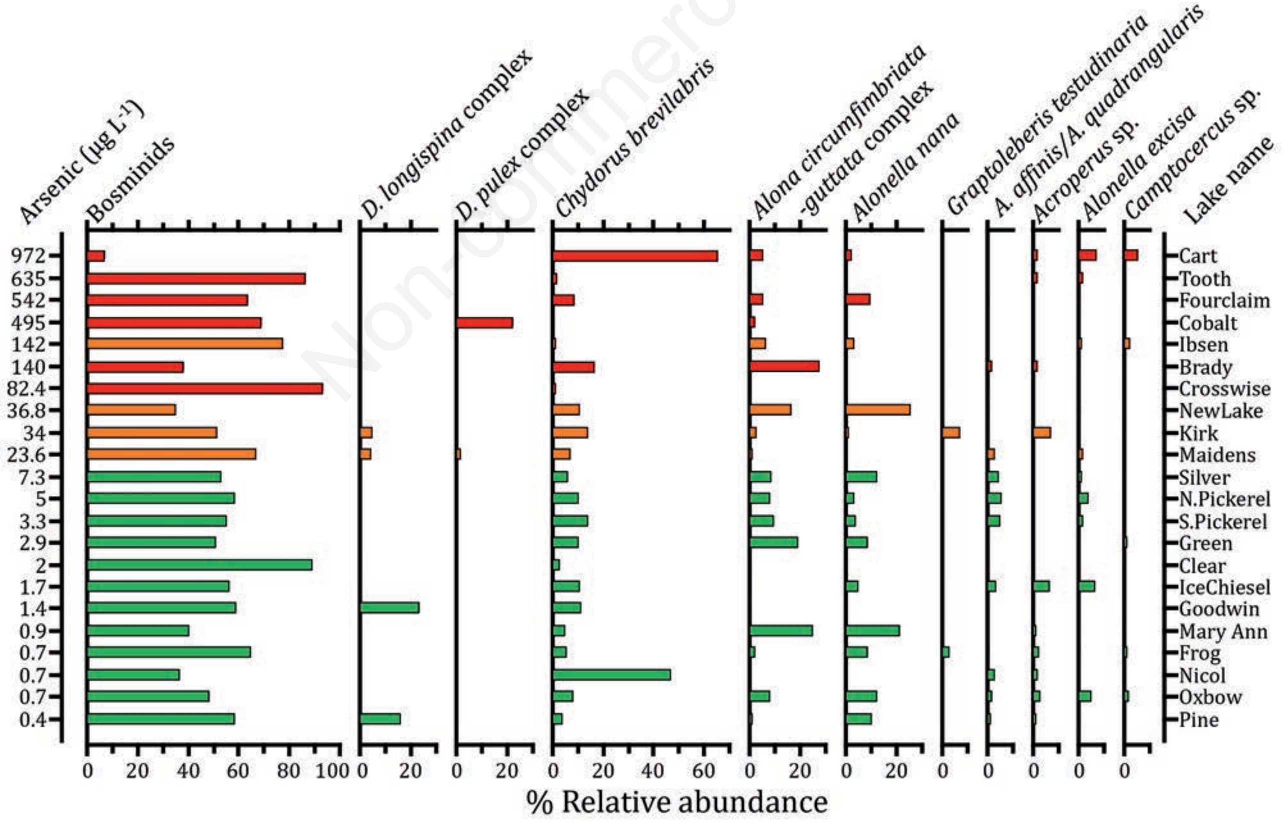

Fig. 4. Histograms of common Cladocera taxa (*i.e.*, taxa that occurred in >5% abundance in at least one lake) from the surface sediments of lakes in the Cobalt area. The sites are arranged according to lakewater As concentrations. Lakes that received direct tailings are represented by red bars while the lakes impacted by migrated tailings are indicated by orange bars. The unimpacted sites are represented by green bars.

DISCUSSION

Environmental determinants of recent limnological characteristics

The Cobalt silver rush took place during the first three decades of the 20th century, although some short-lived smaller operations persisted intermittently (Percival *et al.,* 2004). Nonetheless, more than a century has passed since the initial disposal of As-rich mine tailings began in the region. Yet, when the lakes in this region were sampled in 2015, the mine-impacted sites still had some of the highest [As] in Canada and exceeded the guidelines for the protection of aquatic life (5 µg L^{-1}; CCME 2001) and drinking water quality (10 µg L^{-1}; Health Canada, 2006). The high [As] reported in our study lakes that received tailings are comparable to those from the Yellowknife area where lakes were impacted by the atmospheric deposition of As from regional gold mining activities (Palmer *et al.,* 2015; Sivarajah *et al.,* 2019). The concentrations for Ni, K, and SO_4^2 were also high in the lakes that received direct mine waste and migrated tailings in Cobalt. Even though the majority of the mining activities ended more than eight decades ago, the persistent high concentrations of contaminants in the impacted lakes suggest that the mine tailings on the landscape are still important sources of contaminants (Percival *et al.,* 2004; Sprague and Vermaire, 2018a). Furthermore, the present limnology of

Tab. 2. Changes in the cladoceran species richness, and diversity (Hill's N2) for lakes that received direct and migrated tailings, and unimpacted sites.

	Species richness				Species diversity			
	Top		Bottom		Top		Bottom	
	Range	Median	Range	Median	Range	Median	Range	Median
Direct tailings (n=3)	6.2-15.4	11.7	11.0-13.1	12.6	1.2-3.8	2.3	2.6-5.1	2.7
Migrated tailings (n=3)	10.7-17.5	11.0	10.8-13.7	12.0	1.7-4.3	2.2	1.8-4.7	3.7
Unimpacted (n=10)	7.6-16.0	12.0	9.8-18.0	12.5	1.3-3.7	3.0	1.5-4.0	3.4

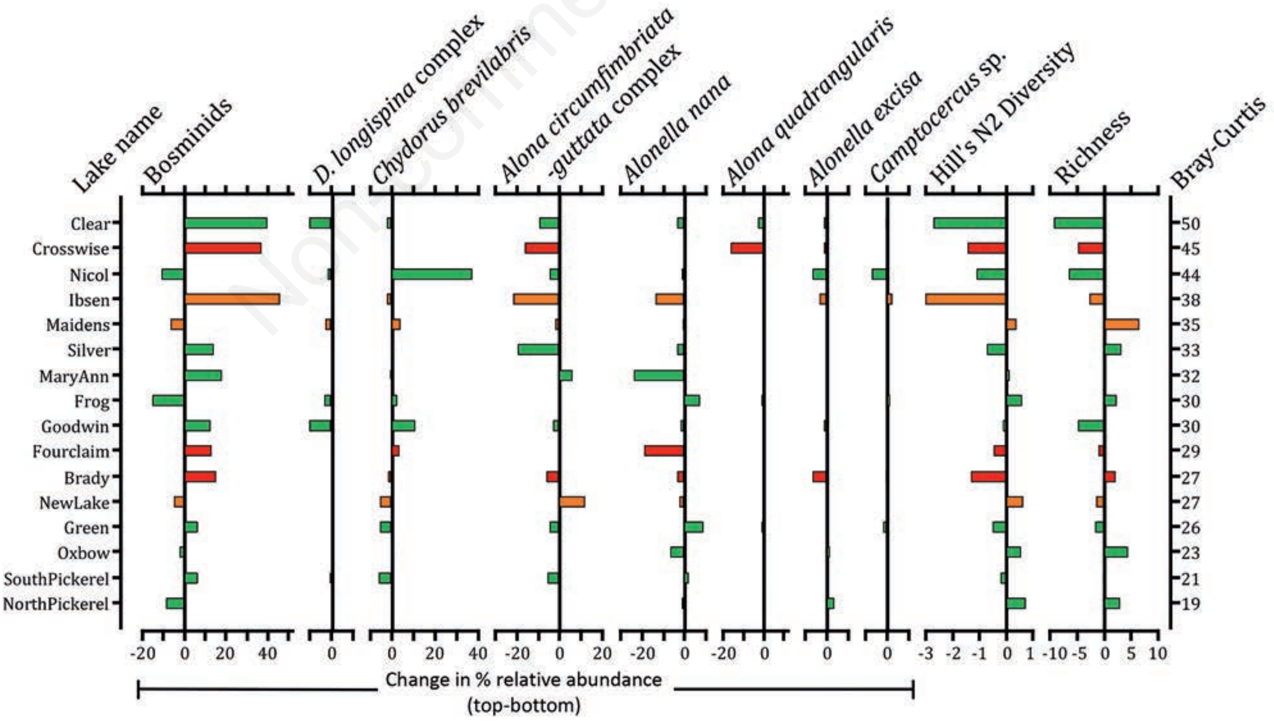

Fig. 5. Histograms of the differences in the relative abundances of the most common Cladocera taxa (*i.e.*, taxa that occurred in >5% abundance in at least one sample) between the recent (top) and older (bottom) sediments from 16 lakes around Cobalt, Ontario. The sites are arranged based on Bray-Curtis dissimilarity coefficients. Lakes that received direct tailings are represented by red bars while the lakes impacted by migrated tailings are indicated by orange bars. The unimpacted sites are represented by green bars.

lakes around Cobalt are also likely impacted by emerging limnological stressors such as run-off from road-salt applications (Valleau *et al.,* 2020). For example, the Na^+ and Cl^{-1} concentrations at 4 study lakes were relatively higher than most other lakes in the region (Supplementary Material 1), likely due to road-salt inputs from the Trans-Canada Highway 11b (Green, Clear, Cobalt) and Ontario Highway 567 (Maidens).

Spatial distribution of cladocerans around Cobalt

Bosminids, the most common pelagic zooplankton in lakes across Canada (Pinel-Alloul *et al.,* 2013), dominated the cladoceran assemblages in Cobalt. Similar to many previous regional surveys, water depth was identified as an important variable structuring the recent cladoceran assemblages (DeSellas *et al.,* 2008; Korosi and Smol, 2011; Griffiths *et al.,* 2019). The strong relationship between cladoceran assemblages and depth exists because lake depth facilitates the availability and complexity of various littoral and pelagic habitats, which have a direct impact on the cladoceran assemblage composition. Specifically, pelagic bosminids and daphniid taxa are often more common in larger and deeper lakes where there are considerable amounts of open-water habitat (Amsinck *et al.,* 2006; Griffiths *et al.,* 2019). Meanwhile Chydoridae taxa, such as *Alona* and *Chydorus* species, often dominate assemblages in small and shallow lakes with considerable amounts of macrophyte cover that provides complex littoral habitats (Korosi and Smol, 2011; Adamczuk, 2014).

Despite the presence of a large arsenic gradient, it was not identified as an important variable influencing the recent cladoceran assemblages around Cobalt and the assemblage composition and diversity indices did not differ significantly among the a priori defined groups. Although this observation was initially unexpected, it was consistent with spatial assessments of diatom-based investigations of As-contaminated lakes in Yellowknife, Northwest Territories (Sivarajah *et al.,* 2019) and Cobalt (Little *et al.,* 2020), where a combination of chemical and physical variables influenced the composition of recent assemblages in lakes around abandoned mines. Yet, downcore paleolimnological (*i.e.*, temporal changes) examinations of As-contaminated lakes have revealed substantial changes in cladoceran assemblage composition through time (Chen *et al.,* 2015; Thienpont *et al.,* 2016; Tenkouano *et al,.* 2019). For instance, the relative abundances of *C. brevilabris* and the *D. pulex* complex have increased

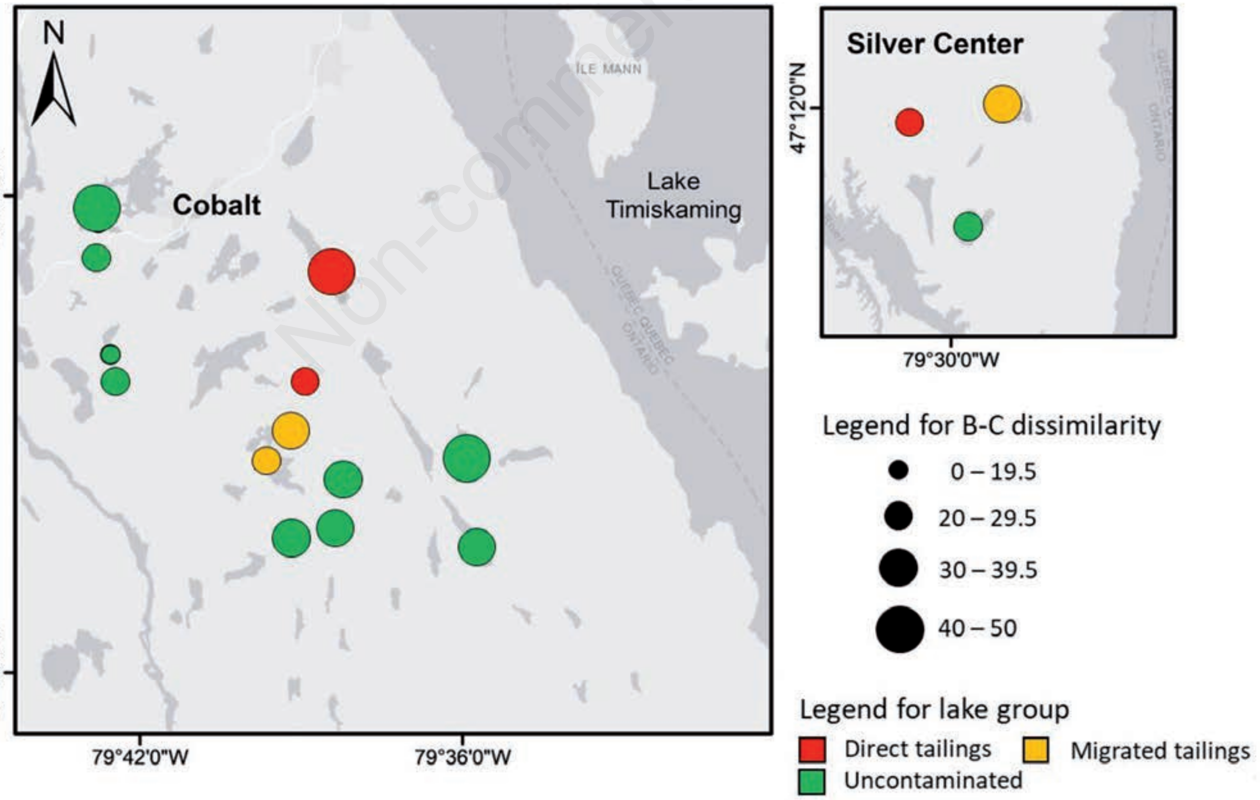

Fig. 6. A regional map visualizing the results of the Bray-Curtis dissimilarity coefficients between the recent (top) and older (bottom) sediments from 16 lakes around Cobalt, Ontario. The size of the circle represents the magnitude of changes and the color of the circle indicate the contamination history of the lakes.

in As-contaminated lakes, at least initially following metal contamination (Thienpont *et al.,* 2016; Tenkouano *et al.,* 2019). In our spatial survey, pelagic *D. pulex* complex was only observed at two deep contaminated sites (Cobalt – 10.4 m and Maidens – 9.4 m) where Na$^+$ and Cl^{-1} concentrations were also high. Meanwhile littoral *C. brevilabris* was observed in most of the study lakes but reached highest relative abundances in the most contaminated shallow site (Cart Lake – As = 972 µg L^{-1}). However, *C. brevilabris* also co-dominated the assemblages at shallow and uncontaminated Nicol Lake where arsenic concentrations were low (0.7 µg L^{-1}). Since these two taxa are known to tolerate a variety of environmental pollutants (*e.g*., metals and road salt; Manca and Comoli, 1995; Labaj *et al.,* 2015; Valleau *et al.* 2020) and have been observed in a broad spectrum of lakes (Griffiths *et al.,* 2019), it is likely that the presence of these taxa in some of the contaminated lakes around Cobalt are influenced by a combination of limnological variables (*e.g*., depth, concentrations of ions) and mining legacies.

Lake-water pH and Ca$^+$ concentrations have also been identified as important drivers of cladoceran assemblages in the soft-water lakes in Ontario where many lakes have acidified (Walseng *et al.,* 2003; DeSellas *et al.,* 2008; Jeziorski and Smol, 2016). In this study, however, most lakes (with the exception of Ice Chiesel Lake) were alkaline and had [Ca$^+$] (range: 3.5-49 mg L^{-1}; median: 20.7 mg L^{-1}) well above the critical thresholds that may induce changes in assemblage composition (Jeziorski and Smol, 2016) and reduce population growth rates (Arnott *et al.,* 2017). Furthermore, at the acidic Ice Chiesel Lake (pH = 4.7), where Ca^{2+}concentrations were relatively low (0.36 mg L^{-1}), assemblages were also dominated by bosminids and thus assemblage composition was not markedly different from the alkaline lakes in this dataset (Fig. 4). However, as expected, daphniid taxa with high Ca^{2+} requirements were not present at Ice Chiesel Lake. Previous investigations have also suggested that predation by fish and other invertebrates are important factors influencing cladoceran assemblages (Manca *et al.,* 2008; Davidson *et al.,* 2010); however, other studies have indicated that physico-chemical limnological variables play a more significant role in shaping assemblages (Amsinck *et al.,* 2006; DeSellas *et al.,* 2008; Kurek *et al.,* 2011). Since we do not have information on zooplanktivorus fish and macroinvertebrates for all the study lakes, we have only focused on the physico-chemical variables that shape the cladoceran assemblages in these lakes.

Differences in cladoceran assemblage between top and bottom sediments

Our exploratory top-bottom paleolimnological analysis suggests that the assemblage composition has changed between the top and bottom sediments in many lakes around Cobalt (Fig. 6). For example, the relative abundances of several littoral taxa (*Alona* species and *Alonella* species) decreased, while one pelagic group (bosminids) increased, which resulted in the decrease in Hill's N2 diversity at the three sites (Brady, Crosswise, Fourclaim) where mine wastes were disposed. Similar increases in the relative abundance of pelagic bosminids were observed at a few unimpacted sites (*e.g*., Clear, Silver) and a lake (Ibsen) impacted by migration of mine tailings from the local landscape. Interestingly, however, the relative abundances of bosminids declined at certain sites (*e.g*., Nicol, Frog) and no major changes were observed at other lakes (*e.g*., North Pickerel, Oxbow). Clearly, the nature and magnitude of cladoceran assemblage changes were not uniform across all lakes in the region.

Earlier paleolimnological assessments of mine-impacted lakes have suggested that metal(loid) contamination results in the loss of several pollution-sensitive species and increases in the relative abundances of a few generalist and/or pollution-tolerant taxa, thus leading to lower diversity (Manca and Comoli, 1995; Leppänen *et al.,* 2017a; Winegardner *et al.,* 2017). Toxicity of mining contaminants may explain the decrease in some of the littoral taxa, as the decreases in taxa such as *A. nana* and the *A. circumfimbriata-guttata* complex have been reported from other metal-contaminated lakes (Thienpont *et al.,* 2016; Pociecha *et al.,* 2020). However, the pelagic bosminids that replaced these littoral taxa in the contaminated lakes are known to be sensitive to As pollution (Passino and Novak, 1984; Chen *et al.,* 2015). This suggests that factors beyond metal(loid) toxicity may be influencing the cladoceran responses to past silver mining activities in Cobalt. For instance, the physical characteristics of some of the Cobalt lakes have been altered by the disposal of mine waste (Dumaresq, 1993; Patterson *et al.,* 1996). Specifically, the length of Crosswise Lake (an impacted lake) has been reduced by about 200 to 300 m as a result of tailings deposition into the lake (Dumaresq, 1993; Patterson *et al.,* 1996). Our top-bottom analysis of this lake revealed that the littoral cladoceran taxa (*A. circumfimbriata-guttata* complex and *A. quadranguilaris*) have decreased substantially while bosminid species have increased in abundance (+37%). Perhaps the shortening of Crosswise Lake has led to substantial reductions in the littoral habitat and contributed, at least in part, to the subsequent decline in the relative abundance of littoral taxa and indirectly favored pelagic taxa, such as bosminids. Similar increases in pelagic taxa were also observed at two other lakes where tailings were deposited directly (Brady and Fourclaim) and one lake where mine tailings migrated into the lake from the local landscape (Ibsen Pond).

The overall increase in pelagic bosminids at the landscape level may also be indicative of climate-mediated changes to lake thermal properties as has been reported in earlier paleolimnological investigations from Ontario (Jeziorski *et al.,* 2015; Hargan *et al.,* 2016) and elsewhere (Leppänen *et al.,* 2017b; Armstrong and Kurek, 2019). Similar to many other parts of Ontario, the annual air temperature around Cobalt has increased (Supplementary Material 4). For instance, warmer temperatures and associated lengthening of ice-free periods may alter the phenology and increase the production of pelagic cladoceran taxa through direct physiological mechanisms and/or indirect pathways such as higher food availability in the pelagic zone due to increases in planktonic production during longer open-water periods (Adrian *et al.,* 2006; Carter and Schindler, 2012; Nevalainen and Luoto, 2012; Nevalainen *et al.,* 2014; Hargan *et al.,* 2016). Furthermore, in two uncontaminated sites (Clear and Goodwin) we observed a switch among pelagic taxa where the relative abundances of the large-bodied *D. longispina* complex decreased while small-bodied bosminids increased. Similar shifts have been reported in temperate lakes from New Brunswick (Canada), where *Bosmina* species were more abundant in recent sediments (Armstrong and Kurek, 2019) because warming-mediated increases in metabolism often favors small-bodied taxa that have lower energy requirement (Moore and Folt, 1993).

Although climate-mediated limnological changes and mining-induced alterations in morphometry could potentially explain the increase in pelagic bosminids in most lakes around Cobalt, there were some exceptions (New Lake, Maidens, North Pickerel, Nicol, and Frog). As we discussed in the previous section, these trends may be due to limnological characteristics that were not measured in this study. For example, long-term changes in nutrient concentrations as well as predation by invertebrates and planktivorous fish could potentially explain some of the observed changes. Furthermore, some of the study sites are impacted by co-occurring stressors (*e.g.*, mining pollution, climatic changes, land-use changes) and so the cumulative biological responses to these stressors may be complex and diverse. Additionally, the relative importance of various environmental stressors usually changes through time, which could lead to novel cladoceran responses over time.

Future directions

The findings of this survey are consistent with a growing number of limnological assessments that suggest biological responses to mining contamination may be influenced by a variety of environmental factors (Little *et al.,* 2020; Persaud *et al.,* 2021). Furthermore, this exploratory study has identified key information gaps that exist in this region (*e.g.*, fish composition data for lakes), which poses challenges to a better understanding cladoceran ecology around Cobalt. The top-bottom paleolimnological analysis has identified notable changes in the cladoceran assemblage composition, and this information can be used to strategically select lakes for detailed paleolimnological analyses in the future. Specifically, the downcore paleolimnological analyses will be necessary to determine the timing of cladoceran assemblage shifts and disentangle the impacts of multiple co-occurring stressors in Cobalt. The downcore biological data can be combined with sedimentary geochemical analyses for metal(loid) contaminants (proxy for mining pollution) and long-term meteorological data (proxy for climate mediated changes) to determine if the relative importance of mining activities and climatic changes on cladoceran assemblage composition through time.

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