

IMPACTS OF LEGACY CONTAMINANTS FROM HISTORIC GOLD MINING
ON LAKES IN DARTMOUTH, NOVA SCOTIA

BY

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Abstract

Historic gold mining activities in Nova Scotia (Canada) occurred prior to modern waste management practices and contaminants were released into the environment. One notable example is the Montague Gold Mine which operated from 1863–1940. Although environmental monitoring data are sparse, nearby freshwaters likely received contaminants from past mining activities through inputs from surface waters and the atmosphere. To investigate the long-term effects of historic gold mining practices on lake ecosystems, I examined zooplankton remains and sediment geochemistry archived in dated sediment cores from impacted (Lake Charles) and reference (Loon Lake) lakes. Sedimentary concentrations of arsenic (As) were used to infer past mining activities. Sedimentary chlorophyll-*a* concentrations were used as a proxy of trends in lake primary production. Cladoceran assemblages of both lakes were significantly different ($p < 0.05$) during the time period of mining, compared to pre- and post-mining periods. Geochemical and zooplankton changes were most extreme in Lake Charles, which recorded levels of As contamination that exceeded sediment guidelines by, on average, 300 times since gold mining began. Despite the Montague Gold Mine closing 80 years ago, modern bioindicator and geochemical measures differ from the pre-mining periods, suggesting that past mining activities and likely also recent anthropogenic stressors, have impacted lake ecosystems.

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Introduction

Historic Gold Mining Activities and Legacy Contaminants

In the early 1800s it was discovered that some geological formations comprising Nova Scotian (NS) bedrock contained gold. In the mid-1800s, gold mines opened all over NS, which expanded the provincial economy. A notable example and the focus of this study is the historic Montague Gold Mine, which operated in Dartmouth, NS from 1863–1940 (Nova Scotia Archives, 2005). Through its 77 years of operation, the Montague Gold Mine expanded to become the 2nd largest gold mine to ever operate in NS (Art Gallery of Nova Scotia, 2013). Unfortunately, early mining operations occurred prior to proper waste management and environmental practices, and toxic mining by-products were released into nearby ecosystems (Brooks et al., 1982; Parsons et al., 2012; LeBlanc et al., 2019). Due to this, many historic NS gold mine sites, such as Montague, are likely polluted and could be negatively affecting nearby ecosystems.

At the Montague Gold Mine, gold was extracted from ore using a mercury (Hg) amalgamation technique (Blakeman, 1978; Dale & Freedman, 1982). This technique first included crushing ore into sand- and silt-sized material. Using water from nearby lakes and streams, the crushed ore was rinsed over Hg-coated copper plates, causing gold grains to amalgamate. It is estimated that for every ounce of gold extracted, one ounce of Hg was required for amalgamation, ultimately releasing massive quantities of Hg, among other contaminants, into the environment (Parsons & Little, 2015; LeBlanc et al., 2019).

Arsenic (As) is another common contaminant associated with historic gold mining activities (Branch, 2009; Plumlee & Morman, 2011). In addition to gold, NS bedrock is enriched with arsenopyrite (Dale & Freedman, 1982; Plumlee & Morman, 2011). At the Montague Gold Mine, when arsenopyrite ore was crushed to extract gold using Hg amalgamation, high concentrations of As were released into the environment (Dale & Freedman, 1982). Furthermore, in the late-1800s, gold extraction methods in NS shifted towards roasting, which includes applying heat to arsenopyrite ore (Bates, 1987; Parsons & Little, 2012). Unbeknownst to mine operators, roasting released hot As dioxide gas to the atmosphere (Branch, 2009; Plumlee & Morman, 2011). Once cooled, As dioxide changes to a solid state and becomes As trioxide, which is the most toxic, water soluble and bioavailable form of As (Plumlee & Morman, 2011). These historic mining techniques practiced across Canada released massive amounts of As.

When Canada's gold mining activities reached peak production in the early to mid-1900s, so did As contamination levels (Wang & Mulligan, 2006). Despite historic gold mines being closed for decades, the hazards of As contamination likely still linger in some terrestrial and aquatic ecosystems near former mines across Canada (Thienpont et al., 2016; Sprague & Vermaire, 2018; LeBlanc et al., 2019).

Throughout historic mining operations at Montague, finely ground mine wastes, or tailings, were deposited into nearby ecosystems (Mudroch & Clair, 1986; Branch, 2009; LeBlanc et al., 2019). Today, tailings at abandoned mines likely remain contaminated. Based on a study performed by DeSito et al. (2011), tailings at Montague contain high levels of As and Hg. At Montague, some tailings were deposited directly into Mitchell's Brook, which lies upstream from Barry's Run (Parsons et al., 2012). Tailings then moved downstream from Mitchells' Brook into Barry's Run and drained into nearby Lake Charles (Brooks et al., 1982; Parsons et al. 2012). Based on water quality monitoring data, As concentrations at Lake Charles are higher than ~50 other lakes in the region and exceed water quality guidelines (Clement et al., 2007).

Arsenic in Aquatic Ecosystems

Once As is removed from bedrock and released into the environment, it may travel large distances before it reaches a sink, such as soils or lake sediments (Dale & Freedman, 1982). Arsenic can also undergo chemical and biological transformations that move the element to and from the atmosphere, soils and water. In soils and sediments, microbial activities facilitate the biotransformation and methylation of As, which may change the element from a non-toxic state to a toxic and more mobile state (Challenger, 1945; Wang & Mulligan, 2006). Furthermore, As can change states through deposition, reduction, sorption, desorption, precipitation and dissolution (Wang & Mulligan, 2006). While As is transported and transformed through the environment, it can accumulate in biota and have toxicological effects (Chen & Folt, 2000). Generally, As accumulates in plants and small primary consumers. These organisms are in turn consumed by larger animals, which can have severe ecological consequences (Wang & Mulligan, 2006).

Arsenic is first taken up by aquatic primary producers, which are in turn consumed by organisms at higher trophic levels (Chen & Folt, 2000; Rahman et al., 2012). Phytoplankton

accumulate As through accidental uptake, whereby cells mistake arsenate for phosphate (Hellweger & Lall, 2004). This occurs due to chemical and structural similarities of phytoplankton arsenate and phosphate uptake mechanisms (Hellweger & Lall, 2004). Furthermore, aquatic primary producers assist in the biotransformation and methylation of As (Hellweger & Lall, 2004). It is debated whether phytoplankton transform As to more toxic forms or to less harmful forms (Petrick et al., 2000; Mass et al., 2001; Murray et al., 2003). Regardless, this has implications for aquatic food webs (Rahman et al., 2012). Contaminated phytoplankton are consumed by grazers such as zooplankton, which are consumed by smaller fish and macroinvertebrates, which are then consumed by predatory fish (Cott et al., 2016). At high concentrations, As negatively impacts fish growth, feeding behaviours, gonad development and fitness (Tierney et al., 2013). Not only is As contamination concerning for phytoplankton, invertebrates and fish but it is likewise a concern for humans (Branch, 2009; Cott et al., 2016).

Arsenic is a class 1 carcinogen that negatively affects the health of millions of people around the world (Ng et al., 2003). Not surprisingly, As-contaminated drinking water raises health concerns (Saint-Jacques et al., 2018). In NS, low concentrations of As are present in some drinking water. This is because NS bedrock contains As, which can be released into groundwater (LeBlanc et al., 2019). At typical levels, As is not of great concern, however, high levels of As and other contaminants in groundwater may pose a range of risks to human health (Saint-Jacques et al., 2018).

Environmental Monitoring and Paleolimnology

Long-term monitoring data are needed to understand how aquatic ecosystems have changed in response to legacy contaminants associated with historic gold mining activities. Unfortunately, water quality is often not directly monitored prior to environmental disturbances and because of this, it is difficult to estimate baseline conditions (Smol, 2008; Smol, 2010). Without baseline conditions, it is nearly impossible to determine the causal mechanisms driving changes in water quality and ecosystem health. Fortunately, lake sediments and paleolimnological techniques can be used to quantify historic changes in water quality (Smol, 2008). These techniques rely on abiotic and biotic measures preserved within sediments and

allow one to investigate how aquatic ecosystems have responded to disturbances or environmental changes in the absence of long-term monitoring data.

Paleolimnological studies use dated lake sediments, which accumulate over time and thus provide archives of past physical, chemical and biological conditions (Smol, 2008). The deposition of sediments in most lakes occurs in a predictable manner, whereby sediments accumulate in the lake's deepest basin. This means that deeper sediments correspond to older time periods, and upper sediments correspond to younger time periods. Using radiometric dating techniques, sediments can be dated and associated with specific time periods. Moreover, the biological and geochemical characteristics of sediments directly reflect allochthonous and autochthonous materials at the time of deposition (Smol, 2008). Allochthonous materials include inputs from sources outside the lake, while autochthonous materials originate inside the lake. Both types of materials preserve well in sediment records and create a continuous record of past environmental conditions. When long-term monitoring data are sparse, paleolimnology is a common approach to study long-term environmental changes.

Many well-preserved indicators are used to understand past lake conditions. Physical indicators are often related to sediment structure and may include grain size and/or colour (Stea & Mott, 1998). Geochemical indicators are crucial in paleolimnology because radiometric dating is used to estimate sedimentary chronologies (Smol, 2008). Other geochemical indicators reflect anthropogenic activities such as heavy metal contamination (Krause-Dellin & Steinberg, 1986). While these physical and geochemical indicators are vital to paleolimnology, they cannot be used to quantify all environmental changes. For example, pH cannot be directly measured in lake sediments and must instead be indirectly inferred through bioindicators (Krause-Dellin & Steinberg, 1986). Due to this, most paleolimnological studies rely on bioindicators to reconstruct environmental changes, such as shifts in food web structure and lake trophic status. Bioindicators are generally subfossil remains made of siliceous or chitinous materials, which preserve well in sediments and can be identified to a reasonable taxonomic level. In paleolimnology, bioindicators are most useful when the organism's tolerance to key environmental conditions, as well as their ecological roles, are known (Smol, 2008). Examples of subfossil remains used to make ecological inferences are diatoms, zooplankton, macroinvertebrates, and pollen grains (Smol, 2008; Lotter et al., 1997; Stoermer, 1998). To reconstruct past ecological conditions,

assemblage compositions and absolute abundances of bioindicators can be measured in conjunction with physical and geochemical indicators preserved in dated sediments.

Paleolimnological approaches are frequently used to determine lake responses to anthropogenic stressors (Smol, 2010). Paleolimnology allows for inferences to be made about baseline or “pre-impact” conditions (i.e. lake conditions prior to disturbance), without having direct monitoring data (Smol, 1992). When establishing baseline conditions, it is important to differentiate ‘noise’ within the physical, chemical, and biological data, from the ecological ‘signal’ of interest (Smol, 2008). Through knowledge of baseline conditions, natural ecosystem variability and environmental changes can be tracked using lake sediments (Smol, 1992; Smol, 2008).

Cladocera as Bioindicators

Cladocera are an order of freshwater crustaceans common to the zooplankton community within lakes. Upon death, cladoceran remains disarticulate and accumulate in sediments. The body parts of Cladocera are composed of chitin, which preserves well in the sediment record, allowing for taxonomic identification of remains to the genus- or species-level (Korhola & Rautio, 2001). Furthermore, Cladocera are widespread and ubiquitous within lakes and their varied habitats. Additionally, Cladocera are abundant in lake sediments, allowing for statistical inferences to be made. Moreover, Cladocera have rapid life cycles and generally reproduce asexually through parthenogenesis. These bioindicators are sensitive to anthropogenic stressors and changes in trophic status or water quality (Korhola & Rautio, 2001). Cladocera are mostly primary consumers that are important to food webs. As primary consumers, Cladocera represent an intermediate trophic level, whereby they are the energetic link between primary producers and secondary consumers. Due to this, Cladocera respond to both bottom-up (e.g. changes in water quality) and top-down (e.g. predation) effects (Korhola & Rautio, 2001; Kurek et al., 2011). These characteristics make cladocerans useful bioindicators in paleolimnological studies.

Cladocerans occupy different habitats within lake ecosystems. Lake habitats are often characterized by the distance from the shore, water depth, light availability, and the presence of macrophytes. Habitat zones include the shallow littoral zone, the open-water pelagic zone, and the deep profundal zone. Cladoceran richness is generally highest in littoral zones (Korhola &

Rautio, 2001; Smol, 2008). A family of Cladocera that is abundant in the littoral zone is Chydoridae (Hofmann, 1987). Families that are more commonly found in the pelagic zone are Bosminidae and Daphniidae (Hofmann, 1987). The tendency of these families to inhabit separate zones makes them useful bioindicators of whole-lake ecosystem changes (Thienpont et al., 2016; Daly et al., 2019).

Cladocerans are increasingly used in paleolimnological studies as bioindicators of anthropogenic stressors. Relative abundances and assemblage compositions can be quantified to understand zooplankton responses associated with stressors. More specifically, cladoceran assemblages have been key in tracking aquatic contaminants, such as pollution associated with historic mining activities (Thienpont et al., 2016; Leppänen et al., 2018; Pocięcha et al., 2019; Little et al., 2020). For example, Thienpont et al. (2016) observed massive shifts in cladoceran assemblages in response to As contamination at the historic Giant Mine, Northwest Territories, Canada. This study found that, following contamination, the cladoceran community shifted away from sensitive littoral taxa, towards As-tolerant pelagic taxa. This showed that pelagic taxa had the competitive advantage in As-contaminated waters, allowing them to exploit a new niche as resident taxa were out-competed (Thienpont et al., 2016). Similar results were observed in Cobalt, Ontario (Little et al., 2020). These studies demonstrate that Cladocera can reliably track environmental changes associated with past gold mining activities.

Thesis Objectives

I used paleolimnological techniques to investigate the long-term impacts of mining at the historic Montague Gold Mine. A timeline of the cumulative environmental effects of legacy contaminants as inferred by paleolimnological measures can establish baseline lake conditions and determine possible water quality declines associated with historic mining activities. Using geochemical measures (i.e. elemental concentrations of As) as proxies of past mining activities, and Cladocera as bioindicators, I answered the following research questions:

1. Do cladoceran assemblages and related paleolimnological measures indicate impacts associated with historic gold mining activities?
2. If so, has recovery occurred, and what are the key drivers of cladoceran assemblages at impact and reference lakes?

This study provides knowledge concerning the ecological effects associated with historic gold mining activities in NS. Findings will contribute to research on abandoned gold mines and remediation plans to reduce contamination into nearby ecosystems.

Methods

Site Description and Core Collection

To understand the long-term ecological consequences associated with legacy contaminants from the historic Montague Gold Mine, two lakes were studied: Loon Lake and Lake Charles. Loon Lake (44.7048; 63.5092) is located upstream of the mining site, while Lake Charles (44.7201; 63.5498) is located downstream of the mining site (Figure 1). A small stream known as Barry's Run drains directly from the mining site into Lake Charles. When legacy contaminants are in a water-soluble state, they may move with surface water from Barry's Run into Lake Charles (Brooks et al., 1982; Wang & Mulligan, 2006). One line of evidence that past mining activities have transported contaminants into Lake Charles is supported by water chemistry data, showing that Lake Charles has the highest As levels of all lakes in the region (Clement et al., 2007). Lake Charles was therefore selected as an impact site, while Loon Lake was selected as a reference site. Loon Lake receives no inputs of surface water from the mining site.

Lake Charles and Loon Lake are located in an urbanized region. Since the opening of the Montague Gold Mine, many land-use changes close to the lakes have occurred. Major changes include the expansion of the Trans-Canada Highway, the development of large businesses, and the construction of waterfront properties. Based on water quality monitoring data, both Lake Charles and Loon Lake are mesotrophic (Clement et al., 2007). Both lakes have relatively neutral pH values, similar conductivity levels, and similar dissolved organic carbon levels (Table 1). Lake Charles is larger, with an average depth of 15m, and Loon Lake is smaller, with an average depth of 5m (Clement et al., 2007). Lake Charles water has As concentrations of 6.4 ppb, whereas Loon Lake water has As concentrations of 0.4 ppb (Clement et al., 2007).

In mid-May 2019, sediment cores were retrieved from the deepest basins of Loon Lake and Lake Charles. Cores were obtained via a Glew gravity corer with a 7.6-cm diameter tube (Glew & Smol, 2001). A 34-cm core was obtained from Lake Charles at a depth of 28.1 m. A 40.5-cm core was retrieved from Loon Lake at a depth of 6.6 m. Following retrieval, sediment cores were photographed, extruded, and sectioned into 0.5-cm intervals using a vertical extruder (Glew & Smol, 2001). Each interval was placed in a labelled Whirl-Pak® bag. During fieldwork, samples were stored in small coolers on ice. Following fieldwork, all samples were brought to

the Environmental Change and Aquatic Biomonitoring (ECAB) Laboratory at Mount Allison University and stored in a freezer.

Processing Samples for Geochemical Measures

To estimate sediment ages, standard radiometric lead 210 (^{210}Pb) dating was performed. Radiometric ^{210}Pb dating is frequently used in paleolimnological studies because it provides reliable and precise depth-time estimates (Appleby et al., 1986). Freeze-dried sediments from 16 intervals at each lake were weighed and placed in labelled vials. Vials were then sealed with epoxy and sent to partners at Queen's University where dating analyses were performed in a gamma counter with a germanium crystal detector (Table 2; Table 3).

Elemental concentrations of total As in lake sediments were obtained through portable x-ray fluorescence (pXRF). All pXRF analyses were performed at Acadia University following methods outlined by Dunnington et al. (2019). Sample preparation included sieving freeze-dried sediments through a 120- μm mesh sieve to ensure homogeneity of sediments, which is essential in pXRF analyses (Boyle, 2000). Approximately 0.5g of freeze-dried sediments were placed in labelled cuvettes. Using Acadia University's pXRF machine, which is calibrated for lake sediments with certified reference materials from the Geological Survey of Canada, 36 sediment intervals were analysed from Lake Charles and 38 sediment intervals were analysed from Loon Lake. Deconvolution and mass attenuation were performed using the BORLAND PASCAL software, which combines the pXRF spectra and generates sedimentary concentrations of total As (Dunnington et al. 2019).

Sedimentary chlorophyll-*a* (chl-*a*) was estimated using visible reflectance spectroscopy (VRS). VRS chl-*a* is used in paleolimnological studies as a proxy of lake primary production (Wolfe et al., 2006). As in pXRF analyses, sediment homogeneity is important for proper VRS analyses and because of this, similar procedures were used. Freeze-dried sediments were sieved through a 120- μm mesh sieve and transferred into labelled cuvettes. Sediments were then sent to partners at Queen's University, where reflectance spectra of the sediments between 650 and 700-nm were obtained using a FOSS NIRSystems model 6500 rapid content analyzer. Through this, the concentration of chl-*a*, including breakdown products, in 36 sediment intervals from Lake

Charles and 38 sediment intervals from Loon Lake, were calculated with a linear regression equation (Michelutti et al., 2005; Wolfe et al., 2006).

Cladoceran Processing, Identification, and Enumeration

Twenty-six samples from Loon Lake were processed for cladocerans, while 28 samples from Lake Charles were processed for cladocerans. Protocols followed modified methods of Korhola & Rautio (2001). To begin, sediments were freeze dried. About 0.2 to 0.3 g of freeze-dried sediments were deflocculated in 80 mL of 10% potassium hydroxide solution and placed on a hotplate set at 90°C for 30 minutes. Next, the solution was poured through a 38- μ m sieve and rinsed with deionized water. The solution was then transferred to a three dram vial and 2 mL of 95% ethanol solution was added to preserve the solution. Furthermore, a drop of 1% Safranin stain was added. Once complete, 100- μ L aliquots of the solution were pipetted onto glass coverslips. Coverslips were placed on a slide warmer at approximately 30°C to facilitate evaporation of the solution. Once dry, coverslips were observed under a dissecting microscope. If coverslips appeared to have low cladoceran abundances, another 100- μ L aliquot was added to the coverslip. Coverslips were then permanently mounted onto glass slides using Entellan® mounting medium.

Cladoceran remains were identified using bright-field microscopy at 200X or 400X magnification. Coverslips were scanned in totality for all identifiable remains, thus reducing biases from the non-random distribution of remains on each coverslip. Following count guidelines of Kurek et al. (2010), a minimum of 70 individuals were counted per sediment interval. Subfossil identifications were made using taxonomic criteria based on lake sediments from northeastern North America (Korosi & Smol, 2012a; Korosi & Smol, 2012b). Identifications were based on post-abdominal claws, carapaces, headshields, exopodite segments, and caudal furca. Each type of remain was tallied separately. The total number of individuals of a taxon was quantified using the most abundant remain in each sediment interval (Frey, 1960; Kurek et al. 2010).

Cladoceran remains were identified to the genus- or species-level. In eastern North America, daphniids of the *Daphnia pulex* complex may include *D. catawba*, *D. pulex*, *D. pulicaria*, and *D. minnehaha*, which are not morphologically distinct and are thus recognized as a complex (Korosi & Smol, 2012a). Similarly, daphniids of the *Daphnia longispina* complex

may include *D. ambigua*, *D. dentifera*, *D. dubia*, *D. longiremus*, and *D. mendotae* (Korosi & Smol, 2012b). Thus, daphniid post-abdominal claws were identified as belonging to either the *D. pulex* or *D. longispina* complex through the presence or absence of four-to-five teeth-like spines on the middle pecten of claws (Korosi & Smol, 2012a). Bosminidae were identified as *Eubosmina* sp. or *Bosmina* sp. based on differing locations of lateral headpores, while carapace remains were undifferentiable (Korosi & Smol, 2012a). Additionally, remains of *Eurycercus* sp., *Camptocercus* sp., *Kurzia* sp., and *Pleuroxus* sp. were identified to the genus-level (Korosi & Smol, 2012b). Occasionally, cladoceran remains were classified as unidentifiable. This usually occurred when debris covered an important identifying feature, or when the remain was fragmented. All other cladoceran remains were identified to the species-level.

Data Analyses

Relative abundances of each taxon were calculated from cladoceran counts using the total number of individuals per taxon present within the sediment interval. Rare taxa were removed prior to further analyses. Taxa were considered rare if they did not occur in at least two intervals at > 2% abundance. Cladocerans were grouped into three time periods based on their ²¹⁰Pb dates: pre-mining (>1863), mining (1863-1940), and post-mining (>1940). These time periods were chosen based on the known mining activities at the historic Montague Gold Mine (Nova Scotia Archives, 2005). All statistical analyses were performed using R (version 1.2.5), with an R Studio interface (RStudio Team, 2019).

Multivariate homogeneity of variances was tested on cladoceran datasets from each lake using the *Betadisper* function in the *vegan* package (Borcard et al., 2011; Oksanen et al., 2019). As neither dataset passed tests for homogeneity of variances, non-parametric analyses were performed. To determine whether cladoceran assemblages differ between time periods, a permutational multivariate analysis of variances (PERMANOVA) was performed in the *vegan* package (Anderson, 2017; Oksanen et al., 2019). PERMANOVA allows for classical partitioning similar to an analysis of variances (ANOVA), thus allowing for estimates of the sizes of main effects, interaction terms, hierarchical structures, and mixed models (Anderson, 2017). This was done through a partitioning of variation across a multivariate cloud, defined in the space of a selected dissimilarity measure, in response to factors in the ANOVA design (Anderson, 2017). PERMANOVA does not have assumptions for the distributions of dependent variables or the

distributions of dissimilarities. The only explicit assumption of PERMANOVA is exchangeability of permutable units established under a null hypothesis (Kempthorne, 1966). To determine where assemblages differ the most, separate pairwise comparisons were done by running PERMANOVAs comparing the pre-mining and mining periods, and the post-mining and mining periods.

To further characterize differences between assemblages, the Indicator Value (IndVal) analysis was performed using the *labdsv* package (Dufrêne & Legendre, 1997; De Cáceres et al., 2010; Legendre, 2013). IndVal combines a taxon's relative abundance with its relative frequency of occurrence in a specific, pre-defined group (Dufrêne & Legendre, 1997). This function outputs indicator values and p-values associated with taxa that are significant indicators of specific groups within the independent variable. To compare overall changes in indicator taxa over time to trends in lake primary production (VRS chl-*a*) and mining activities (total As measured with pXRF), stratigraphic datasets were plotted using C2 graphing software.

Taxon richness in pre-mining, mining, and post-mining periods was calculated for both lakes. To control for different sampling efforts, cladoceran counts of all taxa were first rarefied using the *rrarefy* function in the *vegan* package (Hurlbert, 1971; Heck et al., 1975). This function standardizes the number of taxa in each sample based on the total number of taxa found in the sample with the lowest sampling effort (Hurlbert, 1971). Rarefaction should only be undertaken on assemblages that have been sufficiently and randomly sampled, have consistent sampling methods, and show taxonomic similarities (Gotelli & Chao, 2013). Using rarefied data, taxon richness was then calculated at each sediment interval. Average richness per time period was compared by ANOVA (Scheffé, 1999). To determine where significant differences lie, post-hoc analyses were performed through a Tukey multiple comparison test (Keselman & Rogan, 1977).

Results

Sediment Chronologies

Radiometric ^{210}Pb analyses performed by partners at Queen's University indicated that sedimentation rates were lower in Lake Charles than Loon Lake. Intervals analysed from the Lake Charles core were dateable to ~ 16.5 cm (1852 ± 72 years) (Table 2). Despite the greater uncertainty of dates with increased core depth, all Lake Charles intervals at a depth greater than 16.5 cm were associated with the pre-mining period. Intervals analysed from the Loon Lake core were dateable to ~ 23.0 cm (1873 ± 30 years) (Table 3). Dates below ~ 23.0 cm were associated with the pre-mining period.

Lake Primary Production Trends

Lake Charles VRS chl-*a* trends differed from those of Loon Lake (Figures 2; Figure 3). During the pre-mining period, Lake Charles VRS chl-*a* remained low and stable. VRS chl-*a* declined during the mining period, reaching its lowest concentrations of ~ 0.01 mg/g. During the post-mining period, VRS chl-*a* at Lake Charles increased to ~ 0.03 mg/g. VRS chl-*a* were more stable at Loon Lake than Lake Charles. Although changes in VRS chl-*a* were minimal, Loon Lake VRS chl-*a* were highest (~ 0.02 mg/g) in sediments associated with the post-mining period.

Trends in Arsenic as a Proxy of Past Mining Activities

Lake Charles As concentrations increased over time and were exceptionally high compared to Loon Lake (Figures 2; Figure 3). In pre-mining times, Lake Charles As concentrations ranged between 64 and 172 ppm, while during mining, As concentrations ranged between 266 and 478 ppm. Lake Charles As concentrations peaked at 2702 ppm in the uppermost depth intervals associated with post-mining times. Loon Lake As concentrations also increased over time, however increases were minimal and absolute values were much lower compared to those at Lake Charles. During pre-mining and mining periods, Loon Lake As concentrations averaged 31 ppm. Concentrations then increased to an average of 39 ppm in post-mining periods, with a peak of 45 ppm in the uppermost depth interval.

Cladoceran Assemblages at Lake Charles

A total of 31 taxa were identified throughout the Lake Charles core. After excluding rare taxa, 14 taxa remained and were considered ‘common’ throughout the core (Appendix 1). Based on PERMANOVA results, cladoceran assemblages differed significantly between time periods ($p = 0.001$). The largest differences occurred between the pre-mining and the mining periods ($p = 0.006$) (Table 4; Table 5). The IndVal function identified at least one indicator taxon associated with each time period (Table 6). *Alona costata*, *Chydorus brevilabris*, and *Alona intermedia* were identified as taxa associated with the pre-mining period. *Alona quadrangularis* and *D. pulex* complex were identified as indicators of the mining period. *Bosmina longirostris* was identified as the only taxon associated with the post-mining period. Relative abundances of indicator taxa were, on average, highest in the group they were associated with (Table 6; Figure 2).

Average taxon richness at Lake Charles decreased over time (Figure 4). In the pre-mining period, taxon richness was ~14 taxa, in the mining period it was ~12 taxa, and in the post-mining period it was also ~10 taxa. Based on ANOVA results, these values differed significantly between time periods ($p = 0.004$) (Table 7). The Tukey Test identified that richness in pre- and post-mining periods were significantly different ($p = 0.002$), however richness did not differ significantly between pre-mining and mining ($p = 0.29$) or between mining and post-mining ($p = 0.33$) periods.

Cladoceran Assemblages at Loon Lake

A total of 33 taxa were identified throughout the Loon Lake sediment core (Appendix 2). Among these, 20 taxa were considered ‘common’. Based on PERMANOVA results, cladoceran assemblages differed significantly between pre-mining, mining, and post-mining time periods ($p = 0.001$). The largest differences occurred between the mining and post-mining periods ($p = 0.001$) (Table 4; Table 5). Based on IndVal, each time period was found to have two indicator taxa (Table 6). *Eurycerus* sp. and *Campecerus* sp. were identified as significant taxa associated with the pre-mining period. *Bosmina* sp. and *Eubosmina* sp. were identified as significant taxa associated with the mining period. *D. pulex* complex and *Chydorus brevilabris* were identified as

significant taxa associated with the post-mining period. Relative abundances of indicator taxa were, on average, highest in the group they were associated with (Table 6; Figure 3).

Similar to Lake Charles, average taxon richness at Loon Lake also decreased over time (Figure 5). These changes were smaller than observed at Lake Charles. In the pre-mining period, taxon richness was ~17 taxa, in the mining period it was ~14 taxa, and in the post-mining period it was also ~ 14 taxa. Based on ANOVA results, these values differed significantly between the three time periods ($p = 0.04$) (Table 7). Despite this, the Tukey Test did not identify significant differences between the pre- and post-mining periods ($p = 0.05$), the pre-mining and mining period ($p = 0.10$) or the mining and post-mining periods ($p = 0.94$). Based on Tukey Test results, it is evident that average taxon richness at Loon Lake did not change as much between time periods as it did at Lake Charles.

Discussion

Environmental Changes Related to Historic Mining Activities at Lake Charles

Paleolimnological measures suggest that Lake Charles was impacted by historic gold mining activities at the Montague Gold Mine (Figure 2). Greater As concentrations following the opening of the gold mine in 1863 suggest that sedimentary As contaminants can be linked to past mining activities (Brooks et al., 1982). Furthermore, VRS chl-*a* levels declined during the mining period, which may indicate additional lake responses to historic mining activities. Both bioindicator and geochemical trends demonstrate that Lake Charles has not recovered to a pre-mining state, emphasizing the legacy effects that past mining activities combined with recent stressors (Ginn et al., 2015) can have on nearby aquatic ecosystems.

Prior to historic mining activities, As was observed in Lake Charles sediments at concentrations of ~ 120 ppm, which is elevated compared to sediment guidelines (Canadian Council of Ministers of the Environment, 1999). In NS, some bedrock may have naturally high levels of As, which once weathered, may become incorporated into soils and lake sediments. This explains why Lake Charles pre-mining sediments exceed the Canadian average of 2.5 ppm (Canadian Council of Ministers of the Environment, 1999). Following the opening of the Montague Gold Mine in 1863, As concentrations increased 5 fold, averaging at ~520 ppm. Increases in As concentrations during past mining activities are presumably because mine tailings were transported by surface waters and as wind-blown dust into Lake Charles (Brooks et al., 1982). Since the mine closed in 1940, As concentrations have remained alarmingly high in the lake sediments. In the most-recent sediment intervals, As concentrations are recorded at levels as high as 2702 ppm, which is more than 1000 times higher than sediment guidelines (Canadian Council of Ministers of the Environment, 1999). High As concentrations suggest that legacy contaminants associated with historic mining activities are still an issue of concern at Lake Charles today.

After the Montague Gold Mine closed, it is probable that contaminants were still transported from the mine to Lake Charles, however sedimentary geochemical processes could also explain high As concentrations in modern sediments. These findings require further study. Arsenic exists in several forms that may behave differently under specific environmental conditions. For example, As(V) is a thermodynamically stable state of As that occurs in

oxygenated waters, whereas As(III) is less stable and occurs in reduced redox environments (Azizur et al., 2012). Furthermore, inorganic iAs occurs in anoxic environments and is more toxic than organoarsenic species (Azizur et al., 2012). Studies demonstrate that thermal stratification and resulting oxygen depletion in lakes can cause iAs in lake sediments to mobilize and accumulate near the sediment-water interface (Smedley & Kinniburgh, 2002; Hasegawa et al., 2010). High levels of As in surface sediments at Lake Charles may therefore be the result of redox conditions favouring As mobility, whereby As that accumulated in sediments during the mining period moved up the sediment profile to become incorporated in the modern lake sediments. When dissolved oxygen is reduced in the hypolimnion, lake sediments may even release iAs back into the water column (Smedley & Kinniburgh, 2002; Hasegawa et al., 2010). This is concerning because As that was deposited in sediments decades to centuries ago, may have the ability to be remobilized into lake water and aquatic food webs today. Since pXRF measures do not distinguish specific As forms, it is difficult to identify the potential toxicity associated with As in surface sediments at Lake Charles.

VRS chl-*a* levels from Lake Charles sediments suggest minor changes in lake primary production over time, which is also supported by diatom inferences that quantify total phosphorus (Ginn et al., 2015). Prior to historic mining activities, VRS chl-*a* levels were low and stable, but during mining activities, VRS chl-*a* levels decreased. Various forms of As have been linked to reductions in primary production (Knauer et al., 1999). It is therefore possible that decreases in primary production during the mining period are due to contaminant inputs via mine tailings. This is especially true if tailings contributed toxic forms of As to Lake Charles (Knauer et al., 1999). Another possibility is that tailings and other inorganic materials that entered the lake during mining activities diluted VRS chl-*a* levels. Moreover, VRS chl-*a* levels have increased since mining ended and are highest in modern sediments. Many lakes in the Halifax Region have experienced water quality changes often attributed to climate warming and longer growing seasons, acidification, nutrient inputs, and winter salt inputs (Ginn et al., 2015). Therefore, elevated VRS chl-*a* in modern times could be attributed to climate change, as well as land-use changes in the catchment. Likely, multiple stressors explain current water quality conditions at Lake Charles.

Environmental Changes at Loon Lake

The Loon Lake sediment record showed environmental changes over time that were not related to historic mining activities. Ginn et al. (2015) suggest that Loon Lake diatoms indicate a response to recent climate change and longer growing seasons, similar to many other lakes in the region. Based on VRS measures, Loon Lake chl-*a* trends remained relatively stable in the sediment profile, with a slight increase in recent decades (Figure 3). Sedimentary As concentrations peaked at 58 ppm in modern sediments, which is double its concentration in the pre-mining period, and is also high compared to the Canadian average of 2.5 ppm (Canadian Council of Ministers of the Environment, 1999). While peak sedimentary As concentrations are considered high in this lake, they are almost negligible compared to those observed at Lake Charles. This not only holds true for sediment samples, but also for water samples where Lake Charles records As concentrations of 6.4 ppm and Loon Lake records As concentrations of 0.4 ppm (Clement et al., 2007).

While slight increases in As concentrations could be caused by As moving from the Montague Gold Mine by winds to Loon Lake, it is unlikely that environmental changes in Loon Lake are the result of historic mining activities (Wang & Mulligan, 2006). For example, Highway 118, a freeway linking Dartmouth to the Trans-Canada Highway, was developed between 1970 and 1971. To develop this highway, the NS Department of Transportation expropriated land and blasted through bedrock along the planned highway path. Disturbing bedrock may have released As and other contaminants into nearby environments. Any As that was released via highway construction could have moved into Loon Lake through the atmosphere or surface waters (Wang & Mulligan, 2006). After Highway 118 was established, human activities around Loon Lake likely increased. With this came lakeshore property developments and associated land-use changes. The cumulative effects of climate change and land-use changes surrounding Loon Lake may have caused environmental changes over time (Ginn et al., 2015), explaining minor increases in As concentrations.

Recovery and Drivers of Cladoceran Assemblages

Changes in cladoceran assemblage composition at both Lake Charles and Loon Lake were driven by responses of dominant cladoceran taxa such as *Alona* spp., *Chydorus brevilabris*, *Daphnia pulex* complex, and Bosminidae. How As contamination and other past mining-related pollution affects Cladocera likely varies by taxon. As legacy contaminants increased at Lake Charles, assemblages shifted towards tolerant, mostly pelagic taxa. Similar trends have been observed in other paleolimnological studies, where cladoceran assemblages have exhibited shifts from littoral taxa such as *Alona* spp. and *Chydorus brevilabris* to pelagic taxa such as *Daphnia pulex* complex, immediately following contamination from historic gold mining activities (Thienpont et al., 2016). Because contamination levels and initial assemblages were different between the two lakes, it is not surprising that changes in dominant cladoceran taxa also differed between impact and reference lakes. Ginn et al. (2015) also categorized lake responses inferred from diatoms at Lake Charles and Loon Lake, as likely attributable to nutrient increases and climate change, respectively. Urbanization of the region is also important to consider as a driver of environmental changes in each lake.

Pollution from historic mining activities has been linked to decreases in cladoceran productivity, diversity, community structure, and the stability of brood size (Bozelli, 1996; Kerfoot et al., 1999; Doig et al., 2015; Winegardner et al., 2017). Both Lake Charles and Loon Lake showed significant ($p < 0.05$) decreases in taxon richness over time, however decreases were more extreme at Lake Charles (Figure 4; Figure 5). At Lake Charles, taxon richness decreased by ~20% from the pre-mining to the mining period and was further reduced from the mining to the post-mining period. The initial loss of littoral cladoceran taxa at Lake Charles occurred when sedimentary As concentrations increased, shortly after historic mining activities began at Montague. The reduction in taxon richness at Lake Charles emphasizes that the cladoceran community has not recovered since mining activities ceased ~80 years ago. At Loon Lake, taxon richness decreased by ~14% from the pre-mining period to the mining period. At Loon Lake, initial reductions in taxon richness during the historic mining period could be due to anthropogenic stressors not related to contaminants from historic mining activities, such as land-use changes from urbanization.

The largest cladoceran assemblage changes occurred at different times in each lake. In Lake Charles, assemblage composition changed more from the pre-mining to the mining period than they did from the mining to the post-mining period (Table 5). This suggests that while the Montague Gold Mine was active, legacy contaminants from past mining activities impacted assemblage structure at Lake Charles. After historic mining operations stopped in 1940, community composition continued to shift away from littoral taxa, towards a pelagic-dominated assemblage. In Loon Lake, cladoceran assemblage composition changed more from the mining to the post-mining periods than from the pre-mining to the mining periods. This suggests that most changes to the Loon Lake cladoceran community occurred after mining operations had stopped. It can be assumed that mining activities altered cladoceran assemblages at Loon Lake to a lesser degree than Lake Charles, where large shifts in cladoceran assemblages coincide with the timing of past mining activities and much higher As levels in the sediments.

In Lake Charles, littoral taxa were abundant in the pre-mining period, however their relative abundances decreased when mining activities began in 1863 (Figure 2). *Chydorus brevilabris*, *Alona costata*, and *Alona intermedia* were significant indicators of pre-mining conditions, but were virtually lost in the sediment record after mining began. While *Chydorus brevilabris* is recognized as a taxon that is sensitive to mining-related contaminants (Labaj et al., 2014; Thienpont et al., 2016), some studies have reported that this taxon is tolerant to As contaminants (Little et al., 2020). In Lake Charles, the decline in *Chydorus brevilabris* relative abundance coeval with historic mining activities suggests that contaminants surpassed a threshold some littoral taxa may be responsive to. Moreover, the decline in *Chydorus brevilabris* relative abundance could also be attributed to increases in competitors that were more tolerant to environmental conditions. Relative abundances of *Alona quadrangularis*, which is also a littoral species, increased slightly during mining activities. This indicates that this littoral taxon was more tolerant to environmental conditions associated with historic mining activities than other littoral taxa. Furthermore, relative abundances of *Daphnia pulex* complex increased significantly during the mining period, indicating that this taxon was able to tolerate high sedimentary As concentrations. Although the effects of As contamination on *Daphnia pulex* complex survival has not been well studied, the effects of As contamination on a similar taxon, *Daphnia magna*, has been studied in laboratory settings. The lethal concentration of As at which *Daphnia magna* survival is decreased by 20% (LC₂₀ of *Daphnia magna*), has been established for different forms

of As in water. The LC₂₀ value of *D. magna* to As(III) is 5ppm, whereas the LC₂₀ value of *D. magna* to As(V) is 30ppm (Lim et al., 2009). If LC₂₀ values are generalizable to the genus-level, it can be inferred that As(V) and As(III) concentrations in water at Lake Charles must have remained low during the mining period, allowing taxa from the *Daphnia pulex* complex to survive. Overall, the declines of *Chydorus brevilabris*, *Alona costata*, and *Alona intermedia* following mining activities indicates that these taxa are more sensitive to mining-related contaminants, as compared to *Alona quadrangularis* and *Daphnia pulex* complex.

Shifts in cladoceran assemblages between the pre-mining to mining periods at Loon Lake differ from those at Lake Charles. In Loon Lake, the littoral taxon *Eurycerus* spp. was a significant indicator of pre-mining times, but has since been lost in the sediment record. Loss of this taxon may be due to stressors not associated with past gold mining, such as climate change and/or urbanization (Ginn et al., 2015). *Camptocerus* spp. was also identified as a significant taxon associated with pre-mining times. While *Camptocerus* spp. relative abundances have decreased in post-mining times, it remains fairly common in the sediment record, indicating conditions at Loon Lake are still favorable to this littoral taxon. *Chydorus brevilabris* was abundant throughout the entire sediment record, but its relative abundances increased significantly during the 1960s, and it remains abundant today. *Chydorus brevilabris* is tolerant to low pH conditions, and a closely related taxon, *Chydorus sphaericus*, has been found in acidified lakes (Belyaeva & Deneke, 2007; Labaj et al., 2014). It is possible that Loon Lake has experienced some acidification over time as have many NS lakes, thus accounting for increases in *Chydorus brevilabris* abundance (Ginn et al., 2015). It is also important to note that as *Chydorus brevilabris* relative abundances increased in recent decades, Bosminidae relative abundances dropped. It is not uncommon for *Chydorus brevilabris* to increase markedly at the expense of Bosminidae species (Labaj et al., 2014). Similar to *Chydorus brevilabris*, *Daphnia pulex* complex was rare in pre-mining and mining times, but is a significant indicator taxon of post-mining times at Loon Lake. The relative abundances of these daphniids have been shown to increase as biological production increases in temperate lakes (Bos & Cumming, 2003; Davidson et al., 2011; Chen et al., 2010). Based on VRS chl-*a* trends, primary production has increased slightly in Loon Lake in recent years – possibly leading to greater abundances of daphniids after the 1970s. That said, Ginn et al. (2015) suggest that diatom-inferred water quality trends at Loon Lake can be best explained by climate change.

Taxa that maintained fairly high relative abundances throughout time at both lakes were *Bosmina longirostris* and *Eubosmina longispina* (Figure 2; Figure 3). In Loon Lake, *Bosmina longirostris* and *Eubosmina longispina* were identified by IndVal as significant taxa associated with the historic mining period, but compared to other taxa, they were also abundant in the pre- and post-mining periods. In Lake Charles, *Eubosmina longispina* was not identified as a significant indicator species of any time period, because its abundances remained stable through time. *Bosmina longirostris* on the other hand, was strongly associated with the post-mining period at Lake Charles. The high abundances of *Bosmina longirostris* in both mining and post-mining periods at Lake Charles is slightly surprising because this species is regarded as sensitive to metal contamination (Koivisto et al., 1992; Bossuyt & Jansen, 2005). High abundances of *Bosmina longirostris* during past mining operations could therefore suggest low bioavailability of legacy contaminants. While this may be true, in previous studies, *Bosmina longirostris* remains have increased markedly during anthropogenic stressors and climate change, thus explaining its ability to sustain high abundances in stressed conditions at Lake Charles (Leppänen, 2018; Kurek et al., 2019).

While mining-related contaminants may drive shifts in cladoceran assemblages at Lake Charles, other key drivers may include predation or shifts in food availability (Korhola & Rautio, 2001). Because both lakes showed shifts in large-bodied species such as daphniids, predation could be driving changes in cladoceran community composition. To determine whether predation is driving shifts in cladoceran assemblage composition at Lake Charles or Loon Lake, a future study could examine trends in cladoceran body sizes (Korosi et al., 2013).

While there are many factors that may contribute to changes in cladoceran assemblage composition at these lakes, it is evident that at Lake Charles, legacy contaminants are important in structuring cladoceran assemblages. Shortly after the Montague Gold Mine opened in 1863, As concentrations increased fivefold. With increases in As contamination came declines in taxon richness, as well as significant shifts in assemblage composition, from littoral taxa such as *Alona* spp. and *Chydorus brevilabris* to pelagic taxa such as *Daphnia pulex* complex. Although mining operations ceased 80 years ago, As concentrations in modern Lake Charles sediments remain ~1000 times higher than the 2.5 ppm As sediment guidelines, taxon richness is lower than ever, and several sensitive littoral taxa have been lost from the community.

Implications

Historic mining operations in Canada are often responsible for metal contamination in the form of As and Hg to aquatic ecosystems (Wang & Mulligan, 2016). As demonstrated by bioindicators and geochemical measures in this study, the historic Montague Gold Mine has impacted Lake Charles. While Montague was one of the largest gold mines in NS, it is important to note that hundreds of gold mines were active in NS during the late 1800s to the early 1900s (Art Gallery of Nova Scotia, 2013). Because some NS bedrock contains high concentrations of As, it is presumed that gold extractions at all historic gold mines released vast amounts of As and other toxic contaminants to the environment. Due to differences in the magnitude of past mining operations, the environmental consequences related to smaller mines are likely less concerning. Despite this, there are perhaps legacy contaminants associated with abandoned gold mines across all of NS, which may have legacy effects on aquatic environments (LeBlanc et al. 2019).

In Dartmouth, the abandoned Montague Gold Mine poses environmental concerns. Arsenic concentrations in Lake Charles sediments are alarmingly high in mining and post-mining times. Furthermore, recent water monitoring reports identified Lake Charles water to have As concentrations much higher than ~50 other lakes in the region, including Loon Lake (Clement et al., 2007). Cladoceran assemblages have changed over time in response to this contamination, and it is likely that other aquatic organisms within the lake have also been impacted. If Cladocera are accumulating As, they would pass this contaminant up the food chain. This may result in high concentrations of As in fish. If As is accumulating in fish, consequences may include changes in growth, feeding behaviours, and reproductive success (Tierney et al., 2013). Despite the Montague Gold Mine closing in 1940, the environmental damage associated with historic mining operations remain today and will remain until proper remediation action takes place.

While long-term management strategies must still be put into place, the government of NS recently announced its \$48 million plan to clean up the historic Montague Gold Mine (Willick, 2019). This plan includes excavating contaminated tailings to a depth of 2 meters and redepositing them in a lined containment cell. These cells will be made of berm which is an impermeable liner. Cells will include leachate collection and impermeable cover systems that will control water movement into the cells and prevent contaminants from leaching out (Willick, 2019). This plan also includes the installation of a water treatment system that will treat any

water that leaves the cells. In regions where tailings are less concentrated and where contamination levels are lower, a protective, low-permeability cover will be placed over tailings (Willick, 2019). This cover will prevent precipitation from entering tailings and will limit the redistribution of contaminants within the tailings. This cover will then be enclosed with soil and vegetation. The remediation plan is predicted to take 5 years to finish (Willick, 2019). If the province is able to implement this remediation plan, the adverse environmental effects of legacy contaminants from the historic Montague Gold Mine are expected to decrease. To extend the effectiveness and longevity of this remediation plan, a long-term monitoring plan must also be implemented. Cleaning up the historic Montague Gold Mine will require collaboration and cooperation from scientists, government agencies, and local citizens.

Conclusions

This study provides a long-term perspective on the environmental effects of past mining activities on freshwater ecosystems near the Montague Gold Mine in Dartmouth, NS. In Lake Charles, which is located downstream of Montague, shifts in cladoceran assemblages, and substantial increases in pXRF As concentrations indicate long-term impacts associated with historic gold mining activities. Littoral species were virtually lost in the Lake Charles sediment core as assemblages shifted towards more tolerant, pelagic taxa. In Loon Lake, which is located upstream of Montague, cladoceran assemblages also showed significant changes over time but to a lesser extent than Lake Charles. Because pXRF As levels are low in Loon Lake compared to Lake Charles sediments, it is unlikely that environmental changes in Loon Lake were due to mining activities at Montague. Lake Charles has not recovered since mining activities stopped in 1940. Other non-mining stressors such as anthropogenic activities related to urbanization and climate change could contribute to the lack of recovery at Lake Charles. Considering the many abandoned gold mines in NS, it is possible that mining-related legacy contaminants have impacted multiple watersheds in the province. It is therefore important to quantify the legacy effects of historic mining on other freshwaters in NS.

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Tables

Table 1. Physical, chemical and biological characteristics of Lake Charles and Loon Lake based on data collected by Clement et al. (2007). The values are averages based on pelagic measurements.

	Lake Charles	Loon Lake
As (ppb)	6.4	0.4
pH	6.5	6.2
Temperature (°C)	4	6
Theoretical conductivity (µS)	230	311
Trophic status	Mesotrophic	Mesotrophic
Dissolved organic carbon (mg C/L)	2.5	3.8
Average depth (m)	15	5
Maximum depth (m)	28	6

Table 2. Chronology of the Lake Charles sediment core using standard ^{210}Pb dating techniques performed by Queen's University.

Midpoint Depth (cm)	Age (y)	Year (y)	Standard Error (y)
0	0	2019.4	0.3
0.25	0.2	2019.2	0.3
2.25	6.4	2013.0	0.7
4.25	20.2	1999.2	1.5
6.25	34.5	1984.9	2.4
8.25	47.3	1972.1	3.6
10.25	64.4	1955.0	5.7
12.25	91.2	1928.2	10.8
14.25	121.0	1898.4	22.6
16.25	166.9	1852.5	72.0

Table 3. Chronology of the Loon Lake sediment core using standard ^{210}Pb dating techniques performed by Queen's University.

Midpoint Depth (cm)	Age (y)	Year (y)	Standard Error (y)
0	0	2019.4	0.6
0.25	1.0	2018.4	0.6
2.25	10.1	2009.3	1.0
4.25	20.6	1998.8	1.4
6.25	32.3	1987.1	2.0
8.25	45.7	1973.7	3.0
10.25	60.2	1959.2	4.6
12.25	72.1	1947.3	6.4
14.75	84.5	1934.9	8.4
16.75	92.1	1927.3	9.8
18.75	103.5	1915.9	12.3
20.75	123.1	1896.3	19.4
22.75	145.8	1873.6	30.8

Table 4. Permutational multivariate analysis of variance (PERMANOVA) results comparing cladoceran relative abundances in the pre-mining, mining, and post-mining time periods.

Lake		Df	SS	MS	F. Model	R2	P
Loon	Group	2	0.50	0.25	7.8	0.41	0.001
	Residuals	23	0.73	0.32		0.59	
	Total	25	1.23			1.00	
Charles	Group	2	0.30	0.15	5.1	0.29	0.001
	Residuals	25	0.73	0.03		0.70	
	Total	27	1.04			1.00	

Table 5. Permutational multivariate analysis of variance (PERMANOVA) results of cladoceran relative abundance pairwise comparisons between pre-mining & mining, and post-mining & mining time periods.

Lake	Comparison		Df	SS	MS	F. Model	R2	P-value
Charles	Pre-Mining & Mining	Group	1	0.15	0.15	5.14	0.23	0.006
		Residuals	17	0.50	0.03		0.77	
		Total	18	0.65			1.00	
	Post-Mining & Mining	Group	1	0.10	0.10	4.04	0.25	0.016
		Residuals	12	0.31	0.03		0.75	
		Total	13	0.41			1.00	
Loon	Pre-Mining & Mining	Group	1	0.06	0.06	2.76	0.16	0.01
		Residuals	15	0.34	0.02		0.84	
		Total	16	0.40			1.00	
	Post-Mining & Mining	Group	1	0.88	0.88	18.78	0.54	0.001
		Residuals	16	0.75	0.05		0.45	
		Total	17	1.63			1.00	

Table 6. Cladoceran taxa identified as significant indicators of time periods and their corresponding IndVal coefficients, *p*-values, average abundance per depth interval, and number of occurrences.

Lake	Time Period	Taxon	IndVal	<i>p</i> -value	Average Abundance	Number of Occurrences
Loon	Post-Mining	<i>Daphnia pulex</i> <i>complex</i>	0.97	0.001	17.9	131
		<i>Chydorus brevilabris</i>	0.70	0.001	23.5	176
	Mining	<i>Bosmina</i> sp.	0.48	0.002	17.7	169
		<i>Eubosmina</i> sp.	0.42	0.01	44.3	430
	Pre-Mining	<i>Eurycerus</i> sp.	0.69	0.002	2.4	18
		<i>Camptocerus</i> sp.	0.44	0.04	5.0	38
Charles	Post-Mining	<i>Bosmina</i> sp.	0.46	0.004	29.6	332
	Mining	<i>Alona quadrangularis</i>	0.64	0.005	4.2	20
		<i>Daphnia pulex</i> <i>complex</i>	0.55	0.03	5.7	27
	Pre-Mining	<i>Alona costata</i>	0.64	0.001	2.9	66
		<i>Chydorus brevilabris</i>	0.62	0.005	5.0	108
		<i>Alona intermedia</i>	0.56	0.01	1.7	40

Table 7. Analysis of variance (ANOVA) results comparing the average taxon richness at pre-mining, mining, and post-mining time periods.

Lake		Df	SS	MS	F-value	<i>p</i>-value
Loon	Depth	2	33.5	16.8	3.4	0.04
	(cm)					
	Residuals	23	110.4	4.8		
Charles	Depth	2	131.2	65.6	7.0	0.004
	(cm)					
	Residuals	25	231.7	9.2		

Figures

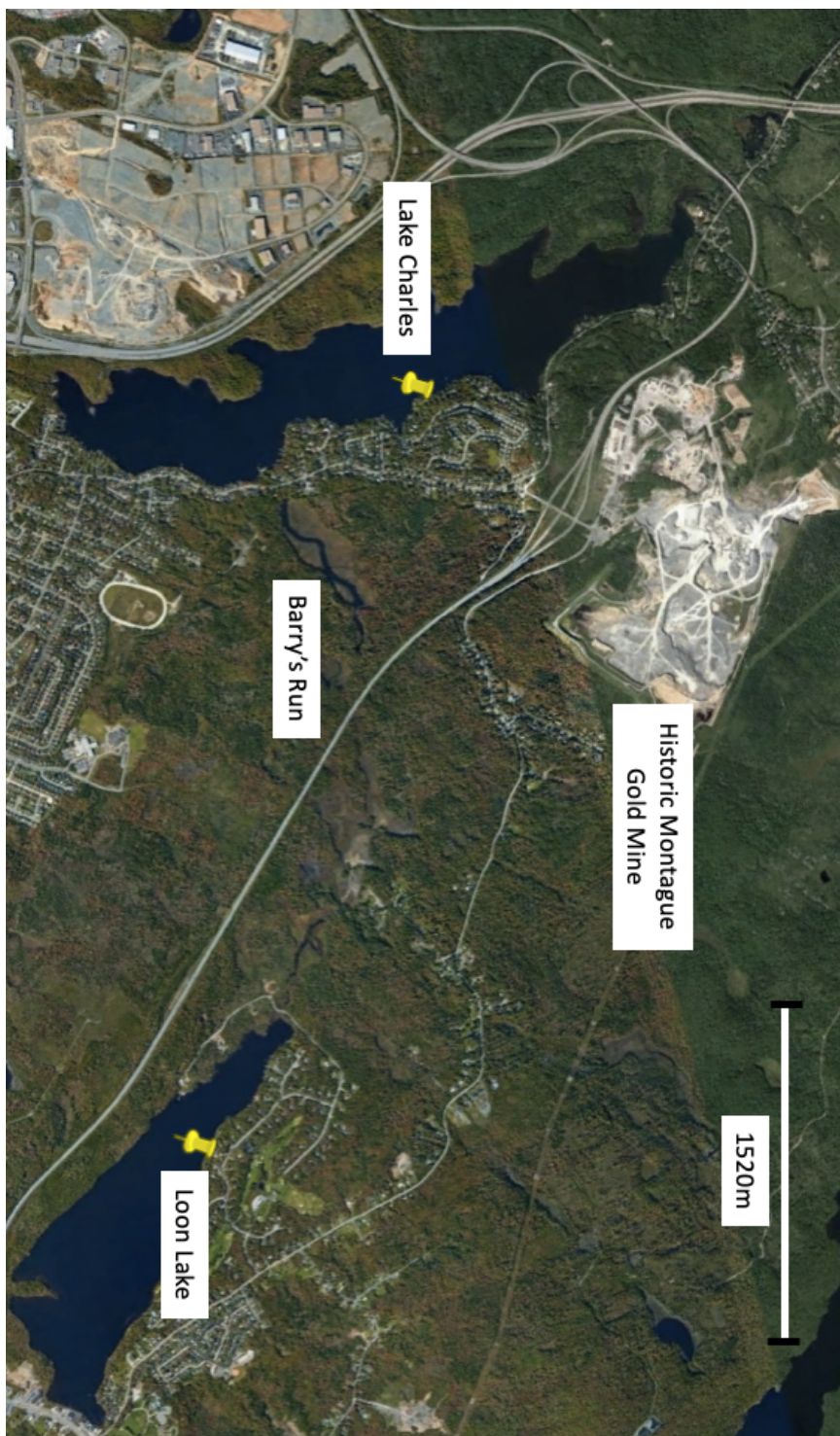


Figure 1. The Montague Gold Mine District and surrounding freshwater ecosystems. Yellow pins indicate the deepest basin of Lake Charles and Loon Lake, where sediment cores were retrieved.

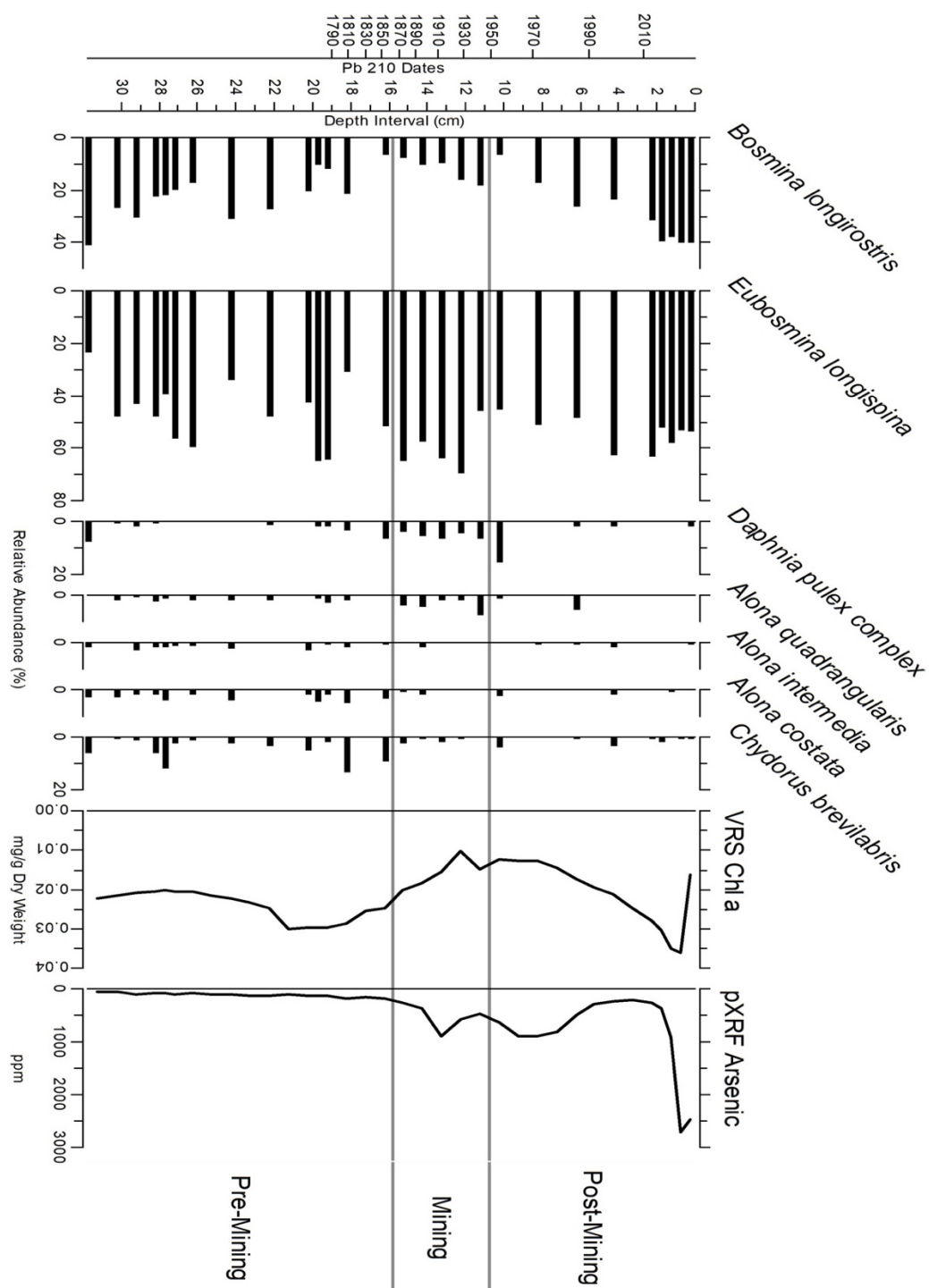


Figure 2. Stratigraphic depth-time visualization of Lake Charles showing cladoceran relative abundances of indicator species, VRS chl-*a* concentrations, and pXRF arsenic concentrations plotted against depth intervals (cm) and coinciding ^{210}Pb dates, with mining time periods outlined.

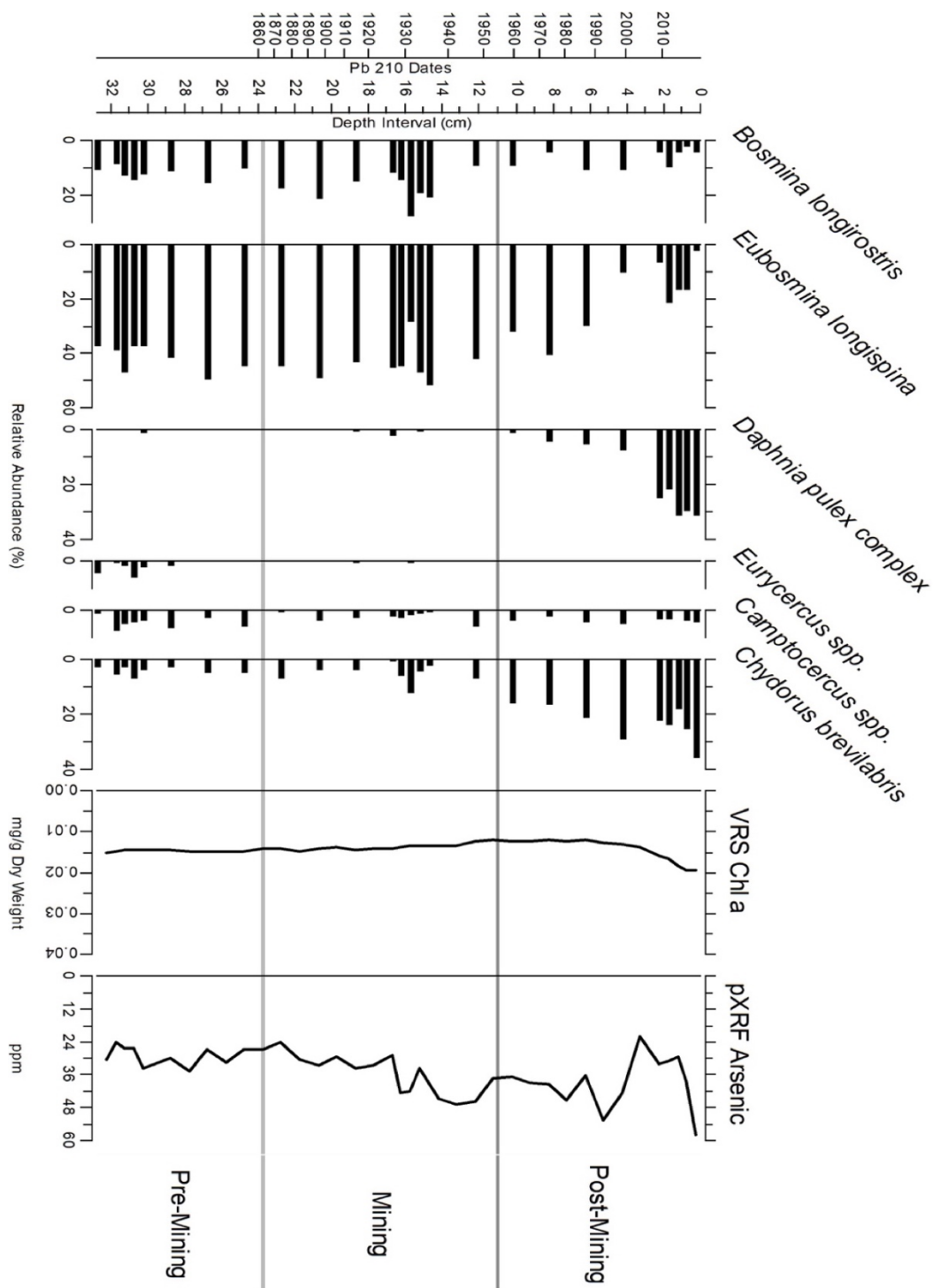


Figure 3. Stratigraphic depth-time visualization of Loon Lake showing cladoceran relative abundances of indicator species, VRS chl-*a* concentrations, and pXRF arsenic concentrations plotted against depth intervals (cm) and coinciding ^{210}Pb dates, with mining time periods outlined.

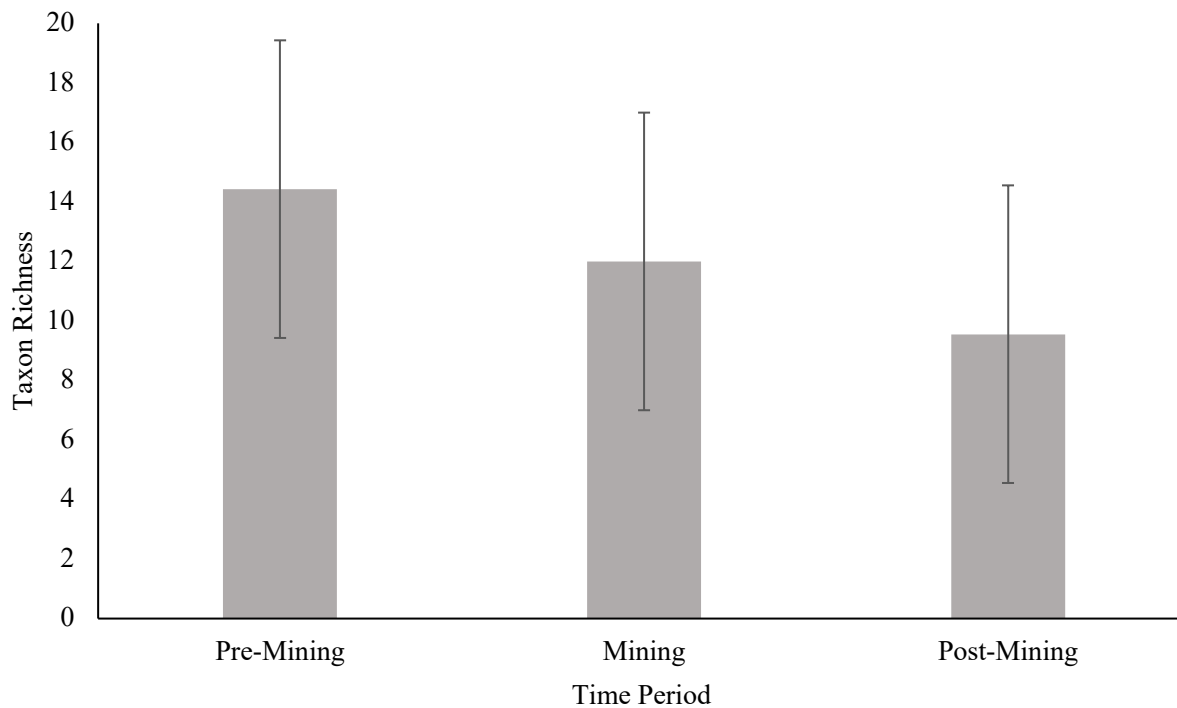


Figure 4. Average cladoceran taxon richness at Lake Charles during pre-mining, mining, and post-mining time periods

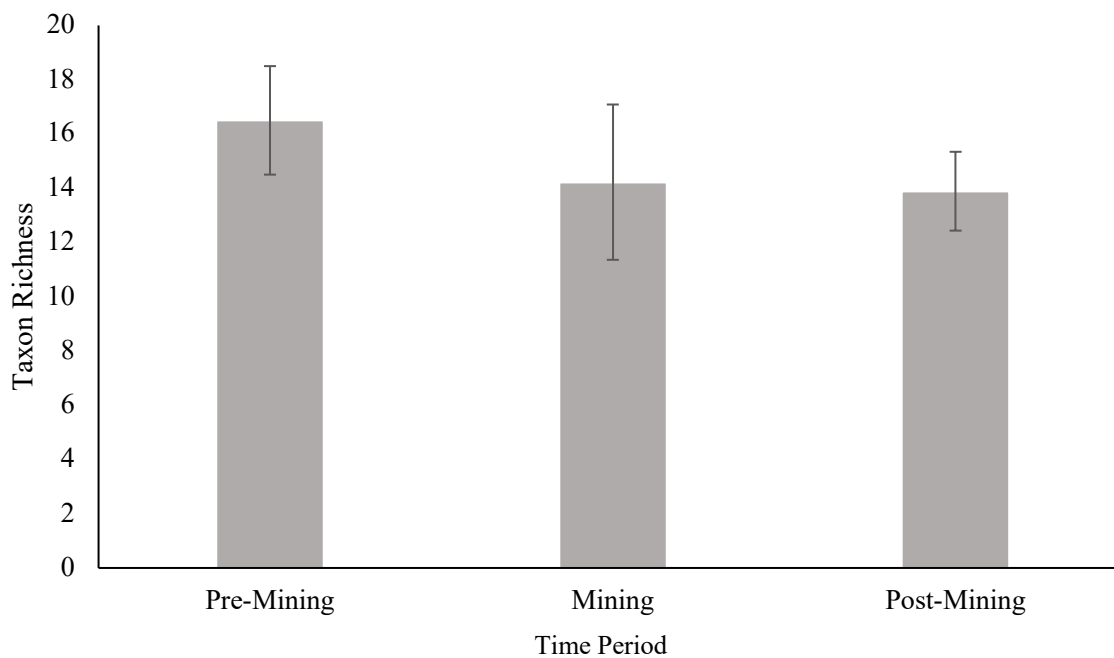


Figure 5. Average cladoceran taxon richness at Loon Lake during pre-mining, mining, and post-mining time periods.

Appendix 1. Lake Charles Raw Cladoceran Count Data

	22-22.5	20-20.5	19.5-20	19-19.5	18-18.5	16-16.5	15-15.5	14-14.5	13-13.5	12-12.5	11-11.5	10-10.5	8-8.5	6-6.5	4-4.5	2-2.5	1.5-2	1-1.5	0.5-1	0-0.5	Depth interval (cm)	
	36	28	16	21	33	7	9	9	9	17	17	8	14	27	25	60	59	67	34	38	<i>Bosmina sp. longirostris</i>	
	63	58	96	111	48	54	74	49	57	73	42	52	41	49	66	120	78	101	45	51	<i>Eubosmina sp. longispina</i>	
	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	<i>D. longispina complex</i>
	2	1	3	4	6	7	5	5	6	5	6	18	0	2	2	0	0	0	0	2	0	<i>D. pullex complex</i>
	1	4	1	0	4	2	2	1	2	0	0	1	1	0	1	0	0	1	0	0	0	<i>Camptocercus sp.</i>
	1	1	1	2	3	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	<i>Acroperus harpae</i>
	1	0	1	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	<i>Alona affinis</i>
	3	1	2	5	3	0	5	4	2	2	7	2	0	6	0	0	0	0	0	0	0	<i>Alona quadrangularis</i>
	0	0	1	0	3	0	2	0	0	0	3	3	0	0	0	0	0	0	1	0	0	<i>Alona guttata</i>
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	<i>Alona circumfimbriata</i>
	0	4	1	2	3	1	0	2	0	0	0	0	1	1	2	0	1	0	0	1	0	<i>Alona intermedia</i>
	1	3	7	4	8	4	1	2	0	0	0	3	0	0	2	0	0	2	0	0	0	<i>Alona costata</i>
	1	3	4	1	1	3	1	0	2	0	2	3	5	0	0	0	1	0	1	0	0	<i>Alona rustica</i>
	0	2	1	0	0	1	4	1	3	0	0	0	2	1	0	2	0	0	0	0	0	<i>Graptoleberis testudinaria</i>
	10	6	3	6	6	5	0	4	2	0	8	5	5	3	0	0	0	0	1	0	0	<i>Rynchotalona falcata</i>
	0	0	1	0	0	3	0	0	1	0	0	2	0	0	0	0	0	0	0	0	0	<i>Monospilus dispar</i>
	1	4	3	3	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	<i>Alonella excisa</i>
	1	5	1	4	2	0	4	4	0	1	0	2	3	5	1	2	2	1	0	0	0	<i>Alonella nana</i>
	0	1	0	2	0	0	0	0	0	0	0	2	0	0	0	0	1	0	0	0	0	<i>Disparalona acutirostris</i>
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	<i>Kurzia</i>
	0	1	0	0	1	0	0	0	0	0	2	2	1	1	0	0	0	0	0	0	0	<i>Pleuroxus sp.</i>
	2	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	<i>Chydorus bicornutus</i>
	1	1	0	0	1	2	0	1	0	0	2	2	0	0	0	0	1	0	0	0	0	<i>Chydorus faviformis</i>
	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	<i>Chydorus linquillabris</i>
	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	1	0	0	<i>Paralona piara</i>
	5	7	1	4	21	10	3	1	2	1	0	5	0	1	4	2	3	1	1	1	1	<i>Chydorus brevilabris</i>
	0	0	1	0	1	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0	<i>Leptodora kindti</i>
	0	1	1	1	3	2	1	0	1	1	0	2	1	0	0	0	1	0	0	0	0	<i>Sida crystallina</i>
	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	<i>Diaphanosoma brachyurum</i>
	0	1	0	0	2	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	<i>Latona setifera</i>
	2	0	1	1	3	1	0	1	0	0	0	1	0	1	1	0	0	0	0	0	0	<i>Polyphemus pediculus</i>
	131	135	147	172	153	104	112	85	89	104	91	114	80	101	105	188	148	174	84	94	SUM	

31-31.5	30-30.5	29-29.5	28-28.5	27.5-28	27-27.5	26-26.5	24-24.5
66	72	67	50	33	42	26	44
38	129	94	107	58	119	88	48
0	1	0	0	0	0	0	0
13	3	4	2	0	0	1	0
4	2	2	3	0	3	2	1
0	1	1	3	3	2	2	1
2	3	2	1	2	0	1	0
0	5	2	6	2	1	3	3
0	0	1	3	1	0	1	0
0	0	0	1	0	0	0	0
3	2	7	5	3	3	2	4
5	9	5	5	6	0	3	6
0	7	1	7	4	6	3	3
2	1	2	1	2	1	0	0
5	16	11	6	5	7	5	13
1	2	1	1	1	2	2	0
1	0	0	1	1	1	1	1
0	2	2	2	2	3	2	1
1	0	1	0	0	0	0	0
0	0	1	0	1	0	0	0
0	2	1	0	1	1	1	0
0	1	1	0	0	1	0	4
0	0	3	0	0	0	0	0
0	1	0	1	0	4	0	2
0	0	0	0	0	0	0	0
10	3	3	14	18	6	2	4
1	0	0	0	0	0	0	0
4	3	1	0	1	3	0	1
3	0	0	0	0	0	0	0
1	0	3	0	1	2	1	1
0	3	1	2	1	2	1	3
160	268	217	221	146	209	147	140

32-32.5	14	8	12	12	9	12	16
31.5-32	47	34	43	31	27	43	50
31-31.5	0	0	0	0	0	0	0
30.5-31	0	0	0	0	1	0	0
30-30.5	6	1	2	5	2	2	0
28.5-29	2	7	5	4	3	7	3
26.5-27	3	1	5	1	3	2	4
	1	2	1	2	0	1	1
	9	7	2	1	3	4	0
	0	0	0	1	1	1	1
	0	0	0	0	0	0	0
	1	1	1	0	0	2	0
	2	1	0	2	1	4	2
	2	2	2	2	1	0	2
	3	3	0	2	2	1	1
	2	2	4	2	1	5	5
	1	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	1	0	1	1	0
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	0	0	0	0	0	2	0
	15	2	3	5	5	4	4
	0	2	0	0	2	1	0
	1	0	2	1	1	1	0
	9	8	4	3	4	4	6
	4	5	3	6	3	3	5
	0	0	0	0	0	0	0
	0	0	0	0	0	0	0
	1	1	0	0	1	2	0
	0	0	1	1	0	0	0
	0	0	0	0	0	0	0
	1	0	0	1	1	1	0
	124	87	91	82	72	103	100