2021-2022

Twelve Mile and Little Boshkung Lakes Benthic Assessment

Prepared for the Twelve Mile and Little Boshkung Lake Association

Brooklynn Jamieson
ERSC 4380Y
BRENDAN HICKIE
LARRY TOMPKINS
SADIE FISCHER

Contents

Introduction

The Community Benthos Biomonitoring Program, coordinated by U-Links and aligned with the Ontario Benthos Biomonitoring Network, was created with the goal of examining the health of lakes in Haliburton County, and communicating the findings to key decision makers such that they can make informed decisions pertaining to lake health. A bioassessment can be defined as evaluating the condition of a water body using biological parameters and direct measurements of resident plants and animals in an aquatic ecosystem (Kenney et al., 2009). Biomonitoring may also be defined as the systematic use of the biological responses of specific indicator organisms (or community of organisms) to evaluate changes in the environment (Bae et al., 2005). Benthic macroinvertebrates are the most commonly used indicator organisms in aquatic biomonitoring studies (Bae et al., 2005; Kenney et al., 2009). This is due in part to the relative ease of benthics sample processing and the lack of sophisticated equipment needed to sample benthic macroinvertebrates (Kenney et al., 2009). Commonly assessed biological responses of benthic macroinvertebrates to environmental changes include measurements of: community composition, species diversity, and individual species abundance (Bae et al., 2005; Cao et al., 2018). These measurements can then be used to estimate the abundance of environmentally-sensitive organisms compared to the abundance of environmentally-tolerant organisms, which in turn can provide insights on the overall health and functioning of an aquatic ecosystem (Menezes et al., 2019). Benthic biomonitoring studies are based on the premise that the benthic communities being studied actually respond to changes in habitat and water quality resulting from environmental disturbance, and that benthic community responses accurately and reliably reflect the state of the water body (Kenney et al., 2009).

Benthic macroinvertebrates are small (0.2 - 0.5 mm) organisms that inhabit the bottom substrates (sediment, debris, logs, etc.) of freshwater habitats for at least part of their life cycle (Bae et al., 2005). "Benthic macroinvertebrate" is an umbrella term, and includes a variety of different insect orders, as well as crustaceans, gastropods (snails, limpets), clams, and worms (Kenney et al., 2009). There are a number of reasons for the popularity of benthic macroinvertebrates in biomonitoring studies. Bae et al. (2005) argued that the high ubiquity and biodiversity of benthic macroinvertebrates, as well as their sedentary lifestyles and long lifespans, make benthic macroinvertebrates a primary candidate for bioassessments. The researchers argued that the ubiquity of benthic macroinvertebrates means that these organisms are found virtually everywhere and thus can be affected by environmental perturbations in many different types of aquatic systems. Also, they argued that the large number of benthic macroinvertebrates offers a spectrum of possible responses to environmental stress. Bae et al. also stated that the relatively sedentary lifestyle of benthic organisms allows researchers to effectively analyze pollutant or disturbance effects over spatial scales, and that the relatively long life cycles of benthic macroinvertebrates enables the observation of benthic community and ecosystem changes over time. Due to these traits, benthic macroinvertebrates make excellent bioindicators. These organisms act as continuous measures of the water body they inhabit, allowing for temporal and spatial analysis of the aquatic environment (Bae et al., 2005; Kenney et al., 2009).

In addition, benthic macroinvertebrates make strong bioindicators because they accurately and reliably respond to changes in their environment (Bae et al., 2005). The structure of benthic macroinvertebrate communities depends on a number of factors including: the quality and quantity of food sources, habitat quality (e.g. the physical structure of the stream/lake bed), water flow regime, water quality, biotic interactions with other organisms, and the condition of the riparian zone (Kenney et al., 2009). If an environmental disturbance occurs and causes changes to any of these factors, benthic community composition and functioning will change as well in response (Kenney et al., 2009). Additionally, there is ample evidence in the scientific literature that suggests that the biological response and community composition of benthic organisms varies between disturbed environments and relatively unaltered environments (Bolam et al., 2017; Menezes et al., 2019). For example, Menezes et al. (2019) found that there was a significant difference in the biological parameters between benthic communities in aquatic ecosystems close to urban development versus those farther away from urban areas. The researchers observed that the total number of taxa, diversity, and the proportion of herbivores to predators was higher in relatively unaltered environments, while the abundance of collectors, filter feeders, and parasites was higher in disturbed environments. These observed community composition changes in benthic macroinvertebrates reflected differences in water quality; the researchers also observed worsening water quality with increased proximity to urban development. This research and other studies support the idea that when environmental disturbance occurs in aquatic habitats there is a transition from a "healthy" state, reflected by many environmentally sensitive species, to a "degraded" state, reflected by many environmentally tolerant species (Bolam et al., 2017).

Benthic community responses to environmental changes also act as reliable indicators of the state of water bodies (Bae et al., 2005). Benthic macroinvertebrates provide many important ecological functions, playing vital roles in decomposition, nutrient cycling, and in the aquatic food web as both consumers and prey (Kenney et al., 2009). The exact role of benthic organisms varies by taxon, thus when benthic community composition shifts in response to environmental disturbance or change, the important ecosystem services provided by benthic organisms may shift or be disrupted as well (Cao et al., 2018). Furthermore, due to the important ecological functions they provide, benthic communities are vital to maintain aquatic ecosystem structure and function as a whole (Kenney et al., 2009; Cao et al., 2018). Therefore, if benthic macroinvertebrate communities are observed to be in a poor state, it is likely that the entire water body is too, as the ecosystem is deprived of the vital functions provided by benthic organisms.

The collection and analysis of benthic macroinvertebrates generates raw data of community composition, species and individual diversity, and similar biological parameters (Cao et al., 2018). These metrics alone do not provide much information on the condition of an ecosystem, thus further analysis is needed to link the raw data to the overall health of a lake or ecosystem. Bae et al. (2005) explains that, over time, biological indices have been developed (both in the scientific literature and in national/provincial programs) which provide estimates of water quality according to the measurements of biological parameters. They state that several indices have been developed for a variety of specific benthic organisms, habitat types, and analysis methods. These include the Trent Biotic Index, the Score System, the Hilsenhoff Biotic Index, and many more. Biological indices can be used to compare benthic communities and water quality between sampling sites and a reference site, to compare among sites, or to compare sampling sites with a set standard (e.g. a water quality standard) (Usseglio-Polatera et al., 2000). Indices provide context to benthic biomonitoring studies, by linking the biological parameters generated by sampling to water quality and overall health of an aquatic ecosystem (Bae et al., 2005; Usseglio-Polatera et al., 2000).

There are many benefits of benthic biomonitoring to the scientific community as well as the public. Benthic biomonitoring is a relatively common practice, and as such is strongly established and present in the scientific literature (Poikane et al., 2016). This strong scientific background and the rigorous testing of analytical and sampling methods involved in benthic biomonitoring equates to accurate, reliable data in most circumstances (Poikane et al., 2016). Additionally, benthic biomonitoring is very cost- and time-effective compared to other techniques to assess water quality (Tubic et al., 2017). Relatively little expertise is required to complete a benthic assessment, yet data generated from benthic biomonitoring studies is generally reliable and accurate despite its simplicity (Resh, 1994; Jones, 2008). In a comparison of two sampling methods commonly used in benthic bioassessments, Tubic et al. (2017) found that the more expensive methods yielded similar results to simpler, more inexpensive methods. Therefore, complex experimental designs are not a necessity for accurate collection of water quality data. Even further, Kenney et al. (2009) argued that benthic biomonitoring projects have the ability to provide an integrated, comprehensive assessment of the health of an entire water body over time. The researchers argued that other methods to assess overall ecosystem health, such as physical and chemical assessments (e.g. dissolved oxygen content, mercury concentration, pH, etc.), can provide snapshots of the condition of a water body, but cannot provide a measure of overall health through time and space.

A major weakness of benthic bioassessments is data analysis of benthic macroinvertebrates. The reliability of data generated by benthic biomonitoring studies is dependent upon the chosen sampling and analysis techniques (Tubic et al., 2017; Resh, 1994; Jones, 2008). In particular, identifying benthic macroinvertebrates to specific taxonomic levels is a major challenge (Kenney et al., 2009). The ability of researchers to identify macroinvertebrates to appropriate taxonomic groups is dependent upon the taxonomic resolution required, level of expertise, and time and cost constraints (Resh, 1994; Jones, 2008). The identification of benthic communities to general taxonomic groups (i.e. family, order) is generally less time consuming, costly, and requires less expertise (Resh, 1994). However, the information that can be gleaned from generic identifications is limited (Jones, 2008). Therefore, advanced scientific studies often seek to classify organisms to the genus or species level (Resh, 1994; Jones, 2008). These identifications take much more time, expertise, and often more money, but the data generated can provide more information on benthic communities in an area which in turn can tell researchers more about overall ecosystem health (Jones, 2008).

In sum, benthic biomonitoring can provide vital information to the decision makers of lakes, by providing a measure of a waterbody through time and space. Despite the possible weaknesses associated with its use, benthic biomonitoring remains a powerful tool in assessing the health of water bodies. Therefore, the intent of this project was to conduct a benthic assessment of Twelve Mile and Little Boshkung (TMLB) Lakes to determine the baseline benthic community in these lakes, as well as possible factors which may be affecting water quality and overall lake health.

Lake History

Twelve Mile Lake is a freshwater lake located between Minden and Carnarvon, ON in the township of Haliburton County, ON. This deep, cold-water lake has a shoreline length of approximately 12.6 km and a surface area of 337 ha (MECP, 2018). Little Boshkung Lake is also a freshwater lake located just west of, and connected to, Twelve Mile Lake. Little Boshkung Lake is the smaller of the two lakes, with a shoreline length of 5.8 km and a surface area of 127 ha (MECP, 2018). TMLB Lakes are connected to the Trent-Severn Waterway, and as a result are in part managed and controlled by Parks Canada.

The mission of the TMLB Lake Association is to ensure the protection and sustainability of land along with the quality of life of the lakes' property owners from environmental, safety, and other community issues (TMLB Lake Association, 2022). The association has completed some prior water chemistry testing of the lakes (including dissolved oxygen and temperature profiles). The TMLB Lake Association is also a member of the Coalition of Haliburton Property Owners Association (CHA), a larger organization which represents over 100 water bodies across Haliburton County, and is committed to the protection and enhancement of lake health (TMLB Lake Association, 2022; CHA, 2022). Thus, the TMLB lakes have been maintained by the TMLB Lake Association since the organization's inception.

Purpose

The Twelve Mile and Little Boshkung Benthics Monitoring Project is aligned with the larger Benthic Biomonitoring Program in Haliburton County, but focused specifically on the needs and concerns of the Twelve Mile and Little Boshkung Lake Association. Therefore, the purpose of this project was to examine the health of Twelve Mile and Little Boshkung Lakes near Carnarvon, Ontario by conducting a benthic assessment. The project is based on the following research questions: (1) what is the baseline composition of the benthic communities in Twelve Mile and Little Boshkung Lakes?, and (2) how are septic systems and other man-made products affecting water quality and overall lake health?. Biomonitoring projects such as these are vital for the maintenance of lake health, as the early identification of negative trends in benthic communities can aid in the conservation of water quality as well as avoiding future issues related to water quality and lake health. The Twelve Mile and Little Boshkung Benthics Monitoring project will provide benefits to residents of the region, along with casual lake users and fishermen. Haliburton County also benefits from benthic biomonitoring projects and maintaining lake health both socially and economically.

Community Concerns

The two main concerns raised by the TMLB Lake Association are a change in water quality and a loss of fish stock. Changes in water quality in lakes are often the result of human development (Bhateria & Jain, 2016). Encroaching human development can result in: high pollution loads, eutrophication, altered turbidity, and physical destruction of the landscape, all of which can degrade water quality (Bhateria & Jain, 2016; Sondergaard et al., 2007). High nutrient loads are a particularly significant problem, as agricultural and urban development both contribute large quantities of nutrients to nearby water bodies (Sondergaard et al., 2007). Damaged or leaking septic systems in particular have the potential to introduce significant pollutant loads to water bodies - this may be especially concerning at TMLB Lakes because of the relatively high number of septic systems in residential areas surrounding the lakes (Withers et al., 2014). Climate change is also a large contributor to changes in water quality, especially in regions with altered ice and snow cover, as flow and erosion regimes are altered, which can increase sedimentation and instigate other processes which contribute to the degradation of water quality (Bhateria & Jain, 2016). Loss of fish stock is often correlated with changes in water quality (Ranjan, 2020). Therefore, the mechanisms behind changes in water quality in TMLB Lakes may be correlated with the loss of fish stocks in the lakes as well. These factors can also directly affect fish stocks; for example, algae blooms spurred by eutrophication can be toxic to fish populations (Ranjan, 2020). Additionally, warmer water temperatures caused by climate change disadvantages cold-adapted fish (Ranjan, 2020). For example, TMLB Lakes houses a population of lake trout, an economically-important species reliant upon large quantities of cold, well-oxygenated water (Dillon et al., 2003). Increased water temperatures therefore pose a significant threat to lake trout populations (Dillon et al., 2003; MECP, 2018). However, long-term monitoring of TMLB Lakes would be necessary to confirm climate change and human development as mechanisms behind changes in water quality or loss of fish stock.

Research Methods and Protocols

Field sampling was conducted on September 28, 2021 at four sample sites in Twelve Mile Lake, and one sample site in Little Boshkung Lake (Appendix A, Figure 1). To account for natural environmental diversity and variation within a site, two replicates were collected at each sampling site. The kick-and-sweep method for collecting benthic macroinvertebrates was used, following Ontario Benthos Biomonitoring Network (OBBN) protocol. After wading out to 1 m depth, the bottom substrate was kicked to disturb benthic insects, which were then caught using a dip net with 0.5 mm mesh. This process continued for three minutes continuously, moving from 1 m depth back toward shore. After three minutes, the contents of the dip nets were emptied into individual jars for each replicate, labelled appropriately, and stored for transport. After all samples/replicates were collected, the jars were filled with an ethanol-based preservative in order to preserve the specimens, and transported to Trent University for laboratory analysis.

Water chemistry measurements (water temperature, conductivity, pH, and DO concentrations) were also collected on September 28, 2021, using a multi-probe field instrument, which requires dipping one end (the probe/sensor) of the device in the water and reading the result from the handheld instrument. Visual observations of the riparian zone, vegetation cover, man-made products, and other significant site descriptions were noted as well.

Laboratory analysis of the benthic invertebrates collected at TMLB Lakes was conducted between October 2021 and November 2021 at the Environmental Science Centre at Trent University. Each replicate was analyzed separately following the OBBN protocol. The jars containing the preserved specimens were emptied into larger containers, stirred with a spoon, and randomly scooped into stage plates for view under a light microscope. Each sub-sample was evaluated under the microscope, removing and identifying any benthic macroinvertebrate found to its taxonomic order/class within the OBBN 27-groups, until all specimens in that subsample were found. The discovered benthic macroinvertebrates were placed in a separate vial, labelled appropriately, and preserved with 70% ethanol. This process was repeated until 100 (+/- 20) benthic macroinvertebrates had been collected for each replicate. The samples/vials were returned to U-Links on November 26, 2021.

Data Analysis Methods

%EOT

The %EOT index refers to the abundance of the taxonomic groups Ephemeroptera (mayflies), Odonata (dragonflies and damselflies), and Trichoptera (caddisflies) found at a site. These orders are sensitive to environmental perturbations and thus evaluating their proportion in the benthic community is a good indicator of pollution levels. In general, areas with low %EOT are more polluted/disturbed than areas with high %EOT. This index was calculated for each sample site by first summing the benthic counts for both replicates for each sample site. The total number of organisms belonging to EOT groups was summed, divided by the total number of benthic organisms in the sample, and multiplied by 100 to get a percentage (**Equation 1**).

$$\%EOT = (\#mayflies + \#dragonflies + \#damselflies + \#caddisflies)/total\,\#benthics \times 100$$

Simpson's Diversity Index

The Simpson's Diversity Index compares the total number of taxonomic groups present at a site to the number of individual organisms. Calculating this provides an idea of the diversity of a sampling site. Values range from 0, which indicates no diversity, to 1, which indicates infinite diversity. Replicates from each sample site were summed to generate an estimate of diversity at every sampling site. To calculate this index, samples were listed according to counts for each taxonomic group. **Equation 2** was used to calculate the index for each respective sample site.

$$D = 1 - \frac{\Sigma n(n-1)}{N(n-1)}$$

where:

D = Simpson's diversity index
n = total number of organisms in a taxonomic group
N = total number of benthic organisms across all groups

% Amphipods vs % Insects

The % amphipods vs % insects index compares the percentage of amphipods, who have high tolerance to pollution, to the percentage of the more pollution-sensitive insects. This ratio can show how many benthic macroinvertebrates have high tolerance to pollution at a specific sample site. Higher amphipod:insect ratios at a site generally reflect a higher proportion of tolerant organisms, indicating a more polluted or disturbed location. To calculate this ratio, the replicates from all samples were summed. The total number of amphipods and insects respectively were counted, along with the total number of benthic macroinvertebrates at each site. The number of amphipods and insects respectively were then divided by the total number of benthics and multiplied by 100. **Equation 3** was then used to calculate the ratio of amphipods to insects.

$$x = \%\,amphipods\,/\,\%\,insects$$

Results

Benthic Data

The most common group of benthic invertebrates was Malacostraca (amphipods and isopods) in both Twelve Mile and Little Boshkung Lakes in 2021, accounting for over 50% of the total benthics present at both lakes (Figures 2 and 3). Diptera (flies) was the second most dominant taxonomic group in Twelve Mile Lake, accounting for 28.5% of benthic organisms, while Mollusca (snails and clams) was the second most dominant taxonomic group in Little Boshkung Lake. Worms were only present at sample sites in Twelve Mile Lake, and in very small proportions (0.4% of all benthic organisms). The raw data generated from the benthic sampling and analysis of Twelve Mile and Little Boshkung Lakes can be found in Appendix B (Table 1).

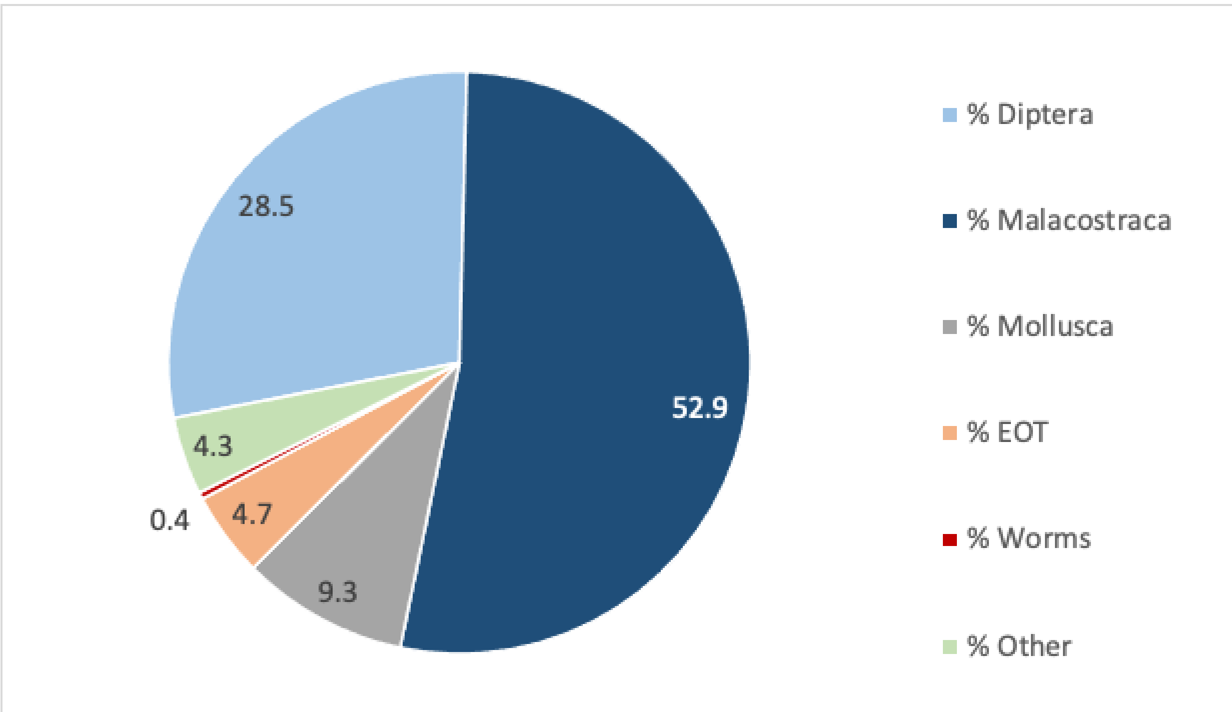

Figure 2. Benthic community composition (% composition) of Twelve Mile Lake (summed total of all samples and respective replicates). % Diptera includes the orders: miscellaneous Diptera (true flies), Chironomidae (midges), and Ceratopogonidae (no-see-ums). % Malacostraca includes the orders Amphipoda (scuds) and Isopoda (sow bugs). % Mollusca includes the orders Pelecypoda (clams) and Gastropoda (snails and limpets). % EOT includes the orders: Ephemeroptera (mayflies), Anisoptera (dragonflies), Zygoptera (damselflies), and Trichoptera (caddisflies). % Worms refers to the order Oligochaeta (aquatic earthworms), and % Other encompasses all other benthic organisms collected and identified in Twelve Mile Lake.

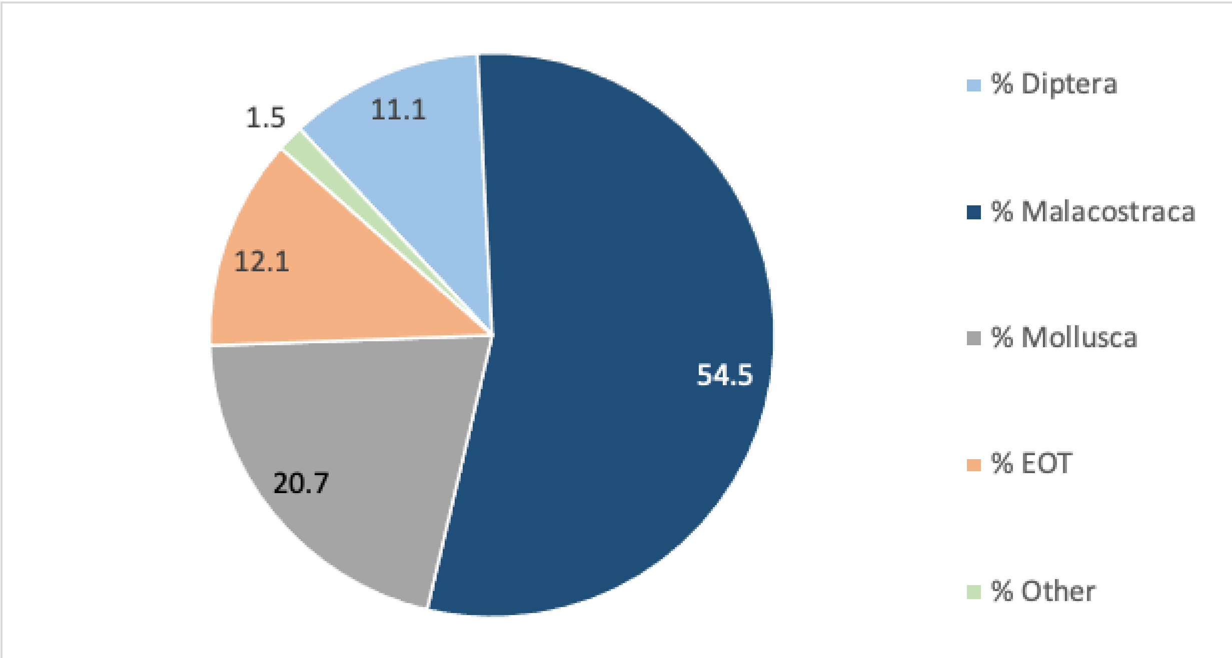

Figure 3. Benthic community composition (% composition) of Little Boshkung Lake 2021 (summed total of replicates). % Diptera includes the orders: miscellaneous Diptera (true flies), Chironomidae (midges), and Ceratopogonidae (no-see-ums). % Malacostraca includes the orders Amphipoda (scuds) and Isopoda (sow bugs). % Mollusca includes the orders Pelecypoda (clams) and Gastropoda (snails and limpets). % EOT includes the orders: Ephemeroptera (mayflies), Anisoptera (dragonflies), Zygoptera (damselflies), and Trichoptera (caddisflies). % Other encompasses all other benthic organisms collected and identified in Little Boshkung Lake.

Water Chemistry and Vegetation

Sample sites TWLV-01 and TWLV-03 were both beach shorelines, with sand being the dominant substrate. Vegetation, woody debris, and detritus were minimal or absent at both of these sites. Sample site TWLV-02 was located south of a public marina and north of a dam. There was plenty of debris, detritus, as well as garbage at this sampling site. Vegetation (emergent and submergent) was plentiful, and the dominant substrate was silt. Site TWLV-04 had clay and cobble substrate, and had some vegetation, woody debris, and detritus. Sampling site LBSK-01 was located just east of a bridge, and had silt and clay substrates. Plenty of woody debris, detritus, vegetation, and algae were observed at this sampling site.

Measures of water temperature, dissolved oxygen (DO) levels, and pH at TMLB Lakes in 2021 had minimal variability between the five sampling sites (Figure 4). Measurements of conductivity were much more variable between the sampling sites, ranging from 3 µS/cm at site TWLV-01 to 70.5 µS/cm at site TWLV-03. The raw data for water chemistry measurements, vegetation cover, and other important site descriptions can be found in Appendix B (Table 2).

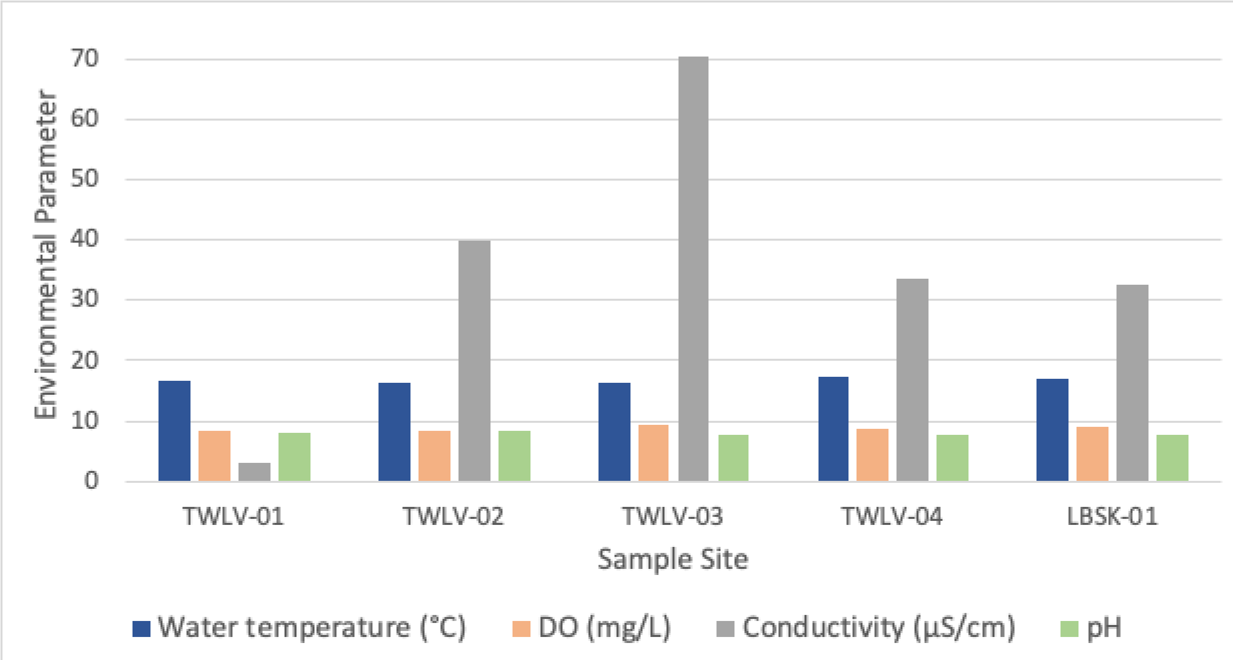

Figure 4. Water chemistry measurements gathered from sampling sites at TMLB Lakes on September 28, 2021.

Benthic Data Indices

%EOT

%EOT values for TMLB Lakes varied across the five sampling sites, with three sites classified as "typical" for Haliburton County, and two sites classified as "extremely atypical" for Haliburton County lakes (Figure 5). Of the three sites classified as "typical", two sites (TWLV-04 and LBSK-01) were well above the regional %EOT average, and one site (TWLV-02) was roughly equivalent to the regional average. Sites TWLV-01 and TWLV-03, however, were well below the regional average for both TMLB Lakes and Haliburton County, hence their classification as "extremely atypical".

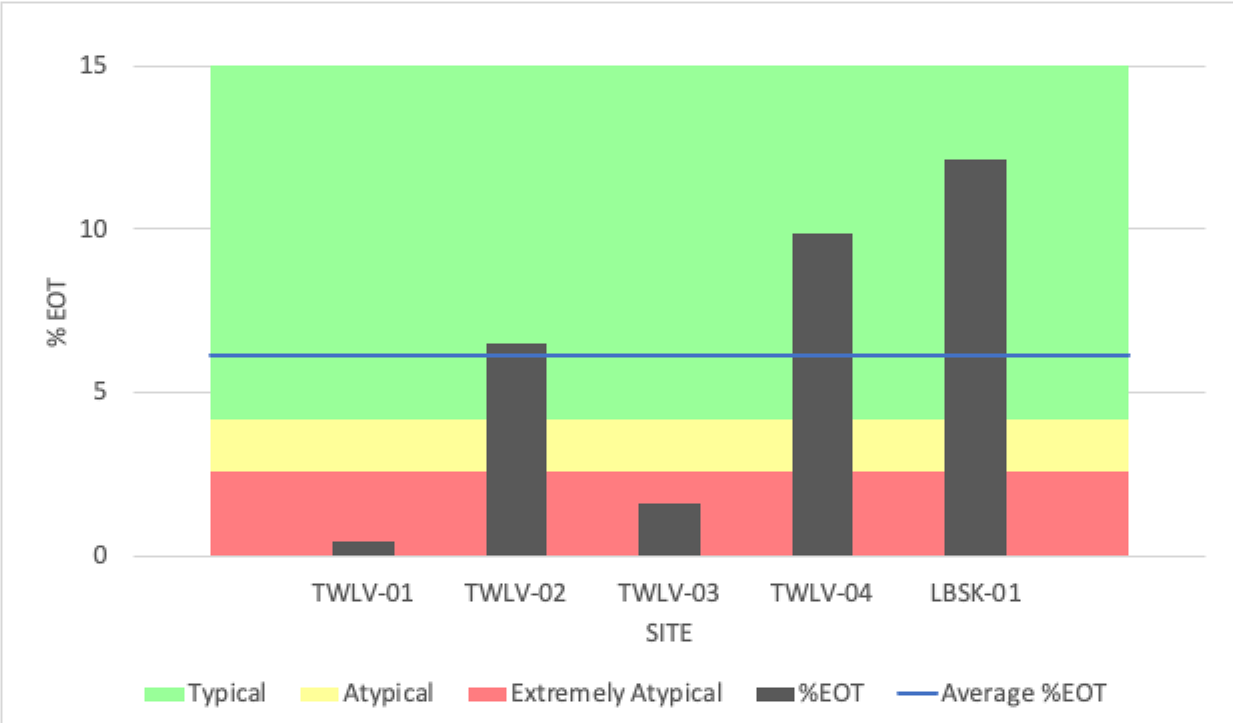

Figure 5. % EOT at each sample site (summed total of replicates) in Twelve Mile and Little Boshkung Lakes 2021 compared to the lower end of the typical, atypical, and extremely atypical %EOT ranges for Haliburton County. The "typical" range indicates %EOT between 4.18 and 37.12. The atypical range indicates %EOT between 2.62 and 4.18. The extremely atypical range indicates %EOT below 2.62.

Simpson's Diversity Index

The average diversity at TMLB Lakes in 2021 using Simpson's Diversity Index was 0.64 (Table 3). Three sites were below this regional average (sites TWLV-01, TWLV-02, and TWLV-03), with the lowest diversity at sampling site TWLV-02. Two sites (TWLV-04 and LBSK-01) were well above the regional average, with the highest diversity at site TWLV-04.

Table 3. Results of the Simpson's Diversity Index for benthic communities at sample sites in Twelve Mile and Little Boshkung Lakes, 2021.

Lake	Twelve Mile				Little Boshkung
Sample	TWLV-01	TWLV-02	TWLV-03	TWLV-04	LBSK-01
Simpson's Diversity	0.616	0.569	0.620	0.710	0.690

% Amphipods vs % Insects

The average %amphipod:%insect value for TMLB Lakes in 2021 was calculated at 1.8 amphipods per insect (Figure 6). Three sites (TWLV-01, TWLV-04, and LBSK-01) were roughly equivalent with this average, while TWLV-02 was well above this average (3.6 amphipods per insect), and TWLV-03 was well below this average (0.3 amphipods per insect).

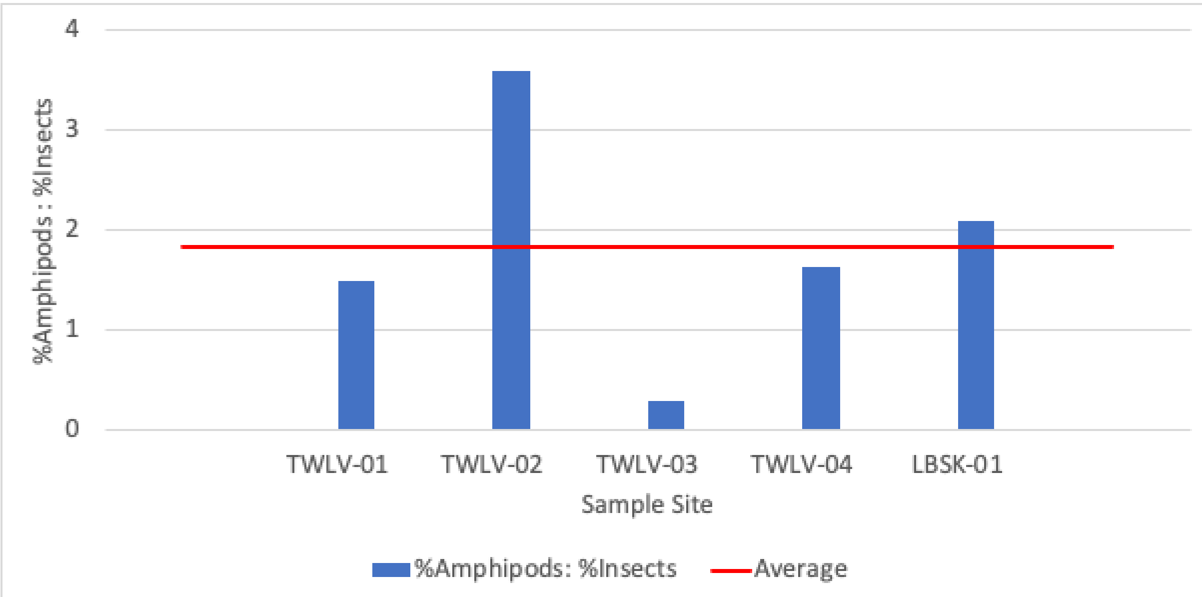

Figure 6. The ratio of amphipods compared to insects at each sample site at Twelve Mile and Little Boshkung Lakes, 2021.

Discussion

Water Chemistry and Vegetation

Measurements of DO levels, conductivity, and pH gathered from TMLB Lakes in 2021 appear to be within normal limits for Haliburton County. The Government of Ontario has determined that dissolved oxygen levels of at least 7 mg/L is required to meet the needs of lake trout in Haliburton County lakes (MECP, 2018). All sample sites at TMLB Lakes measured over 8 mg/L in DO concentrations, and thus are over this minimum value. The "safe" pH range for Ontario lakes according to the Provincial Water Quality Objective is between 6.5 and 8.5 (MECP, 2018). Sample sites at TMLB Lakes ranged in pH from 7.58 to 8.4, and thus are within normal limits. The Ministry of the Environment, Conservation, and Parks (2018) found that the average conductivity for lakes in Haliburton County was 48 µS/cm, ranging from values of 16 µS/cm to 218 µS/cm. The average conductivity of TMLB Lakes was 36 µS/cm, and thus is within the normal range for the Haliburton County region. These environmental parameters, as well as other measurements such as water temperature, should continue to be monitored in future so that trends and/or changes in water chemistry can be observed through time.

Benthic Data Analysis

The most common taxonomic group in TMLB Lakes was Malacostraca, containing the orders: Amphipoda (scuds) and Isopoda (sow bugs). This group had over 50% dominance in both Twelve Mile and Little Boshkung Lakes. In general, the dominance of any one particular group in a water body is reason for concern, as lake conditions may favour the reproduction of only one species, rather than a mix of species (TRCA, 2020). The high proportion of Malacostraca (over 50%) may therefore indicate that the reproduction of this group is being favoured over (or at the expense of) other groups. However, due to the warmer, calmer waters of lake environments which provide ideal habitat for Amphipoda, it is not uncommon to find larger proportions of these organisms in nearshore areas.

%EOT

The sample sites TWLV-01 and TWLV-03 fell into the extremely atypical range for Haliburton County in regards to %EOT. The reason for these low %EOT values is likely the dominant substrate at these sites - both TWLV-01 and TWLV-03 had sand-dominated substrates, which may not correspond with the optimal habitat for EOT taxonomic groups. These low values may also indicate that water quality is relatively poorer at these sample sites compared to other sites at TMLB Lakes. All other sampling sites fell within the typical %EOT range for Haliburton County lakes, and the overall mean %EOT for TMLB Lakes was within the normal range as well. This indicates that water quality in TMLB Lakes is average for Haliburton County.

Simpson's Diversity Index

All sample sites at TMLB Lakes in 2021 had relatively high diversity (mean Simpson's diversity = 0.64), indicating relatively healthy ecosystem functions at these lakes. Sites with the lowest diversity included sites TWLV-01 and TWLV-03, which had sand-dominated substrates not optimal for many benthic species, and TWLV-02, which was a heavily-disturbed site visibly impacted by garbage waste, a nearby marina, and other human influences. Sites with the highest diversity (TWLV-04 and LBSK-01) had relatively fewer disturbances affecting the region and had more-optimal substrates and vegetation conditions to support diverse benthic macroinvertebrate populations.

% Amphipods vs % Insects

The ratio of amphipods to insects in TMLB Lakes varied between the five sampling sites. Three sites (TWLV-01, TWLV-04, and LBSK-01) were relatively close to the regional average for TMLB Lakes (1.8 amphipods per insect). One site (TWLV-02) had a much higher ratio of amphipods to insects than the average, suggesting that this site is less favourable for more sensitive insects and may be of lower quality. This aligns with the visual observations from the sampling site - TWLV-02 was located just south of a public marina and just north of a dam, thus pollution- and disturbance-tolerant species are most likely to colonize and be collected at that location. Sampling site TWLV-03 had a much lower ratio of amphipods to insects than average, suggesting that this location is more favourable for sensitive insects and possibly indicating a higher quality habitat.

Comparison with Literature

Benthic Data

There was a high degree of natural variability in both the water chemistry measurements and the benthic data between the different sampling sites at TMLB Lakes. This is likely normal in lake environments, due to the large geographic area that lakes cover and subsequently the large number of microhabitats and sub-ecosystems which are present within a single lake. For example, benthic communities in the Laurentian Great Lakes vary even within a single lake, largely due to differences in substrate, depth, and vegetation conditions at different points around the lake (Burlakova et al., 2018). These factors impact the community composition of benthic invertebrates because of the vast number of organisms included under the benthic macroinvertebrate umbrella, which each have slightly different habitat requirements and ecosystem functions (Kenney et al., 2009; Burlakova et al., 2018). Some benthic invertebrates may require adequate vegetation cover to find shelter, for example, while other species may require little to no vegetation. The result of these differences is often variable benthic communities between different sampling sites around a single lake (Poikane et al., 2016; Burlakova et al., 2018). Since the Great Lakes are much larger in size than TMLB Lakes, this effect is likely exacerbated due to the relatively higher number of microhabitats and sub-ecosystems in larger lakes (Burlakova et al., 2018). However, multiple different benthic communities may still be present within smaller lakes like TMLB Lakes as well (Poikane et al., 2016).

In this study, the results of the %EOT biotic index and water chemistry data were compared to Haliburton County averages. Further work should be done to ascertain whether the average Haliburton County lake is synonymous with a healthy lake ecosystem. The Ministry of the Environment, Conservation, and Parks (2018) found that many of the lake trout populations in Haliburton County lakes are under stress due to land use changes and other environmental perturbations around lakes in this region. Since lake trout have very specific habitat requirements (clean, deep lakes with well-oxygenated waters) this is indicative that many Haliburton County lakes do not meet these demanding habitat requirements and may indicate a less-than-ideal water quality average in the lakes from this region (MECP, 2018). Despite this potential drawback, comparing the findings from lakes within Haliburton County to the county averages is still a powerful method for comparing water quality and lake health in these lakes, since regional environmental factors affecting the lakes will be similar. Additionally, comparing Haliburton County Lakes to one another will allow for the decision-makers and stakeholders of these lakes to identify the most at-risk lakes in the region, and allow them to adapt their lake management practices in response.

Man-Made Products and Water Quality

Man-made structures like septic systems and other anthropogenic influences are the primary source of disturbance in benthic communities and aquatic ecosystems (Menezes et al., 2019). The main pressures currently affecting global lake health are eutrophication from anthropogenic nutrient inputs, acidification from human-mediated global climate change, and alterations of hydrology and geomorphology from anthropogenic land use changes (Poikane et al., 2016). At Twelve Mile and Little Boshkung Lakes, there are several anthropogenic products around, near, or in the water bodies which may be impacting lake health and water quality. Both lakes are virtually surrounded by roads, and several bridges overpass the lakes at various points. The Twelve Mile Lake Dam is situated at the southernmost end of the lake, and there are multiple septic systems installed around the waterfront. Additionally, the lakes are used quite often for recreational purposes, and thus are subject to plenty of boating and fishing activities, as well as beaches and marinas.

Roads are a major local source of pollutants and can be a significant contributor to the deterioration of water quality in receiving bodies of water (Muller et al., 2020). An extremely diverse number of chemicals may be washed into nearby water bodies from roadways, and many of them are toxic and cause great ecological harm (Muller et al., 2020). Chemicals of concern in road runoff include: gasoline and other fluid leaks, tire and brake wear, trace metals from cars, road salt, and materials associated with vehicle/road washing and construction activities (Muller et al., 2020). In fact, Palmer et al. (2011) indicated road salting as one of the most likely drivers behind increased sodium and chlorine content (as well as increased conductivity) in Muskoka-Haliburton lakes between 1981-2005.

The impact of dams on the aquatic environment is a major concern in water management (Bunea et al., 2010). Dams result in extreme modifications to natural water flow, as well as accumulation of debris upstream of the dam site (Sharma et al., 2005). The Twelve Mile Lake Dam controls water levels in TMLB Lakes and ultimately releases outflows to Mountain Lake, which is in turn connected to the Trent-Severn Waterway. Therefore, some degree of debris accumulation may be occurring in Twelve Mile Lake upstream of the dam site, but these effects are likely minimized by the relatively frequent movement of water through the dam. Another environmental impact of dams in water bodies which is more applicable to TMLB Lakes is the limitation of gene flow and movement for aquatic organisms - dams act as physical barriers which often prevent fish and other aquatic species from moving past the dam site (Sharma et al., 2005; Bunea et al., 2010). For example, Bennett and Litzgus (2014) found that habitat fragmentation for freshwater turtles in the Trent-Severn Waterway was extensive due to the large number of dams installed along the waterway. Dams may also increase injury and/or mortality rates for aquatic organisms, by either directly harming animals caught in the dam, or indirectly due to construction or increased traffic around the dam site (Sharma et al., 2005; Bennett & Litzgus, 2014).

Septic systems have the potential to be a major source of water pollution (Withers et al., 2014). Septic systems are often linked to the addition of excess nutrients (especially nitrogen and phosphorus) to nearby water bodies (Baer et al., 2019). Withers et al. (2014) found that nitrogen enrichment in nearby groundwater is directly related to the density of septic tanks in the area. Extremely high concentrations of nutrients in lakes can lead to the formation of algal blooms and low-oxygen waters (Baer et al., 2019). This is especially concerning in rural areas like Haliburton County, where urban drainage and point source contamination is minimal - in these regions, domestic waste from septic systems may represent the most important anthropogenic source of excess nutrients in water bodies (Paterson et al., 2006). Withers et al. (2014) stated that septic systems are relatively effective at controlling pollutant inputs to water bodies, but failing or damaged septic systems present a large environmental concern. If septic tanks are damaged or full of sludge, tank overflow may occur, leading to high levels of organic matter and excess nutrient additions to nearby water bodies. The researchers also raised concern over the appropriate placement of septic systems. If septic systems are installed in inappropriate locations or on unsuitable soils the chance of leakage and subsequent harmful environmental impacts are much greater compared to properly-installed systems.

Boat activity and associated undertakings have the potential to impact and degrade water quality, as well as alter ecosystem services in aquatic ecosystems (Valdor et al., 2019). Boat activity may result in lakebed alterations, the introduction of various invasive species, changes in water and sediment quality, and habitat loss and degradation (particularly of benthic environments) (Valdor et al., 2019). Pollutants from boats are also a concern, and include trace metals, gasoline, and other fluids from the boats themselves as well as litter from boaters (Muller et al., 2020). Fishing may also pose a threat to lake health, as prolonged, extensive fishing may cause the destruction and disruption of benthic habitats, and a shift in biota community composition in water bodies (Bolam et al., 2017). Overfishing may also result in the collapse of important fisheries, such as lake trout - this is particularly important in TMLB Lakes as lake trout is present in these lakes, and is both an economically important fish and is valuable for preserving natural heritage as well (MECP, 2018).

Perceived State of the Lake

As this is TMLB Lakes' first year of inclusion in the Benthic Biomonitoring Program in Haliburton County, no trends from previous years can be derived. Further study and sampling of these lakes are needed for conclusive findings. That said, from the preliminary findings presented in this study, TMLB Lakes appear to be in the typical range for lake health and water quality in Haliburton County.

Comments and Notes on the Sampling Process

A weakness of benthic biomonitoring projects in lakes specifically is the lack of supporting scientific research in the published literature regarding benthic bioassessments of lake habitats. Most studies advocating the use of benthic biomonitoring have focused on stream habitats, with few addressing the efficacy of using lake benthic macroinvertebrates to assess water quality and ecosystem functioning (Poikane et al., 2016). Poikane et al. (2016) argued that biomonitoring of benthic macroinvertebrates in lakes is particularly difficult. The researchers state that new methods are necessary for benthic sampling in lakes in order to address the different pressures affecting lake habitats as opposed to stream habitats. They also argued that since the use of benthic macroinvertebrates in lakes is relatively new, there are still gaps in scientific knowledge regarding the link between lake macroinvertebrates and water quality. As a result of this knowledge gap, commonly-used biological indices in stream habitats may not be accurate or actual representations of ecosystem health when applied to lake habitats (Usseglio-Polatera et al., 2000). Poikane et al. (2016) also argued that since lakes inhabit far larger geographic areas than streams do, there is much higher natural variability present within lakes which may skew benthic macroinvertebrate data. The researchers state that within a single lake, multiple different microhabitats and sub-ecosystems may exist, each with their own community of benthic organisms. Factors such as dominant substrate in these microhabitats may influence the macroinvertebrate community composition in these smaller areas, and result in dramatically different benthic communities from sampling sites around a single lake (Sharma et al., 2005).

One of the broader goals of the U-Links Community Benthos Biomonitoring Program is to better develop the science of using benthic macroinvertebrates as bioindicators of lake health. Through the analysis of multiple lakes in Haliburton County over the past few years (as well as in coming years), U-Links seeks to develop biotic indices that better reflect the ecological condition of lakes. For example, instead of using the traditional %EPT biotic index (which was designed specifically for stream habitats), %EOT was used in this study. %EOT is similar to %EPT, but %EOT does not include counts of stoneflies since these bugs are found exclusively in stream environments. Adapting biotic indices to account for such differences between lake and stream habitats will strengthen confidence in the findings of benthic biomonitoring studies when applied to lake environments.

Conclusion

In sum, a benthic assessment of Twelve Mile and Little Boshkung Lakes was conducted with the goal of establishing a baseline composition of benthic communities in the lakes and contributing to the evaluation of overall lake health. The primary research questions for this project were: what is the baseline composition of benthic communities in Twelve Mile and Little Boshkung Lakes?, and how are septic systems and other man-made products impacting water quality and lake health?. To answer these questions, benthic macroinvertebrates were gathered following OBBN protocols on September 28, 2021 at five total sample sites in TMLB Lakes. Samples were analyzed and benthic macroinvertebrates were identified through OBBN laboratory analysis, and the generated data was compared against biotic indices for lake health. Ultimately, since TMLB Lakes have never been sampled before as part of the Benthic Biomonitoring Program in Haliburton County, no conclusive results can be determined. Preliminary findings indicate that TMLB Lakes are of average health for Haliburton County, but several more years of sampling and analysis are required for conclusive results. In the meantime, the decision-makers of TMLB Lakes should continue to practice sound lake stewardship and management, including reducing the use of road salts near roads and promoting the maintenance of septic systems and other man-made products which may impact water quality or overall lake health.

Bibliography

Bae, Y.J., Kil, H.K., and Bae, K.S. (2005). Benthic macroinvertebrates for uses in stream biomonitoring and restoration. *KCSE Journal of Civil Engineering, 9*(1), 55-63.

Baer, S., Robertson, W., Spoelstra, J., and Schiff, S. (2019). Phosphorus and nitrogen loading to Lake Huron from septic systems at Grand Bend, ON. *Journal of Great Lakes Research, 45*, 642-650.

Bennett, A.M., and Litzgus, J.D. (2014). Injury rates of freshwater turtles on a recreational waterway in Ontario, Canada. *Journal of Herpetology, 48(2)*, 262-266.

Bhateria, R., and Jain, D. (2016). Water quality assessment of lake water: a review. *Sustainable Water Resources Management, 2,* 161-173.

Bolam, S.G., Garcia, C., Eggleton, J., Kenny, A.J., Buhl-Mortensen, L., Gonzalez-Mirelis, G., van Kooten, T., Dinesen, G., Hansen, J., Hiddink, J.G., Sciberras, M., Smith, C., Papadopoulou, N., Gumus, A., Van Hoey, G., Eigaard, O.R., Bastardie, F., and Rijnsdorp, A.D. (2017). Differences in biological traits composition of benthic assemblages between unimpacted habitats. *Marine Environmental Research, 126*, 1-13.

Bunea, F., Ciocan, G.D., Oprina, G., Baran, G., and Babutanu, C.A. (2010). Hydropower impact on water quality. *Environmental Engineering and Management Journal, 9*(11), 1459-1464.

Burlakova, L.E., Barbiero, R.P., Karatayev, A.Y., Daniel, S.E., Hinchey, E.K., and Warren, G.J. (2018). The benthic community of the Laurentian Great Lakes: Analysis of spatial gradients and temporal trends from 1998 to 2014. *Journal of Great Lakes Research, 44,* 600-617.

Cao, X., Chai, L., Jiang, D., Wang, J., Liu, Y., and Huang, Y. (2018). Loss of biodiversity alters ecosystem function in freshwater streams: Potential evidence from benthic macroinvertebrates. *Ecosphere, 9(*10), e02445.

Coalition of Haliburton Property Owners Association. (2022). About us. *Coalition of Haliburton Property Owners Association.* https://www.cohpoa.org/about-us/.

Dillon, P.J., Clark, B.J., Molot, L.A., and Evans, H.E. (2003). Predicting the location of optimal habitat boundaries for lake trout (*Salvelinus namaycush*) in Canadian Shield Lakes. *Canadian Journal of Fisheries and Aquatic Sciences, 60,* 959-970.

Jones, C.F. (2008). Taxonomic sufficiency: The influence of taxonomic resolution on freshwater bioassessments using benthic macroinvertebrates. *Environmental Reviews, 16,* 45-69.

Kenney, M.A., Sutton-Grier, A.E., Smith, R.F., and Gresens, S.E. (2009). Benthic macroinvertebrates as indicators of water quality: The intersection of science and policy. *Terrestrial Arthropod Reviews, 2,* 99-128.

Ministry of the Environment, Conservation, and Parks. (2018). Water quality and management of lake trout lakes - Haliburton County: 2014 to 2016. *Government of Ontario*. https://static1.squarespace.com/static/5a731fbe914e6b798dad0a38/t/5b75762603ce64e8d58ac947/1534424762475/Haliburton+Lake+Report+All+Sections.pdf.

Menezes, J.P.C., Oliveira, L.F.C., and Salla, M.R. (2019). Metrics of benthic communities and habitat quality associated to different types of land use. *Engenharia Sanitaria E Ambiental, 24*(4), 737-746.

Muller, A., Osterlund, H., Marsalek, J., and Viklander, M. (2020). The pollution conveyed by urban runoff: A review of sources. *Science of the Total Environment, 709,* 136125.

Palmer, M.E., Yan, N.D., Paterson, A.M., and Girard, R.E. (2011). Water quality changes in south-central Ontario lakes and the role of local factors in regulating lake response to regional stressors. *Canadian Journal of Fisheries and Aquatic Sciences*, 68, 1038-1050.

Paterson, A.M., Dillon, P.J., Hutchinson, N.J., Futter, M.N., Clark, B.J., Mills, R.B., Reid, R.A., and Scheider, W.A. (2006). A review of the components, coefficients and technical assumptions of Ontario's Lakeshore Capacity model. *Lake Reservoir and Management, 22(1)*, 7-18.

Poikane, S., Johnson, R.K., Sandin, L., Schartau, A.K., Solimini, A.G., Urbanic, G., Arbačiauskas, K., Aroviita, J., Gabriels, W., Miler, O., Pusch, M.T., Timm, H., and Bohmer, J. (2016). Benthic macroinvertebrates in lake ecological assessment: A review of methods, intercalibration and practical recommendations. *Science of the Total Environment, 543,* 123-134.

Ranjan, R. (2020). Protecting warming lakes through climate-adaptive PES mechanisms. *Ecological Economics, 17,* 106782.

Resh, V.H. (1994). Variability, accuracy, and taxonomic costs of rapid assessment approaches in benthic macroinvertebrate biomonitoring. *Bollettino di Zoologia, 61*(4), 375-383.

Sharma, C.M., Sharma, S., Borgstrom, R., and Bryceson, I. (2005). Impacts of a small dam on macroinvertebrates: A case study in the Tinau River, Nepal. *Aquatic Ecosystem Health and Management, 8,* 267-275.

Sondergaard, M., Jeppesen, E., Lauridsen, T.L., Skov, C., Van Nes, E.H., Roijackers, R., Lammens, E., and Portielje, R. (2007). Lake restoration: successes, failures and long-term effects. *Journal of Applied Ecology, 44,* 1095-1105.

Toronto and Region Conservation Authority (TRCA). 2011. Regional Watershed Monitoring Program: Benthic Macroinvertebrate Summary 2001-2008. 56 pp + appendices.

Tubic, B.P., Popovic, N.Z., Rakovic, M.J., Petrovic, A.S., Simic, V.M., and Paunovic, M.M. (2017). Comparison of the effectiveness of kick and sweep hand net and Surber net sampling techniques used for collecting aquatic macroinvertebrate samples. *Archives of Biological Sciences, 69*(2), 233-238.

Twelve Mile and Little Boshkung Lake Association. (2022). Mission & Focus. *Twelve Mile and Little Boshkung Lake Association.* https://www.tmlb.ca/about-us/mission-focus/.

Usseglio-Polatera, P., Bournaud, M., Richoux, P., and Tachet, H. (2000). Biomonitoring through biological traits of benthic macroinvertebrates: how to use species trait databases? *Hydrobiologia, 422/423,* 153-162.

Valdor, P.F., Gomez, A.G., Juanes, J.A., Kerleguer, C., Steinberg, P., Tanner, E., MacLeod, C., Knights, A.M., Seitz, R.D., Airoldi, L., Firth, L.B., Crowe, T., Chatzinikolaou, E., Smith, A., Arvanitidis, C., Burt, J.A., Brooks, P.R., Ponti, M., Soares-Gomez, A., Ovejaro, A., and Mendez, G. (2019). A global atlas of the environmental risk of marinas on water quality. *Marine Pollution Bulletin, 149*, 110661.

Withers, P.J.A., Jordan, P., May, L., Jarvie, H.P., and Deal, N.E. (2014). Do septic tank systems pose a hidden threat to water quality? *Frontiers in Ecology and the Environment, 12*(2), 123-130.

Appendices

Appendix A: Map of Sampling Site

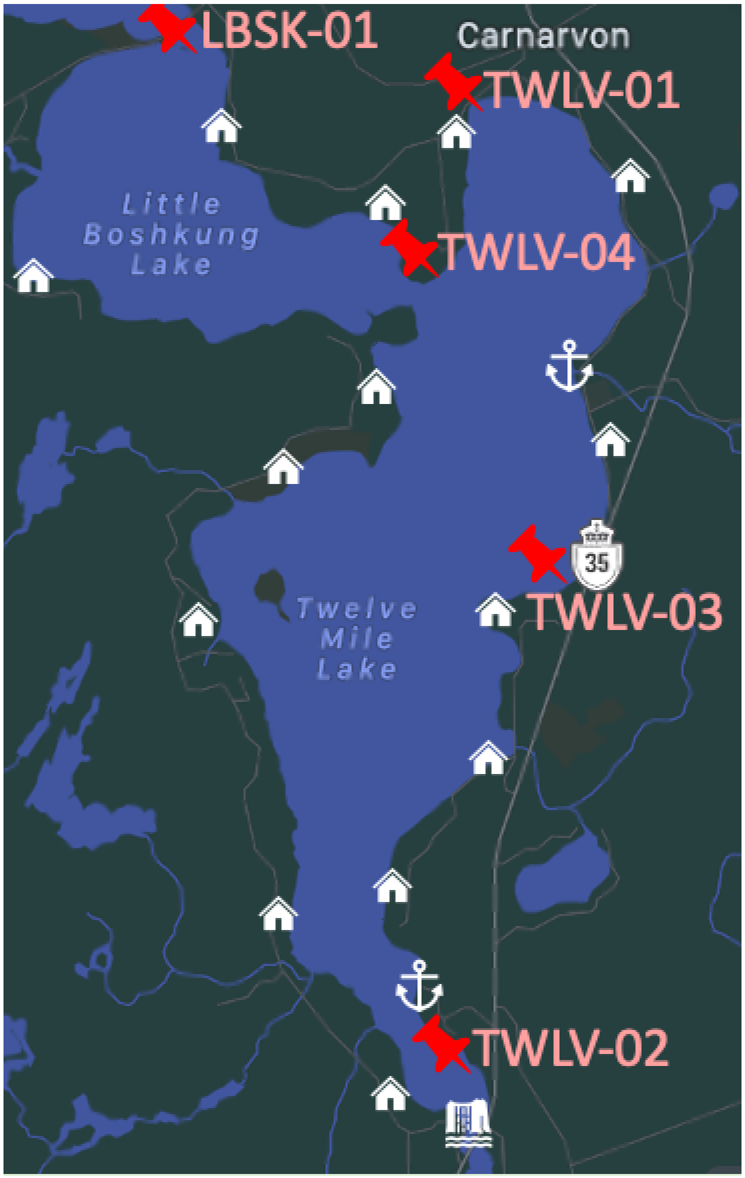

Figure 1. Map of Twelve Mile and Little Boshkung Lakes near Carnarvon, ON. Sampling sites (highlighted in red) are noted in relation to major human inputs (where houses = residential areas, anchor = marinas/public boat launches, grey lines indicate roads, and the waterfall icon = dam).

Appendix B: Raw Data

Table 1. Raw data counts of the community composition of benthic communities in Twelve Mile and Little Boshkung Lakes.

Lake	Twelve Mile								Little Boshkung	
Sample	TWLV-01		TWLV-02		TWLV-03		TWLV-04		LBSK-01	
Replicate	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
Nemotoda (roundworms)					1					
Oligochaeta (aquatic earthworms)			1				1	1		
Pelecypoda (clams)	10	7			8	8	13	3	4	5
Amphipoda (scuds)	48	60	70	56	17	18	54	49	50	52
Trombidiformes-Hydracarina (mites)			2		1			4		
Ephemeroptera (mayflies)	1		1	5	2		5	10	6	13
Anisoptera (dragonflies)				2			1		2	1
Zygoptera (damselflies)			1	3			1	2		
Megaloptera (fishflies, alderflies)							1			
Trichoptera (caddisflies)			1		1			1	2	
Lepidoptera (aquatic moths)								1		
Coleoptera (beetles)							10	14	3	
Gastropoda (snails, limpets)		2	4	1	10	2	4	1	26	6
Chironomidae (midges)	33	28	7	14	36	69	9	7	4	15
Ceratopogonidae (no-see-ums)	6	4	1		6			1		
Misc. Diptera (misc. true flies)						3				3
Isopoda (sow bugs)	2	1	13	18			2	8	3	3
Replicate total	100	102	101	99	82	100	101	102	100	98
Total # of benthics	985									

Table 2. Raw data of water chemistry measurements along with observations of dominant substrate, vegetation conditions, and other sample site descriptions from Twelve Mile and Little Boshkung Lakes, 2021.

Lake	Twelve Mile				Little Boshkung
Sample	TWLV-01	TWLV-02	TWLV-03	TWLV-04	LBSK-01
Water temperature (℃)	16.8	16.5	16.3	17.5	17.0
DO (mg/L)	8.56	8.25	9.36	8.73	8.95
Conductivity (µS/cm)	3	40.0	70.5	33.6	32.7
pH	8.1	8.4	7.58	7.69	7.85
Dominant substrate	sand	silt	sand	clay	silt
Second dominant substrate	gravel	sand	silt	cobble	clay
Woody debris	present	present	absent	present	present
Detritus	present	present	present	present	present
Macrophytes - emergent	present	present	absent	absent	present
Macrophytes - rooted floating	absent	absent	absent	absent	present
Macrophytes - submergent	present	present	absent	present	present
Macrophytes - free floating	absent	absent	absent	absent	absent
Algae - floating	absent	absent	absent	absent	absent
Algae - filamentous	absent	absent	absent	absent	present

Algae - attached	absent	present	absent	present	present
Comments	- beach	- just south of marina - lots of garbage waste at site	- south of public beach - downhill from highway 35	- south of residential / cottage area - boulders along shoreline	- east of bridge - lots of empty snail shells