2022-2023

Benthic Macroinvertebrate Assessment of Koshlong Lake

Prepared for The Koshlong Lake Association

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Introduction

Ontario Benthic Biomonitoring Network (OBBN) macroinvertebrate-based sampling used in this study represents an effects-based approach to understanding ecosystem health as opposed to stressor-based sampling. A stressor is defined as anything that causes strain in an ecosystem. As such, a stressor-based approach would entail identifying what stressors could be present in an environment, what parts of the ecosystem may be sensitive to them, and what pathways facilitate interaction between stressor and organism (Dubé & Munkittrick, 2001). Stressor-based approaches have been used to great effect throughout history, but one of their limitations is that they presume that all stressors in the project are known and their pathways are fully understood. This may lead to an inaccurate analysis of an ecosystem if other stressors remain undetected by researchers, or if the stressors react in unexpected ways in the studied environment.

Alternatively, an effects-based or 'bioassessment' approach can detect adverse effects on an ecosystem even when the pollutant is unknown. This approach is reminiscent of the historic use of canaries in coalmines to detect the presence of poisonous gasses. In this study, the bio-indicators are benthic invertebrates, but studies have used all manner of species as bioindicators ranging from lichens to lizards. For example, Carbal (2010) concluded that indoor fungus can be used as an indicator of the air quality in a given building. Testing water for chemical properties such as pH or phosphorus availability in a stressor-based study often gives researchers an understanding of the ecosystem at the point in time at which they sample it. This means that data may need to be collected over the course of the year to understand if the data collected is indicative of the 'norm' for that given ecosystem. Another benefit of an effects-based approach is that the abundances of macroinvertebrate species give a clearer understanding of what a given ecosystem typically looks like over time than momentary water chemistry values would.

The umbrella category of benthic macroinvertebrate denotes any animal living within the benthic layer of a water system which is large enough to be visible to the naked eye and does not have a spine. OBBN protocol includes the categorization of benthic macroinvertebrate into a 27 group classification of different types of invertebrates. These classifications range across Phylum, Class, Order, and Family levels of taxonomy, with Phylum being the broadest and family being the most specific. For example, all Phylum Coelenterata (or hydras) are included under the same group, while dragonfly and stonefly nymphs are separated during identification despite being included under the same Phylum of arthropod. Notably, smaller creatures like Cladoceran (commonly known as water flea) which are classified as zooplankton often turn up in benthic samples, but are not included in the 27 group taxonomy.

This 27 group taxonomic classification is important because identifying the invertebrates to the species level would not only be more time consuming, but would also make it much more difficult to compare data between reference and study sites. OBBN protocol uses a "reference condition approach" to substitute before/after disturbance data with suitable reference sites, and so comparability across sites is integral to analysis. This reference condition approach is important because without a suitable reference site with minimal disturbance it is impossible to know what a 'healthy' population of benthic invertebrates looks like. The 27 group taxonomy is a compromise between specificity and comparability, allowing study sites to be compared to reference sites to understand if their population displays normal and healthy characteristics.

Within the 27 group taxonomy, some taxa are more sensitive to different stressors than others are. This means the composition of the benthic invertebrate population can be used not only to assess the overall health of the ecosystem, but also to loosely infer what particular stressors may be present. Daphnia or 'water flea' are likely to be present at the sites sampled in this study and are more sensitive than most invertebrates with the exclusion of a few species of Amphipoda (scuds) and Plecopteran (stonefly) (von der Ohe & Liess, 2009). However, they are classified as zooplankton and not considered part of the 27 group taxonomy, so will not be analyzed alongside the 27 groups of invertebrates in the focus of this study. Ephemeroptera (mayfly) have been observed to have high sensitivity to ammonia, metal pollutants and a plethora of other chemicals (Beketov, 2004). Notably there is a wide range in the sensitivity of Ephemeroptera species, but for the purposes of 27 group OBBN taxonomy, they are considered to be one of the most sensitive taxa (Beketov, 2004). Courtney & Clements (2000), corroborated a variation in sensitivity to pH amongst Ephemeroptera species. Appropriately, if Ephemeroptera abundance are well above the normal range for the region, perhaps further levels of taxonomy are necessary to understand if the Ephemeroptera present are from a species which is more resilient, or if the results truly do indicate a lack of stressors.

Chironomidae or midge larvae, are on the other hand fairly resilient to most stressors relative to most other taxa in the OBBN 27 group taxonomy (Chhor et al, 2020). Accordingly, a sample primarily dominated by Chironomidae may suggest that stressors are present. Isopods (sowbugs) show similar resilience to both metals and organic pollutants (von der Ohe & Liess, 2009). Taxa Gastropod (aquatic snails) and Bivalvia (clams and mussels) show an average sensitivity to metal pollution, despite being remarkably resilient to organic stressors (von der Ohe & Liess, 2009). Thus, benthic macroinvertebrate community can suggest what types of stressors may be present.

Lake History

Koshlong Lake is a body of water in the Haliburton Highlands where the partner organization of this project is based: the Koshlong Lake Association. The Koshlong Lake Association represents over 220 shoreline properties and was formed in 1963 (Koshlong Lake Association, 2022). They state their goal as: "to protect, preserve and enhance Koshlong Lake and its watershed for quality use by future generations" (Koshlong Lake Association, 2022). They strive to achieve this through education and information distribution, promotion of safe practices, and environmental protection action "including but not limited to participation in the Lake Partner Program for lake water quality testing" (Koshlong Lake Association, 2022).

There is currently little existing scientific literature regarding Koshlong Lake. In a Ministry of the Environment (1973) report titled "Enrichment status of thirty-five lakes in the Haliburton Highlands region", Secci disk depth and water Chlorophyll content. A Secci disk is an instrument comprised of a metal disk with alternating quarters of black and white paint on its face. The disk is attached to a marked rope and lowered into the water to the depth that it cannot be seen, and that depth is recorded and interpreted as an indicator of turbidity or visibility. Algae growth can influence turbidity, and so examining both turbidity and chlorophyll can indicate if algae is influencing the observed turbidity or if other factors are present. Values in the study were collected from late June to early September, and it was concluded that Koshlong lake had a mean Secci disk depth of 5.7 metres, and a mean chlorophyll (µg/L) value of 2.0. The Secci disk and chlorophyll values for Koshlong Lake collected in 1973 by the Ministry of the Environment were indicative of a mesotrophic environment: containing a normal moderate amount of dissolved nutrients. Had the water been more turbid and the chlorophyll content been higher the environment might have been considered eutrophic.

Another study examined the distribution of crayfish in Southern Ontario lakes, and concluded that Koshlong Lake was the only lake of all examined to have only the species of Cambarus robustus: Robust Crayfish present (Berrill, 1978). However, Koshlong Lake was not the only lake in the study to have only a single species of crayfish present in the samples. Researchers noted that the Robust Crayfish thrive in the rocky fringes of shield lakes, suggesting rocky environments are readily available in the Koshlong Lake ecosystem.

In previous years the Koshlong Lake Association has monitored lake water quality partnering with the Federation of Ontario Cottagers Association and the Ministry of the Environment, taking part in an initiative spanning across the province and over 500 inland lakes (Koshlong Lake Association, 2022). This Lake Partners Program assessment entailed monthly summer measuring of water depth and clarity using a Secchi disk, and taking water samples to send away for lab analysis of phosphorus concentration.

Purpose

The Koshlong Lake Association are interested in monitoring the lake ecosystem to ensure its continued sustainable use. Their website notes Koshlong Lake's role flowing into the Burnt River Watershed, which in turn feeds into the Kawartha Lakes watershed further south (Koshlong Lake Association, 2022); degradations in water quality in Koshlong Lake could potentially impact numerous lakes further downstream in the water system. Furthermore, Koshlong Lake is similar to other lakes in the Haliburton region in that its primary uses entail tourism, boating, and recreation for cottagers. The location of the YMCA Camp Wanakita is also a prominent establishment on the lake which depends on good ecosystem health for its continued operation. As such, sustainable use and continued health of the lake are integral not just for the longevity of its use, but also for the ecosystem services it provides to the local economy.

Key questions established in the project agreement of this study include: What is the baseline composition of the benthic communities of Koshlong Lake? Does the benthic community represent healthy habitat and water quality? How does it compare to other lakes in Haliburton County? What changes to sampling locations and protocol should be implemented in subsequent years of sampling?

Community Concerns

The Koshlong Lake Association has outlined several environmental concerns in a "threats to lake health" section on their website. Concerns were highlighted regarding the impacts of shoreline development and septic leeching, as anthropogenic actions which could alter lake health and benthic community composition (Koshlong Lake Association, 2022). As comparing septic leaching to the health of the benthic invertebrate community is difficult without adding extra investigative steps like surveying of cottage properties, this study will not be including analysis of septic leaching. Sampling sites were chosen to reflect shoreline variation, but conclusions based on the correlation between ecosystem health and shoreline may require the addition of more testing locations with more variation in altered shorelines. Climate change and invasive species were also included as threats to lake health, but fall beyond the primary scope of the project. Blue/green algae was another key concern identified because of its ability to bloom in eutrophic conditions and negatively impact human and canine health when consumed, even if the water is boiled (Koshlong Lake Association, 2022).

Research Methods and Protocols

All samples were taken using modified OBBN methodology at as outlined by Fischer, Porter, Solti. (2022) in the Woodlands and Waterways EcoWatch Aquatic Monitoring Protocol Manual. Samples were collected at Koshlong lake on October 14th, 2022, between 10 AM and 2:40 PM. Site characteristics were recorded regarding algae, macrophytes (aquatic vegetation), primary and secondary substrate, riparian vegetation, and detritus/woody debris. Next, water chemistry readings were recorded. Dissolved Oxygen (DO), water temperature, conductivity, and pH were recorded with multi-probe field instruments. Latitude and longitude coordinates for sample sites were recorded with a GPS unit.

Lastly, the benthic invertebrate samples were taken using the OBBN kick and sweep method. The sampler waded out into the water until they reached a water depth of 1 metre or the closest depth possible. The distance of the sampler from the shoreline was recorded. The sampler kicked up the lake bottom, disrupting the benthic layer to a depth of 5cm, while using a d-net with a mesh size of 500 microns to constantly scoop and sweep back and forth just above the lake bottom. The sampler slowly made their way back to the shoreline, kicking and sweeping for a total time of 3 minutes, and only removing the net from the water when the time was up and they were at the shoreline. Samples were rinsed in the d-net to remove fine sediment (silt, clay). Samples were stored in jars, and for sites where there was too much material to fit, two jars were used for one sample. A second replicate sample was taken two metres along the shoreline from the first sample. Sampling protocol was repeated for each of the four sample sites, KOSH-01, KOSH-03, KOSH-04, KOSH-06. See Appendix B Figure 9 for sample site locations. Site Codes follow the formula: Lake name, site number, replicate number. At the end of the day, all samples were strained using a 500-micron sieve to remove lake water from the sediment and invertebrates, and were filled with non-toxic antifreeze as a preservative. Straining the samples was important so the antifreeze preservative was not diluted by lake water.

The main deviation from intended methodology was the use of paper to record data in the field. Originally, the plan was to enter data into a tablet device, but upon arrival at sample sites the tablet would not function as intended. Data was later entered into the OBBN database and digitized for the purposes of this study's analysis in Microsoft Excel. This data entry method did not alter any of the data values for this project, and simply added an extra step to data analysis.

Lab analysis of the benthic invertebrate samples took place in the later months of 2022 at the facilities of Trent University. Each sample jar was sorted by mixing up the sediment and preservative in the jar and taking a tablespoon worth at a time onto a white dissection tray. Care was taken to sample in an un biased way: larger and more visible or floating specimens were not prioritized. The tablespoons worth of sample was picked through without visual aid, and collected invertebrates were identified with the aid of a dissection microscope. Taxonomy followed the OBBN 27 group system, in which invertebrates are identified into 1 of the 27 groups of varying levels of the taxonomic hierarchy.

Sample jars for each replicate were sorted to the first 100 invertebrates. If 100 invertebrates had been reached but the current tablespoons worth of sample had not been fully examined, it was completely dissected even if it put the invertebrate count past 100. Identified invertebrates were preserved in vials of 70% Ethanol Alcohol and handed over to Ulinks staff for their taxonomy to be verified. The verified benthic data was analysed and indices were calculated in Google Sheets.

Data Analysis Methods

%EOT measures the percentage of Ephemeroptera (Mayfly), Odonata, and Trichoptera (Caddisfly), all pollution sensitive taxa, found at a site. The label of Odanata includes Anisoptera (dragonfly) and Zygoptera (damselfly). The EOT index is the proportion of the invertebrate belonging to these taxa to the population of the whole sample. The ratio can be a good indicator of pollution levels. EPT (Ephemeroptera, Plecoptera, and Trichoptera) is a similar index commonly used in the analysis of streams, but EOT is used here because Plecoptera (stonefly) thrive primarily in streams rather than lake systems, where Odonata species are more common. As such, in an environment like this an %EPT score may be lower than an %EOT score, potentially resulting in a misleading analysis of ecosystem health and stressors.

$$Percent\ EOT = \frac{Total\ individuals\ in\ EOT\ taxa}{Total\ sample\ population\ size} * 100\%$$

Simpson's Diversity Index compares the number of groups present at a site and their abundances to the number of individual organisms as a measure of the biotic diversity of a population. Values range from 0 to 1, with 0 indicating absolutely no biodiversity and 1 indicating infinite diversity. Accordingly, a higher value indicates higher biodiversity and a healthier ecosystem (Fischer, Porter, Solti. 2022).

$$Simpson's\ Diversity\ Index\ (D) = 1 - (\frac{\sum n(n-1)}{N(N-1)}$$

n = the number of individuals of a given species in a sample
N = the total number of individuals in the sample population

Shannon Wiener Diversity Index is a biotic diversity index similar to Simpson's. However, Simpsons Diversity Index places a higher weight on dominant species and is often described as a "dominance index" (University of Florida, n.d.). Because of this, Shannon Wiener is preferred by some institutions and is proposed as being a more accurate depiction of diversity.

$$Shannon\ Weiner\ Diversity\ Index\ (H) = -\sum Pi(lnPi)$$

P_i = Proportion of each species within entire sample

Results

Benthic Data

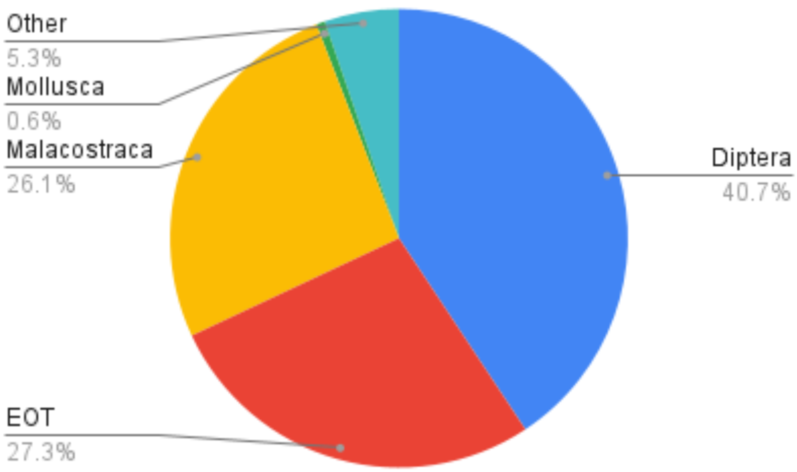

Figure 1: Average percent composition across all sites on Koshlong Lake.

The average most dominant taxa by far were Diptera with a value of 40.7%, a group which includes Chironomidae, Tabanidae, Culicidae, Ceratopogonidae, Tipulidae, Simuliidae and other Miscellaneous Diptera. In the Diptera group Chironomidae were the most abundant taxa. The second most abundant group was EOT at 27.3% closely followed by Malacostraca at 26.1%. "Other" was the least abundant group at 5.3%, indicating Colenterata, Turbellaria, Nematoda, Olegochaeta, Hirudinea, Hydracarnia, Plecoptera, Hemiptera, Megaloptera, Lepidoptera, and Coleoptera.

Water Chemistry and Vegetation

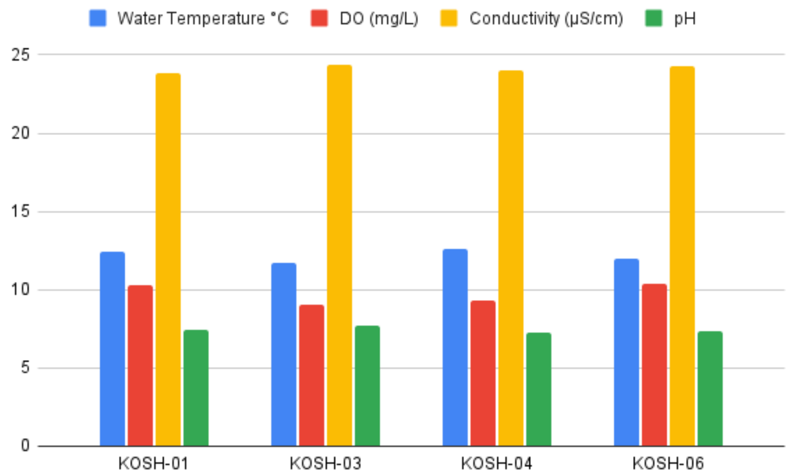

Figure 2: Water chemistry for all sites on Koshlong Lake (indicating replicate sites is unnecessary testing was only conducted once at each location).

The water chemistry data shows similar values between sites for all aspects of water chemistry examined: water temperature, Dissolved Oxygen (DO), conductivity, and pH. DO is highest at site KOSH-06 with a value of 10.35 (mg/L), which is closely followed by KOSH-01 with a value of 10.26. pH is highest at site KOSH-R3 with a value of 7.66.

Site KOSH-01-R1 is a cottage front beach with a sandy shoreline and a dominant substrate material of sand with an abundance of pine needles in the benthic layer. The KOSH-01-R2 replicate on the other hand is primarily a steep cobble substrate at the shoreline, transitioning to sand moving away from the shoreline. Riparian zones transition from no vegetation, to grass, to forest. KOSH-03 replicates are both located in a much narrower portion of the water system with abundant submergent macrophytes and the forest beginning right at the shoreline. The benthic substrate was primarily silt-based. KOSH-04 replicates are located on a wide stretch of sandy beach with present submergent macrophytes and scrubland transitioning to forest riparian zones. KOSH-06 replicates are located in an inlet which had shrunk considerably by late season October with the lowering of the water level. The benthic layer was primarily composed of silt and submergent macrophytes were present.

Table 1: Vegetation data for all sample sites on Koshlong Lake. Color coding is not representative of "normal" or "healthy" vegetation expectations and is only present to make the table more visually readable.

	KOSH-01-R1	KOSH-01-R2	KOSH-03-R1	KOSH-03-R2	KOSH-04-R1	KOSH-4-R2	KOSH-06-R1	KOSH-06-R2
Macrophytes - Emergent	0 - absent	0 - absent	0 - absent	0 - absent	1 - present	1 - present	0 - absent	0 - absent
Macrophytes - Rooted Floating	0 - absent	0 - absent	0 - absent	0 - absent	0 - absent	0 - absent	0 - absent	0 - absent
Macrophytes - Submergent	0 - absent	0 - absent	2 - abundant	2 - abundant	1 - present	1 - present	1 - present	1 - present
Macrophytes - Free Floating	0 - absent	0 - absent	0 - absent	0 - absent	0 - absent	0 - absent	0 - absent	0 - absent

Benthic Data Indices

Percent EOT

Table 2: Percent EOT ranges for the Haliburton Region. Values provided by Fischer, Porter, Solti (2022) in the Woodlands and Waterways EcoWatch Manual.

Typical	Between 4.18 and 37.12
Atypical	Between 2.62 and 4.18 Between 37.12 and 54.41
Extremely Atypical	Less than 2.62 and greater than 54.41

Table 3: Colour coding legend for %EOT figure

EOT colour grading key		
Extremely Atypical	Atypical	Typical

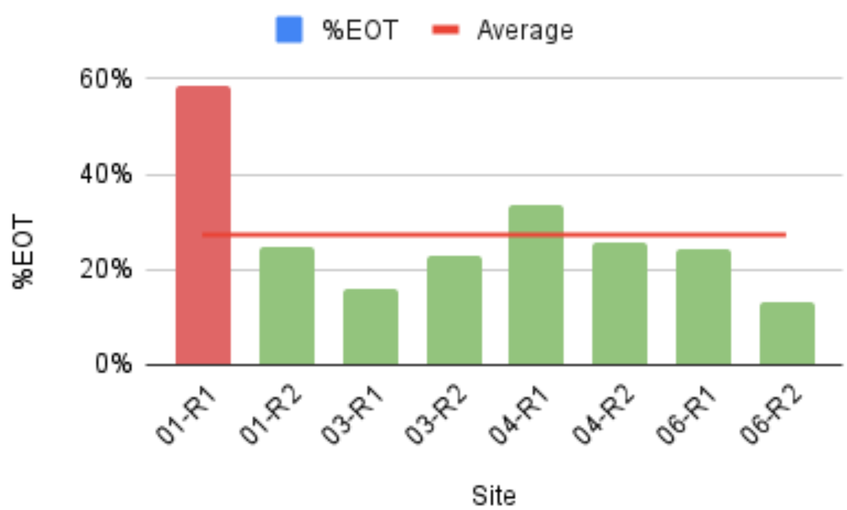

Figure 3: Percent EOT at Koshlong Lake across all sites and replicates.

Values for percent EOT are highest at 01-R1 with a value of 58.4% and KOSH-04-R1 with a value of 33.3%. Values are lowest at 03-R1 and 06-R2 with values of 16.0% and 13.0% respectively. All sites but KOSH-01-R1 fall into the range of typical EOT for the Haliburton region. None of the sites fall within the lower range of atypical or extremely atypical EOT abundance. The average EOT value is 27.3%, which falls within the typical range for Haliburton.

Simpson's Diversity Index

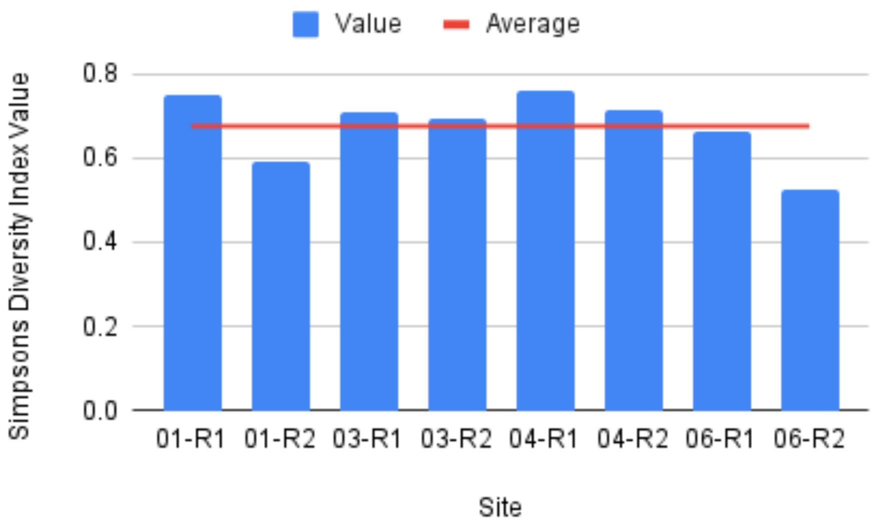

Figure 4: Simpsons Diversity Index for all Koshlong Lake sample sites.

All sites had values of over 0.6 with the exception of 01-R2 and 06-R2, with 04-R1 having the highest Simpsons Diversity Value of 0.76. KOSH-03 sites were the most homogenous within replicates, with a difference in value of only 0.02. The average Simpsons Diversity Index value is 0.68.

Shannon Wiener Index

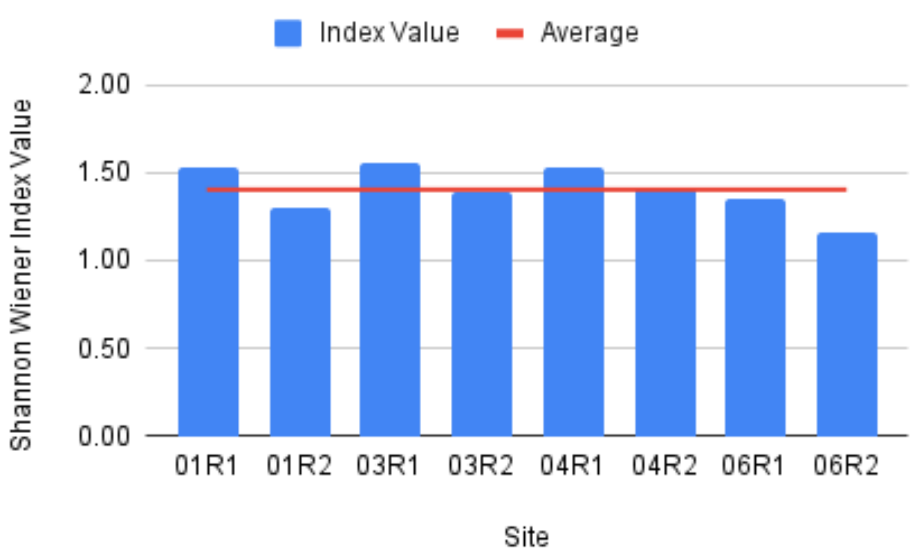

Figure 5: Shannon Wiener Index values for all Koshlong Lake sample sites.

Shannon Wiener Index displays similar visual trends as Simpsons Diversity index, although the 03-R1 appears to have a higher value relative to other sites in Shannon Wiener Index.

Discussion

Water Chemistry and Vegetation

Dissolved Oxygen (DO) values for Koshlong Lake vary between 9.06 mg/l and 10.35 mg/l with an average value of 9.76 mg/l. This is slightly higher than the average DO value of 9.34 mg/l for Halls Lake; a slightly larger lake in the Halibutron Highlands area (McBain. 2020). Koshlong lake also has a higher average DO value than the 8.58 mg/l recorded at Big Hawk Lake in 2021 which is another lake in the region (Schweighardt, 2021). Interestingly, site KOSH-03 with the lowest DO was also the site with the most abundant submergent macrophytes. pH data ranged from 7.23 to 7.66, which is well within the habitable pH range of 7.0-9.0 recommended for the majority of fresh water fish by the Michigan Sea Grant (2023). Koshlong Lake had an average pH of 7.40, which is more acidic than Big Hawk Lake which had an average pH value of 7.68 (Schweighardt, 2021).

The Koshlong Lake average water temperature was 12.2^{o}C, which is colder than the average temperature of 17.8°C Big Hawk Lake (Schweighardt, 2021). Both lakes were sampled in late October, however Koshlong lake was sampled in 2022 and Big Hawk Lake was sampled in 2020, which may have been a colder year. The coldest temperature at Koshlong Lake was at KOSH-03 which was the narrow river site, and the warmest temperature was at KOSH-06, the shallow inlet site. The heightened temperature at KOSH-06 may be because of its shallower depth; 06-R1 was the only site where a maximum depth of 100cm could not be met, and so samples were taken at a depth of 66cm.

Conductivity is influenced by the presence and abundance of dissolved solids like Phosphate ions and Aluminum cations, but is also influenced by temperature (EPA, 2012). As the temperature of water increases, so does the conductivity. However, as the highest and lowest site values for conductivity and temperature do not align at the same sites and do not appear to have a linear relationship. This suggests that the conductivity in Koshlong Lake is influenced more by dissolved solids than by regional differences in temperature across the lake. Water conductivity at Koshlong Lake was an average of 24.13 (µs/cm), lower than 32.5 (µs/cm) at Big Hawk Lake in 2021 (Schweighardt, 2021). All sites at Koshlong Lake were devoid of free-floating vegetation and floating algae. Subsequent years of data should tell if that is indicative of normal conditions in October.

Benthic Data Analysis

Ephemeroptera (mayfly) have been observed to have high sensitivity to ammonia, metal pollutants and a plethora of other chemicals (Beketov, 2004). Their abundance in “typical” levels expected in the Haliburton area as evidenced by the %EOT index suggests that these types of pollution are at low levels in Koshlong lake. %EOT in Koshlong lake was at average 27.3%. Finding other lakes in the region to compare %EOT to is difficult because many other studies have opted to use an EPT calculation for the index rather than EOT. However, average %EOT was only 4.7% at Twelve Mile Lake and 12.1% at Little Boshkung Lake, both values that Koshlong Lake surpasses. Furthermore, as most values at Koshlong Lake remain within the range of “typical” for the Haliburton region, EOT would suggest levels of stressors typical for the region. However, KOSH-01-R1 %EOT is abnormally high.

There is a wide range in the sensitivity of Ephemeroptera species, but for the purposes of 27 group OBBN taxonomy, they are considered to be one of the most sensitive taxa (Beketov, 2004). Courtney & Clements (2000), corroborated a variation in sensitivity to pH amongst Ephemeroptera species. As Ephemeroptera abundance for KOSH-01-R1 is extremely atypically high for the region, perhaps further levels of taxonomy are necessary to understand if the Ephemeroptera present are from a species which is more tolerant, or if the results truly do indicate a lack of stressors at that site. However, many of the samples should have been rinsed more heavily when sampling, and were very silty and murky when preserved and picked. Ephemeroptera are relatively large and pale coloured taxa which are easier to see with the naked eye compared to smaller and darker taxa like Ceratopogonidae and Chironomidae, especially in dark and silty samples. Considering this, it is a more likely possibility that the unusually high EOT score for sample at KOSH-01-R1 is influenced by a sampling visual bias, as that sites sample seemed to have the most sediment while picking in the lab.

On the other hand, Chironomidae (midge larvae) are fairly resilient to most stressors relative to most other taxa in the OBBN 27 group taxonomy (Chhor et al, 2020); a sample entirely dominated by Chironomidae may suggest that stressors are present. While Chironomidae are abundant in the Koshlong Lake samples with an average of 39% composition, their abundance compared to other lakes in the area should be taken into consideration. Twelve Mile Lake had an average percent Chironomidae of 25% (Jamieson, 2022), and as such the Chironomidae proportion at Koshlong Lake may simply be indicative of normal conditions for the region.

Isopods (sowbugs) show a similarly high level of resilience to both metals and organic pollutants (von der Ohe & Liess, 2009). They are absent from all sites at Koshlong lake, which was a surprising test result. This is especially strange as Isopod represented a considerable portion of the benthic population at Big Hawk Lake, which had a high average Isopod percentage of 23.84% (Schweighardt, 2021). Twelve Mile Lake had a much smaller percent population of Isopods at 5.08% (Jamieson, 2022). Subsequent years of study should monitor if Isopods are absent at Koshlong lake. Isopods are similarly resilient to most stressors as Amphipods (which are present at Koshlong Lake) according to Hilsenhoff tolerance values (Hilsenhoff, 1988). As Amphipods are abundant at Koshlong Lake, if the absence of Isopod in samples continues in subsequent years of study, perhaps an unforeseen factor or predator is at play influencing Isopod population.

Misc. Diptera were only found at Site KOSH-03-R1, one of four sites with “abundant” detritus (KOSH-01-R1/2, KOSH-03-R1/2), with the other four sample sites (KOSH-04-R1/2, KOSH-06-R1/2) having “present” detritus. However, without the presence of Misc. Diptera at the other “abundant” detritus sites, it is unlikely that detritus is the primary factor influencing the distribution of Misc. Diptera in the lake. Similarly, the sites with the highest percent EOT composition tended to have a sandy primary substrate. As the four highest %EOT values are at sites 01-R1/R2 and 04-R1/R2 this may suggest benthic ecosystems with sandy bottoms are healthier in Koshlong Lake. However, as the difference in %EOT between 01-R2 (sandy) and 06-R1 (silty) is only 0.22%, further years of data will be needed to support this connection, and other larger factors may be shaping this apparent relationship. Furthermore, there do not appear to be any clear trends between low %EOT and substrate, as sites with low %EOT are split between primary substrate of silt and cobble.

Simpson's Diversity Index at Koshlong lake averages at 0.68 which is similar to other lakes in the region. Little and Big Hawk lakes both came in with the same slightly lower diversity score at 0.63 (Schweighardt, 2021). Twelve Mile lake also had an average Simpsons Diversity Index of 0.63 (Jamieson, 2022). Halls Lake was also in a similar range with a rounded average value of 0.6 (McBain, 2020). This data suggests that Koshlong Lake surpasses its peers with regards to diversity by a thin margin. Shannon Wiener Index values display similar trends between sites, with the primary exception of 03-R1 having higher relative to other sites in Shannon Wiener compared to Simpsons. As Simpson's Index is often considered a "dominance index" (University of Florida, n.d.), this minimal discrepancy suggests that there is a lower level of domination at Koshlong Lake, and a slightly more diverse ecosystem at KOSH-03-R1.

Perceived State of the Lake

At least five years of consecutive data are needed before meaningful trends can be identified. However, EOT and Simpson's Diversity Index for this year's data meet or surpass values for lakes in the area in previous years. As such, the results of this first year of study suggest that Koshlong Lake is a healthy ecosystem with low levels of stressors. If the results after the conclusion of five years study appear to be in good standing, sampling schedule can be revised to an as-needed approach or with gap year(s).

Zooplankton

As previously mentioned, zooplankton are not the primary focus of this study: benthic macroinvertebrates are. However, it is worth noting the presence of Spiny Waterflea; an invasive zooplankton introduced into The Great Lakes through the ballast water of trans-Atlantic ships in the 1980s (Hay, 2016). Their negative impact on ecosystems relates to their long spiny tail, which make it difficult for indigenous fish species to prey on them, allowing them to outcompete indigenous water flea. Three individuals of Spiny Waterflea were identified at KOSH-06, and were present in both of the site's replicates. See Appendix A figure 6.

Within this year's data it cannot be verified that Spiny Waterflea were not present in other regions of the lake, because they may have remained unseen while sample picking. As such, perhaps it would be useful for future years of this study to monitor the presence of Spiny Waterflea, within the established system of OBBN samples which are processed in tablespoon sized subsamples. This would allow their abundance relative to macroinvertebrate species to be understood, and would likely take little extra effort on the part of samplers. Although there does not appear to be previous record of their presence in Koshlong Lake, they are known to be present in the Haliburton region (Hay, 2016). Preventative action can be taken to limit the spread of Spiny Waterflea, but their discovery is likely not a cause for great concern.

Spiny Waterflea typically spread to other lakes on boats, anchor chains, nets, fishing line and tackle (Hay, 2016). They can often form clumps on fishing equipment, resembling a mass of gelatin. Drying through air exposure is likely the most efficient way for lake users to limit the spread, as exposure to air for longer than six hours will normally kill Spiny Waterflea eggs

(Branstrator et al, 2013). The Lake Champlain Basin Programme (2014) recommends a system of "clean, drain, and dry"; letting any equipment dry for at least five days while switching water systems or lakes, and washing equipment with hot water if drying for that duration is not possible. The Georgian Bay Biosphere (2020) recommends power washing with at least a pressure of 250 PSI and hot water. However, some sources dispute the effectiveness of heat against Spiny Waterflea and drying in direct sunlight appears to be the consensus of the most effective way to prevent spread (Hay, 2016). Another common recommendation is to limit use to one lake per day to make drying periods easier to manage.

Microplastic

As with zooplankton, microplastics are not a prime focus of this study but may be useful metadata in this paper in leu of the conclusions that multiple years of study can provide. Some samples of brightly coloured fibres (Appendix A Figure 7) which resembled microplastics and blue objects in the shells of several caddisfly larvae were discovered at KOSH-01 (Appendix A Figure 8). Unfortunately, these samples were not preserved for further analysis, and photographs are the only evidence of their presence. Burn tests are commonly used to determine if a fiber is plastic, and without further testing the true composition of fibers cannot be known. However, if the observed fibers and objects were microplastic, their absence in sites other than the cottage front KOSH-01 could suggest that there is some level of microplastic in more cottage dense areas of the lake.

It may be beneficial to save and test fibers found while sorting through benthic samples in subsequent years of study, should it be determined a worthwhile use of time. In any case, a recent study found that the rate of atmospheric deposition of microplastic in the Haliburton-Muskoka region was on average five particles per metre squared per day (Welsh. 2022). It was also noted that lakes in the region retain between 30-45% of plastics introduced through atmospheric deposition. As such, it cannot be known if the level of potential microplastic observed is from local sources or is simply representative of the baseline amount atmospherically deposited into lakes in the region.

Recommendations

The biggest field protocol recommendation for students sampling in subsequent years is to sift the samples while still in the d-net incredibly thoroughly, as sifting of this year's samples wasn't done vigorously enough. This made identifying the invertebrates through the remaining silt difficult the during the picking process. Furthermore, it may be beneficial to monitor benthic samples during the picking process for Spiny Waterflea and microplastic to generate understanding of their abundances in Koshlong lake. Preserving those samples would likely require minimal extra effort during the picking process, if it is decided that those aspects of ecosystem health should be included in future iterations of this study.

While picking through the benthic samples it was useful to use a lamp bent downwards directly above the dissection tray to make smaller taxa like Ceratopogonidae easier to see. It was also useful while transferring samples from the dissection scope tray to the sample preservation vial to dip the picking tweezers back into the water in the dissection tray after coming out of the ethanol filled sample vial. This helped to stop the liquid in the scope tray from swirling around as the ethanol and water quickly mixed, making it easier to grab the invertebrates. These tricks may be useful to other student samplers in the future.

Conclusion

In October of 2022, Koshlong Lake was sampled using Ontario Benthic Biomonitoring Network protocol with the collaboration of the Koshlong Lake Association. The aim of this study was to understand the level of stressors and ecosystem health in Koshlong lake by examining water chemistry and the benthic macroinvertebrate community. The invasive Spiny Waterflea were discovered, but further analysis of the zooplankton is beyond the scope of this project. Proportion of sensitive taxa Ephemeroptera Odanata Tricoptera (EOT) suggests satisfactory ecosystem health for the Haliburton area, as most values are within the “typical” range for the region, and roughly equal or surpass those of other lakes. Diversity Index values at Koshlong Lake are also consistent with what has been observed at other lakes in the region in recent years. These results suggest a healthy ecosystem. This year marks the first year of study, and at least five years of data will be required to understand if these values are indicative of a typical year at Koshlong Lake.

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Appendices

Appendix A: Photos

Figure 6: Spiny Waterflea specimen from KOSH-06-R1 under the dissection scope.

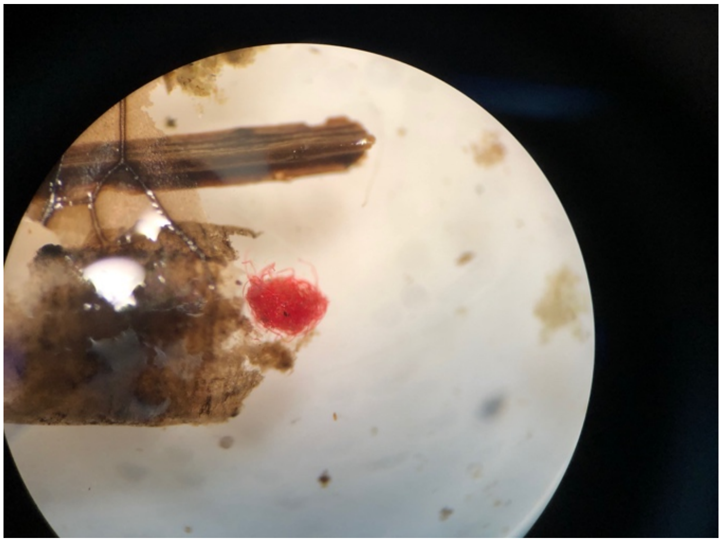
Figure 7: Red filament from sample KOSH-01-R1

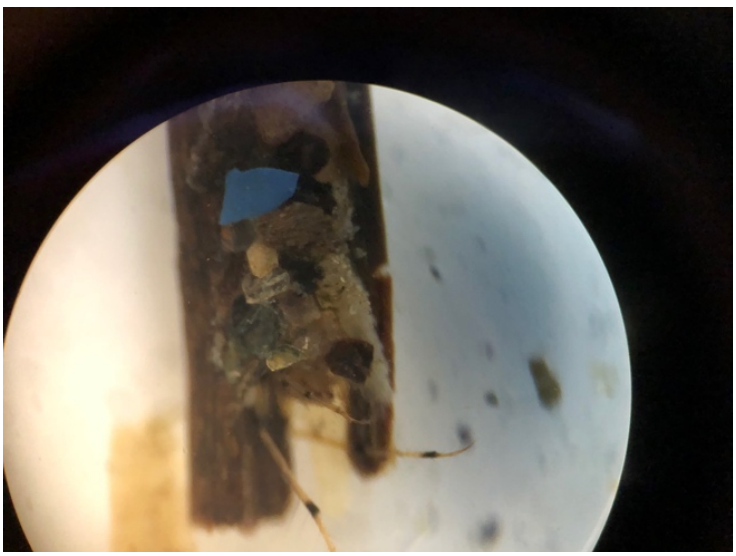
Figure 8: Blue material in the shell of caddisfly from sample KOSH-01-R1

Appendix B: Maps

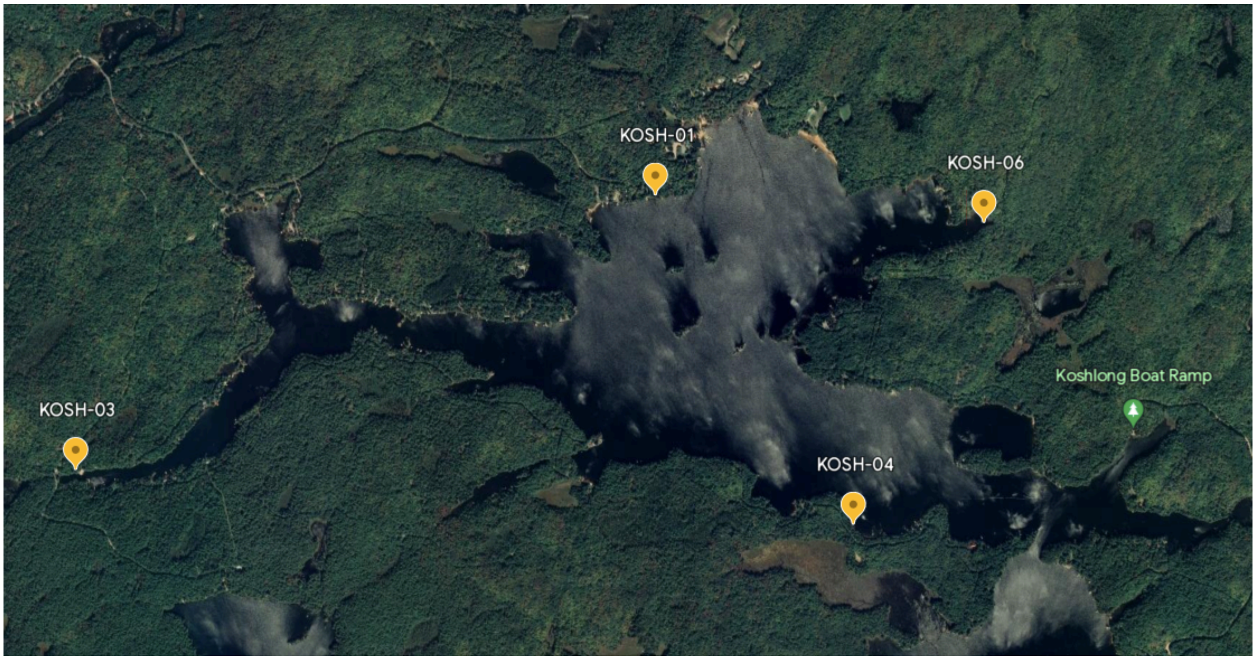

Figure 9: Map of Koshlong Lake sampling locations: KOSH-01 at 44.97725, -78.48864, KOSH-03 at 44.96575, -78.52293, KOSH-04 at 44.96347 -78.4769, and KOSH-06 44.9761 -78.4692.

Appendix C: Tables

Table 4: Raw data for benthic samples across all sites and replicates.

Lake		Koshlong Lake	Koshlong Lake	Koshlong Lake	Koshlong Lake	Koshlong Lake	Koshlong Lake	Koshlong Lake	Koshlong Lake	
Site Code		**01-R1**	**01-R2**	**03-R1**	**03-R2**	**04-R1**	**04-R2**	**06-R1**	**06-R2**	
OBBN Sample ID		KOSH-01-R1	KOSH-01-R2	KOSH-03-R1	KOSH-03-R2	KOSH-04-R1	KOSH-04-R2	KOSH-06-R1	KOSH-06-R2	
Sample Date:		14/10/2022	14/10/2022	14/10/2022	14/10/2022	14/10/2022	14/10/2022	14/10/2022	14/10/2022	
Indices										
Shannon Wiener		1.53	1.30	1.56	1.38	1.52	1.42	1.35	1.16	
Simpson's Diversity Index		0.747	0.593	0.711	0.693	0.759	0.715	0.663	0.524	
# of Unique Taxa		7	8	10	6	7	7	8	10	**Average**
%Diptera		20.79%	65.35%	27.00%	18.10%	30.39%	41.58%	53.77%	68.00%	40.7%
%Malacostraca		16.83%	3.96%	47.00%	48.57%	28.43%	28.71%	19.81%	15.00%	27.3%
%Mollusca		0.00%	0.00%	3.00%	0.00%	0.98%	0.00%	0.00%	1.00%	26.1%
%EOT		58.42%	24.75%	16.00%	22.86%	33.33%	25.74%	24.53%	13.00%	0.6%
%Other		3.96%	5.94%	6.00%	10.48%	6.86%	3.96%	1.89%	3.00%	5.3%
%Worms		0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.0%
	Tolerance Values									**Total**
Coelenterata [Hydras]	5.00									0
Platyhelmintes [Flatworms]	**4.00**									0
Nematoda [Roundworms]	5.00									0
(Oligochaeta) [Aquatic Worms]	8.00									0
(Hirudinea) [Leeches]	**10.00**	1		1		1		1		4
Isopoda [Sow Bugs]	**8.00**									0
(Bivalvia) [Clams]	**8.00**									0
Amphipoda [Scuds]	**4.00**	17	4	47	51	29	29	21	15	213
Decapoda [Crayfish]	**8.00**									0
Acarina [Mites]	**6.00**		1					1		2
Ephemeroptera [Mayflies]	**5.00**	41	16	13	19	26	18	19	9	161
Anisoptera [Dragonflies]	5.00	3	2	2	2		1	2	2	14
Zygoptera [Damselflies]	7.00				3		1		1	5
Plecoptera [Stoneflies]	**1.00**									0
Hemiptera [True Bugs]	**5.00**									0

Megaloptera [Dobson/Alderflies]	**4.00**								1	1
Trichoptera [Caddisflies]	**4.00**	15	7	1		8	6	5	1	43
Lepidoptera [Moths]	**6.00**								2	2
Coleoptera [Beetles]	**4.00**	3	5	5	11	6	4			34
(Gastropoda) [Snails]	**7.00**			3		1			1	5
Chironimade [Midges]	6.00	21	62	23	19	31	42	55	67	320
Tabanidae [Deer/Horse Flies]	6.00									0
Culicidae [Mosquitos]	8.00									0
Ceratopogonidae [No-see-ums]	6.00		4	4				2	1	11
Tipulidae [Crane Flies]	3.00									0
Simuliidae [Black Flies]	6.00									0
Other Diptera	**7.00**			1						1
	Σ =	101	101	100	105	102	101	106	100	816