Bear Brook Watershed Study – Surface Water Quality Characterization Report

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Prepared for:

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Summary of Findings

Protecting water quality in the Bear Brook Watershed requires an integrated approach that addresses both natural and human-induced sources of pollution. The watershed's surface water quality has been shaped by its geological history, particularly the vast clay plains formed by glacial activity and the remnants of an ancient inland arm of the Atlantic Ocean. Geological processes have led to naturally elevated levels of phosphorus, chlorides, and iron in the watershed's surface water; elements that, while essential in small quantities, can significantly influence water quality and ecosystem health.

- Historical settlement patterns, guided by the region's geology, have shaped current land use practices, impacting water quality across the watershed. The high agricultural capability of the clay plains has resulted in approximately 37% of the land area being used for agriculture. This has contributed to increased sedimentation and nutrient loading in surface waters, exacerbating naturally high phosphorus levels and raising the risk of eutrophication and oxygen depletion in local water bodies.
- Urbanization is a significant stressor on the watershed. Urban growth in the headwaters of the Bear Brook Watershed has led to elevated levels of chlorides, nutrients, *E. coli,* and metals, particularly in areas where natural habitats have been disturbed or removed, where vegetative and riparian cover is lacking, and during periods of runoff following precipitation events.
- The Canadian Water Quality Index (WQI) scores for the Bear Brook Watershed reflect frequent impairments in water quality.
- Chloride levels have shown consistent increases across the watershed, with the highest concentrations observed in the more urbanized areas.
- Some headwater tributaries of the Bear Brook exhibited cold to cool water characteristics when sampled between 2020-2024. These areas represent sensitive and less common habitats in the Bear Brook Watershed.
- A DNA-based benthic invertebrate sampling approach was employed and detected 27 species of mayflies, 13 species of stoneflies, 24 species of caddisflies and 2 species of mussels. An excellent baseline of species presence was established.
- Traditional benthic invertebrate sampling was conducted across the study area, identifying sites that range from excellent to poor condition.
- The Black Creek Subwatershed appears to be in excellent condition and can be used as a reference of environmental health for the rest of the Bear Brook Watershed.

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Surface Water Quality Characterization

Water quality monitoring programs establish baseline conditions and detect spatial and temporal changes in streams that may indicate changing environmental and human factors. Studying temporal trends in water quality is a critical component of characterizing and understanding the condition of a water body over time. Analyzing water quality data over time helps identify long-term trends, such as gradual improvements or deteriorations, and helps identify whether new issues are emerging.

Several different indicators were used to characterize surface water quality in the Bear Brook Watershed to better understand past and current conditions and trends. Indicators include surface water chemistry, surface water temperature, and benthic macroinvertebrate community assemblages. The analysis of this data aims to achieve the following goals:

1. Establish baseline water quality conditions and identify trends.

By using data from these monitoring programs, baseline conditions can be established and used for future comparisons. This is key to identifying long-term trends and distinguishing between short-term fluctuations and more persistent shifts in water quality.

2. Determine variability between sites.

This data helps to identify spatial variability in water quality across different parts of the Bear Brook Watershed. This includes comparing upstream and downstream sites and assessing the impact of different land uses. By analyzing data from multiple sites, it becomes possible to determine which areas are most affected by specific pollutants or stressors.

3. Identify factors influencing water quality in the Bear Brook Watershed.

To support the development of a future implementation strategy for the Bear Brook Watershed, it is important to identify the key factors that lead to declines in condition. This allows appropriate actions to be recommended and supported.

1. Surface Water Chemistry

Key Findings

- The Canadian Water Quality Index (WQI) scores for the Bear Brook Watershed range from fair to poor, reflecting frequent impairments in water quality due to pollutants exceeding recommended levels.
- Geological processes have led to naturally elevated levels of phosphorus, chlorides, and iron in the watershed's surface water. Current land uses such as agriculture and urbanization are exacerbating high levels through increased sedimentation and nutrient loading, raising the risk of eutrophication and oxygen depletion in local water bodies.
- Chloride levels have shown consistent increases across the watershed, with the highest concentrations observed in the more urbanized areas such as the headwaters of the South Bear Brook and McKinnon’s Creek.

South Nation Conservation operates two long-term water quality monitoring programs within the Bear Brook Watershed. They are the Watershed Characterization Network in partnership with the Ministry of Environment, Conservation and Parks (MECP) and the Baseline Surface Water Quality Monitoring Program in partnership with the City of Ottawa. Both programs routinely sample chemical parameters that have potential impacts on aquatic life, recreational activities, and agricultural practices.

The Watershed Characterization Network has been in operation since 1997, and the Baseline Surface Water Quality Monitoring Program has been in operation since 1998. Additional sites were added to the Baseline Program in 2010 and again in 2021 to capture data in rivers that are under increased human influence due to urbanization. The addition of these stations aims to provide valuable insights into how urbanization is affecting water quality. This includes understanding the impacts of increased impervious surfaces, stormwater runoff, and changes in hydrology due to urban development.

Both the Watershed Characterization Network Program and the Baseline Surface Water Quality Monitoring Program are in-stream sampling programs that collect monthly samples during ice-free months. These programs follow standardized protocols outlined by MECP and the Canadian Council for the Ministers of the Environment (CCME) (CCME, 2011). Once samples are collected, they are stored on ice and delivered to accredited laboratories where they are analyzed for a suite of water quality parameters. Additional details on laboratories and sampling protocols can be provided upon request.

Data records were obtained for all sample stations within the Bear Brook Watershed. Table 1 identifies station locations, the monitoring program, and the length of data records. Figure 1 depicts all stations by program.

Table 1. Water quality monitoring programs and locations in the Bear Brook Watershed and the length of data record available.

Station and Location	Program	Data Record
South Bear Brook at Hawthorne Rd. (BB-HAW)	City of Ottawa Baseline Program	2020-2023
South Bear Brook at Hall Rd. (BB-HALL)	City of Ottawa Baseline Program	2020-2023
South Bear Brook at Boundary Rd. (CK31-04)	City of Ottawa Baseline Program	1998-2023
Bear Brook at Russell Rd. (CK31-01)	City of Ottawa Baseline Program	1998-2023
McKinnon's Creek at Wall Rd. (CK74-050)	City of Ottawa Baseline Program	2010-2023
McKinnon's Creek at Smith Rd. (CK74-01)	City of Ottawa Baseline Program	2010-2023
South Indian Creek at South Indian Creek Rd. (CK80-001)	City of Ottawa Baseline Program	2010-2023
Bear Brook at Ettyville Rd.	MECP Watershed Characterization Network	2002-2023

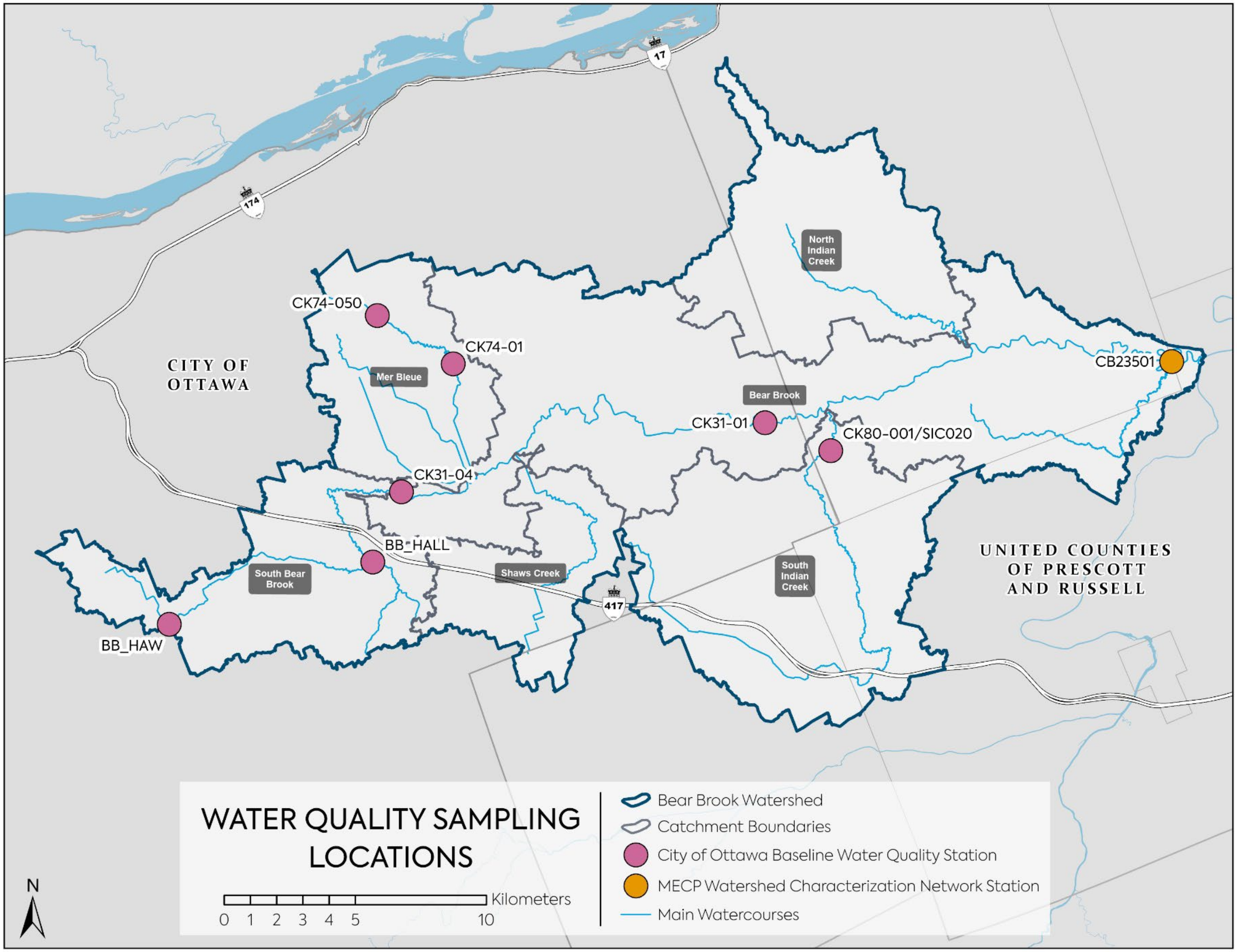

Figure 1. South Nation Conservation water quality sampling locations in the Bear Brook Watershed by program.

1.1. Water Quality Parameters of Interest

Several parameters were chosen to establish existing conditions, relate water quality results to specific non-point source influences, and identify potential trends of concern within the Bear Brook Watershed. Parameter results collected over a long period of time were compared to established provincial or Canadian benchmarks to determine if they are potentially impacting the health of water systems.

The benchmarks used in the analysis of this report included Canadian Water Quality Guidelines for the Protection of Aquatic Ecosystems (CWQG; CCME, 2011) and Provincial Water Quality Objectives (PWQO; Ministry of Environment, Conservation and Parks, 1994). CWQGs are national benchmarks developed by the federal government of Canada through the CCME that are focused on the protection of aquatic life across Canada. They are designed to ensure that water bodies across the country support healthy ecosystems, including fish, invertebrates, and plants.

When available, PWQOs were used in place of CWQGs because they are numerical criteria that represent satisfactory parameter levels in surface waters that protect all forms of aquatic life, as well as recreational water uses based on public health and aesthetic considerations. While both benchmarks are designed to protect water quality, PWQOs are tailored to the specific needs and conditions of the Province of Ontario, and they are used by the MECP and other provincial ministries in regulatory and management decisions. Table 2 summarizes indicator parameters, including sources, potential impacts, and published guideline values.

Table 2. Indicator parameters and their associated guidelines used in the application of the water quality index (WQI) for the Provincial Water Quality Monitoring Network and the City of Ottawa Baseline Water Quality Monitoring Program.

Parameter	Recommended Guideline	Source	Description
pH	>6.5, <8.5	PWQO*	pH indicates the alkalinity of water and is measured using a scale that ranges from 0-14. A pH of 7 is neutral, values below 7 are acidic, and values above 7 are basic or alkaline. Freshwater pH values should remain between 6.5 and 8.5. Conditions outside of this range lead to impacts on aquatic life, as well as recreational use. Notable impacts to aquatic and plant life can include changes to life cycles and metabolisms, impacting population dynamics and habitat characteristics (EPA, 2024). Factors influencing pH values along a system can include temperature, road and agricultural runoff, substrates, and varying sources of pollution (EPA, 2024). Shifts in pH can also influence water quality parameters including toxicity chemicals and the solubility of metals. A higher pH increases the toxicity of substances such as ammonia (Erickson, 1985), while lower pH increases the solubility of metals (EPA, 2024).
Chloride (Cl)	< 120 mg/L	CWQG◊	While negatively charged chloride can be found in most surface waters, chloride ions can also be bound in compounds commonly used for road salts and fertilizers such as sodium chloride, potassium chloride, magnesium chloride, and calcium chloride (CCME, 2011), entering water systems through road and agricultural runoff. While chloride is necessary to fuel aquatic animal and plant life, elevated levels of chloride salts are toxic to aquatic organisms (CCME, 2011).
Total Suspended Solids (TSS)	< 30 mg/L	CWQG◊	Total suspended solids are a measure of free-floating solids suspended in a water column that are larger than 2 microns (EPA, 2012), consisting of silt particles, clay, organic matter, inorganic matter, or algae, and are often used as an indicator of agricultural activity (EPA, 2012). Although TSS can increase turbidity and absorb light resulting in a temporary cooling of a system, preventing light from entering a system can negatively impact aquatic life by decreasing photosynthesis rates and hindering oxygen production (EPA, 2012). The current recommended guideline for total suspended solids indicates no increases greater than 25mg/L from background TSS concentrations (CCME, 2002). For this report, background concentrations in all systems were assumed to be no greater than 5 mg/l, resulting in a recommended guideline for TSS of <30mg/L.

Parameter	Recommended Guideline	Source	Description
Escherichia Coli (*E. coli*)	< 100 CFU (Coliform Forming Units)/100mL	PWQO*	*Escherichia coli*, or *E. coli* is a bacterium commonly used to indicate the presence of human and animal by-products such as sewage outflow, animal excrement and agricultural runoff (manure). The PWQO is a recreation guideline that if exceeded is deemed unsuitable for swimming and bathing (PWQO, 2016).
Nitrate (NO_3)	< 13 mg/L	CWQG◊	Nitrate is a naturally occurring compound essential for plant growth (CCME, 2012) and is also used extensively in fertilizers, food processing, metals, and paper. It is present in agricultural and urban runoff as well as sewage and exhaust emissions (CCME, 2012). Elevated concentrations of nitrates result in excessive plant and algae growth leading to eutrophication which may lead to reduced oxygen in a system and can impact the growth and metabolism of aquatic organisms (CCME, 2012).
Total Phosphorus (TP)	< 0.03 mg/L	PWQO*	Phosphorus is naturally occurring and essential for all living organisms (CCME, 2004). It is found in 3 different forms (inorganic phosphorus, particulate bound phosphorus and dissolved organic phosphorus), and while some systems may be naturally rich in phosphorus through sediment or organic matter contributions, others may receive excess phosphorus from external sources (CCME, 2004). As an essential plant nutrient, phosphorus is commonly used and applied to landscapes through fertilizers and manures. Urban and agricultural runoff are often common sources of excessive phosphorus within a system. Excessive phosphorus accelerates algae growth, causing eutrophication and reductions in oxygen within water systems (CCME, 2004).
Copper (Cu)	< 0.005 mg/L	PWQO*	Copper is a naturally occurring metal found in sediment, rocks, and water (CCME, 1999). Copper tends to be associated with urban impacts and is found in road and stormwater runoff (i.e., brake pad dust; Environment Canada, 1998, NASA, 2022). It accumulates in sediment where it can negatively affect aquatic life (CCME, 1999). Copper is also affected by pH where lower pH increases the solubility of copper.
Iron (Fe)	< 0.3 mg/L	PWQO*	Iron is a metal essential to all living organisms and commonly found in rock minerals. Typically, iron is present in surface water at a pH above 7 (Government of Canada, 2009). When present in elevated concentrations in aquatic systems, iron can accumulate in essential organs of aquatic organisms causing significant health impacts (Cadmus et al., 2018). Iron is also known to

Parameter	Recommended Guideline	Source	Description
			enter aquatic systems through rust, landfills and sewage treatment plants. It can leach into nearby systems through surface and stormwater runoff (Government of Canada, 2009).
Lead (Pb)	<0.005 mg/L	PWQO*	Lead is a heavy metal that is considered toxic to many species. Sources of lead include fossil fuels, wastewater, and stormwater runoff (CCME, 1999; Kumar et al., 2022). Once in an aquatic system, lead can be taken up by aquatic organisms and in some cases can be lethal. Lead is also known to inhibit plant growth (Pourrut et al., 2011). Its solubility within an aquatic ecosystem is often influenced by pH with acidic environments increasing its solubility.
Zinc (Zn)	< 0.03 mg/L	PWQO*	Zinc is present in water systems through the erosion of soils and through stormwater runoff. Common sources include fertilizers, pesticides, sewage effluents, building runoff, and vehicle wear and tear (CCME, 2018). As with many metals, zinc solubility in water is influenced by pH levels with higher pH levels decreasing solubility. Increasing dissolved organic carbon concentrations has also been known to increase its toxicity (CCME, 2018).
*Provincial Water Quality Objective ◊Canadian Water Quality Guideline			

1.2. Canadian Water Quality Index

The Canadian Water Quality Index (CWQI) is a tool used to summarize large amounts of water quality data into one simple, easily understandable score. It was developed by the CCME and is used across Canada to assess the quality of water in lakes, rivers, and other water bodies. CWQI scores were calculated for each site during two different time intervals (2012-2014 and 2021-2023).

The CWQI is calculated using three main factors:

1. **Scope (F1)**: The number of water quality variables that do not meet water quality objectives. For this assessment the ten variables listed in Table 2 were used.
2. **Frequency (F2)**: The frequency with which the water quality objectives are not met over a specified time period. For this assessment, two different time periods were used (2012-2014 and 2021-2023).
3. **Amplitude (F3)**: The amount by which failed tests exceed the water quality objectives.

The index is calculated using the following formula:

$$CWQI = 100 - \sqrt{\left(\frac{F1^2 + F2^2 + F3^2}{3}\right)}$$

F3 is calculated using the sub-formula:

$$F3 = \frac{\text{nse}}{0.01 \times (0.01 + nse)}$$

Where **nse** (normalized sum of excursions) is calculated as:

$$\text{nse} = \frac{\sum(\text{excursions})}{\text{number of tests}}$$

An excursion is defined as:

- If the test value must not exceed the objective:

$$\text{excursion} = \frac{\text{failed test value}}{\text{objective}} - 1$$

- If the test value must not fall below the objective:

$$\text{excursion} = \frac{\text{objective}}{\text{failed test value}} - 1$$

The resulting CWQI value is a number between 0 and 100. The index number is then used to classify water quality into one of five categories: Excellent, Good, Fair, Marginal, or Poor. CWQI values and their interpretation is provided in Table 3.

Table 3. Canadian Water Quality Index Rankings, score ranges, and an interpretation of rankings.

CWQI Ranking	CWQI Score	Interpretation
Excellent	**95-100**	Water quality is almost always protected. The water body meets the water quality objectives virtually all the time, with only minor deviations.
Good	**80-94**	Water quality is protected with only a few minor exceptions. The water body meets the water quality objectives most of the time, but there are occasional minor deviations.
Fair	**65-79**	Water quality is usually protected, but there are occasional issues. The water body sometimes fails to meet water quality objectives, with some moderate deviations.
Marginal	**45-64**	Water quality is frequently threatened or impaired. The water body often fails to meet water quality objectives, and the deviations are significant.
Poor	**0-44**	Water quality is almost always threatened or impaired. The water body regularly fails to meet water quality objectives, with significant deviations from the desired levels.

CWQI scores are presented in Figure 2 (2021-2023 scores) and Table 4 (2012-2024 and 2021-2023 scores) for eight river reaches throughout the Bear Brook Watershed. Results vary widely across the watershed from fair to poor. Individual site results are discussed in section 4.2.

Table 4. Calculated Canadian Council of Ministers of the Environment Canadian Water Quality Index scores within the Bear Brook Watershed based on collected samples as part of the City of Ottawa Baseline Water Quality Monitoring Program.

Site	Location	2012-2014 WQI Score	2012-2014 WQI Ranking	2021-2023 WQI Score	2021-2023 WQI Ranking
BB-HAW	South Bear Brook at Hawthorne Rd	-	n/a	67.3	Fair
BB-HALL	Bear River at Hall Rd	-	n/a	56.8	Marginal
CK31-04	South Bear Brook at Boundary Rd	49	Marginal	50.8	Marginal
CK31-01	Bear Brook at Russell Rd	45	Marginal	44.5	Poor
CK74-050	McKinnon's Creek at Wall Rd	44	Poor	38.1	Poor
CK74-01	McKinnon's Creek at Smith Rd	45	Marginal	56.3	Marginal
CK80-001	South Indian Creek at Indian Creek Rd	52	Marginal	53.7	Marginal
BB-WCN	Bear Brook at Robillard Rd	58.9	Marginal	54.4	Marginal

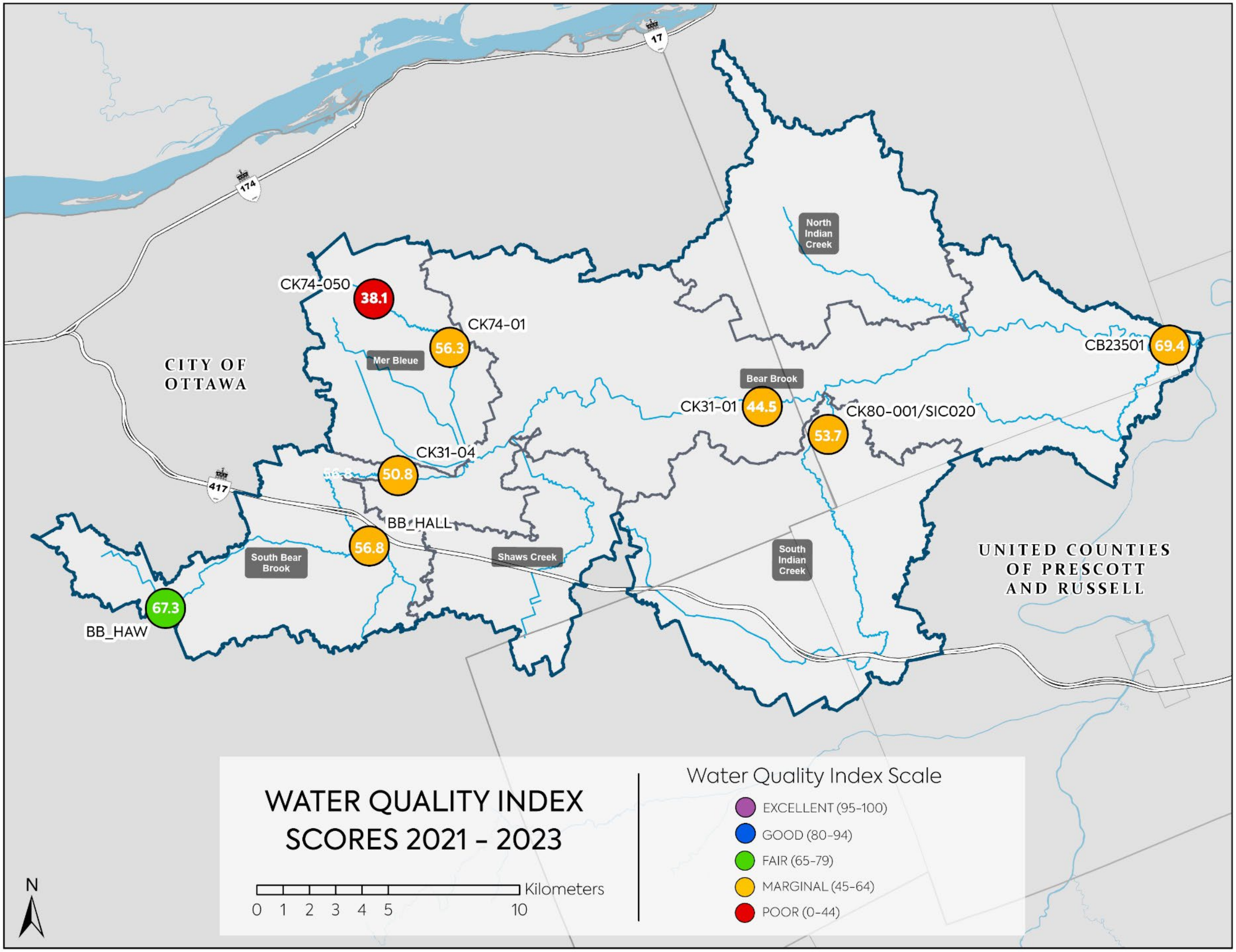

Figure 2. Canadian Water Quality Index scores from 2021-2023 in the Bear Brook Watershed.

1.3. Site Summaries and Trends

Water quality indicators were summarized for each site using two periods of record: 1998-2023 and 2018-2023. The 75th percentile is recognized as the upper threshold of the normal range in water quality assessments. These were calculated for each indicator using two different periods of record and were compared to published Provincial Water Quality Objectives (PWQO) (Ministry of Environment and Climate Change, 1994) or CWQGs for the Protection of Aquatic Life (Canadian Council of Ministers of the Environment, 2011). Frequencies of exceedances (count and percentage) were evaluated for each parameter using the published objectives and guidelines and are presented for each sample site.

For this study, results from sites with greater than 5 years of data were assessed for temporal trends using their entire period of record, as well as the last 5 years of data. Trend over time analysis was conducted using 75th percentile indicator values. A non-parametric trend test (Mann-Kendall) that determines if a data record is significantly trending up or down in amplitude was completed (Mustapha, 2013). Trends were considered statistically significant if they presented a p value of less than 0.05 (p value < 0.05).

1.3.1. South Bear Brook at Hawthorne Road (BB-HAW)

The headwaters of the Bear Brook River arise out of the Lester Road Wetland Complex east of Conroy Road, before travelling southeast of Davidson Road near the Canadian Forces Station Leitrim. The river upstream of Hawthorne Road is near the urban boundary of the City of Ottawa. Built-up areas are mostly located within the urban boundary of Ottawa, or along roadways. Land cover in the headwaters is largely in wetland, forest cover, or open thickets.

This station has the highest CWQI score (67.3) across all sample stations, receiving a fair assessment. Water quality results are presented for this site in Table 5. The South Bear Brook River at Hawthorne Road meets water quality objectives for most parameters. However, it fails to meet water quality objectives for chlorides, total phosphorus and iron in most sample events (i.e., 89.5%, 94.7%, 78.9% respectively). The amplitude of deviations is moderately high for all parameters, and total phosphorus observed displays a significant increasing trend over the 1998 to 2023 data record. Chloride exceedances are likely due to road salts and proximity to urban transportation corridors, while phosphorus and iron exceedances are likely due to industrial and natural occurrences.

Table 5. Parameter results and trends for South Bear Brook at Hawthorne Road (BB-HAW) from 2021-2023.

	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
Param-eter	**1998-2023**	**2021-2023**	**1998-2023**	**2021-2023**	**1998-2023**	**2021-2023**	**1998-2023**	**2021-2023**
pH	n/a	8.17	n/a		n/a	0 (0)	n/a	
Cl (mg/L)	n/a	215.20	n/a	19	n/a	17 (89.5)	n/a	
TSS (mg/L)	n/a	4.50	n/a	19	n/a	0 (0)	n/a	
E. coli (cfu/100mL)	n/a	150	n/a	19	n/a	7 (36.8)	n/a	
NO3 (mg/L)	n/a	0.10	n/a	19	n/a	0 (0)	n/a	
TP (mg/L)	n/a	0.07	n/a	19	n/a	18 (94.7)	n/a	↑
Cu (mg/L)	n/a	0.005	n/a	19	n/a	0 (0)	n/a	
Fe (mg/L)	n/a	0.610	n/a	19	n/a	15 (78.9)	n/a	
Pb (mg/L)	n/a	0.001	n/a	19	n/a	0 (0)	n/a	
Zn (mg/L)	n/a	0.025	n/a	19	n/a	1 (5.3)	n/a	

*Trends were calculated to a significance level of $p < 0.05$

1.3.2. Bear River at Hall Road (BB-HALL)

A water quality station was added to Bear River at Hall Road in 2021 to provide a reference station within the South Bear Brook Subwatershed and to fill a data gap on the watercourse identified in the recent South Bear Brook Catchment Study (SNC, 2022). Bear River is a small catchment (13.7 km-sq), located in the southeast section of the South Bear Brook Subwatershed. Over half of the landcover is comprised of Provincially Significant Wetlands and forests, with agriculture and settlement largely making up the rest. Bear River flows in a northeast direction, just south of Highway 417 before changing direction and joining the main Bear Brook channel west of Hall Road.

CWQI results at this station are assessed as marginal (56.8). Water quality results are presented for this site in Table 6. Like Bear Brook at Hawthorne Road, this station experienced regular exceedances in chlorides (71.4%), total phosphorus (85.7%) and iron (78.6%). An additional parameter that experienced regular exceedances includes *E. coli* (50%). The 75th percentile for total phosphorus is especially high at 0.157 mg/L, likely due to non-point sources from natural and agricultural land cover. Increasing trends were observed for pH, total phosphorus (TP), and iron (Fe).

Table 6. Parameter results and trends for South Bear Brook at Hall Road (BB-HALL) from 2021-2023.

	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
Parameter	1998-2023	2018-2023	1998-2023	2018-2023	1998-2023	2018-2023	1998-2023	2018-2023
pH	n/a	8.18	n/a	14	n/a	0 (0)	n/a	↑
Cl (mg/L)	n/a	159.8	n/a	14	n/a	10 (71.4)	n/a	
TSS (mg/L)	n/a	18.25	n/a	14	n/a	0 (0)	n/a	
E.coli (cfu/100 mL)	n/a	205	n/a	14	n/a	7 (50)	n/a	
NO3 (mg/L)	n/a	0.36	n/a	14	n/a	0 (0)	n/a	
TP (mg/L)	n/a	0.152	n/a	14	n/a	12 (85.7)	n/a	↑
Cu (mg/L)	n/a	0.003	n/a	14	n/a	0 (0)	n/a	
Fe (mg/L)	n/a	1.956	n/a	14	n/a	11 (78.6)	n/a	↑
Pb (mg/L)	n/a	0.001	n/a	14	n/a	0 (0)	n/a	

Parameter	75th percentile 1998-2023	75th percentile 2018-2023	Number of Observations 1998-2023	Number of Observations 2018-2023	Number of Exceedances (%) 1998-2023	Number of Exceedances (%) 2018-2023	Trend* 1998-2023	Trend* 2018-2023
Zn (mg/L)	n/a	0.025	n/a	14	n/a	0 (0)	n/a	
*Trends were calculated to a significance level of $p < 0.05$								

1.3.3. South Bear Brook at Boundary Road (CK31-04)

South Bear Brook at Boundary Road is located further downstream in the South Bear Brook Subwatershed, near Carlsbad Springs. Agriculture accounts for approximately a quarter of the landcover in the upstream catchment and is predominantly located in clay plains where soil types are rated high for agricultural capability. Much of the remaining landcover comprises of wetlands, wooded areas, or grasslands upstream of the Boundary Road station.

South Bear Brook at Boundary Road observed marginal CWQI scores, ranging between 49 (2012-2014) and 50.8 (2021-2023) over the last decade. Water quality results are presented in Table 7. Chloride levels are elevated at this station, and regularly exceeded the PWQO (74% 1998 - 2023 and 89% 2018-2023). Although no significant increase in trends was detected for this station, the 75th percentiles increased from 249 mg/L (1998-2023) to 278 mg/L (2018-2023).

Phosphorus levels exceeded the PWQO in every sample event at this station and observed a 75th percentile over three times the PWQO at 0.095 mg/L. Comparatively, nitrate levels were low in the samples, with nitrate levels never exceeding the PWQO. Exceedances in *E. coli* (CFU/100 mL) were observed in about half of all sample events and the 75th percentile levels were observed at 220 CFU/100 mL over the entire period of record (1998 to 2023) and 160 CFU/100 mL over the past 5 years (2018 to 2023). Although the 75th percentile for *E. coli* has been lower in the past 5 years, the Mann-Kendall trend test observed an increasing trend in *E. coli* across the sampling period on record (1998 to 2023).

Of all the metals analyzed, only iron regularly exceeded the recommended guideline (85% 1998-2023 and 69% 2018-2023). However, results indicate that iron concentrations decreased over time (1998-2023). It should be noted that although lead and zinc saw few exceedances in the PWQO, an increasing trend in lead and zinc concentrations were observed in the last 5 years (2018-2023).

Table 7. Parameter results and trends for South Bear Brook at Boundary Rd. (CK31-04) from 1998-2023 and 2018-2023.

	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
Parameter	**1998-2023**	**2018-2023**	**1998-2023**	**2018-2023**	**1998-2023**	**2018-2023**	**1998-2023**	**2018-2023**
pH	8.15	8.14	199	36	0 (0)	0 (0)		
Cl (mg/L)	249	277.6	199	36	147 (73.9)	32 (88.9)		
TSS (mg/L)	15	9.25	199	36	9 (4.5)	3 (8.3)	↓	
E. coli (cfu/100 mL)	220	160	209	35	93 (44.5)	15 (42.9)	↑	
NO3 (mg/L)	0.3	0.15	149	36	0 (0)	0 (0)		
TP (mg/L)	0.099	0.095	199	36	100 (88)	36 (100)		
Cu (mg/L)	0.005	0.005	196	36	42 (21.4)	1 (2.8)		
Fe (mg/L)	1.34	0.89	191	36	162 (84.8)	25 (69.4)	↓	
Pb (mg/L)	0.001	0.001	196	36	2 (1)	0 (0)	↓	↑
Zn (mg/L)	0.01	0.02	196	36	4 (2)	1 (2.8)		↑

*Trends were calculated to a significance level of $p < 0.05$

1.3.4. Bear Brook at Russell Road (CK31-01)

The Bear Brook River continues to flow in an easterly direction crossing the City of Clarence-Rockland Boundary at Russell Road. Results at this station (CK31-01) were slightly lower when compared to upstream conditions with the station observing a marginal CWQI score of 45 in 2012 to 2014 and a poor CWQI score of 44.5 in 2021-2023.

Water quality results are presented for this site in Table 8. Notably, chloride concentrations were lower at this station compared to upstream results, with fewer exceedances and lower background levels (2018-2023, 75th percentile: 107.9 mg/L). This is likely due to the distance from urban influences, as well as dilution of chloride concentrations from additional tributaries flowing into the Bear Brook upstream. Like upstream conditions, regular exceedances of the recommended guidelines and elevated 75th percentiles were observed for total phosphorus and *E. coli*.

Increasing trends across the sampling period on record (1998-2023) were observed in 3 of 10 indicator parameters, including pH, chloride, and *E. coli*, while increases in lead and zinc concentrations were observed in the last 5 years (2018-2023).

Table 8. Parameter results and trends for Bear Brook at Russell Rd. (CK31-01) from 1998-2023 and 2018-2023.

Parameter	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
	1998-2023	2018-2023	1998-2023	2018-2023	1998-2023	2018-2023	1998-2023	2018-2023
pH	8.3	8.37	212	34	0 (0)	0 (0)	↑	
Cl (mg/L)	104.1	107.9	213	35	23 (10.8)	5 (14.3)	↑	
TSS (mg/L)	22	13	213	35	36 (16.9)	4 (11.4)	↓	
E. coli (cfu/100 mL)	530	550	249	35	182 (73.1)	26 (74.3)	↑	
NO3 (mg/L)	1.45	1.45	152	33	0 (0)	0 (0)		
TP (mg/L)	0.11	0.11	213	35	208 (97.7)	33 (94.3)		
Cu (mg/L)	0.007	0.004	211	35	90 (42.7)	3 (8.6)	↓	

Parameter	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
	1998-2023	2018-2023	1998-2023	2018-2023	1998-2023	2018-2023	1998-2023	2018-2023
Fe (mg/L)	1.6	0.87	206	35	164 (79.6)	20 (57.1)	↓	
Pb (mg/L)	0.001	0.001	211	35	4 (1.9)	0 (0)	↓	↑
Zn (mg/L)	0.01	0.01	210	34	3 (1.4)	1 (2.9)		↑
*Trends were calculated to a significance level of $p < 0.05$								

1.3.5. Bear Brook at Robillard Road in Ettyville (Watershed Characterization Network)

The Bear Brook at Ettyville Road in The Nation Municipality is the furthest downstream sample site, located approximately 3 km upstream of the Bear Brook and South Nation River confluence. Water quality results are presented for this site in Table 9. Unfortunately, due to the differences in sampling programs, there are gaps in key parameters including chloride, total suspended solids, and limited data available for *E. coli*.

Results at this station were slightly higher when compared to upstream conditions, with the station observing marginal CWQI scores of 58.9 in 2012-2014 and 54.4 in 2021-2023. This may be because the CWQI was calculated using a reduced number of parameters.

Total phosphorus was lower at this station compared to upstream conditions using the 2018-2023 time period and a declining trend in total phosphorus was observed for the 1998-2023 time period.

Although elevated and regularly in exceedance of the PWQO, iron levels exhibit a decline over time (1998-2023). Lead and zinc saw few exceedances but increasing trends were observed for both parameters.

Table 9. Parameter results and trends for Bear Brook at Robillard Road near Ettyville from 1998-2023 and 2018-2023.

Parameter	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
	1998-2023	2018-2023	1998-2023	2018-2023	1998-2023	2018-2023	1998-2023	2018-2023
pH	8.32	8.25	161	35	0 (0)	0 (0)		
Cl	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
TSS	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
E. coli (cfu/100 mL)	152.5	n/a	36	n/a	12 (33.3)	n/a		n/a
NO3 (mg/L)	n/a	1.81	n/a	20	n/a	0 (0)	n/a	
TP (mg/L)	0.123	0.091	161	33	159 (98.8)	33 (100)	↓	
Cu (mg/L)	0.004	0.003	145	33	8 (5.5)	0 (0)	↓	
Fe (mg/L)	1.03	0.69	145	33	139 (95.9)	30 (90.9)	↓	
Pb (mg/L)	0.003	0.007	145	33	29 (20)	19 (57.6)		↑
Zn (mg/L)	0.011	0.010	145	33	4 (2.8)	1 (3)	↑	
*Trends were calculated to a significance level of $p < 0.05$								

1.3.6. McKinnon’s Creek at Wall Rd. (CK74-050)

McKinnon's Creek is approximately 11.48 km in length, beginning at the Avalon West (NH5) storm water management pond and outflowing to the Bear Brook just north of Russell Road. The section of the creek from the Prescott-Russell Recreation Trail downstream to the confluence with the Bear Brook is classified as a Class ‘E’ Municipal Drain (Richard Clark Municipal Drain) by Fisheries and Oceans Canada (DFO).

CWQI scores for McKinnon’s Creek at Wall Road were generally poor, with scores of 44 (poor; 2012-2014) and 38.1 (poor; 2021-2023). The lowest CWQI scores are observed in the 2021-2023 period of record which corresponds to increasing nutrient and chloride levels, likely resulting from increased urban development in the headwaters of the catchment.

Water quality results are presented for this site in Table 10. Chloride levels are variable, with a greater number of exceedances being observed in the 2018-2023 period of record (69.4%) compared to the 2010-2023 period (57.4%). Nutrient enrichment is an issue within McKinnon’s Creek as 100% of all samples collected exceed the PWQO for total phosphorus, with recent data (2018–2023) revealing an increasing trend in phosphorus levels. Most samples collected at Wall Road were three times greater than the recommended guideline of 0.03 mg/L.

Other parameters experiencing regular exceedances include *E. coli* (80.6%; 2018-2023) and iron (91.7%; 2018-2023). It should be noted that the 75th percentile for *E. coli* is markedly high for the 2018-2023 period of record (890 CFU/100mL), representing the highest *E. coli* levels across all the Bear Brook water quality monitoring sites. The high concentration is potentially due to manure runoff from surrounding agricultural lands and the presence of waterfowl in storm water ponds upstream.

Table 10. Parameter results and trends for McKinnon's Creek at Wall Rd. (CK74-050) from 2010-2023 and 2018-2023.

Parameter	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
	2010-2023	2018-2023	2010-2023	2018-2023	2010-2023	2018-2023	2010-2023	2018-2023
pH	8.23	8.34	108	36	0 (0)	0 (0)	↑	
Cl (mg/L)	187.7	184.9	108	36	62 (57.4)	25 (69.4)		
TSS (mg/L)	30.5	29	107	35	27 (25.2)	8 (22.9)	↓	↑
E. coli (cfu/100 mL)	535	890	63	36	50 (79.4)	29 (80.6)		
NO3 (mg/L)	0.5	0.21	108	36	0 (0)	0 (0)		
TP (mg/L)	0.129	0.125	107	35	107 (100)	35 (100)		↑
Cu (mg/L)	0.005	0.005	107	36	29 (27.1)	4 (11.1)	↓	
Fe (mg/L)	1.43	1.17	107	36	103 (96.3)	33 (91.7)	↓	
Pb (mg/L)	0.001	0.001	107	36	1 (0.9)	1 (2.8)	↓	↑
Zn (mg/L)	0.01	0.02	107	36	4 (3.7)	3 (8.3)		↑

*Trends were calculated to a significance level of p < 0.05

1.3.7. McKinnon’s Creek at Smith Rd. (CK74-01)

CWQI scores slightly improve at the downstream site in McKinnon’s Creek near Smith Road in the Village of Navan, with scores ranging from 45 (marginal; 2012-2014) to 56.3 (marginal; 2021-2023). Water quality is improved through the sandy ridge upstream of the community of Navan where forest cover is higher, groundwater enters the stream, and there is likely an increased assimilative capacity for nutrients.

Water quality results are presented for this site in Table 11. Although phosphorus enrichment seems more prevalent in the upper reaches of McKinnon’s Creek, it is still an issue at Smith Road with 100% of all samples collected exceeding the PWQO and an observed 75th percentile of 0.129 mg/L. Similar to the upstream site, chloride levels are variable. Exceedances are more prevalent in the 2018–2023 time span and the Smith Road site (CK74-100) displays an increasing trend in chloride levels over the 2010–2023 period of record. This is likely a result of increased road salts accumulating in the stream due to increased development within the catchment.

Like the upstream site, *E. coli* concentrations are high with 75th percentiles of 485 CFU/mL (2010-2023) and 445 CFU/mL (2018-2023), regularly exceeding the 100 CFU/100 mL PWQO.

Both sites display high iron levels with most samples in exceedance of the PWQO. Overall, copper, lead and zinc levels were low, exceeding the PWQO only on a few occasions over the period of sampling. An increasing trend was observed for lead and zinc, which should be considered in future assessments.

Table 11. Parameter results and trends for McKinnon's Creek at Smith Rd. (CK74-01) from 2010-2023 and 2018-2023.

Parameter	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
	2010-2023	2018-2023	2010-2023	2018-2023	2010-2023	2018-2023	2010-2023	2018-2023
pH	8.26	8.31	106	35	0 (0)	0 (0)	↑	
Cl (mg/L)	160.93	173.75	106	35	58 (54.7)	30 (85.7)	↑	
TSS (mg/L)	20	15.5	106	35	7 (6.6)	1 (2.9)	↓	
E. coli (cfu/100 mL)	485	445	71	35	63 (88.7)	29 (82.9)		
NO3 (mg/L)	1.0	0.72	106	35	0 (0)	0 (0)		
TP (mg/L)	0.115	0.114	106	35	106 (100)	35 (100)		↑
Cu (mg/L)	0.005	0.005	105	35	32 (30.5)	5 (14.3)	↓	↓
Fe (mg/L)	1.6	1.15	105	35	99 (94.3)	30 (85.7)	↓	
Pb (mg/L)	0.001	0.001	105	35	1 (1)	0 (0)	↓	↑
Zn (mg/L)	0.01	0.02	105	35	3 (2.9)	1 (2.9)		↑

*Trends were calculated to a significance level of $p < 0.05$

1.3.8. South Indian Creek (CK80-001)

South Indian Creek is a catchment within the Bear Brook Watershed, that is contained within the City of Ottawa and United Counties of Prescott and Russell, including Russell Township, Nation Municipality and the City of Clarence-Rockland. The South Indian Creek headwaters arise out of a series of wetlands south of Highway 417 and the river flows north through the town of Limoges, joining the Bear Brook south of Russell Road near Cheney. The watercourse is approximately 84 km in length and has a drainage area of approximately 97 square kilometers, including all tributaries.

Land cover is a mix of woodlands, wetlands, and agricultural cropland, with several small settlements scattered across the watershed. The town of Limoges is the largest settlement within the subwatershed and is experiencing growth and development in recent years. Despite the large amount of natural cover, water quality conditions are marginal according to CWQI scores, with scores ranging from 52 (marginal; 2012-2014) to 53.7 (marginal; 2021-2023).

Water quality results are presented for this site in Table 12. An increasing trend in chlorides and pH are observed over the entire period of record for South Indian Creek (CK80-001). Although chlorides and pH are increasing, they had few exceedances compared to other stations within the Bear Brook Watershed.

Similar to other catchments within the Bear Brook Watershed, total phosphorus and *E. coli* are elevated and regularly exceed the PWQO's. Background total phosphorus concentrations are over two times the PWQO at 0.074 mg/L (2010-2023) and 0.069 mg/L (2018-2023), exceeding the guideline in over 89% of all samples. Background *E. coli* concentrations are 435 CFU/mL (2018-2023) and 262.5 CFU/mL (2018-2023), with exceedances observed in over 80% of all samples.

Most metals observe few exceedances, with the exception of iron, which exceeds the PWQO in almost all samples.

Table 12. Parameter results and trends for South Indian Creek at South Indian Creek Rd. (CK80-001) from 2010-2023 and 2018-2023.

Parameter	75th percentile		Number of Observations		Number of Exceedances (%)		Trend*	
	2010-2023	2018-2023	2010-2023	2018-2023	2010-2023	2018-2023	2010-2023	2018-2023
pH	8.18	8.26	95	28	0 (0)	0 (0)	↑	
Cl (mg/L)	63.2	68.8	95	28	3 (3.2)	0 (0)	↑	
TSS (mg/L)	25	17.3	95	28	20 (21.1)	5 (17.9)		
E. coli (cfu/100 mL)	435	262.5	59	28	49 (83.1)	24 (85.7)		
NO3 (mg/L)	1.31	1.11	95	28	0 (0)	1.31		
TP (mg/L)	0.074	0.069	95	28	85 (89.5)	25 (89.3)		
Cu (mg/L)	0.004	0.031	94	28	13 (13.8)	3 (10.7)	↓	
Fe (mg/L)	1.47	1.19	94	28	92 (97.9)	28 (100)		
Pb (mg/L)	0.001	0.001	94	28	1 (1.1)	0 (0)	↓	
Zn (mg/L)	0.01	0.01	94	28	3 (3.2)	1 (3.6)		
*Trends were calculated to a significance level of $p < 0.05$								

1.4. Parameter Summaries

Key Findings

- pH levels are within acceptable levels across all sites. Most sites in the study area observed increasing pH trends.
- Chloride values regularly exceeded standard guidelines and show consistent increasing trends across the Bear Brook Watershed, especially in urbanizing areas such as the headwaters of South Bear Brook and McKinnon's Creek.
- Total phosphorus levels regularly exceeded guideline values, in some cases 4 to 5 times the guideline value. Total phosphorus levels are decreasing in rural areas but increasing in urbanizing areas such as the headwaters of South Bear Brook and McKinnon's Creek.
- All sites observed regular exceedances in *E. coli*, while McKinnon's Creek, South Indian Creek and the Bear Brook at Russell Road show extreme values, likely resulting from contamination during runoff events.
- There are few exceedances in lead and zinc in the existing data record. Significant increasing trends were observed for both parameters across most stations in the study area.

Parameter statistics were derived for each site, including minimum, percentiles (i.e., 10th, 25th, 50th, 75th, 90th), maximum, and average to further study inter-station variability. The variability of water quality data for parameters in regular exceedance of guideline values is displayed using 75th percentile data and is themed to display natural breaks in the data using an ArcMap classification method called natural breaks or "jenks". Jenks classification seeks to minimize the variance within classes and maximize the variance between classes. It identifies "natural breaks" in the data, where there are significant jumps in values, and creates classes accordingly. This classification method is more useful for data with a skewed distribution and helps emphasize natural groupings in the data.

1.4.1. General Chemistry

pH levels are within acceptable levels across all sites within the Bear Brook Watershed. However, most sites in the study area display increasing significant trends. Stations with greater variability in pH include the Bear Brook at Russell Road and Bear Brook at Ettyville Road. pH plays a critical role in the solubility and availability of nutrients and metals in water. A shift in pH can change the availability of essential nutrients like phosphorus and nitrogen, potentially leading to either nutrient deficiencies or harmful algal blooms, which can further degrade water quality.

Many aquatic species, including fish, amphibians, and invertebrates, have evolved to thrive within a specific pH range. Even small changes in pH stresses organisms, potentially reducing their ability to reproduce, grow, or survive. A shift toward more alkaline conditions (higher pH) can impair physiological processes like respiration and metabolism in some species. If pH levels continue to rise, sensitive species may decline, leading to a loss of biodiversity. The disappearance of key species can disrupt food webs, alter predator-prey relationships, and change the structure of aquatic communities. While the current pH levels are not alarming, the observed increasing trend across the watershed should be monitored.

Background (75^{th} percentile) levels of TSS were within recommended levels across the Bear Brook Watershed. All stations were under 25 mg/L, except for McKinnon’s Creek at Hall Road (CK74-050) at 30.5 mg/L. This station also observed one of the highest 90^{th} percentile and maximum levels (42.8 mg/L, 261 mg/L), indicating that run-off is carrying TSS material during rain events. During rainfall events, stormwater runoff from urban areas picks up and transports various particles, including soil, sand, debris, and pollutants directly into nearby water bodies. This runoff often contains high levels of TSS, leading to increased turbidity and sedimentation. TSS carries attached pollutants, including nutrients, pesticides, and metals.

These pollutants can be released into the water as the particles settle, leading to long-term contamination of the waterbody and affecting the health of aquatic ecosystems. Other stations with high levels of TSS during runoff events (90^{th} percentile, maximum levels) include Bear Brook at Russell Road (CK31-01; 54.2 mg/L, 260 mg/L) and South Indian Creek (SIC-080; 48.6 mg/L, 319 mg/L).

1.4.2. Chlorides

Chloride levels have shown consistent increasing trends across the Bear Brook Watershed, with the highest concentrations observed in urbanized areas, such as the headwaters of the South Bear Brook and McKinnon's Creek (Figure 3, Figure 4). This trend is documented in other regions across Ontario experiencing increased urbanization and growth; Concentrations are higher where populations are dense, and they are increasing over time (Sorichetti et al., 2022).Evidence shows that the primary anthropogenic source of chloride to aquatic environments is runoff from deicers and road salts (Environment Canada and Health Canada, 2001).

In the Bear Brook Watershed, naturally occurring chloride comes from ancient marine clays that blanket large portions of the watershed to variable depths. While negatively charged chloride can be found in most surface waters, chloride ions can also be found bonded to other elements in compounds including sodium chloride, potassium chloride, magnesium chloride, and calcium chloride (CCME, 2011), commonly found in road salts and fertilizers.

Summary statistics depicted in Figure 3 highlight that there is variability in chloride concentrations across different stations, with some stations showing more extreme conditions than others. Stations located in urban areas, including South Bear Brook at Hawthorne Road (BB-HAW), McKinnon's Creek at Wall Road (CK74-050), as well as Bear Brook downstream of the 417 (CK31-04) exhibit more extreme conditions (i.e., 90th percentile levels of 307, 255 and 342 mg/L; Maximum levels of 318, 1178 and 774 mg/L). These observations are consistent with chloride trends across southern Ontario in areas experiencing urbanization and population growth.

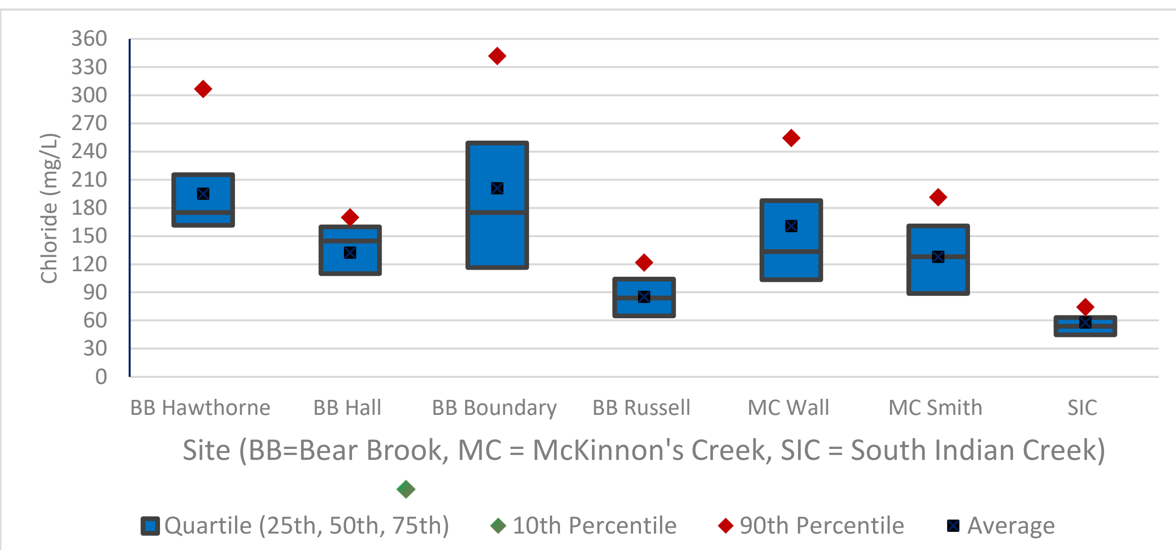

Figure 3. Box and whisker plots of chloride levels in the Bear Brook Watershed.

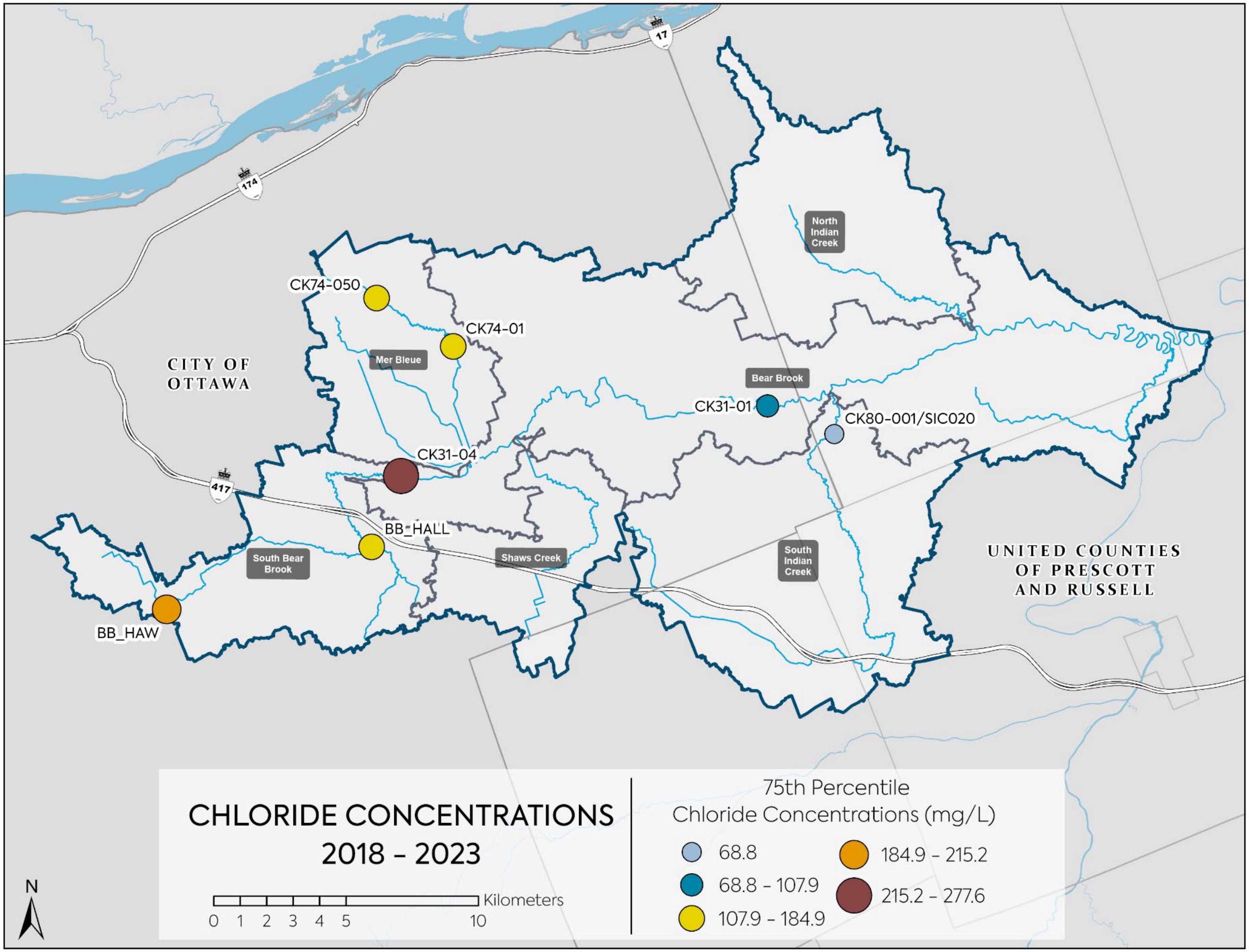

Figure 4. 75th percentile chloride (mg/L) levels in the Bear Brook Watershed.

1.4.3. Nutrients

Nutrients, such as nitrogen and phosphorus, are essential for the growth of aquatic plants and organisms. When these nutrients are present in excessive amounts due to human activities, they can have detrimental effects, including algal blooms (possibly leading to increased toxins in the water), oxygen dead zones, and shifts in species composition.

Nitrates are consistently low across the Bear Brook Watershed, with levels that are well below the PWQO. Total phosphorus is present in naturally high background levels, which is typical for rivers located in clay plains where phosphorus particles are bound to soil particles (Schneider et al. 2022). During rain events, spikes in phosphorus result from soil particles washing into surface water. This is especially evident in areas where soil has been disturbed (i.e., construction, agriculture) and is lacking vegetative cover or riparian buffers.

Sites that have excessive phosphorus include Bear River at Hall Road, McKinnon's Creek at Wall Road and Bear Brook at Robillard Road in Ettyville, with background levels that are 4 to 5 times the guideline value (Figure 5, Figure 6). While phosphorus levels are declining in Bear Brook at Robillard Road over time, they are significantly increasing in sites that have urban influences (i.e., South Bear Brook and McKinnon's Creek).

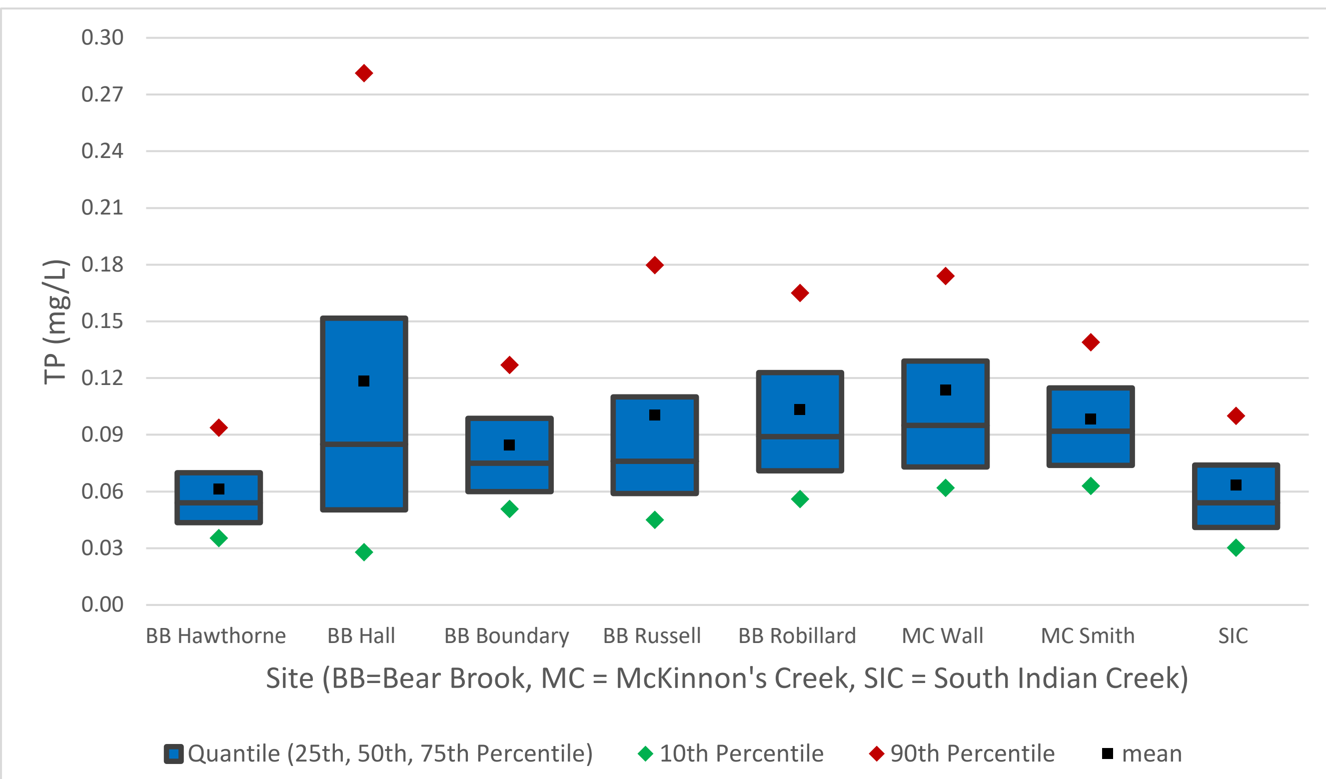

Figure 5. Box and whisker plots of total phosphorus levels in the Bear Brook Watershed.

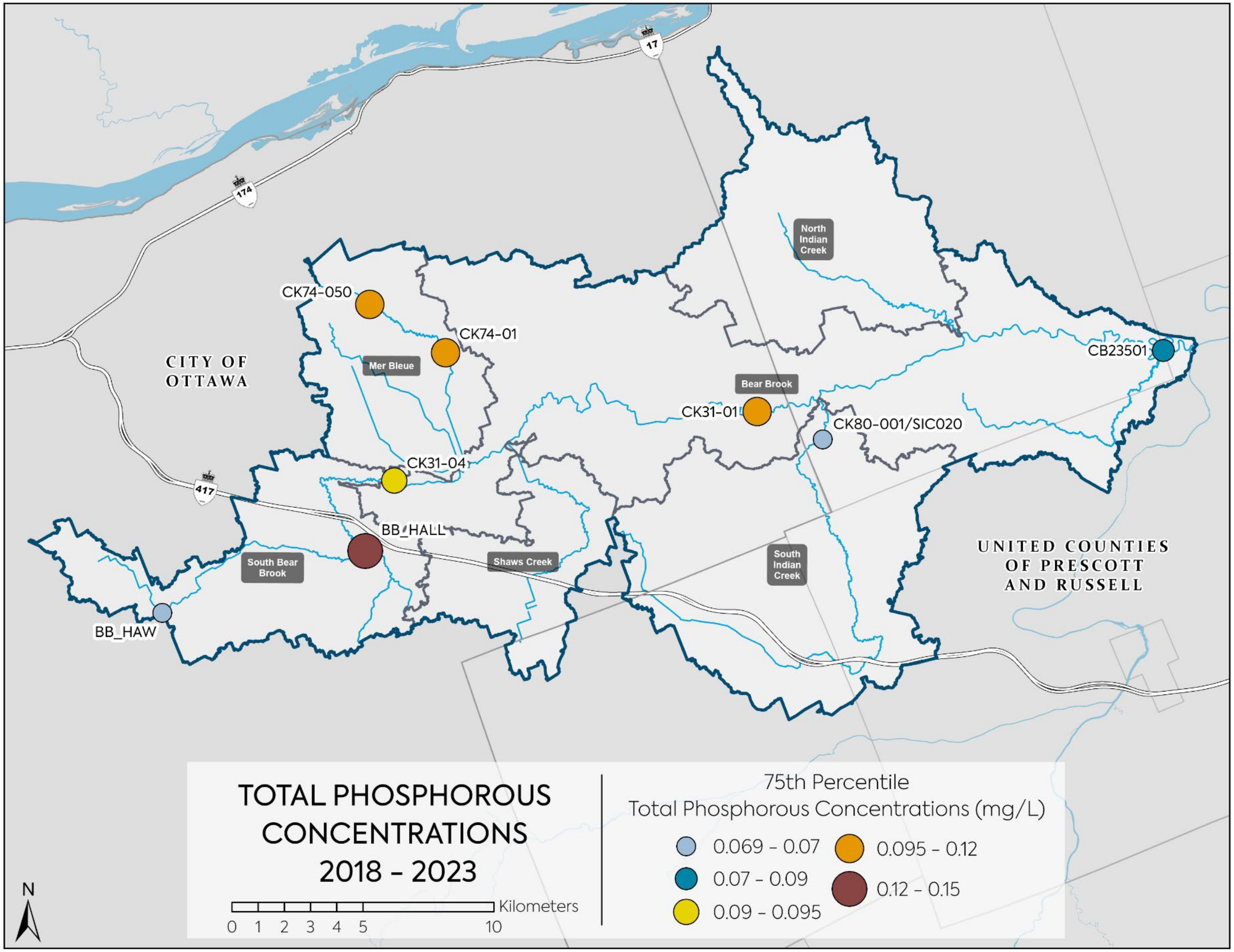

Figure 6. 75th percentile total phosphorus (mg/L) levels in the Bear Brook Watershed.

1.4.4. E. coli

Escherichia coli (*E. coli*) is a type of bacteria commonly found in the intestines of warm-blooded animals, including humans. While most strains of *E. coli* are harmless, some can cause serious illness. *E. coli* is used as an indicator organism to assess the level of fecal contamination in water bodies. High levels often indicate the presence of other pollutants, such as nutrients, pathogens, and organic matter.

All sites across the Bear Brook Watershed observed regular exceedances in *E. coli*, however several sites observed particularly high 75th percentile background levels, including McKinnon's Creek at Wall Rd and Smith Road, Bear Brook at Russell Road, and South Indian Creek (Figure 8, Figure 9). These same stations have excessive 90th percentiles, indicating *E. coli* contamination during runoff events.

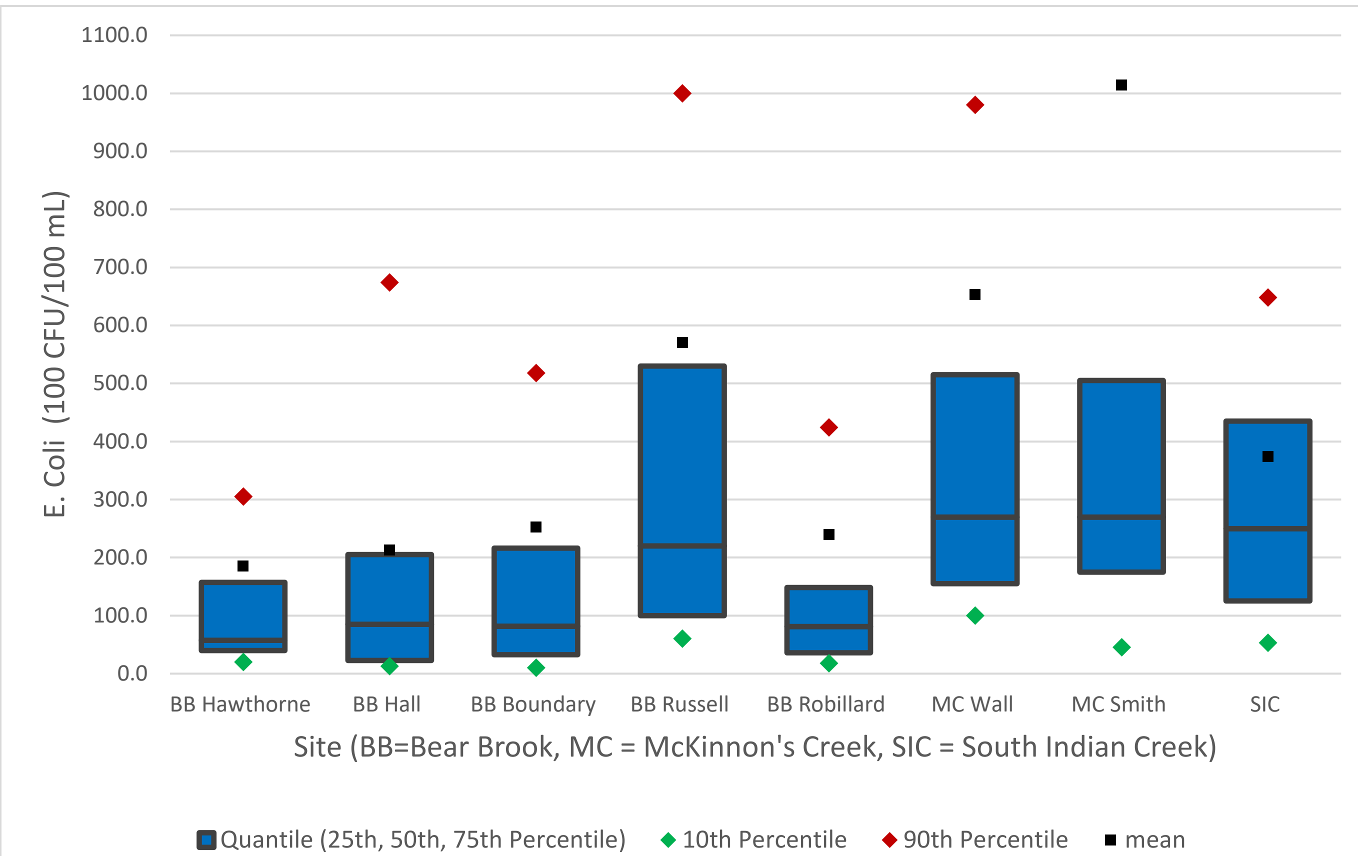

Figure 7. Box and Whisker Plot of *E. coli* Levels (CFU/100ml) in the Bear Brook Watershed.

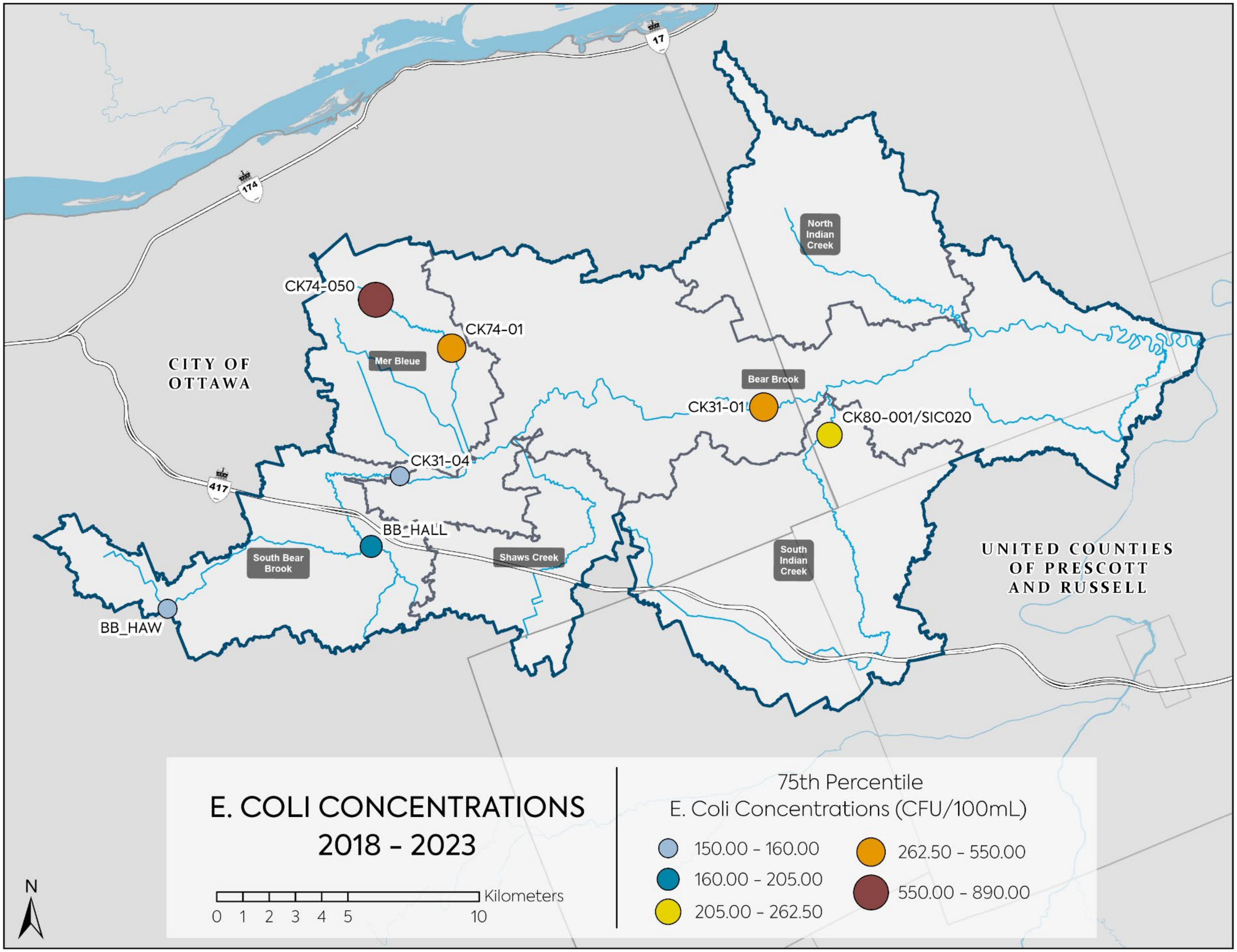

Figure 8. 75th Percentile *E. Coli* Levels (CFU/100ml) in the Bear Brook Watershed.

1.4.5. Metals

Trace amounts of metals are often essential for aquatic ecosystems. Elevated levels of metal contaminants in water can pose serious threats to aquatic life. The impacts of these metals vary widely, depending on the type and concentration, the bioavailability, and the sensitivity of the organisms exposed.

In many instances, the presence and concentration of metal contaminants are influenced by the natural geology of the landscape and region. For example, in the Bear Brook Watershed, iron concentrations are high due to the gleysolic nature of clay soils across the watershed. Regular exceedances in iron were observed in all stations, with the exception of South Bear Brook at Hawthorne Road, likely due to the underlying geology upstream of Hawthorne Road being sand and silt, as opposed to clay. Although elevated, iron concentrations are declining over time.

Human activities such as mining, industrial discharges, and urban runoff can lead to metal levels that exceed the natural background and potentially harm aquatic species. Metals like lead and zinc can accumulate in the tissues of aquatic organisms, leading to toxic effects that can impair growth, reproduction, and survival. There are few exceedances in lead and zinc in the existing data record. However, significant increasing trends were observed for both parameters across most stations in the study area. Monitoring lead and zinc should continue into the future to determine if management strategies need to be developed.

2. Water Temperature

Key Findings

- The headwaters of the main tributaries of the Bear Brook historically displayed cold to cool water stream characteristics.
- Some headwater tributaries of the Bear Brook exhibited cold to cool water characteristics when sampled between 2020-2024. These areas represent sensitive and less common habitat in the Bear Brook Watershed.

Changes in water temperature can affect the composition and function of aquatic ecosystems as organisms adapt to specific temperatures. Therefore, it is important to understand the water temperature regime of a stream and its response to weather, land use, and physiography. In general, areas of cold to cool water in headwater streams is indicative of groundwater upwellings.

Stream thermal characteristics and permanence of the flows in the Bear Brook Watershed were first studied in the 1940's as part of the South Nation Valley Interim Report. While exact details and methods of how or when this study was undertaken are unavailable, the results illustrated in Figure 10 show areas of permanent-flowing, cold water streams concentrated in the headwaters of the Bear Brook and its tributaries. Compared to the rest of the South Nation River Watershed (at the time of study in the 1940's), the headwaters of the Bear Brook Watershed had the highest concentration of permanent flow, cold water streams in the entire South Nation River Watershed.

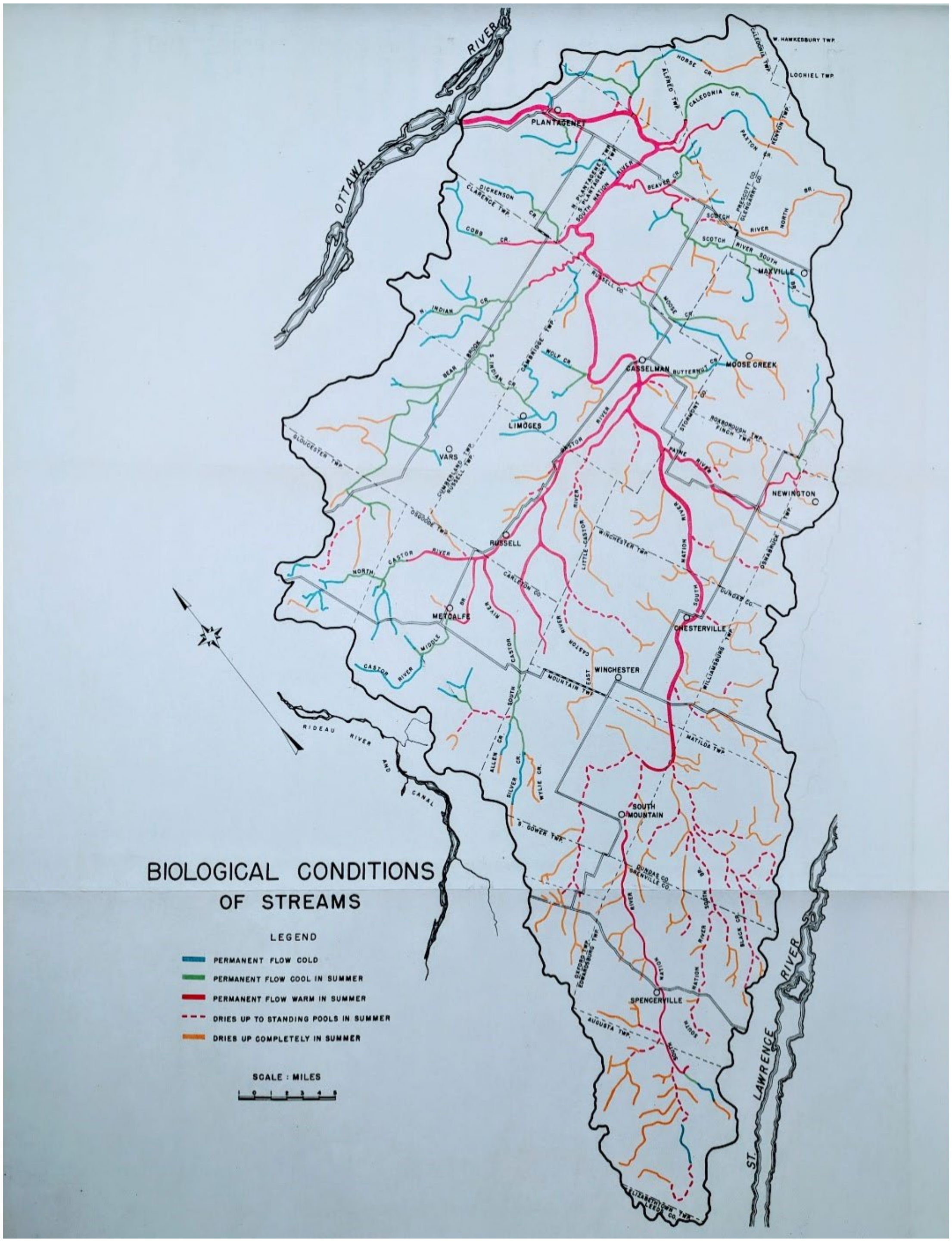

Figure 9. The permanence of flow and stream thermal regime based upon data collected in the late 1940's in the South Nation River Watershed (South Nation River Valley Interim Report, 1948).

Today, mass deployment of water temperature loggers allows accurate thermal regime studies and land use decisions based upon the thermal sensitivity of streams and rivers. Buffer widths and riparian area management can be based upon stream characteristics and the sensitivity of aquatic habitat at exact locations in the Bear Brook Watershed.

Continuous water temperature data were collected across the Bear Brook Watershed over the years 2020-2024. Data was collected over the summer months (Approximately June to October) and included hourly readings of water temperatures. The daily maximum air temperature and total rainfall as measured at Ottawa International Airport were used for analysis. Water temperatures were only included for the dates where the air temperature reached at least 24.5 degrees Celsius and no rain fell for at least two preceding days.

A thermal stability analysis was completed for all water temperature sites with requisite amounts of data by plotting the air and water temperatures between 1600 and 1800 hours according to Chu et al. (2009). To reduce the potential for error, multiple observations were used during the summer months and the arithmetic mean of the data was used for classification. Thermal stability classification confidence increases the further the arithmetic mean of the data is from the boundary of a thermal regime on a thermal classification graph. The closer the site is to a regime boundary, the more likely it will fall into either regime depending on annual weather conditions (Chu et al., 2009). Thermal stability graphs for all temperature sites across all years were created.

The results of all thermal stability graph classifications are presented in Table 13 by the year of data collection. Results by year and across the entire Bear Brook Watershed from 2020-2024 are shown in Figure 11.

Table 13. Thermal stability classifications at each site monitored for stream water temperatures 2020-2023. The UTM location of each site sampled is provided. Protocols for classification followed Chu et al., 2009.

Location Description	SNC OSAP Site Code*	2020	2021	2023	UTM East	UTM North	Notes
Bear Brook Trib. at Russell Rd.	CB37571			Cool	478417.5	5027635	
Bear Brook Trib. at Russell Rd.	CB01577			Warm	477871	5027394	
South Indian Creek at Indian Creek Rd., First	CB39689			Cool-warm	480508.3	5023414	

Location Description	SNC OSAP Site Code*	2020	2021	2023	UTM East	UTM North	Notes
Crossing of Saumure Rd.							
Johnston Municipal Drain at Hall Rd.	UB243004			Cool-warm	462756.2	5022146	
Johnston Municipal Drain at Hall Rd.	UB253004			Cool-warm	462703.7	5022084	
Bear Brook Creek at Hall Rd.	UB03643			Cool-warm	461756	5024780	
South Bear Brook Creek at Boundary Rd.	UB08644			Cool-warm	464047	5024715	
Bear Brook Municipal Drain at Milton Rd.	MB013271			Cool-warm	466896	5025241	
Savage Municipal Drain at Milton Rd.	MB07625			Cool	466668.8	5025452	
McKinnon's Creek at Milton Rd	MB06625			Cool-warm	466699	5025508	
Bickerton Municipal Drain at Milton Rd.	MB05615			Cool-warm	466671	5025645	
McKinnon's Creek at Wall Rd.	MB04434			Warm	463019	5031399	
McKinnon's Creek at Smith Rd.	MB03503			Cool-warm	465977	5029581	

Location Description	SNC OSAP Site Code*	2020	2021	2023	UTM East	UTM North	Notes
McFadden Municipal Drain at Delson Dr.	CB30492			Cool	467182.6	5029883	
Nelson Charlebois at the Prescott/Russell Rec. Trail	CB2901			Cool-warm	467862.9	5029151	
McFadden Municipal Drain at Frank Kenny Rd.	CB28582			Cool-warm	469060.6	5027269	Site went dry. Data may have inaccuracies.
Findlay Creek at Blais Rd.	UC122135			Cool-warm	454774	5018519	
Findlay Creek at Albion Rd.	UC24000			Cool-cold	451792	5017578	
Scott Extension Municipal Drain at Watson Rd.	CB11468			Cool-warm	473499	5030275	
Scott Extension Municipal Drain at Dunning Rd.	CB263274			Cool-warm	472654.1	5029388	
Bear Brook Trib. at Sarsfield Rd.	CB34553			Cool	474524.2	5028080	
South Indian Creek at Devine Rd.	CB3504			Cool-warm	479725.7	5024837	
South Indian Creek Trib. at Grant Side Rd.	CB3605			Cold	481626.9	5023231	

Location Description	SNC OSAP Site Code*	2020	2021	2023	UTM East	UTM North	Notes
South Indian Creek near Savage St.	CB3806			Cool-warm	481309.9	5021835	
South Indian Creek Trib. at Racette Side Rd.	CB12717			Cool-warm	481875	5021332	
South Indian Creek Trib. at Despins St.	CB13787			Cool-warm	481539	5020258	
South Indian Creek Trib. at Main St.	CB14806			Cool	480237	5019941	
Bear Brook Creek at Robillard Rd.	CB23501			Cool-warm	493391.2	5029659	
Black Creek at Clarence Cambridge Rd.	CB16559			Cool	492034	5027996	
Black Creek Trib. at Clarence Cambridge Rd.	CB31562			Cool-cold	491952.2	5027908	
Black Creek Trib. at Delatour Rd.	CB3202			Cool	489567.5	5026788	
Black Creek Trib. at Delatour Rd.	CB3303			Cool-cold	489672.1	502638.9	
Bear Brook Creek at Boileau Rd.	CB24480			Cool-warm	490924.3	5030051	

Location Description	SNC OSAP Site Code*	2020	2021	2023	UTM East	UTM North	Notes
North Indian Creek at Russell Rd.	NIC06457			Cool	485464	5030468	
Bear Brook Creek at Bouvier Rd.	CB25494			Cool-warm	485520.9	5029788	
North Indian Creek Trib. at Lacroix Rd.	NIC072742			Cool-warm	481938.8	5032341	
Bear Brook Trib. at St. Felix Rd.	CB40578			Cool	481733.3	5027308	
South Indian Creek at Indian Creek Rd.	CB22610			Cool-warm	480388	5026257	
Johnston Municipal Drain at Ramsayville Road		Cool			457825	5018531	
Johnston Municipal Drain at Anderson Road	UB202143	Cool-cold			459559	5019530	
Smith Gooding Municipal Drain at Ramsayville Road	UB042156	Cool-warm			456744	5021168	
Tributary of Bear Brook at Thunder Road - West		Cool			462280	5020753	
Smith Gooding Municipal Drain	UB102147	Cool			458459	5022242	

Location Description	SNC OSAP Site Code*	2020	2021	2023	UTM East	UTM North	Notes
at Anderson Road							
South Bear Brook at Hall Road	UB143004	Cool-warm			462869	5022007	
Smith Gooding Municipal Drain at Hawthorne Road	UB092158	Cool-warm			455177	5019634	
Tributary of Smith Gooding Municipal Drain at Davidson Road			Cool		452038	5021615	
Tributary of South Bear Brook at Farmers Way			Cool		460806	5021468	
Smith Gooding Municipal Drain at Farmers Way			Cool		460708	5022054	
Tributary of South Bear Brook at Thunder Road - East			Cool-cold		463862	5021569	

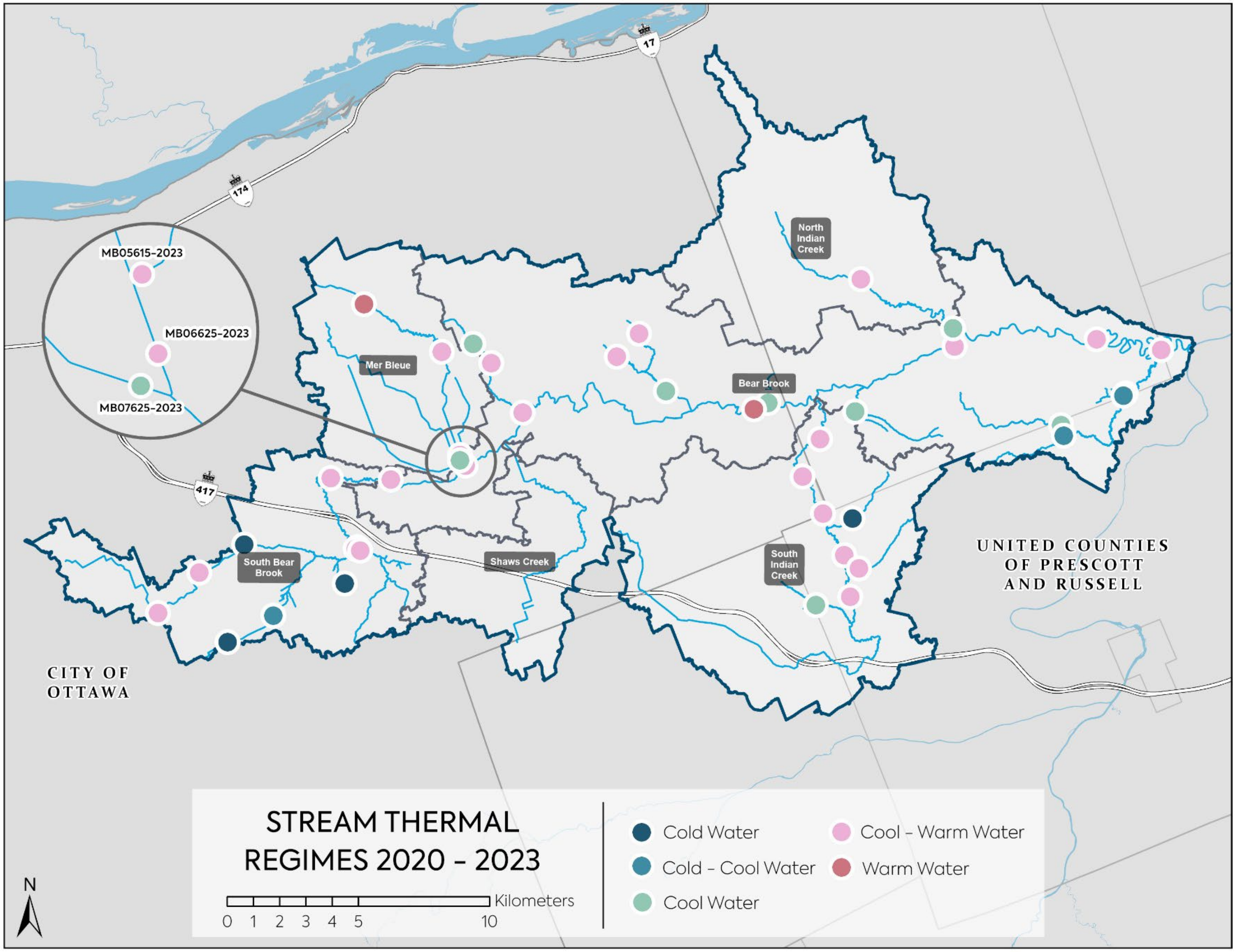

Figure 10 Stream thermal regimes in Bear Brook Watershed as measured between 2020-2023.

In general, thermal classifications and their influence on species distribution are somewhat predictable based upon physiographic areas and land use. Cooler surface water can be expected to occur where groundwater upwelling and seepage occurs, which is typically in the more forested headwaters of watersheds and river systems. In these areas, fish and benthic macroinvertebrates of the Bear Brook Watershed that prefer cool, pristine waters can be found (i.e., Northern Pearl Dace and Stoneflies). Land use, riparian shading and tree cover also influence thermal characteristics of streams, so warmer water temperatures in the heavily modified and developed areas of the watershed are consistent with expectations. In these areas, species more adapted to warm water temperatures are found (i.e., Bluntnose Minnows and blood worms).

The Bear Brook and Watershed can be considered a cool-warmwater system with specific areas exhibiting cool to cold water surface water temperatures. Not unlike results in the 1940's, the very headwater tributaries of the watershed are still exhibiting cold water thermal characteristics and in general, these areas have cooler water temperatures than that of the larger tributaries and streams further downstream in the watershed. Some of these headwater areas have been drastically modified, with headwater streams straightened and managed as Municipal Drains, some exhibiting year-round flows when they dried up in the past, and some drying up when they used to provide permanent, cold flowing water.

3. Benthic Macroinvertebrate Communities

<u>Key Findings</u>

- A new DNA-based approach used at 15 locations in the study area detected 27 species of mayflies, 13 species of stoneflies, 24 species of caddisflies and 2 species of mussels. No invasive bivalve mussel species were detected, providing an excellent baseline for monitoring biodiversity impacts and invasive species colonization over time.
- Traditional sampling and morphology identification by family level was conducted across the study area, identifying sites that range from excellent to poor condition according to a biotic index used to assess nutrient impairment (i.e., Hilsenhoff Family Biotic Index).
- Indices reveal a healthier benthic macroinvertebrate community in subwatersheds with higher forest and wetland cover compared to the urbanized areas of the watershed.

Freshwater benthic macroinvertebrates ("bugs") are the insects, worms, mollusks (i.e., mussels) and crustaceans (i.e., crayfish) that live at the bottom of a stream. Benthic macroinvertebrates occupy important roles in the functioning of freshwater ecosystems. They complete nutrient cycling within aquatic food webs and influence numerous processes including microbial production (Covich et al., 1999; Schmera et al., 2017 in Silva, 2023). These bugs are routinely used as indicators in freshwater studies because they are relatively sedentary, have high species richness, and display a range of responses to different environmental stressors and contaminants including temperature (Curry et al. 2018; Geest et al., 2010; Rosenberg and

Resh, 1993; Sidney et al., 2016 in Silva, 2023). Some groups of macroinvertebrates (mayflies, Ephemeroptera; stoneflies, Plecoptera; and caddisflies, Trichoptera), commonly referred to as EPT groups, are more sensitive to change in the aquatic environment and are deemed important bioindicator taxa for assessing freshwater quality (Curry et al., 2018; Hajibabaei et al., 2012, 2011 in Silva, 2023).

SNC utilized two different sampling techniques to assess benthic macroinvertebrate condition across the Bear Brook Watershed. Sample techniques and results are summarized below.

3.1. DNA Data Assessment

Traditionally, macroinvertebrates are identified to family level through morphological identification using microscopy. A new DNA-based approach is now available by combining bulk-tissue DNA collection (i.e. benthos) and next-generation sequencing (NGS), to produce high-quality data that allows identification to a finer resolution than traditional methods (Baird and Hajibabaei, 2012; Hajibabaei et al., 2012 in Silva, 2023).

SNC partnered with STREAM (Sequencing The Rivers for Environmental Assessment and Monitoring) in 2023 to sample several sites across the Bear Brook Watershed. STREAM is a biomonitoring project that involves the combination of community-based monitoring and DNA metabarcoding technologies. STREAM is a collaboration between World Wildlife Fund (WWF) Canada, Living Lakes Canada (LLC) and Environmental and Climate Change Canada (ECCC), led by the Hajibabaei Lab at the Centre for Biodiversity Genomics (University of Guelph, Canada).

Appendix B provides the methodology and results from a Preliminary DNA Data Assessment completed by STREAM on several sites across the Bear Brook Watershed (2023).

A range of macroinvertebrate species were detected. Traditional bioindicator EPT species were detected across the sampling sites, including 27 species of Ephemeroptera (mayflies), 13 species of Plecoptera (stoneflies) and 24 Trichoptera (caddisflies). Some families of these EPT groups are typically sensitive to many pollutants in the stream environment and are therefore associated with clean water (Gresens et al., 2009; Laini et al., 2019; Loeb and Spacie, 1994 in Silva, 2023).

Two freshwater mussel species (*Elliptio complanata* and *Pyganodon grandis*) were identified in six sampled sites. No Dreissenidae invasive species (such as the Zebra and Quagga mussel) were identified in the sampled areas.

This data will provide a good baseline for future water quality monitoring to track changes over time.

3.2. Ontario Benthos Biomonitoring Network

49 sites were sampled across the Bear Brook Watershed in 2020-2023 using the Ontario Benthos Biomonitoring Network Transect Kick Methodology (Jones *et al.*, 2007). Invertebrates were preserved for archiving purposes in 95% ethanol. Samples were processed and randomly sub-sampled to obtain an approximate 100-animal fixed count and identified to family level by a certified taxonomist.

Several metrics were chosen to assess the condition of the aquatic environment across the Bear Brook Watershed according to benthic macroinvertebrate community assemblages (Table 14).

Table 14. Metrics used to assess aquatic condition in the Bear Brook Watershed using benthic macroinvertebrates as indicators of ecosystem health.

Metric	Calculation	Description
Hilsenhoff Family Biotic Index (HBI)	Refer to Hilsenhoff (1988) Family Biotic Index (FBI) as modified by New York State (Smith et al., 2009). The ranges considered in this study were selected through watershed reporting initiatives by conservation authorities. 0.00 - 4.25 Excellent 4.26 - 5.00 Good 5.01 - 5.75 Fair 5.76 - 6.50 Poor 6.51 - 10.00 Very Poor	HBI is used to detect and assess the presence of organic pollutants such as agricultural runoff and sewage on aquatic ecosystems. Higher scores are typically associated with community assemblages that have a higher tolerance to nutrient enrichment and may be indicative of organic pollution at a monitoring site.
% EPT (Percent of Ephemeroptera, Plecoptera, Trichoptera)	Total EPT Count/Total Taxa Count Refers to the proportion of mayflies, stoneflies and caddisflies in a sample.	EPT are considered to be sensitive to pollution and declines in oxygen. High proportions of these taxa may be indicative of good environmental conditions at a monitoring site.
% CIGH (Percent of Chironomidae,	Total CIGH Count/ Total Taxa Count	CIGH represents a group of taxa that are generally tolerant of

Metric	Calculation	Description
Isopoda, Gastropoda, Hirudinea)	Refers to the proportion of midge larvae, sowbugs, aquatic snails and leeches.	nutrient enrichment, pollution and habitat disturbances. Large proportions may indicate fair or poor conditions.
Taxa Richness	Total number of unique taxa families present at a monitoring site.	The greater the diversity of an ecosystem, the better it can maintain balance and productivity and withstand environmental stressors. Richness declines as a monitoring site experiences altered flow regimes, habitat loss, invasive species presence, and changes in water chemistry.
EPT Richness	Total number of mayfly stonefly and caddisfly Families present at a monitoring site.	A high diversity or richness of EPT species in surface water often correlates with lower pollution levels, stable habitat conditions, and good oxygen availability.

Table 15 presents the results for all sites monitored in the Bear Brook Watershed from 2020 to 2023 and Figure 11 depicts Hilsenhoff Family Biotic Index scores by site. Similar to water chemistry monitoring stations, impairment is more concentrated in areas experiencing urban influences compared to sites further downstream.

South Bear Brook and Mer Bleue subcatchments observed a greater number of sites in poor condition compared to other catchments and received an overall average poor rating. Sites located in the Main Bear Brook, and North Indian Creek catchments range from good to fair condition, observing poor conditions in locations lacking in vegetative cover. The average catchment rating is fair for Main Bear Brook and good for North Indian Creek. South Indian Creek Catchment ranged from excellent to fair condition, and received an overall good rating, while the Black Creek catchment is in reference condition and largely observed excellent results, with one stem of the river experiencing fair ratings. The Black Creek catchment provides an excellent opportunity as a reference condition since land ownership is under the United Counties of Prescott and Russell and land cover is largely made up of forest and wetlands within Larose Forest.

Table 15. Benthic macroinvertebrate metric scores for sample sites across the Bear Brook Watershed.

Watercourse	Site	Year	HBI	Grade	% EPT	% CIGH	Richness	EPT Richness
South Bear Brook								
South Bear Brook	UB03643	2023	5.25	C	6%	29%	22	5
Johnston MD	UB253004	2023	5.6	C	23%	16%	20	5
South Bear Brook	UB13643	2021	5.75	C	25%	9%	28	5
Johnston MD	UB243004	2023	5.82	D	31%	15%	30	8
Smith Gooding Municipal Drain	UB11715	2021	6.37	D	18%	26%	23	3
Bear River	SBB_Fwthunder	2021	6.12	D	7%	17%	19	1
Smith Gooding Municipal Drain	UB042156	2021	5.95	D	16%	26%	25	5
Smith Gooding Municipal Drain	SBB_Davidson	2021	6.24	D	1%	19%	15	2
Smith Gooding Municipal Drain	UB092158	2021	5.85	D	36%	7%	24	4
Smith Gooding Municipal Drain	UB102147	2020	6.82	D	1%	36%	18	2
Tributary of South Bear Brook	UB02731	2020	6.15	D	20%	28%	28	5
Smith Gooding Municipal Drain	UB122145	2020	6.41	D	4%	45%	20	4
South Bear Brook	UB08644	2020	5.99	D	21%	13%	33	8
South Bear Brook	UB143004	2020	5.91	D	17%	14%	23	3
Mer Bleue								
McKinnon's Creek	MB03503	2023	4.78	B	35%	4%	28	9
Bickertine MD	MB05625	2023	5.74	C	5%	13%	32	7
McKinnon's Creek	MB013271	2023	5.97	D	21%	29%	33	7
McKinnon's Creek	MB04434	2023	6.21	D	3%	30%	15	2
McKinnon's Creek	MB06625	2023	5.85	D	4%	11%	33	8
Savage MD	MB07625	2023	6.43	D	22%	34%	17	4

Watercourse	Site	Year	HBI	Grade	% EPT	% CIGH	Richness	EPT Richness
Main Bear Brook								
Central Bear Brook	CB01577	2023	4.45	B	19%	3%	27	8
Bear Brook MD	CB25494	2023	4.25	B	11%	5%	28	9
Bear Brook MD	CB27582	2023	4.51	B	9%	35%	23	3
Scott Extension MD	CB11468	2023	5.75	C	0%	33%	16	0
Nelson Charlebois	CB2901	2023	5.08	C	33%	5%	21	4
Tributary of Bear Brook	CB34553	2023	5.28	C	38%	11%	26	8
Main Bear Brook	CB37571	2023	5.5	C	53%	13%	19	3
Scott Extension MD	CB263274	2023	5.76	D	13%	27%	27	5
McFadden MD	CB28582	2023	5.99	D	24%	12%	24	5
McFadden MD	CB30492	2023	5.97	D	2%	1%	8	2
South Indian Creek								
South Indian Creek	CB3605	2023	3.7	A	67%	1%	24	11
South Indian Creek	CB12717	2023	4.75	B	19%	0%	20	7
South Indian Creek	CB13787	2023	4.68	B	42%	10%	33	11
South Indian Creek	CB22610	2023	4.37	B	42%	10%	28	9
South Indian Creek	CB3806	2023	4.81	B	46%	1%	31	11
South Indian Creek	CB40578	2023	4.35	B	17%	2%	26	7
South Indian Creek	CB14806	2023	5.01	C	52%	5%	22	7
South Indian Creek	CB3504	2023	5.57	C	12%	6%	24	7
South Indian Creek	CB39689	2023	5.33	C	40%	5%	20	7

Watercourse	Site	Year	HBI	Grade	% EPT	% CIGH	Richness	EPT Richness
Black Creek								
Black Creek	CB23501	2023	3.7	A	8%	12%	23	8
Tributary of Black Creek	CB31526	2023	2.91	A	3%	9%	17	3
Black Creek	CB3303	2023	4.12	A	8%	7%	14	3
Black Creek	CB16559	2023	5.56	C	9%	7%	23	8
Black Creek	CB3202	2023	5.18	C	7%	7%	24	7
North Indian Creek								
Tributary of North Indian Creek	NIC05389	2023	3.24	A	53%	1%	24	8
North Indian Creek	NIC06457	2023	4.54	B	33%	3%	27	7
Louis Lafleur Municipal Drain	NIC082745	2023	4.97	B	67%	1%	28	7
Tributary of North Indian Creek	NIC072742	2023	5.42	C	18%	10%	34	7
Tributary of North Indian Creek	NIC093100	2023	6.4	D	19%	21%	29	5

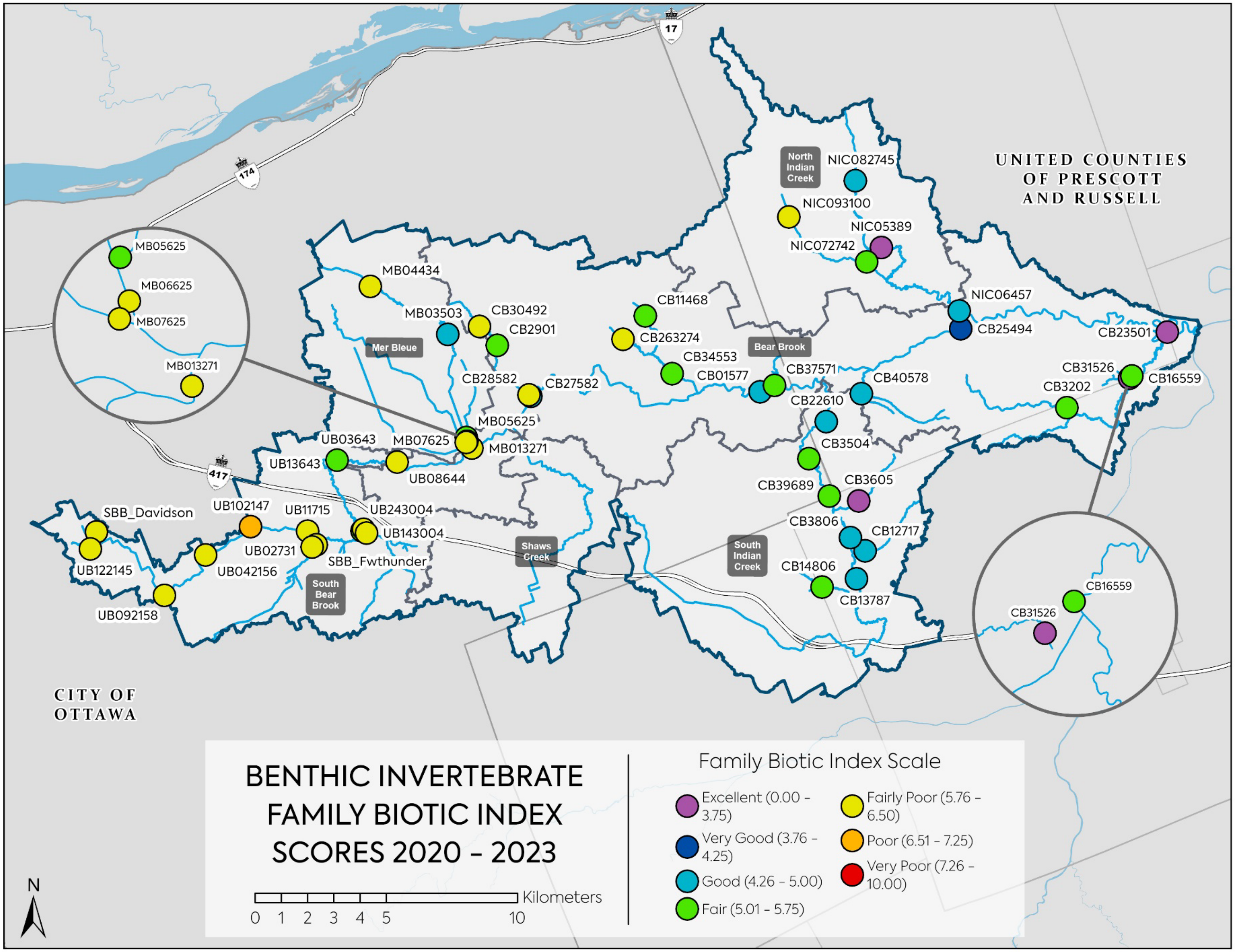

Figure 11. Benthic invertebrate Family Biotic Index scores in Bear Brook Watershed, 2020-2023.

4. Summary

Surface water quality in the Bear Brook Watershed is shaped by a combination of natural geological processes and human activities. The weathering and erosion of soil material is a major determinant of background surface water chemistry, while several other factors include inputs associated with the amount, type and distribution of precipitation, catchment hydrology and surrounding land use and vegetation (Hamid et al. 2020).

The Bear Brook Watershed's water quality is deeply influenced by its geological history, particularly the vast clay plains formed by glacial activity and the remnants of an ancient inland arm of the Atlantic Ocean. These processes have resulted in naturally elevated levels of phosphorus, chlorides, and iron in the watershed's surface water (Schneider et al., 2022, Colgrove, 2016). Understanding these natural contributions is critical for distinguishing between background levels and anthropogenic impacts when managing the Bear Brook Watershed.

As glaciers advanced and retreated in the Bear Brook Watershed, they sculpted the landscape, depositing vast amounts of sediment. The movement of glaciers also led to the creation of glacial lakes and rivers, which left behind large plains of fine-grained clay after their retreat. Clay-rich soils are known for their ability to retain water and nutrients. The high surface area of clay particles allows them to hold onto phosphorus, which can slowly release into water bodies through erosion or leaching, resulting in high background levels of this nutrient in the watershed.

Additional parameters that have naturally high background levels include chloride and iron. The Bear Brook Watershed was once part of an inland arm of the Atlantic Ocean. This ancient marine environment left behind marine sediments rich in chloride ions. Over time, as the sea retreated, these sediments were buried under glacial deposits. These deposits still contribute to the chemistry of the groundwater and surface water in the region. The naturally elevated background levels of chloride are a legacy of the watershed's marine past and are present in the groundwater that feeds into streams and rivers.

The combination of glacial deposits and marine sediments also contributes to the naturally high levels of iron in the watershed's soils and waters. Iron is a common element in the Earth's crust and is often found in significant quantities in both glacial till and marine deposits. The iron present in these deposits can be mobilized into water bodies through natural processes, particularly under conditions where the oxygen levels in the water or soil are low (reducing conditions). This process is influenced by the watershed's hydrology and the interactions between groundwater and surface water.

Recognizing these natural background levels is essential before assessing the impact of human activities and developing effective watershed management strategies. Historical settlement patterns have also been influenced by the geologic history of the Bear Brook Watershed and have contributed to current land practices. The vast clay plains of the Bear Brook Watershed have high agricultural capability. Over the years forests and wetlands have been converted to support cropping systems, with approximately 36% of the watershed currently under agricultural land use, including 17% that is tile drained, leading to increased nutrient loading in the watershed. Fertilizers used in crop production contribute additional phosphorus to the already nutrient-rich soils, exacerbating the risk of eutrophication in nearby water bodies. Agricultural activities (i.e., tillage) can lead to increased soil erosion. During precipitation events, nutrient rich soil is transported into streams and rivers, increasing the sediment load and nutrient concentrations in the water.

Urbanization is another significant stressor on the watershed. The proximity of the Bear Brook Watershed to the City of Ottawa and major transportation corridors like the 417 has driven urban expansion, particularly in the headwaters of the South Bear Brook and McKinnon's Creek catchments. This urban growth has led to elevated levels of chlorides, nutrients, *E. coli* and metals, particularly in areas where natural habitats have been disturbed or removed, where vegetative and riparian cover is lacking, and during periods of runoff following precipitation events.

The Canadian Water Quality Index (WQI) scores for the Bear Brook Watershed range from fair to poor, reflecting frequent impairments in water quality due to pollutants exceeding recommended levels. The WQI considers a variety of parameters, including nutrients, chlorides, and pathogens like *E. coli*, all of which are affected by both natural processes and human activities within the watershed.

Chloride levels, in particular, have shown consistent increases across the watershed, with the highest concentrations observed in the more urbanized areas, including the headwaters of South Bear Brook and McKinnon's Creek. Addressing road salt application in an area that will significantly increase in population will be a key management strategy that will need to be addressed.

Naturally elevated levels of chlorides and phosphorus present challenges that are compounded by agricultural and urban activities. Biological community sampling identified that sites closer to urban influences are on average in poor condition, while sites that are located in areas with good natural cover (i.e., Black Creek) are faring better. Locations such as Black Creek act as important future reference sites when facing challenges determining if fluctuations in stream temperature, chemistry, and biological community composition are due to changes in climate or due to urban influences.

As urban expansion continues, it is crucial to implement strategies that mitigate these impacts, protecting water quality for the long term. This involves a combination of sustainable land use planning, the adoption of best management practices in agriculture, and ongoing monitoring to ensure that the watershed remains a healthy and viable ecosystem for future generations.

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Appendix A Preliminary DNA Data Assessment Completed by STREAM

Preliminary DNA Data

Bear Brook subwatershed - South Nation watershed, ON
South Nation Conservation – Water Monitoring
October 2023

Photo: Braulio Silva, Credit: STREAM project

STREAM project

Hajibabaei Lab, Centre for Biodiversity Genomics, University of Guelph

STREAM

WWF Canada
Environment and Climate Change Canada
Living Lakes Canada

Table of Contents

DISCLAIMER: This report is a preliminary report based on the samples and information collected in the field. Identifications of taxa are based on best available information at time of analysis and reporting.

1. INTRODUCTION

1.1. Benthic Macroinvertebrates

Freshwater benthic macroinvertebrates are typically insect orders, as well as crustaceans (e.g. crayfish), gastropods (e.g. snails), bivalves (e.g. freshwater mussels) and oligochaetes (e.g. worms), which are located on or within the benthic substrate of freshwater systems (i.e. streams, rivers, lakes; (Covich et al., 1999; Schmera et al., 2017). Benthic macroinvertebrates occupy important roles in the functioning of freshwater ecosystems, namely nutrient cycling within aquatic food webs and also influence numerous processes including microbial production and release of greenhouse gases (Covich et al., 1999; Schmera et al., 2017).

Biological monitoring (biomonitoring), referring to the collection and identification of particular aquatic species is an effective method for measuring the health status of freshwater systems. Currently, macroinvertebrates are routinely used for biomonitoring studies in freshwater habitats because they are relatively sedentary, have high species richness and a range of responses to different environmental stressors and contaminants, including temperature (Curry et al., 2018; Geest et al., 2010; Rosenberg and Resh, 1993; Sidney et al., 2016). Some groups of macroinvertebrates (mayflies, Ephemeroptera; stoneflies, Plecoptera and caddisflies, Trichoptera), commonly referred to as EPT groups, are more sensitive to change in the aquatic environment and are deemed important bioindicator taxa for assessing freshwater quality (Curry et al., 2018; Hajibabaei et al., 2012, 2011).

Traditionally, macroinvertebrates are identified to family level (**Figure 1**) through morphological identification using microscopy, however there has been a shift from this labour-intensive methodology to a DNA-based approach (Curry et al., 2018; Hajibabaei et al., 2012, 2011). 'Biomonitoring 2.0' combines bulk-tissue DNA collection (i.e. benthos) with next-generation sequencing (NGS), to produce high-quality data in large quantities and allows identification to a finer resolution than traditional methods (Baird and Hajibabaei, 2012; Hajibabaei et al., 2012).

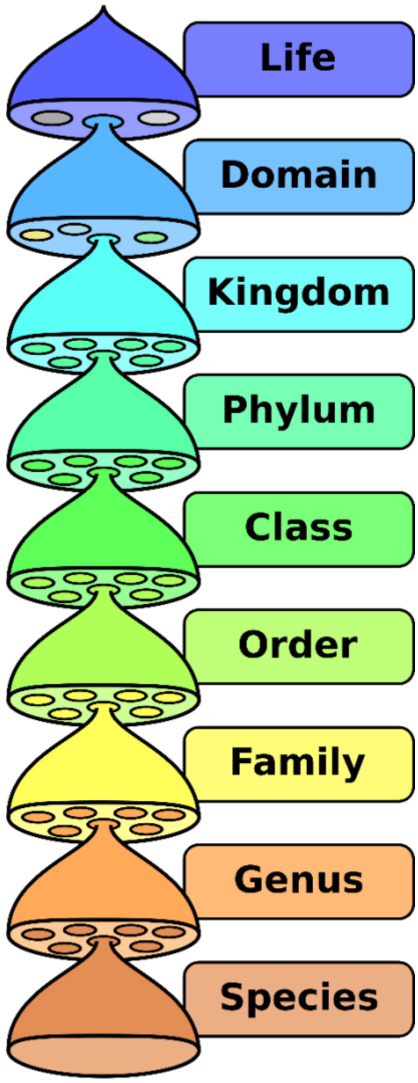

Figure 1. Graphical representation of the classification of organisms.

1.2. Background of STREAM

STREAM (Sequencing The Rivers for Environmental Assessment and Monitoring), is a biomonitoring project, which involves the combination of community based monitoring and DNA metabarcoding technologies to assess the benthic macroinvertebrate communities in watersheds across Canada (**Figure 2**). STREAM is a collaboration between World Wildlife Fund (WWF) Canada, Living Lakes Canada

(LLC) and Environmental and Climate Change Canada (ECCC), led by the Hajibabaei Lab at Centre for Biodiversity Genomics (University of Guelph, Canada). STREAM is integrated with the Canadian Aquatic Biomonitoring Network (CABIN) programme, through the implementation of existing nationally standardized protocols for freshwater monitoring. The aquatic biodiversity data generated in STREAM will be added to the existing CABIN database, to improve our understanding of the health of Canadian watersheds.

The main objective of STREAM is to generate baseline benthic macroinvertebrate DNA data from across Canada. To understand the health status of freshwater systems, we first need to understand the natural fluctuations and trends of benthic macroinvertebrates, especially in locations which are data deficient. By building this baseline, in years to come we can investigate the longer-term trends and begin to understand the impact of issues, such as climate change, on freshwater systems. STREAM was established with the main premise of fast-tracking the generation of benthic macroinvertebrate data from 12-18 months to ~2 months, while increasing the taxonomic resolution of the data produced.

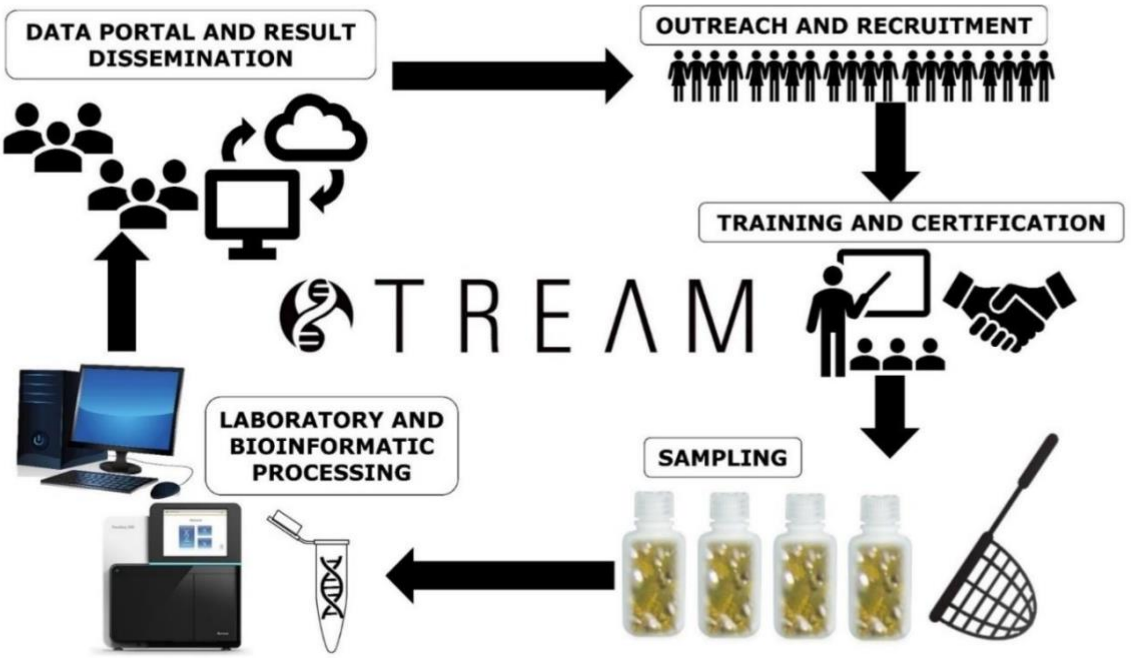

Figure 2. Graphical representation of the STREAM collaborative workflow for DNA biomonitoring of benthic invertebrates.

1.3. Objective of Report

Data and information included in this report is a preliminary examination of results from the Bear Brook subwatershed, within the South Nation Watershed (ON), which consists of a list of the macroinvertebrate and other metazoan taxa detected within the samples analyzed. This report aims to highlight the different macroinvertebrate taxa to assess the subwatershed health in 2023.

2. METHODOLOGY

2.1. Study Area

This study was conducted from 16 to 19 October 2023 at fifteen sampling locations within the Bear Brook subwatershed (ON; **Figure 3**). Braulio Silva and Andrew Riley (Centre for Biodiversity Genomics – University of Guelph) conducted the sampling for benthic macroinvertebrate monitoring with STREAM.

Additional site information, including coordinates is provided in the Excel file submitted.

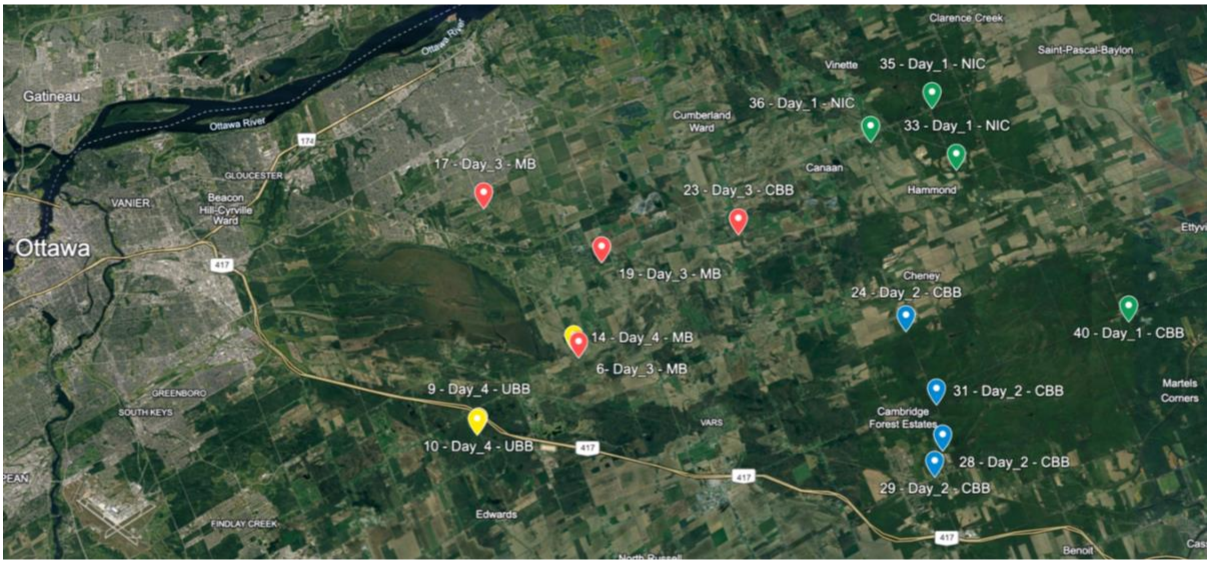

Figure 3. Map of sampling locations within the Bear Brook subwatershed (ON). Collection days are also shown.

2.2. DNA Sampling and Processing Methods

2.2.1. Measures to Avoid DNA Contamination

Prior to sampling, kick-nets were sanitized in bleach for 45 minutes and kept in clean garbage bags until they were used in the field. Gloves were used when handling all sampling materials to avoid contamination. During the kick-netting, the surveyor in the water wore two pairs of gloves while handling the kick-net. The outer pair of gloves was removed prior to transferring the contents into sampling containers so that the gloves used when contacting the sample were guaranteed to be clean. Each sampling container was individually sealed in a Ziploc bag prior to placing them in the cooler.

2.2.2. Benthic Macroinvertebrate Field Sampling Protocol

Benthic macroinvertebrate DNA samples were collected following the STREAM Procedure for collecting benthic macroinvertebrate DNA samples in wadeable streams (v1.0 June 2019) and the CABIN Field Manual for Wadeable Streams (2012). The STREAM procedure outlines steps to minimize DNA contamination and preserve DNA samples and was employed in conjunction with sampling steps outlined in the CABIN manual. All samples collected were transported to the Centre for Biodiversity Genomics (University of Guelph, Guelph, ON) for laboratory processing.

2.2.3. Laboratory Methods

Benthic samples were preserved in antifreeze and stored at -20°C until processing. Benthic samples were coarsely homogenized in a sterile blender and DNA was extracted using a DNeasy® PowerSoil® Pro kit (Qiagen, CA) kit. Extracted DNA was then processed following the standard Hajibabaei Lab protocol for Next-Generation Sequencing (NGS). Sequences were then processed through the MetaWorks (v1.13.0) pipeline using the COI classifier (RDP_COIv5.1.0) for the COI marker: https://github.com/terrimporter/MetaWorks.

3. RESULTS

3.1. Overview

The raw data output from NGS produced sequences for a range of taxa. This taxa list was reduced to only sequences that identified macroinvertebrates associated with freshwater and riparian ecosystems, and that were of high enough quality to match reference sequences. These results consisted of **6 Classes, 19 Orders, 80 Families, 166 Genera, and 295 Species of invertebrates**. A full taxonomic list of macroinvertebrates identified to the raw genus and species level is included as a separate Excel spreadsheet.

3.2. Taxonomic Coverage

A range of macroinvertebrate species were detected. Traditional bioindicator EPT species were detected across the sampling sites, including **27** species of Ephemeroptera (mayflies), **13** species of Plecoptera (stoneflies) and **24** Trichoptera (caddisflies; **Table 1**). Some families of these EPT groups are typically sensitive to many pollutants in the stream environment and are therefore associated with clean water (Gresens et al., 2009; Laini et al., 2019; Loeb and Spacie, 1994).

Please refer to the '**Macroinvertebrate Bioindicator Families Guide v1.2**' attached with your data or visit the corresponding website here for more information on approximate tolerances for the species detected in your sites.

Note: The benthic macroinvertebrate kick-net sample procedure often results in collection of both aquatic and terrestrial taxa, however terrestrial taxa are not identified using the traditional taxonomic identification methods. Due to the nature of DNA metabarcoding, both terrestrial and aquatic macroinvertebrates are identified and described using the DNA approach in this report.

Table 1. List of Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa identified to the species level. P = present. Grey cells indicate absence. Site names for each column refer to site code (see Excel spreadsheet). Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included.

Order	Family	Genus	Species	Bear Brook site														
				SN-6	SN-9	SN-10	SN-14	SN-17	SN-19	SN-23	SN-24	SN-28	SN-29	SN-31	SN-33	SN-35	SN-36	SN-40
Ephemeroptera	Baetidae	Acerpenna	Acerpenna_macdunnoughi								P		P	P		P	P	
Ephemeroptera	Baetidae	Baetis	Baetis_flavistriga								P		P					
Ephemeroptera	Baetidae	Baetis	Baetis_phoebus								P							
Ephemeroptera	Baetidae	Baetis	Baetis_tricaudatus			P						P						
Ephemeroptera	Baetidae	Callibaetis	Callibaetis_fluctuans	P		P											P	
Ephemeroptera	Baetidae	Centroptilum	Centroptilum_sp._BOLD:AAD6070		P	P											P	
Ephemeroptera	Baetidae	Cloeon	Cloeon_dipterum		P												P	
Ephemeroptera	Baetidae	Procloeon	Procloeon_fragile		P	P							P					
Ephemeroptera	Baetidae	Procloeon	Procloeon_rivulare												P			
Ephemeroptera	Baetidae	Procloeon	Procloeon_rubropictum			P												
Ephemeroptera	Baetidae	Procloeon	Procloeon_viridoculare		P	P												
Ephemeroptera	Caenidae	Caenis	Caenis_amica	P	P	P												
Ephemeroptera	Caenidae	Caenis	Caenis_latipennis	P	P	P	P		P	P	P		P		P	P	P	P
Ephemeroptera	Caenidae	Caenis	Caenis_youngi	P	P	P												
Ephemeroptera	Heptageniidae	Maccaffertium	Maccaffertium_luteum								P							
Ephemeroptera	Heptageniidae	Maccaffertium	Maccaffertium_smithae								P	P	P	P	P		P	
Ephemeroptera	Heptageniidae	Maccaffertium	Maccaffertium_vicarium								P					P		
Ephemeroptera	Heptageniidae	Stenacron	Stenacron_gildersleevei		P						P		P				P	P
Ephemeroptera	Heptageniidae	Stenacron	Stenacron_interpunctatum		P						P		P				P	P
Ephemeroptera	Heptageniidae	Stenonema	Stenonema_femoratum		P	P			P				P					P
Ephemeroptera	Leptophlebiidae	Habrophlebiodes	Habrophlebiodes_americana								P	P	P		P	P	P	
Ephemeroptera	Leptophlebiidae	Leptophlebia	Leptophlebia_cupida								P	P			P			P
Ephemeroptera	Leptophlebiidae	Leptophlebia	Leptophlebia_nebulosa								P	P	P	P	P	P	P	P
Ephemeroptera	Leptophlebiidae	Leptophlebia	Leptophlebia_sp._BOLD:AAX5673											P	P	P		P
Ephemeroptera	Leptophlebiidae	Leptophlebia	Leptophlebia_sp._BOLD:ACL6896												P			P
Ephemeroptera	Leptophlebiidae	Paraleptophlebia	Paraleptophlebia_heteronea			P								P				
Ephemeroptera	Leptophlebiidae	Paraleptophlebia	Paraleptophlebia_sp._BOLD:AAC5651											P		P		
Plecoptera_stoneflies	Capniidae	Allocapnia	Allocapnia_vivipara				P		P		P					P	P	
Plecoptera_stoneflies	Capniidae	Paracapnia	Paracapnia_angulata								P							
Plecoptera_stoneflies	Nemouridae	Amphinemura	Amphinemura_nigritta											P				
Plecoptera_stoneflies	Nemouridae	Amphinemura	Amphinemura_varshava											P				
Plecoptera_stoneflies	Nemouridae	Nemoura	Nemoura_trispinosa											P				
Plecoptera_stoneflies	Nemouridae	Prostoia	Prostoia_similis									P			P			
Plecoptera_stoneflies	Nemouridae	Soyedina	Soyedina_vallicularia									P		P				
Plecoptera_stoneflies	Perlidae	Doroneuria	Doroneuria_theodora								P							
Plecoptera_stoneflies	Perlodidae	Isoperla	Isoperla_frisoni								P			P				
Plecoptera_stoneflies	Perlodidae	Megarcys	Megarcys_signata								P							
Plecoptera_stoneflies	Perlodidae	Skwala	Skwala_americana								P							
Plecoptera_stoneflies	Taeniopterygidae	Taeniopteryx	Taeniopteryx_burksi								P		P					
Plecoptera_stoneflies	Taeniopterygidae	Taeniopteryx	Taeniopteryx_nivalis								P							
Trichoptera	Dipseudopsidae	Phylocentropus	Phylocentropus_lucidus									P		P				
Trichoptera	Hydropsychidae	Ceratopsyche	Ceratopsyche_bronta								P							
Trichoptera	Hydropsychidae	Ceratopsyche	Ceratopsyche_slossonae						P									
Trichoptera	Hydropsychidae	Ceratopsyche	Ceratopsyche_sparna								P							
Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche_analis		P		P		P		P	P	P	P		P	P	
Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche_smithi						P		P		P	P		P	P	
Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche_sp._BOLD:ACF3382						P									
Trichoptera	Hydropsychidae	Cheumatopsyche	Cheumatopsyche_wrighti						P									
Trichoptera	Hydropsychidae	Hydropsyche	Hydropsyche_betteni						P		P	P	P			P	P	
Trichoptera	Hydropsychidae	Hydropsyche	Hydropsyche_betteni_group_BOLD:AAA1669						P		P							
Trichoptera	Hydropsychidae	Hydropsyche	Hydropsyche_sp._BOLD:AAA2528							P	P							
Trichoptera	Hydropsychidae	Hydropsyche	Hydropsyche_sp._BOLD:AAA3450								P							
Trichoptera	Lepidostomatidae	Lepidostoma	Lepidostoma_bryanti									P						
Trichoptera	Leptoceridae	Oecetis	Oecetis_inconspicua			P		P									P	
Trichoptera	Leptoceridae	Triaenodes	Triaenodes_marginatus														P	
Trichoptera	Limnephilidae	Hydatophylax	Hydatophylax_argus								P			P	P			P
Trichoptera	Limnephilidae	Pycnopsyche	Pycnopsyche_aglona			P												
Trichoptera	Limnephilidae	Pycnopsyche	Pycnopsyche_antica									P		P				
Trichoptera	Limnephilidae	Pycnopsyche	Pycnopsyche_guttifera									P		P	P			P
Trichoptera	Limnephilidae	Pycnopsyche	Pycnopsyche_lepida						P			P	P		P			P
Trichoptera	Phryganeidae	Ptilostomis	Ptilostomis_ocellifera											P		P		P
Trichoptera	Polycentropodidae	Plectrocnemia	Plectrocnemia_cinerea	P														
Trichoptera	Psychomyiidae	Lype	Lype_diversa									P						
Trichoptera	Psychomyiidae	Psychomyia	Psychomyia_flavida								P							

3.3. Mussels Detection

Considering the collaborator's interest, we also performed a specific search to detect mussel species in the sampled sites. After our analyses, we detected two freshwater mussel species (*Elliptio complanata* and *Pyganodon grandis*) in six sampled sites (SN-6, SN-9, SN-10, SN-35, SN-28, and SN-33) (**Table 2**). Additionally, we present results for other metazoan species that do not pertain to the Arthropoda phylum (see Excel spreadsheet). **It is important to highlight that we did not identify any Dreissenidae invasive species (such as the Zebra and Quagga mussel) in the sampled areas using the COI marker.**

Table 2. List of freshwater mussels identified to the species level. P = present. Grey cells indicate absence. Site names for each column refer to the site code (see Excel spreadsheet). Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) are included.

						Bear Brook site														
Phylum	Class	Order	Family	Genus	Species	SN-6	SN-9	SN-10	SN-14	SN-17	SN-19	SN-23	SN-24	SN-28	SN-29	SN-31	SN-33	SN-35	SN-36	SN-40
Mollusca	Bivalvia	Unionida	Unionidae	Elliptio	Elliptio_complanata									P			P			
Mollusca	Bivalvia	Unionida	Unionidae	Pyganodon	Pyganodon_grandis	P	P	P										P		

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5. GLOSSARY

Term	Meaning
Benthic/benthos	The ecological region at the lowest level of a body of water such as an ocean, lake, or stream, including the sediment surface and some sub-surface layers.
Biomonitoring	The science of inferring the ecological condition of an ecosystem (i.e. rivers, lakes, streams, and wetlands) by examining the organisms that live there.
Bootstrap support	Statistical methods used to evaluate and distinguish the confidence of results produced.
Bulk-tissue DNA sample	This refers to the collection and removal of a reasonable quantity of representative material (including organisms such as river bugs) from a location (i.e. river bed).
DNA extraction	Isolation of DNA from either the target organism (i.e. DNA from an insect leg) or from an environmental sample (i.e. DNA from a water or benthos sample).
DNA Metabarcoding	Amplification of DNA using universal barcode primers (e.g. universal for invertebrates) to allow sequencing of DNA from target organisms (e.g. invertebrates) from environmental samples (e.g. river water or benthos).
Environmental DNA (eDNA)	The DNA released into the environment through faeces, urine, gametes, mucus, etc. eDNA can result from the decomposition of dead organisms. eDNA is characterized by a complex mixture of nuclear, mitochondrial or chloroplast DNA, and can be intracellular (from living cells) or extracellular. Environmental DNA: DNA that can be extracted from environmental samples (such as soil, water, or air), without first isolating any target organisms.
EPT groups	The three orders of aquatic insects that are common in the benthic macroinvertebrate community: Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies).
Macroinvertebrate	Organisms that lack a spine and are large enough to be seen with the naked eye. Examples of macro-invertebrates include flatworms, crayfish, snails, clams and insects, such as dragonflies.
Next-generation sequencing (NGS)	Use of next-generation sequencers (i.e. Illumina) to millions or billions of DNA strands in parallel.
Riparian	Relating to or situated on the banks of a river.
Sample homogenization	The process of making an environmental sample (i.e. benthos) uniform. For liquid/benthos samples, this often involves mixing using a blender so that DNA is evenly distributed within the sample.
Taxa	Unit used in the science of biological classification, or taxonomy.