AN ADAPTIVE MANAGEMENT FRAMEWORK FOR ENVIRONMENTAL SUBSTANCES OF CONCERN IN WASTEWATER

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Final Public Report

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Prepared by University of Calgary and InnoTech Alberta

Co-authored by: Kelly Munkittrick[1], Fred Wrona[1], Kerry Black[1], Rajiv Tanna[1,2], Patricija Marjan[1], David Barrett[1], Maricor Arlos[3,4], Karen Kidd[5] and Mark Servos[4]

[1]University of Calgary; [2]InnoTech Alberta; [3]Unversity of Alberta (Current address [4]University of Waterloo); [5]McMaster University

Submitted by: Kelly Munkittrick, CAIP Research Chair in Aquatic Ecosystem Health
Kelly.munkittrick@ucalgary.ca ; 226 789-4835

UNIVERSITY OF CALGARY

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Executive Summary

This project was designed to characterize environmental responses to environmental substances of concern (ESOCs) introduced via The City's wastewater in the Bow River Watershed. Impacts of ESOCs on aquatic ecosystem structure and function are not fully understood. The study utilized collections from the Bow River as well as controlled studies conducted at the Advancing Canadian Wastewater Assets (ACWA) at the Pine Creek wastewater facility; the ACWA facility is a shared research infrastructure between The City of Calgary and the University of Calgary. The Pine Creek waste treatment facility produces wastewater that includes tertiary treatment and the effluent quality meets all current regulatory criteria and frequently exceeds minimum requirements. Research using replicate experimental streams at the facility at Pine Creek Wastewater Treatment Plant was conducted in parallel to the Bow River, to allow development and testing of new monitoring technologies and environmental prediction models to evaluate the current state of the river, to set a current baseline for future studies, and to develop interim thresholds for identifying changes in the accumulated environmental state.

The first objective was to establish effective ecosystem health indicators for evaluating wastewater effluent impacts that can allow evaluation and provide guidance for optimization of wastewater treatment investments. The project included further identification and quantification of environmental substances of concern (ESOCs) from The City's existing monitoring plan as well as targeted sampling to support the exposure indicators, as well as lower trophic ecosystem indicators reflecting productivity, benthic macroinvertebrate indicators, and measures of small-bodied whole organism indicators. It also evaluated emerging tools including microbiome measures and transcriptomic measures of gene expression.

The ESOCs studies resulted in a prioritization of the existing suite of chemicals used to reflect exposures, removing compounds from the suite of indicators that were consistently below detection limits. Method development focused on the ability to measure ESOCs in sediment, benthic invertebrates, and fish tissue, and showed accumulation of some ESOCs residues. These results provide an initial baseline for future studies, and demonstrated that biofilm and benthic invertebrates were accumulating detectable levels of some chemicals.

The lower trophic level studies showed that microbial communities downstream of MWWE are heterotroph dominant; heterotrophic ecological endpoints are generally not measured, and are essential for understanding downstream productivity along with autotrophic responses (ie, chlorophyll-a measures). Current productivity measures in field studies concentrate on detecting chlorophyll-a levels which are a measure of autotrophic production. The studies proposed that a better indicator of downstream productivity could be an autotrophic index, which is a ratio of chlorophyll-a versus ash-free dry mass, to capture the productivity associated with heterotrophic production.

Not necessarily surprising, there was a dramatic increase in benthic invertebrate numbers between upstream and downstream locations in the Bow River, particularly below MWWE nutrient-enriched reaches. Physicochemical parameters linked to MWWE appear to drive changes to benthic macroinvertebrate communities more notably than other potential urban drivers within The City, and metrics related to specific types of taxa present in samples (and their relative abundances) were far better at identifying the presence of MWWE than more general metrics, such as richness or diversity, that did not capture community turnover.

The research also demonstrated the importance of the application of standardized artificial substrates (rock baskets), *in-situ* cotton-strip decomposition bioassays, and nutrient diffusing substrates as an integrated monitoring tool box to further tease apart and assess complex nutrient-ESOC related MWWE exposure effects on the structure and function of the basal aquatic food web. These studies are instrumental in informing future MWWE and stormwater monitoring program designs and choice of biological/ecological indicators to assess aquatic ecosystem health.

A second major objective was the identification of areas of potential concern in the Bow River Watershed within The City. While the studies confirmed the contribution of wastewater effluents to changes in water quality, periphyton, benthic invertebrate, and fish responses, the influence of stormwaters on responses is an understudied area. We did confirm the potential for stormwater exposure to be contributing to responses in the river and the utility of the downstream site (Highwood confluence) for assessing the cumulative effects of upstream contributions to the river.

Wild and caged fish studies in the river evaluated both wastewater exposure and stormwater exposure, and confirmed the ability of both to impact small-bodied fish responses. With the exception of a storm event in Nose Creek driving down dissolved oxygen levels, none of the other exposures suggested lethal consequences over 28 d of exposure. But fish clearly showed whole organism and physiological responses. Caged fish responses over a 28 d exposure period showed changes that were usually consistent with wild fish, although they were muted and not all always statistically significant.

A key objective was to evaluate the potential of the ACWA facility to support studies reflecting prioritization and pilot scale testing for wastewater effluents. The benthic macroinvertebrate communities in the ACWA streams were poor representations of the Bow River in both control and treatment streams. The overall communities were largely made up of pollution tolerant organisms and lacked most representatives of EPT organisms that were more present in the adjacent mainstem Bow River. In spite of the differences, the ability to control exposures and manipulate exposures is a key characteristic reflecting the capability of the ACWA streams to play a key role in future evaluations of technologies for wastewater treatment, and to better understand the potential impacts of climate change. This information can help guide The City in setting future investment priorities to assist them in optimizing existing infrastructure performance.

One challenge of the ACWA streams is that the Bow River contributes flow to the headpond as a source of dilution water to the streams; the Bow River at that point has already received the effluents of upstream Bonnybrook and Fish Creek wastewater effluents, as well as significant stormwater inputs. More realistic reflection of Bow River results would require a pre-treatment of the dilution water to remove the influence of upstream sources. The ability to source additional effluent sources from upstream in the Pine Creek treatment line would expand the relevance of results at ACWA to a broader audience.

Two types of emerging tools were evaluated for their ability to play a role in monitoring MWWE. Gut microbiomes of larval and adult insects invertebrates are impacted by wastewater discharges to the Bow River and confirms that baseline microbiome and stable isotope data of effluent-exposed insects can be used to assess effluent exposure in a monitoring framework.

Four sets of caging experiments in the ACWA streams, and exposure studies in the Bow River and Nose Creek evaluated transcriptomic indicators. Results are still being finalized, but the studies demonstrated that the techniques can identify gene markers that could be utilized more frequently, potentially reducing costs for future stormwater monitoring efforts. Analyses of the data are ongoing to determine whether there are specific markers or patterns that can be used to identify and separate MWWE and stormwater stressors.

The final objective was related to engagement with multiple departments within The City, stakeholders and local Indigenous communities to develop communication and knowledge translation tools specific to evaluating effects of municipal wastewater effluent. This work is ongoing, and future work with Indigenous communities will focus on developing the capacity and continued development of partnerships with local and regional Indigenous communities will play a significant role in future phases of this project.

1 Introduction

As part of its Integrated Watershed Management Plan, The City of Calgary (The City) endeavours to protect water supplies and manage use to maintain healthy ecosystems. Operational expenditures and performance are evaluated to identify risks and opportunities as they relate to plan objectives, and to identify capital investment requirements. Key threats to sustainable delivery of water services identified by The City include drought mitigation, contamination to drinking water, and water security & supply scenarios. These threats serve as the basis for The City's core Integrated Watershed Management goals of:

1) Protecting our water supply;
2) using our water wisely; and
3) keeping our rivers healthy.

Supporting objectives under these goals enable material contributions to urban water sustainability by establishing risk-prioritized prediction and prevention capacity. The Bow River through The City serves as a net aggregator of environmental management decisions. This recognition has formed the basis of an ongoing in-stream water quality monitoring program that has been maintained since 1982. Landscape/footprint characteristics, aerial deposition and precipitation represent indirect factors influencing aquatic receiving environments, while wastewater and stormwater management are direct influences. More recent concerns about environmental substances of concern (ESOC) have prompted sample collection and analysis to understand the potential influence on conditions and impacts as defined by the Integrated Watershed Management Plan.

Program Investments provided by Alberta Innovates to InnoTech Alberta between 2011-2012 and 2018-2019 under the Water for Life program, enabled initial evaluation of in-situ receiving environment conditions downstream of the Bonnybrook Wastewater Treatment Plant, and within areas of major stormwater influence, establishing a basis for continued engagement by InnoTech with the City of Calgary. In 2014, The City of Calgary began a decade long $1.2 Billion upgrade and expansion program at the Bonnybrook Wastewater Treatment aimed largely to accommodate the city's future growth. In addition, The City identified other challenges including: increasing regulatory standards, fiscal responsibility, reduced energy, smaller footprints, climate resiliency, innovation and resource recovery. The City recognizes that research the value of research for advancing solutions a broad range of challenges. In 2019, the City put out a call through their University of Calgary partnership Advancing Canadian Water Assets (ACWA) to solicit research projects to advance 3 priority area through scientific study: Treatment Process and Capacity, Resource Recovery and Public Health and River Impacts. Twelve potential projects were submitted to the City of Calgary through the ACWA partnership for review. The City selected this project, committed funding and invited the research team to codevelop a project for the priority Public Health and River impacts.

Following meetings to discuss current commitments and plans, knowledge, technology and policy gaps were defined. The depth and breadth of existing unprocessed data and desired ecosystem health understanding provided the basis for establishing a team of globally recognized experts spanning structure, function and processes of aquatic ecosystem health (Bow River

Ecosystem Health Assessment team, BREHA). This purposeful establishment of a project team provided the capacity for natural science activities to define adaptive risk management parameters, and evaluation of existing safeguards, and/or establishing the basis for new or modified safeguards (in the form of administrative or engineered controls). Defined gaps represent strategic risks and/or opportunities for Calgary and other Albertan municipalities.

1.1 Strategic Priorities/Gaps

Knowledge Gap: Evaluation of current adequacy of existing monitoring, mitigation and management of ecosystem health as influenced by release of tertiary treated municipal wastewater effluent.

- Relevance, essentiality, resilience of environmental substances of concern (ESOCs), where previous research has shown evidence of responses in the river;
- Verification of leading indicators to enable optimized water resources business planning, with validation of observations achieved via proposed surveillance;
- Framework-based evaluation of areas of potential concern which are the subject of routine sampling and assessment (i.e. existing State of Watershed activities).

Technology Gap: Undefined adequacy and capacity of existing infrastructure to evaluate current and future ecosystem health parameters, and outcome-positive management solutions (i.e. opportunities).

- ACWA streams infrastructure value in evaluation, validation of environmental performance, and by extension;
- ACWA laboratory and capacity for assessing cross-sector health and environmental resilience;
- ACWA biotreatment systems within existing infrastructure to assess stormwater /wastewater risks and optimization advantages.

This final public report establishes progress to date as demonstrated via a concurrent cross-ecosystem and cross-water management infrastructure evaluation stewarded and delivered by Principal Investigators and trainees. These evaluations form the framework parameters and enable the application of data insights into other monitoring programs developed to be leveraged in assessment of local-scale ecosystem resilience.

2 Project Description

This project was designed to characterize the water quality and associated biological and ecological responses in key components of the aquatic food web (ie.,microbial, algal and benthic macroinvertebrate assemblages, genomic and eco-physiological endpoints in targeted fish populations) to water quality parameters (nutrient regimes) and environmental substances of concern (ESOCs) introduced via The City's wastewater in the Bow River Watershed. Impacts to aquatic ecosystem structure and function are not fully understood. The City of Calgary and the University of Calgary share research infrastructure at the Advancing Canadian Water Assets (ACWA) at the Pine Creek wastewater facility. The Pine Creek facility produces wastewater that includes tertiary treatment and the effluent quality meets or exceeds all current regulatory criteria. Research in a semi-controlled setting at the facility at Pine Creek Wastewater Treatment Plant was conducted in parallel to the Bow River, to allow development and testing of new monitoring technologies and environmental prediction models to evaluate the current state of the river, to set a current baseline for future studies, and to develop interim thresholds for identifying changes in the accumulated environmental state. This information will help guide The City in setting future investment priorities to assist them in operating and managing infrastructure in a cost-optimized manner.

The project continues into the winter of 2025 with continued emphasis on additional capacity building/training, on the land discussion and co-learning, and sampling support to form the basis for future phases.

2.1 Project Goal

The main goals of this program included:

1) Establishment of effective ecosystem health indicators for evaluating wastewater effluent impacts on core aquatic ecosystem biological and ecological processes that can guide optimization of wastewater treatment investments, with the consideration of the role that climate change (increasing flood/drought conditions) may play in influencing the validity of selected indicators;
2) Identification and verification of areas of potential concern in the Bow River Watershed within The City, in a templated manner that can be replicated by other Albertan municipalities; and;
3) Engagement with multiple departments within The City, stakeholders and local Indigenous communities to develop communication and knowledge translation tools specific to evaluating effects of municipal wastewater effluent.

2.2 Tactics

1) Synthesis of previous data and characterization of environmental fate/transport of ESOCs;
2) Evaluation of responses to municipal wastewater effluent across a range of indicators;
3) Enhanced baseline environmental characterization; and
4) Evaluation of the potential for new tools to contribute to monitoring effects.

2.3 Changes to objectives

Engaging with Indigenous Peoples in Canada requires a significant investment of time in order to build genuine, trust-based relationships. This process is essential for understanding and respecting the distinct cultural, historical, and social contexts of each community. Indigenous engagement involves meaningful collaboration and co-creation, which includes listening to community members, incorporating their perspectives, and ensuring their voices are integral to research co-development processes. This approach is not only respectful but necessary to foster true partnerships and achieve sustainable outcomes.

The project is guided by Indigenous research methodologies, and the principles of OCAP®. Engagement with surrounding Indigenous communities occurred once the appropriate ethics approvals were obtained. Working with the Treaty 7 Nations surrounding the Bow River presents a complex landscape due to the diversity and distinctiveness of each Nation and its culture. This diversity requires a nuanced approach to engagement, ensuring that the specific needs and perspectives of each Nation are addressed. While this project focused on downstream Indigenous communities, upstream Nations were also incorporated during early discussions. All of this takes time – and is essential to a successful project.

At the start of the project, the ongoing delays due to COVID-19 and ongoing health epidemics affecting Blackfoot First Nations, in particular, required a careful and slow approach. Rather than a short-term output-focused project, this research is part of a long-term outcome-focused research collaborative that will investigate the ecological health of the Bow River through community-determined indicators. This Phase of the project focussed on the initiation of the development of partnerships for meaningful engagement with Indigenous peoples and communities to help prioritize issues, sites, and the integration of Indigenous Knowledge. The original proposed Bow River Ecosystem Health Assessment project included a Phased Design, with engagement with downstream communities representing Phase 1.

In Phase 1, we proceeded with interviews with 5-7 downstream Indigenous community members from Siksika Nation and/or other members of the Blackfoot Confederacy. In addition, Phase 1 included the co-creation of research methodology, and the development of place-based narrative mapping. The interviews held in Phase 1 will inform the subsequent focus group/interviews. They will be targeted to understanding the core elements that we need to have a better understanding to shape the events, and group sessions. This initial phase was a request from community partners to ensure we have a fulsome understanding before jumping into the next stage of interviews.

3 Methodology

The methodology, equipment and facilities used to execute and complete the project are described relative to the work completed on Environmental Substances of Concern (ESOCs), biological responses (including basal food web, benthic macroinvertebrates and fish), and new tools (including microbiome assessment and transcriptomics).

3.1 ESOCs Fate and Transport

The evaluation of ESOCs fate/transport involved two parts: **(1)** assessing the distribution and occurrence of 55 ESOCs in 11 sites within and around The City of Calgary boundary (Figure 1) and **(2)** investigating ESOCs partitioning in environmental and biological matrices collected in replicate artificial streams (ACWA). The team worked closely with The City of Calgary (Dr. Norma Ruecker and Victoria Arnold, MSc) to process and analyse the data collected by The City of Calgary from 2018 - 2022. Detailed methodology can be found in Arlos et al. (2023).

The second part of this study involved the assessment of the partitioning and uptake of select ESOCs, including pharmaceuticals and personal care products in the following environmental compartments: (i) water, (ii) sediments, (iii) invertebrates, (iv) biofilm, and (v) fish. The study focused on improving sample preparation methods in these complex environmental matrices so defensible analytical data (*via* liquid chromatography, triple quadrupole mass spectrometry) on trace concentrations can be obtained. After evaluating different sampling preparation techniques for the solid matrices (accelerated solvent extraction [ASE], liquid-liquid extraction), the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) for sediment, biofilm, invertebrate (*Gammaridae* spp.), and fish (longnose dace [*Rhinichthys cataractae*] and spoonhead sculpin [*Cottus ricei*]) tissues were found to be an appropriate sample extraction method (i.e., analytical recoveries from 70% to 120% for most of the compounds analyzed). Once the methodology was evaluated, the ACWA streams receiving different levels of wastewater treatment (i.e., ultrafiltration, ozonation, reverse osmosis) was evaluated for impact on the occurrence and partitioning of these compounds.

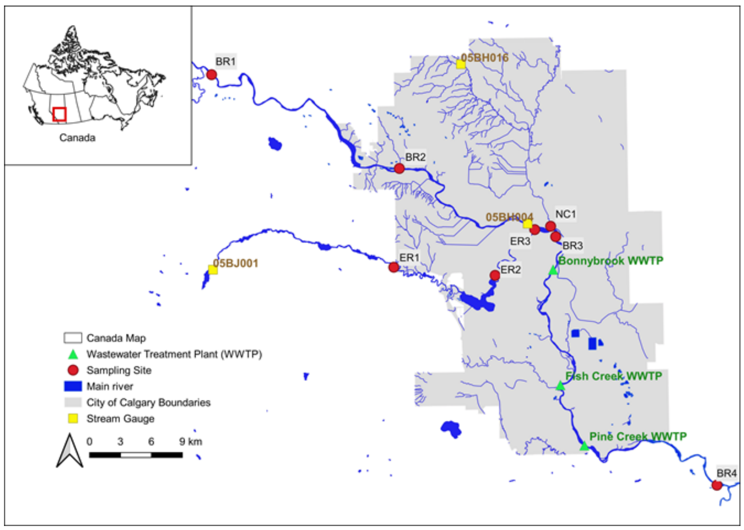

Figure 1 Site locations of ESOCs monitoring by The City of Calgary. Modified from Arlos et al. 2023. BR1 = Cochrane, BR2 = Bearspaw Reservoir, BR3 = Cushing Bridge, BR4 = Highwood Confluence, NC1 = Nose Creek mouth, ER1 = Elbow River Twin Bridge, ER2 = Glenmore Source Water

3.2 Biological Responses

3.2.1 Basal Food Web

Benthic Algae/Heterotrophic Production was evaluated from February to July 2022 using various methods for quantifying endpoints in all ACWA raceways including: organic matter decomposition (cotton strips and leaf litter bags), periphyton algal standing crop (via chlorophyll-a concentration) and biomass production (via ash free dry mass) utilizing different substrate types (natural river rock, ceramic briquette, and glass slides) across specified time series and seasonal variation.

A separate sampling campaign took place in Fall 2022 (August-October) in the Bow River and ACWA raceways to deploy nutrient diffusing substrata, cotton strips, and rock baskets containing artificial substrates over timeframes spanning 14-42 days in the Bow River (upstream site in Canmore, downstream site at Policeman's Flats) and in the ACWA raceways (control, 5% effluent exposure, 15% effluent exposure). Chlorophyll a was quantified using acetone extractions. Ash free dry mass (AFDM) was assessed using a series of drying, combusting, and weighing. Nutrient diffusing substrata were quantified via chlorophyll-a using methanol extractions and the same process for AFDM. Decomposition rates were quantified by measuring the tensile strength loss of the cotton strips. Alongside the biological endpoints, nutrient (analyzed by Bureau Veritas), ESOC (analyzed by Arlos[UofA]), and water quality samples were taken. All samples have been processed and data analyzed except for environmental DNA. DNA extractions have been conducted on biofilm from the nutrient diffusing substrata, but bioinformatics on the sequencing has yet to be completed.

3.2.2 Benthic Invertebrates

Benthic macroinvertebrate samples were collected from ACWA control and tertiary-treatment wastewater exposed streams in late October 2020. Conventional Surber-style samples were taken from each ACWA stream replicate, as well as triplicate experimental artificial substrate rock basket samplers, which were allowed to colonize over a six-week exposure. Additional physicochemical measurements were taken over this time, including conductivity and temperature loggers and substrate profiles. All benthic macroinvertebrate samples (both sampling methods) were subsampled to reach a minimum count of organisms and all taxa was identified to the lowest practical level (usually family). Communities were characterized using common community metrics.

Triplicate benthic macroinvertebrates samples were collected using 3-minute travelling kicknets (400 µm) from the Bow River throughout the open water season of 2021 (May, September, and November). Sites included two sites bracketing the Canmore wastewater treatment plant (WWTP), between one and four Calgary sites upstream of Calgary's WWTPs, and 1-2 sites downstream of at least one WWTP. A suite of physiochemical parameters, including dissolved oxygen, conductivity, nutrient concentrations, and average substrate size, was collected for at each site in each month. Samples were subsampled to reach a minimum count of organisms and all taxa were identified to the lowest practical level (usually family). Sites were combined into reaches for subsequent analysis using cluster analysis. Reach communities were characterized using common community metrics. Benthic macroinvertebrate samples have also been collected from similar sites on the Bow River in 2023 but these samples are currently archived.

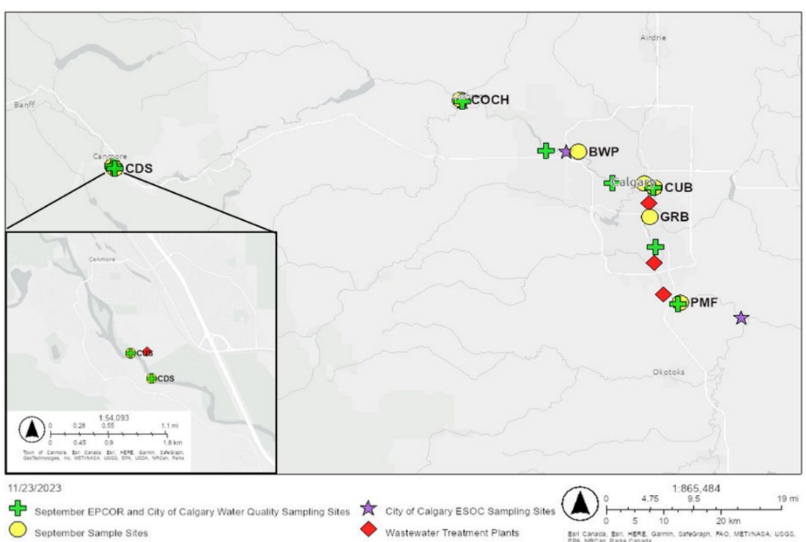

Figure 2 Bow River benthic macroinvertebrate sampling sites in the open water season of 2021. Wastewater treatment plant outfalls are also marked, as are sites of regular water quality sampling by The City of Calgary and EPCOR and sites where ESOC concentrations were monitored as part of a City of Calgary pilot program.

3.2.3 Fish

Signal exposure involved quantification of endpoints at locations at/around municipal wastewater outfalls via naturally present longnose dace, as well as translocation of reference location fish (used to quantify natural variability) using fish cages. Evaluating the impact of exposures also involved translocation of fish found naturally at wastewater treatment outfalls to reference/unexposed locations. Wild longnose dace (*Rhinichthys cataractae*) were collected at reference sites and downstream of municipal waste outfalls in the fall of 2021, and in the fall of 2022 collections were simultaneous with caging of wild longnose dace from a reference site at exposure sites (Figure 3).

Wild fish used for all caging experiments were captured using backpack electrofishers from two reference sites: Bowness Park on the Bow River and Jumpingpound Creek. Minnow traps or bait buckets were used as cages in the ACWA and Bow River caging experiments. In the spring of 2022, longnose dace were captured and cage sites were placed downstream of significant stormwater sites. In the fall of 2022, longnose dace were collected in the areas of the confluence of the Elbow and Nose Creek tributaries as well as within the tributaries to examine the potential impacts of stormwater exposure. In the spring and fall of 2023, fathead minnow (*Pimephales promelas*) were collected at sites throughout the Nose Creek tributary to examine for impacts of stormwater exposure.

Endpoints of evaluation included both gene expression (initiating event) and energy allocation endpoints (health outcomes) in each of the experiments. Measurements of fish length, weight, gonad weight, and liver weight were taken to calculate health indices such as condition factor, gonadosomatic index, and liversomatic index. External validation was achieved via exposure of fish to stormwater to understand and account for potential synergistic, additive, and/or antagonistic signals which would preclude objective evaluation of wastewater-induced changes.

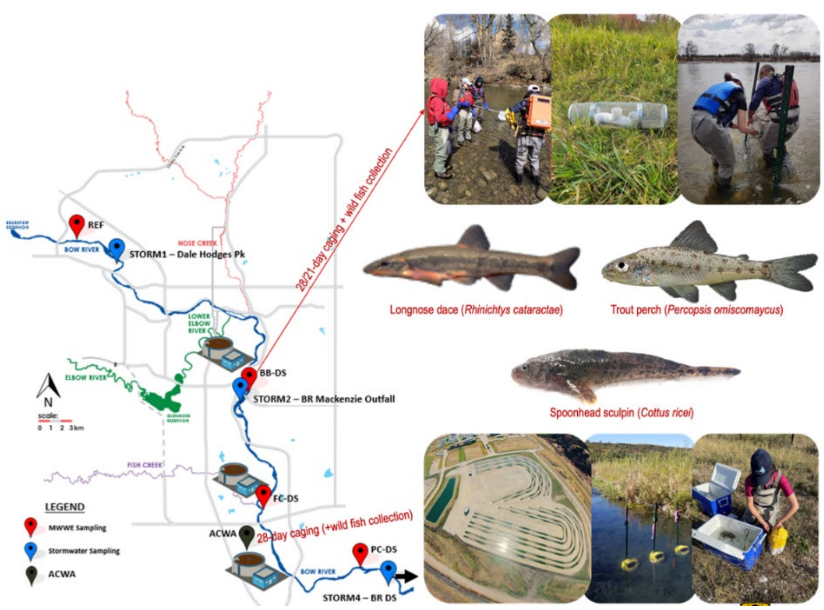

Figure 3 Study locations for Bow River fish sampling and caging for MWWE responses (red) and stormwater (blue).

3.3 New Tools

3.3.1 Microbiome Studies

Larval insects, adult insects, and riparian spiders were collected from 5 sites on the Bow River, 3 upstream of wastewater discharges and 2 downstream of wastewater discharges (Figure 5). Larval and adult insects were also collected from 3 experimental streams from the ACWA facility with varying concentrations of effluent additions entering from the Pine Creek wastewater treatment facility (Bow River control, 5%, 15%) after ~ 3 weeks of exposure. These samples were sorted to family level and analyzed as below for microbiome (bacterial community) composition and stable carbon and nitrogen isotopes (along with biofilms and riparian leaves) to understand food web structure. All samples collected for host microbiome were flash-frozen on dry ice and kept at –80ºC until analysis, and samples for stable isotopes were kept at -20ºC. Nutrient excretion experiments were conducted in the Bow River and in the ACWA streams by incubating caddisflies from the family Hydropsychidae in tubes filled with filtered river water for an hour. Caddisflies were then removed and the water frozen until analysis.

Microbiome samples were processed at the Farncombe Institute at McMaster University in Hamilton, ON. The DNA of bacteria living on or in the invertebrates was extracted, amplified with nested PCR amplification, and was sequenced on the 16S rRNA gene on an Illumina MiSeq instrument. The data were trimmed for quality assurance and bacterial taxonomy was assigned before performing downstream analysis in RStudio.

The stable isotope samples were freeze dried and homogenized and sent to the Stable Isotopes in Nature Laboratory in Fredericton, New Brunswick to be analyzed for $\delta^{13}C$ and $\delta^{15}N$ on a mass spectrometer. The incubated water samples were sent to the CRASR facility in Waco, Texas to be analyzed for nitrogen (NH3-N) and phosphorus (PO4-P) using a Lachat QuikChem 8500 flow-injection autoanalyzer.

3.3.2 Transcriptomic assessments

Caging experiments with fish were conducted at ACWA to better understand the effects of urban effluent exposure on fish hepatic transcriptomic responses and identify candidate gene markers that characterize such complex mixtures. Four sets of 28-day caging experiments involved 3 different fish species in the ACWA raceways (set at 5% effluent concentration) with longnose dace in the fall of 2020, trout perch (*Percopsis omiscomaycus*), and spoonhead sculpin (*Cottus ricei*) in the fall of 2021 and longnose dace in the fall of 2022 exposed to increased effluent concentration (15%) . Concurrently with the caging experiments, water samples from the raceways were collected to analyze selected ESOCs to characterize the exposure. In the spring of 2024, juvenile rainbow trout (*Oncorhynchus mykiss*) were caged at reference sites as well as downstream of Cushing bridge, the Bonnybrook outfall, and at ACWA to compare the impacts of exposure to stormwater and MWWE on transcriptomic responses.

Samples from wild fish collections and Bow River caging experiments were processed similarly. Liver tissue samples were snap-frozen in liquid nitrogen and stored at −80°C for subsequent RNA extraction for either RNA sequencing or EcoToxChip analysis (https://www.ecotoxchip.ca/).

Fish sampling and most sample preparations were conducted at the University Research Center (UofC). A portion of the samples was prepared at Innotech AB, with quality assurance and quality control (QA/QC) conducted at UofA. RNA extracts were sent to Novogene (Sacramento, CA) or Genome Quebec (Montreal) for RNA sequencing analysis. RNA extracts for EcoToxChip analysis were analyzed at the University of Saskatchewan. Sex steroid production analysis was performed externally by Environment and Climate Change Canada (Burlington), and fish aging analysis was conducted by North/South Consultants Inc.

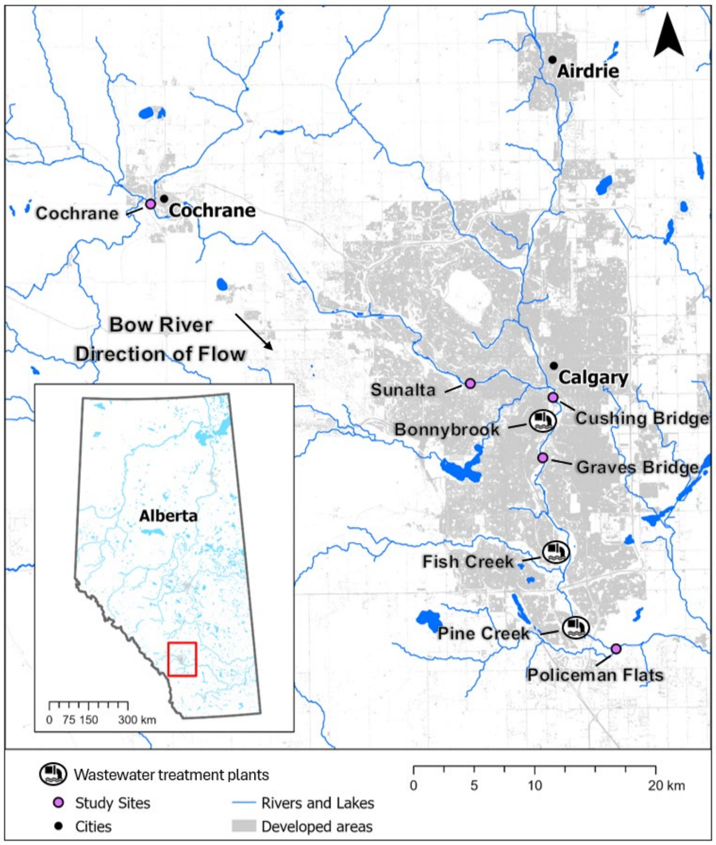

Figure 4. Bow River sampling sites for microbiome, stable isotopes, and nutrient excretion analyses relative to wastewater treatment plants along an ~75 km stretch.

3.4 Indigenous Engagement

Research co-creation began through principles of OCAP® - respecting community voice and autonomy. Building on existing relationships within Treaty 7, research team members engaged in early discussions to determine key areas of potential interest. Following this, ethics approval was obtained from the University of Calgary. Research team members then utilized snowball sampling to identify key individuals to engage with. The team has undertaken targeted interview discussions that have focused on identifying an appropriate framework for meaningful participation of community-members.

Our research methodology adheres to the OCAP® principles—Ownership, Control, Access, and Possession—to engage with downstream Indigenous Nations on the health of the Bow River. As part of this, many members of the research team undertook OCAP® principles training. OCAP®

prioritizes the self-determination of these Nations by ensuring they retain ownership and control over all data collected. Access to the data is granted as per their guidelines, and they possess the physical data throughout the research process. Engagement involves collaborative and respectful dialogue to incorporate traditional knowledge and community insights, ensuring the research aligns with their cultural values and priorities. This approach fosters a partnership that is transparent, equitable, and beneficial to the health of the Bow River and its communities.

A specific focus of engagement included Blackfoot Youth Water council members that was co-developed in 2022 and has informed Phase 1 of this research project. Further discussions have also been initiated with team members on potential avenues of knowledge mobilization, focused on communicating findings from the western science components of the project. This work is expected to continue beyond the conclusion of the initial phase of the project and includes:

- Developing a framework for meaningful participation of Indigenous communities as the Project develops
- Further inclusion of community members, leadership, administration, elders and youth, to help support the development of conceptual models for practical integration of traditional and scientific knowledge
- Developing mechanisms to bridge the gap between traditional and technical knowledge and aid in developing toolkits for successful engagement, as well as highlighting the role of land-based reconnection activities in enhancing community participation in decision-making
- Engagement of upstream communities in the Bow River
- Providing capacity building opportunities in western science techniques

4 Project Results

Project results are described relative to the work completed on Environmental Substances of Concern (ESOCs), biological responses (including basal food web, benthic macroinvertebrates and fish), and new tools (including microbiome assessment and transcriptomics).

4.1 ESOCs Fate and Transport

Seasonality, occurrence, and distribution: There was significant collaboration with The City of Calgary for the interpretation of their ESOCs monitoring program (2018 – 2021) on the Bow and Elbow River historic data on ESOCs monitoring, including the summarization, visualization, and analyses of >25,000 data points (Arlos et al. 2023). The work provided general insights on the effectiveness of the current wastewater technology employed within The City (i.e., Biological Nutrient Removal [BNR]) as well as additional research questions and hypothesis related to ESOCs and their role in water quality management frameworks within The City (e.g., source water protection, stormwater). Although not included in the current transboundary water quality guidelines, this work attracted attention from the Committee on Water Quality – Prairie Provinces Water Board who coordinates the water quality monitoring program and addresses issues about the water quality of water crossing the interprovincial border with Saskatchewan.
Partitioning of ESOCs in other environmental matrices: Method development (sample preparation and extraction) for ESOCs presence in various matrices (sediment, biofilm, fish, and invertebrates) were completed between December 2021 – May 2023. This work was completed via a research exchange between UofA and UWaterloo. The outputs (e.g., Figure 5) were also

part of an Pulgarin-Zapata (2023, MSc thesis) and portions were published in Pulgarin-Zapata et al. (2024).

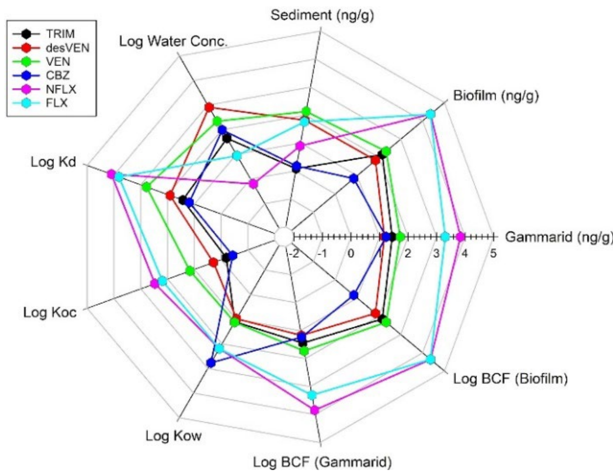

Figure 5 Comparison of matrix concentration (water, sediment (SED), gammarids (GAM), biofilm (BF)) and their BFCs (bioconcentration factor) for biofilm (BF) and gammarids (GAM) and sediment/water partition coefficient (log Kd) and log Koc for sediment and Log Kow for water in S-WWTP-D 15%. Fluoxetine (FLX) was not found in the water column at high levels but partitioned to the solid matrices at higher concentrations that the rest of the target substances.

Toxicokinetics of ESOCs uptake: The rate of uptake/depuration may influence the bioaccumulation of substances in aquatic organisms. The 5% effluent contribution from Pine Creek WWTP (ACWA stream) showed little to no changes in stream concentrations (almost same as the background) (Pulgarin-Zapata, 2023, MSc thesis), but we saw a proportional increase when the effluent contribution was increased by 15% (e.g., venlafaxine [antidepressant] and carbamazepine [antiepileptic]). Even with the 15% increase, we found no substantial differences in temporal changes when whole-body concentrations were collected monthly (Figure 6). Instead, the bioconcentration factor (BCF), carbon-water partition coefficient (Koc) and sorption coefficient (Kd) were calculated (steady-state, equilibrium assumptions) to assess likelihood of ESOCs to partition in different environmental matrices.

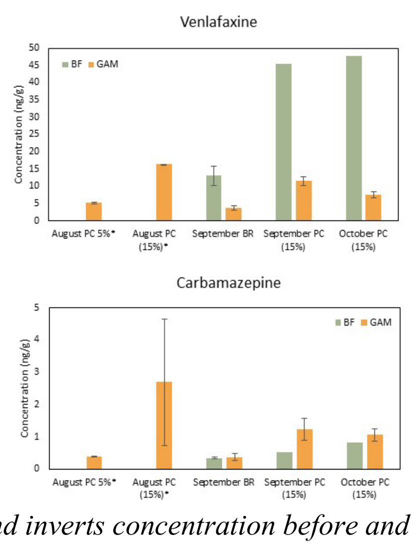

*Figure 6. Comparison of biofilm and inverts concentration before and after the increase (5% to 15%). BR= Bow River, PC 5%= Pine Creek 5% effluent, Pine Creek 15%= Pine Creek 15% effluent. *Rock baskets for biofilm samples were deployed in August, so there are not data points for this month.*

4.2 Biological Responses

4.2.1 Basal Productivity

Characterization/Assessment of Sampling Methods: New data and published theses (Sutherland 2024; Sayles 2024) have been produced comparing the efficacy of various sampling and analytical protocols in assessing the effects of MWWE exposure on autotrophic and heterotrophic productivity and benthic macroinvertebrate assemblage responses. Controlled experiments were conducted February to July 2022 in all ACWA raceways to assess various methods for quantifying autotrophic and heterotrophic biological endpoints. The main objective was to compare the standardization and repeatability of method application, and the efficacy, variability, and efficiency in method interpretation. Methods compared were organic matter decomposition (cotton strips and leaf litter bags), periphyton algal standing crop (via chlorophyll-a concentration) and biomass production (via ash free dry mass) utilizing different substrate types (natural river rock, ceramic briquette, and glass slides) across specified time series and seasonal variation. When comparing leaf bags and cotton strips for decomposition methods, both showed the same measures after a six-week exposure period. However, after 12-week and 42-week exposures, cotton strips exhibited consistently higher decomposition rates than leaf bags suggesting these measures are only comparable after shorter term exposures. Decomposition results need to be reassessed using degree-day data. Assessing chlorophyll-a standing crop between different substrates showed that after 6-week exposures, natural river rocks had higher chlorophyll-a concentrations than ceramic briquettes and glass slides. The results suggest after 6-weeks, ceramic briquettes and/or glass slides do not provide comparable results to natural river rocks.

ACWA/Bow River Responses and Intercomparison: Sampling campaigns took place in Fall 2022 (August-October) in the Bow River and ACWA raceways to examine the relationship between nutrients (nitrogen and phosphorous) and complex mixtures of ESOCs on basal decomposition, periphyton standing crop (measured as chlorophyll-a) and production rates, biofilm biomass (measured as ash free dry mass), biofilm accumulation, and community composition. In the Bow River, endpoints suggested increased cumulative MWWE exposure (i.e. Policeman's Flats) promotes heterotrophic microbial community activity. It was concluded that nutrient enrichment seems to be a large driver in basal microbial community changes and functioning; however, the seasonal discharges and drought conditions should be noted as a potential factor. Higher heterotrophic microbial activity was witnessed through increased decomposition rates and heterotroph dominant biofilm communities at Policeman's Flats relative to Canmore.

ACWA streams showed both autotrophic and heterotrophic components of the basal food web were influenced by increasing MWWE, but in different ways. With more controlled settings, it was determined that there was a nutrient-ESOC interaction present for the endpoints assessed. Autotrophs showed suppressed biomass after a 5% addition of MWWE, with no nutrient limitation, suggesting ESOC complex mixtures influence photosynthetic functioning. Heterotrophs maintained similar organic matter decomposition rates across effluent treatments but exhibited a subsidy-stress (bell-shaped curve with increasing MWWE concentrations) response for biofilm AFDM, potentially indicating induced tolerance for functional endpoints. Observations reflect the importance of assessing both autotrophic (green) and heterotrophic (brown) energy pathways in response to MWWE, to better understand contributing factors that led to heterotroph dominant biofilms.

4.2.2 Benthic Macroinvertebrates

Characterization/Assessment of Sampling Methods: Initial characterization survey of benthic macroinvertebrate community composition from conventional grab samples (Surber samples) and experimental longer-term samplers (artificial substrate baskets) was completed in the control and 5% effluent ACWA raceways in the fall of 2020. The overall health of the ACWA streams was mid-range, according to metrics such as the HBI index (rated most streams as Fair). Streams were typically dominated by common Diptera taxa and more pollution tolerant Trichoptera taxa, which were also identified as influential in differentiating streams statistically. Control and treatment streams were consistently significantly different but the patterns differentiating stream communities were inconsistent between different sampling methods. Additionally, a targeted indicator caddisfly taxon was sampled and processed for biomass and density endpoints in the control, 5%, and 15% effluent raceways at ACWA in the fall of 2022.

ACWA/Bow River Responses and Intercomparison: In the spring and fall of 2021, benthic macroinvertebrate community composition was sampled through conventional grab samples (kicknets) three times at relevant sites in the Bow River in Canmore and Calgary, also alongside relevant physicochemical parameters and periphyton standing crop (estimated via chlorophyll-a concentration). Experimental long-term samplers (artificial substrate baskets) and DNA barcoding methods were also utilized in fall sampling and additional years of conventional kicknet sampling were completed to better characterize natural variation in the system. Overall, benthic macroinvertebrate communities were observed to show greatest differences (based on Bray-Curtis cluster analysis) where there were significant inputs of MWWE, such as below the Bonnybrook WWTP. Generally, the river could be grouped into reaches where communities remained largely similar, delineated by landscape (i.e. moving between ecozones) and significant point source inputs (i.e. WWTPs) (Figure 8). However, community metrics suggested that the system was in good health in all reaches and dominated by EPT taxa, aside from the south part of Calgary where more pollution tolerant taxa (i.e. Diptera) were observed in higher proportions, although overall raw abundance also massively increased in this reach. Similarly, most differences between sites and reaches could be contributed to EPT and Diptera taxa and turnover between these taxonomic groups. These differences, as assessed by a dbRDA (Figure 9), were highly correlated with nutrient presence, supporting the role of MWWE in driving system changes in reaches around The City of Calgary. Environmental DNA samples of macroinvertebrates remain to be further analyzed for potential application.

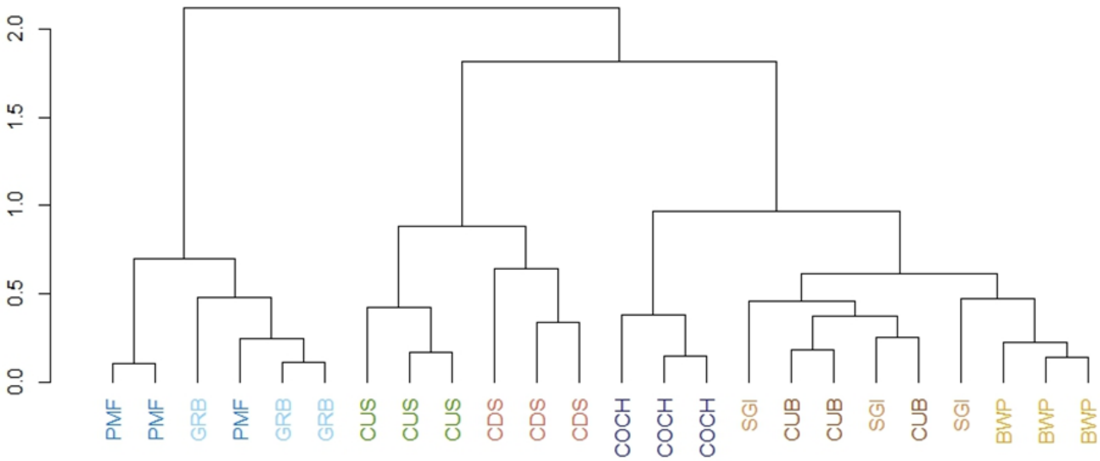

Figure 7. Cluster analysis of all site replicates of benthic macroinvertebrates Bray-Curtis differences sampled in September 2021

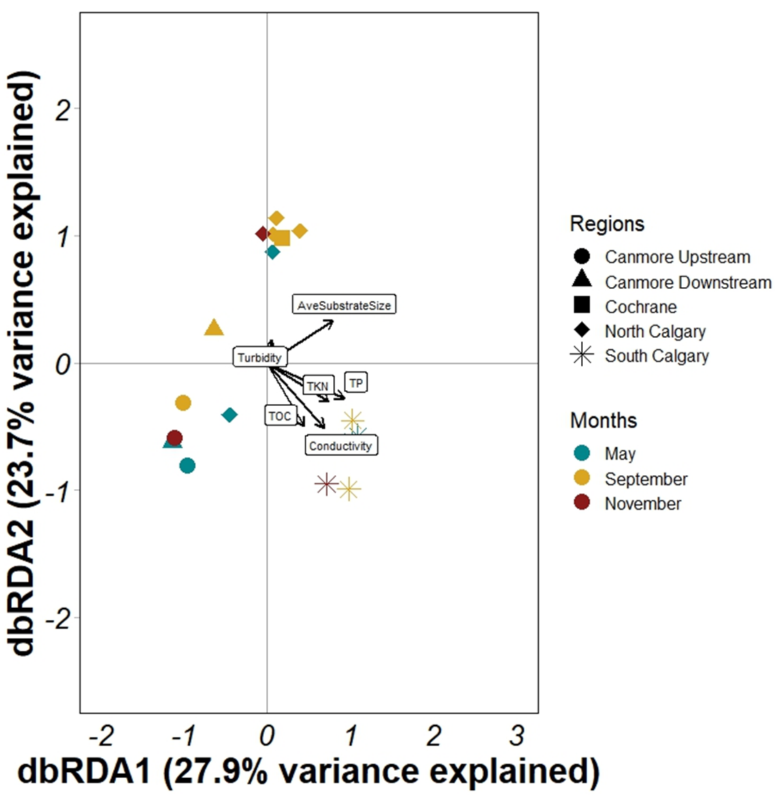

Figure 8 dbRDA describing correlations in differences between benthic macroinvertebrate communities and key physicochemical measures across the open water season of 2021

4.2.3 Fish

Health was assessed in wild longnose dace in the Bow River in the fall of 2021 and 2022 downstream of MWWE outfalls and at reference sites, and in May of 2022 at reference sites and downstream of stormwater outfalls to determine long-term exposure effects to municipal wastewater effluents and/or stormwater. Caging exposures of reference longnose dace were also conducted concurrent with the wild fish collections to determine the suitability of short-term caging exposures to predict wild fish responses, and to account for confinement effects. In the Fall of 2022, an additional caging experiment with longnose dace was conducted to determine depuration/recovery over 21 days after chronic exposure to municipal effluent.

Wild longnose dace showed increased liver size and condition downstream of Calgary, but gonad sizes were not different or smaller (Tanna, in prep.). Caged fish showed a similar response but it was muted, not always statistically significant, and in short term exposure the gonad size effect could be opposite, suggesting that (not surprisingly), caging periods of up to 28 d did not do a very good job of replicating wild fish responses at the whole organism level (Tanna, in prep.). Physiological analyses are still in progress to evaluate the capability of caged exposures to predict wild fish responses at that level.

Additional collections in fall 2022 focused on understanding the potential impacts of stormwater on fish responses, examining the potential influence of Nose Creek, including West Nose Creek, and their impact on the Bow River. Collections included longnose dace in the lower Elbow River, and downstream in the Bow including below Cushing Bridge and the Bonnybrook municipal wastewater discharge. Three rounds of wild fish collections were conducted along the Nose Creek/Bow River gradient, including longnose dace (Fall 2022) and fathead minnow (Spring 2023/Fall 2023). Results showed clear responses in Nose Creek to exposure, including a eutrophication-type response where fish had higher condition, larger livers and increased gonad size relative to the Bow River. Transcriptomic approaches were used to try and differentiate responses associated with MWWE versus stormwater exposure; analyses are currently underway.

4.3 Emerging Tools

4.3.1 Microbiome studies

Caged fish and macroinvertebrates for evaluation of microbiome changes at ACWA Microbiome samples have been collected from the caged fish exposures in the streams at ACWA and from a major sampling campaign in July 2022. There were minimal shifts in microbiome compositions and nutrient excretion rates of larval and adult aquatic insects exposed to varying concentrations of municipal effluents in the ACWA streams and bacterial communities were different from the same insect taxa collected in the Bow River with similar effluent exposures (Diesbourg, 2024)

Microbiome responses to wastewater effluents were found in downstream larval and adult insects but minimal impacts to riparian spiders. Specifically, we found decreases in abundances of an endosymbiont bacteria that may have a role in manipulating host reproduction downstream of wastewater discharges, shifts in the overall composition of bacteria between the upstream and downstream sites for most aquatic insects (larval and adult), an increase in municipal wastewater-associated bacteria in aquatic insects downstream of wastewater discharges, and differences in bacterial communities between larval and adult insects. However, there were minimal shifts in spider microbiomes along the river and no increase in wastewater-associated bacteria in spiders collected from downstream of the municipal wastewater discharges. Despite this, downstream of the Bonnybrook wastewater treatment facility we found that all aquatic insects and spiders from the Bow River were enriched in $\delta^{15}N$, indicating exposure to effluent nutrients - consistent with the eutrophication response observed in basal productivity and fish - and subsequent transfer to terrestrial ecosystems (Diesbourg, 2024). Although invertebrate $\delta^{15}N$ values and aqueous nutrient concentrations increased at downstream sites, nitrogen and phosphorus excretion by larval aquatic insects was not correlated with wastewater exposure.

4.3.2 Transcriptomics

Health was assessed in caged fish in the Bow River and at ACWA to better understand the effects of urban effluent exposure on fish hepatic transcriptomic responses and to identify candidate gene markers that characterize such complex mixtures. To achieve this objective, we conducted 4 sets of 28-day caging experiments involving 3 different fish species in the ACWA raceways (set at 5% effluent concentration) with longnose dace (*Rhinichthys cataractae*) in the fall of 2020, trout perch (*Percopsis omiscomaycus*) and spoonhead sculpin (*Cottus ricei*), both in the fall 2021, and the fourth exposure with longnose dace in the fall of 2022 exposed to a higher effluent concentration (i.e., 15%). Concurrently with the caging experiments, water samples from the raceways were collected for select ESOC analysis to characterize the exposure. The first set of data analyses that involves transcriptomics responses in longnose dace caged at 5% effluent is completed (Marjan et al. 2023). The remainder of samples have recently completed sequencing; upon completion of the bioinformatics, we will have four datasets, encompassing three species exposed to either 5% or 15% municipal effluent.

Preliminary bioinformatics analysis suggests that the duration of exposure had a more pronounced impact on the activation or suppression of significantly different genes compared to the effluent exposure itself. Additionally, the results indicate minimal differences between the raceways containing effluent and those with Bow River water (control), consistent with our previously published findings. Using pathway analysis, we aim to gain insights into the specific genes and biological processes predominantly affected. Our earlier data highlighted compromised immune and stress responses, as well as susceptibility to pathogen challenges.

The responses of caged fish species at ACWA at the whole organism level indicate that trout perch exhibited the highest sensitivity, followed by longnose dace and then spoonhead sculpin. Notably, the whole organism responses of longnose dace exposed to 15% effluent were like those exposed to 5% effluent.

The whole organism responses highlighted significant impacts from stormwater exposure, particularly on the liver somatic index. These effects were less pronounced in longnose dace exposed or caged downstream of major municipal wastewater discharges in Calgary. Analysis of liver RNA sequencing results aims to reveal differences in affected genes and pathways, potentially distinguishing between the effects of stormwater and municipal wastewater. Additionally, we aim to explore the differences between short-term and long-term exposures in the Bow River and compare these findings with caging experiments conducted at ACWA using 5% and 15% concentrations of longnose dace.

Whole organism data analysis and EcoToxChip analysis of fathead minnow liver samples related to the stormwater studies in Nose Creek have been completed. EcoToxChip is a qPCR tool containing 384 genes that have been carefully selected in key vertebrate species of relevance in ecological and chemical risk assessment. It can be used to screen and prioritize compounds based on gene expression. The goal is to better understand the type of contaminants that cause effects on fish in stormwater dominated stream compared to municipal wastewater affected sites. A final caging experiment was conducted in summer of 2024 across eight sites in Nose Creek, Bow River, and ACWA, involving rainbow trout (*Oncorhynchus mykiss*). Results from these experiments are still pending.

4.4 Indigenous Engagement

At the project's inception, COVID-19 and ongoing health challenges affecting Treaty 7 First Nations necessitated a careful and gradual approach. This research is part of a long-term collaborative effort to assess the ecological health of the Bow River using community-determined indicators, rather than a short-term project. This phase of the project concentrated on establishing partnerships for meaningful engagement with Indigenous peoples and communities, prioritizing issues, sites, and the integration of Indigenous Knowledge. The original project design included multiple phases, with Phase 1 focusing on engaging with downstream communities.

In Year 1 of the Project, an ethical approach and early-engagement plan was made. During this time, pre-engagement occurred. Pre-engagement, which often requires a significant time investment, is crucial in research involving Indigenous communities because it lays the foundation for trust, mutual understanding, and respect. Before the research even begins, pre-engagement allows researchers to build genuine relationships with community members, understanding their values, needs, and concerns. This phase ensures that the research agenda is co-developed with the community, aligning with their priorities and cultural protocols.

During pre-engagement, researchers can learn about the community's historical context, cultural practices, and traditional knowledge, which are vital for designing a culturally sensitive and relevant research methodology. This process also provides an opportunity for the community to express their expectations, consent, and potential reservations, fostering transparency and ethical conduct throughout the research. This process continued into Year 2 and 3 as well.

In Year 2 a community-based researcher based out of the surrounding First Nations was hired to support engagement. Hiring a First Nations community-based researcher is invaluable for this work due to their deep cultural knowledge, lived experience, and established relationships within the community. Such a researcher brings an authentic perspective that enhances the relevance and sensitivity of the research process, ensuring it aligns with the community's values and priorities. Their presence fosters trust and open communication, which are crucial for meaningful engagement and accurate data collection. Moreover, they can navigate cultural protocols and traditions effectively, facilitating respectful and appropriate interactions. This approach not only enriches the research with local insights but also empowers the community by involving them directly in the research process, promoting ownership and long-term sustainability of the project outcomes.

Following the successful hiring of a community-based researcher, the research team engaged in interviews with targeted Individuals identified through snowball sampling. However, given the nature of the topics and some elements involving sensitive information, it was decided to proceed with deep-dive interviews with specific individuals. During these deep-dive interviews, significant historical and culturally-specific context was shared, resulting the creation of a draft Community-Based Bow River Water Health Mind Map in Year 3. This Mind Map is a visual tool and aid that is supporting subsequent discussions with community members, helping to trigger more detailed discussions on specific topics of interest. A community-developed mind map of all potential Bow River water health impacts would significantly support this project by capturing a comprehensive and holistic view of the river's ecological status as perceived by the community. This collaborative tool will integrate diverse perspectives, including traditional ecological knowledge and contemporary scientific insights, ensuring a more inclusive understanding of water health issues. The mind map would highlight critical areas of concern, identify interconnections between various factors, and prioritize issues based on community input. This process not only fosters community engagement and ownership but also guides the research focus towards the most relevant and pressing concerns. Additionally, the mind map serves as a visual and interactive representation that can facilitate communication and collaboration among stakeholders, ensuring that all voices are heard and considered in decision-making processes.

The Mind Map exercise is considered an important and critical component to facilitating discussions moving forward.

Place-based narrative mapping helped to clearly articulate areas and places of concern. Place-based narrative mapping is a collaborative approach that integrates storytelling with geographical data to capture the lived experiences and cultural significance of specific locations for Indigenous communities. In this method, community members share narratives about their relationships with water and the environment, which are then mapped onto a geographical framework to highlight areas of concern and significance. This process not only respects and values Indigenous knowledge systems but also facilitates the identification of water quality issues from a community perspective, ensuring that research priorities are aligned with the values, needs, and aspirations of the community. By visualizing these narratives on a map, researchers and community members can better understand the spatial dimensions of water concerns, allowing for more effective co-development of research projects that are contextually relevant and culturally appropriate.

Another important activity that occurred following initial pre-engagement was a historical deep-dive into the potential impacts on the Bow River from downstream First Nations, through a methodology that was aimed at being comprehensive, multidisciplinary, and community-centered. This included:

- A literature review and archival research search to gather any existing knowledge;
- Community consultations and oral histories in order to understand Indigenous knowledge perspectives;
- Historical mapping to visualize historical changes to the area to support community engagement discussions; and,

- Cross-referencing with historical events to understand any links between existing environmental data and historical events.

The intention is to utilize this historical deep-dive in community discussions as a validation step. This has already been done during the deep-dive interviews that created the Mind Map and will be continued as the upcoming interviews are completed.

Throughout this entire time, community capacity-enhancing was being developed. Based on discussions with the Blackfoot Youth Water Council (see above), there was a desire to investigate several Western-based science training elements in order to support their full and meaningful participation in the remainder of the larger Bow River project. Additional funding was obtained to support this activity and this training (CABiN), has been arranged to be delivered in July 2024. This has also included an open-ended community-driven capacity building workshop, scheduled for July 2024.

Current discussions are focused on knowledge mobilization and better knowledge co-creation environments through the Mind Map, and will be ongoing. Based on these discussions, additional sampling at sites identified by community members will be undertaken in summer 2024, with support from community members. Sampling will be focused on endpoints that a) integrate with the western science sampling, and b) are identified by community members as priority.

5 Key Learnings

5.1 ESOCs Fate and Transport

Seasonality, occurrence, and distribution: Working closely with The City of Calgary allowed for mutually beneficial collaborations and discussions that were meaningful for their decisions related to ESOCs monitoring. The collaboration further initiated a subsequent MSc project (Jaime Hicks -comparison of microbial source-tracking and chemical source-tracking tools) to assist The City with their research needs related to their source water protection objectives. ESOCs results from the ACWA streams indicate that there was no difference in micropollutant concentration among the Bow River, Reverse Osmosis (RO), and Ozonation (O3) streams suggesting that they exhibit similar traits. Given that the Bow River contributes 95% of the total stream volume, it is likely that the concentrations in the RO and O3 streams are driven by the Bow River background concentrations from upstream WWTPs. However, there was a significant increase in venlafaxine (antidepressant) concentrations in Pine Creek WWTP streams compared to BR, RO, and O3.

Partitioning of ESOCs in other environmental matrices: Samples from solid matrices (sediment, biofilm, gammarids, fish) were analysed at the University of Waterloo (Servos Lab) as part of the student research exchange (MSc student, Daniela Pulgarin Zapata). Research exchange with the University of Waterloo was valuable in determining best extraction method for sediments and biota. Frequent interaction with other students allowed for additional insights related to the analytical data. Extraction methods for biological tissues were improved which resulted in better detection limits and higher recoveries. A literature review was completed for determination of the best approach to detect the ESOCs in fish tissues. Literature findings were validated in the lab and QuEChERS was found to be the most effective method for isolation of

ESOCs from biota as well as sediment samples. Also, water concentrations are not always sufficient to accurately assess their impact on fish and invertebrates. For this study, triclosan and triclocarban (antibacterials) were always detected in the sediments. Fluoxetine (antidepressant) and its metabolite (norfluoxetine) were found the highest concentration in biological matrices (fish, gammarids and biofilm) although it only appears in the water column at very low levels. Working with an interdisciplinary group allowed the students to cover the topics deeply considering different perspectives.

Toxicokinetics of ESOCs uptake: There was very little change with the monthly assessments with the concentrations of ESOCs in biofilm and amphipods suggesting that the target substances may have achieved steady-state in these environmental compartments relatively quickly. Whether the time-scale factor is of primary importance (i.e., sampling daily or weekly, timescale refinement) can be assessed together with other items from this toolbox (basal productivity, biological responses, Indigenous research questions prioritization).

5.2 Biological Responses

5.2.1 Autotrophic/Heterotrophic Production

In the Bow River, increased MWWE exposure was found to enhance heterotrophic microbial community activity and nutrient enrichment was identified to be a main driver of the observed responses. Decomposition rates increased with increasing MWWE loadings. Ash-free dried mass (AFDM) and AFDM accumulation rates showed higher values at the higher MWWE loading site in the first 21 d of exposure; however, after 42 d, rates were the same at both sites. Chlorophyll *a* and production rates were higher at the lower MWWE loading site after 21 d, but after 42 d endpoints were the same at each site. Nutrient limitation was only observed at 1.0 mol/L NDS concentrations for the low MWWE loading site.

ACWA raceway stream experiments and results suggest autotrophs may be suppressed compared to heterotrophs with increasing MWWE concentrations. Increasing nutrient and ESOC concentrations, in the absence of nutrient limitation, showed decreases in chlorophyll a concentration in the rock baskets and NDS assays. Collectively, this suggests increasing ESOC concentrations may be suppressing autotrophic activity. Both river and experimental stream (mesocosm) exposures were essential in assessing the autotrophic and heterotrophic basal responses to MWWE. All biological endpoints in combination revealed the mechanisms at play and are relevant and suitable endpoints for biomonitoring.

5.2.2 Benthic Macroinvertebrates

Mesocosm and Methods Experiments: The benthic macroinvertebrate communities in the ACWA streams were poor representations of the Bow River in both control and treatment streams. The overall communities were largely made up of pollution tolerant organisms and lacked most representatives of EPT organisms that were more present in the adjacent mainstem Bow River. Most measures of ecosystem health stemming from benthic macroinvertebrate community metrics suggested that the ACWA streams were less diverse, less rich, and less healthy than the Bow River. However, even with notably different communities to the mainstem, the control stream communities were significantly different than the treatment streams, suggesting that MWWE exposure is still exerting pressures on ACWA benthic communities.

Bow River Biomonitoring: When sampling similar habitats in the Bow River within Calgary, there are substantial shifts in the benthic macroinvertebrate assemblages that closely align with exposure to point source MWWE. Physicochemical parameters linked to MWWE also appear to drive changes to benthic macroinvertebrate communities more notably than other potential urban drivers within The City, as supported by multivariate analysis across the whole open water season (May – November). Metrics related to specific types of taxa present in samples (and their relative abundances) were far better at identifying the presence of MWWE than more general metrics, such as richness or diversity, that did not capture community turnover.

Shifts in benthic macroinvertebrate community assemblage composition and abundance were largely related to a few small dominant groups, suggesting that sampling could be refined to focus on particular taxa, rather than processing and describing entire community samples. Standardized macroinvertebrate sampling and data analytical approaches such as CABIN may not have the sensitivity to adequately couple observed responses to potential effects associated with specific ESOC classes. While changes in benthic invertebrate community structure is a good indicator of reach-specific nutrient-related eutrophication processes, their use as indicators of ESOC exposure and acute or chronic toxicological effects requires additional research.

5.2.3 Fish

Wild fish responses in the Bow River and tributaries: Wild fish studies, including longnose dace and fathead minnow, showed changes relative to upstream reference and progressive changes throughout the urban reach of the Bow River and the tributaries, both in association with cumulative impacts (MWWE and stormwater) and stormwater exposure. The changes in fish health indices indicated effects consistent with the eutrophication pattern (increases relative body size, liver and gonad size) in longnose dace in both spring and fall, and the fathead minnow in Nose Creek. There were suggestions of reduced reproductive investment; although condition and liver size were significantly increased, there was not a concomitant increase in gonad size.

Caged fish responses in the Bow River and tributaries: Caged fish (longnose dace) responses to cumulative effects were comparable with those observed in wild fish (eutrophication pattern), especially in the spring (wild vs caged). Stormwater had inconsistent effects on caged longnose dace and rainbow trout. Data on condition factor (K), liver somatic index (LSI), and gonadosomatic index (GSI) were determined for all 3 species in all 4 caging experiments at ACWA. The results indicate that there are no consistent responses among fish species or selected morphometric parameters. Trout perch were the most sensitive fish species, potentially displaying effects on GSI in the PC treatment (5% effluent; fall 2021). Spoonhead sculpin were the least affected during the 28-day caging experiment (5 %, fall 2021). Longnose dace responded differently in the 2 experiments, namely LSI was affected in the 5% caging experiment while the 15% exposure had no effects on the LSI. Minor effects were noticed on the condition factor in both experiments.

Changes in gonad and liver size in wild longnose dace beyond critical effect levels as defined by industrial federal effluent guidelines have been verified via fish translocation studies (i.e. moving fish from unexposed areas to exposed areas) across Calgary's wastewater outfalls and to stormwater exposed sites. These results have been further validated by moving naturally exposed fish to unexposed areas, generating RNA sequencing that enable objective parsing of genetic markers of exposure and relevant toxicant response pathways. As determined via reverse-exposure (depuration/recovery) of wild fish in reference locations, the ability to adapt and change to new conditions suggests resilience (i.e., sustained health of fish). The study design also addresses the utility of such indicators in routine surveillance monitoring of urban effluents. Changes in molecular (transcriptomic/EcoToxChip) signals will provide additional insight into impacted biological pathways and aid in potentially distinguishing the effects of MWWE and/or stormwater exposure.

5.3 Emerging Tools

5.3.1 Microbiome Studies

The ACWA stream larval and adult insect microbiomes and nutrient excretion rates showed minimal shifts across effluent exposures. These results at ACWA using standardized exposure conditions differed from results in Bow River for the same taxa collected downstream of WWTP discharges. This suggests that shifts in microbiomes of aquatic insects may occur under different exposure scenarios in the ACWA streams.

We found alterations in microbiomes of most larval and adult insects exposed to wastewater effluents, and differences in microbiomes through metamorphosis. Riparian spiders had minimal shifts in their microbiomes, suggesting that they are either not exposed to effluent-derived contaminants, including bacteria, or that they are less susceptible to such exposures. There were also no consistent shifts in functional profiles of bacteria within macroinvertebrates collected downstream of wastewater discharges, suggesting that there may be no physiological effect on exposed invertebrates, although physiological endpoints would be better directly assessed. While riparian spider microbiomes were not affected by proximity to wastewater effluents, adult emergent insects retained effluent-derived bacteria and transported them across the aquatic – riparian boundary which may have implications for other terrestrial predators.

We also found an $\delta^{15}N$ enrichment and shifts in $\delta^{13}C$ in freshwater primary producers (rock scrapings), freshwater consumers, and riparian spiders collected at a site downstream of the Bonnybrook wastewater treatment facility, indicating that effluent-derived nutrients are being incorporated into receiving ecosystems and transferred to these terrestrial predators. These results are consistent with the eutrophication responses observed in basal resources and fish at MWWE-impacted sites. Stable isotope analyses can be used as a measure of exposure of effluent-derived contaminants and may be easy to implement as part of a regulatory monitoring framework. The key function of nutrient (N, P) excretion by macroinvertebrates did not seem to be affected by exposures to wastewater effluents in the Bow River.

As this study is the first to assess microbial changes in effluent-impacted aquatic insects in the Bow River, our data provides a baseline for future studies and may help evaluate impacts of wastewater as the population in The City of Calgary increases or as treatment processes evolve. Additional sampling downstream of Nose Creek would help delineate impacts of stormwater from municipal wastewater inputs to the Bow River and improve the use of microbiome data in monitoring programs to evaluate areas of concern.

5.3.2 Caged fish responses in the raceways at ACWA

RNA sequencing suggested that the longnose dace hepatic transcriptome response reflected urban effluent exposure in both treatments (Bow River and 5% Pine Creek effluent raceways) and the number of differentially expressed transcripts increased with the duration of exposure (7d<14d<28d), suggesting responses at the molecular level may be predictive of exposure duration. The sex of the fish was not a significant factor in quantitative changes in the hepatic transcriptome, suggesting that either sex may be used for monitoring effluent changes in the field. Hierarchical analysis of the liver transcriptome response, called gene set enrichment analysis, revealed that both male and female longnose dace were responding to inflammation and pathogen challenge. It remains a high priority to complete the interpretation of RNA sequencing for all 3 fish species to understand species-specific differences, which will further contribute to selecting candidate gene markers or relevant pathways of effluent exposure. In addition, comparison of transcriptomics in ACWA exposures with caging experiments in the Bow River will aid in determining differences in responses and further the effects of confounding factors.

5.4 Indigenous Engagement

The most substantive efforts to-date have been focused on relationship building and knowledge translation from western science components of the project.

Key learnings from the initial phase of the project as they relate to Phase 1 & Pre-Engagement:
1. Collaborative Research Enhances Relevance: Engaging with downstream First Nations communities through engagement and workshops at the inception of the project is essential in co-developing research questions and identifying key historical events and current indicators of ecosystem health. This collaborative approach ensures that the research focus is aligned with community priorities and addresses their most pressing concerns. This can be supported through pre-engagement that supports mutual learning and built trust, making the research process more inclusive and the findings more relevant to the community, and the research more relevant both locally and globally.

2. Importance of Meaningful Inclusion of Indigenous Knowledge: One of the most significant learnings from our historical deep-dive into the Bow River's impacts is the importance of ensuring significant time and resources to ensure there is meaningful contribution of Indigenous knowledges. Oral histories and traditional ecological knowledge provided by Elders and community members offer insights that are not available in written records. Preliminary engagements highlighted water management and historical observations of ecological changes, emphasizing the need to include Indigenous perspectives in environmental research for a more comprehensive understanding.

3. Complex Interplay of Historical Impacts: The historical analysis revealed a complex interplay of factors affecting the Bow River. Industrial developments, agricultural practices, and policy changes over the decades have all contributed to the current state of the river which is well documented. Less so is this interplay with surrounding Indigenous communities and the political, social and economic ramifications. This complexity underscores the necessity of multidisciplinary approaches in environmental research.

4. Value of Historical and Environmental Data Correlation: Cross-referencing environmental data with historical events will provide a clearer picture of the Bow River's ecological trajectory. Sediment and water sample analyses will help detect historical pollutants and changes in sediment composition, which, when correlated with documented industrial and agricultural activities can support community narratives on environmental degradation, which allow for more meaningful discussion on future/forward-looking assessment. This retro-active approach was deemed necessary by community to enable a forward-looking approach.

5. Empowerment Through Community Co-Creation and Involvement: Finally, involving First Nations communities directly in the research process empowers and ensures that their knowledge and experiences are central to the study. This involvement not only enhances the quality of the research but also promotes community ownership of the findings. By validating and reporting the results back to the community, the research process will continue to foster transparency, accountability, and a sense of shared purpose in protecting the Bow River as the project evolves.

6 Outcomes and Impacts

6.1 ESOCs fate and transport

General ESOCs trends: The work produced baseline data for ESOCs occurrence within Bow River and Elbow. A major outcome related to the 4 years of data resulted in streamlining the suite of analytical chemicals from 55 chemicals in the ESOCs list down to ~10 priority chemicals to allow refocusing of resources on other types of analysis (e.g., PFAS analysis). The streamlining process was based on detection levels (i.e., remove substances that were rarely detected), presence of surface water quality guidelines, and interest in relative persistence (e.g., antidiabetic, metformin). Focused monitoring of ESOCs is now being done at fewer sites (Glenmore and Bearspaw raw water intakes, and Cushing bridge and Highwood confluence). Conventional water quality parameters are still embedded in various monitoring programs within The City. This work also found relationships in Glenmore Reservoir with seasonal occurrence of chemicals in source waters for drinking water (DEET), which directed additional questions related to recreation use of the reservoir and its connection with existing source water protection plans.

Regulatory impacts: Eight of the 55 ESOCs have surface water quality guidelines (SWQG). The concentrations detected in the downstream Bow River (Highwood Confluence) were below the surface water quality guidelines (Alberta, Federal Quality Guidelines, and CCME). There are no ESOCs guidelines for other matrices (sediments, biota, biofilm, amphipods) except for triclocarban in the sediments. Triclocarban detections at the artificial streams (ACWA) were below these guidelines. Although it does not have SWQG, diclofenac was found to exceed the EU proposed limit (50 ng/L) and hence this substance is included in the priority ESOCs monitoring list. This study also evaluated estrogenic chemicals with *in vitro* bioassays (level with chemical 0.8 ng/L and guidelines are 0.5 ng/L) to determine whether there is a need to improve EE2 detection. The total estrogenicity via the Yeast Estrogen Screen (YES) assay in the influent and effluents showed ~20 ng/L and at ~2 ng/L, but based on dilution, the estrogencity is likely not an issue at this time. This can be revisited during low flow conditions/drought to assess potential impacts of wastewater on the receiving environment. Reallocation of resources allowed more focus by The City on PFAS, and other potential chemicals of concern (DEET, sunscreen chemicals [e.g., benzophenones). The City's ESOCs monitoring is outside of regulatory compliance. However, the outcomes of first study (Arlos et al 2023) are being considered by the Prairie Provinces Water Board (Committee on Water Quality) via the Committee on Water Quality Group, looking at whether to include new WQG for ESOCs.

6.2 Biological Responses

6.2.1 Autotrophic/Heterotrophic Production:

Impacts on Methods: Both autotrophic and heterotrophic components important to measure for assessing MWWE and display there are interactions between the basal groups. Our results showed that microbial communities downstream of MWWE are heterotroph dominant, which has implications for higher trophic levels. Heterotroph dominant food sources would be lower quality food source and may impact invertebrate communities. In terms of the Bow River, heterotrophic ecological endpoints are generally not measured and are essential for understanding productivity along with autotrophic responses (ie, chlorophyll-a measures). A proposed measure for productivity could be the autotrophic index, which is a ratio of chlorophyll-a versus ash-free dry mass. The index is useful identifying the relative responses and shifts in autotrophic (algal) and heterotrophic (microbial) assemblages and related trophic energy pathways in responses to MWWE exposure.

Cotton strips were measured as indicator of microbial heterotrophic decomposition rate and compared Bow River and ACWA. The ideal timeframe to remove cotton strips are when they reach 50% tensile strength loss, which is typically 21-28 days for most published studies. For the Bow River, 16-18 days is an ideal exposure time frame and provides a good timepoint for heterotrophic decomposition rate. Future studies needs to examine the shift in benthic community needs to be investigated relative to shifts in productivity. ACWA streams (Bow river water only, 5% (v/v) MWWE, and 15% (v/v) MWWE) showed biofilm responses and higher MWWE volumes suggests algal suppression.

Environmental monitoring recommendations: There needs to be more emphasis on heterotrophic measures in monitoring– eutrophication effects downstream of MWWE are not being manifested as higher autotrophic production (measured as chlorophyll-a). Our studies also did not measure macrophyte productivity - need to be able to integrate different measures of productivity to get a better measure of overall production. Higher rates of decomposition are going to be enhanced downstream and may create a risk for higher rates of under-ice oxygen depletion – oxygen sags overwinter need to be looked at to evaluate risk.

Artificial substrates (rock baskets) could be deployed to get more consistent measures; timing of incubation is important because communities change over time biofilm succession and time frames.

6.3 Benthic macroinvertebrates:

Baseline for future studies: the planned and produced baseline data has improved basin understandings, as historical data does not exist or is not readily available, especially within City limits. Overall, benthic macroinvertebrate assemblages appear to be primarily driven by nutrient-related effects (particularly from a spatial perspective regarding MWWE inputs) and assemblage changes match expected nutrient effects such as increased abundance of taxa, generally, and pollution-tolerant taxa, specifically. Alternatively, there is little evidence at a community-level that ESOCs are driving changes in communities (if there are specific species-level impacts from specific modes of action, those are not evident at community level). Benthic macroinvertebrate community responses to stormwater were also not distinguishable from other reference sites (i.e. Zoo site and Cushing Bridge not distinguishable despite multiple tributary and stormwater inputs but Graves Bridge below Bonnybrook is highly divergent). The next major outcome will be to sample additional years of data to complete a baseline sample to identify ranges of how treatment plants impact the basal food web in the river, especially given projected population growth and potential changes to flows and treatments.

Benthic macroinvertebrates impacts: Identified similarities between areas of the river and lack of response to cumulative stormwater inputs (the other major urban input) likely means that sampling sites within identified river reaches can be studied with reduced sampling. Also, while eDNA samples were collected, the most representative metrics required abundance. Other indicators, such as richness, were poor indicators compared to %EPT or %Diptera but eDNA cannot be used for these endpoints. Overall, there were also consistent but varied differences in streams between control and treatment streams at ACWA. Current experiments that will inform the toolbox include identifying specific physicochemical Trichoptera responses to examine whether future monitoring should focus on specific taxonomic groups.

6.4 Fish

Baseline data: While there has been considerable work done in the Bow River on larger fish species, and assemblages, there is not an abundance of information for small-bodied species in terms of whole organism responses. Whole organism endpoints, like those used in the Canadian Environmental Effects Monitoring (EEM) program, provide an integrated response to the cumulative experiences of fish, and the smaller home range of many small-bodied fish species provides the ability to separate different local stressors. Differing responses as sampling progressed downstream through Calgary highlighted the ability of small-bodied species to identify the presence of localized stressor sources. The studies provide important baseline data for future studies.

Study design: Together, the collection of wild longnose dace and caging experiments conducted in the Bow River, alongside those conducted in raceways at ACWA, provided valuable insights into fish responses to acute versus chronic exposure to tertiary treated effluents. These experiments were carefully designed to consider both time and confinement effects, aspects often overlooked in many caging studies. It was confirmed that stormwater had systemic effects on the organism, such as altering the liver somatic index.

The study design involving reverse-exposure (depuration/recovery) and addresses the utility of such indicators in routine surveillance monitoring of urban effluents. Identifying interspecies sensitivity through whole organism responses and liver transcriptomics in caging experiments provides insights into how species, influenced by their ecologies, habitat preferences, food sources, and reproductive stages, cope with exposure to municipal wastewater effluent. This has implications for the selection of fish species for future monitoring to study effects of municipal effluents and contributes to our knowledge of data extrapolation across multiple fish species. Additionally, examining the effects of the 15% effluent exposure can simulate realistic scenarios of climate change impacts, offering valuable information on adaptation mechanisms employed by fish species.

6.5 Emerging Tools

6.5.1 Microbiome

Our results show that larval and adult insect microbiomes are impacted by wastewater discharges to the Bow River but that these effects are not transferred to riparian spiders. Although there were some shifts in aquatic insect microbiomes, this did not seem to change their ability to excrete N and P. This work provides baseline microbiome and stable isotope data of effluent-impacted insects and riparian spiders in the Bow River and can be used to assess effluent exposure in a monitoring framework. Our results also show that Hydropsychid caddisflies may serve as useful bioindicators for effluent exposure in the Bow River since their microbiomes showed greater responses to municipal wastewater effluents compared to other aquatic insects, they are abundant at all sites in the Bow River and ACWA streams, and they are optimal as an organism to effectively extract and amplify DNA from.

6.5.2 Transcriptomic tools

RNAseq and EcoToxChip offer distinct insights into how stormwater affects molecular responses in fish. While RNAseq examines thousands of transcripts, EcoToxChip provides a streamlined approach. Together, they can identify gene markers that could be utilized more frequently, potentially reducing costs for future stormwater monitoring efforts. Analyses of the data are ongoing to determine whether there are specific markers or patterns that can be used to identify and separate MWWE and stormwater stressors.

6.6 Indigenous-Centered Research

Future phases of this project, or projects of similar design, need to prioritize the co-creation of knowledge and community engagement *from the onset of (and throughout) the project* to be successful.

- Phase 1 discussions will be critical for developing meaningful relationships and collaborative opportunities with local Treaty 7 communities prior to the development of future Phases.
- Mechanisms will need to be designed to:
  - develop parallel, transferable and scalable methods to foster participation of communities and inclusion of Indigenous knowledges;
  - acknowledge context specificity in upstream and downstream watershed quality and ecosystem changes; and
  - steer and support education around traditional and scientific knowledge within the community, particularly with youth.

Through community co-leads, future work will focus on leveraging the information gathered in Phase 1 to select parameters of concern, key locations and develop a plan of action.

7 Program Specific Metrics:

7.1 Clean Resources Metrics

Clean resource metrics existed for field pilots, new products and services, practices informed and influenced, and knowledge mobilization. Each is discussed in terms of the project target, the commercialization target, and the outcome.

7.1.1 Field pilots/ demonstrations

Use of new and unique Alberta infrastructure (Project target): this project was the first to use the replicate ACWA treatment streams (raceways) for their designed purposes in assessing complex aquatic food web responses to MWWE exposure gradients and tertiary treatment options. The project conducted four sets of fish caging studies in the ACWA raceways, as well as basal food web inter-seasonal method comparisons, 21 d and 42 d exposures of varying MWWE exposures to assess relative autotroph-heterotroph community responses, and one set of benthic invertebrate natural and artificial substrate studies, and completed chemical characterizations for ESOCs. The ACWA streams were instrumental in demonstrating a proportional increase in ESOCs in sediments, biofilms, and amphipods with percent effluent contribution, and in comparing the bioaccumulation potential between chemicals and across trophic levels. The streams allowed for a level of control on exposure that is critical to identifying mechanisms of impact. Although the benthic invertebrate communities in the raceways differed from the Bow River, taxon-specific, exposure-response patterns were identified.

Increase use of ACWA facility due to characterization of stream mesocosm infrastructure (Commercialization target): the project was successful at demonstrating a foundation for innovative use and to establish the potential to use the facilities as a basis for technology developers to quantify benefits. The studies characterized the streams in terms of chemistry, basal productivity, benthic invertebrate communities and responses of fish placed in cages. It was useful for characterizing chemistry, evaluate the potential chemical benefits of alternate treatment technologies, to manipulate exposure conditions to simulate future exposure or temperature scenarios, to compare methodologies under standardized exposure conditions, and to compare responses across trophic levels. The ability to control MWWE exposure concentrations across treatment streams under controlled flow and temperature conditions was critical to assessing the ecological effects of complex chemical mixtures (nutrients, pharmaceuticals, metals) on aquatic food web responses.

Program used as basis for design of cumulative effects studies (Outcome): analyses are still underway for detailed comparisons but the ability to control exposures is critical for sorting out causative factors in cumulative effects studies. In both the fish exposures in the river and the ACWA exposures to higher effluent concentrations (15%), there are suggestions of impairments beyond simple eutrophication effects. Facilities like the ACWA streams are essential to being able to disentangle impacts.

7.1.2 New products/services created

*Developing foundation for innovative use of unique Alberta infrastructure: u*nder BREHA, we further developed and validated a suite of integrated assessment approaches that utilized a combination of biomonitoring endpoints involving both conventional and novel sampling systems. These included: 1) assessing changes in the diversity and abundance of benthic macroinvertebrates along an MWWE exposure gradient in the Bow River and in the ACWA experimental streams using both conventional (CABIN) and standardized artificial substrates (Rock Baskets); 2) responses of autotrophic (algal) and heterotrophic biofilm communities to MWWE exposure using standardized cotton strips, nutrient diffusing substrates and artificial substrates (Rock Baskets); 3) in collaboration with Innotech Alberta, preliminary analyses examining the application of eDNA approaches to assess biological community responses; and, 4) utilization of The City of Calgary ACWA streams for controlled MWWE exposures.

Establish basis for water treatment technology developers to quantify benefits to aquatic resources: understanding the biological and ecological effects and interactions of complex chemical mixtures in municipal wastewater effluents requires a multi-faceted approach involving various monitoring and experimental techniques. Only by employing a combination of these approaches is it possible to better understand and mitigate the impacts of complex chemical mixtures in municipal wastewater effluents on aquatic ecosystems.

Outcome: ACWA streams were characterized as an artifact of work conducted, which presents opportunities for modular water treatment system at facility to be utilized for piloting of new technologies that are general/targeted to primary ESOCs and other environmental stressors (e.g., flow, temperature regimes). The project was successful at demonstrating the ability to quantify the benefits in the streams. Project outcomes would be enhanced by additional treatment on the Bow River head pond water for the streams, to reduce the incoming load associated with upstream sources of nutrients and ESOCs. The project also provided fundamental genomics information to advance molecular tools for wastewater surveillance and the assessment of biological impacts from exposure for the Bow River watershed, and more broadly to Alberta.

7.1.3 Practices informed/influenced

Federal wastewater regulations: work conducted, as well as a literature review on 150 case studies of fish exposed to MWWE demonstrated the capability of the endpoints to monitor wastewater impacts. Federal regulations for monitoring and assessment of MWWE are still being considered by provincial and federal regulators. An early draft of the Canadian Wastewater Systems Effluent Regulations (2012) included a requirement for environmental effects monitoring (EEM), but it was not included in the final regulation for a variety of reasons. The literature review demonstrated the need for including whole organism endpoints to be able to differentiate mechanisms of impact when looking at potential wastewater impacts. Although >90% of the studies reviewed concluded a reproductive impact, <35% of studies monitored whole organism endpoints, and of those studies, <20% suggested a whole organism reproductive disruption associated with ESOC-like compounds (Marjan et al. 2024).

Knowledge generated from multiple-MWWE outfall work in natural and semi-controlled systems will generate practical guidance for monitoring impacts of tertiary treated effluent at regional, national, and international scales. Knowledge generated from work in natural and artificial systems has generated practical guidance for approaches and methodology. The City of Calgary underwent a prioritization exercise based on ESOCs trends/patterns from 2018 – 2022. The City refocused monitoring efforts to target ESOCs to complement other monitoring campaigns occurring within The City (e.g., microbial indicators).

Outcome: Federal regulations for monitoring and assessment of MWWE are being developed. We are working to communicate results to regulators updating Environmental Effects Monitoring regulations, including recommendations for the selection of integrated biological/ecological endpoints to be assessed under an adaptive monitoring and water management framework.

7.1.4 Knowledge mobilization

Project outcome: 5+ scientific publications*:* in addition to the 4 articles already submitted, there will be >6 additional articles originating in theses which are still being completed (see list below in Appendix I). There are also 5 completed Masters projects (2 PhD still underway), 1 Engineering capstone projects and 4 undergraduate research theses (see Appendix I for listing). There are additional papers published directly related to municipal effluent assessment published out of the University of Waterloo.

The research program as designed is intended to provide practical guidance in the form of direct communication with The City of Calgary and stakeholders: we have had a number of direct meetings with partners and held an annual open house to share outcomes. The project team focused the study to ensure that best practices are developed and that environmental quality monitoring tools are calibrated to the environmental stressors (MWWE) being investigated. In addition, recent work has focused on examining the relative roles that stormwater inputs and MWWE inputs may be having in the river.

In addition to the publications and theses outlined above, there have been several public media publications and >50 conference presentations (see Appendix I).

The project team is focused on undertaking this study to inform best practices and ensure that environmental quality monitoring tools are calibrated to the environmental stressor (MWWE) being investigated.

7.2 Program Specific Metrics

The objective of the Project was to provide tools that could be used to improve overall water use efficiency (treatment plant optimization). The project advanced the scientific ability to measure a variety of ESOCs in wastewater, surface waters and biological tissues and link this exposure to potential effects on aquatic biota. The project integrated several approaches to detect change associated with wastewater exposure and link to biological changes under controlled and wild populations. This included examination of responses across different levels of biological organization (gene expression to whole organism) in fish, algal, microbial and macroinvertebrate communities. This work advanced the application of existing and novel tools to isolate effects providing an improved ability to identify and separate various stressors.

There was evidence of some improvement in removal of some ESOCs using advanced effluent treatments available at ACWA (Arlos et al. 2023), but interpretation could be enhanced by treatment of the dilution water used for the streams to reduce background signatures from upstream sources. The existing ESOCs data for individual facilities was used to help interpret differences between treatment plants.

The results of these studies support the conclusion that subtle effects on fish continue to be possible downstream of even well treated wastewater outfalls. Although eutrophication related effects remain evident, biological changes may also be associated with other trace contaminants. Wastewater treatment meeting or exceeding the current federal regulation are likely to reduce exposure to many ESOCs, although many important contaminants remain recalcitrant to treatment. The controlled ACWA stream exposure studies identified possible nutrient/ESOC interactions affecting the autotrophic/heterotrophic food web pathways, with potential implications for higher trophic levels. Further studies would also be required to determine how hydraulic retention can be modified relative to other important treatment parameters such as solids retention time. This project has created a foundation for further studies to better assess the potential impacts of wastewater treatment options (optimizations). Using controlled experimental streams allowed for separation of potential effects of the complex mixtures in wastewater from other stressors (e.g., contaminants) and confounding factors in the watershed (e.g., habitat, water quality, other contaminants). The project emphasizes the importance of the consideration of cumulative effects at the watershed scale that can mask subtle changes associated with wastewater exposure.

7.3 Project Success Metrics

7.3.1 Cumulative effects assessment

The studies clearly demonstrated the ability to use EEM-derived approaches for fish and benthic macroinvertebrates to evaluate effects of multiple stressors (MWWE and stormwater) in urban settings. Despite the push towards novel approaches like eDNA, the benthic studies showed the need to have measures of benthic macroinvertebrate abundance to improve interpretation. The studies also highlighted the importance of examining the responses of both algal and microbial communities responses to understanding cascading implications for higher trophic levels. In terms of fish studies, caged fish show the same whole organism responses, but muted and not always statistically significant.

Existing approaches for quantifying productivity do not adequately consider heterotrophic production, especially downstream of MWWE outfalls where productivity shifts.

The ability to discern effects of MWWE and stormwater are still being evaluated in ongoing analyses of the data obtained. However, the availability of baseline data is critical to considering cumulative effects at a local scale, and EEM approached have been shown to provide regional consistency critical for the evaluation of effects at regional scales to better manage Alberta's water resources.

7.3.2 Building capacity

Training of Highly Qualified Professionals in Alberta to apply tools and approaches developed: HQP training exceeded the targets proposed and included 3 PhD students currently in course approaching completion, 5 MSc students, 3 MSC Eng and Env Eng students, and 3 undergraduate theses (see Appendix II for details). It also included 6 undergraduate technicians, 5 postdocs, and 5 Technicians and biologists. The graduating HQP bring novel technologies and approaches to Alberta for efficient and targeted evaluation of largest point-source pollutant source (MWWE).

7.3.3 Building Collaborations

Our project engaged with various groups during the Project to understand concerns and establish channels of communication, including the Bow River Trout Foundation, Nose Creek Watershed Partnership, Jumping Pound Community Association, and the Bow River Basin Council (BRBC). We also held a community science workshop on biomonitoring in the Bow, made Presentations to Alberta Environment and Protected Areas Community of Practice, and the Committee on Water Quality for the Prairie Provinces Water Board. We actively participated in Annual Science fora with the BRBC, participated in focused workshops with the BRBC's Biomonitoring Workshop, and worked on data visualization approaches with the State of the Watershed Water Quality Technical Committee.

Indigenous community collaborations with members of the Blackfoot Confederacy are critical to future phases of the Project, in addition to surrounding and upstream Indigenous partners.

We held Open Houses at ACWA, held an annual lunch time and coffee Open House at The City of Calgary's Water Centre, and invited partners from The City of Calgary, RiverWatch, the BRBC, EPCOR, and the Jumping Pound Community Association to take part in our annual One Health Summer Institute at Kananaskis, Alberta.

Our collaborations included academic partners at 6 Institutions, and the original project team included The City of Calgary, NSERC and Alberta Innovates. During the project the team included expansions to include Innotech Alberta for eDNA analyses and transcriptomic sample preparation, Environment and Climate Change Canada for CABIN data and eDNA analysis, and connections with other Bow River researchers.

We actively collaborated with the Molecular Biology Facility (MBSU) at the University of Alberta, and the Toxicology Centre at the University of Saskatchewan for RNAseq bioinformatics analysis, and Alberta EPA for the macrophyte sampling program.

7.3.4 Maintaining healthy aquatic ecosystems

The ESOCs analyses allows the optimization of The City of Calgary's ESOCs monitoring program. The baseline data developed for periphyton, benthic invertebrate communities and small-bodied fish will be crucial for future assessment as Calgary grows.

Our advice to the BRBC's Watershed Water Quality Technical Committee for their development of their State of the Watershed assessment for data analysis and visualization impacted the analysis and presentation of data to generate "state of watershed" information that considers site-specific changes within the context of regional change. The baseline data allows the development of thresholds to identify future change, not just for water quality, but also for other indicators of ecosystem health employed. These site-specific thresholds are essential for identifying whether ecosystem health is "improving", "unchanged" or "degrading".

Bow River historic data on ESOCs monitoring has been summarized and analyzed and methods for extracting ESOCs from various matrices (sediment, biofilm, fish, and invertebrates) have been developed. the first draft manuscript has been submitted. Experiments to assess various methods for quantifying autotrophic and heterotrophic biological endpoints were compared for the standardization and repeatability of method application, and the efficacy, variability, and efficiency in method interpretation. Microbiome samples in larval insects, adult insects and riparian spiders were studied to assess the effect of wastewater exposure on the microbiome communities and to trace the assimilation of wastewater derived nitrogen into nearshore food webs.

Baseline data for periphyton, benthic macroinvertebrate assemblages and fish have been collected and analyzed. Responses will be used to develop a current baseline for future comparisons. The assessment of sustainability requires that the current accumulated state be thoroughly understood, with the development of thresholds that will enable the detection of further degradation and establish targets for improving the health of the Bow River ecosystem.

Molecular responses in fish and microbiome tools are being examined to identify markers of exposure to wastewater and stormwater. These tools will help establish cause of ecosystem changes. The regulatory environment will benefit from the baseline assessments, the development of thresholds, the evaluation of new tools, and a framework for future health assessments.

8 Benefits

Assessment tools developed as part of this project will inform monitoring program design and state-of-environment reporting, identify potential indicators of MWWE and stormwater exposure, and prioritize future areas for study. This project created opportunities for proactive identification of environmental degradation and created opportunities for treatment technology development for the protection of Alberta's freshwater resources. It demonstrated the full potential of ACWA's globally unique experimental stream infrastructure in Alberta to allow for more robust risk assessment of complex organic pollutants in these conditions. Additional infrastructure would allow manipulation of temperature and flow regimes to simulate future climate scenarios to fully evaluate potential future changes in the Bow River system.

8.1 Economic:

Prioritization of the ESOC monitoring framework deployed by The City has led to economic savings, and reprioritization of concerns. Employment and capacity building benefits are outlined below. The project's emphasis on hiring a community-based researcher from the surrounding First Nations not only created employment but also built local capacity, providing skills and experience in research methodologies. That work will be ongoing. The recent Western-based science training (CABiN) and the community-driven capacity-building workshops empower community members with skills that enhance employability and foster potential future economic opportunities in environmental monitoring and management.

8.2 Environmental

Much of the environmental benefits are associated with contributions to improving environmental monitoring systems, including comparing methodologies, developing environmental baselines, and building new tools and evaluating approaches for detecting impacts of MWWE.

The place-based narrative mapping and the community-based Bow River Water Health Mind Map help identify specific areas of concern that might not be evident through traditional scientific approaches. This facilitates targeted actions to mitigate environmental impacts, ensuring the preservation and restoration of important ecosystems. The project continues to work to develop community-determined indicators and integrating Indigenous Knowledge, to provide a more nuanced understanding of the ecological health of the Bow River. This can lead to more effective water management strategies that address both contemporary and traditional concerns.

The historical deep-dive, which includes mapping and cross-referencing historical events with environmental data, supports a holistic understanding of the river's health, enabling better identification of environmental impacts and trends.

8.3 Social

The analyses of ESOCs data collected for the raw (Bow and Elbow) and treated waters (Bearspaw and Glenmore) aid in the communication of results for public perception and notions of risks improved (i.e., drinking water contains ESOCs but will require large volumes of water to achieve the same therapeutic effects of pharmaceuticals and personal care products). Analyses also provided The City of Calgary another line of evidence to ensure the source water is protected (i.e., Glenmore Reservoir) by exploring tools to assist in evaluating the potential impacts of recreational activities that are allowed on reservoirs.

The project’s emphasis on pre-engagement and continuous collaboration with community members fosters trust and strengthens the relationship between researchers and the community. This collaborative approach ensures that the research is culturally relevant and aligns with the community’s values and priorities. By involving community members directly in the research process, the project empowers them to take an active role in environmental stewardship and decision-making, fostering a sense of ownership and responsibility for local water resources. Through activities like narrative mapping and deep-dive interviews, the project documents and validates Indigenous perspectives on water health, contributing to the preservation of cultural heritage and knowledge. This also promotes intergenerational knowledge transfer, ensuring that traditional wisdom is passed down to future generations.

The Indigenous engagement component of the project was initiated as part of a multi-year plan, with initial research activities, focusing future phases of the work on meaningful participation of Indigenous communities and incorporation of priority issues, sites, and methodologies that will help address concerns that these communities are facing.

8.4 Building Innovation Capacity

In terms of capacity building and employment opportunities, more than 30 HQP were employed and trained as part of this program, with significant numbers originating outside of the province. Recent graduates are working in consulting in Alberta (3) and elsewhere in Canada (3), with government agencies in Alberta, with the US government (1), employed at the University under other programs (6), and continuing education elsewhere (4).

The project has significantly contributed to the training of highly qualified and skilled personnel (HQSP) in Alberta by hiring and training a community-based researcher from the surrounding First Nations, equipping them with skills in community engagement, traditional ecological knowledge integration, and advanced water quality monitoring techniques. This role has provided valuable, context-specific research experience, fostering the retention of local talent and creating a model for attracting HQSP from outside the province who are interested in collaborative, community-driven research approaches that integrate Indigenous and Western scientific methodologies.

This project was the first major initiative to utilize the artificial stream components at the ACWA Pine Creek facilities. We conducted significant ESOC measurement campaigns, deployed various periphyton and benthic invertebrate sampling approaches and successfully deployed 4 fish caging experiments. The facilities represent a major resource for conducting controlled experiments and for testing the environmental impacts. The Advancing Canadian Water Assets (ACWA) facility commissioned in 2015 is a $37M research partnership between UofC and The City of Calgary's Pine Creek Wastewater Treatment Facility that serves as a critical research hub. This foundational investment includes 12 x 320 m constructed, naturalized, replicated experimental stream raceways. It is a globally-unique infrastructure within an operating full scale wastewater treatment facility to test treatment options capable of understanding the downstream impacts in parallel.

9 Recommendations and Next Steps

The current project is ongoing and the current Phase 1 continues into early summer 2025. The City has already modified its regular ESOC program, and the current baseline will be critical for evaluating future ecological sustainability of the river system. As the population of The City of Calgary continues to grow, and flows are modified with the changing climate, a good baseline of responses as the river flows through The City will be required to prioritize issues. Studies are continuing to examine the relative contribution of stormwaters to the changes seen in the river, and the potential for endocrine disruption suggested by the studies.

Important partnerships have been developed with the City of Calgary, Creekwatch/Riverwatch, Innotech Alberta and the Bow River Basin Council. There is a need to formalize the baseline data into a series of interim monitoring triggers that can be used in future phases of monitoring within the city limits. The monitoring triggers would serve to identify whether significant change has happened. As the project expands in Phase 2 outside of the City limits, partnerships with AEPA and downstream rural and Indigenous communities will be critical for influencing the South Saskatchewan Regional Plan and local water management plans.

Based on the learnings from this project, the following actions are recommended for the coming years to more formally integrate Indigenous communities into the monitoring program and to continue advancing the innovations developed:

a) Expansion of Place-Based Narrative Mapping to include additional First Nations communities along the Bow River and other watersheds in Alberta. This will enhance the understanding of regional water quality issues and promote broader collaboration.

b) Pilot Testing and Validation of Community-Determined Indicators at key sites identified in the mind map. This will involve collaborative sampling and analysis with community members to validate these indicators against traditional and contemporary scientific metrics.
c) Establishment of a Knowledge Exchange Platform to create a digital knowledge exchange platform that facilitates the sharing of data, stories, and research findings between community members, researchers, and policymakers. This platform would support ongoing dialogue and the co-creation of solutions to water quality challenges.

Continued development of partnerships with local and regional Indigenous communities will play a significant role in future projects in several strategic ways. The community-developed indicators and water health assessments can be integrated into local and regional water management plans, providing a culturally relevant framework for ongoing monitoring and decision-making. The goal is to share these back with the Nations and the Confederacy. There are a variety of additional next steps which will require the development of funding proposals to fulfill, including

a) Expanding educational, training, and capacity-building initiatives, such as the Western-based science training (CABiN) and workshops with the Blackfoot Youth Water Council. Expansion of training will work to empower community members to take active roles in environmental stewardship and research.
b) Development of a Community-Led Water Monitoring Program is a key long-term goal may be to establish a sustainable, community-led water monitoring program that leverages both Indigenous Knowledge and Western science, ensuring continuous and autonomous water quality monitoring and management by community members.

Several partnerships are being developed to support the advancement of this project and the implementation of its findings, including with:

a) Partnerships with provincial and federal government agencies will ensure that the community-determined indicators and findings are integrated into policy frameworks, contributing to more inclusive and culturally relevant water management policies.
b) Relationships with Indigenous organizations and leadership bodies within Treaty 7 and other First Nations communities need to be strengthened to expand the scope of the project, promote knowledge sharing, and support the scaling of community-led water management initiatives.

10 Knowledge Dissemination

The knowledge gained from this project is being disseminated through a combination of traditional methods such as peer-reviewed publications, conference presentations, and workshops (publications and workshops are found in Appendix I). Workshops included specific open houses at The City of Calgary, presentations to the Bow River Basin Council Science Forum, participation in annual meetings of the Nose Creek Watershed Partnership, and the Jumpingpound Community Association. As well, each year we organized a One Health Summer Institute at Kananaskis: in 2022 it was focused on a One Health view of agricultural and ecosystem relationships, specifically focusing on cattle operations; in 2023 it was focused on glacier contributions to the Bow River and climate change impacts from a One Health perspective, and in 2024 it was focused on water users and discharges to the Bow River basin from a One Health perspective.

With respect to Indigenous engagement, meetings included innovative approaches like the use of interactive community-developed digital maps, participatory storytelling sessions, and collaborative platforms that enable real-time sharing of research findings with community members, government agencies, and industry stakeholders.

11 Conclusions

This project was designed to characterize environmental responses to environmental substances of concern (ESOCs) introduced via The City's wastewater in the Bow River Watershed. Impacts of ESOCs on aquatic ecosystem structure and function are not fully understood. The study utilized collections from the Bow River as well as controlled studies conducted at the Advancing Canadian Wastewater Assets (ACWA) at the Pine Creek wastewater facility; the ACWA facility is a shared research infrastructure between The City of Calgary and the University of Calgary. The Pine Creek waste treatment facility produces wastewater that includes tertiary treatment and the effluent quality meets all current regulatory criteria and frequently exceeds minimum requirements. Research using replicate experimental streams at the facility at Pine Creek Wastewater Treatment Plant was conducted in parallel to the Bow River, to allow development and testing of new monitoring technologies and environmental prediction models to evaluate the current state of the river, to set a current baseline for future studies, and to develop interim thresholds for identifying changes in the accumulated environmental state.

The first objective was to establish effective ecosystem health indicators for evaluating wastewater effluent impacts that can allow evaluation and provide guidance for optimization of wastewater treatment investments. The project included further identification and quantification of environmental substances of concern (ESOCs) from The City's existing monitoring plan as well as targeted sampling to support the exposure indicators, as well as lower trophic ecosystem indicators reflecting productivity, benthic macroinvertebrate indicators, and measures of small-bodied whole organism indicators. It also evaluated emerging tools including microbiome measures and transcriptomic measures of gene expression.

The ESOCs studies resulted in a prioritization of the existing suite of chemicals used to reflect exposures, removing compounds from the suite of indicators that were consistently below detection limits. Method development focused on the ability to measure ESOCs in sediment, benthic invertebrates, and fish tissue, and showed accumulation of some ESOCs residues. These results provide an initial baseline for future studies, and demonstrated that biofilm and benthic invertebrates were accumulating detectable levels of some chemicals.

The lower trophic level studies showed that microbial communities downstream of MWWE are heterotroph dominant; heterotrophic ecological endpoints are generally not measured, and are essential for understanding downstream productivity along with autotrophic responses (i.e., chlorophyll-a measures). Current productivity measures in field studies concentrate on detecting chlorophyll-a levels which are a measure of autotrophic production. The studies proposed that a better indicator of downstream productivity could be an autotrophic index, which is a ratio of chlorophyll-a versus ash-free dry mass, to capture the productivity associated with heterotrophic production.

Not necessarily surprising, there was a dramatic increase in benthic invertebrate numbers between upstream and downstream locations in the Bow River, particularly below MWWE nutrient-enriched reaches. Physicochemical parameters linked to MWWE appear to drive changes to benthic macroinvertebrate communities more notably than other potential urban drivers within The City, and metrics related to specific types of taxa present in samples (and their relative abundances) were far better at identifying the presence of MWWE than more general metrics, such as richness or diversity, that did not capture community turnover.

The research also demonstrated the importance of the application of standardized artificial substrates (rock baskets), *in-situ* cotton-strip decomposition bioassays, and nutrient diffusing substrates as an integrated monitoring tool box to further tease apart and assess complex nutrient-ESOC related MWWE exposure effects on the structure and function of the basal aquatic food web. These studies are instrumental in informing future MWWE and stormwater monitoring program designs and choice of biological/ecological indicators to assess aquatic ecosystem health.

A second major objective was the identification of areas of potential concern in the Bow River Watershed within The City. While the studies confirmed the contribution of wastewater effluents to changes in water quality, periphyton, benthic invertebrate, and fish responses, the influence of stormwaters on responses is an understudied area. We did confirm the potential for stormwater exposure to be contributing to responses in the river and the utility of the downstream site (Highwood confluence) for assessing the cumulative effects of upstream contributions to the river.

Wild and caged fish studies in the river evaluated both wastewater exposure and stormwater exposure, and confirmed the ability of both to impact small-bodied fish responses. With the exception of a storm event in Nose Creek driving down dissolved oxygen levels, none of the other exposures suggested lethal consequences over 28 d of exposure. But fish clearly showed whole organism and physiological responses. Caged fish responses over a 28 d exposure period showed changes that were usually consistent with wild fish, although they were muted and not all always statistically significant.

A key objective was to evaluate the potential of the ACWA facility to support studies reflecting prioritization and pilot scale testing for wastewater effluents. The benthic macroinvertebrate communities in the ACWA streams were poor representations of the Bow River in both control and treatment streams. The overall communities were largely made up of pollution tolerant organisms and lacked most representatives of EPT organisms that were more present in the adjacent mainstem Bow River. In spite of the differences, the ability to control exposures and manipulate exposures is a key characteristic reflecting the capability of the ACWA streams to play a key role in future evaluations of technologies for wastewater treatment, and to better understand the potential impacts of climate change. This information can help guide The City in setting future investment priorities to assist them in optimizing existing infrastructure performance.

One challenge of the ACWA streams is that the Bow River contributes flow to the headpond as a source of dilution water to the streams; the Bow River at that point has already received the effluents of upstream Bonnybrook and Fish Creek wastewater effluents, as well as significant stormwater inputs. More realistic reflection of Bow River results would require a pre-treatment of the dilution water to remove the influence of upstream sources. The ability to source additional effluent sources from upstream in the Pine Creek treatment line would expand the relevance of results at ACWA to a broader audience.

Two types of emerging tools were evaluated for their ability to play a role in monitoring MWWE. Gut microbiomes of larval and adult insects invertebrates are impacted by wastewater discharges to the Bow River and confirms that baseline microbiome and stable isotope data of effluent-exposed insects can be used to assess effluent exposure in a monitoring framework.

Four sets of caging experiments in the ACWA streams, and exposure studies in the Bow River and Nose Creek evaluated transcriptomic indicators. Results are still being finalized, but the studies demonstrated that the techniques can identify gene markers that could be utilized more frequently, potentially reducing costs for future stormwater monitoring efforts. Analyses of the data are ongoing to determine whether there are specific markers or patterns that can be used to identify and separate MWWE and stormwater stressors.

The final objective was related to engagement with multiple departments within The City, stakeholders and local Indigenous communities to develop communication and knowledge translation tools specific to evaluating effects of municipal wastewater effluent. This work is ongoing, and future work with Indigenous communities will focus on developing the capacity and continued development of partnerships with local and regional Indigenous communities will play a significant role in future phases of this project.

12 References

Arlos, M.J., V. Arnold, J.S. Bumagat, J. Zhou, K. Cereno, A. Deas, K. Dai, N.J. Ruecker, and K.R. Munkittrick. 2023. Combining chemical, bioanalytical and predictive tools to assess persistence, seasonality, and sporadic releases of organic micropollutants within the urban water cycle. Water Research 244 Article 120454

Marjan, P., C.J. Martyniuk, M.J. Arlos, M.R. Servos, N.J. Ruecker, and K.R. Munkittrick. 2024. Identifying transcriptomic indicators of tertiary treated municipal effluent in longnose dace (*Rhinichthys cataractae*) caged under semi-controlled conditions in experimental raceways. Sci. Tot. Env. 923 (2024) 171257. doi.org/10.1016/j.scitotenv.2024.171257

Diesbourg, Emilie. 2024. Microbiomes of Freshwater Insects and Riparian Spiders Downstream of Municipal Wastewater Discharges in the Bow River, AB. MSc Thesis. Biology. McMaster University. 103 p.

Pulgarin-Zapata D, Bragg L, Cardenas Soraca D, Marjan P, Arnold V., Munkittrick K, Servos M, and Arlos M. 2024. A multi-compartment examination of micropollutant partitioning in replicate artificial streams highlights the limitations of assessing water matrices alone. ES&T – Water, 4 (9) 4165-4174.

Pulgarin Zapata, Daniela. 2023. Investigating micropollutant partitioning in five environmental and biological matrices collected in replicate artificial streams. MSc Thesis. Civil and Environmental Engineering. University of Alberta

Sayles, Breanna. 2024. Basal autotrophic and heterotrophic foodweb responses to municipal wastewater effluent exposure in the Bow River, Alberta. M.Sc. University of Calgary

Sutherland, Aphra. 2024. Evaluating the Potential Impacts of Municipal Wastewater Effluent on Benthic Macroinvertebrates in the Upper Bow River. M.Sc. Thesis, Biological Sciences, University of Calgary. 194 p.

13 Appendices

13.1 Appendix I. Scientific Publications

13.1.1 Journal Articles

Marjan, P., C. Cunada, A. Mahaffey, S. Marshall, L. Snook, F. Taridashti, K. Munkittrick. A global review to examine study design considerations for detecting impacts of municipal effluents on fish reproduction. Submitted Sci Tot Env (May 2024)

Pulgarin-Zapata D, Bragg L, Cardenas Soraca D, Marjan P, Arnold V., Munkittrick K, Servos M, and Arlos M. A multi-compartment examination of micropollutant partitioning in replicate artificial streams highlights the limitations of assessing water matrices alone. ES&T - Water *In Press.*

Marjan, P., C.J. Martyniuk, M.J. Arlos, M.R. Servos, N.J. Ruecker, and K.R. Munkittrick. 2024. Identifying transcriptomic indicators of tertiary treated municipal effluent in longnose dace (*Rhinichthys cataractae*) caged under semi-controlled conditions in experimental raceways. Sci. Tot. Env. 923 (2024) 171257. doi.org/10.1016/j.scitotenv.2024.171257

Arlos, M.J., V. Arnold, J.S. Bumagat, J. Zhou, K. Cereno, A. Deas, K. Dai, N.J. Ruecker, and K.R. Munkittrick. 2023. Combining chemical, bioanalytical and predictive tools to assess persistence, seasonality, and sporadic releases of organic micropollutants within the urban water cycle. Water Research 244 Article 120454

13.2 Theses

13.2.1 Completed Student Theses

<u>Master's Thesis</u>

Breanna Sayles. 2024. Basal autotrophic and heterotrophic foodweb responses to municipal wastewater effluent exposure in the Bow River, Alberta. M.Sc. University of Calgary

Aphra Sutherland. 2024. Evaluating the Potential Impacts of Municipal Wastewater Effluent on Benthic Macroinvertebrates in the Upper Bow River. M.Sc. Thesis, Biological Sciences, University of Calgary. 194 p.

Emilie Diesbourg. 2024. Microbiomes of Freshwater Insects and Riparian Spiders Downstream of Municipal Wastewater Discharges in the Bow River, AB. MSc Thesis. Biology. McMaster University. 103 p.

Daniela Pulgarin Zapata. 2023. Investigating micropollutant partitioning in five environmental and biological matrices collected in replicate artificial streams. MSc Thesis. Civil and Environmental Engineering. University of Alberta

Stephanie Marshall. 2023. The Influence of Temporal and Spatial Variability in Trout-perch (Percopsis omiscomaycus) Whole Organism Characteristics on Monitoring Strategies. M.Sc. Thesis, Biological Sciences, University of Calgary.107 p.

<u>M.Eng. Capstone Project</u>

Alison Deas. 2022. Comparison of conventional water quality indicators with spatiotemporal trends of emerging substances of concern in the Bow River basin. M.Eng. Civil and Environmental Engineering. University of Alberta

Undergraduate Theses

Zach Fernandes. 2023. Reproduction, growth, and survival of *Hyalella azteca* exposed to municipal wastewater effluent. Undergraduate Zoology Thesis, Biological Sciences, University of Calgary. 34 p.

Kyle M. Paradis, 2022. Fish and macroinvertebrate community structure in reconstructed fish compensation habitat located in an urban setting of the Bow River, Alberta. Undergraduate Ecology Thesis, Biological Sciences, University of Calgary. 60 p.

Angela Burk. 2022. Assessment of hydrologic and geomorphic features of fish habitat suitability in a Bow River compensation channel: A preliminary study. Undergraduate Ecology Thesis, Biological Sciences, University of Calgary.

13.3 Media Presentations

Munkittrick, K and F. Wrona. 2022. The UCalgary Bow River Ecosystem Health Assessment Project: understanding the impacts of municipal wastewater and stormwater. Preserving Our Lifeline (Bow River Basin Council newsletter, March 2022) p 4-5. (available: https://www.brbc.ab.ca/our-activities/newsletters).

Barrett, D and Black, K. 2024. Water woes in southern Alberta could spell disaster for aquatic ecosystems, and the people who rely on them. The Conversation. March 2024. (available: https://theconversation.com/water-woes-in-southern-alberta-could-spell-disaster-for-aquatic-ecosystems-and-the-people-who-rely-on-them-222941).

13.4 Presentations and Workshops

13.4.1 Conference Presentations

1) Diesbourg, E., Perrotta, B.G., Kidd, K.A. 2024. Microbiomes of freshwater insects and riparian spiders downstream of wastewater effluents. June 2024. Laurentian SETAC 28th annual general meeting. Hamilton, ON.
2) Kowalczyk, S.J.*, D.M. Cárdenas-Soracá*, L.M. Bragg*, S. Jamal*, M.R. Servos. Development of the optimal extraction method of chiral pharmaceuticals from rainbow darter. World Water Day 2024. March 22, 2024. Presentation Award People's Choice Award
3) Kowalczyk, S.J.*, D.M. Cárdenas-Soracá*, L.M. Bragg*, S. Jamal*, M.R. Servos. Development of the optimal extraction method of chiral pharmaceuticals from rainbow darter. World Water Day 2024. March 22, 2024. Presentation Award People's Choice Award
4) Sayles, B., Sutherland, A., Pietsch, W., Black, K., Barrett, D. 2024. City of Calgary Open house – water concerns mapping exercise. March 7, 2024. Calgary, AB.
5) Brown, C., T. Arciszewski, A. Curry, S. Smith and K. Munkittrick. 2024. Developing Fish Population Forecast Triggers For Adaptive Monitoring Programs. Society of Canadian Aquatic Science, February 21-24, 2024, Fredericton, NB
6) Taridashti, F., K. Munkittrick and M. Hecker. 2024. Transcriptomic profiles of fathead minnow (*Pimephales promelas*) from a stormwater-dominated tributary of the Bow River. Society of Canadian Aquatic Science, February 21-24, 2024, Fredericton, NB
7) Pulgarin-Zapata D*, Bumagat J, Bragg L, Marjan P, Munkittrick K, Servos M, Arnold V, Ruecker N, Arlos M. Assessing the bioaccumulative potential of micropollutants in replicate artificial streams facility. 44th SETAC North America Annual Meeting. Louisville, Kentucky. November 12-16, 2023.

8) Arlos M (2023) "H2 Uh-Oh: Emerging Water Contaminants and Our Urban Environment". Edmonton Public Library Public Lecture Series. Oct 18, 2023.
9) Diesbourg, E., Perrotta, B.G., Kidd, K.A. 2023. Effects of wastewater effluents on exposed freshwater insects and nearshore spiders. 49th Canadian Ecotoxicity Workshop, Oct 2-5, 2023, Ottawa, ON.
10) Taridashti, F., P. Marjan, C. Chan and K. Munkittrick. 2023. Responses of Longnose Dace and Fathead Minnow to Stormwater Exposure in the Nose Creek Watershed in Calgary, AB. 49th Canadian Ecotoxicity Workshop, Oct 2-5, 2023, Ottawa, ON
11) Mahaffey, A., M. Fischer, B. Hunter, B. Sayles, P. Siwik and K. Munkittrick. 2023. Workshop on best practices for recognizing and including multiple ways of knowing in State of Environment reporting. 49th Canadian Ecotoxicity Workshop, Oct 2-5, 2023, Ottawa, ON
12) Kowalczyk, S.J.*, D.M. Cárdenas-Soracá*, L.M. Bragg*, S. Jamal*, M.R. Servos. Development of the optimal extraction method of chiral pharmaceuticals from rainbow darter (*Etheostoma caeruleum*). Canadian Ecotoxicity Workshop, Ottawa, Ontario, Oct 2-5, 2023.
13) Marjan, P., C.J. Martyniuk, M.J. Arlos, M.R. Servos, N.J. Ruecker, M. Hecker, and K.R. Munkittrick. 2023. How does increased percentage of tertiary treated municipal effluent affect hepatic transcriptome in longnose dace (*Rhinichthys cataractae*) caged under semi-controlled conditions in artificial raceways. 49th Canadian Ecotoxicity Workshop, Oct 2-5, 2023, Ottawa, ON
14) Kowalczyk, S.J.*, D.M. Cárdenas-Soracá*, L.M. Bragg*, S. Jamal*, M.R. Servos. Development of the optimal extraction method of chiral pharmaceuticals from rainbow darter (*Etheostoma caeruleum).* Canadian Ecotoxicity Workshop, Ottawa, Ontario, Oct 2-5, 2023.
15) Pulgarin-Zapata D*, Bumagat J, Bragg L, Marjan P, Munkittrick K, Servos M, Arnold V, Ruecker N, Arlos M. Investigating micropollutant partitioning in five environmental and biological matrices collected in replicate artificial streams. 49th Canadian Ecotoxicity Workshop. Ottawa, Canada. October 2-6, 2023.
16) Pulgarin-Zapata D*, Bumagat J, Bragg L, Marjan P, Munkittrick K, Servos M, Arnold V, Ruecker N, Arlos M. Assessing the bioaccumulative potential of micropollutants in replicate artificial streams facility. 75th Western Canada Water Conference. Saskatoon, Canada. September 25-28, 2023.
17) Pulgarin-Zapata D*, Bragg L, Marjan P, Munkittrick K, Servos M, Arnold V, Arlos M. A multi-compartment examination of PPCP partitioning in replicate artificial streams highlights the limitation of assessing water matrices alone. Poster. SETAC PNC Annual Meeting. June 16-18, 2023. Edmonton, Alberta
18) Hicks, J*, Arlos M, Camm E, Ruecker N. The potential for micropollutants as indicators of domestic sewage contamination. Poster. SETAC PNC Annual Meeting. June 16-18, 2023. Edmonton, Alberta
19) Fernandes, Z., A. Bartlett and K. Munkittrick. 2023. Survival, growth, and reproduction of *Hyalella* exposed to tertiary-treated municipal wastewater. SETAC Prairie Northern Chapter Annual General Meeting, June 18-20, 2023. Saskatoon, SK
20) Taridashti, F., R.N. Tanna, P. Marjan, C. Chan, S. Marshall and K.R. Munkittrick. 2023. Does the stormwater contaminant load in Nose Creek play a role in fish responses in the Bow River in Calgary, AB. SETAC Prairie Northern Chapter Annual General Meeting, June 18-20, 2023. Saskatoon, SK
21) Mahaffey, A. and K. Munkittrick. State of Environment Workshop Overview. Mackenzie River Basin Board Meeting (virtual), June 7 2023

22) Munkittrick, K. and F. Wrona. 2023. Developing a framework for monitoring the health of the Bow River: Bow River Ecosystem Health Assessment (BREHA). World Water Day, University of Calgary, March 21, 2023
23) Brown, C., R.A. Curry, G. Yamazaki and K. Munkittrick. 2023. Consider What Information You Need to Effectively Monitor Post-Development Before Collecting Baseline Data for Your Environmental Impact Assessments. Can. Soc. Aquatic Sciences, Feb 23-25, 2023. Montreal PQ.
24) Munkittrick, K. 2023. The history of the environmental monitoring of pulp mill effluents in Canada. Invited Presentation to Centro EULA, Universidad de Concepcion, Chile, January 24, 2023
25) Wrona, F.J., K. Black, T. Stadnyk, A. Pietroniro, and K. Munkittrick. 2023. The CWA: A "One-Water" approach to research, development & knowledge mobilization. CWRA National Mid-Term Workshop: Canada Water Agency – Facilitating Collaboration on Freshwater Coordination and Stewardship, January 27 2023, Ottawa, ON
26) Sayles, B., A. Sutherland, D. C. Barrett, and F. J. Wrona. 2022. Quantifying impacts of point source municipal wastewater effluent on benthic macroinvertebrate community composition in the Bow River near Calgary, AB, Alberta Society of Professional Biologists Annual Conference, December 1-2 2022, Lake Louise, AB.
27) Munkittrick, K., R. Tanna, S. Marshall, and P. Marjan. 2022. Bow River Ecosystem Health Assessment (BREHA): Fishes of Jumpingpound Creek. Jumpingpound Creek Watershed Partnership Annual Fall Gathering, Nov. 22, 2022
28) Marjan, P., C.J. Martyniuk, M.J. Arlos, M.R. Servos, N.J. Ruecker and K.R. Munkittrick. 2022. Development of molecular indicators for municipal effluent exposure responses in longnose dace (*Rhinichthys cataractae*) caged in artificial streams. Poster at North Amer. Soc. Environ. Toxicol. Chem., Nov 13-17, 2022, Pittsburgh, PA
29) Pulgarin-Zapata, D., J.S. Bumagat, L.M. Bragg, P. Marjan, K. R. Munkittrick, M.R. Servos, V. Arnold, N. Ruecker, and M. J. Arlos. 2022. Assessing the bioaccumulative potential of micropollutants in replicate artificial streams system. Poster at North Amer. Soc. Environ. Toxicol. Chem., Nov 13-17, 2022, Pittsburgh, PA
30) Marshall, S., T. Clark, P. Marjan, M. McMaster and K. Munkittrick. 2022. The influence of annual variability on the interpretation of whole organism characteristics in Troutperch (Percopsis omiscomaycus) to inform design of long term monitoring programs. Platform and poster at North Amer. Soc. Environ. Toxicol. Chem., Nov 13-17, 2022, Pittsburgh, PA
31) Patricija Marjan and Cristine O'Grady. 2022. Bow River ecosystem health assessment: an urban alliance collaboration. Presentation to the National Water and Wastewater Conference; November 6-9, 2022, Halifax, Nova Scotia, Canada
32) Munkittrick, K.R. 2022. Developing linked hydrological and ecological prediction systems to drive cumulative effects management. Canadian Water Resources Association Annual Conference, June 5-8, 2022, Canmore, AB (also Session Chair and Member of Program Committee)
33) Sutherland, A., D. Barrett, K. Munkittrick and F. Wrona. 2022. Quantifying impacts of point source municipal wastewater effluent on benthic macroinvertebrate community composition in the Bow River near Calgary, AB. Poster and platform at CWRA National Conference, June 5-8, 2022, Canmore, AB.
34) Bumagat, J.S. and D. Pulgarin. 2022. Protecting our water: investigating the spatio-temporal trends and bioaccumulative potential of emerging substances of concern in the bow river in

Calgary. Canadian Water Resources Association SYP Webinar, November 24, 2022. (Virtual)

35) Sutherland, A., D. Barrett, and F. Wrona. 2021. Aquatic Invertebrate Field Sampling Comparison: Developing Adaptive Methods. Entomological Society of Alberta Virtual Conference October 15-16, 2021 (Virtual)

13.4.2 BREHA-related Workshops

1) Barrett, D and Sutherland, A. 2024. Water quality and CABIN monitoring. Bow River Basin Council Biomonitoring Workshop. March 25, 2024, Calgary, AB.
2) Munkittrick et al. 2024. City of Calgary staff open house – broad engagement. March 7, 2024. Calgary, AB.
3) Arlos M (2023), Arnold V, and Ruecker M. "The City of Calgary's Emerging Substances of Concern (ESOCs) Strategy". Committee on Water Quality Meeting. February 6, 2024.
4) Munkittrick, K.R. 2023. Feedback on approach to SOE reporting for water quality. Bow River Basin Council, State of the Watershed Water Quality Technical Committee (Virtual) December 6, 2023.
5) Marjan, P., R. Tanna, S. Marshall, F. Taridashti, K. Munkittrick and A. Mahaffey. 2023. An update on the Bow River Ecosystem Health Assessment (BREHA) project: Jumping Pound Creek, Jumpingpound Creek Watershed Partnership Annual Fall Gathering November 28, 2023
6) Blackfoot Crossing Historical Park Staff. 2023. Reconciliation in research day. November 15. Siksika Nation, AB.
7) Crow Chief, D. et al. 2023. Formation and ongoing support of the Blackfoot Youth Water Council. Ongoing 2023-2024. Siksika Nation, AB.
8) Taridashti, F. and K. Munkittrick. 2023. Using fish as biological indicator to monitor areas with concerns of multiple stressors: measuring fish responses of Nose Creek Watershed. 25th Anniversary of Nose Creek Watershed Partnership, Oct 12, 2023, Airdrie, AB
9) Munkittrick, K. 2023. The role of monitoring in SOE reporting, and approaches for incorporating monitoring data. Bow River Basin Council Science Committee, May 18, 2023, Calgary, AB
10) Wrona, F., A. Pietroniro, K. Black, T. Stadnyk, K. Munkittrick, C. Ryan, J. He, and Andre Buret. 2023. A "One-Water" approach to advancing knowledge mobilization through improving linkages between societal needs, research, and policy relevant decision-making. Canadian Water Resources Assoc., Alberta Branch Conference, April 26, 2023, Calgary, AB
11) Marjan, P., K. Munkittrick, F. Wrona et al. 2023. Bow River Ecosystem Health Assessment (BREHA) Bow River Basin Council Science Forum, Wednesday, April 26, 2023, Calgary, AB
12) Sayles, B., A. Sutherland, D. Barrett, and Frederick Wrona. 2023. Assessing Methods for Quantifying Potential Impacts of Municipal Wastewater Effluent on the Basal Aquatic Food Web in the Bow River, AB Bow River Basin Council Science Forum, Wednesday, April 26, 2023, Calgary, AB
13) Tanna, R.N., F. Taridashti, P., Marjan, C. Chan, S. Marshall, and K.R. Munkittrick. 2023. Using fish to identify areas of concern in the Bow River system. Bow River Basin Council Science Forum, Wednesday, April 26, 2023, Calgary, AB

14) Munkittrick, K. and F. Wrona. 2023. Research towards a tiered and adaptive regional monitoring planning framework. City of Calgary Water Centre, February 21, 2023, Calgary, AB
15) Munkittrick, K. and C. Davidson. Adaptive Monitoring. Lakes Technical Advisory Committee, Oil Sands Monitoring, March 17, 2023, Calgary, AB
16) Taridashti, F., K. Munkittrick and P. Marjan. 2023. Bow River Ecosystem Health Assessment (BREHA): Fishes of Nose Creek. Nose Creek Community Partnership Meeting, January 19, 2023, Airdrie, AB
17) Munkittrick, K.R. and F.J. Wrona. 2022. Bow River Ecosystem Health Assessment (BREHA). Water Innovation Webinar, Nov 8, 2022, Alberta Innovates
18) Marjan, P., Rajiv Tanna, Stephanie Marshall, Cherrie Chan, Fateme Taridashti, Kyle Paradis, Maricor Arlos, Fred Wrona, Chris Martyniuk, Norma Ruecker, Kelly Munkittrick. 2022. Bow River Ecosystem Health Assessment Project. ACWA Public Meeting, September 28, 2022, Calgary, AB

13.5 Appendix I HQP Training

Table 1. HQP training

Level			Status	Previous	Current	Sector
PhD Students	Rajiv Tanna	Biological Sciences, U Calgary	Current	AB	AB	
	Fateme Taridashti	Biological Sciences, U Calgary	Current	Iran	AB	
	Sondus Jamal	Civil and Environmental Engineering, UWaterloo	Current	ON	ON	
MSc Students	Aphra Sutherland	Biological Sciences, U Calgary	Completed, Winter 2024	AB	AB	Academic
	Breanna Sayles	Biological Sciences, U Calgary	Scheduled Summer 2024	AB	AB	Academic
	Stephanie Marshall	Biological Sciences, U Calgary	Completed, Fall 2023	ON	AB	Consulting
	Emilie Diesbourg	Biology, McMaster	Completed, Winter 2024	NB	ON	Government
	Sarah Kowalczyk	Biology, UWaterloo	Current			
MSc Env Eng Students	Daniela Pulgarin Zapata	Environmental Engineering, UAlberta	Completed, Fall 2023	Colombia	AB	Academic
	Jaime Hicks	Environmental Engineering, UAlberta	Scheduled Fall 2024	AB	AB	
MEng Students	Alison Deas	Environmental Engineering, UAlberta	Completed, Spring/Summer 2022	AB	AB	Industry
Undergraduate Honours theses	Kyle Paradis	Biological Sciences, U Calgary	Completed August 2022	AB	AB	Consulting
	Angela Burk	Biological Sciences, U Calgary	Completed, August 2022	AB	AB	Academic
	Zach Fernandes	Biological Sciences, U Calgary	Completed May 2023	AB	AB	Academic
Undergraduate students	Cherrie Chan	Biological Sciences, U Calgary	Completed May 2022	AB	ON	Academic

	Seth Bumagat	Chemical Eng, UAlberta	Completed May 2024	AB	AB	Academic
	Alex Zhou	Environmental Eng, UAlberta	Completed May 2023	AB	AB	Government
	Katrina Cereno	Civil Eng, UAlberta	Current	AB	AB	
	Demi Meier	(Animal Health), UAlberta	Completed May 2022	AB	AB	Academic
	Sarah Kowalczyk	Biology, UWaterloo	Current	ON	ON	
Research Associates and Postdocs	David Barrett	Biological Sciences, U Calgary	Current	BC	AB	Academic
	Patricija Marjan	Biological Sciences, U Calgary	Current	ON	AB	Academic
	Brittany Perrotta	Biology, McMaster	Completed July 2023	USA	USA	Government
	Diana Cardenas	Biology, U Waterloo	Current			
Technicians/ Biologists	Cherrie Chan	Biological Sciences, U Calgary	August 2023	AB	ON	Academic
	Shemar Williams	Biological Sciences, U Calgary	December 2023	ON	AB	Consulting
	Leslie Bragg	UWaterloo	Current			
	Wylie Pietsch		Current	AB	AB	Community
	Disa Crow Chief		Current	AB	AB	Community