Integrated Ecological Research

Pre- restoration biomonitoring of Six Mile Slough

Prepared for
BC Forest, Lands and Natural Resource Operations and Rural Development
and Creston Valley Wildlife Management Area

Prepared by:

Darcie Quamme, MSc., R.P.Bio., Integrated Ecological Research
Rhia MacKenzie, MSc. Candidate, BArch., BIT
And Ryan Durand, MSc., R.P.Bio., EcoLogic

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SUMMARY

The goal of the current project was to establish baseline conditions and a monitoring design (pre and post restoration) by which to evaluate large scale restoration and potential reestablishment of hydrological connectivity of Six Mile Slough located within the Creston Valley Wildlife Management Area (CVWMA), Creston, BC with the Kootenay River. The Six Mile Slough Wetland Restoration Project proposes to restore up to 1260 hectares of wetlands for Northern Leopard frog, *Lithobates pipiens*, White sturgeon, *Acipenser transmontanus*, burbot, *Lota lota*, other native fish species and migratory birds (Biebighauser and Annschild 2016).

We plan to track the effects wetland restoration in Six Mile Slough potentially affected by varying levels of hydrological change that may result from floodplain reconnection in pre- and post- restoration scenarios. In the present 2019 study, we used quantitative measures such as biogeochemical parameters, nutrient levels, and monitoring using methods embedded in the Canadian Aquatic Biomonitoring Network (CABIN) for wetland protocols to examine the pre-restoration baseline. These indicators were also used to characterize Northern Leopard Frog habitat near breeding locations within Six Mile Slough, a species of highest priority and conservation concern in the Columbia Basin.

Floodplain reconnection could also re-establish off-channel rearing habitat for juvenile white sturgeon, and burbot, British Columbia Ministry of Environment Red Listed species (MOE 2020, Biebighauser and Annschild 2016) and provide improvements to ecosystem health of Kootenay River through seasonal inundation and flooding (Statistical Consulting Services 2017). Thus, the mechanisms and the links between wetland inundation and downstream transport of nutrients, carbon, and other physiochemical parameters with associated implications to higher trophic levels are crucial to assess and understand. The current monitoring complements pre-restoration fisheries assessments carried out of Six Mile Slough in 2020.

In 2019, our first year of data collection, we identified reference sites that can be used to compare to trends following changes due to restoration activities in Six Mile Slough over time. Outputs from the project include evaluations of water and sediment quality, nutrient status, habitat parameters, macroinvertebrate enumeration, macroinvertebrate richness calculations, and wetland mapping and classification.

The primary use of data collected in 2019 was to aid in the decision to move forward with reconnection of Six Mile Slough to the Kootenay River. To this end, we focussed on evaluating the water and sediment physiochemistry within Six Mile Slough to evaluate the nutrient status of the wetland but also to quantify the production and biodiversity of macroinvertebrates within the wetland. Macroinvertebrates were used to provide inference to wildlife populations and habitat which may be difficult to assess directly because of appropriate scale and population movements. In addition, we identified potential projected changes in ecological productivity, and nutrient/organic matter export. Recommendations from this

project included actions that encourage the development of a diverse macroinvertebrate community providing a base for higher trophic levels in wetland ecosystems.

1 Introduction

This project used the Environment Canada's Canadian Aquatic Monitoring Protocols (CABIN) for wetlands (Env. Canada 2018) to carry out pre-restoration monitoring of Six Mile Slough in 2019. We collected samples from six locations within the wetland for analyses of both traditional macroinvertebrate taxonomic methods and DNA collection for metabarcoding to assess baseline conditions of the Six Mile Slough. We plan to use quantitative indicators summarized from the data outputs to evaluate baseline conditions prior to planned restoration of the wetland. Abiotic and biotic indicators developed from the monitoring will serve as benchmarks and aid management decisions for the restoration work. These indicators can then be used to assess trends at wetland sites over time following restoration and providing a baseline for future comparisons.

The goals of the project are to (1) establish baseline conditions at Six Mile Slough to aid with decision making around wetland restoration, (2) track wetland restoration over time using quantitative measures of wetland stress and biological health (3) compare sites at Six Mile Slough to previously established sites in the West Kootenays (n=58 samples), (4) evaluate the nutrient status of Six Mile Slough through water quality monitoring, (5) and help to characterize Northern Leopard Frog habitat near breeding locations, a species of highest priority and conservation concern in the Columbia Basin.

This project aligns with larger plans in the Columbia Region including the Columbia Basin Trust's Environmental Program including goals such as: (1) enhancing or conserving ecosystems and/or species of conservation concern, (2) supporting the protection, enhancement or restoration of water resources that are important for species and/or ecosystems of conservation concern and (3) carrying out water quality and/or quantity research that is scientifically sound and will contribute knowledge to the management of water resources for the benefit of ecosystems, communities and watershed stakeholders.

The project also addresses the FWCP Wetlands and Riparian Actions (FWCP 2019) including monitoring and evaluation, species-based and habitat-based actions priorities in the Creston Valley described here.

- **Monitoring and Evaluation:** (Action 21, Priority 1) Monitor and evaluate the effectiveness of previous FWCP wetland and riparian restoration. Include an approach for adaptive management, documenting and assessing ecological conditions and parameters (pre- and post-restoration), information sharing and collaboration among agencies and the public stakeholders to increase the efficacy of conservation action.
- **Species-based Actions:** (Action 23, Priority 1.) Support strategies and initiatives outlined in the BC Recovery Plan for Northern Leopard Frog that relate to compensation for dam impacts. Where possible, link project work to the connectivity of this species across ecosystems and collaborate with recovery team specialists.

- **Habitat-based Actions:** (Action 16, Priority 2.) Implement habitat-based actions to conserve/restore/enhance water levels and water quality in wetland habitats. Ensure alignment with relevant actions in Rivers and Riparian Areas and Reservoirs and Large Lakes ecosystem plans.

In addition, this work supports increased knowledge of the ecology of wetlands in the Columbia Basin with important management outcomes for the community, funders, and supporters.

In 2019 this project was funded by the Creston Valley Wildlife Management Area, BC Ministry of Forests, Lands, Natural Resource Operations and Rural Development. This work builds on past funding streams from 2014 to 2020. Support for this work has been obtained from the Environment Canada's National Wetland Conservation Program (NWCF), the Columbia Basin Trust (CBT), the Columbia Basin Watershed Network, BC Ministry of Forest Lands and Natural Resource Operations and Rural Development, Fish and Wildlife Compensation Program (FWCP). The evaluation of Six Mile Slough is a collaborative project because previously monitored sites from multiple funding sources will serve as reference sites for comparison to Six Mile Slough and provide inference to current work.

Current Columbia Basin Trust funds are matched with a major in-kind contribution and/or support from the Royal BC Museum (RBCM), as well as groups such as Slocan Solutions, Slocan River Streamkeepers Society (SRSS), BC Wildlife Federation, and Integrated Ecological Research. Forty-two percent of matching funds from 2014-2020 have come from provincial or federal agencies outside of the Columbia Basin. In addition, the proposed project also overlaps with previously CBT-funded projects or candidates including: Crooked Horn Farm Restoration, Meadow Creek conservation lands (FLNRO), Bonanza wetland (Valhalla Wilderness Society) and the Goulden-Thurston Property (SRSS).

Adam Martens from Environment Canada has provided guidance with respect to the developing CABIN for wetland protocols. Living Lakes, World Wildlife Fund and the provided in-kind funding for logistics, shipping, and DNA laboratory support through the STREAM program centered in the Hajibabaei Lab at the Center for Genomic Biodiversity at the University of Guelph. Chloe Robinson from the Hajibabaei Lab authored a companion report that summarizes the DNA meta-barcoding work (STREAM 2019). The Royal BC Museum in Victoria continues to provide support on the order of $10,000 per year to voucher and house samples in their entomology and invertebrate collections, in perpetuity.

2 Methods

The primary goal of using CABIN for wetland protocols will be to document changes to physiochemistry, macroinvertebrates and the diversity and complexity of plant species and habitat over time. CABIN methods for wetlands (Environment Canada 2018) is a National Canadian protocol that has been tested in Quebec (Tall et al. 2016 and 2008), the Yukon (Baily and Reynoldson 2009), and prairie provinces including Saskatchewan and Alberta (pers com. Adam Martens 2019). Other similar protocols have used macroinvertebrates to assess wetland health (Uzarski et al. 2017, Kovalenko 2014, Mazzacano 2011, Adama et al. 2013 and Miller and Hawkes 2013, Archer et al. 2010, U.S EPA 2002 and Apfelbeck 2000) in the U.S and Canada. In addition, CABIN for wetlands protocols have been successfully used to track restoration success in the West Kootenays (Quamme et al. 2016, 2018 and 2019).

With respect to macroinvertebrate data, our main objective will be to examine biodiversity and abundance of macroinvertebrates from Six Mile Slough before and after reconnection to the Kootenay River. However, we will also examine these indicators relative to other reference sites previously collected from 2014-2018 in the West Kootenays.

Pre-restoration monitoring will take place in 2019 and 2020 and post restoration monitoring will occur following floodplain reconnection - onward. We will have at least two years of pre-restoration monitoring to compare to post restoration monitoring. We plan to have a balanced design with some of the sites affected by changes in hydrology and other sites serving as reference sites from compartments with stabilized hydrology within the slough.

In 2019, the focus of our fieldwork was to initiate the collection of pre-restoration data so as to review water and sediment chemistry within the wetland and assess the potential for entrainment of nutrients at peak flows with delivery to the Kootenay River. As well, monitoring will help to characterize two possible Northern Leopard frog breeding locations. We also collected macroinvertebrates from twelve quadrats to capture the variance of the abundance/biodiversity of macroinvertebrates within the wetland and assess the range of taxonomic composition of the slough in Compartments 2-5 (Figure 1). We selected sites at six locations with paired samples for DNA and taxonomy at each location for a total of twelve samples. In the current report, we reported the biodiversity and abundance of macroinvertebrates within Six Mile Slough relative to other wetlands in the West Kootenays to provide greater inference to the 2019 monitoring.

Clockwise. (1) Dragonfly on quadrat stake.(2) Burweed. (3) Katherine McGlynn in kayak (4) jars with sample sediment. (4) Darcie Quamme collecting a samlple

Photo 1: Sampling equipment used at Six Mile Slough.

Clockwise. (1) Darcie Quamme with CABIN collection net.(2) Canoeing to site wit Rhia MacKenzie. (3 and 4) Canoe and sampling jars and other equipment.

Photo 2: Sampling equipment used at Six Mile Slough.

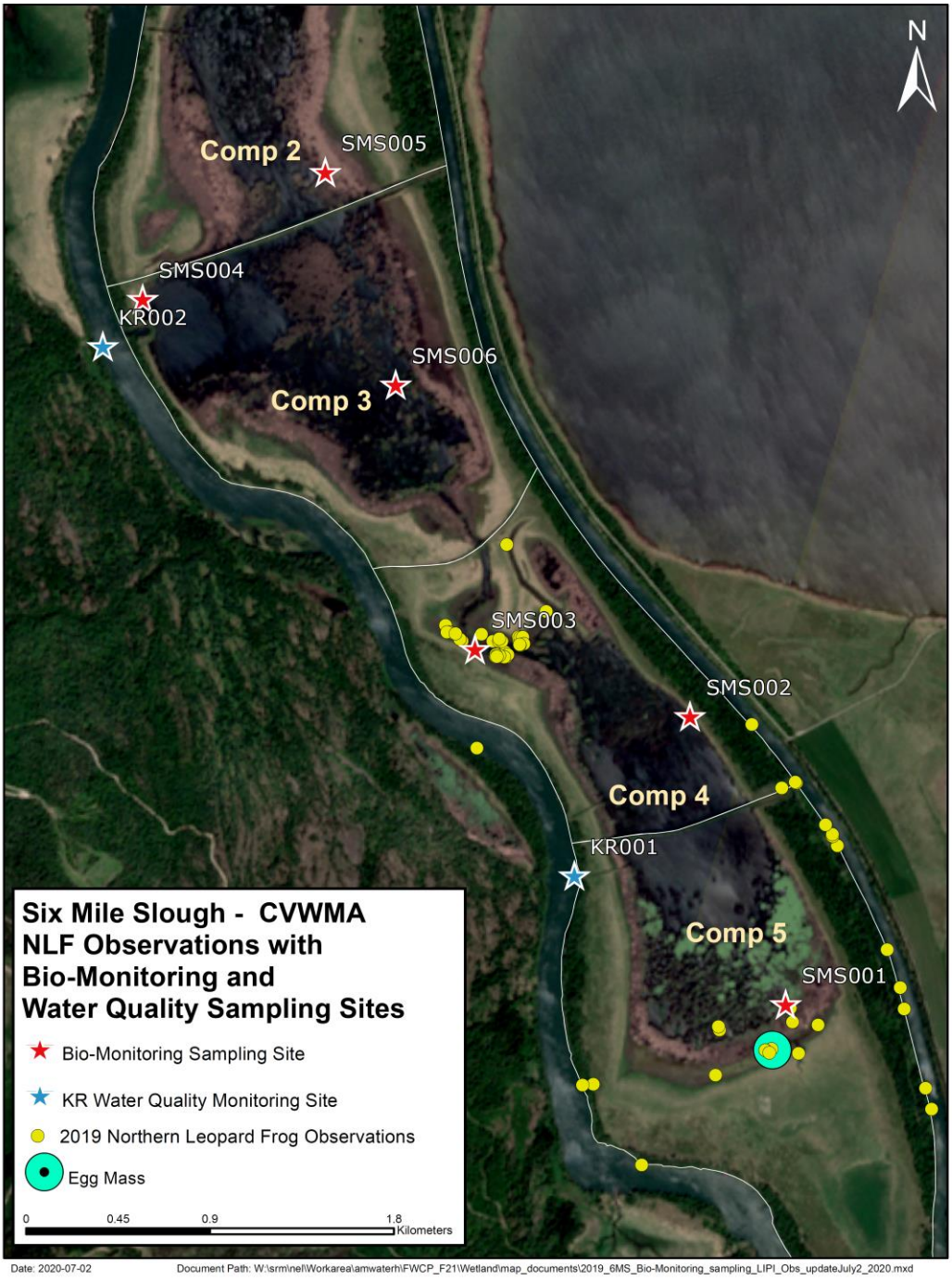


Figure 1: Location of monitoring of monitoring sites at Six Mile Slough. Red stars indicate CABIN for wetland. Blue stars indicate water quality monitoring only. Yellow circles indicate Northern Leopard frog (LIPI) observations and green circles indicate LIPI egg masses found in 2019. Comp = Compartments separated by dikes. Yellow dots indicate observations of Northern Leopard Frogs. Large green dot indicates egg mass. Breeding sites are in Compartments 4 and 5. Two biomonitoring sites were placed near breeding sites.

Clockwise. (1) Northern Leopard frog (LIPI) in sampling net, (2) CABIN sampling near breeding location at SMS003 (3) LIPI near SMS001 and breeding location within Compartment 5, (4) Marc-André Beaucher at NLF breeding location in Compartment 4 adjacent to SMS003.

Photo 3: Sampling of Northern Leopard frog habitat within Six Mile Slough.

2.1.1 Geospatial measures

Base orthophotos were collected from DataBC Imagery Web Map Service (DataBC 2019) with a resolution of one-meter ranging of the Six Mile Slough area. Mapping was completed in ESRI ArcMap 9.3 using heads up delineation adjusted to fit natural features as needed. Mapping procedures followed provincial methods including Ecosystem Classification Methods (Province of BC 2016 and 2010), Terrestrial Ecosystem Mapping (TEM) (RISC 1998), BC Ministry of Environment, Lands and Parks and Ministry of Forests (1998), Standard for Mapping Ecosystems At Risk in British Columbia (RISC 2006), and Mackenzie and Moran (2004). Other sources of information include the BC Vegetation Resources Inventory Mapping, BC Bio geoclimatic Ecosystem Classification, Provincial base layers for lakes, streams, contours, and roads.

2.1.2 Macroinvertebrate collection and processing

CABIN for wetland protocols characterize the macroinvertebrate community that inhabit the emergent and submergent zones of the wetlands where the macroinvertebrate diversity is greatest (De Szalay and Resh 2000). The kick sampling procedure in wetlands involves a gentle disturbance of bottom sediments and three-minute sweeps of the water column in a zig-zag pattern over a 5 m by 5 m quadrat. Thus, macroinvertebrates are collected from the water column, bottom sediments and aquatic plants at each site

Macroinvertebrates were sampled from the near shore of the emergent zone at a depth of approximately 0.5-1 m using a CABIN kick-net of length 45.7 cm, width 25.4 cm, and depth 25.4 cm with a 400 µm mesh

net (Environment Canada 2007, Tall et al 2008). Emergent plants represented at least 50% of the plot area.

The samples were collected from a 25 m^2 area in a timed three-minute sweep sample (Environment Canada 2018). This technique involves a gentle disturbance of bottom sediments and sweep in a zig-zag pattern within the water column quadrat at each site. Sampling was timed for mid-July where possible to coincide with optimal water levels prior to draw-down and the presence of mature macrophytes at temporary, seasonal wetlands and permanent wetlands. Estimates of the relative proportion of vegetation were made within the quadrat within the emergent zone. The 25 m^2 quadrat was marked with cedar stakes following water collection, assessments of percent composition of wetland plants were made prior to macroinvertebrate collection so as not to disturb or damage emergent plants. Quadrats for taxonomy and DNA collection were located side by side at each location.

Field sheets provided by Environment Canada's CABIN program were used as a basis for field measurements (Environment Canada 2018) including:(1) percent disturbance within a 50 m buffer around the site, (2) percent zones of wetland based on a visual estimate, (3) percentage of marginal zone vegetation, 50 m buffer zone around quadrat and (4) percent composition of plant type, periphyton, open water and large woody debris within the 25 m^2 sampling quadrat as well as other estimates.

2.1.2.1 Morphology-based taxonomy

In the case of sampling for macroinvertebrates for identification by taxonomy, the volume of sediment/vegetative matter in each sample was reduced by gently washing the nets in water well away from sampling area or sample can be taken back to the laboratory and further reduced. Material was gently poured through a 400 µm sieve. The sampling net, cup and sieve were carefully check for macroinvertebrates clinging to equipment. Large pieces of plant material were inspected and rinsed and then removed from the net.

Sample material was transferred to one litre wide mouth Nalgene jars with 80% ethanol used as a preservative as recommended by the Royal BC Museum. Sample material comprised no more than 50% of the jar. Ethanol was replaced with fresh 80% ethanol at least once before shipping because water from unsorted organics tends to dilute the preservative over time (Mazzacano 2011, Jepsen et al. 2007). Prior to shipping for taxonomy large pieces of vegetation were inspected, rinse and removed in the laboratory if necessary, to reduce samples. All samples were checked with a hydrometer to verify preservation at 80% ethanol prior to shipping and Rhithron Associates Inc. (taxonomist) reported that the samples were well preserved when they arrived and reassessed with a hydrometer.

For shipping, all wide mouth Nalgene sample jars were sealed with electrical tape and 'Glad Stretch and Seal'. In addition, the samples were placed inside separate zip lock bags to prevent leaks and sample loss in case of breakage.

Samples were shipped in coolers with a Chain-of-Custody form to Rhithron Associates Inc, taxonomists based in Missoula, Montana specializing in identifying wetland invertebrates. Rhithron invertebrate taxonomists collectively hold 34 Level-II certifications from the Society for Freshwater Science.

Samples collected for the CABIN database were sent to a certified taxonomist that follow procedures outlined in Environment Canada (2012) and follow Level 2 Standard Taxonomic Effort (STE) for Pacific Northwest Freshwater Macroinvertebrate Samples (2013) also see Section 5.1.2 for Rhithron's Technical report. STE Level 2 is the lowest practical and cost-effective level of identification and is the target level for harmonizing data sets across the region for comparison. Identifications are typically to genus for the common and diagnostic taxa.

All laboratory techniques and quality control (Section 5.1.2) were carried out according to CABIN methods (Environment Canada 2018 and 2012). Preservative levels within the sample were maintained at the laboratory until sorting and samples were processed within a few months to prevent accidental degradation of the sample.

In addition, voucher specimens were shipped to the Royal BC Museum in 80% ethanol following identification by taxonomist to add to our understanding of wetlands in Interior BC where there are currently knowledge gaps. All project methods met museum specifications for collection, taxonomic identification, and storage of specimens (Environment Canada 2018, 2012 and 2007).

2.1.2.2 DNA meta-barcoding

New this year was sample collection for DNA analysis of macroinvertebrate species. Sample collection for DNA was carried out as recommended by the Hajibabaei Lab, University of Guelph, Center for Genomic Biodiversity STREAM protocols for DNA collection. Gloves were used so as not to contaminate the sample and no attempt to reduce the sample was made to handle the sample as little as possible. Sample was filled to under 50% of the jar to facilitate sample preservation. Thus, a greater number of jars was required to contain the entire sample. No reduction of samples was carried out post-sampling to minimize handling of the sample.

Samples were shipped to the University of Guelph and stored in freezers at -20°C in the lab until they could be processed. Samples consisting of mud, vegetation and invertebrates were coarsely homogenized in a sterile blender and DNA was extracted using a DNeasy® PowerSoil® kit (Qiagen, CA) kit. Extracted DNA was then processed following the standard Hajibabaei Lab protocol for Next-Generation Sequencing (NGS), using Illumina that allows sequencing billions of DNA strands in parallel. Methods and results from DNA metabarcoding are reviewed in detail in the STREAM (2019) companion report from the University of Guelph produced by project manager, Chloe Robinson.

In addition, we requested (1) species-level identifications from the Hajibabaei Lab to provide a deeper understanding of the macroinvertebrate communities in Six Mile Slough and (2) that the raw data be inspected for any trace of invasive invertebrate species.

The raw output from NGS produced invertebrate and vertebrate sequences that were then reduced to sequences that were of high enough quality to match reference sequences (STREAM 2019). Only species taxonomically assigned with high confidence (bootstrap support >= 0.70) were included to indicate species present in Six Mile Slough.

2.1.2.3 Amphibian protocols for safe handling

An inspection for amphibians at each site was made prior to sampling to avoid collection of amphibians. Our protocol calls for quick removal of amphibians from the CABIN net following sampling according to Ministry of Environment (2008) protocol for safe handling of amphibians. However, no amphibians were collected in CABIN nets in 2019 at Six Mile Slough. At the time of sampling most Northern Leopard frogs (LIPI) had transformed and were found in terrestrial areas.

2.1.3 Water and sediment physiochemistry

Prior to sampling for water and sediment quality, all jars were labeled, packed, and transported to sites in a field cooler in Ziplock bags by site. At each site field personnel labeled all sample jars with site code, time, and all other relevant information.

Field measurements of water quality and surface water samples were collected prior to other sampling using methods of Environmental Canada (2018), Duncan and Duncan (2012), Clark (2013) and Cavanagh et al (1997). Metering of water quality included: temperature, pH, conductivity, and dissolved oxygen carried out using field meters.

Surface water and sediment were collected at each site. Samples were taken wearing latex gloves in a non-disturbed area free of large amounts of vegetation prior to completing invertebrate sampling. Surface water samples were collected immediately after field measurements for the following parameters including, low level nutrients (total phosphorus, total Keldhal nitrogen, nitrate, nitrite, and ammonia), alkalinity, major ions (Ca, Mg, Na, K), total suspended solids, sulfate, chlorine, and dissolved organic carbon. A subset of these parameters was monitored in the 2014 pilot study when funding was limited. Grab samples of surface sediment were collected following invertebrate sampling in an undisturbed location using methods described in Environment Canada (2018), Duncan and Duncan (2012), Marvin-DiPasquale (2009), and Clark (2013). Total metals were measured in sediment only in 2014 and in both water and sediment from 2015-2018.

The sample jars were wrapped in bubble wrap and immediately put in a cooler with ice packs and sent to laboratories within 24 hours of collection. CARO Analytical Services was used to analyse water and sediment quality in 2019.

2.1.4 Quality Control

Duplicate sampling was carried out on one of the six biomonitoring sites (SMS005) for water and sediment samples to CARO. All data was screened, and quality control measures were conducted to assess field and laboratory data collection methods according to quality assurance and quality control field sampling protocols in Clark (2013). Duplicate values that were greater than five times the method reporting limit

(MRL) with RPD values of 20-50% (Clark 2013) were inspected and values of greater than 25% were further considered as alerts on possible contamination or lack of representativeness. All internal quality control for laboratory methods and results provided by the labs were reviewed and evaluated. The quality control information on the macroinvertebrate sorting and subsampling is presented in the technical report by Rhithron Associates Inc. (see Section 5.0).

2.2 Results

2.2.1 Geospatial measures

In the present study, the mapping of the entire wetland boundaries of Six Mile Slough and classification was carried out by Ryan Durand (Figure 2). These products are available for other collaborative work including: Northern Leopard Frog assessment, future biomonitoring, fish studies and vegetation evaluations.

In addition, "buffer zones" of 100 m circular radii around biomonitoring site locations were selected as the most relevant size to quantify disturbance affecting macroinvertebrates and physiochemistry at point locations. We selected this scale to minimize the incorporation of areas of the Kootenay River which are presently isolated from Six Mile Slough. Buffer zones of 100 m have also been used to evaluate landcover in previous work by Environment Canada CABIN for wetlands (Tall et al. 2008 and 2016). Recommendations for geospatial measures within the CABIN for wetlands protocol for quantifying landcover are project specific (pers. com. Martens 2020).

We used the disturbance coding within the provincial Terrestrial Ecosystem Monitoring (TEM) protocols and Sensitive Ecosystem Inventory methods (RISC 2006) to quantify landcover classes at a site level within Six Mile Slough similar to Quamme and Durand (2019), Quamme et al. (2019). In previous work (Durand 2013, 2014, Quamme et al. 2018), the disturbance variables or stressors were simply categorized as "non-sensitive" or coded as NS. However, this type of coding does not categorize the type of stressors which has been shown to be more predictive of biotic indices than total disturbance (Rooney et al 2012). In 2019, we reviewed the disturbance codes in the Terrestrial Ecosystem mapping protocol (RISC 1998) in collaboration with Amy Waterhouse and Deb MacKillop of FLNRO and determined that these codes could function to quantify stressors (Quamme et al 2019, Section 5.1).

We used disturbance categories (TEM) to create additional variables to identify reference sites versus test sites and other purposes similar to CABIN for streams protocols (see BCMOE 2012) that can be calculated from Ecosystem Classification and TEM methods (Section 5.1). Additional notes for residential or urban development or other needed codes (X. Miscellaneous) were suggested where required.

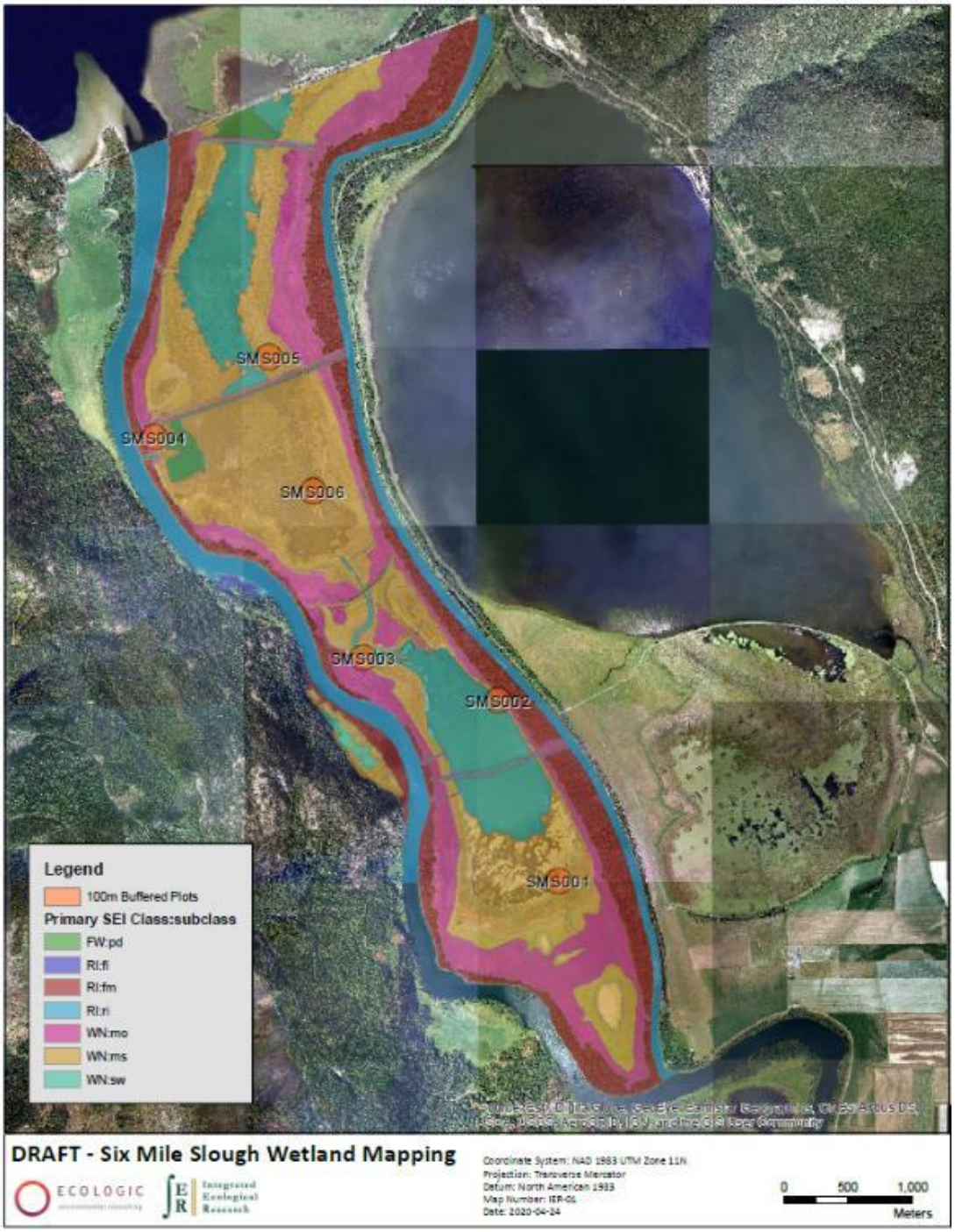


Figure 2. Sensitive Ecosystem Inventory Mapping (SEI) of 500m buffer zones around plot centers of biomonitoring sites in Six Mile Slough. SEI mapping was carried out for all wetland sites. Biomonitoring sites SMS001-SMS006. FW:pd = Open water >2m deep and generally <50 ha, RI:fi=River and creeks including gravel bars, RI:fm =medium bench, flooded wetlands, WN:mo =modified wetlands (Canary Reed grass), WN:ms = graminoid or forb dominated wetlands, WN:sw = shrub dominated wetlands.

Scales of 100 m and 500 m have been shown to be predictive of biotic indices for plants and birds, respectively by Rooney et al. (2012) who assessed scales of 100-3000 m radii. In contrast, the influences of wetland cover and impervious cover on wetland quality and benthic invertebrates may also be important at larger scales of 0.8–1.8 km (Patenaude et al. 2015). In other work, a 50 m buffer around wetland areas was used to evaluate the presence of natural terrestrial vegetation on the perimeter of wetland areas as a protection from external stressors in work on the St Lawrence River by Jean Martin and collaborators with Environment and Climate Change Canada (pers. com Adam Martens 2020).

However, it may be that landscape variables are more predictive at different or varying scales. Herlihy et al. 2019 modelled and tested scales of 200, 500 and 1000 m radii which were correlated in data collected across the United States. They suggested that site level disturbance as well as landscape level disturbance are both important in predicting wetland responses. To incorporate the importance of variables at varying scales, multi-scale habitat models have been developed based on predictive power of each habitat layer. For example, foraging bats were most strongly associated with variables measured at smaller spatial scales of 100-500 m although variables were evaluated up to 6 km and some of these were incorporated into the model to improve performance (Bellamy et al. 2012 and 2013). Perhaps, this could be a future application of a large wetland database such as CABIN.

Table 1. Wetland classification of 100m buffer zones of biomonitoring sites in Six Mile Slough.

Wetland Name	Sensitive Ecosystem Class: Subclass	Description	%	Disturbance Class
SMS001	WN:ow	OW-Open water	4	
	WN:ms	Wm05-Typha	63	
	WN:ms	Wm06-Bulrush	33	
SMS002	RI:fm	Mid-bench Floodplain	19	S.e/W.d= 18.9%
	WN:ow	OW-Open water	9	
	WN:ms	Reed canary grass marsh	22	B.v =22%
	WN:ms	Wm05-Typha	50	
SMS003	WN:ow	OW-Open water	26	S.e/W.d = 24%
	WN:ms	Reed canary grass marsh	24	B.v =24%
	WN:ms	Wm05-Typha	50	
SMS004	RI:fm	Mid-bench Floodplain	9	S.e/W.d = 0.3
	WN:ow	OW-Open water	13	
	WN:ms	Reed canary grass marsh	22	B.v = 22%
	WN:ms	Wm05-Typha	56	
SMS005	WN:ms	Wm05-Typha	95	
	WN:ms	Wm06-Bulrush	5	
SMS006	WN:ow	OW-Open water	50	
	WN:ms	Wm05-Typha	50	

WN:ms = graminoid or forb dominated wetlands, WN:sw = shrub dominated wetlands, WN:ow permanently flooded shallow wetland, RI:fm =medium bench flooded wetlands or dike, Wm05 = Cattail, Wm06 = Bulrush, S.e =Soil disturbance, excavation, W.d = dike, B.v = aggressive vegetation , (Reed canary grass).

2.2.2 Comparisons to previously established reference sites

We used data from previously sampled wetlands (CABIN for wetlands) in the West Kootenays (Slocan Valley and Meadow Creek), monitored (2014-2018, Table 2), in order to compare to Six Mile Slough with the goal of assessing the relative status of the slough to the range of wetlands in this area. This was carried out in the first year of reporting to aid with management decisions. However, pre-restoration monitoring (before restoration controls) as well as the sites not affected or less affected by changes to floodplain restoration and changes in hydrology (post-restoration controls) will serve as reference sites to elucidate the changes of floodplain reconnection and restoration in the future.

Six Mile Slough includes 1,260-hectares of wetlands within the CVWMA located on the floodplain of the Kootenay River as it enters Kootenay Lake. The wetlands have been impacted by agriculture, draining, flood control, channeling, and rail. Other disturbances to the wetland in the past, include historical grazing of the area which was called Lewis Island and later became part of Six Mile Slough. There were as many of 250 cows and calves grazed there, and a house and slaughterhouse owned by Ike Lewis (Biebighowser and Annschild, 2016). In 1974-75 diking was carried out to stabilize water levels for wildlife purposes. Currently, the water control structures are non-functioning.

Twelve samples were collected from Six Mile Slough wetland at n=6 locations, collected from 523-533 m elevation. We found that Six Mile Slough was dominated by cattail (*Typha latifolia*) and cattail mats (Wm05), reed canary grass and bulrush (Wm06) or shallow water (OW) (Table 1). The wetland also had a compacted bottom overlain with organic soils (10-20 cm deep) that developed since 1975 when flooding was stabilized by dikes. Diking is extensive and comprises 6.7-hectares of Six Mile Slough (Biebighowser and Annschild 2016).

Mapping of 100m buffers zones around biomonitoring sites in our project demonstrated that *Typha* comprised 50-95% of the circular areas (Table 1). *Typha* also dominated the emergent vegetation in quadrats (median =50%) while submergent vegetation dominated bottom coverage of quadrats (90%) with little development of periphyton (Table 4). The disconnection of the wetland from the natural flooding regime allowed cattails to invade the wetland and resulted in the loss of plant diversity (Biebighowser and Annschild, 2016).

Table 2: Number of CABIN for wetland samples collected in the West Kootenays to date.

	Lentic[1]		Lotic[1]				Total
Year	Lacustrine[2]	Palustrine[2]	Riverine[2] Streams	Riverine[2] Floodplain			No. of Samples
				Natural	Constructed	Six Mile Slough	
2014	**1**		3				4
2015[4]	**5**	**4**	5	6			20
2016	**2**	**1**	2	2	3[3]		10
2017	**1**			4[3]	4[3]		9
2018	**1**			4[3]	4[3]		9
2019					3[5]	12[6]	15
Total	**10**	**5**	**10**[4]	**16**	**14**	**12**	**67**

[1] Wetland classifications from Hansen et al. 2000.[2] Wetland classifications from Env Canada 2018. [3] Repeat visits.
[4] Four sites affected by historical mining not included in the present study. [5] DNA only, [6] Paired DNA and taxonomy at 6 locations.

Six Mile Slough had a basic pH (median=8.25), a median conductivity of 211 uS/sec. Hardness values (median=116 mg/L) and total nitrogen (median=0.782 mg/L) were higher than other wetlands sampled in the West Kootenays. Median values and ranges for water quality are given in this section with further discussion in Section 2.2.3.

In comparison, reference wetlands from the Slocan Valley and Meadow Creek areas included wetlands of elevations from 470-1580 m associated with lentic (lacustrine and palustrine) and lotic (riverine/stream and floodplain) hydrology (Figure 3, Table 3-4). Reference sites in this study are defined as least-impacted sites with moderate levels of human impacts rather than "in-reference condition". Low to moderate impacts to sites included historical agriculture, forestry, impoundment, nearby roads, residential. But also included possible impacts from road salt at one floodplain site in the Slocan Valley and aerial or ground spraying of *Bacillus thuringiensis subspecies israelensis*, BTi, for mosquitoes at the six locations in Meadow Creek.

Lacustrine wetland (n=9) sites were associated with inflows and outflows of lake habitat at Little Slocan Lakes, Summit Lake, Bonanza wetland (Slocan lake), Little Wilson Lake, and Cooley Lake at elevations of 534 to 1515 m. The emergent vegetation at these sites (25m^2) was dominated by sedges, grasses, cattail, horsetail, and these wetlands were classified primarily as Marsh (Wm01) or Shallow water (OW). Lacustrine wetlands had neutral pH (median=7.5), conductivity (median=140 uS/sec), and hardness value (median=69.34 mg/L).

Palustrine wetland (n=5) sites occurred at mid-bench to upper elevations were from 976m to 1580 m. These locations were dominated by sedges, grasses, cattail, horsetail and were classified as marsh (Wm01, Wm02, Wm05 and Wm06) or shallow water (OW). Palustrine wetlands in our study had the lowest median pH (6.5), conductivity (39.3 uS/sec) and hardness values (21.5 mg/L).

Riverine wetlands (n=10) situated along streams or within river valleys were located at elevations of 567-1080 m. These sites were dominated by sedges, cattails and grasses and were classified as marsh (Wm01, Wm02) or shallow water (OW). Complexes of these types of habitats were typically associated with treed swamp habitats (Durand 2016). Upper elevation riverine wetlands had neutral pH (median=7.5), conductivity (median =75.3 uS/sec) and hardness values (median =29.7 mg/L).

Floodplain wetlands (n=16) included small ponds or side-channels located at low elevations (470-558 m) on the floodplain of the Slocan or Duncan Rivers. Five of these sites were constructed wetlands. These wetland sites (25m^2) were dominated by sedges, cattails and grasses and were classified as marsh (Wm01, Wm02, Wm05) or shallow water (OW). Floodplain habitats were frequently dominated by canary reed grass and/or treed swamp habitats (Durand 2016).

Table 3: Description and classification of reference sites.

	n[2]	Elevation (m)	Dominant emergent	Classification[1]	Locations
Lacustrine	10	534-1515	Sedges, grasses, cattail, horsetail	Marsh (Wm01, WM05), Shallow water (OW)	Little Slocan Lakes, Summit Lake, Snk'mip/Bonanza Marsh, Little Wilson Lake, Cooley Lake
Palustrine	5	976-1580	Sedges, grasses, cattail, horsetail	Marsh (Wm01, Wm02, Wm05 and Wm06) or Shallow water (OW).	Mid-bench wetlands in Winlaw Creek woodlot, private land Paradise Road, Goose Creek FSR above Cooley Lake
Riverine, Stream	10	567-1080	Sedges, cattails, and grasses	Marsh (Wm01, Wm02) or shallow water (OW)	Pass Creek wetland, Beaver Lakes complex, Bear Lake outflow
Riverine, Floodplain	30	470-558	Sedges, cattails, and grasses	Marsh (Wm01, Wm02, Wm05) or shallow water (OW)	Small ponds and side-channels on the floodplain of the Slocan or Duncan Rivers including natural (n=16 samples) and 5 constructed wetlands (n=14 samples)
Six Mile Slough	12 (6-paired)	523-533	Cattail, bulrush, and Reed canary grass dominated wetland (See Table 5)	Marsh (Wm05 and Wm06) or Shallow water (OW). Deep pool/small lake in Compartment 3 is >5m (Figure 1).	1,260-hectares of wetlands within the CVWMA historically impacted by agriculture, ditching/draining, flood control, channeling, and rail. Divided into 5 compartments in 1974 and 75 by diking for wildlife purposes. currently non-functioning water control structures.

[1] Wetland classification, MacKenzie W. and J. Moran (2004), [2] n=number of samples

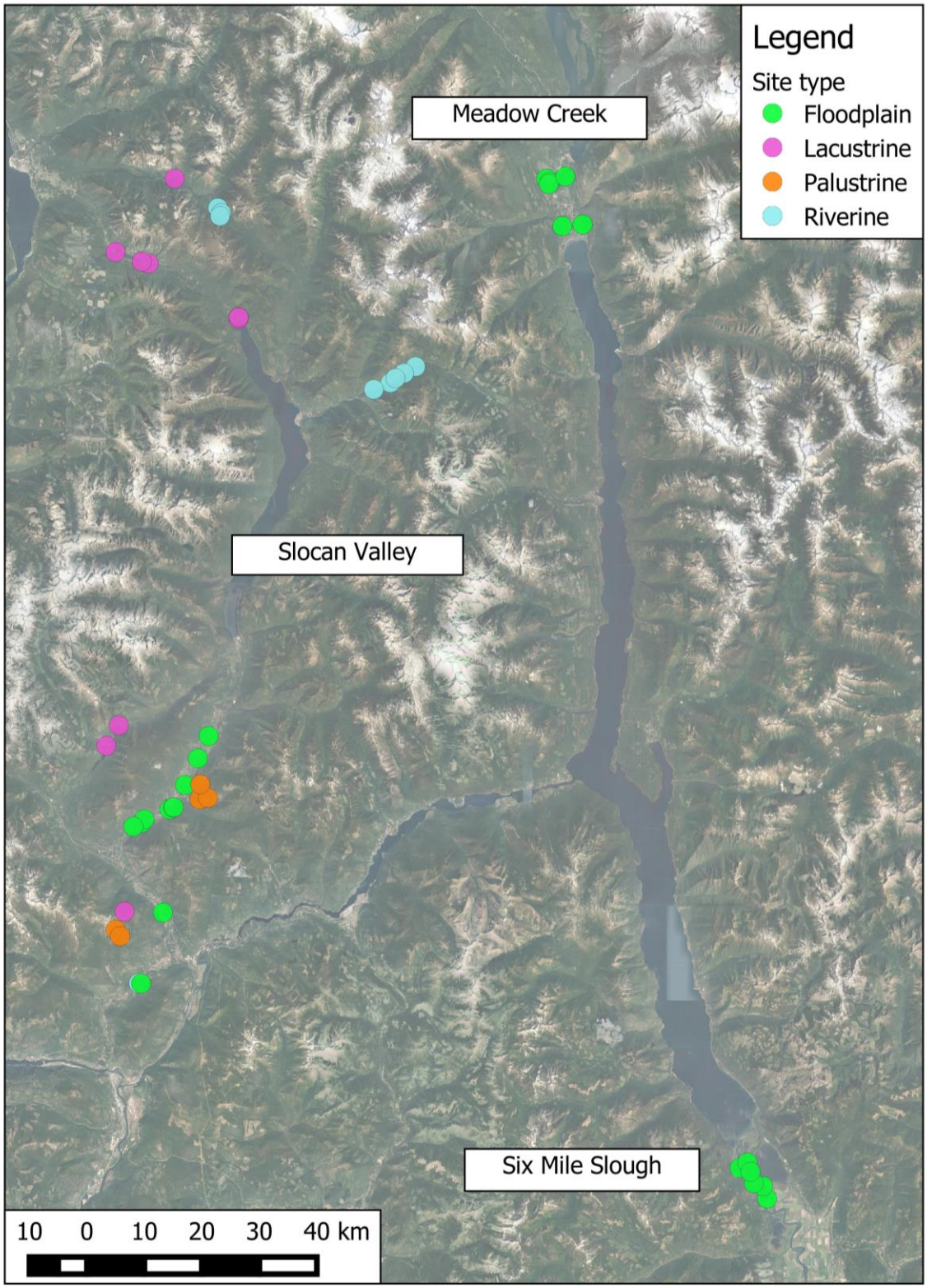


Figure 3: Location of monitoring of CABIN sites in the West Kootenays by wetland type.

Table 4: Selected site characteristics from Six Mile Slough and reference sites in the West Kootenays 2014-2019.

Variables in percent area	Lotic_Floodplain Six Mile Slough		Lotic_Floodplain Reference		Lotic_Riverine Reference		Lentic_Lacustrine Reference		Lentic_Palustrine Reference	
	mean	min-max	mean	min-max	mean	min-max	mean	min-max	mean	min-max
% Zones of wetland										
Emergent vegetation -Visual	52.1	30-75	80	30-100	57.9	10-95	46.1	1-90	51.0	25-70
Submergent vegetation- Visual	85.0	60-100	34.6	0-85	27.9	1-50	23.7	0-80	29.0	0-75
Open Water- Visual	23.9	10-50	11.8	0-40	11.4	0-40	17.0	0-80	24.0	0-60
% Margin disturbance (0-50m)										
Disturbance - none	100.0	0-100	46.2	0-100	55.7	0-100	61.0	20-100	76.0	50-100
Disturbance - filling	0.0	0.0	3.5	0-25	0.0	0-0	2.0	0-20	0.0	0-0
Disturbance - grazing	0.0	0.0	6.9	0-90	0.0	0.0	0.0	0-0	0.0	0-0
Disturbance - road	0.0	0.0	30.4	0-50	35.7	0-100	30.0	0-60	24.0	0-50
Disturbance - farm yard	0.0	0.0	8.1	0-50	0.0	0.0	0.0	0-0	0.0	0-0
Disturbance - urban	0.0	0.0	5.0	0-40	1.4	0-10	7.0	0-30	0.0	0-0
Disturbance - mining	0.0	0.0	0.0	0-0	7.1	0-50	0.0	0-0	0.0	0-0
Percentage of marginal zone vegetation (0-50m)										
Woody riparian	7.1	0-25	32.9	0-90	28.7	1-90	28.5	0-80	66.0	20-95
Typha	57.1	5-95	25.7	0-100	0.1	0-1	9.0	0-80	0.0	0-0
Scirpus	21.4	5-50	0.4	0-5	4.4	0-30	12.3	0-60	0.0	0-0
Grass/sedge	14.4	1-45	41.1	1-90	53.0	1-90	50.3	18-90	34.0	5-80
Percentage of quadrat vegetation ($25m^2$)										
Emergent	82.1	80-90	79.6	50-100	61.4	45-100	73.3	50-98	56	30-80
Floating plants	2.9	0-12	2.5	0-15	11.6	0-25	4.8	0-25	16.2	1.1-30
Open water	15.3	3-20.0	12.9	1 to 30	27.3	1-55	21.0	0-40	29	5-55
Periphyton	0.0	0.0	16.9	0-90	27.3	0-80	13.2	0-40	1.2	0-5
Submergent plants	90.0	65-100	46.1	0-90	33.7	0-100	22.8	0-90	19.2	0-65
Woody debris	0.0	0.0	3.4	0-15	13.7	0-20	0.8	0-5	12.8	0-60

Historical Mine sites were excluded

2.2.3 Physiochemistry of Six Mile Slough and Kootenay River

2.2.3.1 Water quality: Nutrients

Analyses of nitrogen and phosphorus were undertaken to aid the evaluation of possible restoration decisions regarding Six Mile Slough including reconnection of the wetland to the mainstem Kootenay River (Figure 4). Aquatic organisms require nitrogen and phosphorus for basic metabolic processes. Nutrient status is an important indicator in regard to the productivity of a wetland or body of water.

We also used water quality to help assess the results from the macroinvertebrate collection within Six Mile Slough. Our priority in 2019 was the water quality of the wetland rather than a full assessment of the restoration impacts on the Kootenay River. Thus, only two sites were collected from the Kootenay River as a preliminary verification of work done in other monitoring (Hoyle et al. 2013, Swain 2017 and Bassett et al. 2018). Further possible pre-restoration planning and recommendations for this approach will be discussed for 2020.

Measurements of the total nutrients in water were higher in Six Mile Slough than the Kootenay River. The total nitrogen was 4.7 times higher in Six Mile Slough (SMS001-6, n=6) than in the Kootenay River (KR001-2, n=2) when measured July 29-30, 2019 while the total phosphorus was 4.0 times higher in Six Mile Slough (SMS001-6, n=6) than in the Kootenay River (KR001-2, n=2).

The mean level of total nitrogen within Six Mile Slough wetland was 0.826 mg/L N (n=6, median=0.782, 25th-75th percentile = 0.687-0.819 mg/L) whereas the mean of the Kootenay River samples was 0.175 mg/L (n=2). The median values of total nitrogen from Six Mile Slough were within the ranges of reference wetlands (n=36, mean= 0.640, median=0.430, 25th-75th percentile = 0.243-0.862 mg/L, Table 5).

The mean level of total phosphorus within the wetland was 0.034 mg/L N (n=6, 25th-75th percentile = 0.024-0.033 mg/L) whereas the mean of the Kootenay River samples was 0.0085 mg/L (n=2). Six Mile Slough is a mesotrophic system because total phosphorus falls within the limits of 0.01-0.035 mg TP/L for assessment of trophic status (Ready and Deleune 2008). In contrast, the Kootenay River is an oligotrophic system because total phosphorus is less than 0.01 mg TP/L. The median values of total phosphorus from Six Mile Slough were within the ranges of reference wetlands in Meadow Creek and the Slocan Valley (n=40, mean=0.068, median=0.024, 25th-75th percentile = 0.011-0.073 mg/L, Table 5). Values for reference wetlands suggest that the trophic status of wetlands in the Slocan and Meadow Creek areas ranged from mesotrophic to eutrophic (Ready and Deleune 2008).

Total nutrient measurements included dissolved and organic fractions. The total organic phosphorus comprised 63.5% of the total phosphorus within Six Mile Slough relative to 56.7% within Kootenay River. While the organic fraction of nitrogen, total organic nitrogen, comprised 87.2% of the total nitrogen within Six Mile Slough while the samples collected from Kootenay River were below detection. In contrast, in Kootenay River the dominate forms of nitrogen were ammonia and nitrate-N which comprised over 98% of the total nitrogen (n=2) while these forms of nitrogen only comprised 14% in Six Mile Slough (n=6).

Typically, nitrogen and phosphorus within wetlands predominate in organic forms due to the high organic fraction within wetlands soils and overlying water.

For the purposes of this study, we defined the term total organic phosphorus as the difference between total phosphorus and inorganic phosphorus in order to approximate the total organic phosphorus (TOP) within Six Mile Slough (similar to Ready and DeLaune 2008). We justified this terminology due to the predominance of organics and the lack of clays in Six Mile Slough that might bind phosphorus. The surface organic soils (15-25cm) are underlain by sand and silt laid down prior to the isolation of the slough from Kootenay River in the 1970s through diking and restoration for migratory birds. In water quality analyses, some mineral forms of phosphorus may not have been solubilized during the extraction process with a strong acid if strongly bonded to inorganic particulates. As a result, an alternative terminology that is likely slightly more accurate would be "insoluble phosphorus".

Nitrogen is the typically limiting nutrient in wetlands with most of nitrogen and phosphorus stored in the form of organic nitrogen and phosphorus (Ready and DeLaune 2008). If we use the N:P ratio of biologically available dissolved inorganic nitrogen (DIN) to total dissolved phosphorus (TDP) where <14:1 (weight to weight) indicative of nitrogen limitation and >14:1 as indicative of phosphorus limitation (Koerselman and Meuleman. 1996) like Basset et al. 2018. The mean DIN:TDP ratio for the six sites at Six Mile Slough was 10.06 (n = 6, median = 10.38, 25-75th percentile = 7.8-12.3, Table 5) which suggests that DIN-nitrogen may be limiting relative to total dissolved phosphorus concentrations. These trends were confirmed by 36 samples from lacustrine, palustrine, riverine and small floodplain wetlands where the median N:P ratio was 4.3 (n = 36, median = 3.35, 25-75th percentile = 2.7-12.4, Table 5) in the Slocan and Meadow Creek areas (Quamme et al. 2019).

In contrast, the two samples from Kootenay River suggests that phosphorus is limiting because the DIN:TDP is 46.7 (n = 2). Low levels of nitrogen and phosphorus monitored in Kootenay River and Kootenay Lake have been well documented in a large body of work in the USA (Hoyle et al. 2013) and on-going monitoring in Canada (Swain, 2007, Bassett et al. 2018, Schindler et al. 2011). Lower nutrients within Kootenay River and Kootenay Lake are thought to result from upstream habitat alteration from diking and draining of floodplain wetlands, channelization, impoundment from Libby Dam, and deforestation of the historical floodplain (Hoyle et al. 2013, Bassett et al. 2018).

The reducing conditions found at the bottom of Six Mile Slough wetland (mean dissolved oxygen 2.8 mg/L, range 0.4-5.4 mg/L, 10cm from bottom) promote denitrification, typically, caused by uptake by aquatic vegetation, microbial activity, and mineralization of organic matter. As a result, nitrates (NO_3^-) are converted to ammonia (NH_4^-) in lower oxygen environments such as Six Mile Slough. Total dissolved phosphorus is also released under reducing conditions likely accounting for higher phosphorus levels and ammonia within the wetland. This may account for the higher concentrations of phosphorus at SMS001 had the second lowest level of dissolved oxygen (0.5 mg/L) measured within Six Mile Slough for all forms of phosphorus (Figure 4).

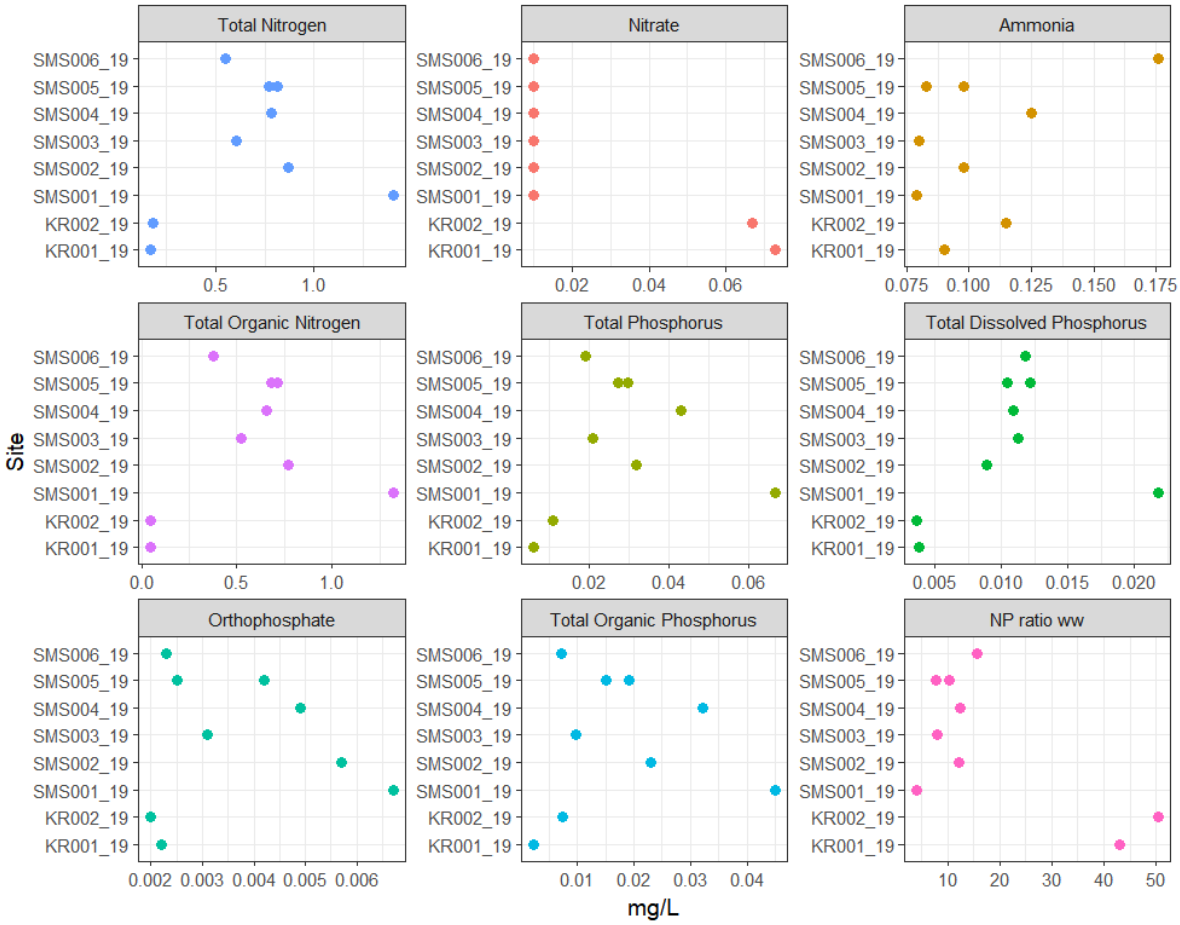


Figure 4. Nutrient concentrations (mg/L) for surface water samples collected from six sites within Six Mile Slough (SMS001-6) and two sites within the Kootenay River (KR001-2). NP ratio (weight:weight) is the ratio of dissolved inorganic nitrogen (nitrite, nitrate and ammonia) to dissolved phosphorus. Nitrate levels in Six Mile Slough were below detection. Method reporting levels included: total and total organic nitrogen (0.05 mg/L), nitrate (0.01 mg/L), ammonia (0.02 mg/L), all forms of phosphorus (0.002 mg/L). Nitrite was below detection at all sites (0.01 mg/L).

Further work and planning regarding the downstream effects of reconnection of Six Mile Slough to the Kootenay River could involve greater water quality sampling and downstream evaluation of nutrient spiralling during peak flows and throughout the growing season. Calculation of nutrient loadings at key locations may aid this evaluation. Work up of Environment Canada time-series data from Kootenay River at Creston would also be useful as well as downstream monitoring. Further review of nutrient levels or comparisons to the South Arm of Kootenay Lake nutrient monitoring station may provide additional inference.

2.2.3.2 Water quality: alkalinity, carbon, turbidity, pH, and silicon

Basic water quality parameters for surface water samples was collected from six sites within Six Mile Slough (SMS001-6) and two sites within the Kootenay River (KR001-2) including: alkalinity, total and total organic carbon, turbidity, and total silicon (Figure 5).

Alkalinity is a property of water which is the buffering capacity or ability to resist changes in pH and is dependent on the concentration of bicarbonates, carbonates, and hydroxides in water (Swensen and Baldwin 1965). Measurements of alkalinity in water in Six Mile Slough (SMS001-6, n=6) and the Kootenay River (KR001-2, n=2) ranged from 91-208 mg/L when measured July 29-30, 2019. The mean alkalinity within the Six Mile Slough wetland was 136.7 mg/L N (n=6, median=124 mg/L, 25th-75th percentile = 103.4-173.0 mg/L) whereas the mean of the Kootenay River samples was 91.4 mg/L (n=2). The median alkalinity from reference wetlands in the Slocan and Meadow Creek area was 91.8 mg/L and ranged from 13.7 to 427mg/L (25th-75th percentile = 34.1-74.8 mg/L, n=38, Table 5).

Carbon in water was measured as both, total and dissolved organic carbon. Carbon acts as an energy sources for living systems and is a key component in living systems and cells structure also serving as an electron acceptor in microbial processes (Ready and DeLeune 2008). The mean level of total organic carbon within Six Mile Slough was 8.62 mg/L N (n=6, median= 7.8, 25th-75th percentile = 6.17-8.66 mg/L). The mean of the Kootenay River samples was 2.35 mg/L (n=2). The mean level of total dissolved organic carbon within Six Mile Slough was 8.60 mg/L N (n=6, median= 8.41, 25th-75th percentile = 5.97-10.40 mg/L). The mean of the Kootenay River samples was 1.29 mg/L (n=2). The median total dissolved organic carbon from reference wetlands in the Slocan and Meadow Creek area was 4.0 mg/L ranging from 0.5-35.7 mg/L (25th-75th percentile = 3.0-10.45 mg/L, n=38, Table 5).

Total organic carbon and dissolved organic carbon were remarkably similar in value. This is because dissolved organic carbon, typically, accounts for more than 90% of the total organic matter in wetlands (Ready and DeLeune 2008). Dissolved organic carbon is released under the reducing conditions such as observed in Six Mile Slough during organic matter decomposition and breakdown with microbial activity (Scott et al 2014).

The mean level of turbidity within Six Mile Slough was 2.9 NTU (n=6, median= 2.5 NTU, 25th-75th percentile = 1.8-3.9 NTU). The mean of the Kootenay River samples was 0.9 NTU (n=2). The median turbidity from reference wetlands in the Slocan and Meadow Creek area was 3.7 NTU ranging from 0.5-19.3 NTU (25th-75th percentile = 0.5-2.0 NTU, n=39, Table 5). These values are reflected of seasonally receding water levels and baseline flows on the Kootenay River. Increased wind on July 30, 2019, compared to the previous sampling day may have cause a slight increase in turbidity in the sampling of SMS005 and SMS006, relative to other sites (Figure 2).

The pH of within Six Mile Slough was 8.3 (n=6, median= 8.3, 25th-75th percentile = 8.1-8.3). The mean of the Kootenay River samples was 8.1 (n=2). The median pH level from reference wetlands in the Slocan and Meadow Creek area was 7.4 ranging from 6.0-8.3 (25th-75th percentile = 6.9-7.4, n=40, Table 5).

Thus, Six Mile Slough and the Kootenay River were slightly basic (greater than pH 7), a pH of 7 is considered neutral.

Total silicon concentrations were monitored in the present study. Silicon is important to wetland plants (Schaller et al. 2014) and diatomaceous algae (Wetzel 2001). Dissolved reactive silica (monomeric) is the form of silicon most available for uptake by diatoms and thus is often monitored in an indicator of silica limitation for diatoms (0.5 mg/L), (Wetzel 2001, Bassett et al. 2018).

Total silicon is comprised of dissolved, colloidal, and particulate forms of silicon. We did not monitor these components as separate fractions of total silicon. However, in initial water quality assessments we used total silicon as an initial parameter to examine the difference in total silicon levels between Six Mile Slough and the Kootenay River. In the laboratory, colloidal and dissolved polysillcic acids were analyzed in water passed through a 0.45 µm filter while the particulate fraction is retained on the filter. Laboratory methods, then, differentiate the monomeric (reactive) from colloidal fractions (non-reactive) that pass through this filter size. In summary, the total silicon levels measured in our study (mg/L) as part of the metals scan carried out by CARO Laboratories included particulate forms of silicon, dissolved reactive (monomeric) silicates and non-reactive (colloidal polysilicic acids).

The mean level of total silicon in water within Six Mile Slough was 8.4 mg/L (n=6, median= 8.7 mg/L, 25th-75th percentile = 6.4-10.0 mg/L). The mean of the Kootenay River samples was more than 4 times lower at 2.0 mg/L (n=2). The median silicon level from reference wetlands in the Slocan and Meadow Creek area ranged from below detection (<5 or < 1 mg/L to 16.7, n= 35).

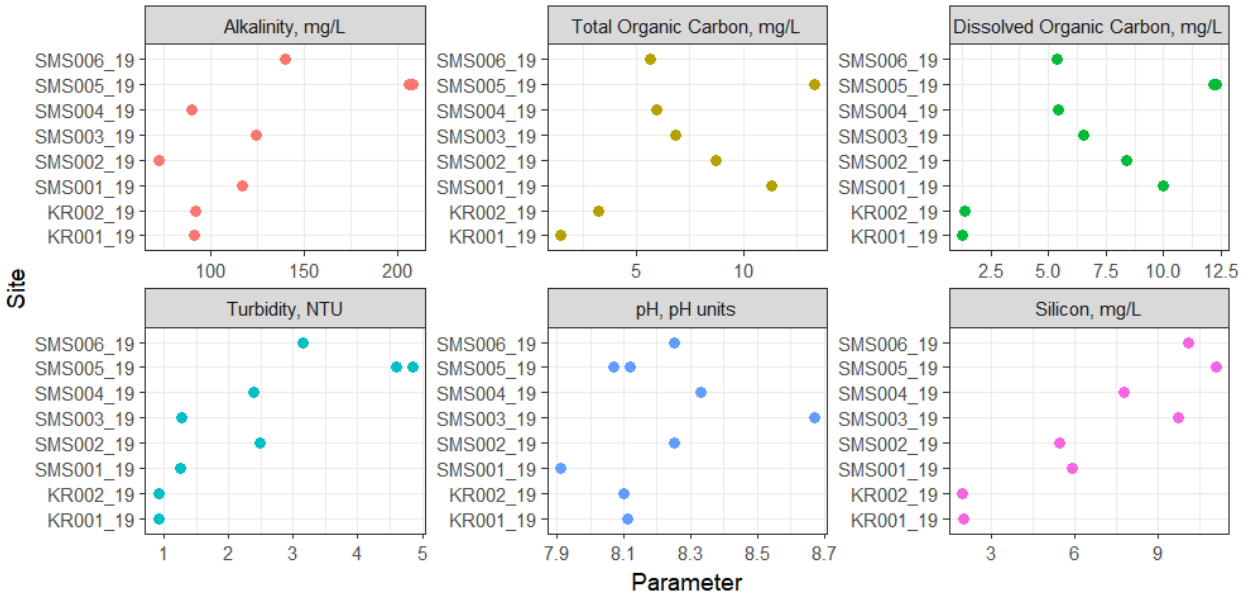


Figure 5. Basic water quality parameters (mg/L) for surface water samples collected from six sites within Six Mile Slough (SMS001-6) and two sites within the Kootenay River (KR001-2). Method reporting levels include: alkalinity (1 mg/L), total and total organic carbon (0.5 mg/L), turbidity (0.1 NTU), and total silicon (0.1 mg/L).

Table 5: Selected physiochemical variables from water and sediment by habitat type 2014-2019.

	Lotic_Floodplain Six Mile Slough		Lotic_Floodplain Reference		Lotic_Riverine Reference		Lentic_Lacustrine Reference		Lentic_Palustrine Reference		
Variables	**median**	**min-max**	**median**	**min-max**	**median**	**min-max**	**median**	**min-max**	**median**	**min-max**	**Units**
Water											
Chloride	0.3	0.15-0.59	1.88	<0.10-76.9	4.97	0.34-76.9	1.85	<0.10-5.89	1.8	<0.10-2.29	mg/L
Sulfate	<1.0	<1.0-<1.0	3.7	1.1-18.5	3.7	<1.0-18.7	11	<1.0-90.8	1.0	<1.0-5.2	mg/L
Alkalinity, Total as $CaCO_3$	124.0	72-208	88.4	20.52-427	34.0	20.52-175	61.6	16-216	27.0	13.7-87	mg/L
Carbon, Dissolved Organic	7.5	5.37-12.2	5.5	0.5-20	5.12	1-11.8	2.6	1.2-5.18	7.3	3.2-35	mg/L
Total Nitrogen	0.782	0.551-1.40	0.206	<0.05-0.989	0.697	<0.05-2.9	0.272	0.05-0.97	0.580	0.35-1.08	mg/L
Dissolved Inorganic Nitrogen	0.108	0.089-0.186	0.034	<0.05-0.949	0.085	0.03-0.271	0.034	0.03-0.14	0.044	0.031-0.058	mg/L
DIN:TDP ratio (wt:wt)	10.4	4.08-15.8	6.1	2.1-38.0	2.80	0.46-26.5	4.6	2.14-38.0	2.5	0.45-5.5	mg/L
Phosphorus, Total	0.030	0.019-0.06	0.1	0.0023-0.101	0.050	0.0023-0.129	0.011	0.003-0.035	0.040	0.011-0.82	mg/L
Phosphorus, Total Dissolved	0.011	0.009-0.022	0.006	<0.002-0.016	0.021	<0.002-0.426	0.006	0.002-0.016	0.013	0.008-0.115	mg/L
Solids, Total Suspended	4.8	<2.0-10.4	10.0	<2.0-80.0	11.0	4-42	2.4	<2.0-8.0	10.5	<2.0-186	mg/L
Turbidity	2.49	1.26-4.6	1.8	0.40-19.3	1.8	0.4-4.9	0.55	6.7-8.18	1.2	0.65-47.4	NTU
pH	8.25	7.91-8.67	7.6	6.73-8.29	7.2	6.73-8.2	7.6	6.7-8.18	6.5	0.65-2.0	pH units
Conductivity (EC)	211.0	126-363	173.0	38.9-738	82.0	38.9-620	143.0	16.4-565	39.3	8.6-165	µS/cm
Hardness, Total ($CaCO_3$)	116.0	70.5-189	92.8	17.1-417	31.8	17.1-204	77.0	5-261	21.5	<5.0-86.7	mg/L
Calcium, Total	26.25	12.6-47.8	30.5	12.9-97.9	9.90	6.3-55.4	26.4	<2.0-72.5	7.2	<2.0-23.5	mg/L
Magnesium, Total	11.9	9.16-16.9	3.3	0.6-26.3	2.00	0.6-15.9	2.80	0.4-21.7	0.8	0.1-6.8	mg/L
Potassium, Total	0.71	0.20-1.77	1.4	<0.2-5.4	2.85	<0.2-5.1	0.54	0.2-0.9	0.3	<0.2-1.5	mg/L
Sediment											
>75um	67.5	45-72.9	18.3	2.8-81.9	27.8	16.3-60.6	42.6	27.6-82.9	17.5	11.2-52.6	
Size class	Coarse		Fine		Fine		Fine		Fine		Fine/Course
Phosphorus, Total P	697.0	657-767	828.0	282-1580	500.0	394-1070	680	397-1110	639	262-1090	mg/kg
Antimony (Sb)	0.58	0.44-0.83	0.5	<0.1-2.24	1.2	<0.1-3.04	1.05	0.13-4.8	0.9	0.2-4.4	mg/kg
Arsenic (As)	4.92	3.86-9.17	3.56	1.5-15.8	1.2	0.6-10.4	4.7	<0.4-8.06	2.2	0.8-4.2	mg/kg
Cadmium (Cd)	0.68	0.60-0.88	2.46	0.373-7.28	1.45	0.08-4.44	2.085	0.15-7.29	0.89	0.38-5.82	mg/kg
Chromium (Cr),	18.45	15.4-21.9	29.5	5.8-43.1	23.5	11.5-29.7	29.65	3.1-69.8	7.5	2.5-14.3	mg/kg
Cobalt (Co)	7.74	7.04-9.02	7.3	2.8-16.3	2.98	1.3-11	5.45	0.8-14	1.2	0.4-2.9	mg/kg
Copper (Cu),	19	18.2-20.8	28.9	3.7-38.9	11.4	5.77-45.9	15.15	5.7-63.1	19.6	2-61	mg/kg
Lead (Pb)	52.05	33.7-110.0	25.3	5.9-145	36.7	3.8-77.4	16.05	7-204	26.8	7.4-61.3	mg/kg
Nickel (Ni),	19.2	17.3-19.9	21	5.9-40.1	9.9	7.7-47.9	17.35	3.5-50	7.5	1.1-16.1	mg/kg
Silver (Ag)	0.19	0.14-0.33	0.26	<0.20-0.5	0.7545	<0.050-1.4	0.27	<0.050-0.4	<0.2	<0.2-<0.2	mg/kg
Tin (Sn)	0.51	0.39-0.89	0.8	0.36-1.5	0.7	0.29-1.4	0.85	0.23-1.6	0.5	0.3-1.3	mg/kg
Vanadium (V)	16.8	14.2-17.3	29.3	12.3-43.9	20.4	9.7-23	32.5	3-105	15.6	3.5-18.5	mg/kg
Zinc (Zn)	125	103-202	140	100-494	77.0	25-275	88.5	41-298	33	5-63	mg/kg

2.2.3.3 Water and sediment quality: metals

All water and sediment samples were reviewed in accordance with applicable provincial and federal guidelines (see Section 5.5 for summarized water and sediment guidelines). Water and sediment quality indicated non-significant metals contamination and low impacts from anthropogenic activity at all sites.

Provincial and federal water quality guidelines for the protection of aquatic life were reviewed and of the fifty-seven water quality parameters monitored fifteen parameters have associated guidelines including: chloride, nitrate, nitrite, sulfate, alkalinity, ammonia, total phosphorus (for lakes), pH, aluminum, antimony, arsenic, barium, beryllium, boron, cadmium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, selenium, silver, mercury and zinc. In water, sites varied from 0-3 exceedances of at least one guideline including: nickel (5 of 6 wetland sites), iron (3 of 6 sites), ammonia (1 of 6 sites) (Section 5.2.3) for the lowest guidelines which required sampling over 5-times over 30 days. Here we used these guidelines as an alert.

Provincial and federal sediment quality guidelines for the protection of aquatic life were reviewed and of the thirty-seven sediment quality parameters monitored twelve parameters have associated guidelines including: arsenic, cadmium, chromium, cobalt, copper, iron, lead, magnesium, manganese, mercury, nickel, selenium, silver, mercury and zinc. Sediment guidelines were exceeded in Six Mile Slough for 2-5 parameters including arsenic (3 of 6 wetland sites), cadmium (6 of 6 sites.), lead (5 of 6 sites), nickel (6 of 6 sites) and zinc (4 of 6 sites) (see Section 5.2.6).

We used metals in sediment as an indicator of human activity (Nahlik et al. 2019 and US EPA 2016) and to evaluate possible impacts to the macroinvertebrate community present at each site (Clements et al. 2000). Sediment from CABIN sites in Six Mile Slough were all at or less than two cumulative toxic units (CTUs) indicating non-significant pollution (Clements et al 2000) including the sites that were located adjacent to know breeding locations for the Northern Leopard frog (SMS001 and SMS003, Figure 1).

Cumulative toxic units were calculated using criteria based on Canadian Council of Ministers of the Environment, Probable Effect Levels (CCME PEL) for zinc, lead, arsenic, copper, and cadmium. Seven other wetlands in the Slocan and Meadow Creek had levels between two to ten times criterion values indicating that metals levels may influence benthic community structure and cause mortality in sensitive species at these sites (Figure 6). No reference or constructed wetlands had values that were ten times criterion value which is considered significantly polluted (Clements et al. 2000). As a comparison, five contaminated sites were affected by legacy mining had 100-fold higher CTUs than other reference sites and sites in Six Mile Slough with levels ranging 50-102 CTU (See Section 5.2.7 and Quamme et al. 2016).

In addition, Table 5 summarizes key heavy metals in wetland soils identified as “indicative of anthropogenic activities” rather than toxicity in wetlands across the U.S. (Nahlik et al. 2019 and US EPA 2016). However, we did not include tungsten unlike Nahlik et al. (2019) and US EPA (2016) because this was not included in the metals scan carried out by CARO Analytical Services.

While collection methods varied between our study and the U.S study, all samples within Six Mile Slough were below stress-level thresholds developed for individual heavy metals except for lead. The median concentrations of lead in Six Mile Slough was above background (>35 mg/kg) but below risk of aquatic toxicity (>120 mg/kg). Lead levels were higher in Six Mile Slough than other wetlands in Meadow Creek and the Slocan Valley (Table 5). For example, median lead levels were two times higher in Six Mile Slough than other lower valley "Lotic Floodplain" wetlands in Meadow Creek and the Slocan Valley. Other metals were within the range of concentrations observed in these sites (Table 5). Lead levels across the US were more likely to exceed thresholds than other metals in these studies similar to Six Mile Slough but were also thought to be commonly bound to wetland sediments with resulting lower toxicity to aquatic life (Nahlik et al. 2019).

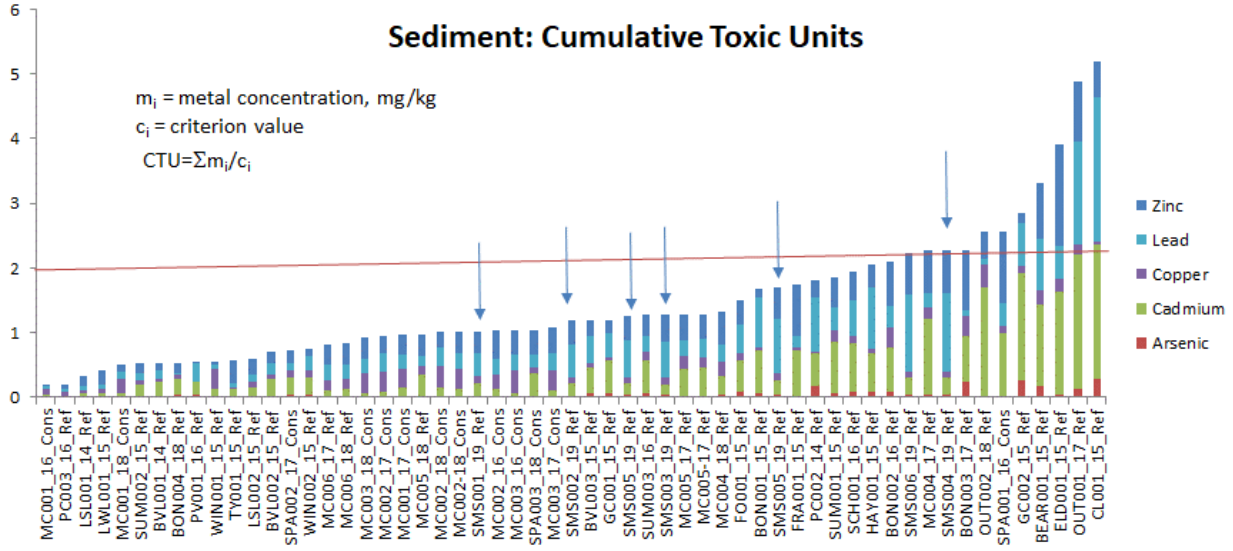


Figure 6. Graph of cumulative toxic unit of zinc, lead, copper, cadmium, and arsenic in sediment. Line in red indicates two times criterion values above which metals levels may influence macroinvertebrate community structure and cause mortality in sensitive species. Blue arrows indicate sites located within Six Mile Slough (SMS001-6). Cumulative toxic units are the sum of metals divided by the guideline (CCME PEL). Sites are indicated with Site name_Year_Type, Type = Reference (Ref) or constructed (Cons) wetlands.

2.2.4 Ecological productivity and diversity of Six Mile Slough

This study is the first project in BC to use both traditional taxonomy and DNA meta-barcoding to assess wetland biodiversity of invertebrates to assess restoration and management actions. This project provided a unique opportunity to obtain data using two different methods in a paired sample approach to evaluate the biodiversity and invertebrate productivity of Six Mile Slough.

DNA meta-barcoding provided species identification and a deeper understanding of indicator organisms present at the species level. This data will be used as a baseline to effectively evaluate pre-restoration years (2019 and 2020) and to post-restoration monitoring (2021-) in comparison analyses. Next Generation Sequencing also provided new information on wetland invertebrate species inhabiting Six Mile Slough. This is important because knowledge on wetland invertebrates at the species level is limited in BC and because Six Mile Slough within the Creston Valley Wildlife Management Area is a designated RAMSAR site and wetland of international importance.

With respect to assessing productivity of Six Mile Slough, traditional taxonomy outputs based on morphology included the numerical abundance and biodiversity measures at the genus-level for diagnostic species. In addition, voucher specimens housed at the Royal BC Museum could be used to analyze for food web analyses that involve biomass estimates in the future if costs permit. For the present study, we focussed on numerical abundance of invertebrates in Six Mile Slough as a measure of production. Importantly, traditional taxonomy also allowed us to make comparisons to previous sites sampled in the West Kootenay Region to date.

2.2.4.1 Quantifying the abundance and biodiversity: morphology-based taxonomy

The abundance and biodiversity of macroinvertebrates identified by morphology from sites in Six Mile Slough relative to previously collected samples from the Slocan Valley and Meadow Creek area from 2015-2018 were compared as benchmarks for the present data. However, the main goal of this project will be to compare future post-restoration monitoring at Six Mile Slough relative to pre-restoration monitoring at control and restored sites.

The total abundance and richness were grouped by Chironomidae (midges), Other Diptera (flies), Segmented worms (Annelida), Arachnids (aquatic mites), OET (Odonata, dragonflies, Ephemeroptera, mayflies, Trichoptera, caddisflies) and BGA (Bivalves, Amphipods, freshwater shrimp, and Gastropod, snails) and Arachnids (aquatic mites) (Figure 7 and 8) at constructed wetlands was compared graphically to reference sites.

Counts of the abundance of chironomids comprised 33% (1-91%) of the total counts 40% (2-92%) of total counts were dipteran on average (Figure 7). Other groups excluding Diptera comprised 60% (8-98%) of total counts. Chironomids were the most diverse group at the genus level comprising 15-53% of the number of genus across all wetland types among these groups while total dipterans (flies including chironomids) comprised 26-65%. Other groups not including Diptera comprised 35-74% of the total number of genus across all wetland, respectively. At Six Mile Slough, chironomids comprised 21-33% of

the number of genus across all wetland types among these groups while total dipterans (flies including chironomids) comprised 26-64%. Other groups not including Diptera comprised 46-74%

Counts of the number of total dipteran genus (chironomids plus other dipterans) comprised 39-72% of the total counts relative to constructed sites 50-% of total counts were dipteran on average (Figure 8). Other groups including OET, annelids and BGA, together, comprised 28-61% of total counts at reference sites and 25-50% at constructed sites over the 3-years (Figure 8).

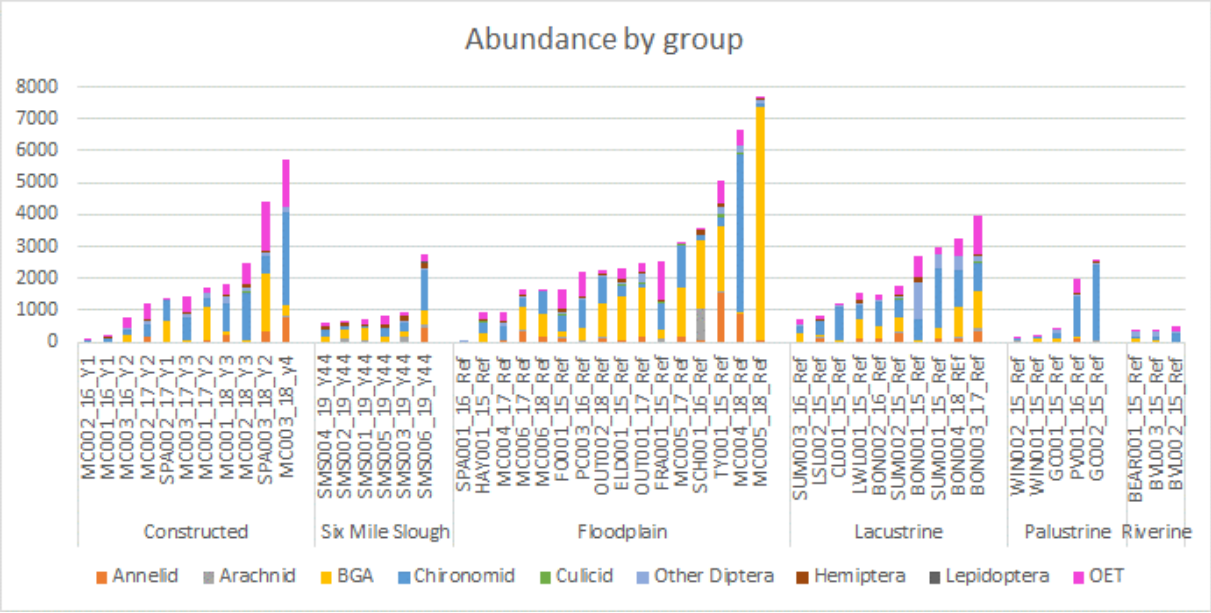


Figure 7. Abundance at wetlands monitored in 2019 from Six Mile Slough and 2015-18 in Meadow Creek and Slocan areas. Annelid (orange)= segmented worms), Arachnid (grey)= Aquatic mites, BGA (yellow) = Bivalves, gastropods plus amphipods, Chironomidae (bright blue), Culicidae (green), Other Diptera (light blue), Coleoptera (dark red), Hemiptera (Dark blue), Lepidoptera (brown), and OET (pink) = Odonata, Ephemeroptera and Trichoptera (dragonflies, mayflies and caddisflies). Site name is followed by year monitored, YXX= Year following restoration. Ref =Reference site.

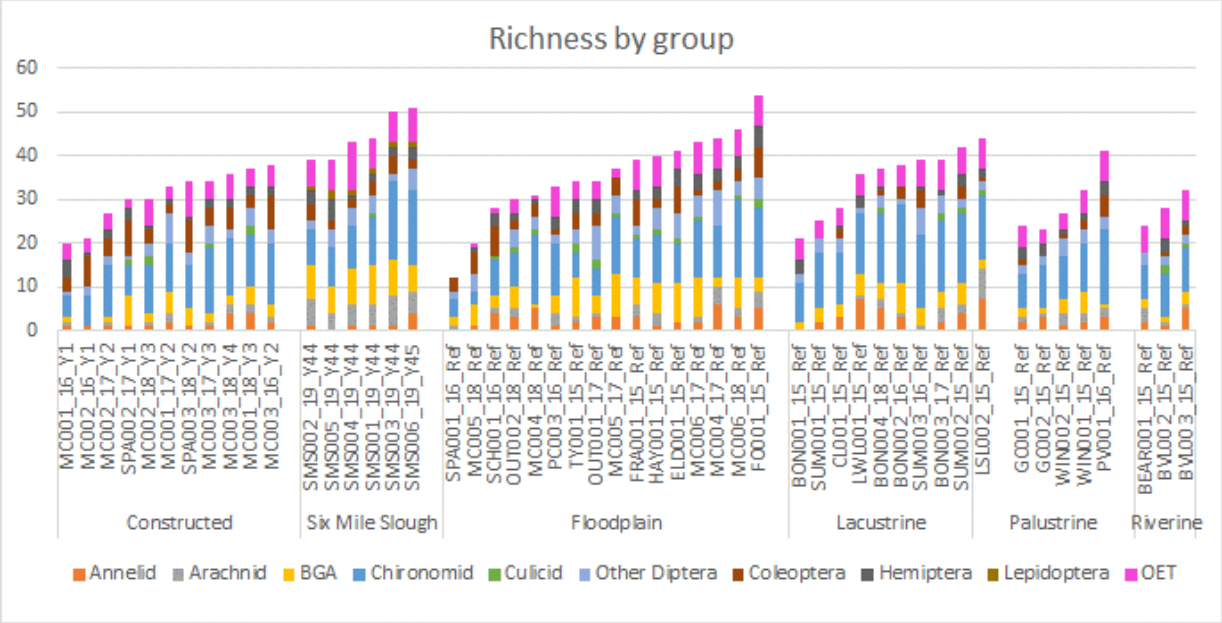


Figure 8. Richness (total count of genus) for samples analyzed by traditional taxonomy at wetlands monitored in 2019 from Six Mile Slough and 2015-18 in Meadow Creek and Slocan areas. Annelid (orange)= segmented worms), Arachnid (grey)= Aquatic mites, BGA (yellow) = Bivalves, gastropods plus amphipods, Chironomidae (bright blue), Culicidae (green), Other Diptera (light blue), Coleoptera (dark red), Hemiptera (Dark blue), Lepidoptera (brown), and OET (pink) = Odonata, Ephemeroptera and Trichoptera (dragonflies, mayflies and caddisflies),. Site name is followed by year monitored, YXX= Year following restoration. Ref =Reference site.

Pirate plots (R Development Core Team. 2018) were used to display the total counts and richness of macroinvertebrates, graphically for a subset of reference sites including floodplain and lacustrine sites used as the best benchmarks for Six Mile Slough (Kampstra 2008) at present (Figures 9 and 10).

Pirate Plots display 95% Highest Density Intervals (HDIs) of the mean of each group. HDIs indicate that there is a 95% probability that the true population mean falls within that interval. In the pirate plots, 95% HDIs are shown as solid bands around the sample mean.

Sites monitored at Six Mile Slough suggest that average values on macroinvertebrate abundance were lower (sqrt(mean) of 31.9 and HDIs of 21.2-40.7) than at Lacustrine (sqrt(mean) of 31.9 and HDIs of 21.2-40.7) and Floodplain wetlands (sqrt(mean) of 31.9 and HDIs of 21.2-40.7) but that this trend was non-significant at a 95% probability.

However, the biodiversity of invertebrates at Six Mile Slough were higher than average values (sqrt(mean) of 31.9 and HDIs of 21.2-40.7) at Lacustrine (sqrt(mean) of 31.9 and HDIs of 21.2-40.7) and Floodplain wetlands (sqrt(mean) of 31.9 and HDIs of 21.2-40.7) again that this trend was non-significant at a 95% probability.

Figure 9.

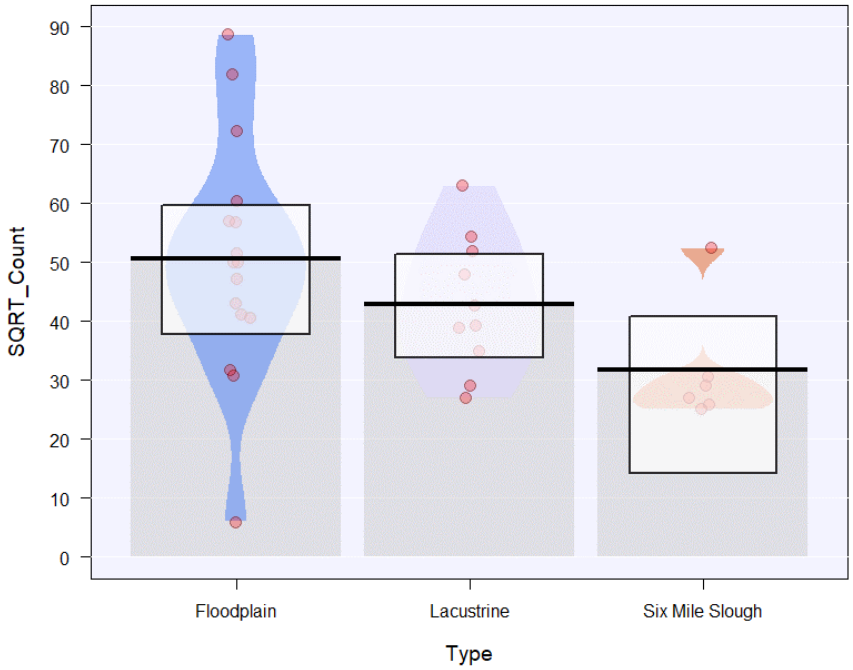
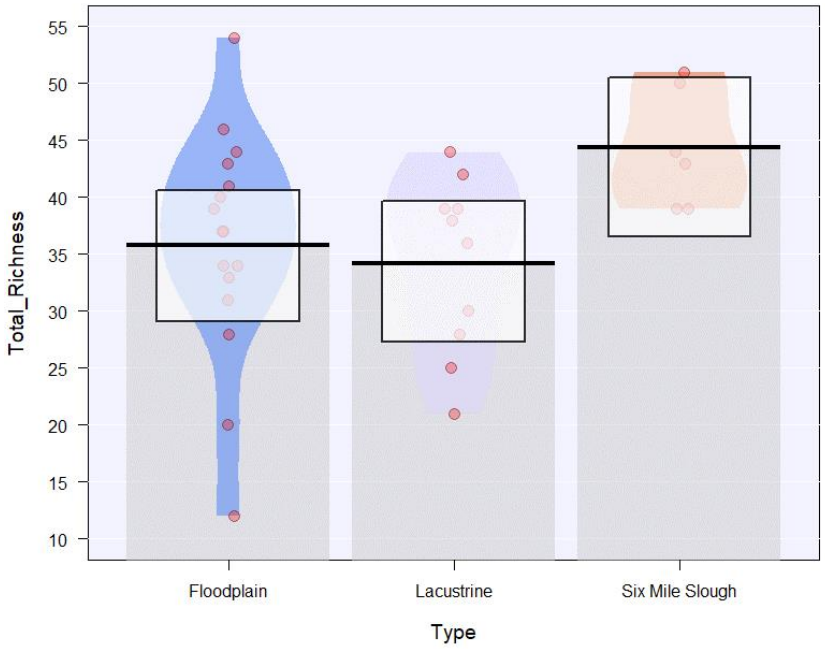


Figure 10.



Figures 9 and 10. Pirate plots of total count (Figure 9) and total richness (Figure 10) for Floodplain and Lacustrine reference wetlands relative to Six Mile Slough using BEST (Bayesian Estimation Supersedes the T-Test) with 95% Highest Density Intervals (HDIs) of the mean of each group. White boxes indicate that there is a 95% probability that the true population mean falls within that interval. Horizontal black lines indicate mean. (n= 16, 10, 6 and 4 for, Floodplain, Lacustrine and Six Mile Slough, respectively). All data was transformed using a square root transformation (Zar 1984).

2.2.4.2 Quantifying the species biodiversity of Six Mile Slough: DNA meta-barcoding

DNA meta-barcoding provided important information on the species diversity of invertebrates within Six Mile Slough. DNA meta-barcoding provided rapid and accurate species identifications compatible for comparisons with taxonomic data previously collected (see STREAM 2020 for data summary).

Meta-barcoding of DNA from Six Mile Slough resulted in 21 Orders, 58 Families, 127 Genera, and 139 species of macroinvertebrates from six sites within the slough from the six samples. Dominant groups namely the orders Diptera (flies), Odonata (dragonflies and damselflies), Ephemeroptera (mayflies) and Trichoptera (caddisflies) comprised 52% of the species present in Six Mile Slough (Figure 11).

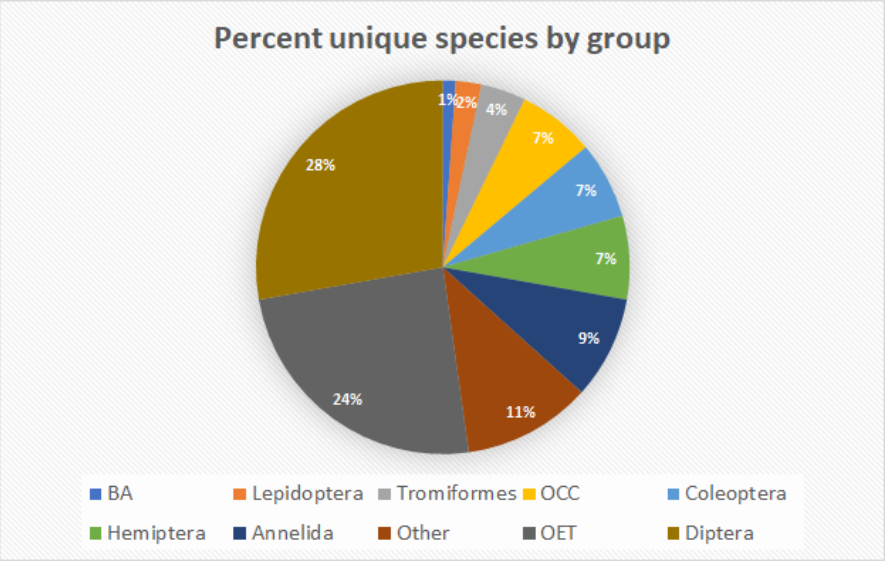


Figure 11. Percent unique species by group from DNA meta-barcoding within Six Mile Slough (all sites pooled). BA=Bivalves + Amphipods, Annelid (dark blue), Annelida= segmented worms (dark blue), Trombiformes (grey)= Aquatic mites, BA (blue) = Bivalves, plus amphipods, Diptera (light brown), Coleoptera= beetles (light blue), Hemiptera = True bugs (light green), Lepidoptera = moths (orange), and OET (dark grey) = Odonata, Ephemeroptera and Trichoptera (dragonflies, mayflies and caddisflies). Note DNA metabarcoding did not detect gastropods so %BGA was not calculated.

Across all six sites, species richness (number of species present) ranged from 38 species at SMS001 to 86 species at SMS005 with 54 species and SMS002, 68 species at SMS003 and 39 species at SMS004 and 67 species at SMS006 (Figure 11). A species list is included as a separate Excel spreadsheet also see Section 5.3). DNA meta-barcoding incorporated terrestrial and semi-aquatic species as well as target aquatic invertebrates. These species represent the ranges of species found associated with marsh habitat at Six Mile Slough. However, our focus in future analyses will be diagnostic aquatic species (CABIN 2018).

Results from the DNA analyses for the six sites showed similar occurrences of key families when compared to taxonomic identification (Figures 12 and 13), particularly the orders Diptera (flies), Odonata (dragonflies and damselflies), Ephemeroptera (mayflies) and Trichoptera (caddisflies) and other families

within the Class Insecta. The diversity of these groups and others are important because they provide a food source for other wetland vertebrate species and act as biological indicators for wetland health and productivity.

DNA meta-barcoding was also used to assess the occurrence of invasive species in Six Mile Slough. This was particularly important with respect to reconnecting the slough to the Kootenay River. We found that there were no occurrences of the aquatic invasive species, *Mysis relicta* (Mysid shrimp introduced to Kootenay Lake in the 70s), *Dreissena polymorpha* (Zebra mussels), or *Dreissena rostriformis bugensis* (Quagga mussels) in raw data output from Next Generation Sequencing. This result comes with the caveat that results were based on the CABIN collection protocols, selected locations, wadable depths, seasonality, and time of day sampled. For instance, the large deep pool (lake) in Compartment 3 near SMS004 was not sampled and samples were collected during daylight hours.

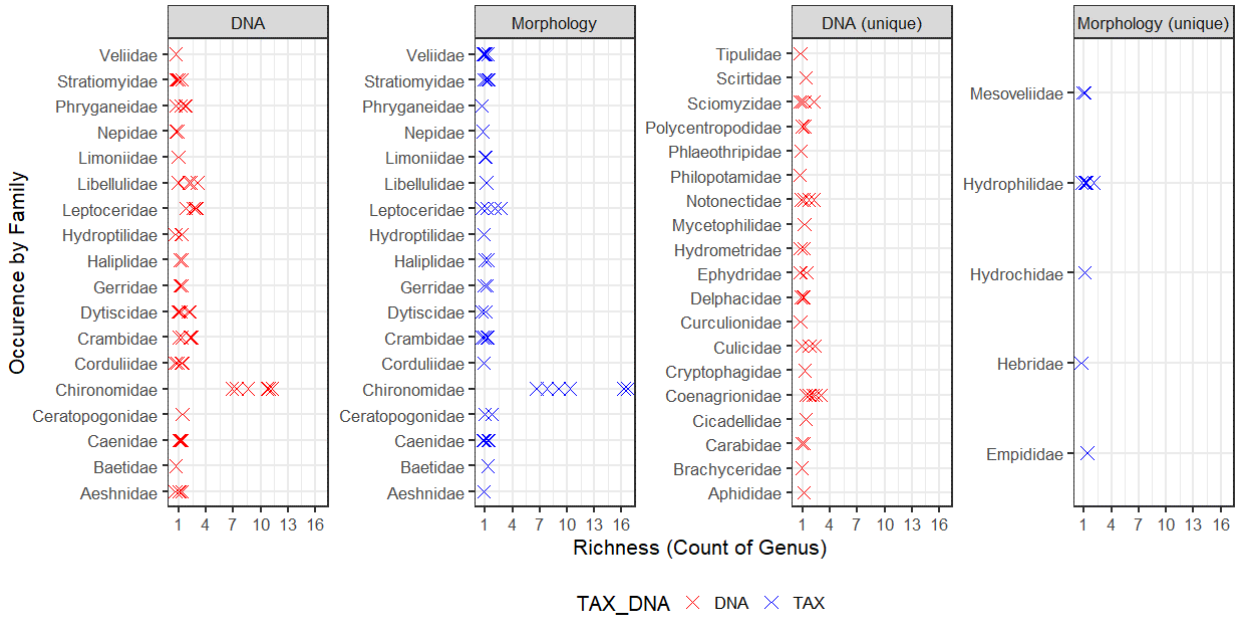


Figure 12. Richness (count of genus) by family and site within Six Mile Slough for Class Insecta. Paired samples were analyzed for DNA and traditional taxonomy (morphology), n=12 (6 paired samples x 2 methods). Facets identify cases where both DNA and traditional taxonomy methods detected similar occurrences within a family and unique detections by method (indicated by unique).

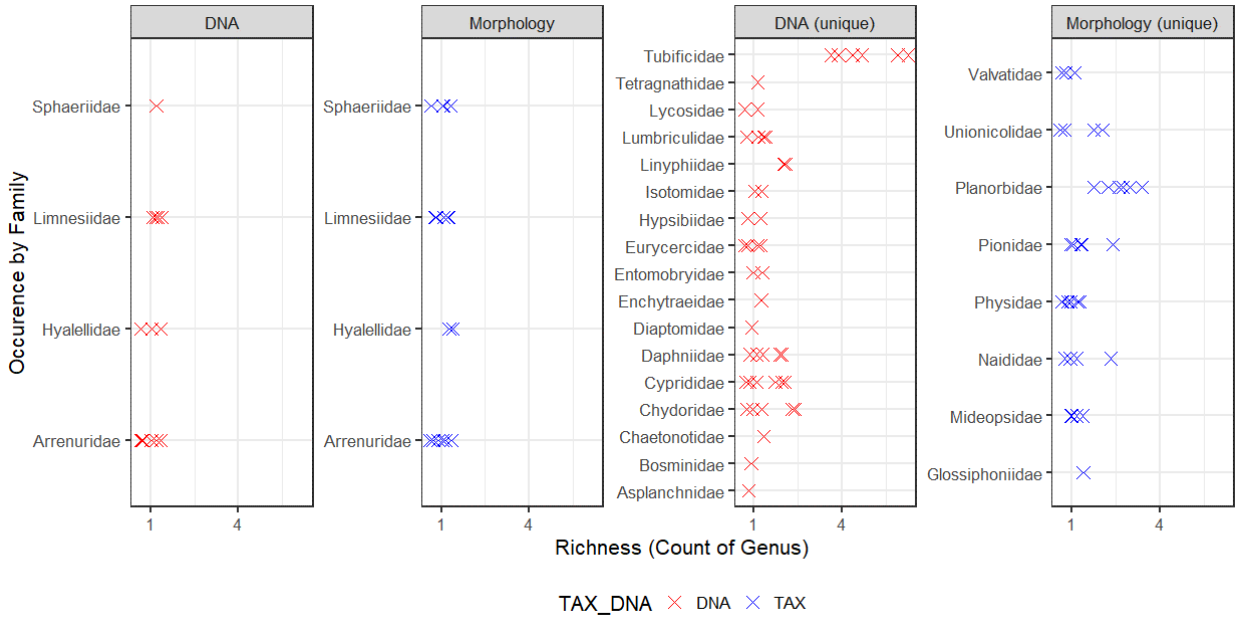


Figure 13. Richness (count of genus) by family and site within Six Mile Slough for other groups including: Ostracods, Cladocerans, Copepods, Bivalves, Gastropods, Amphipods and Arachnids. Paired samples were analyzed for DNA and traditional taxonomy (morphology), n=12 (6 paired samples x 2 methods). Facets identify cases where both DNA and traditional taxonomy methods detected similar occurrences within a family and unique detections by method (indicated by unique).

2.2.4.3 Review of paired samples

We used DNA meta-barcoding in conjunction with traditional morphology as an effective monitoring approach that will allow for future detection and changes in biodiversity due to the re-establishment of the natural water regime within Six Mile Slough.

Paired samples were analyzed using traditional taxonomy based on morphology and DNA meta-barcoding including 6 paired locations for the two analytical methods for a total of twelve samples. Morphological methods use CABIN protocols (2014) and follow Level 2 Standard Taxonomic Effort (STE) for Pacific Northwest Freshwater Macroinvertebrate Samples (2013) (also see Section 5.1.2 for Rhithron's Technical report) while analytical methods from DNA metabarcoding are reviewed in detail in the STREAM (2019) companion report. We examined richness at the genus level grouped by family to compare the two different taxonomic techniques and to evaluate how best to use both techniques in paired statistical analyses as further data accumulates in future years.

In total, using both methods there were seventy-one unique families identified with occurrences of at least one genus in Six Mile Slough. DNA meta-barcoding identified from 58 families and while morphology identified 35 families.

Richness (count of genus) was calculated for family and site (Figures 12 and 13). There were 18 families in the Class Insecta and 3 families in "Other groups" that were detected by both methods for at least one

site within Six Mile Slough. In addition, there were 19 families in the Class Insecta and 17 families from other groups that were detected by DNA meta-barcoding only. Finally, there were 8 families in the Class Insecta and 5 families from other groups that were detected by only by morphology.

The families detected by both traditional taxonomy and DNA methods at 12 sites included the: (1) true bugs, Velidae (broad-shouldered water striders), Nepidae (water scorpian), Dytiscidae, Gerridae, (2) flys, Stratiomyidae (soldier fly), Limoniidae, Ceratopogonidae, Chironomidae (midge), (3) caddisflys, Phryganeidae (giant casemakers), Hydroptilidae (micro-caddis), (4) dragonflys, Lebellulidae, Corduliidae, Aeshnidae. (5) mayflys, Leptoceridae, Caenidae, Baetidae (6) beetles, Haliplidae (crawling water beetle). Four non-insect families were identified by both DNA and morphology including the: sphaerid clam (Sphaeriidae), aquatic mites (Limnesiidae, Arrenuridae) and amphipod (Hyallelidae).

Three insect families were only identified to family using morphology which typically occurs with small or damaged individuals. These families were Notonectidae (backswimmer), Culicidae (mosquitoes), and Ephrydidae (shorefly). However, Figures 11 and 12 only show instances where individuals within these Families were identified to genus.

Other variance around the number of aquatic families identified either only by DNA or morphology were likely due to natural differences between paired samples because of the low occurrence of the following families: Tipulidae (craneflies), Scirtidae (marsh beetle), Sciomyzidae (marshfly), Polycentropodidae (trumpet net caddisfly), Philopotimidae (fingernet caddis), Hydrometridae (marsh treader), Coenagrionidae (narrow winged damsel), Mesovelidae (water treader), Hydrophilidae (water scavengers), Hydrochidae, Hebridae (Velvet water bug) and Empididae (aquatic dance flies) (Figures 12 and 13).

Some taxa were not identified by morphology but were identified by DNA meta-barcoding because of CABIN and STE protocols do not include terrestrial species. In Six Mile Slough, this included terrestrial insects such as thrips (Phlaeothripidae), fungus gnat (Mycetophilidae), planthopper (Delphicidae), semi-aquatic true weevil (Curculionidae), silken fungus beetle (Cryptophagidae), leaf hopper (Cicadellidae), ground beetle (Carabidae), weevil (Brachyceridae), aphids (Aphidae), and semi-aquatic moth (Crambidae) (Figure 12). It also included terrestrial arachnids including long-jawed orb weaver (Tetragnathidae), wolf spider (Lycosidae), dwarf spider Linyphiidae (Figure 13).

Many non-insect groups identified by DNA were not identified to genus by morphology because of the instructions by CABIN and Level 2 STE protocols for morphological-based taxonomy. This included (1) springtails, Isotomidae (long-bodied springtail), Entomobryidae (slender springtails), (2) tardigrades, Hypsibiidae (water bears), and (3) crustaceans, Eurycercidae (cladoceran), Diaptomidae (pelagic copepod), Daphniidae (cladoceran), Cypridae (ostracod), Chydoridae (cladoceran), Bosminidae (cladoceran) and (4) Chaetonidae (gastrotricha),and (5) Asplanchidae (rotifer).

There were also a number of groups that were identified using morphology but not by DNA meta-barcoding including: (1) the gastropods, Valvatidae (valve snail), Planorbidae (ramshorn snails), and Physidae (bladder snail), (2) aquatic mites (Unionicolidae, Pionidae, and Mideopsidae) and (3) jawless

leeches (Glossiphonidae). This may be due to the lack of markers for these groups or the presence of species of these families in the reference library. This may be due to the data quality filtering process, which removes poor quality data/identifications to ensure a high 95-99% accuracy of identifications across all taxonomic levels. Additionally, for these groups or the presence of species of these families, there may not be any reference DNA in the reference libraries that are used to classify the sequences (pers. com Chloe Robinson).

In addition, the genus Dero sp. was classified as Naididae using STE taxonomic methods in morphological reporting but was reported as Tubificidae which is an older classification of the genus in DNA outputs. Standard Taxonomic Effort was used to harmonize data for all analyses in the present report. There is also error within both methods that may account for some differences. The family Lumbriculidae, small aquatic worms, was identified by DNA methods but not by morphology and is a case that requires follow up by taxonomists and genomic specialists to determine why this may occur.

Finally, when DNA meta-barcoding was reduced and subjected to Level 2 STE rules, then both methods identified twenty-two families in common with morphology identifying seven unique families and DNA metabarcoding identifying eight unique families. Families within the Class Insecta would be most applicable for paired statistics using both traditional taxonomy and DNA-metabarcoding.

2.1 Conclusions and recommendations

This study is the first project in BC to use both traditional taxonomy and DNA meta-barcoding of macroinvertebrates to assess restoration and management actions using the CABIN for wetlands protocols. This data will be used to effectively evaluate pre-restoration years (2019 and 2020) compared to post-restoration trends (2021-).

We quantified the numerical abundance and diversity of taxa within the wetland. Traditional taxonomy and DNA meta-barcoding together identified seventy-one unique macroinvertebrate families in Six Mile Slough. Our analyses suggested that Six Mile Slough may be relatively high in the “kinds” of taxa relative to other wetlands in the West Kootenays but may be lower in numerical abundance of these taxa. A likely cause of this observation is the in-filling by *Typha latifolia* in Six Mile Slough, the high density of thick root mats resulting in the lack of plant diversity. *Typha* has been shown to be associated with lower densities and biomass of macroinvertebrates and reduced macroinvertebrate habitat quality (Lawrence et al 2016).

In addition, common dominants such as sedges (*Carex sp.)* have reduced seedling survival in stable wet conditions such as observed in Six Mile Slough which favor vegetative propagation by *Typha latifolia* (Bansal et al. 2019, Hall and Zedler 2010). Sedges comprised <1% of the composition of CABIN plots in Six Mile Slough. The reduced numbers aquatic macroinvertebrates could have negative effects on higher trophic-levels and important wildlife species that inhabit Six Mile Slough. Pre-restoration monitoring (2019 and 2020) of the abundance and biodiversity of invertebrates will provide a basis for evaluation of change due to restoration of the natural flooding regime in plant and macroinvertebrate communities over time.

DNA meta-barcoding provided a list of indicator aquatic organisms present at the species level within the wetland but also a list of semi-aquatic and terrestrial species associated with the marsh. DNA meta-barcoding also allowed us to make a preliminary assessment of aquatic invasive invertebrate species. We found that there were no occurrences of the following aquatic invasive species, *Mysis relicta* (mysid shrimp introduced to Kootenay Lake in the 70s), *Dreissena polymorpha* (Zebra mussels), or *Dreissena rostriformis bugensis* (Quagga mussels). Paired samples using traditional taxonomy and DNA will help us understand the patterns of biodiversity within Six Mile Slough, particularly as restoration actions proceed.

Currently, there is meager coverage of wetland invertebrate species in the Interior BC as well as across Canada (pers. com. C. Copely). Filling gaps in knowledge on the occurrence of species from sensitive freshwater wetland habitats is a high priority of both the Royal BC Museum and the STREAM project because wetlands are at the forefront of ecological change with regards to climate warming. We submitted voucher samples from traditional taxonomy and the results from DNA meta-barcoding to the Royal BC Museum in Victoria, BC for their collection and records. Voucher samples were also inspected in a tour of the Royal BC Museum in September 2019.

A key advantage to using the CABIN methods to assess wetland response to restoration is the inference these indicators provide to wildlife populations and habitat which may be difficult to assess directly because of appropriate scale and population movements. The composition and abundance of macroinvertebrates integrates effects on aquatic biota over time and can capture evidence of multiple disturbance events, termed the Integrative Ecological Condition (CABIN 2019).

The current monitoring complements pre-restoration amphibian and fisheries assessments carried out of Six Mile Slough. For instance, water and sediment quality indicated non-significant metals contamination and low impacts from anthropogenic activity at breeding locations for the Northern Leopard frog. However, *Typha latifolia* appears to be limiting the abundance of macroinvertebrates within Compartments 4 and 5 at sites near the breeding locations for Northern Leopard frogs as indicated by surveys and mapping. At this threshold of infilling, invasive *Typha* may also restrict the extent of possible breeding locations or reduce habitat for larval development, and movement (reviewed in Bansal et al. 2019). In addition, observational experience with previous drawdown and mechanical management in *Typha* within breeding areas of Northern Leopard Frog at other locations within the CVWMA have appeared to improve breeding success (pers. com. Marc-Andre Beaucher). The reconnection of compartments 4 and 5 to the floodplain is not planned at this time to maintain remaining breeding and rearing locations for Northern Leopard frog. These compartments will also serve as reference sites for restoration work.

One of the goals of the project is to re-establish the natural hydrological regime and restore natural floodplains which historically existed in the valley. It is expected that re-establishing the natural hydrological regime should reduce dense stands of *Typha* over the long-term (Biebighauser and Annschild, 2016) and increase the diversity of plant species within Compartments 2 and 3. Increasing plant and

structural heterogeneity within these compartments will likely increase macroinvertebrate abundance as well as improve habitat for higher trophic levels over a long-term time scale (Bansal et al 2019).

Importantly, reconnection to the mainstem Kootenay River will improve key habitat that will benefit all native fish species including sturgeon and burbot (SARA 2020, EKBSWG 2019). Long-term projections suggest that the restored compartments (ponds 2 and 3) in Six Mile Slough will have higher nutrient levels, warmer spring temperatures, and higher primary and secondary productivity than the mainstem river. Increased off-channel habitat and the downstream effects of floodplain reconnection could address an important bottleneck to the burbot population in the lower Kootenay River (pers com. Valerie Evans). Restoration of Six Mile Slough could provide additional early food resources at this time of year to help establish self-sustaining burbot populations.

Water quality monitoring in July 2019 demonstrated that nitrogen and phosphorus and dissolved organic carbon concentrations were higher in Six Mile Slough than Kootenay River. The total nitrogen was 4.7 times higher in Six Mile Slough than in the Kootenay River when measured while the total phosphorus was 4.0 times higher in Six Mile Slough than in the Kootenay River. In addition, dissolved organic carbon was 3.6 times higher in Six Mile Slough than the Kootenay River. Water and sediment quality indicated low impacts from metals contamination at all sites. We plan to track these parameters over at least three years to assess restoration activities and improvements relative to these baseline conditions.

Reconnection of the floodplain to compartments 2 and 3 will result in wetland inundation at freshet by oligotrophic riverine water from the mainstem Kootenay River. A hydrological assessment of the wetland reconnection would help to assess the percent of riverine water potentially moving off-channel at different peak flows. Intermediate water quality values will likely predominate during the freshet as water from the river and wetland mix, particularly, on the rising limb to the peak of the hydrograph (Scott et al 2014). As the flows decline and the wetland becomes isolated form the river, reducing conditions should eventually re-establish resulting in a return to mesotrophic conditions as total nitrogen, phosphorus, and dissolved organic carbon are solubilized. This process is dependent on the restoration design details, seasonal climate and the extent and timing of river-floodplain connectivity for varying peak flows.

We used existing provincial TEM methods to quantify 100 m buffer areas for the biomonitoring sites and Northern Leopard frog breeding sites as wells as the entire wetland. Populations of Northern Leopard frogs that breed within Six Mile Slough are likely affected by immigration and emigration to and from the wetland on a larger scale than documented here. Mapping and monitoring of these migration corridors are likely important to Northern Leopard frog populations in Six Mile Slough and recommended for future work.

Reconnection of the Six Mile Slough to the Kootenay River will result in greater hydrological connectivity between Six Mile Slough and the Kootenay River, particularly at peak flows. This could result in downstream export of nutrients and carbon in dissolved and particulate forms (Scott et al. 2014). Planned

hydrological monitoring would help the evaluation of the potential for delivery of particulates, nutrients, and carbon to the Kootenay River if this information is prioritized under current funding.

- Understanding the percent of river water moving into and out of the wetland at various stage levels based on reconnection design would be particularly useful both at peak and low flow. For instance, nutrient (Scott et al. 2014) or carbon concentrations may be solubilized if there is seasonal isolation between riverine and wetland habitats that promotes reducing conditions within the wetland while delivery mechanisms to Kootenay River would occur at peak flows.
- Calculation of nutrient loadings within Kootenay River at key locations may also aid this evaluation if this is a project priority.

If it is a priority to understand the ecological implications and transport dynamics of nutrients, carbon and particulates following restoration of lentic floodplain habitat such as Six Mile Slough to the Kootenay River then the following are recommended for evaluating downstream effects.

- Further work and planning regarding the downstream effects of reconnection of Six Mile Slough to the Kootenay River could involve greater water quality sampling and downstream evaluation of nutrient spiralling during peak flows and trophic-level status throughout the growing season.
- Work up of Environment Canada time-series data from Kootenay River at Creston would also be useful as well as downstream monitoring.
- Further review of nutrient levels and trophic-level comparisons to the South Arm of Kootenay Lake nutrient monitoring station may provide further inference.
- It might be wise to also consider how the large pool or small lake near the potential site of reconnection (near site SMS004, Figure 1 and 2) may act as a settling pond for sediments entrained in floodwaters, thus, potentially counteracting particulate and nutrient delivery to the Kootenay River at peak flow.

Pre- and post- restoration monitoring of large-scale restoration projects is important to inform management actions using an iterative “adaptive management process” (Stelk et al 2017). It is crucial to continue monitoring the same specific variables over time to evaluate trend and ecological response to management actions. The indicators established in the current report will inform the process of adaptive management over time so that the Creston Valley Wetland Management area can make small iterative adjustments and carry out effective restoration and long-term management of these globally important wetlands.

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5 Appendices

5.1 Mapping: Disturbance

Typical disturbance codes found in reference wetlands the West Kootenays

Disturbance	Disturbance codes		
Biotic effects	B	b	b. beaver tree cutting
		d	d. domestic grazing/browsing
		v	Aggressive vegetation (Canary Reed grass) or other
Forest Harvesting	L	l	l. land clearing (includes abandoned agriculture)
Plant or site modification effects	M	g	g. seeded or planted to grasses
		i	i. irrigation
Soil disturbance	S	a	a. cultivation (agricultural)
		f	f. sidecast/fill
		r	r. road bed, abandoned
		t	t. railway, abandoned
		e	e. excavation
Water-related effects	W	d	d. water table control (diking, damming) ** project specific code to include ditching
		i	i. inundation (including temporary inundation resulting from beaver activity)
		s	s. temporary seepage (artificially induced; excludes intermittent seepage from climatic conditions)
Miscellaneous	X		undefined (just X)
		.r	road * project specific code
		.b	buildings (residential, farm, etc.) Lawn and out-buildings *project specific

5.2 Physiochemistry

5.2.1 Water quality guidelines for BC and the Canadian Council of Ministers

Analyte	Units	BC WQG Max A Life	BC WQG 30-day	BC WQG Wildlife	BC Drinking WQG Max	WQ CCME Short term	WQ CCME Long term
Chloride	mg/L	600	150	600	250	640	120
Nitrate as N	mg/L	32.8	3	100	10	550	13
Nitrite as N	mg/L	0.06 when Cl^-≤ 2, 0.12 when Cl^- 2- 4, 0.6 when Cl^->10	0.02 when Cl^- ≤ 2, 0.04 when Cl^- 2- 4, 0.2 when Cl^->10	10	1		60
Sulfate	mg/L	100 [1]	Sulfate calc[1]		500		
Alkalinity, Total as $CaCO_3$	mg/L		<10 when Ca2+<4, 10-20 when Ca2+ 4-8, >20 when Ca2+ >82				
Ammonia-N	mg/L	3.62 - 23.8 temp & pH			None		3.62-23.8 temp & pH
pH	pH units	6.5-9.0					
Nitrate+Nitrite	mg/L				10		
Aluminum, total (diss)	mg/L	0.1		5 (total)	0.2		0.005 if pH <6.5, 0.1 if pH>6.5
Antimony	mg/L	0.009 (Sb III only)[1]					
Arsenic, total	mg/L		0.005	0.025[2]	0.025		0.005
Barium, total	mg/L		1				
Beryllium, total	mg/L		0.13				
Boron, total	mg/L		1.2	5	5	29	1.5
Cadmium, total	mg/L	Cd calc[2]	Cd calc[3]			0.001	0.00009
Chromium, total	mg/L		0.009 (III), 0.001 (VI)				
Cobalt, total	mg/L	0.11	0.004				
Copper, total	mg/L	Cu Calc[4]	≤2 hardness ≤ 50 Cu Calc[5] if Hardness ≥ 50	300			Cu Calc[6]
Iron, total	mg/L	1				0.3	

[1]Sulphate 128mg/L if hardness(0-30), 218 if hardness=(31-75), 309 if hardness=(76-180), 49 if hardness=(181-250)

[2]WQG Cd(mg/L) = $e^{[1.03 \times \ln(\text{hardness}^{**}) - 5.274]}$/1000, short-term max

[3]WQG Cd(mg/L)= $e^{[0.736 \times \ln(\text{hardness}^{*}) - 4.943]}$/1000, long-term average

[4]WQG Cu (mg/L)≤ (0.094 hardness(mg/L) + 2)/1000

[5]WQG Cu (mg/L) ≤ 0.04 (mean hardness)/1000

[6]When the water hardness is 0 to < 82 mg/L, the WQG is 0.002 mg/L, At hardness ≥82 to ≤180 mg/L, WQG (mg/L) =(0.2 * $e^{[0.8545[\ln(\text{hardness})]-1.465]}$)/1000, At hardness >180 mg/L, the WQG is 0.004 mg/L

Water quality guidelines for British Columbia, BC, and the Canadian Council of Ministers (CCME) continued

Analyte	Units	BC WQG Max Aquatic Life	BC WQG 30-day	BC WQG Wildlife	BC Drinking WQG Max	WQ CCME Short term	WQ CCME Long term
Lead, total	mg/L	Pb Calc[7]	≤2Hardness ≤ 50 Pb Calc[8] If Hardness ≥ 50			Pb Calc[9]	
Manganese, total	mg/L	Mn Calc[10]	Mn Calc[11]			Mncalc[12]	Mncalc[13]
Molybdenum, total	mg/L	2	<1	0.05		0.73	
Nickel, total	mg/L		0.025 Hardness 0-60mg/L 0.110 >60 ≤180mg/L 0.150 >180mg/L				Ni Calc[12]
Phosphorus, total	mg/L		0.015 for lakes				
Potassium, total	mg/L						
Selenium, total	mg/L	0.001 (alert) 0.002			0.002 (water), 0.006 (bird egg)	0.01	
Silver, total	mg/L	$0.0001_{Hardness\ \leq 100mg/L}$ $0.003_{>100mg/L}$	0.0015 Hardness ≤100mg/L 0.005Hardness>100mg/L				
Thallium, total	mg/L		0.008 [13]				0.0008
Uranium, total	mg/L					0.033	0.015
Zinc, total	mg/L	0.0075 Hardness ≤90mg/L Zncalc>90mg/L[15]	0.033 Hardness ≤90mg/L Zncalc>90mg/L[16]			Zncalc[17]	Zncalc[18]

[7]WQG Pb (mg/L) ≤ $e^{[1.273\ ln\ (hardness^*)\ -1.460]}/1000$

[8]WQG Pb (mg/L) ≤≤ 3.31 + $e^{[1.273\ ln\ (hardness^*)\ -\ 4.704]}/1000$

[9]When hardness is 0 to ≤ 60 mg/L, the WQG is 0.001 mg/L, At hardness >60 to ≤ 180 mg/L WQG (mg/L)= $(e^{\{1.273[ln(hardness)]-4.705\}})/1000$, At hardness >180 mg/L, the CWQG is 0.007 mg/L

[10]Mn Calc (mg/L) ≤ 0.01102* hardness+ 0.54/1000

[11]Mn Calc(mg/L)≤ 0.0044* hardness + 0.605/1000

[12]Mn Calc (ug/L)= exp(0.878[ln(hardness)] + 4.76) where the benchmark is expressed in dissolved manganese concentration (go/L), and hardness is measured as CaCO3 equivalents in mg/L.

[13]The CWQG for manganese (i.e. long-term guideline) is found using the CWQG calculator in Appendix B of the Scientific Criteria Document for the Development of the Canadian Water Quality Guidelines for the Protection of Aquatic Life: Manganese.

[14]When the water hardness is 0 to ≤ 60 mg/L, the WQG is 0.025 mg/L , At hardness > 60 to ≤ 180 mg/L WQG Ni (mg/L)=$(e^{\{0.76[ln(hardness)]+1.06\}})/1000$

At hardness >180 mg/L, the WQG is 0.150 mg/L

[15]WQG Zn (mg/L) ≤33 + 0.75(hardness - 90)/1000

[16]WQG Zn (mg/L)≤ 7.5 + 0.75 (hardness - 90))/1000

[17]Zncalc (ug/L) =exp(0.833[ln(hardness mg/L)] + 0.240[ln(DOC mg/L)] + 0.526). For 50 mg CaCO3/L hardness and 0.5 mg/L dissolved organic carbon (DOC). Valid between hardness 13.8 and 250.5 mg CaCO3/L and DOC 0.3 and 17.3 mg/L.

[18]Zncalc (ug/L) = exp(0.947[ln(hardness mg/L)] - 0.815[pH] + 0.398[ln(DOC mg/L)] + 4.625). For 50 mg CaCO3/L hardness, pH of 7.5 and 0.5 mg/L DOC. Valid between hardness 23.4 and 399 mg CaCO3/L, pH 6.5 and 8.13 and DOC 0.3 to 22.9 mg/L.

5.2.2 Raw water quality data analyzed by CARO Analytical Services

			SMS001	SMS002	SMS003	SMS004	SMS005	SMS006	SMS005-2 (	KR001	KR002
			2019-07-29	2019-07-29	2019-07-29	2019-07-30	2019-07-30	2019-07-30	2019-07-30	2019-07-29	2019-07-29
			2019-08-01	2019-08-01	2019-08-01	2019-08-01	2019-08-01	2019-08-01	2019-08-01	2019-08-01	2019-08-01
Analyte	Units	MRL	Water	Water	Water	Water	Water	Water	Water	Water	Water
Chloride*	mg/L	0.1	0.15	0.22	0.26	0.59	0.32	0.33	0.29	1.79	1.77
Nitrate (as N)*	mg/L	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.073	0.067
Nitrite (as N)*	mg/L	0.01	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010
Phosphate (as P)	mg/L	0.002	0.0067	0.0057	0.0031	0.0049	0.0042	0.0023	0.0025	0.0022	<0.0020
Sulfate*	mg/L	1	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	18.6	18.7
Hardness, Total (as CaCO3)	mg/L	0.1	115	70.5	117	78.8	189	121		99.5	99.2
Nitrate+Nitrite (as N)	mg/L	0.01	<0.0100	<0.0100	<0.0100	<0.0100	<0.0100	<0.0100	<0.0100	0.0732	0.0667
Nitrogen, Total	mg/L	0.05	1.4	0.868	0.606	0.782	0.811	0.551	0.767	0.171	0.179
Nitrogen, Organic	mg/L	0.05	1.32	0.77	0.526	0.657	0.713	0.375	0.684	<0.0500	<0.0500
Alkalinity, Total (as CaCO3)*	mg/L	1	117	72	124	89.7	208	140	206	91	91.7
Alkalinity, Phenolphthalein (a	mg/L	1	<1.0	<1.0	6.6	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Alkalinity, Bicarbonate (as CaC	mg/L	1	117	72	111	89.4	208	140	206	91	91.7
Alkalinity, Carbonate (as CaCO	mg/L	1	<1.0	<1.0	13.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Alkalinity, Hydroxide (as CaCO	mg/L	1	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Ammonia, Total (as N)*	mg/L	0.02	0.079	0.098	0.08	0.125	0.098	0.176	0.083	0.09	0.115
Carbon, Total Organic	mg/L	0.5	11.3	8.68	6.85	5.94	13.3	5.64		1.45	3.25
Carbon, Dissolved Organic	mg/L	0.5	10	8.41	6.51	5.42	12.2	5.37	12.3	1.24	1.34
Nitrogen, Total Kjeldahl	mg/L	0.05	1.4	0.868	0.606	0.782	0.811	0.551	0.767	0.098	0.112
Nitrogen, Dissolved Kjeldahl	mg/L	0.05	0.69	0.659	0.567	0.452	0.622	0.392	0.696	0.084	<0.050
Phosphorus, Total (as P)*	mg/L	0.002	0.0669	0.0318	0.021	0.0431	0.0296	0.019	0.0272	0.0061	0.011
Phosphorus, Total Dissolved	mg/L	0.002	0.0218	0.0089	0.0112	0.0109	0.0104	0.0118	0.0121	0.0038	0.0036
Solids, Total Suspended	mg/L	2	9.2	10.4	<2.0	8.2	4.8	<2.0	3.8	2.8	2
Turbidity	NTU	0.1	1.26	2.49	1.28	2.39	4.6	3.15	4.85	0.92	0.91
pH*	pH units	0.1	7.91	8.25	8.67	8.33	8.07	8.25	8.12	8.11	8.1
Conductivity (EC)	uS/cm	2	201	126	211	158	363	243	354	211	211
Aluminum, total*	mg/L	0.002	0.005	0.007	0.0024	0.0109	0.0028	0.002		0.0173	0.0144
Antimony, total*	mg/L	0.00005	0.000067	0.000088	0.000076	0.000074	0.000068	0.000061		0.000098	0.000096
Arsenic, total*	mg/L	0.00005	0.000805	0.00103	0.00154	0.00276	0.00213	0.00207		0.000531	0.000534
Barium, total*	mg/L	0.0001	0.027	0.0155	0.0326	0.0147	0.0486	0.0316		0.0386	0.0391
Beryllium, total*	mg/L	0.00001	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010		<0.000010	<0.000010
Bismuth, total	mg/L	0.00001	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010		<0.000010	<0.000010
Boron, total*	mg/L	0.002	0.006	0.0062	0.0034	0.003	0.003	0.0039		0.0046	0.0048
Cadmium, total*	mg/L	0.000002	0.000003	0.0000029	0.0000022	0.0000029	<0.0000020	<0.0000020		0.0000088	0.0000081
Calcium, total	mg/L	0.04	29	13.1	24.6	12.6	47.8	27.9		26.3	26.1
Chromium, total*	mg/L	0.0001	0.00024	0.0002	0.00021	0.00027	0.00023	0.00026		0.00034	0.00029
Cobalt, total*	mg/L	0.000005	0.0000967	0.0000479	0.0000652	0.0000552	0.000118	0.0000307		0.0000429	0.0000376
Copper, total*	mg/L	0.0002	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020		0.00042	0.00041
Iron, total*	mg/L	0.002	0.425	0.313	0.304	0.286	1.63	0.618		0.0403	0.0367
Lead, total*	mg/L	0.00005	0.000078	0.000082	0.000052	0.000212	0.000063	<0.000050		0.000158	0.000159
Lithium, total	mg/L	0.00005	0.0022	0.0024	0.00314	0.00249	0.00376	0.00279		0.00181	0.00184
Magnesium, total*	mg/L	0.005	10.4	9.16	13.5	11.5	16.9	12.3		8.18	8.26
Manganese, total*	mg/L	0.00005	0.0824	0.0573	0.00718	0.0695	0.101	0.0152		0.00528	0.00542
Molybdenum, total	mg/L	0.00001	0.000047	0.00021	0.000084	0.000069	0.000076	0.000081		0.000569	0.000573
Phosphorus, total	mg/L	0.01	0.091	0.035	0.028	0.043	0.028	0.021		<0.010	<0.010
Potassium, total	mg/L	0.01	0.226	0.203	0.786	2.62	0.639	1.77		0.51	0.516
Selenium, total*	mg/L	0.0001	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010	<0.00010		0.00078	0.00078
Silicon, total	mg/L	0.1	5.92	5.48	9.72	7.77	11.1	10.1		2	1.96
Silver, total*	mg/L	0.00001	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010		<0.000010	<0.000010
Sodium, total	mg/L	0.02	0.388	1.7	2.43	3.85	4.39	3.64		2.67	2.68
Strontium, total	mg/L	0.0001	0.0954	0.045	0.111	0.0625	0.177	0.118		0.106	0.106
Sulfur, total	mg/L	1	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0		6.5	6.7
Tellurium, total	mg/L	0.00005	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050	<0.000050		<0.000050	<0.000050
Thallium, total	mg/L	0.000004	<0.0000040	<0.0000040	<0.0000040	<0.0000040	<0.0000040	<0.0000040		<0.0000040	0.0000042
Thorium, total	mg/L	0.00001	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010	<0.000010		<0.000010	<0.000010
Titanium, total	mg/L	0.0002	<0.00020	0.0003	<0.00020	0.00039	<0.00020	<0.00020		0.00065	0.00036
Tungsten, total	mg/L	0.0002	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020		<0.00020	<0.00020
Uranium, total	mg/L	0.000001	0.0000284	0.0000432	0.000133	0.0000538	0.0000131	0.0000384		0.000617	0.000619
Vanadium, total	mg/L	0.0002	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020	<0.00020		<0.00020	<0.00020
Zinc, total*	mg/L	0.001	0.0013	0.001	<0.0010	0.001	<0.0010	<0.0010		<0.0010	<0.0010
Zirconium, total	mg/L	0.00002	<0.000020	<0.000020	<0.000020	<0.000020	<0.000020	<0.000020		0.000021	<0.000020
Nickel, total*	ug/L	0.04	0.119	0.127	0.132	0.084	0.086	0.067		0.213	0.201
Tin, total	ug/L	0.05	0.053	<0.050	<0.050	<0.050	<0.050	<0.050		<0.050	<0.050

Starred parameters were evaluated against the BC water quality guidelines and Canadian Council of Ministers (CCME) for water. Numbers highlighted in grey exceeded at least one criterion. Lower long-term criteria (5 sample monitoring requirement) were used as an alert rather than a true evaluation.

5.2.3 The number of water quality parameters exceeding at least one guideline

Site	% of parameters exceeding guidelines	Exceedance (source*)
SMS001	4.3	Iron (aq. Life CCME) Nickel (aq. Life CCME)
SMS002	4.3	Iron (aq. Life CCME) Nickel (aq. Life CCME)
SMS003	4.3	Iron (aq. Life CCME) Nickel (aq. Life CCME)
SMS004	0	
SMS005	2.1	Iron (aq. Life WQG & CCME)
SMS006	4.3	Ammonia (aq. Life and WQG) Iron (aq. Life CCME)
KR001	2.1	Nickel (aq. Life CCME)
K2002	2.1	Nickel (aq. Life CCME)

Legend:
*Source:
aq. Life CCME= Canadian Water Quality Guidelines
aq. Life CCME*= Canadian Water Quality Guidelines long term
aq. Life WQG = BC Water Quality Guidelines
aq. Life WQG* = BC Water Quality Guidelines long term
WQGwild = BC Water Quality Guidelines for wildlife
BCdw = BC Drinking Water Guidelines

5.2.4 Sediment guidelines for British Columbia and the Canadian Council of Ministers

		Sediment			
Analyte	**Units**	**BC SQ**	**BC SQ PEL**	**CCME ISQG**	**CCME PEL**
Arsenic	mg/kg dry	5.9	17		
Cadmium	mg/kg dry	0.6	3.5	0.6	3.5
Chromium	mg/kg dry	37.3	90	37.3	90
Copper	mg/kg dry	35.7	197	35.7	197
Iron	mg/kg dry	21,200	43,766		
Lead	mg/kg dry	35	91.3	35	91.3
Manganese	mg/kg dry	460	1100		
Mercury	mg/kg dry	0.17	0.486		
Nickel	mg/kg dry	16	75		
Selenium	mg/kg dry	2			
Silver	mg/kg dry	0.5			
Zinc	mg/kg dry	123	325	123	315

SQ=sediment quality, ISQG=Interim Sediment Quality Guidelines., PEL=Probable effects levels

https://catalogue.data.gov.bc.ca/dataset/water-quality-guidelines-of-b-c-

5.2.5 Raw sediment quality data analyzed by CARO Analytical Services

			9080079-02	9080079-04	9080079-06	9080079-08	9080079-10	9080079-12	9080079-14
			SMS001	SMS002	SMS003	SMS004	SMS005	SMS006	SMS005-2 (Duplicate)
			2019-07-29	2019-07-29	2019-07-29	2019-07-30	2019-07-30	2019-07-30	2019-07-29
			2019-08-01	2019-08-01	2019-08-01	2019-08-01	2019-08-01	2019-08-01	2019-08-01
		MRL	Solid	Solid	Solid	Solid	Solid	Solid	Solid
> 80 mm	%	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
> 56 mm	%	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
> 40 mm	%	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
> 25 mm	%	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
> 19 mm	%	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
> 12.5 mm	%	0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
> 4.75 mm	%	0.1	<0.1	<0.1	<0.1	<0.1	0.3	<0.1	0.2
> 2.36 mm	%	0.1	4.9	6	2	2.6	6.7	3.9	3.6
> 2.0 mm	%	0.1	3.2	3.8	1.4	1.4	3.1	2.5	2.5
> 1.18 mm	%	0.1	11.7	13.6	4.4	7.5	12.8	10.8	10.4
> 600 µm	%	0.1	14.8	16.5	6.7	7.8	15	15.6	13.7
> 425 µm	%	0.1	7.1	7.5	3.8	11.2	6.7	7.9	6.8
> 300 µm	%	0.1	6.8	6.5	4.3	6.8	6.2	7.9	6.7
> 150 µm	%	0.1	10.7	10.1	8.1	10.6	9	13.8	10.4
> 75 µm	%	0.1	8.3	8.8	14.2	8	10.1	10.5	9.9
< 75 µm	%	0.1	32.5	27.2	55	44.1	30.2	27.1	35.8
Carbon, To	% dry	0.05	24.3	5.62	4.68	6.21	6.66	4.38	6.65
Aluminum	mg/kg dry	40	9480	12300	9310	10900	11100	11200	11200
Antimony	mg/kg dry	0.1	0.44	0.5	0.58	0.8	0.58	0.83	0.75
Arsenic*	mg/kg dry	0.3	5.47	4.34	4.36	3.86	6.82	9.17	11.7
Barium	mg/kg dry	1	109	117	82.2	108	118	108	114
Beryllium	mg/kg dry	0.1	0.49	0.64	0.45	0.53	0.55	0.57	0.55
Bismuth	mg/kg dry	0.1	0.3	0.37	0.34	0.54	0.36	0.51	0.43
Boron	mg/kg dry	2	3.8	4	2.7	3	3.3	3.1	2.9
Cadmium*	mg/kg dry	0.04	0.671	0.69	0.606	0.88	0.637	0.888	0.783
Calcium	mg/kg dry	100	58300	10700	38900	57300	56300	50600	56600
Chromium	mg/kg dry	1	15.4	18.7	18.2	21.9	17.5	21.1	19.3
Cobalt	mg/kg dry	0.1	7.98	7.25	7.51	7.04	9.02	7.96	9.7
Copper*	mg/kg dry	0.4	20.8	18.2	18.3	20.6	18.2	19.7	19.7
Iron*	mg/kg dry	20	16600	19100	16100	18000	20200	20200	20600
Lead*	mg/kg dry	0.2	33.7	45.3	52.1	110	52	109	76.8
Lithium	mg/kg dry	0.1	20	25.2	18.7	22.8	22.8	23.6	24
Magnesium	mg/kg dry	10	14200	11800	16200	18200	16500	16800	18000
Manganese	mg/kg dry	0.4	275	193	242	289	479	446	365
Mercury*	mg/kg dry	0.04	0.054	0.056	0.043	0.061	0.043	0.072	0.096
Molybdenu	mg/kg dry	0.1	0.47	0.3	0.24	0.19	0.38	0.3	0.72
Nickel*	mg/kg dry	0.6	17.8	19.4	17.3	19	19.6	19.9	20.5
Phosphoru	mg/kg dry	10	702	767	673	692	657	715	691
Potassium	mg/kg dry	40	983	981	828	997	1170	1180	1220
Selenium*	mg/kg dry	0.2	0.41	0.44	0.29	0.33	0.35	0.38	0.4
Silver*	mg/kg dry	0.1	0.14	0.17	0.18	0.33	0.19	0.32	0.27
Sodium	mg/kg dry	50	169	92	101	94	99	95	106
Strontium	mg/kg dry	0.2	100	20.8	71.5	116	104	97.9	91.7
Sulfur	mg/kg dry	1000	1600	<1000	<1000	<1000	<1000	<1000	1180
Tellurium	mg/kg dry	0.1	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10
Thallium	mg/kg dry	0.1	0.12	0.1	0.12	0.14	0.13	0.14	0.16
Thorium	mg/kg dry	0.5	3.49	4.53	4.35	5.17	5.07	4.85	5.02
Tin	mg/kg dry	0.2	0.43	0.52	0.49	0.89	0.39	0.77	0.61
Titanium	mg/kg dry	1	83.3	83.4	161	154	122	124	102
Tungsten	mg/kg dry	0.2	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20	<0.20
Uranium	mg/kg dry	0.05	0.903	0.725	0.646	0.808	0.678	0.717	0.872
Vanadium	mg/kg dry	1	14.2	17.3	14.8	17.2	16.4	17.2	18.1
Zinc*	mg/kg dry	2	103	116	129	212	121	202	158
Zirconium	mg/kg dry	2	<2.0	<2.0	<2.0	2.1	<2.0	<2.0	<2.0

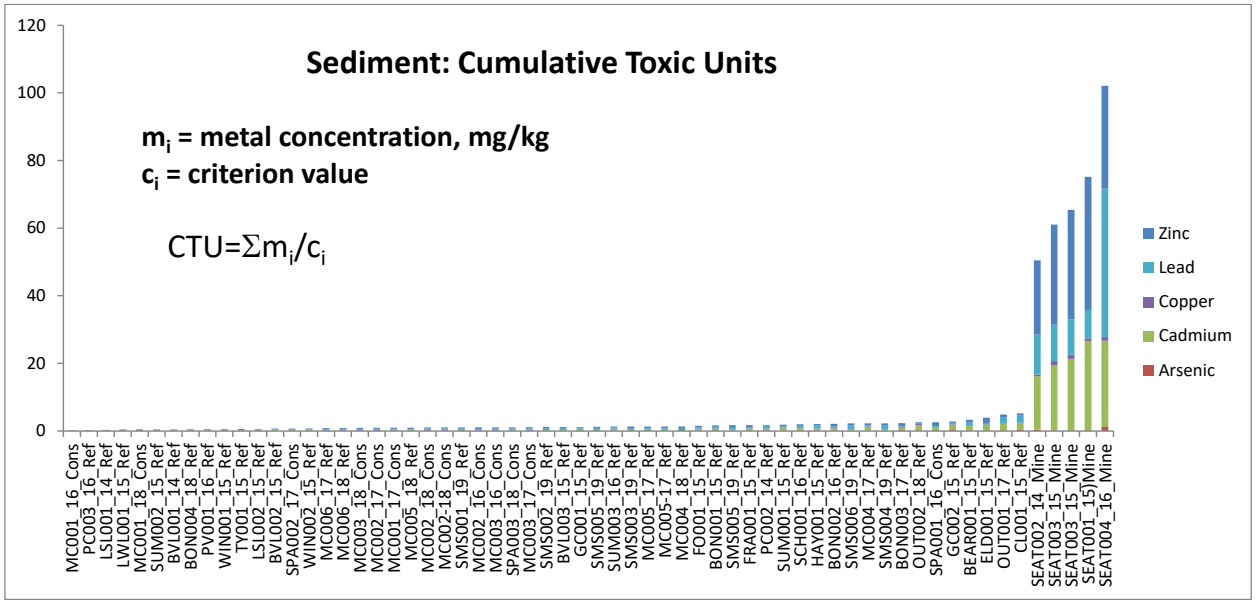
Starred parameters were evaluated against the Canadian Council of Ministers (CCME) for interim sediment quality (ISQG) high-lighted in grey adopted by BC

5.2.6 The number of sediment quality parameters greater than at least one guideline

Site	% of parameters exceeding guidelines	Exceedance (source*)
SMS001	16.6	Cadmium (aq. Life ISGQ and CCME) Nickel (aq. Life ISQG)
SMS002	25	Cadmium (aq. Life ISGQ and CCME) Lead (aq. Life ISGQ and CCME) Nickel (aq. Life ISQG)
SMS003	33.3	Cadmium (aq. Life ISGQ and CCME) Lead (aq. Life ISGQ and CCME) Nickel (aq. Life ISQG) Zinc (aq. Life ISGQ and CCME)
SMS004	33.3	Cadmium (aq. Life ISGQ and CCME) Lead (aq. Life ISGQ, PEL and ISGQ, PEL CCME) Nickel (aq. Life ISQG) Zinc (aq. Life ISGQ and CCME)
SMS005	41.6	Arsenic (aq. Life ISGQ) Cadmium (aq. Life ISGQ and CCME) Lead (aq. Life ISGQ and CCME) Manganese (aq. Life ISGQ) Nickel (aq. Life ISQG)
SMS005-2	41.6	Arsenic (aq. Life ISGQ) Cadmium (aq. Life ISGQ and CCME) Lead (aq. Life ISGQ and CCME) Nickel (aq. Life ISQG) Zinc (aq. Life ISGQ, ISGQ CCME)
SMS006	41.6	Arsenic (aq. Life ISGQ) Cadmium (aq. Life ISGQ and CCME) Lead (aq. Life ISGQ, PEL and ISGQ, PEL CCME) Nickel (aq. Life ISQG) Zinc (aq. Life ISGQ, ISGQ CCME)

Legend:
*Source: aq. Life (ISQG or PEL) CCME = Canadian Sediment Quality Guidelines
aq. Life (ISQG or PEL) = BC Sediment Quality Guidelines

5.2.7 Cumulative toxic units in sediment including sites affected by historical mining



Graph of cumulative toxic unit of zinc, lead, copper, cadmium, and arsenic in sediment including sites affected by historical mining. Two times criterion is the value above which metals levels may influence macroinvertebrate community structure and cause mortality in sensitive species. Cumulative toxic units are the sum of metals divided by the guideline (CCME PEL). Sites are indicated with Site name_Year_Type, Type = Reference (Ref), constructed wetlands (Cons), impacted by legacy mining (Mine).

5.2.8 Quality Assurance: water and sediment quality

Twenty-four of the twenty-five water quality parameters analysed from SMS005 Six Mile Slough in duplicate samples were below the RPD limit of 25% in duplicate except the parameters, Phosphate which was less than two times the method reporting level. However, phosphate was below the additional criteria that the difference between duplicates should be less than two times the method detection limit when duplicates are less than five times detection (Clark 2013).

In 2019, 52 out 54 sediment quality parameters analysed from SMS005 Six Mile Slough in duplicate samples were below the RPD limit of 25% in duplicate except the parameters, Antimony and Manganese.

5.3 Macroinvertebrates

5.3.1 Quality assurance: morphology-based taxonomy

Technical Report, Rhithron: Macroinvertebrate quality assurance procedures:
By W. Bollman, Chief Biologist, Rhithron Associates, Inc., Missoula, Montana

Sample processing: All samples arrived in good condition. A chain-of-custody document containing sample identification information was provided by the Integrated Ecological Research (IER) Project Manager. Upon arrival, samples were unpacked, examined, and checked against the IER chain-of-custody. An inventory spreadsheet was created which included project code and internal laboratory identification numbers and was uploaded into the Rhithron database prior to sample processing.

Sorting protocols consistent with CABIN standard operating procedures (Environment Canada: CABIN Laboratory Methods: Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples: May 2014) were applied to achieve representative subsamples of a minimum of 300 organisms. A Marchant Box was used for subsampling and sorting. Subsampling of each sample began with a random selection of 5 Marchant Box cells. All ostracods, copepods and cladocerans were picked from the first selected cell and placed in a separate vial; these organisms were not assigned a count and did not contribute to the 300-organism target. Subsequent sorting did not include these organisms. The initial 5 cells were completely sorted of all organisms. The contents of each grid were examined under stereoscopic microscopes using 10x-30x magnification. All aquatic invertebrates from each selected grid were sorted from the substrate and placed in 80% ethanol for subsequent identification. Grid selection, examination, and sorting continued until at least 300 organisms were sorted. If more than 50% of the sample was required to obtain the minimum 300 organism count, the entire sample was sorted. All unsorted sample fractions were retained and stored at the Rhithron laboratory.

Organisms were individually examined by certified taxonomists, using 10x – 80x stereoscopic dissecting scopes (Leica S8E) and identified to target taxonomic levels specified by the IER Project Manager, using appropriate published taxonomic references and keys. Chironomids and oligochaetes were carefully morphotyped using 10x – 80x stereoscopic dissecting microscopes (Leica S8E) and representative specimens were slide mounted and examined at 200x – 1000x magnification using an Olympus BX 51 or Leica DM 1000 compound microscope.

Identification, counts, life stages, and information about the condition of specimens were recorded on electronic bench sheets. Organisms that could not be identified to the taxonomic targets because of immaturity, poor condition, or lack of complete current regionally applicable published keys were left at appropriate taxonomic levels that were coarser than those specified. Organisms designated as “unique” were those that could be definitively distinguished from other organisms in the sample. Identified organisms were preserved in 80% ethanol in voucher labeled vials (by taxon and life stage), and shipped to the Royal BC Museum in Victoria, British Columbia.

Quality control procedures: Quality control procedures for initial sample processing and subsampling involved checking sorting efficiency. These checks were conducted on 15% of the samples (minimum of 3 samples from the project) by independent observers who microscopically re-examined sorted substrate from each sample. Quality control procedures for each sample proceeded as follows: the quality control technician poured the sorted substrate from a processed sample out and all substrate was re-examined under 10x – 30x magnification. All organisms that were missed were counted and this number was added to the total number obtained in the original sort. Sorting efficiency was evaluated by applying the following calculation, where: SE is the sorting efficiency, expressed as a percentage, n1 is the total number of specimens in the first sort, and n 2 is the total number of specimens in the second sort.

$$SE = \frac{n_1}{n_1 + n_2} \times 100$$

Quality control procedures for taxonomic determinations of invertebrates involved checking accuracy, precision, and enumeration. Three samples were randomly selected, and all organisms re-identified and counted by an independent taxonomist. Taxa lists, and enumerations were compared by calculating a Bray-Curtis similarity statistic (Bray and Curtis 1957), Percent Taxonomic Disagreement (PTD) and Percent Difference in Enumeration (PDE). Routinely, discrepancies between the original identifications and the QC identifications are discussed among the taxonomists, and necessary rectifications to the data are made. Discrepancies that cannot be rectified by discussions are routinely sent out to taxonomic specialists for identification.

Data analysis: Taxa and counts for each sample were entered into Rhithron's customized database software. A taxonomic flat file including site information, taxonomic hierarchy, taxonomic identifications, counts, life stages and other information was formatted in Microsoft Excel.

Results: Results of internal quality control procedures for subsampling and taxonomy are given in Table 1. Sorting efficiency varied from 96-100%. Taxonomic precision for identification and enumeration ranged from 96-99% (Bray-Curtis), with a range of 0.6-4% for percent taxonomic disagreement and 0-1.2% for percent difference in enumeration for the randomly selected taxonomic QC samples, and data entry efficiency averaged 100% for the project. These similarity statistics fall within acceptable industry criteria (Stribling et al. 2003). An electronic spreadsheet was provided to the IER Project Manager via e-mail. Voucher labeled vials were shipped to the Royal BC Museum.

Table 1. Results of internal quality control procedures for subsampling and taxonomy for 2019.

Rhithron ID	Station ID	Date Collected	Sorting efficiency	Bray-Curtis similarity for taxonomy and enumeration	Percent Taxonomic Disagreement (PTD)	Percent Difference in Enumeration (PDE)
IER19DQ001	SMS001	7/29/2019	0.9884	0.9739	0.0335	0.0077
IER19DQ002	SMS002	7/29/2019				
IER19DQ003	SMS003	7/29/2019	0.9879			
IER19DQ004	SMS004	7/30/2019		0.9550	0.0481	0.0032
IER19DQ005	SMS005	7/30/2019	0.9702			
IER19DQ006	SMS006	7/30/2019		0.9900	0.0133	0.0033

5.3.1 Outputs from morphology-based taxonomy

Taxonomic list and results from Rhithron Associates Inc.

Site	Class	Order	Family	Genus	Taxon	Subsample	%Sorted
SMS001	Insecta	Diptera	Chironomidae	Ablabesmyia	Ablabesmyia	1	41.00%
SMS001	Insecta	Odonata	Aeshnidae		Aeshnidae	1	41.00%
SMS001	Insecta	Odonata			Anisoptera	1	41.00%
SMS001	Arachnida	Trombidiformes	Arrenuridae	Arrenurus	Arrenurus	8	41.00%
SMS001	Bivalvia				Bivalvia	18	41.00%
SMS001	Insecta	Ephemeroptera	Caenidae	Caenis	Caenis Diminuta Gr.	51	41.00%
SMS001	Insecta	Diptera	Ceratopogonidae		Ceratopogoninae	10	41.00%
SMS001	Insecta	Odonata	Coenagrionidae		Coenagrionidae	8	41.00%
SMS001	Insecta	Hemiptera	Corixidae		Corixidae	1	41.00%
SMS001	Insecta	Diptera	Culicidae		Culicidae	1	41.00%
SMS001	Insecta	Diptera	Ceratopogonidae	Dasyhelea	Dasyhelea	2	41.00%
SMS001	Insecta	Diptera	Chironomidae	Dicrotendipes	Dicrotendipes	1	41.00%
SMS001	Insecta	Coleoptera	Dytiscidae		Dytiscidae	1	41.00%
SMS001	Insecta	Coleoptera	Dytiscidae		Dytiscidae	22	41.00%
SMS001	Insecta	Coleoptera	Hydrophilidae	Enochrus	Enochrus	5	41.00%
SMS001	Insecta	Coleoptera	Hydrophilidae	Enochrus	Enochrus	2	41.00%
SMS001	Arachnida	Trombidiformes	Pionidae	Forelia	Forelia	1	41.00%
SMS001	Gastropoda				Gastropoda	2	41.00%
SMS001	Insecta	Diptera	Chironomidae	Glyptotendipes	Glyptotendipes	1	41.00%
SMS001	Insecta	Diptera	Chironomidae	Guttipelopia	Guttipelopia	7	41.00%
SMS001	Gastropoda	Basommatophora	Planorbidae	Gyraulus	Gyraulus	102	41.00%
SMS001	Clitellata	Rhynchobdellida	Glossiphoniidae	Helobdella	Helobdella	4	41.00%
SMS001	Clitellata	Rhynchobdellida	Glossiphoniidae	Helobdella	Helobdella stagnalis	1	41.00%
SMS001	Insecta	Trichoptera	Hydroptilidae		Hydroptilidae	2	41.00%
SMS001	Insecta	Coleoptera	Dytiscidae	Ilybius	Ilybius	1	41.00%
SMS001	Insecta	Diptera	Chironomidae	Larsia	Larsia	1	41.00%
SMS001	Insecta	Diptera	Chironomidae	Lauterborniella	Lauterborniella agrayloides	2	41.00%
SMS001	Insecta	Odonata	Libellulidae		Libellulidae	1	41.00%
SMS001	Arachnida	Trombidiformes	Limnesiidae	Limnesia	Limnesia	2	41.00%
SMS001	Insecta	Diptera	Chironomidae	Microtendipes	Microtendipes Pedellus Gr.	3	41.00%
SMS001	Insecta	Hemiptera	Veliidae	Microvelia	Microvelia	11	41.00%
SMS001	Insecta	Diptera	Stratiomyidae	Odontomyia / Hedriodiscus	Odontomyia / Hedriodiscus	4	41.00%
SMS001	Insecta	Trichoptera	Leptoceridae	Oecetis	Oecetis	1	41.00%
SMS001	Insecta	Lepidoptera	Crambidae	Parapoynx	Parapoynx	1	41.00%
SMS001	Gastropoda	Basommatophora	Physidae	Physella	Physella	4	41.00%
SMS001	Arachnida	Trombidiformes	Pionidae	Piona	Piona	2	41.00%
SMS001	Gastropoda	Basommatophora	Planorbidae	Planorbella	Planorbella	1	41.00%
SMS001	Gastropoda	Basommatophora	Planorbidae		Planorbidae	6	41.00%
SMS001	Insecta	Diptera	Chironomidae	Polypedilum	Polypedilum	7	41.00%
SMS001	Insecta	Diptera	Chironomidae	Procladius	Procladius	1	41.00%
SMS001	Gastropoda	Basommatophora	Planorbidae	Promenetus	Promenetus	5	41.00%
SMS001	Insecta	Diptera	Chironomidae	Pseudochironomus	Pseudochironomus	1	41.00%
SMS001	Bivalvia	Veneroida	Sphaeriidae	Sphaerium	Sphaerium	13	41.00%
SMS001	Insecta	Diptera	Chironomidae		Tanypodinae	1	41.00%
SMS001	Insecta	Diptera			Tipuloidea	1	41.00%
SMS001	Gastropoda	Heterostropha	Valvatidae	Valvata	Valvata	2	41.00%
SMS001	Arachnida	Trombidiformes	Mideopsidae	Xystonotus	Xystonotus	3	41.00%
SMS002	Insecta	Diptera	Chironomidae	Ablabesmyia	Ablabesmyia	4	45.00%
SMS002	Insecta	Odonata			Anisoptera	2	45.00%
SMS002	Arachnida	Trombidiformes	Arrenuridae	Arrenurus	Arrenurus	32	45.00%
SMS002	Bivalvia				Bivalvia	3	45.00%
SMS002	Insecta	Ephemeroptera	Caenidae	Caenis	Caenis Diminuta Gr.	1	45.00%
SMS002	Insecta	Diptera	Ceratopogonidae		Ceratopogonidae	2	45.00%
SMS002	Insecta	Diptera	Ceratopogonidae		Ceratopogoninae	4	45.00%
SMS002	Insecta	Diptera	Chironomidae	Chironomus	Chironomus	2	45.00%
SMS002	Insecta	Diptera	Chironomidae	Corynoneura	Corynoneura	1	45.00%
SMS002	Insecta	Coleoptera	Dytiscidae	Desmopachria	Desmopachria	2	45.00%
SMS002	Insecta	Coleoptera	Dytiscidae		Dytiscidae	1	45.00%
SMS002	Insecta	Coleoptera	Hydrophilidae	Enochrus	Enochrus	1	45.00%
SMS002	Insecta	Coleoptera	Hydrophilidae	Enochrus	Enochrus	3	45.00%
SMS002	Gastropoda				Gastropoda	8	45.00%
SMS002	Insecta	Hemiptera	Gerridae	Gerris	Gerris	1	45.00%
SMS002	Insecta	Diptera	Chironomidae	Guttipelopia	Guttipelopia	1	45.00%
SMS002	Gastropoda	Basommatophora	Planorbidae	Gyraulus	Gyraulus	68	45.00%
SMS002	Insecta	Coleoptera	Haliplidae	Haliplus	Haliplus	3	45.00%
SMS002	Insecta	Coleoptera	Haliplidae	Haliplus	Haliplus	3	45.00%
SMS002	Gastropoda	Basommatophora	Planorbidae	Helisoma	Helisoma	12	45.00%
SMS002	Insecta	Diptera	Limoniidae	Helius	Helius	5	45.00%
SMS002	Insecta	Trichoptera	Hydroptilidae		Hydroptilidae	2	45.00%

Site	Class	Order	Family	Genus	Taxon	Subsample	%Sorted
SMS003	Insecta	Diptera	Chironomidae		Thienemannimyia Gr.	3	31.00%
SMS003	Insecta	Coleoptera	Hydrophilidae	Tropisternus	Tropisternus	1	31.00%
SMS003	Arachnida	Trombidiformes	Unionicolidae	Unionicola	Unionicola	1	31.00%
SMS003	Gastropoda	Heterostropha	Valvatidae	Valvata	Valvata	3	31.00%
SMS003	Arachnida	Trombidiformes	Mideopsidae	Xystonotus	Xystonotus	1	31.00%
SMS004	Insecta	Diptera	Chironomidae	Ablabesmyia	Ablabesmyia	7	48.00%
SMS004	Insecta	Odonata	Aeshnidae		Aeshnidae	7	48.00%
SMS004	Insecta	Odonata			Anisoptera	1	48.00%
SMS004	Arachnida	Trombidiformes	Arrenuridae	Arrenurus	Arrenurus	8	48.00%
SMS004	Insecta	Trichoptera	Phryganeidae	Banksiola	Banksiola crotchi	4	48.00%
SMS004	Insecta	Ephemeroptera	Caenidae	Caenis	Caenis	7	48.00%
SMS004	Insecta	Ephemeroptera	Baetidae	Callibaetis	Callibaetis	1	48.00%
SMS004	Insecta	Diptera	Ceratopogonidae		Ceratopogonidae	16	48.00%
SMS004	Insecta	Diptera	Ceratopogonidae		Ceratopogoninae	4	48.00%
SMS004	Insecta	Odonata	Coenagrionidae		Coenagrionidae	12	48.00%
SMS004	Insecta	Diptera	Chironomidae	Cricotopus	Cricotopus (Isocladius)	1	48.00%
SMS004	Insecta	Coleoptera	Hydrophilidae	Enochrus	Enochrus	2	48.00%
SMS004	Gastropoda				Gastropoda	18	48.00%
SMS004	Insecta	Diptera	Chironomidae	Glyptotendipes	Glyptotendipes	6	48.00%
SMS004	Gastropoda	Basommatophora	Planorbidae	Gyraulus	Gyraulus	17	48.00%
SMS004	Insecta	Coleoptera	Haliplidae	Haliplus	Haliplus	4	48.00%
SMS004	Gastropoda	Basommatophora	Planorbidae	Helisoma	Helisoma	3	48.00%
SMS004	Insecta	Diptera	Limoniidae	Helius	Helius	1	48.00%
SMS004	Insecta	Diptera	Empididae	Hemerodromia	Hemerodromia	1	48.00%
SMS004	Malacostraca	Amphipoda	Hyalellidae	Hyalella	Hyalella	5	48.00%
SMS004	Insecta	Diptera	Chironomidae	Labrundinia	Labrundinia	1	48.00%
SMS004	Insecta	Diptera	Chironomidae	Larsia	Larsia	1	48.00%
SMS004	Insecta	Diptera	Chironomidae	Lauterborniella	Lauterborniella agrayloides	2	48.00%
SMS004	Insecta	Odonata	Libellulidae		Libellulidae	2	48.00%
SMS004	Insecta	Hemiptera	Veliidae	Microvelia	Microvelia	47	48.00%
SMS004	Clitellata	Tubificida	Naididae	Nais	Nais	1	48.00%
SMS004	Insecta	Trichoptera	Leptoceridae	Nectopsyche	Nectopsyche	1	48.00%
SMS004	Arachnida	Trombidiformes	Unionicolidae	Neumania	Neumania	1	48.00%
SMS004	Insecta	Diptera	Stratiomyidae	Odontomyia / Hedriodiscus	Odontomyia / Hedriodiscus	2	48.00%
SMS004	Insecta	Trichoptera	Leptoceridae	Oecetis	Oecetis	8	48.00%
SMS004	Arachnida	Sarcoptiformes			Oribatida	2	48.00%
SMS004	Insecta	Lepidoptera	Crambidae	Parapoynx	Parapoynx	6	48.00%
SMS004	Insecta	Diptera	Chironomidae	Paratanytarsus	Paratanytarsus	18	48.00%
SMS004	Gastropoda	Basommatophora	Physidae	Physella	Physella	4	48.00%
SMS004	Arachnida	Trombidiformes	Pionidae	Piona	Piona	3	48.00%
SMS004	Insecta	Trichoptera	Polycentropodidae		Polycentropodidae	1	48.00%
SMS004	Insecta	Diptera	Chironomidae	Procladius	Procladius	3	48.00%
SMS004	Gastropoda	Basommatophora	Planorbidae	Promenetus	Promenetus	11	48.00%
SMS004	Insecta	Diptera	Chironomidae	Pseudochironomus	Pseudochironomus	16	48.00%
SMS004	Insecta	Diptera	Chironomidae	Pseudochironomus	Pseudochironomus	3	48.00%
SMS004	Bivalvia	Veneroida	Sphaeriidae		Sphaeriidae	3	48.00%
SMS004	Bivalvia	Veneroida	Sphaeriidae	Sphaerium	Sphaerium	1	48.00%
SMS004	Insecta	Diptera	Chironomidae		Thienemannimyia Gr.	44	48.00%
SMS004	Insecta	Trichoptera	Leptoceridae	Triaenodes	Triaenodes	4	48.00%
SMS004	Arachnida	Trombidiformes	Mideopsidae	Xystonotus	Xystonotus	2	48.00%
SMS005	Insecta	Diptera	Chironomidae	Ablabesmyia	Ablabesmyia	69	39.00%
SMS005	Insecta	Odonata	Aeshnidae		Aeshnidae	4	39.00%
SMS005	Insecta	Odonata	Aeshnidae	Anax	Anax	1	39.00%
SMS005	Insecta	Odonata			Anisoptera	7	39.00%
SMS005	Arachnida	Trombidiformes	Arrenuridae	Arrenurus	Arrenurus	7	39.00%
SMS005	Insecta	Ephemeroptera	Caenidae	Caenis	Caenis Diminuta Gr.	90	39.00%
SMS005	Insecta	Diptera	Ceratopogonidae		Ceratopogonidae	1	39.00%
SMS005	Insecta	Diptera	Ceratopogonidae		Ceratopogoninae	8	39.00%
SMS005	Insecta	Odonata	Coenagrionidae		Coenagrionidae	9	39.00%
SMS005	Insecta	Lepidoptera	Crambidae		Crambidae	1	39.00%
SMS005	Insecta	Diptera	Chironomidae	Cricotopus	Cricotopus (Isocladius)	1	39.00%
SMS005	Insecta	Diptera	Ceratopogonidae	Dasyhelea	Dasyhelea	6	39.00%
SMS005	Insecta	Diptera	Chironomidae	Dicrotendipes	Dicrotendipes	1	39.00%
SMS005	Insecta	Coleoptera	Dytiscidae		Dytiscidae	1	39.00%
SMS005	Insecta	Coleoptera	Hydrophilidae	Enochrus	Enochrus	1	39.00%
SMS005	Insecta	Diptera	Ceratopogonidae	Forcipomyia	Forcipomyia	1	39.00%
SMS005	Gastropoda	Basommatophora	Planorbidae	Gyraulus	Gyraulus	12	39.00%
SMS005	Gastropoda	Basommatophora	Planorbidae	Helisoma	Helisoma	1	39.00%
SMS005	Insecta	Odonata	Libellulidae	Leucorrhinia	Leucorrhinia	3	39.00%
SMS005	Arachnida	Trombidiformes	Limnesiidae	Limnesia	Limnesia	1	39.00%

Site	Class	Order	Family	Genus	Taxon	Subsample	%Sorted
SMS005	Gastropoda	Basommatophora	Lymnaeidae		Lymnaeidae	2	39.00%
SMS005	Insecta	Hemiptera	Hebridae	Merragata	Merragata	2	39.00%
SMS005	Insecta	Hemiptera	Mesoveliidae	Mesovelia	Mesovelia	1	39.00%
SMS005	Insecta	Diptera	Chironomidae	Micropsectra	Micropsectra	3	39.00%
SMS005	Insecta	Hemiptera	Veliidae	Microvelia	Microvelia	19	39.00%
SMS005	Arachnida	Trombidiformes	Unionicolidae	Neumania	Neumania	1	39.00%
SMS005	Insecta	Hemiptera	Notonectidae		Notonectidae	3	39.00%
SMS005	Insecta	Diptera	Stratiomyidae	Odontomyia / Hedriodiscus	Odontomyia / Hedriodiscus	1	39.00%
SMS005	Arachnida	Sarcoptiformes			Oribatida	1	39.00%
SMS005	Insecta	Lepidoptera	Crambidae	Parapoynx	Parapoynx	2	39.00%
SMS005	Insecta	Diptera	Chironomidae	Paratanytarsus	Paratanytarsus	19	39.00%
SMS005	Insecta	Trichoptera	Phryganeidae		Phryganeidae	2	39.00%
SMS005	Gastropoda	Basommatophora	Physidae	Physella	Physella	22	39.00%
SMS005	Insecta	Diptera	Chironomidae	Psectrocladius	Psectrocladius	5	39.00%
SMS005	Insecta	Diptera	Chironomidae	Pseudochironomus	Pseudochironomus	10	39.00%
SMS005	Insecta	Diptera	Chironomidae	Pseudochironomus	Pseudochironomus	2	39.00%
SMS005	Insecta	Hemiptera	Nepidae	Ranatra	Ranatra	1	39.00%
SMS005	Bivalvia	Veneroida	Sphaeriidae		Sphaeriidae	8	39.00%
SMS005	Bivalvia	Veneroida	Sphaeriidae	Sphaerium	Sphaerium	4	39.00%
SMS005	Insecta	Diptera	Chironomidae		Tanypodinae	1	39.00%
SMS005	Insecta	Diptera	Chironomidae	Tanytarsus	Tanytarsus	2	39.00%
SMS005	Insecta	Diptera	Chironomidae		Thienemannimyia Gr.	1	39.00%
SMS006	Insecta	Diptera	Chironomidae	Ablabesmyia	Ablabesmyia	9	11.00%
SMS006	Insecta	Odonata	Aeshnidae		Aeshnidae	3	11.00%
SMS006	Insecta	Odonata			Anisoptera	1	11.00%
SMS006	Arachnida	Trombidiformes	Arrenuridae	Arrenurus	Arrenurus	6	11.00%
SMS006	Insecta	Ephemeroptera	Caenidae	Caenis	Caenis diminuta	2	11.00%
SMS006	Insecta	Ephemeroptera	Caenidae	Caenis	Caenis Diminuta Gr.	14	11.00%
SMS006	Insecta	Diptera	Ceratopogonidae		Ceratopogonidae	3	11.00%
SMS006	Insecta	Diptera	Ceratopogonidae		Ceratopogoninae	6	11.00%
SMS006	Branchiopoda	Diplostraca			Cladocera	4	11.00%
SMS006	Insecta	Odonata	Coenagrionidae		Coenagrionidae	8	11.00%
SMS006	Maxillopoda				Copepoda	1	11.00%
SMS006	Insecta	Odonata	Corduliidae	Cordulia	Cordulia	1	11.00%
SMS006	Insecta	Diptera	Chironomidae	Corynoneura	Corynoneura	6	11.00%
SMS006	Insecta	Diptera	Chironomidae	Cricotopus	Cricotopus (Isocladius)	1	11.00%
SMS006	Clitellata	Tubificida	Naididae	Dero	Dero	38	11.00%
SMS006	Insecta	Diptera	Chironomidae	Dicrotendipes	Dicrotendipes	2	11.00%
SMS006	Insecta	Coleoptera	Dytiscidae		Dytiscidae	1	11.00%
SMS006	Insecta	Coleoptera	Hydrophilidae	Enochrus	Enochrus	1	11.00%
SMS006	Insecta	Hemiptera	Gerridae		Gerridae	1	11.00%
SMS006	Insecta	Hemiptera	Gerridae	Gerris	Gerris	1	11.00%
SMS006	Clitellata	Rhynchobdellida	Glossiphoniidae		Glossiphoniidae	2	11.00%
SMS006	Insecta	Diptera	Chironomidae	Glyptotendipes	Glyptotendipes	1	11.00%
SMS006	Gastropoda	Basommatophora	Planorbidae	Gyraulus	Gyraulus	13	11.00%
SMS006	Insecta	Diptera	Limoniidae	Helius	Helius	1	11.00%
SMS006	Malacostraca	Amphipoda	Hyalellidae	Hyalella	Hyalella	8	11.00%
SMS006	Insecta	Diptera	Chironomidae	Labrundinia	Labrundinia	5	11.00%
SMS006	Insecta	Diptera	Chironomidae	Labrundinia	Labrundinia	1	11.00%
SMS006	Insecta	Diptera	Chironomidae	Larsia	Larsia	4	11.00%
SMS006	Insecta	Diptera	Chironomidae	Lauterborniella	Lauterborniella agrayloides	3	11.00%
SMS006	Insecta	Odonata	Libellulidae		Libellulidae	1	11.00%
SMS006	Arachnida	Trombidiformes	Limnesiidae	Limnesia	Limnesia	1	11.00%
SMS006	Insecta	Diptera	Limoniidae		Limoniidae	1	11.00%
SMS006	Insecta	Diptera	Chironomidae	Microtendipes	Microtendipes Rydalensis Gr.	1	11.00%
SMS006	Insecta	Hemiptera	Veliidae	Microvelia	Microvelia	12	11.00%
SMS006	Clitellata	Tubificida	Naididae		Naididae	3	11.00%
SMS006	Clitellata	Tubificida	Naididae	Nais	Nais	5	11.00%
SMS006	Insecta	Diptera	Stratiomyidae	Odontomyia / Hedriodiscus	Odontomyia / Hedriodiscus	2	11.00%
SMS006	Arachnida	Sarcoptiformes			Oribatida	2	11.00%
SMS006	Ostracoda				Ostracoda	4	11.00%
SMS006	Insecta	Trichoptera	Hydroptilidae	Oxyethira	Oxyethira	7	11.00%
SMS006	Insecta	Lepidoptera	Crambidae	Parapoynx	Parapoynx	6	11.00%
SMS006	Insecta	Diptera	Chironomidae	Paratanytarsus	Paratanytarsus	32	11.00%
SMS006	Insecta	Diptera	Chironomidae	Phaenopsectra	Phaenopsectra	1	11.00%
SMS006	Gastropoda	Basommatophora	Physidae	Physella	Physella	5	11.00%
SMS006	Arachnida	Trombidiformes	Pionidae	Piona	Piona	5	11.00%
SMS006	Insecta	Diptera	Chironomidae	Polypedilum	Polypedilum	2	11.00%
SMS006	Insecta	Diptera	Chironomidae	Procladius	Procladius	3	11.00%

Site	Class	Order	Family	Genus	Taxon	Subsample	%Sorted
SMS006	Gastropoda	Basommatophora	Planorbidae	Promenetus	Promenetus	4	11.00%
SMS006	Insecta	Diptera	Chironomidae	Psectrocladius	Psectrocladius	2	11.00%
SMS006	Insecta	Diptera	Chironomidae	Pseudochironomus	Pseudochironomus	16	11.00%
SMS006	Bivalvia	Veneroida	Sphaeriidae		Sphaeriidae	14	11.00%
SMS006	Bivalvia	Veneroida	Sphaeriidae	Sphaerium	Sphaerium	2	11.00%
SMS006	Insecta	Diptera	Tabanidae		Tabanidae	1	11.00%
SMS006	Insecta	Diptera	Chironomidae		Tanypodinae	1	11.00%
SMS006	Insecta	Diptera	Chironomidae		Tanytarsini	1	11.00%
SMS006	Insecta	Diptera	Chironomidae	Tanytarsus	Tanytarsus	51	11.00%
SMS006	Insecta	Diptera	Chironomidae	Tanytarsus	Tanytarsus	1	11.00%
SMS006	Insecta	Diptera	Chironomidae		Thienemannimyia Gr.	12	11.00%
SMS006	Insecta	Trichoptera			Trichoptera	1	11.00%
SMS006	Arachnida	Trombidiformes	Mideopsidae	Xystonotus	Xystonotus	1	11.00%

5.3.2 Outputs from DNA meta-barcoding

Results from DNA meta-barcoding by the Center for Genomic Biodiversity (STREAM 2019)

Order	Family	Species	SMS001	SMS002	SMS003	SMS004	SM005	SMS006
Arhynchobdel	Erpobdellidae	Motobdella montezuma						
Haplotaxida	Enchytraeidae	Globulidrilus riparius			P			
Haplotaxida	Tubificidae	Aulodrilus pluriseta						
Haplotaxida	Tubificidae	Chaetogaster diaphanus	P		P	P	P	P
Haplotaxida	Tubificidae	Chaetogaster diastrophus						
Haplotaxida	Tubificidae	Chaetogaster limnaei	P	P			P	
Haplotaxida	Tubificidae	Dero digitata	P		P	P	P	P
Haplotaxida	Tubificidae	Dero obtusa	P		P	P	P	P
Haplotaxida	Tubificidae	Limnodrilus hoffmeisteri						
Haplotaxida	Tubificidae	Nais christinae		P	P	P	P	
Haplotaxida	Tubificidae	Nais communis			P			
Haplotaxida	Tubificidae	Nais variabilis					P	
Haplotaxida	Tubificidae	Pristina longiseta	P	P	P	P	P	P
Haplotaxida	Tubificidae	Slavina appendiculata			P		P	
Haplotaxida	Tubificidae	Stylaria lacustris	P	P	P	P	P	P
Haplotaxida	Tubificidae	Tasserkidrilus kessleri						
Lumbriculida	Lumbriculidae	Lumbriculus variegatus			P	P	P	P
Araneae	Linyphiidae	Erigone aletris		P				P
Araneae	Linyphiidae	Microlinyphia impigra		P				P
Araneae	Lycosidae	Pirata piraticus	P				P	
Araneae	Tetragnathidae	Tetragnatha caudata			P			
Araneae	Tetragnathidae	Tetragnatha elongata						
Trombidiform	Arrenuridae	Arrenurus americanus	P	P	P	P	P	P
Trombidiform	Arrenuridae	Arrenurus intermedius		P	P	P	P	
Trombidiform	Arrenuridae	Arrenurus megalurus		P	P		P	
Trombidiform	Arrenuridae	Arrenurus reflexus		P		P	P	
Trombidiform	Arrenuridae	Arrenurus wardi	P	P				
Trombidiform	Limnesiidae	Limnesia marshallae		P				
Trombidiform	Limnesiidae	Limnesia undulata	P	P	P		P	P
Diplostraca	Bosminidae	Bosmina longirostris						P
Diplostraca	Chydoridae	Alona circumfimbriata					P	
Diplostraca	Chydoridae	Camptocercus rectirostris				P		P
Diplostraca	Chydoridae	Chydorus brevilabris			P			P
Diplostraca	Chydoridae	Pseudochydorus globosus	P		P			
Diplostraca	Daphniidae	Ceriodaphnia dubia	P	P	P			
Diplostraca	Daphniidae	Simocephalus punctatus			P	P		
Diplostraca	Daphniidae	Simocephalus serrulatus		P	P			P
Diplostraca	Eurycercidae	Eurycercus longirostris		P	P		P	P
Diplostraca	Polyphemidae	Polyphemus pediculus						
Entomobryom	Entomobryidae	Entomobrya nivalis					P	P
Entomobryom	Isotomidae	Isotomurus stuxbergi	P				P	
Coleoptera	Brachyceridae	Tanysphyrus lemnae					P	
Coleoptera	Carabidae	Agonum nigriceps					P	P
Coleoptera	Cryptophagidae	Telmatophilus typhae		P				

Shaded species are of diagnostic interest in assessing wetland health (CABIN 2018)

Order	Family	Species	SMS001	SMS002	SMS003	SMS004	SM005	SMS006
Coleoptera	Curculionidae	Euhrychiopsis lecontei					P	
Coleoptera	Dytiscidae	Agabus anthracinus	P					
Coleoptera	Dytiscidae	Hygrotus sayi	P		P		P	P
Coleoptera	Dytiscidae	Liodessus obscurellus				P		
Coleoptera	Dytiscidae	Rhantus wallisi			P			
Coleoptera	Haliplidae	Haliplus immaculicollis			P		P	
Coleoptera	Hydrophilidae	Berosus sayi						
Coleoptera	Hydrophilidae	Tropisternus columbianus						
Coleoptera	Scirtidae	Contacyphon laevipennis	P					
Diptera	Ceratopogonidae	Bezzia nobilis	P					
Diptera	Ceratopogonidae	Stilobezzia antennalis						
Diptera	Chironomidae	Ablabesmyia americana	P	P	P		P	
Diptera	Chironomidae	Ablabesmyia illinoensis					P	P
Diptera	Chironomidae	Chironomus acidophilus					P	
Diptera	Chironomidae	Chironomus atrella						
Diptera	Chironomidae	Chironomus atroviridis	P			P		
Diptera	Chironomidae	Chironomus maturus						
Diptera	Chironomidae	Corynoneura arctica		P		P	P	
Diptera	Chironomidae	Corynoneura scutellata						
Diptera	Chironomidae	Cryptochironomus digitatus			P			
Diptera	Chironomidae	Dicrotendipes modestus		P				P
Diptera	Chironomidae	Dicrotendipes tritomus	P		P		P	P
Diptera	Chironomidae	Glyptotendipes lobiferus			P	P		
Diptera	Chironomidae	Lauterborniella agrayloides	P	P	P		P	P
Diptera	Chironomidae	Monopelopia tenuicalcar					P	P
Diptera	Chironomidae	Orthocladius smolandicus					P	P
Diptera	Chironomidae	Paraphaenocladius impensus		P				
Diptera	Chironomidae	Paratanytarsus laccophilus	P	P	P	P		P
Diptera	Chironomidae	Phaenopsectra punctipes						P
Diptera	Chironomidae	Procladius culiciformis	P		P	P	P	P
Diptera	Chironomidae	Psectrocladius conjungens					P	
Diptera	Chironomidae	Psectrocladius obvius					P	
Diptera	Chironomidae	Psectrocladius platypus						
Diptera	Chironomidae	Psectrocladius sordidellus		P	P		P	P
Diptera	Chironomidae	Tanytarsus aigos			P	P	P	P
Diptera	Chironomidae	Tanytarsus buckleyi						P
Diptera	Chironomidae	Tanytarsus innarensis			P		P	P
Diptera	Chironomidae	Tanytarsus mendax			P	P	P	P
Diptera	Chironomidae	Tanytarsus recurvatus		P	P	P	P	P
Diptera	Chironomidae	Tanytarsus striatulus					P	P
Diptera	Chironomidae	Tanytarsus usmaensis						
Diptera	Chironomidae	Tanytarsus wirthi						P
Diptera	Chironomidae	Thienemanniella lobapodema		P	P			
Diptera	Chironomidae	Chironomidae 8H-20279-1	P		P	P	P	P

Order	Family	Species	SMS001	SMS002	SMS003	SMS004	SM005	SMS006
Diptera	Culicidae	Anopheles earlei		P			P	
Diptera	Culicidae	Anopheles punctipennis						
Diptera	Culicidae	Coquillettidia perturbans	P	P			P	
Diptera	Culicidae	Culex territans						
Diptera	Ephydridae	Hydrellia tenebricosa		P		P		
Diptera	Ephydridae	Notiphila olivacea					P	
Diptera	Limoniidae	Dicranomyia haeretica					P	
Diptera	Mycetophilidae	Mycetophila lunata		P				
Diptera	Sciomyzidae	Elgiva solicita					P	
Diptera	Sciomyzidae	Sepedon fuscipennis		P	P		P	P
Diptera	Stratiomyidae	Hedriodiscus vertebratus	P		P			
Diptera	Stratiomyidae	Odontomyia cincta				P	P	P
Diptera	Syrphidae	Lejops lunulatus						
Diptera	Tachinidae	Exorista larvarum						
Diptera	Tipulidae	Angarotipula illustris					P	
Ephemerop	Baetidae	Baetis tricaudatus	P					
Ephemerop	Baetidae	Callibaetis ferrugineus						
Ephemerop	Caenidae	Caenis diminuta	P	P	P	P	P	P
Ephemerop	Caenidae	Caenis punctata	P				P	P
Ephemerop	Caenidae	Caenis youngi	P					
Hemiptera	Aphididae	Rhopalosiphum oxyacanthae		P				
Hemiptera	Cicadellidae	Macrosteles quadrilineatus					P	
Hemiptera	Cicadellidae	Macrosteles sordidipennis					P	
Hemiptera	Corixidae	Hesperocorixa laevigata						
Hemiptera	Delphacidae	Javesella atrata					P	
Hemiptera	Delphacidae	Javesella dolera		P	P			
Hemiptera	Gerridae	Gerris buenoi			P		P	P
Hemiptera	Hydrometridae	Hydrometra martini					P	P
Hemiptera	Nepidae	Ranatra fusca		P				P
Hemiptera	Notonectidae	Buenoa confusa					P	
Hemiptera	Notonectidae	Buenoa macrotibialis					P	P
Hemiptera	Notonectidae	Notonecta undulata		P	P		P	P
Hemiptera	Veliidae	Microvelia buenoi		P				
Hymenopte	Vespidae	Dolichovespula media						
Lepidoptera	Crambidae	Elophila obliteralis		P		P	P	P
Lepidoptera	Crambidae	Parapoynx allionealis	P	P	P	P	P	P
Lepidoptera	Noctuidae	Apamea unanimis						
Lepidoptera	Noctuidae	Helotropha reniformis						

Order	Family	Species	SMS001	SMS002	SMS003	SMS004	SM005	SMS006
Odonata	Aeshnidae	Aeshna canadensis					P	P
Odonata	Aeshnidae	Aeshna constricta						
Odonata	Aeshnidae	Aeshna crenata			P		P	
Odonata	Aeshnidae	Aeshna eremita			P	P	P	
Odonata	Aeshnidae	Aeshna interrupta	P		P	P	P	P
Odonata	Aeshnidae	Aeshna sitchensis						
Odonata	Aeshnidae	Aeshna umbrosa						
Odonata	Coenagrionidae	Amphiagrion abbreviatum						
Odonata	Coenagrionidae	Enallagma hageni						P
Odonata	Coenagrionidae	Ischnura kellicotti	P	P	P	P	P	P
Odonata	Coenagrionidae	Ischnura verticalis			P			
Odonata	Coenagrionidae	Nehalennia irene		P	P	P	P	P
Odonata	Corduliidae	Cordulia aenea	P		P		P	
Odonata	Corduliidae	Tetragoneuria cynosura		P		P		
Odonata	Lestidae	Lestes congener						
Odonata	Libellulidae	Leucorrhinia glacialis						P
Odonata	Libellulidae	Leucorrhinia hudsonica						
Odonata	Libellulidae	Leucorrhinia intacta	P	P	P	P	P	P
Odonata	Libellulidae	Leucorrhinia proxima			P		P	
Odonata	Libellulidae	Libellula quadrimaculata			P		P	P
Odonata	Libellulidae	Plathemis lydia						
Odonata	Libellulidae	Plathemis subornata						
Odonata	Libellulidae	Sympetrum corruptum						
Odonata	Libellulidae	Sympetrum costiferum			P			
Odonata	Libellulidae	Sympetrum vicinum			P	P		
Orthoptera	Acrididae	Stethophyma lineatum						
Thysanopte	Phlaeothripidae	Cephalothrips monilicornis					P	
Trichoptera	Hydroptilidae	Oxyethira michiganensis		P	P		P	P
Trichoptera	Hydroptilidae	Oxyethira verna		P	P		P	P
Trichoptera	Leptoceridae	Nectopsyche albida		P	P	P		P
Trichoptera	Leptoceridae	Oecetis cinerascens		P	P			
Trichoptera	Leptoceridae	Oecetis inconspicua		P	P	P	P	P
Trichoptera	Leptoceridae	Triaenodes marginatus						P
Trichoptera	Leptoceridae	Triaenodes nox			P			
Trichoptera	Leptoceridae	Triaenodes tardus		P	P	P	P	P
Trichoptera	Limnephilidae	Glyphopsyche irrorata						
Trichoptera	Limnephilidae	Limnephilus externus						
Trichoptera	Philopotamidae	Wormaldia gabriella						P
Trichoptera	Phryganeidae	Banksiola crotchi		P	P		P	P
Trichoptera	Phryganeidae	Phryganea cinerea		P				P
Trichoptera	Polycentropodidae	Polycentropus flavus	P	P			P	
Amphipoda	Hyalellidae	Hyalella azteca		P		P		P
Calanoida	Diaptomidae	Aglaodiaptomus leptopus					P	
Cyclopoida	Cyclopidae	Acanthocyclops robustus						
Podocopid	Cyprididae	Cypridopsis vidua	P	P	P	P	P	P
Podocopid	Cyprididae	Notodromas monacha			P		P	P
Chaetonoti	Chaetonotidae	Lepidodermella squamata					P	
Veneroida	Sphaeriidae	Musculium kashmirensis					P	
Ploima	Asplanchnidae	Asplanchna sieboldi			P			
Ploima	Synchaetidae	Synchaeta pectinata						
Parachela	Hypsibiidae	Hypsibius dujardini						
Parachela	Hypsibiidae	Isohypsibius pushkini	P				P	