

**Ecosystem impact of nutrient enrichment by  
Kokanee in the Williston Reservoir Watershed  
(PEA-F17-F-1471)**

**Final Report**

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**10 May 2017**



*Prepared with financial support of the Fish and Wildlife Compensation Program on behalf of its program partners BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations, and public stakeholders.*



## Executive Summary

The Williston Reservoir and its tributaries are highly nutrient limited. From 1990 to 1998 over 3,000,000 Kokanee were stocked into rivers that flow into the Williston Reservoir in an attempt to increase the productivity of the reservoir ecosystem, and to also create a potential sport fishery for Kokanee and the fish that eat them. Aerial counts a decade later showed major runs of Kokanee in the Finlay, Ingenika, Omineca, Ossilinka, and Germanson Rivers. Kokanee in these rivers potentially can provide a major new source of nutrients, impacting other fish species, stream-living macroinvertebrates, and the adjacent riparian zone. The goal of the project is to determine the impact of the introduction of Kokanee into the Williston Reservoir on the nutrient dynamics and the complex web of interactions between Kokanee, stream-living macroinvertebrates (aquatic insects), and the surrounding riparian zone (lichen communities). This project aligns with the Peace Fish and Wildlife Compensation Program's Stream Action Plan Objective 2a – "Understand the effects of Kokanee introductions on the aquatic food web", and Objective 2a-1 – "Undertake a Kokanee assessment study to summarize status, trends, and aquatic and terrestrial ecosystem impacts and potential risks of Kokanee introductions - Develop appropriate recommendations for actions, as needed."

Our survey questionnaire provided a qualitative assessment of Kokanee presence / absence and relative abundance and informed our selection of the specific stream reaches for data collection activities. The historic pattern of distribution in tributaries to the Williston Reservoir is extensive - but it is clear that one of the few watersheds where Kokanee have not colonized is the Parsnip River system, although there are reports of Kokanee spawning in tributaries to the system that are close to the reservoir.

We used stable isotope analysis to track the potential delivery of nutrients from the reservoir to tributary streams and their riparian ecosystems. Data from samples collected in 2016 reveals two patterns; pelagic stream resident species such as salmonids and minnows showed much more variability in stable isotope signature than benthic species such as slimy sculpin, and there was more variability in larger rivers than smaller streams. Although there was variation in the stable isotope signatures within each system, signatures from stream resident fish collected in systems with high numbers of Kokanee spawners showed significant separation from signatures of fish collected from streams with no Kokanee. Our findings are strongly suggestive that Kokanee provide a significant source of nutrients to tributary streams where they spawn.

We surveyed seven streams and rivers in the Williston system for aquatic macroinvertebrates and used DNA barcoding to obtain putative identifications, often to a species level. We sent 667 specimens for barcoding and received back 601 useful sequences from which we were able to discern approximately 115 species – 28 Ephemeroptera, 32 Plecoptera, 16 Trichoptera, 37 Diptera, and two Coleoptera. One of the Trichoptera may represent a new species record for

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Canada. This checklist represents the most comprehensive listing of species found in the Williston system that we are aware of. We also surveyed museum and other collection records from 10 different institutions and databases and developed a combined geographically-labeled database of 17,879 specimens covering most of western Canada and some of the adjacent US. Of those 6,989 records were from BC, including 620 Ephemeroptera, 1,716 Plecoptera, and 4,653 Trichoptera. The specimens – now vouchered in a curated collection – and the database provide an excellent foundation for further research in the region and will also be a useful resource for others.

Our 2016 lichen surveys on non-Kokanee-run rivers reveal a rich lichen flora in east-side riparian habitats of the Table, Hominka, and Parsnip Rivers, including several significant rare species. The abundance of 2 key groups of lichens, the Teloschistaceae and Physciaceae, often regarded as indicators of nitrogen enriched habitats, will be a key part of comparisons with Kokanee-run rivers in 2017.

*Cover: Ospika River – photo by Dezene Huber.*

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## Introduction

Temperate freshwater systems are highly oligotrophic. Flooding of land following the building of a dam and the creation of a reservoir results in leaching of nutrients from the newly submerged soils and productivity can be quite high during the early life of a reservoir. Reservoir dynamics in temperate regions, however, lead to a gradual loss of nutrients. Consequently over time reservoirs become less productive and are often characterized as ultra-oligotrophic. Attempts to increase productivity in the Williston Reservoir watershed have been limited, but a fertilization project on the Mesilinka River during the 1990's met with limited success. Kokanee (*Oncorhynchus nerka*) were stocked into Williston Reservoir from 1990 to 1998. During this time, over 3,000,000 juvenile Kokanee were stocked into Carbon Creek, Davis River, Dunlevy Creek, Manson River, and Nation River.

An aerial enumeration study conducted from 2002 to 2006 found that the distribution and abundance of Kokanee in tributaries to the Williston Reservoir poorly reflected the stocking patterns from the 1990's. Systems with the greatest abundance of Kokanee were found to be Russell Creek (Finlay River tributary), Ingenika River, Omineca River, Osilinka River, and Germanson River – some years with up to 250 000 spawners within a single river. Spawning Kokanee, therefore, have selected tributaries in the Williston watershed that flow into the northwestern portion of the reservoir – not the regions originally stocked. The introduced Kokanee to the Williston Reservoir watershed have the potential to dramatically affect the flow of nutrients due to their semelparous life history and associated migrations. The impacts of these changes in Kokanee populations will be assessed in two lines of study:

*Kokanee-associated nutrient transfer.* The fate of nutrient transfer from the Williston Reservoir to reaches of rivers where Kokanee spawn is unknown, but could be appreciable. The first objective of our study will be to characterize the magnitude of the annual influx of mature Kokanee to spawning streams compared to streams where Kokanee do not or have not been documented to spawn, both in terms of number of fish spawning and the biomass and nutrient transfers this represents within the watershed.

*Impact on other taxa.* The transfer of nutrients into regional stream systems in the Williston watershed potentially may also have large impacts on a range of other biota and ecosystem processes. These will be assessed using two target groups, namely aquatic insects and riparian lichen communities. Both of these groups have previously been used as biological indicators and have well-developed biomonitoring methodologies published in the literature.

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Aquatic insect feeding activities process organic matter affecting stream productivity and other characteristics. Aquatic insects, particularly in their immature stages, form the major portion of the diet of resident fish and also alter and maintain stream characteristics that provide habitat for fish. Emergent adult aquatic insects influence surrounding riparian areas through substantial deposition of nutrients.

Epiphytic lichens obtain their nutrients directly from through-flow precipitation that flows over their surface, much of which derives from leachates released from bark and leaf surfaces. As a result, they are highly sensitive to changes in the nutrient status of the host trees upon which they grow. Additionally, measurements of nutrient status within lichen thalli will provide a direct indication of changes in factors such as nitrogen content.

Our results will provide both baseline data (e.g., species lists, abundance, and diversity measures) and new tools (e.g., biodiversity indicators, monitoring methods, forecasting models) for fisheries and forest managers working in the Williston Reservoir watershed region.

## **Goals and Objectives**

The goal of the project is to determine the impact of the introduction of Kokanee into the Williston Reservoir on the nutrient dynamics and the web of interactions between Kokanee, aquatic insects, and the surrounding riparian zone. This project aligns with the Peace Fish and Wildlife Compensation Program's Stream Action Plan Objective 2a – "Understand the effects of Kokanee introductions on the aquatic food web", and Objective 2a-1 – "Undertake a Kokanee assessment study to summarize status, trends, and aquatic and terrestrial ecosystem impacts and potential risks of Kokanee introductions - Develop appropriate recommendations for actions, as needed."

In year 1 of the project we used a questionnaire to determine Kokanee occurrence in tributaries of the Williston Reservoir. The questionnaire was distributed to local FNs in the region by our FN project partners. We also distributed the survey to guide outfitters within the region and recreational anglers. The survey provided a qualitative assessment of Kokanee presence/absence and relative abundance. The information gathered successfully informed our choice of study sites for sampling resident fish, aquatic macroinvertebrates, and lichens in two systems without, and five systems with Kokanee for species checklists (insects) and to use stable isotope methods to track nutrient flow (fish and insects).

In year 2 we will continue to investigate nutrient inputs from Kokanee through the stream and riparian ecosystems using stable isotope analysis to assess source, sink, and process

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relationships. We will continue our survey of records and collections to develop an historical understanding of aquatic insect biodiversity in the area. Iterative quantitative sampling of insects will be conducted in the systems to temporally refine checklists and to compare diversity between streams with and without Kokanee.

Lichen community composition in the stream riparian areas will be assessed using time-limited survey approaches within fixed plots. Collection and stable isotope analysis of fish, insects, and lichens in the systems will help to refine our understanding of Kokanee nutrient transfers.

Our results will provide baseline data (e.g., species lists, abundance, diversity) and new tools (e.g., biodiversity indicators, monitoring methods, predictive models) for fisheries and forest managers working in the region.

Working in partnership with Chu Cho Environmental (Tsay Keh Dene First Nation) this research provides important training opportunities for both local community members and UNBC students alike.

## **Study Area**

Specific stream and river reaches were chosen based on Kokanee spawner abundance from surveys conducted by Chu Cho Environmental and results from our survey questionnaire. Locations that were a part of the study in 2016 that represented sites with high Kokanee spawner abundance were Aley Creek, Stevenson Creek, and Bruin Creek (Figure 1). Although we initially planned to target the Osilinka River as a site with high numbers of spawning Kokanee, escapement to the Osilinka River and tributaries was lower for 2016. Consequently, our study locations on the Osilinka River above Tenakihi Creek and Tenakihi Creek represent sites where potential nutrient input from Kokanee was low. Control sites where Kokanee have not been documented and were not observed in 2016 were the Upper Parsnip River above Wichcika Creek, Missinka River, the Table River, and the Hominka River (Figure 1).

## **Methods**

*Nutrient Input to Streams.* To assess Kokanee abundance within tributaries to the Williston Reservoir we developed a survey questionnaire and in collaboration with our research partners estimated abundance in key watersheds. The survey questionnaire and consent form are attached. Surveys were distributed to local First Nations, guide outfitters within the region, and recreational anglers. Our survey questionnaire provided a qualitative assessment of Kokanee presence / absence and relative abundance (Table 1). The historic pattern of distribution in tributaries to the Williston Reservoir is extensive and many respondents indicated that Kokanee



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are “everywhere”. In fact, establishing control sites where Kokanee do not spawn was a concern – but it is clear that one of the few watersheds where Kokanee have not colonized is the Parsnip River system, although there are reports of Kokanee spawning in tributaries to the system that are close to the reservoir. The information gathered was complementary to the previous work by the Fish and Wildlife Compensation Program and we selected tributaries to the Parsnip River for control sites and tributaries to the Omineca River as our treatment sites.

In September 2016, Chu Cho Environmental collected Kokanee at the fence on the Osilinka River set up by the Freshwater Fisheries Society of BC to capture broodstock. We selected Tenakihi Creek as a second site where Kokanee spawn. Water levels were high in September and water was turbid, but it was also a poor year for Kokanee spawners in the Osilinka system. From the Osilinka River 13 kokanee were collected at the 8-km canyon bridge location. Numbers of spawners were also low in Tenakihi Creek; no fish were collected and only three were spotted. For other systems where Kokanee are known to spawn, flights over the Davis River over two days found few kokanee and very limited signs of redds. Consequently, it looked like it might be quite a poor year for spawning Kokanee. Aerial surveillance of the Ingenika River and tributaries, however, found some Kokanee and redds were visible. Flights over the Pelly River revealed that the Kokanee run was high – although access for sampling was very poor. Kokanee spawner abundance was also found to be high in tributaries to the Ospika River where access was good. Consequently, Kokanee were collected from Aley Creek (18) and Stevenson Creek (10) in September 2016. Due to the poor Kokanee runs in the Osilinka River system, we changed our sampling design; two control systems with no Kokanee, two treatment systems with low Kokanee spawner abundance, and three treatment systems with high Kokanee spawner abundance.

*Biotic Response to Nutrient Inputs.* We tested whether nutrient inputs from Kokanee can be tracked through the stream and riparian ecosystems using stable isotope analysis. Dual-isotope approaches have proven useful in assessing nutrient inputs with nitrogen isotopes measurements functioning as trophic level indicators and carbon isotope measurements indicating sources of nutrition (Peterson and Fry, 1987). Such approaches can be helpful in identifying allochthonous inputs to aquatic food webs. Values for  $\delta^{13}\text{C}$  are useful because of the wide range of  $\delta^{13}\text{C}$  of algae at the base of food webs. The other major source of energy in river food webs, terrestrial detritus, has a much more constrained  $^{13}\text{C}$  signature, so that these two carbon sources are often isotopically distinct (Finlay, 2001). Hydrogen stable isotope ratios ( $\delta^2\text{H}$ ) have recently been used as endogenous markers to improve the ability to quantify the relative importance of allochthonous input of organic material into aquatic ecosystems (Voigt et al., 2015). Oxygen stable isotope ratios ( $\delta^{18}\text{O}$ ) in combination with  $\delta^2\text{H}$  have increasingly been used to determine the origin and movement of animals (Bowen et al. 2005). Using a

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multiple-isotope approach, we examined whether food webs differed in stable isotope ratios ( $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$ ) according to the ecosystem (river with large numbers of Kokanee spawners, river with few Kokanee spawners, and river without Kokanee). The ratios of isotopes will reveal the source of nutrients, but does not indicate the quantity of nutrients introduced to the system. Aquatic invertebrates, and small stream resident fish were sampled from the study rivers after the spawning period in October 2016. Aquatic invertebrates were collected from each system by kick netting; sampling procedures are described in more detail below. Fish were caught by electrofishing using a Smith-Root backpack electrofisher (model L24). The sampling location, species and numbers of fish caught for each river systems are shown in Table 2.

Stable isotope analysis was conducted on pooled aquatic invertebrate samples and individual fish samples collected from our seven study streams. Dried, ground and homogeneous samples were weighed into tin capsules and analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  by an Elemental Analyzer (EA) coupled to a DeltaPlus XP – Conflo III Continuous Flow-Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany). Samples for  $\delta^2\text{H}$  were weighed into silver capsules and loaded into a Costech Zeroblank autosampler. Samples were converted to hydrogen ( $\text{H}_2$ ) gas by pyrolysis using a Thermo-Finnigan High Temperature Conversion Elemental Analyzer (TC/EA). All analyses were conducted at the Stable Isotopes in Nature Laboratory at the University of New Brunswick.

*Invertebrate Sampling.* Kick-netting for invertebrates was carried out in the same reaches of the same systems as was the electrofishing. Kick-netting was done in an upstream direction for about 10 minutes and about 15 - 30 m of stream bed, or until the kick net was substantially full. Three samples were taken at each location. One sample was placed immediately into a sealed, labelled container of 100% ethanol. One sample was placed immediately into a sealed, labelled container of stream water. The final sample was placed into a white, deep-sided tray of stream water and moving macroinvertebrates were removed by hand, forceps, or plastic pipette and placed alive into a labelled vial of 100% ethanol. All containers were labeled on the outside, and an identical paper-and-pencil label was also placed inside to ensure correct curation of samples. Samples were placed into a freezer in our truck in the field and were immediately transferred to a lab freezer upon return to UNBC.

The two ethanol samples for each system were sorted by three personnel who are all well-acquainted with aquatic entomology. They collected morphospecies from each system sample and, if there was space in each DNA barcoding tray (up to 95 samples per system), they added replicates of morphospecies. The completed trays were sent to the Canadian Centre for DNA Barcoding at the University of Guelph. There DNA was extracted in a nondestructive manner from each specimen and the “barcode” region of the cytochrome oxidase 1 gene was

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sequenced. DNA sequences were deposited in the Barcode of Life Database (BOLD) and physical specimens are now vouchered at the Biodiversity Institute of Ontario with a curated link to their DNA barcode.

DNA barcodes were assessed by a technician who has done a great deal of similar work in the past. Where BOLD analysis gave unambiguous calls (usually at least 2% variance from any similar species) to previously annotated specimens, an operational taxonomic unit (OTU) was given a specific binomial identification. If there was ambiguity and/or if there was no previously annotated sequence in the database (e.g. many of the Diptera), the OTU was only identified to genus or family as suggested by the DNA match. In all cases, conservative choices were made for DNA-based identifications. Species identified to genus or family level are labeled as sp.1, sp.2, etc. within a particular genus or family.

Invertebrate samples collected into water were processed and sent for stable isotope analysis. Please refer to the previous section “Biotic Response to Nutrient Inputs” for specific details on the protocol used.

We also surveyed historical checklists and 10 museum collections and online public databases across Canada for their curated and digitized records pertaining to aquatic macroinvertebrates (specifically Ephemeroptera, Plecoptera, and Trichoptera; EPT) in BC and other parts of western Canada to develop an historical understanding of aquatic insect biodiversity in the area. The museum and database sources were: the Royal British Columbia Museum, Royal Saskatchewan Museum, Royal Ontario Museum, Strickland Museum, University of Alberta Collection, Canadian National Collection, BioBus 2016 data, Beaty Biodiversity Collection, Global Biodiversity Information Facility, and the Canadensys database. Data were consolidated into an Excel spreadsheet. In instances where a geographic location was given without GPS coordinates, a GPS coordinate in the immediate area was input into the spreadsheet. For instance, if a lake were given as the location, a GPS coordinate approximately in the middle of the lake was given. Such instances were highlighted in the spreadsheet.

Museum records provide a physical spacial-temporal record of biodiversity. The area around the Williston Reservoir is mainly unsurveyed for aquatic invertebrates (and invertebrates in general). More detailed experiments in years 2 and 3 will provide more data and information about species distribution and inferred ecosystem services. A comprehensive museum record database allows us to complement our physical collections to develop a catalog of known and likely species in the area. We now have a comprehensive record of what is likely vast majority of specimen records for the area, as well as spatial-temporal data for each individual specimen.

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*Lichen Community Response.* Lichen community composition was assessed using time-limited survey approaches within fixed plots, an approach previously used to assess the response of central interior British Columbia lichen communities to regional gradients in air pollution (Coxson et al., 2013). Streamside habitats were chosen to minimize other environmental co-variates, i.e. assessments were conducted within similar successional stages. The riparian habitat alongside major rivers lends itself well to this approach, as other disturbances such as fire and forest harvesting are typically absent from this zone and forest age can be controlled by choice of microsites within the floodplain. The 2016 assessments were from east-side stream and river reaches with few or no known kokanee, on the Upper Parsnip River, Table River, and the Hominka River. At each of these locations between three and five sub-plots were chosen, depending on their availability, each sub-plot located in a separate late successional riparian forest stand.

Each species observed was given an overall abundance rating based on the assessment of their general abundance within the search area using a five-point scale (after Goward and Arsenault 1997): 1, two or fewer thalli per tree (and associated branches); 2, three to five thalli per tree; 3, six thalli or up to 20% cover; 4, from 21% to 50% cover; and 5, 51% cover or greater.

## **Results**

*Nutrient Input to Streams.* To quantify trophic links in the different rivers, we used the stable isotope data to estimate diet sources. Aquatic invertebrate samples differed among the study streams; streams where Kokanee spawned were depleted in  $^{13}\text{C}$  and also had lower  $\delta^{15}\text{N}$  than the control streams where no Kokanee spawned (Figure 2; top panel). Streams with low escapement of Kokanee spawners were generally intermediate to the control and high spawner streams.

Pelagic stream resident species caught in our sampling program were mainly salmonids and minnows and the stable isotope signatures did not show a clear distinction among the different study streams (Figure 2; middle panel). The benthic fish sampled were mainly slimy sculpin and there were substantive differences in stable isotope signatures among the study streams (Figure 2; bottom panel). Differences among the systems for sculpins tended to reflect the pattern observed for the benthic invertebrates; ratios for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were both lower in systems where large numbers of Kokanee had spawned.

The 2016 stream fish data reveals two patterns; pelagic stream resident species such as salmonids and minnows showed much more variability in stable isotope signature than benthic species such as slimy sculpin, and there was more variability in larger rivers than smaller

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streams (Figure 1; middle and bottom panels). Variation in stable isotope signature was found for sculpin collected within each river system (Figure 1; bottom panel). For example, slimy sculpin from the Missinka River ranged in  $\delta^{13}\text{C}$  by approximately 2‰. In contrast, sculpin from the Parsnip River had  $\delta^{13}\text{C}$  that ranged more than 4‰. Streams with high numbers of Kokanee spawners, however, clustered together and streams with no Kokanee clustered together.

Figure 3 shows stable isotope signatures for Kokanee and sculpin caught in our seven study streams. Kokanee signatures are higher for  $\delta^{15}\text{N}$  than for the sculpins and show no overlap with nitrogen signatures for the sculpins.

Incorporation of additional stable isotope signatures did not alter the relationship appreciably. Signatures for  $\delta^2\text{H}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\delta^{18}\text{O}$  incorporated into a discriminant function analysis are shown in Figure 4. Data for Kokanee from all three systems clustered together and there was good separation of sculpin from the different river systems.

***Invertebrate Diversity.*** Our DNA barcode-assisted rapid assessment of aquatic macroinvertebrate biodiversity in the seven systems yielded a total of 115 OTUs of which we were able to provide species-level identifications for 54 (Tables 3 - 7). We detected 28 Ephemeroptera (14 to species-level ID), 32 Plecoptera (19), 16 Trichoptera (15), 37 Diptera (6), and 2 Coleoptera (0).

Detected diversity on a per-stream basis for **Ephemeroptera, Plecoptera, Trichoptera, Diptera, and Coleoptera** were as follows:

- Missinka: 8E, 6P, 3T, 5D, 2C for a total of 24 species.
- Parsnip: 8E, 6P, 3T, 2D, 1C for a total of 20 species.
- Aley: 10E, 14P, 3T, 11D, 0C for a total of 39 species.
- Stevenson: 11E, 11P, 3T, 5D, 0C for a total of 30 species.
- Bruin: 14E, 11P, 6T, 9D, 0C for a total of 40 species.
- Tenekih: 8E, 10P, 7T, 8D, 0C for a total of 33 species.
- Osilinka: 8E, 8P, 5T, 9D, 0C for a total of 30 species.

Only three species were found in all seven systems: *Zapada cinctipes* (Plecoptera), *Drunella grandis* (Ephemeroptera), and *Ephemerella* sp.3 (Ephemeroptera). A substantial number of species were found in only one system each (see Tables 1-5). One of those latter species – identified as *Apatania comosa* – may represent a first record for BC, and possibly for all of Canada. All other OTUs identified via DNA barcode to species level are known to occur in BC.

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Our museum and online-record compiled database contains 17,879 records including: 12,293 Trichoptera records, 3,475 Plecoptera records, and 2111 Ephemeroptera records. Of these records, 11,043 (61.8% of all records) had been identified down to species level at the reporting institution. A total of 8,323 Trichoptera, 1,946 Plecoptera, and 774 Ephemeroptera records were available with species-level identification. There were a total of 887 different species within the database comprised of 665 Trichoptera species, 142 Plecoptera species, and 80 Ephemeroptera species. Within British Columbia, the database contained 6,989 records in total, comprised of 4,653 Trichoptera records, 1,716 Plecoptera records, and 620 Ephemeroptera records. The excel spreadsheet file containing these compiled data is appended with this report.

*Lichens.* A total of 181 lichen taxa were observed in the 3 non-kokanee river runs that were examined in 2016 (Table, Hominka, and Parsnip) (Appendix 1). The 2016 assessments revealed a previously undocumented population of one federally listed rare species, *Collema coniophilum* (listed under the Species at Risk Act, or SARA, ranked by the Committee on the Status of Endangered Wildlife in Canada, or COSEWIC). Additionally, *Collema quadrifidum* was found, red-listed by the B.C. Conservation Data Centre, and *Nephroma isidiosum*, Blue-listed species by the BC CDC.

Two groups of lichen taxa in these watersheds bear special attention. Both the *Teloschistaceae* and *Physciaceae* are often regarded as indicators of nitrogen enriched habitats, taxa which may provide an indication of Kokanee enrichment. Species of *Physciaceae* found in the study plots were *Rinodina capensis*, *R. colobina*, *R. degeliana*, *R. disjuncta*, *R. flavosoralifera*, *R. griseosoralifera*, *R. metaboliza*, *R. orculata*, *R. oregana*, *R. pyrina*, *R. stictica*, and *R. trevisanii*. *Teloschistaceae* species were *Xanthomendoza fallax*, *X. fulva*, *Xanthoria fallax*, *X. fulva*, and *Xanthoria kaernefeltii*. The year 2 data from the project (Kokanee-run sites) will provide critical comparisons for the abundance of these taxa.

## Discussion

Nitrogen isotopes measurements function as trophic level indicators (Peterson and Fry, 1987) and indicate that Kokanee are eating at a higher trophic level than the sculpins. Carbon isotope measurements indicate sources of nutrition (Peterson and Fry, 1987) and there was considerable difference among sculpins sampled from the different systems – but sculpin sampled from streams where high numbers of Kokanee spawned had  $\delta^{13}\text{C}$  values that were depleted and similar to the values for Kokanee. Decomposition of Kokanee introduces nutrients to streams. Additionally, intragravel flow of water carries nutrients to riparian vegetation,

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aquatic insects and stream resident fish feed on eggs and dead fish, which in turn feed numerous other organisms.

Resident species of fish caught in rivers where high Kokanee spawners were present, however, showed a wide range in  $\delta^{13}\text{C}$ , but this was primarily due to the variation seen in the pelagic fish species. The higher  $\delta^{13}\text{C}$  values for pelagic species may indicate supplementation of the diet with terrestrial food items. Benthic species may indicate an isotopic signature more strongly influenced by nutrients from benthic invertebrates as sculpins tend to have a more restricted diet composed primarily of aquatic insect nymphs, but pelagic species such as trout and char feed on benthic invertebrates and terrestrial insects. Generally, the isotopic signatures for benthic fish did not differ appreciably within a watershed. Benthic fish species caught in one system, however, also showed considerable variation. It is not clear why there is such a large variation in  $\delta^{13}\text{C}$ , however, the Parsnip River was the largest system sampled in 2016 and it is possible that nutrients for the system are not dominated by allochthonous inputs – but autochthonous algae production.

The overlap in  $\delta^{13}\text{C}$  values for sculpin from streams where Kokanee spawn and Kokanee spawners indicates that a potential source of nutrients in these streams is from the reservoir – suggesting that Kokanee are an important source of nutrients to tributary streams where they spawn. The classification matrix is shown in Table 3 and reveals considerable overlap among the signatures for Kokanee, but no Kokanee were incorrectly assigned to any of the sculpin groups. Sculpin collected from tributaries near the Ospika River system were assigned only to these streams, except for one fish assigned to the Parsnip River. Sculpin from Tenakihi Creek and the Osilinka River were also assigned to the Osilinka system or the Parsnip River. Sculpin from Missinka River were all assigned to the Missinka system. Sculpins from the Parsnip River showed more variation in assignment and the results were similar to those using just  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ .

Based on our results from 2016, benthic stream resident fish will be sampled again for each system in 2017. To determine the temporal variation in stable isotope signatures, we will also sample each system twice in 2017 – in July before the spawning period for Kokanee and October after the spawning period for Kokanee. The clustering of stable isotope signatures for Kokanee would suggest little variation in stable isotope signature from the reservoir. The three river systems where Kokanee were sampled were from a similar latitude, although on different sides of the Reservoir (Osilinka River on the west side; Aley and Stevenson Creeks on the east side). The migration patterns for Kokanee are unknown in the reservoir and stable isotope signatures may differ for different parts of the reservoir due to latitude. Consequently, we plan to expand our sampling further north and south in 2017. Large numbers of spawners were

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observed in the Ingenika River and tributaries last year. Historically, Kokanee have also been found to spawn in tributaries to the Nation River. We plan, therefore, to expand our sampling program for Kokanee to systems further north and south for 2017.

Aquatic invertebrates provide numerous ecosystem services to the systems in which they reside including nutrient processing and as food for fish. In turn, their assemblages are directly and rapidly affected by shifts in conditions in their systems, including changes in nutrient inflow. We hypothesize that the nutrient dynamics and the complex web of interactions between Kokanee; streamliving macroinvertebrates (Aquatic insects) and the ecosystem services that they provide; and the surrounding riparian zone (lichen communities) have shifted as result of the introduction of Kokanee into the Williston Reservoir. We aimed to complete a baseline record of aquatic insect taxa to help with subsequent assessment of the taxonomic and functional diversity responses to Kokanee abundance.

Our DNA barcode-based rapid survey of aquatic macroinvertebrate biodiversity in the Williston region, while only at one site in each of seven lotic systems, uncovered a substantial level of biodiversity in the area with a total of 115 species. Diptera were the most diverse group followed by Plecoptera and Ephemeroptera. While there were a substantial number of existing species-level annotation matches in BOLD for Ephemeroptera, Plecoptera, and particularly Trichoptera, the database did not provide much high-resolution identification for Diptera. This is typically the case as dipteran larvae are difficult to identify by morphological means and, as such, often lack reliable annotations in barcode databases. It should be noted that due to the rapid nature of our survey, these numbers represent substantially conservative estimates for total biodiversity in the system. However aquatic macroinvertebrates are particularly apt for use in this type of work because their immature stages are almost always present in the systems in which they reside; and this is particularly the case in the spring and autumn, the latter being when we were sampling.

Very few species were found in all seven streams, and quite a few were found in only one or a small subset of streams even though we sampled in reasonably similar situation in each stream (riffles or rapid flow and rocky bottoms). This is indicative of a high level of beta-diversity within the region and our catalog of species will assist us in developing data to refine such estimates.

Most of the species that were identified down to species via DNA barcode annotation have previously been collected in British Columbia. One species, *Apatania comosa*, has not been previously recorded in British Columbia, and according to database searches, is a potential new record for Canada. It should be noted that the specimen which we have labelled as *Apatania comosa* provides a 99.46% match to another published specimen in the BOLD database (in BIN BOLD:AAJ1546, Zhou et al. 2016), however it also provides a 98.57% match to a published



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specimen of *A. chasica*. (BOLD:ABZ878, Zhou et al. 2016). If the latter, this would still represent a new Canadian record. If the former, its range is currently considered so limited (portions of ID, MT, UT, WY) that it is ranked as “G2 - Imperiled” by NatureServe with a current estimated range extent - obviously only in suitable habitat within that range - of just 250-1000 km<sup>2</sup>. If *A. comosa* exists in the Williston region, it would greatly expand that range extent and would perhaps indicate a more secure conservation rank.

Specimen barcodes that did not give a reliable match at the level of species either represent species that have so far not been morphologically identified and deposited at BOLD, or they represent currently undescribed species. With more than half of the OTUs that we detected having no reliable BOLD match, it is likely that at least a few of the specimens represent undescribed species. However, work on that aspect of these specimens will require substantial time and also collaboration with taxonomic group experts. Vouchering at BIO will allow long-term access for ourselves or for workers with particular expertise in some of the groups represented in this collection. In other words, our work has left a lasting physical legacy which will be useful not just for us but for others, and may result in new discoveries in time.

Our museum-and-database compiled EPT record is extremely substantial and covers much of western Canada and some neighbouring areas of the United States, although more than a third of the records are from British Columbia because that is where we mainly focused our search. This database likely represents the most complete compilation of digitized records of EPTs for BC, and perhaps for all of western Canada. During our discussions with collection curators we found that while a substantial number of specimens and their records are digitized, a much greater number remain undigitized and thus much less accessible to contemporary analysis methods. As can be seen in the appended XLS file, even the digitized records often do not contain full collection information; in particular many lack accurate geographical information or, in some cases, any geographical information at all beyond the larger provincial or state jurisdiction.

While a large number of records were from BC, most of those were from regions much further south in the province than the Williston system. Those records still provide useful information as many of these species tend to have rather wide distributions, particularly along the Rocky Mountains. However, it does highlight the fact that the Williston region is minimally explored, at best, for aquatic macroinvertebrates. This database, and the rapid DNA barcode-based biodiversity survey are evidence of a plethora of new questions to explore and provide a good foundation for further work on the effects of Kokanee on stream and riparian biodiversity and food webs.

## Recommendations

This report is based on the findings from Year 1 results only. Our year 2 project results will provide a clearer picture of trends in nutrient transfer between kokanee and non-kokanee stream and river reaches. Our Year 3 research is designed to provide a synthesis of project findings and develop major recommendations. However, based on results to date we would recommend that the sampling program be expanded to increase both temporal and spatial resolution. Consequently, we plan to expand our sampling program to cover a greater area, but also sample earlier in the season before Kokanee spawn and then again following the spawning period. In collaboration with our research partners, we also plan to identify potential study streams where Kokanee spawn in lower reaches, but are unable to access higher reaches of the system – thereby limiting any potential geographic influence on the stable isotope signals. For each of the locations, we will sample for aquatic invertebrates, benthic stream dwelling fish, and lichen within the riparian areas adjacent to the streams.

## Acknowledgements

We thank our research partners Luke Gleeson, Shawna Case, Mike Tilson, Curtis Bjork, and Trevor Goward. We would also like to acknowledge the technical assistance of Lindi Anderson, Daemon Cline, Adam O’Dell, and Claire Shrimpton. Also thank you to the Fish and Wildlife Compensation Program on behalf of its program partners BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations, and public stakeholders for the financial support of this project.

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Table 1. Summary of responses to survey on Kokanee presence in the Williston Reservoir watershed. A total of 62 individuals provided responses to the survey questions.

REACH	WATERSHED	RIVER	RESPONSE		
FINLAY		Bruin Creek	√		
		Chowika River	√√		
		Collins Creek	√		
		Davis River	√√		
		Factor Ross Creek	√		
		Hydro Creek	√√		
		Ivor Creek	√		
		Lafferty Creek	√		
		Police Creek	√		
		Rubyred Creek	√		
		Finlay River	Finlay River	√√	
			Akie River	√√	
			Bower Creek	√	
			Del Creek	√	
			Fox River	√	
			McGraw Creek	√√	
			Paul River	√	
			Pesika River	√√	
			Ingenika River	Ingenika River	√√
				Pelly Creek	√
				Ravenal Creek	√
				Swannell River	√
Ospika River	Ospika River	√√			
	Aley Creek	√			
	Gauvreau Creek	√			
	Stevenson Creek	√			
OMINECA	Mesilinka River	Mesilinka River	√		
		Omineca River	√		
	Omineca River	Germansen River	√		
		Osilinka River	√√		
		Tenakihi Creek	√		
PARSNIP	Parsnip River	Crooked River	×		
		McLeod River	×		
		Manson River	√		
		Nation River	√		
		Parsnip River	√		
		Anzac River	×		
		Colbourne Creek	×		
		Hominka River	×		
		Misinschinka River	×		
		Missinka River	×		
		Reynolds Creek	√		
		Table River	×		
		Wichcika Creek	×		

√ indicates Kokanee observed in the system by at least one respondent; √√ indicates Kokanee observed in the system by at least 10 respondents; × indicates respondents specifically mentioned never observing Kokanee in the system.

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Table 2. Sampling locations and sample date where aquatic invertebrates and stream resident fish were collected in October 2016. Species of stream fish caught at each site are also listed. Number of each species caught are given in parentheses.

River	Latitude	Longitude	Date	Species collected
Missinka	54° 37' 1"	121° 57' 49"	6 Oct 2016	<i>Cottus cognatus</i> (14)
Parsnip	54° 35' 51"	122° 4' 52"	6 Oct 2016	<i>Cottus cognatus</i> (2) <i>Cottus asper</i> (1) <i>Thymallus arcticus</i> (1) <i>Prosopium williamsoni</i> (1) <i>Richardsonius balteatus</i> (5)
Aley Creek	56° 28' 24"	123° 55' 22"	12 Oct 2016	<i>Cottus cognatus</i> (4)
Stevenson Creek	56° 20' 45"	123° 56' 27"	12 Oct 2016	<i>Cottus cognatus</i> (10) <i>Oncorhynchus mykiss</i> (2)
Bruin Creek	56° 21' 24"	123° 57' 16"	12 Oct 2016	<i>Cottus cognatus</i> (7) <i>Oncorhynchus mykiss</i> (1) <i>Salvelinus confluentus</i> (3)
Tenekihi Creek	56° 8' 41"	125° 8' 20"	13 Oct 2016	<i>Cottus cognatus</i> (3) <i>Lota lota</i> (1)
Osilinka River	54° 8' 38"	125° 8' 22"	13 Oct 2016	<i>Cottus cognatus</i> (10) <i>Lota lota</i> (2)

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Table 3. Presence or absence of Trichoptera (caddisflies) as detected by DNA barcoding for seven systems in the Williston region.

<u>Barcode-based ID</u>	<u>Missinka</u>	<u>Parsnip</u>	<u>Aley</u>	<u>Stevenson</u>	<u>Bruin</u>	<u>Tenekihi</u>	<u>Osilinka</u>
<i>Apatania comosa</i>						√	
<i>Arctopsyche grandis</i>			√	√		√	√
<i>Brachycentrus americanus</i>	√	√				√	√
<i>Ecclisomyia conspersa</i>						√	
<i>Lepidostoma cascadenense</i>		√				√	
<i>Lepidostoma pluviale</i>							√
<i>Micrasema bactro</i>	√						
<i>Oligophlebodes sierra</i>					√		
<i>Onocosmoecus unicolor</i>		√					
<i>Parapsyche elsis</i>			√	√	√	√	
<i>Rhyacophila brunnea</i>			√	√	√		√
<i>Rhyacophila hyalinata</i>					√		
<i>Rhyacophila narvae</i>						√	
<i>Rhyacophila pellisa</i>					√		√
<i>Rhyacophila vaccua</i>			√		√		
Rhyacophilidae sp.1	√						

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Table 4. Presence or absence of Plecoptera (stoneflies) as detected by DNA barcoding for seven systems in the Williston region.

<u>Barcode-based ID</u>	<u>Missinka</u>	<u>Parsnip</u>	<u>Aley</u>	<u>Stevenson</u>	<u>Bruin</u>	<u>Tenekihi</u>	<u>Osilinka</u>
<i>Alloperla severa</i>	√	√					
<i>Bolshecapnia milami</i>			√				√
<i>Capnia coloradensis</i>					√		
<i>Capnia nana</i>			√			√	
<i>Capnia</i> sp.1					√		
<i>Capnia</i> sp.2			√		√		
<i>Capnia</i> sp.3	√						
<i>Doddsia occidentalis</i>			√	√			√
<i>Doddsia</i> sp.1					√		
<i>Eucapnopsis brevicauda</i>			√		√		
<i>Hesperoperla pacifica</i>		√					√
<i>Isoperla petersoni</i>		√	√			√	√
<i>Isoperla sobria</i>			√	√			
<i>Kogotus modestus</i>				√		√	√
<i>Megarcys signata</i>			√	√			
<i>Paraleuctra occidentalis</i>			√	√	√		
<i>Perlodidae</i> sp.1						√	
<i>Plumiperla diversa</i>		√		√	√		
<i>Plumiperla</i> sp.1			√				
<i>Prostoia besametsa</i>					√		
<i>Sweltsa borealis</i>			√	√	√		
<i>Sweltsa coloradensis</i>	√	√				√	√
<i>Sweltsa</i> sp.1						√	

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<i>Sweltsa</i> sp.2						√	√
<i>Taenionema pallidum</i>	√					√	
<i>Utacapnia columbiana</i>	√						
<i>Zapada cinctipes</i>	√	√	√	√	√	√	√
<i>Zapada</i> sp.1						√	
<i>Zapada</i> sp.2				√			
<i>Zapada</i> sp.3				√		√	
<i>Zapada</i> sp.4			√	√			
<i>Zapada</i> sp.5			√				



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Table 5. Presence or absence of Ephemeroptera (mayflies) as detected by DNA barcoding for seven systems in the Williston region.

<u>Barcode-based ID</u>	<u>Missinka</u>	<u>Parsnip</u>	<u>Aley</u>	<u>Stevenson</u>	<u>Bruin</u>	<u>Tenekihi</u>	<u>Osilinka</u>
<i>Ameletus celer</i>			√	√			
<i>Ameletus oregonensis</i>	√						√
<i>Ameletus velox</i>				√			
<i>Ameletus</i> sp.1				√		√	
<i>Baetis bicaudatus</i>			√	√	√	√	
<i>Baetis tricaudatus</i>	√	√	√		√		√
<i>Baetis</i> sp.1					√		
<i>Baetis</i> sp.2				√			
<i>Cinygmula</i> sp.1					√		
<i>Cinygmula</i> sp.2			√		√		
<i>Cinygmula</i> sp.3		√					
<i>Cinygmula</i> sp.4			√	√	√	√	√
<i>Drunella coloradensis</i>					√		
<i>Drunella doddsii</i>	√	√			√	√	
<i>Drunella grandis</i>	√	√	√	√	√	√	√
<i>Drunella</i> sp.1						√	
<i>Epeorus deceptivus</i>			√				
<i>Epeorus grandis</i>				√	√		
<i>Epeorus longimanus</i>					√		
<i>Ephemerella</i> sp.1		√					
<i>Ephemerella</i> sp.2	√	√					

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<i>Ephemerella</i> sp.3	√	√	√	√	√	√	√
<i>Paraleptophlebia heteronea</i>							√
<i>Paraleptophlebia</i> sp.1							√
<i>Rhithrogena hageni</i>	√	√		√	√		
<i>Rhithrogena robusta</i>	√		√	√			
<i>Rhithrogena</i> sp.1			√				
<i>Rhithrogena</i> sp.2					√	√	√

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Table 6. Presence or absence of Diptera (true flies) as detected by DNA barcoding for seven systems in the Williston region.

<u>Barcode-based ID</u>	<u>Missinka</u>	<u>Parsnip</u>	<u>Aley</u>	<u>Stevenson</u>	<u>Bruin</u>	<u>Tenekihi</u>	<u>Osilinka</u>
Agromyzidae sp.1							√
Ceratopogonidae sp.1			√		√		√
<i>Chelifera</i> sp.1	√						
Chironomidae sp.1						√	
Chironomidae sp.2	√						
Chironomidae sp.3					√		
Chironomidae sp.4						√	√
Chironomidae sp.5							√
Chironomidae sp.6			√				
Chironomidae sp.7							√
Chironomidae sp.8			√				
Chironomidae sp.9					√		
Chironomidae sp.10				√			
Chironomidae sp.11			√			√	
Chironomidae sp.12							√
Chironomidae sp.13			√	√			
Chloropidae sp.1				√			
<i>Conchapelopia pallens</i>	√		√				
<i>Diamesa</i> nr. <i>hyperborea</i>			√				
<i>Eukiefferiella claripennis</i>			√				
<i>Helodon pleuralis</i>					√		
<i>Helodon onychodactylus</i>					√		
Limoniidae sp.1				√	√	√	

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Limoniidae sp.2	√						
Limoniidae sp.3						√	
Limoniidae sp.4			√	√	√	√	√
Limoniidae sp.5	√	√					
<i>Microspectra</i> sp.1			√				
<i>Microspectra</i> sp.2							√
<i>Neoplasta megorchis</i>		√					
<i>Oreogeton scopifer</i>					√		
Oreoleptidae sp.1						√	
Oreoleptidae sp.2					√		
Pediciidae sp.1							√
Psychodidae sp.1							
Sphaeroceridae sp.1			√				
Sphaeroceridae sp.2						√	

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Table 7. Presence or absence of Coleoptera (beetles) as detected by DNA barcoding for seven systems in the Williston region.

<u>Barcode-based ID</u>	<u>Missinka</u>	<u>Parsnip</u>	<u>Aley</u>	<u>Stevenson</u>	<u>Bruin</u>	<u>Tenekihi</u>	<u>Osilinka</u>
Dytiscidae sp.1	√						
Elmidae sp.1	√	√					

***Ecosystem impact of nutrient enrichment by Kokanee in the Williston Reservoir Watershed.***

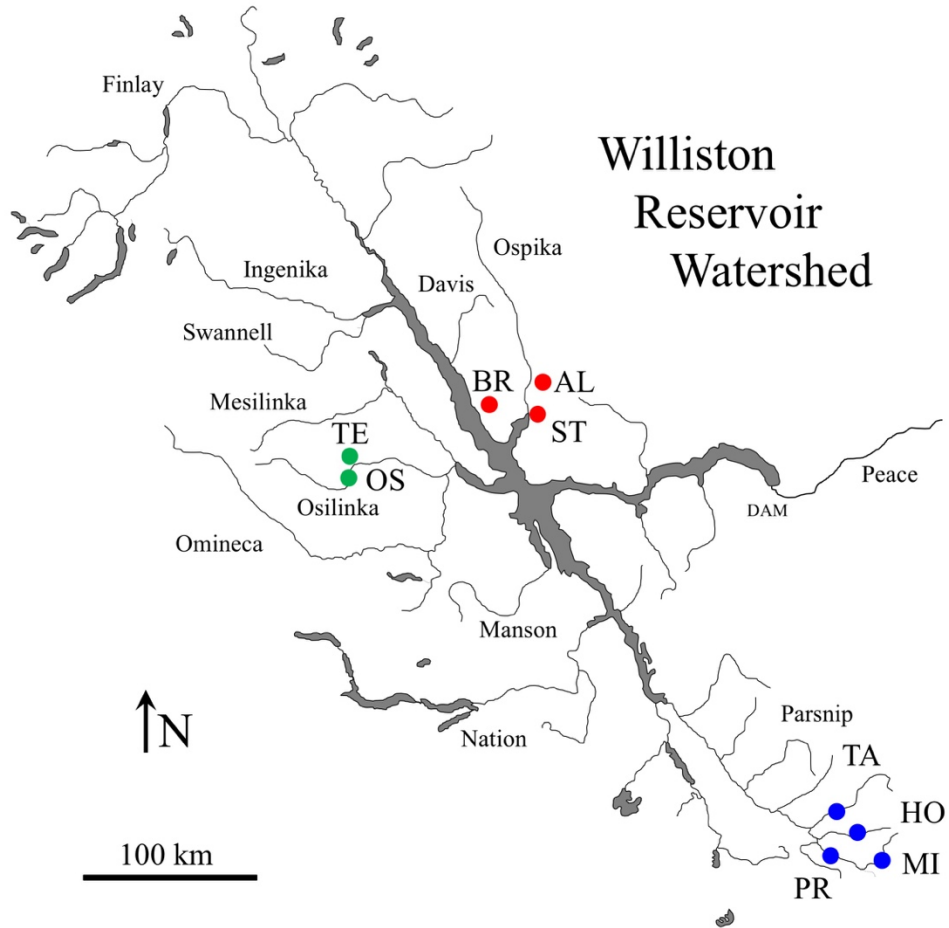


Figure 1. Map of sampling locations in the Williston Reservoir watershed for 2016.

Red and green circles indicate sites where sampling was conducted for Kokanee spawning systems;

- Green symbols for Osilinka River (OS) and Tenakihi Creek (TE) that had low escapement in 2016.
- Red symbols for Aley Creek (AL), Stevenson Creek (ST) and Bruin Creek (BR) that had high escapement in 2016.

Blue circles indicate control sites where no Kokanee spawned; Table River (TA), Hominka River (HO), Missinka River (MI), and upper Parsnip River (PR).

**Ecosystem impact of nutrient enrichment by Kokanee in the Williston Reservoir Watershed.**

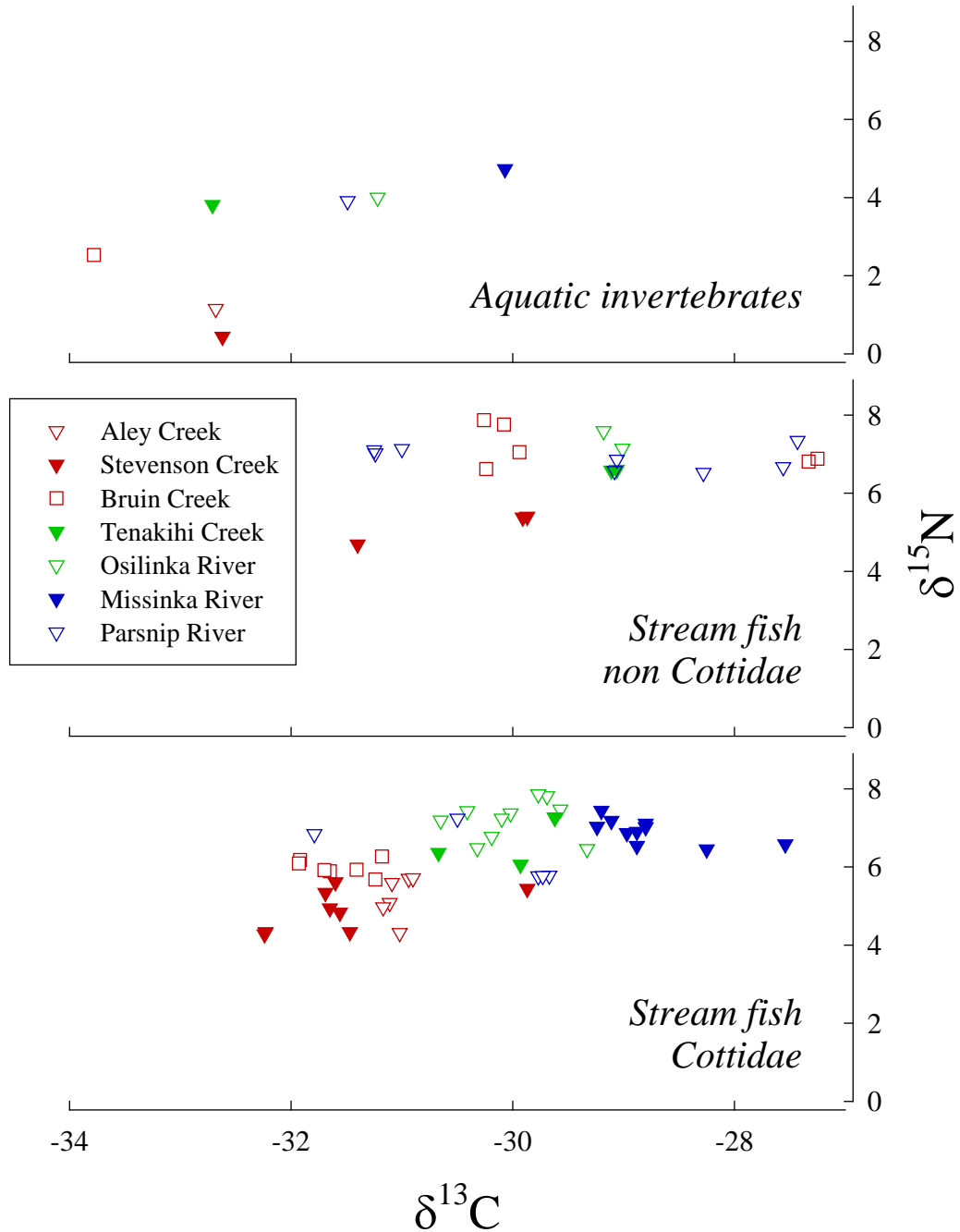


Figure 2. Plot of stable isotope signatures for carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) from aquatic invertebrates (top panel), stream fish not belonging to the family Cottidae (middle panel), and stream fish belonging to the family Cottidae (bottom panel) for seven study streams in the Williston watershed. In 2016, Kokanee spawned in high abundance in Aley, Stevenson and Bruin Creeks, low abundance in the Osilinka River and Tenakihi Creek, and there were no spawners in the Parsnip River and Missinka River.

**Ecosystem impact of nutrient enrichment by Kokanee in the Williston Reservoir Watershed.**

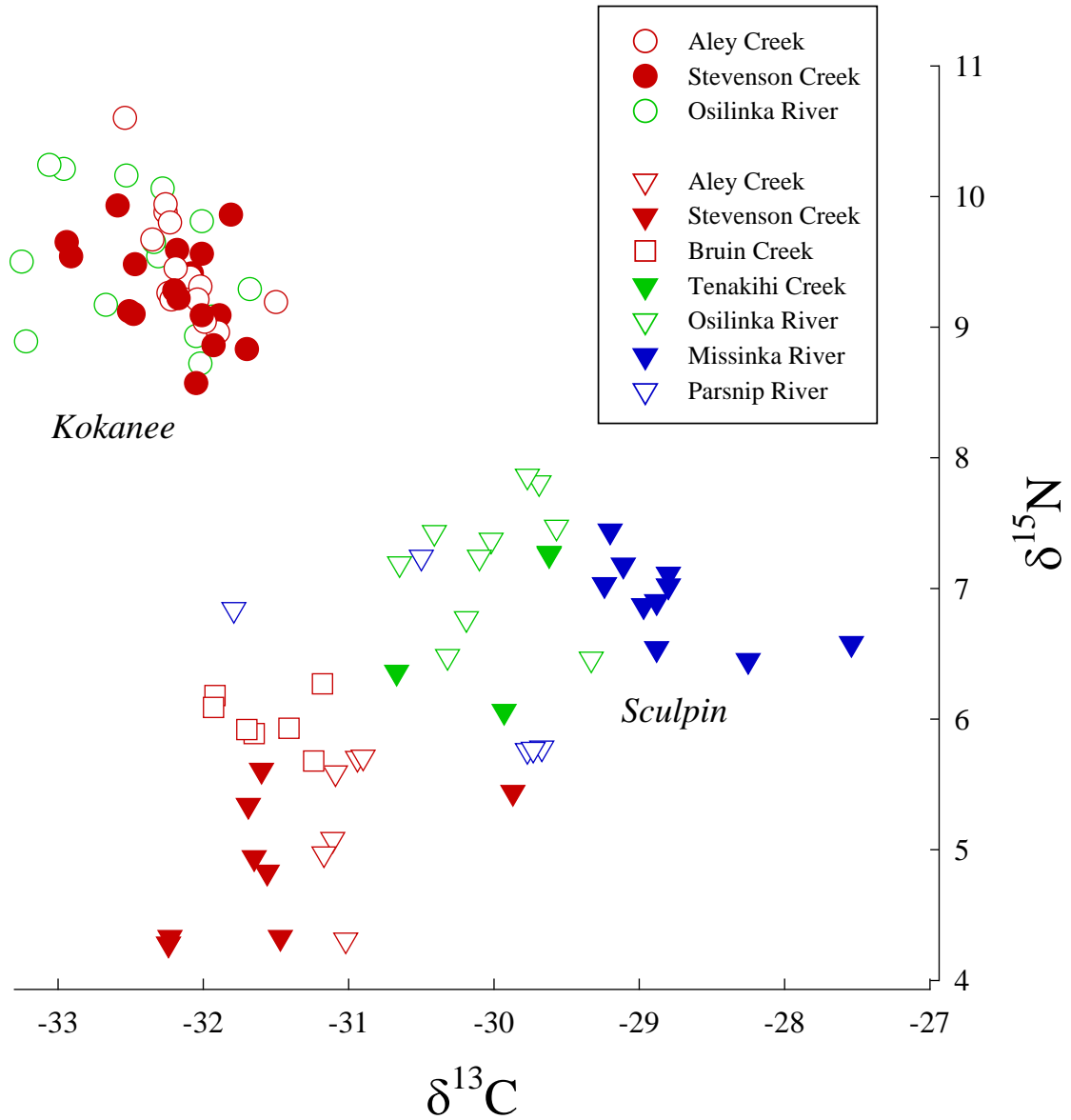


Figure 3. Plot of stable isotope signatures for carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) comparing values for Kokanee collected in tributaries where they were spawning compared to stream resident fish belonging to the family Cottidae.



**Ecosystem impact of nutrient enrichment by Kokanee in the Williston Reservoir Watershed.**

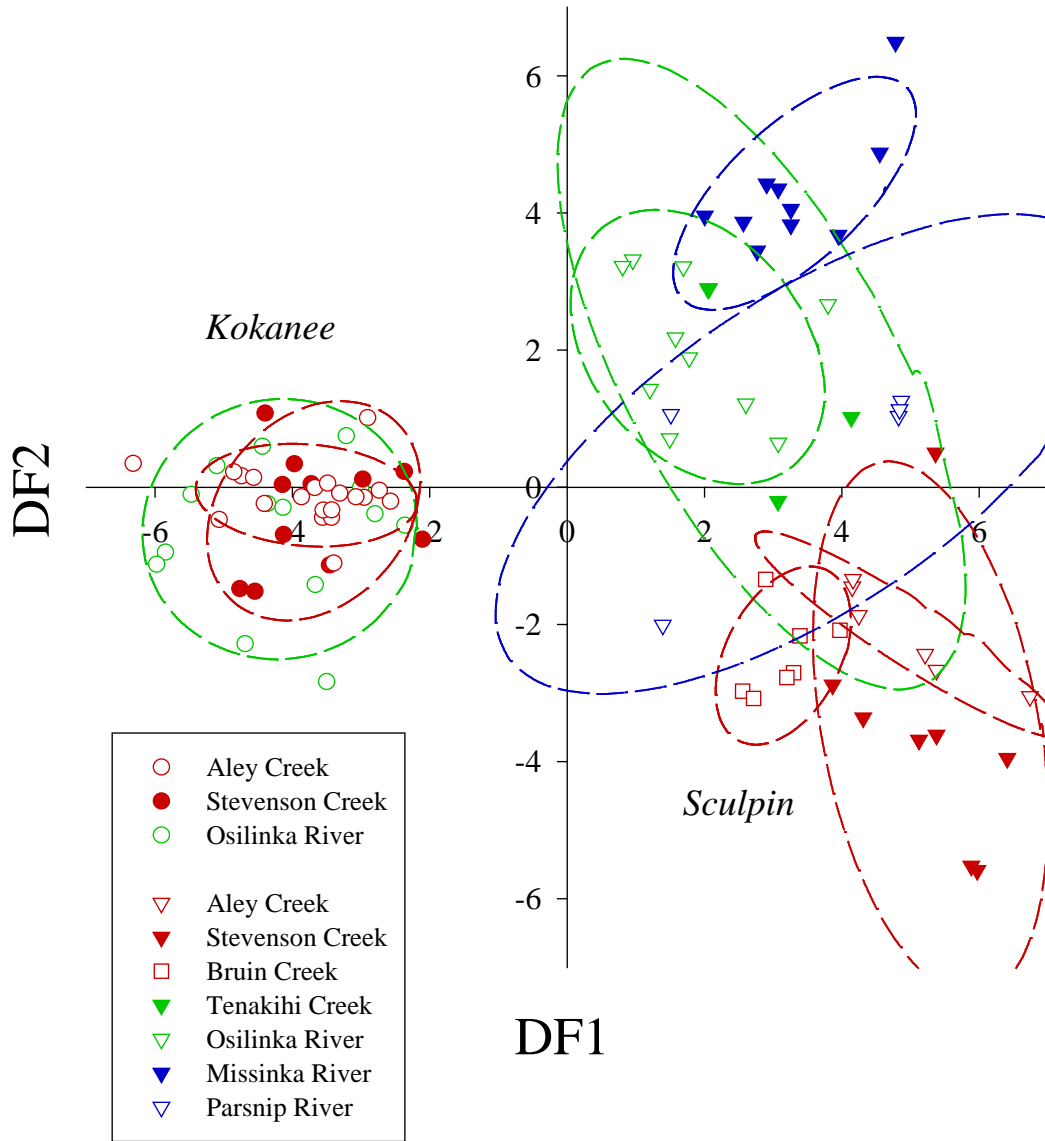


Figure 4. Canonical discriminant function scores for slimy sculpin and Kokanee collected from tributaries to the Williston Reservoir in fall 2016. Values for each river system are defined by 95% confidence ellipses.

*Ecosystem impact of nutrient enrichment by Kokanee in the Williston Reservoir Watershed.*

Appendix 1. Lichen abundance values by species for Table River (Site 1), Hominka River (Site 2), and Parsnip River (Site 3).

Species	Group	Subgroup	Abundance by Plot													
			Site									Plot				
			1			2			3			1	2	3		
			1	2	3	4	5	1	2	3	4	5	1	2	3	
Arthonia apatetica	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Arthonia didyma	Microlichen	Trentepohlioid	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Arthonia edgewoodensis	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Arthonia leptalea ined.	Microlichen	Trebouxioid	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Arthonia lignariella	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Arthonia radiata	Microlichen	Trentepohlioid	0	0	1	0	1	0	0	0	0	1	0	0	0	0
Bacidia beckhausii	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	1	0	0	0	0	0
Bacidia circumspecta	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Bacidia laurocerasi s. str.	Microlichen	Trebouxioid	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Bacidia rosellizans	Microlichen	Trebouxioid	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Bacidia subincompta	Microlichen	Trebouxioid	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Bacidia vermifera	Microlichen	Trebouxioid	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Biatora efflorescens	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Biatora pallens	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Biatora subduplex	Microlichen	Trebouxioid	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Biatora vacciniicola	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Biatoridium delitescens	Microlichen	Trebouxioid	1	1	1	0	0	0	1	0	0	0	1	0	0	0
Bilimbia sabuletorum	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Bryoria chalybeiformis (aurea form)	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Bryoria fuscescens	Macrolichen	Chloro	1	1	1	1	1	0	1	1	2	0	1	1	1	1
Bryoria glabra	Macrolichen	Chloro	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Bryoria pikei	Macrolichen	Chloro	2	1	1	1	1	0	1	0	2	2	1	1	0	0

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Bryoria yellow soralia	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	0	1	0
Buellia arborea	Microlichen	Trebouxioid	0	1	0	0	0	0	0	0	0	0	0	0	0
Buellia disciformis	Microlichen	Trebouxioid	0	0	0	0	1	0	0	0	0	2	0	0	0
Buellia griseovirens	Microlichen	Trebouxioid	0	0	0	1	0	1	0	0	0	0	0	1	0
Buellia nicolaensis ined.	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	0	0	0	0
Buellia penichra	Microlichen	Trebouxioid	2	3	3	1	5	0	0	2	0	1	0	1	1
Buellia punctata	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	0	0
Buellia unknown species (erumpent)	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	0	0
Calicium adaequatum	Microlichen	Calicioid	0	0	0	0	0	0	0	0	0	0	0	1	0
Calicium parvum	Microlichen	Calicioid	0	0	0	0	0	0	0	0	0	0	1	0	0
Calicium viride	Microlichen	Calicioid	1	0	0	1	0	0	1	1	0	2	2	1	2
Caloplaca ahtii	Microlichen	Trebouxioid	0	1	0	0	0	0	0	0	0	0	0	0	0
Caloplaca atosanguinea	Microlichen	Trebouxioid	1	1	0	0	0	0	0	1	0	0	2	0	0
Caloplaca cf. oleicola	Microlichen	Trebouxioid	1	0	0	0	0	0	0	0	0	0	0	0	0
Caloplaca flavorubescens	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	1	0	0	0
Caloplaca pyracea sensu lato	Microlichen	Trebouxioid	0	1	0	0	0	0	0	0	0	0	0	0	0
Caloplaca sorocarpa	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	0	0
Caloplaca tricolor	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	0	1	0	0
Candelariella efflorescens	Microlichen	Trebouxioid	0	1	0	0	0	0	1	0	0	1	0	0	0
Candelariella reflexa	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	1	0	0	0
Catinaria atropurpurea	Microlichen	Trebouxioid	0	1	1	0	0	0	0	0	0	0	0	0	0
Chaenotheca cinerea	Microlichen	Calicioid	0	0	0	0	0	0	1	1	1	0	0	0	0
Chaenotheca hispidula	Microlichen	Calicioid	0	0	0	1	0	0	0	0	0	0	0	0	0
Chaenotheca trichialis	Microlichen	Calicioid	0	0	0	0	0	0	0	0	0	1	0	0	0
Chaenothecopsis populicola	Microlichen	Calicioid	0	0	0	0	0	0	1	0	0	0	0	0	0
Chaenothecopsis sp. unknown	Microlichen	Calicioid	0	0	0	0	0	0	0	0	0	1	0	0	0

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Chrysothrix candelaris	Microlichen	Trebouxioid	1	0	0	1	0	0	1	1	0	0	0	1	0
Cladonia cenotea	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	0	0	1
Cladonia coniocraea	Macrolichen	Chloro	0	0	0	0	0	0	1	1	0	1	1	1	1
Cladonia fimbriata	Macrolichen	Chloro	0	0	0	0	0	1	0	1	1	1	1	0	0
Cladonia sulphurea	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	0	0	1
Cliostomum griffithii	Microlichen	Trebouxioid	1	0	0	1	0	1	0	0	0	1	1	1	1
Cliostomum spribillei	Microlichen	Trebouxioid	1	0	0	0	1	0	1	0	0	1	0	0	0
Collema coniophilum	Macrolichen	Cyano	1	1	0	0	0	0	0	1	0	0	0	0	0
Collema quadrifidum?	Macrolichen	Cyano	0	0	0	0	0	0	0	0	1	0	0	0	0
Collema striatum	Macrolichen	Cyano	0	0	1	0	0	0	0	0	0	0	0	0	0
Collema subflaccidum	Macrolichen	Cyano	0	1	1	1	0	0	0	1	0	0	1	0	0
Collema subfuscum	Macrolichen	Cyano	1	0	0	0	0	0	0	0	0	0	0	0	0
Cyphelium inquinans	Microlichen	Trebouxioid	0	0	0	1	1	1	2	0	0	0	0	1	1
Hypogymnia recurva (coll)	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	2	0	0
Hypogymnia occidentalis	Macrolichen	Chloro	1	1	1	1	1	1	0	1	1	1	1	1	1
Hypogymnia physodes	Macrolichen	Chloro	3	0	2	1	1	1	1	1	1	1	1	1	1
Hypogymnia protea	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	1	0	0
Hypogymnia rugosa	Macrolichen	Chloro	1	1	1	1	1	0	0	0	0	0	0	1	0
Hypogymnia tubulosa	Macrolichen	Chloro	1	1	2	1	1	1	0	1	0	0	0	1	1
Hypogymnia vittata	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	0	0	1
Hypogymnia wilfiana	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	1	0	0
Japewia subaurifera	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	0	0	0	0
Japewia tornoensis	Microlichen	Trebouxioid	0	0	0	0	1	0	0	0	0	0	0	0	0
Lecania cyrtellina	Microlichen	Trebouxioid	0	0	0	0	0	0	2	0	0	0	0	0	0
Lecanora allophana f. allophana	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	0	0
Lecanora allophana f. soralifera	Microlichen	Trebouxioid	0	1	0	0	0	1	1	2	1	1	1	0	0
Lecanora cadubriae	Microlichen	Trebouxioid	0	0	0	0	0	0	1	0	0	0	0	0	0
Lecanora chlarotera	Microlichen	Trebouxioid	3	4	3	1	2	2	1	2	2	2	2	2	1
Lecanora farinaria	Microlichen	Trebouxioid	1	2	0	1	0	0	1	1	1	2	1	3	1

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Lecanora farinaria cfr. (cottonwood form)	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	1	0	0	0
Lecanora fuscescens	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	0	0	0	1
Lecanora hybocarpa	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	1	0	0	0	0
Lecanora impudens	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	1	1	0	0
Lecanora symmicta	Microlichen	Trebouxioid	1	2	0	0	1	1	0	1	1	0	1	1	1
Lecidea albohyalina	Microlichen	Trebouxioid	0	0	1	0	0	0	0	0	0	0	0	0	1
Lecidea betulicola	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	0	1	0	0
Lecidea erythrophaea	Microlichen	Trebouxioid	0	1	0	1	0	0	0	1	1	1	0	0	0
Lecidea hypnorum	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	1	0	0	0	0
Lecidea leprarioides	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	0	1	0	0
Lecidea nylanderii	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	0	0	0	0
Lecidea pullata	Microlichen	Trebouxioid	0	0	0	1	2	0	0	1	0	0	1	1	1
Lecidella euphorea	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	0	1	0	0
Lepraria torii	Microlichen	Trebouxioid	0	0	0	0	0	0	1	0	0	0	0	0	0
Leptogium cellulosum	Macrolichen	Cyano	1	0	1	0	0	1	0	1	1	1	1	1	0
Leptogium compactum	Macrolichen	Cyano	0	1	0	0	0	0	0	0	0	0	0	0	0
Leptogium saturninum	Macrolichen	Cyano	1	2	1	1	0	1	1	1	1	0	1	1	0
Leptogium teretiusculum	Macrolichen	Cyano	0	2	1	1	0	0	0	1	0	1	0	0	0
Leptorhaphis epidermidis	Microlichen	Trebouxioid	1	1	1	0	1	2	0	1	0	1	1	0	1
Lobaria hallii	Macrolichen	Cyano	1	1	1	1	0	1	1	2	0	0	1	1	0
Lobaria pulmonaria	Macrolichen	Cyano	1	2	1	1	0	3	2	3	3	3	1	1	1
Lobaria scrobiculata	Macrolichen	Cyano	0	0	0	0	0	1	0	1	0	1	0	1	0
Lopadium disciforme	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	1	0	1	0
Melanelixia glabratula	Macrolichen	Chloro	1	2	2	1	1	0	0	1	0	0	0	1	2
Melanelixia subaurifera	Macrolichen	Chloro	0	1	0	0	1	0	0	1	0	1	1	1	0
Melanohalea exasperatula	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	1	0	0
Melanohalea glabratula	Macrolichen	Chloro	0	0	0	0	0	0	0	1	0	0	0	0	0
Melanohalea multispora	Macrolichen	Chloro	1	1	0	0	1	0	0	1	1	1	1	1	0

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Melanohalea septentrionalis	Macrolichen	Chloro	0	0	0	0	1	0	0	0	0	0	0	1	0
Micarea paupercula ined.	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	1	0
Micarea prasina group	Microlichen	Trebouxioid	0	0	1	0	0	1	0	0	1	0	0	1	0
Mycobilimbia carneoalbida	Microlichen	Trebouxioid	0	1	0	0	0	1	1	1	1	1	0	1	0
Mycobilimbia epixanthoides	Microlichen	Trebouxioid	0	1	1	1	0	1	1	1	1	1	1	0	0
Mycobilimbia tetramera	Microlichen	Trebouxioid	0	0	0	0	0	0	1	0	0	0	0	0	0
Mycoblastus affinis	Microlichen	Trebouxioid	1	0	2	1	2	0	1	1	0	0	1	2	1
Mycoblastus alpinus cfr.	Microlichen	Trebouxioid	0	0	0	0	0	0	2	0	0	0	0	0	0
Mycoblastus sanguinarioides	Microlichen	Trebouxioid	0	0	0	0	0	0	1	0	0	0	0	0	1
Mycoblastus sanguinarius	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	2	0	1	0	0
Nephroma bellum	Macrolichen	Cyano	1	0	0	0	0	2	1	1	1	1	0	0	1
Nephroma isidiosum	Macrolichen	Cyano	0	0	0	0	0	0	0	0	0	0	0	0	1
Nephroma parile	Macrolichen	Cyano	2	1	1	1	1	2	2	1	1	1	2	1	1
Nephroma resupinatum	Macrolichen	Cyano	0	0	0	0	0	1	0	0	1	0	2	1	1
Ochrolechia androgyna	Microlichen	Trebouxioid	0	0	0	0	1	0	1	0	0	0	0	1	0
Ochrolechia szatalaensis	Microlichen	Trebouxioid	0	1	1	1	0	1	1	1	0	1	2	0	0
Opegrapha varia	Microlichen	Trentepohlioid	0	0	0	0	0	0	0	0	0	1	0	0	0
Parmelia hygrophila	Macrolichen	Chloro	2	1	2	1	1	1	1	2	1	0	0	1	1
Parmelia sulcata	Macrolichen	Chloro	4	4	4	2	5	2	1	2	1	1	2	1	2
Parmelia sulymae	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	1	0	0
Parmeliella triptophylla	Macrolichen	Cyano	0	0	0	0	0	0	0	0	1	0	0	0	0
Parmeliopsis ambigua	Macrolichen	Chloro	0	1	1	0	1	0	2	1	2	2	1	2	1
Parmeliopsis hyperopta	Macrolichen	Chloro	0	1	1	1	1	1	2	1	2	2	1	2	1
Peltigera aphthosa	Macrolichen	Cyano	0	0	0	0	0	0	1	0	0	0	0	0	0
Peltigera collina	Macrolichen	Cyano	0	1	1	1	0	2	0	2	0	2	1	1	0
Peltigera membranacea	Macrolichen	Cyano	0	0	1	0	0	0	1	0	1	0	0	0	1
Pertusaria amara	Microlichen	Trebouxioid	0	0	0	1	0	0	0	0	0	0	0	0	1

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Pertusaria carneopallida	Microlichen	Trebouxioid	0	1	1	0	1	3	0	0	1	0	0	1	2
Pertusaria multipuncta	Microlichen	Trebouxioid	0	0	0	0	0	3	0	0	0	0	0	0	0
Pertusaria ophthalmiza	Microlichen	Trebouxioid	1	1	2	1	2	1	3	1	1	2	1	1	1
Pertusaria sommerfeltii	Microlichen	Trebouxioid	0	0	0	0	0	1	0	0	0	0	0	0	0
Pertusaria stenhammari	Microlichen	Trebouxioid	0	0	0	0	0	0	0	1	0	0	0	0	1
Phaeocalicium compressulum	Microlichen	Calicioid	0	0	1	0	0	0	0	1	0	0	0	0	0
Phaeophyscia kairamoi	Microlichen	Calicioid	0	0	0	0	0	0	0	1	0	1	0	0	0
Phlyctis argena	Microlichen	Trebouxioid	1	0	0	1	0	0	1	1	1	1	0	1	0
Phlyctis speirea	Microlichen	Trebouxioid	0	0	0	0	1	0	0	0	0	0	0	0	0
Physcia alnophila	Macrolichen	Chloro	1	1	0	1	0	0	0	1	1	0	1	0	0
Physcia caesia	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	1	0	0	0
Physconia perisidiosa	Macrolichen	Chloro	1	0	0	0	0	2	0	1	1	1	0	0	0
Platismatia glauca	Macrolichen	Chloro	2	3	2	1	2	2	3	1	2	3	2	1	2
Pseudocyphellaria anomala	Macrolichen	Cyano	0	1	0	0	0	1	0	1	0	0	0	0	1
Ramalina dilacerata	Macrolichen	Chloro	0	1	0	0	0	0	0	1	0	0	0	0	0
Ramalina intermedia	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	1	0	0	0
Ramalina obtusata	Macrolichen	Chloro	0	0	0	1	0	0	0	0	0	1	1	1	0
Ramalina thrausta	Macrolichen	Chloro	3	2	2	1	2	1	3	0	3	3	2	2	2
Ramboldia cinnabarina	Microlichen	Trebouxioid	1	1	1	1	1	2	1	1	0	0	2	1	1
Rinodina capensis	Microlichen	Trebouxioid	0	1	1	1	0	0	0	0	0	1	1	0	0
Rinodina colobina	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	1	0	0	0
Rinodina degeliana	Microlichen	Trebouxioid	1	0	0	1	1	1	0	0	1	1	0	0	0
Rinodina disjuncta	Microlichen	Trebouxioid	1	0	0	1	1	0	0	0	0	0	0	1	0
Rinodina flavosoralifera	Microlichen	Trebouxioid	0	0	0	0	0	0	0	1	0	0	0	1	0
Rinodina griseosoralifera	Microlichen	Trebouxioid	0	1	1	1	1	0	0	0	0	0	0	1	0
Rinodina metaboliza (coll)	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	0	1	0	0
Rinodina orculata	Microlichen	Trebouxioid	0	1	0	0	0	0	0	0	0	1	1	0	0
Rinodina oregana	Microlichen	Trebouxioid	0	0	0	0	0	0	0	0	0	1	1	0	0
Rinodina pyrina	Microlichen	Trebouxioid	1	1	0	0	1	0	0	0	0	0	0	0	0

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Rinodina stictica	Microlichen	Trebouxioid	0	1	0	0	0	0	0	0	0	1	0	0	0
Rinodina trevisanii	Microlichen	Trebouxioid	1	1	1	0	1	0	0	0	0	1	0	0	0
Santessoniella saximontana	Macrolichen	Cyano	0	0	0	0	0	1	0	0	0	0	0	0	0
Schaereria corticola	Microlichen	Trebouxioid	1	1	1	0	1	0	0	0	1	0	0	1	1
Stenocybe pullatula	Microlichen	Calicioid	0	0	0	0	0	0	0	1	0	0	0	0	0
Sticta fuliginosa	Macrolichen	Cyano	1	0	0	0	0	1	0	0	1	1	0	0	0
Sticta sylvatica	Macrolichen	Cyano	0	0	0	0	0	0	0	1	0	0	0	0	0
Toensbergia leucococca	Microlichen	Trebouxioid	0	0	0	1	0	1	0	0	0	0	0	0	0
Tuckermannopsis chlorophylla	Macrolichen	Chloro	1	1	1	1	2	1	1	1	1	2	1	1	1
Usnea barbara f. isidiomorpha	Macrolichen	Chloro	0	0	1	0	1	0	0	0	0	1	0	1	1
Usnea barbata f. scabrata	Macrolichen	Chloro	1	1	0	1	1	1	1	1	1	1	0	1	1
Usnea dasopoga	Macrolichen	Chloro	0	0	0	0	0	1	0	0	0	0	0	0	0
Usnea glabrata	Macrolichen	Chloro	0	0	1	0	1	0	0	0	0	0	0	0	0
Usnea lapponica	Macrolichen	Chloro	1	0	1	0	0	0	0	0	0	1	0	0	0
Usnea substerilis	Macrolichen	Chloro	1	1	1	0	0	0	0	0	0	0	0	0	0
Vulpicida pinastri	Macrolichen	Chloro	0	1	1	0	1	1	1	0	1	0	0	0	0
Xanthomendoza fallax	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	1	0	0
Xanthomendoza fulva	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	1	0	0	0
Xanthoria fallax	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	0	1	0
Xanthoria fulva	Macrolichen	Chloro	0	0	0	0	0	1	0	0	0	0	0	0	0
Xanthoria kaernefeltii	Macrolichen	Chloro	0	0	0	0	0	0	0	0	0	0	0	1	0