

Ecosystem impact of nutrient enrichment by Kokanee in the Williston Reservoir Watershed (PEA-F19-F-2624)

Final Report

Prepared for:

Fish and Wildlife Compensation Program – Peace Region

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2 March 2020



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, Osilinka, 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 Reservoir 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."

The distribution of spawning Kokanee 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. In 2016, we sampled the Missinka River and Upper Parsnip River as control sites where Kokanee have not been observed to spawn. Sites where Kokanee spawn that were sampled were Tenakihi Creek and Osilinka River in the Omineca River watershed, Aley and Stevenson Creeks in the Ospika River watershed, and Bruin Creek in the Finlay Reach of the reservoir. Due to the wide spatial separation of our control and experimental sites in 2016, we selected additional control and experimental sites for both 2017 and 2018. These included the Wichcika River, an upper Parsnip River tributary as a control stream. We also included the Wooyadilinka River, a tributary to the Parsnip River closer to the Williston Reservoir than our other control streams, as an additional control stream. Additionally, in 2018, we added Bill's and Lamonti Creeks to our control stream sampling. We spent considerable effort to find additional experimental (Kokanee present) streams that were closer geographically to our control streams. Consequently, in addition to experimental streams in the Ospika River watershed, Aley Creek and Stevenson Creek, and streams in the Omineca River watershed, Osilinka River and Tenakihi Creek, we sampled streams closer in proximity to the control streams. We sampled two streams that flow into the Parsnip Reach of the Williston Reservoir. Mugaha Creek, a stream that was stocked with Kokanee through the salmonids in the classroom program, and Cut Thumb Creek, where Kokanee had previously been observed to spawn were included as experimental streams. To assess temporal variation in our study and control streams, we sampled streams in both summer and fall 2017. In 2018, Patsuk and Scott Creeks, which were documented to have spawning Kokanee were added as experimental streams.

The transfer of nutrients into streams in the Williston watershed was assessed using two target groups, namely aquatic insects and riparian lichen communities to assess differences in abundance and community composition. Diversity of aquatic invertebrates within tributaries to

the Williston Reservoir and lichen in riparian areas adjacent to these streams is extensive, but little difference existed between streams where Kokanee did and did not spawn. Our analysis of aquatic insect diversity revealed differences due to season and no pattern related to presence of Kokanee spawners. Incidental to these studies, we found undocumented species from our taxonomic surveys extending the known range for these organisms to central BC.

We also collected samples for stable isotope analysis to track the potential delivery of nutrients from the reservoir to tributary streams and their riparian ecosystems using five functional groups;

1. Kokanee (*Oncorhynchus nerka*), spawning in tributary streams,
2. slimy sculpin (*Cottus cognatus*), a stream resident fish,
3. aquatic insects that dominated the stream macroinvertebrate community belonging to the Orders Ephemeroptera, Plecoptera, and Trichoptera (EPT),
4. a stem dwelling foliose green algal lichen *Parmelia squarrosa*, and
5. periphyton scraped from rock substrate of streams.

Carbon varied by study streams, but was depleted in $\delta^{13}\text{C}$ from streams where Kokanee spawn and highly variable in control streams where Kokanee have not been documented to spawn. This pattern was seen in benthic stream resident fish, aquatic invertebrates – primarily Ephemeroptera, and the lichen samples. Nitrogen also varied among the samples – $\delta^{15}\text{N}$ was enriched in Kokanee from the Williston Reservoir that were spawning in tributary streams and depleted in samples collected from sculpin, aquatic insects and lichen. Stable isotopes of hydrogen were variable among sculpin samples, but generally depleted in $\delta^2\text{H}$ for samples from streams where Kokanee spawn and with increasing latitude. Oxygen signatures were strongly correlated with latitude and do not appear to reflect nutrient contribution, but rather reflect the spatial distribution of our sampling effort that overlapped Kokanee spawning distribution; a pattern of Kokanee spawning in more northerly tributaries in the Williston Reservoir watershed. For benthic invertebrates and stream resident fish, our findings suggest that Kokanee do not have an effect on stable isotope signatures or indicators of ecosystem integrity. Such findings suggest that the introduction of Kokanee to the Williston Reservoir watershed has had a limited impact on the aquatic food web and poses a low risk to ecosystem function. The lichen (*Parmelia squarrosa*), however, showed a strong separation between control and experimental streams for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, suggesting transfer of nutrients beyond the stream and into the adjacent riparian area, irrespective of geographic location. Differences may be due to differences in turnover rate for the different functional groups examined in our study. Escapement of spawning Kokanee to Williston Reservoir tributary streams, however, is highly variable from year to year, potentially providing an inconsistent nutrient pulse from the reservoir.

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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, can lead to a gradual loss of nutrients. Consequently, over time reservoirs in temperate regions become less productive and are often characterized as ultra-oligotrophic (Ross 1997). Fertilization projects for reservoirs in the Canadian portion of the Columbia River watershed have shown considerable success in enhancing productivity (Schindler et al. 2009); but the size of the reservoirs created in the Columbia Basin are relatively small. . In this context, the introduction of Kokanee into some streams in the Williston system could facilitate the transfer of nutrients from the reservoir to the surrounding tributaries.

The Williston Reservoir was created in 1968 following the construction of the WAC Bennett Dam and impounding the Peace River in the canyon near Hudson's Hope, BC for hydroelectric generation. With a surface area of 1,779 km², the Williston Reservoir is the largest lentic freshwater system in British Columbia. Attempts to increase productivity in the watershed have been limited, but a fertilization project on the Mesilinka River during the 1990's met with moderate success. Additionally, Kokanee (*Oncorhynchus nerka*) were stocked into Williston Reservoir from 1990 to 1998 to create a Kokanee sport fishery and a prey source for large piscivorous fish species. During this time, over three million juvenile Kokanee were stocked into five rivers that flow into the Williston Reservoir. The five systems were: Carbon Creek, Davis River, Dunlevy Creek, Manson River, and Nation River; three systems on the east side of the reservoir and two rivers that flow into the southwest portion of the reservoir (Langston 2012).

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 (Langston 2012). Spawning Kokanee, therefore, have selected tributaries in the Williston watershed that flow into the north-western portion of the reservoir – not the regions originally stocked. Estimated number of spawners was over 1 million from the 2006 survey of 99 streams (Langston 2012); the number of Kokanee spawners increased to almost 1.7 million from a survey of 48 streams conducted in 2010 (Fish & Wildlife Compensation Program – Peace Region, unpublished data). Enumeration of Kokanee spawner was not repeated again until 2018, however, aerial enumeration estimated Kokanee escapement at approximately 184 000 spawners in 28 rivers assessed (DWB 2019). The introduced Kokanee to the Williston Watershed have the potential to dramatically affect the flow of nutrients due to their semelparous life history and a complex web of interactions between the fish, stream-living macroinvertebrates, and the surrounding riparian zone.

Anadromous Pacific salmon are a source of nutrients from the marine environment that are released to spawning streams after they die (Naiman et al. 2002), enriching not only the streams, but also the surrounding riparian areas (Quinn et al. 2018). The potential transfer of nutrients into regional stream systems in the Williston watershed via Kokanee migration could

also have large impacts on a range of other biota and ecosystem processes, both aquatic and terrestrial. Nutrient transfer and its effect on riparian habitat and Kokanee populations were assessed using two target groups, namely aquatic insects and riparian lichen communities. Both of these assemblages have previously been used as biological indicators of ecosystem integrity and function and have well-developed monitoring methodologies published in the literature (McCabe and Gotelli 2000; McCune 2000; Giordani et al. 2012).

All three groups of organisms – Kokanee, terrestrial lichens, and aquatic insects – affect each other in a complex web of interactions. Aquatic insects, particularly in their immature stages, provide food for fish. Aquatic insect feeding activities – often processing allochthonous (i.e. terrestrial material [e.g., plant leaves and needles, etc.] that has fallen into the stream) organic matter – affect stream productivity and other characteristics (Vannote et al. 1980). Aquatic insects alter and maintain stream characteristics that provide habitat for the fish. Emergent adult aquatic insects influence surrounding riparian areas through substantial deposition of nutrients (Dreyer et al. 2015). In addition to affecting surrounding forests, aquatic insect assemblages are also themselves affected by the riparian forest characteristics and the resulting allochthonous input into their streams (Compson et al. 2013). The fate of nutrient transfer from the Williston Reservoir to reaches of rivers – including terrestrial habitat (Helfield and Naiman 2001) – where Kokanee spawn is unknown, but considering previous research on the topic, are likely to be appreciable. 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 three target groups, stream resident fish, aquatic insects and riparian lichen communities. These groups have previously been used as biological indicators and have well-developed biomonitoring methodologies published in the literature.

Stream resident fish are represented by the slimy sculpin (*Cottus cognatus*) has the widest North American distribution among fish belonging to the family Cottidae, but exhibits limited movement as they get older, thus integrating environmental conditions of localized areas. Diet of Cottidae in streams where semelparous Pacific salmon spawn has previously been shown to shift from aquatic invertebrates and small stream fishes to salmon eggs during the spawning season (Swain et al. 2014). Thus, Kokanee may provide a direct source of nutrients to stream resident fish in addition to the potential influx of reservoir-derived nutrient contributions to the stream ecosystem. This would be reflected in changes to the stable isotope signature of the stream resident fish (i.e, slimy sculpin).

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. Development of a baseline record of commonly assessed insect taxa [Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)] is important both for further research in this area and for ongoing and future monitoring activities. Quantitation and comparisons of aquatic insect taxonomic diversity and functional diversity among streams with or without active Kokanee populations will provide information on the

effect of Kokanee in watersheds on aquatic insect diversity and macro invertebrate assemblage functional ecology and ecosystem services.

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 provided a direct indication of changes in factors such as nitrogen content.

GOALS AND OBJECTIVES

The goal of the project was 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 in streams where Kokanee return to spawn. The project aligned with the Peace Fish and Wildlife Compensation Program's Reservoir and Stream Action Plans 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 results provide both baseline data (e.g., species lists, abundance, and diversity measures) and new tools (e.g., biodiversity indicators, monitoring methods) for fisheries and forest managers working in the Williston Reservoir watershed region. Working in partnership with Chu Cho Environmental (Tsay Keh Dene First Nation) and Northern Spruce Contracting (McLeod Lake Indian Band) this research provided important training opportunities for both local community members and University of Northern British Columbia (UNBC) students alike.

STUDY AREA

Sampling locations

The results of our survey questionnaire conducted in 2016 revealed that Kokanee distribution in tributaries to the Williston Reservoir is extensive. In fact, establishing control sites where Kokanee do not spawn remains 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.

Due to the wide spatial separation of our control (non-Kokanee) and experimental (Kokanee) sites in 2016, we selected additional control and experimental sites for 2017 and 2018 (Figure 1). We included the Wichcika River (WI), an upper Parsnip River tributary as a control stream. We also included the Wooyadilinka River (WO), a tributary to the Parsnip River closer to the Williston Reservoir than our other control streams, as an additional control stream. Additionally, in 2018, we added Bill's (BI) and Lamonti (LA) Creeks to our control stream sampling. We spent considerable effort to find additional experimental streams that were closer geographically to our control streams. Consequently, in addition to experimental streams

in the Ospika River watershed, Aley Creek and Stevenson Creek, and streams in the Omineca River watershed, Osilinka River (OS) and Tenakihi Creek (TE), but closer in proximity to the control streams. We sampled two streams that flow into the Parsnip Reach of the Williston Reservoir. Mugaha Creek (MU), a stream that was stocked with Kokanee through the salmonids in the classroom program, and Cut Thumb Creek (CT), where Kokanee had previously been observed to spawn (Langston 2012) were included as experimental streams. To assess temporal variation in our study and control streams, we sampled streams in both summer and fall 2017. In 2018, Patsuk (PT) and Scott (SC) Creeks, which were documented to have spawning Kokanee were added as experimental streams.

METHODS

In year 1 (2016-17) of the project we used a questionnaire to determine Kokanee occurrence in tributaries of the Williston Reservoir. The questionnaire was distributed to local First Nations in the region by our First Nations 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 Kokanee, and five systems with Kokanee for species checklists (insects) and to use stable isotope methods to track nutrient flow (fish and insects). Preliminary sampling of the identified study sites was conducted in the late summer/early fall of 2016.

In year 2 (2017-18) we continued to investigate nutrient inputs from Kokanee through the stream and riparian ecosystems using stable isotope analysis to assess source, sink, and process relationships, but expanded our sampling to assess both temporal and spatial variation in isotope signatures. We continued our survey of records and collections to develop an historical understanding of aquatic insect biodiversity in the area. Iterative quantitative sampling of insects was 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 was assessed using 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.

In year 3 (2018-19) of the project additional sampling was conducted to fill in data gaps and limit spatial bias in our metrics to assess Kokanee nutrient transfer, analysis of the collected samples was completed, and we began the development and delivery of extension activities (e.g., publications, an extension note, posters, and presentations).

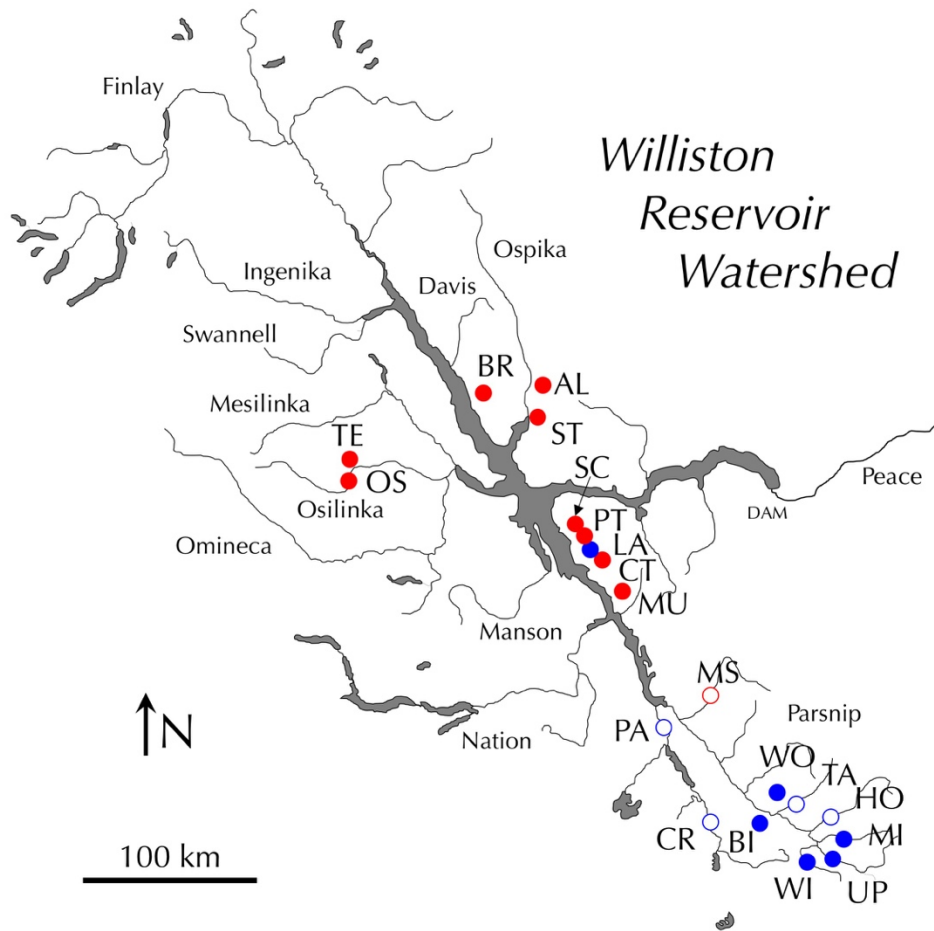


Figure 1. Map of sampling locations for benthic fish and aquatic macroinvertebrates in fall 2016, summer and fall 2017, and summer and fall 2018. Missinka (MI), Wichcika (WI), Wooyadilinka (WO), Table (TA), Hominka (HO), Upper Parsnip (UP), Crooked (CR), and Pack (PA), Bill's (BI), and Lamonti (LA) watersheds (blue symbols) were control systems where Kokanee have not been observed to spawn. Missinchinka (MS), Cut Thumb (CT), Mugaha (MU), Osilinka (OS), Tenakihi (TE), Aley (AL), Stevenson (ST), Patsuk (PT), and Scott (SC) watersheds (red symbols) were systems where Kokanee spawned. Closed symbols are where benthic invertebrates, fish and lichen were sampled; open symbols are locations where only lichen were surveyed.

Kokanee escapement

An aerial reconnaissance flight on 11 September 2017 was conducted to document spawning Kokanee in our experimental streams. High water levels and turbidity in tributaries to the Parsnip Reach, Mugaha Creek and Cut Thumb Creek, prevented observation of spawners in these two streams. Spawner abundance was high in Aley and Stevenson Creek in the Ospika River watershed. Kokanee were not observed in the Ospika River, however, water clarity in the mainstem was low and limited our ability to detect fish. We also flew rivers on the west side of the Williston Reservoir. Our aerial flight revealed large numbers of Kokanee in the Osilinka River from approximately km 8 on the Osilinka FSR to km 25. Few Kokanee were observed above this location and none were observed in Tenakihi Creek. Kokanee were not observed in the lower section of the Mesilinka River, Manson River or Nation River. On 18 September 2017, we surveyed streams flowing into the east side of the Parsnip reach on the ground. Kokanee spawners were observed in Cut Thumb Creek, however, they were not abundant. We did not observe spawners in the other streams surveyed. We repeated the ground survey on 25 September 2017 to the east side Parsnip reach tributaries, but did not observe Kokanee.

Sample collection

Sampling in 2016 was designed to examine whether tributary streams where Kokanee spawned differed from non-Kokanee streams. Number of samples by type is provided in Table 1. Due to the spatial separation of locations where Kokanee spawn (OS, TE, AL, ST, BR) from non-Kokanee streams (UP, MI, TA, HO), experimental Kokanee streams were further north than the control non-Kokanee streams (Figure 1).

We tested for temporal changes in species composition and nutrient signatures in 2017 and targeted most of our sampling in tributaries to the Parsnip River and the Parsnip Reach. Macroinvertebrates, and stream resident fish were collected from all study locations in July and late October/early November (Table 1). Access in the fall was difficult due to weather and high-water levels which protracted our sampling dates considerably later in fall 2017 than we had originally planned. Consequently, Osilinka River and Tenakihi Creek were sampled on October 14, and Cut Thumb and Mugaha Creeks were sampled on October 15 – but precipitation resulted in substantial increase in flows and we could not sample the Parsnip River tributaries the next day. Due to the early snowfall, we planned to fly to the Ospika River tributaries on October 17 – but low cloud cover made it unsafe to fly. We sampled the Parsnip River tributaries on October 28 and the Ospika River tributaries on November 2.

In late June 2018, aquatic invertebrates were sampled, and in late September 2018, stream resident fish were sampled from three control sites (Wooyadilinka, Bill's, and Lamonti Creeks) and three sites documented to have spawning Kokanee (Cut Thumb, Patsuk and Scott Creeks). The 2018 sampling was an attempt to look for control and Kokanee streams that were closer geographically and determine whether some of the previous year findings were related to geographic location (Table 1). Sampling was planned for other streams but was not able to be conducted due to high water levels and deactivated access roads.

Fish sampling

We targeted benthic fish based on our results from 2016 where pelagic species showed greater range of stable isotope signatures for each river system. Fish were captured using a Smith Root L24 backpack electrofisher (Vancouver, WA). We progressed upstream while electrofishing until eight specimens were collected or for approximately 500 m. After capture fish were transferred to a bucket containing $100 \text{ mg} \cdot \text{L}^{-1}$ tricaine methanesulfonate buffered with $200 \text{ mg} \cdot \text{L}^{-1}$ sodium bicarbonate for sampling. Length (to 0.1 cm) and mass (to 0.01 g) were measured for each fish prior to being placed in a bag on ice or in a portable freezer until transfer to a freezer for storage. A summary of fish collected in the study is given in Table 2. In July 2017, Chu Cho Environmental collected native Kokanee from Thutade Lake and donated some to this study. These fish were tested to see if the stable isotope signatures were consistent between Lake and Reservoir fish. Kokanee were also collected from Aley Creek (10) and Stevenson Creek (10) in September 2017 to replicate the sampling performed in 2016.

Aquatic invertebrate survey

Kick-netting for invertebrates was carried out in years 1, 2 and 3, 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. Each sample was placed immediately into a sealed, labelled container of stream water. 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.

At each stream, Surber samples were collected from five sites. Surber sampling was performed in years 2 and 3 of the project. Sample collection started downstream and proceeded upstream to minimize disturbance and avoid contamination between sites, with each replicate Surber samples collected at each of the five sites. Benthic invertebrates were sampled using a Surber net (Dynamic Aqua Supply, Surrey, BC) with a 30 x 30 cm frame (0.09 m^2) and 250-micron mesh size, using methods adapted from the Canadian Aquatic Benthic Invertebrate Network (CABIN) Field Manual (Reynoldson et al. 2003).

For aquatic invertebrates collected by Surber sample, all analyses were conducted on abundance data (Family-level sorting) of Plecoptera collected in the summer and autumn of 2017. Analyses were conducted in R (R Core Team, 2019) with the vegan package (Oksanen et al., 2019) in RStudio (RStudio Team, 2015) using specaccum (accumulation curve), vegdist and hclust (dendrogram), metaMDS and stressplot (stress plot), radfit (rank-abundance curves), and vegdist "bray" and ordiplot (non-metric multidimensional scaling plot). Kick-netting samples were hand-sorted using dichotomous keys and DNA barcode data. This data provides information on shifts in aquatic invertebrate communities potentially due to the introduction of Kokanee into the system.

Periphyton sampling

In June 2018, 6 streams were sampled for periphyton. Periphyton sampling involves rubbing rocks to dislodge algae and other primary producers attached to the rocks into a bucket and then filtering the water to trap the organisms. Samples were analyzed for stable isotope signatures as outlined below.

Lichen survey

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-variables, 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 assessments from 2016 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 was 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 lichens 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. We used an ordination approach to determine the ecological similarity of the entire lichen community. Non-metric multidimensional scaling (NMS) is an ordination method that is well suited to data that are non-normal or have discontinuous or otherwise arbitrary data sets (McCune & Mefford 1999). NMS is a distance-based ordination technique where a solution is based on minimizing stress, this is defined as a measure of the poorness of fit between the ordination and measured ecological distances. NMS was run using the ordination routines of PC-ORD Version 6.0 (MJM Software Design, Gleneden Beach, OR).

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 isotope 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}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 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. 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, individual muscle samples from fish, lichen, and periphyton collected from our control and experimental 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 $\delta^2\text{H}$ and $\delta^{18}\text{O}$ were weighed into silver capsules and loaded into a Costech Zeroblank autosampler. Samples were converted to hydrogen (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.

We used several approaches to assess factors that influenced the variation in stable isotope signatures. A multivariate combination of the four stable isotope ratios, $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$, were used to discriminate locations using the different sample types. Stable isotope signatures for fish, aquatic invertebrates and lichen were differentiated using discriminant function analysis with jack-knife re-sampling. Discriminant function analysis is used to predict group membership based on a linear combination of interval predictor variables and indicates how well the study sites differ from each other.

We also assessed the effect of latitude on $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$ for each of the different taxonomic groups collected from the different tributary streams to the Williston Reservoir. Our final analytical approach was restricted to just $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ to compare differences between streams where Kokanee have been observed to spawn (experimental) to non-spawning streams (control) using a restricted maximum likelihood (REML) analysis of variance (ANOVA). Treatment (experimental and control) were fixed effects and location (stream) were random effects in the models.

Table 1. Summary of samples collected by date, location and type. “K–” indicates rivers where Kokanee do not spawn and “K+” indicates rivers where Kokanee have been observed to spawn. Abbreviations are: KO = Kokanee collection by dip net or electrofishing, EF = electrofishing for stream resident fish, KN = kick net sampling for benthic invertebrates for stable isotope analysis and DNA barcoding, LS = lichen biodiversity survey within fixed plots, LC = lichen collection from riparian vegetation for stable isotope analysis, SS = Surber sample collection for quantitative assessment of benthic aquatic invertebrates, and PP = periphyton sample collection from stream substrate. Sample size provided in parentheses or sampling effort in seconds for electrofishing.

	Date	River	Site Type	Sample type	
Fall 2016	Sep-2016	Oslinka	K+	KO (10)	
	Sep-2016	Aley	K+	KO (13)	
	Sep-2016	Stevenson	K–	KO (10)	
	Oct-2016	Missinka	K–	EF [558 s], KN (3), LS, LC (6)	
	Oct-2016	Upper Parsnip	K–	EF [788 s], KN (3), LS, LC (6)	
	Oct-2016	Hominka	K–	LS, LC (6)	
	Oct-2016	Table	K–	LS	
	Oct-2016	Osilinka	K+	EF [156 s], KN (3)	
	Oct-2016	Tenakihi	K+	EF [543 s], KN (3)	
	Oct-2016	Aley	K+	EF [887 s], KN (3)	
	Oct-2016	Stevenson	K+	EF [637 s], KN (3)	
	Oct-2016	Bruin	K+	EF [368 s], KN (3)	
Summer 2017	Jul-2017	Wichcika	K–	EF [1203 s], KN (3), SS (5)	
	Jul-2017	Missinka	K–	EF [275 s], KN (3), SS (5), LC (5)	
	Jul-2017	Pack	K–	LS	
	Jul-2017	Crooked	K–	LS	
	Jul-2017	Missinchinka	K+	LS	
	Jul-2017	Cut Thumb	K+	EF [809 s], KN (3), SS (5)	
	Jul-2017	Mugaha	K+	EF [209 s], KN (3), SS (5), LC (4)	
	Jul-2017	Tenakihi	K+	EF [302 s], KN (3), SS (5), LC (5)	
	Jul-2017	Osilinka	K+	EF [533 s], KN (3), SS (5)	
	Jul-2017	Aley	K+	EF [891 s], KN (3), SS (5), LC (5)	
	Jul-2017	Stevenson	K+	EF [938 s], KN (3), SS (5), LC (3)	
	Jul-2017	Thutade	K+	KO (10)	
	Fall 2017	Sep-2017	Aley	K+	KO (10)
		Sep-2017	Stevenson	K+	KO (10)
Oct-2017		Wichcika	K–	EF [785 s], KN (3), SS (5)	
Oct-2017		Wooyadilinka	K–	EF [257 s], KN (3), SS (5)	
Oct-2017		Cut Thumb	K+	EF [501 s], KN (3), SS (5)	
Oct-2017		Mugaha	K+	EF [121 s], KN (3), SS (5)	
Oct-2017		Tenakihi	K+	EF [575 s], KN (3), SS (5)	
Oct-2017		Osilinka	K+	EF [539 s], KN (3), SS (5)	
Nov-2017		Aley	K+	EF [746 s], KN (3), SS (2)	
Nov-2017		Stevenson	K+	EF [465 s], KN (3), SS (5)	
Fall 2018	Sep-2018	Bill’s	K-	EF [217 s], KN (3), SS (5), PP (3)	
	Sep-2018	Wooyadilinka	K-	EF [780 s], KN (3), SS (5), PP (3)	
	Sep-2018	Lamonti	K-	EF [279 s], KN (3), SS (5), PP (3)	
	Sep-2018	Cut Thumb	K+	KN (3), SS (5), PP (3)	
	Sep-2018	Patsuk	K+	EF [585 s], KN (3), SS (5), PP (3)	
	Sep-2018	Scott	K+	EF [593 s], KN (3), SS (5), PP (3)	

RESULTS

Fish

Slimy sculpin were caught in all river systems sampled and were often the only species captured (Table 2). Catch per unit effort varied among the watersheds, but there was no apparent pattern in relation to streams where Kokanee did or did not spawn.

Aquatic invertebrates

Kick net sampling revealed that aquatic invertebrates predominantly belonged to the orders Ephemeroptera, Plecoptera and Trichoptera (EPT); although there was considerable variation among samples ranging from 18 to 97%, the average EPT was 78%. Number of specimens also varied among orders, but generally mayflies were the most abundant, followed by stoneflies, and in some samples no caddis flies were found. Specimens from each sample were separated into their respective orders and submitted for stable isotope analysis. Not all kick net samples contained sufficient number of individuals for analysis (particularly Trichoptera), and within a stream some samples were pooled.

Our DNA barcode-assisted rapid assessment of aquatic macroinvertebrate biodiversity in the seven systems yielded a total of 115 Operational Taxonomic Units (OTUs) of which we were able to provide species-level identifications for 54 (Appendix I). 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.
- Tenekihi: 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 (Appendix I). 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.

We collected aquatic invertebrates by Surber sample as a better method to quantify differences among the sample sites. Surber sample analysis collected at each of the study sites also indicated considerable diversity within and among the samples collected. Our sampling was determined to be adequate in terms of detecting Families as indicated by the plateau of the

accumulation curve (Figure 2). Within approximately 15 sampling events (chosen randomly) we would usually capture at least one specimen from each of the stonefly Families.

Given that our sampling effort was robust, we used a variety of approaches to examine potential differences among the study sites based on samples collected from locations where Kokanee spawned and where no Kokanee have been observed to spawn. The cluster dendrograms were used to illustrate similarities and differences in the invertebrate communities among the different streams. Aquatic invertebrates collected in 2017 from the two non-Kokanee systems cluster near to each other, indicating similarity (Figure 3). The two non-Kokanee streams, however, also cluster near to some Kokanee systems (Figure 3). Notably the Kokanee systems break into two overall clusters, and those clusters may be at least in part geographically based (e.g. Osilinka and Tenakihi, Aley and Stevenson). Including season in the analysis emphasized that temporal variation had a greater effect on differences than presence or absence of Kokanee. When stonefly abundances are measured on a seasonal basis, there is a reasonably good differentiation by season (autumn vs. summer) with some additional likely geographic similarities as well (Figure 4).

The stress plot is a diagnostic plot used to explore both, dimensionality and interpretative value for the NMDS plot. Our analysis showed a high R^2 value and indicates that non-metric multidimensional scaling is a robust method of assessing the 2017 Plecoptera collection results (Figure 5). Similar to the cluster dendrogram, the NMDS plot shows some differentiation in stonefly samples based upon the collection season (summer vs. autumn) in 2017 (Figure 6). As with the dendrogram, the separation is not complete at this level of sampling.

The rank abundance plots are a useful way to summarize both equitability in abundance between species and species richness. Each plot in the matrix shows an RA curve for Plecoptera families in the different samples systems either in total (summer + autumn; Figure 7) or in each of the two seasons in 2017 (Figure 8). Steeper curves indicate sites with high abundance of a few Families, longer curves indicate sites with many Families present in lower numbers. Some systems (e.g. AL) consistently show similar abundance and number of Families in both autumn and summer. Most systems show some RA variation between the two seasons. Most systems contained at least seven of the eight Plecoptera Families, although not all of those families were detected in both seasons. There is variation in Plecoptera biodiversity around the reservoir, but the sampled systems are fairly consistent on the Family level.

Lichens

A total of 229 lichen taxa were observed in the 6 Peace-Williston river- and stream-side riparian forests that were examined in 2016 and 2017 (Appendix II). A relatively small proportion of the lichen species were found at all study sites (14.9%), but many were found at multiple locations; 10.5% found at 5 sites, 9.65% found at 4 sites, 9.65% found at 3 sites, and 15.8% found at just two sites. A large number of lichen were only found at a single site (39.5%).

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. The 2017 sampling found 3 additional populations of *Collema coniophilum* that previously were unknown. This species has now been found at all sites, except the upper Parsnip. The other conservation-ranked species found in 2017 is *Phaeophyscia nigricans*, found at the Missinchinka site (Appendix II).

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. Species of nutrient indicating Physciaceae found in the study plots were *Rinodina capensis*, *R. colobina*, *R. degeliana*, *R. disjuncta*, *R. efflorescens*, *R. flavosoralifera*, *R. griseosoralifera*, *R. metaboliza*, *R. orculata*, *R. oregana*, *R. pyrina*, *R. stictica*, and *R. trevisanii*. Teloschistaceae species were *Caloplaca ahtii*, *Caloplaca atosanguinea*, *Caloplaca cf. oleicola*, *Caloplaca flavorubescens*, *Caloplaca pyracea*, *Caloplaca sorocarpa*, *Caloplaca tricolor*, *Caloplaca urceolata*, *Chaenotheca chrysocephala*, *Chaenotheca phaeocephala*, *Xanthomendoza fallax*, *X. fulva*, *Xanthoria Candelaria*, *X. fallax*, *X. fulva*, and *Xanthoria kaernefeltii*. The study plot with the highest number of the Teloschistaceae and Physciaceae was the Pack River at 19 (Table 3), followed closely by the Hominka and Missinchinka (17 and 18 respectively). The upper Parsnip and Table were intermediate (14 and 15), while the Crooked River was much lower, at 8 species. The one Kokanee-run stream (Missinchinka) fell among the richest set of sites. The Crooked River was the most species impoverished site for Teloschistaceae and Physciaceae. Interesting, there is a strong separation of the Pack River samples from all others along Axis 1 of the ordination (Figure 9). The Pack and Crooked River sites are the two most impoverished sites for total number of lichen species.

Stable Isotopes

Multivariate analysis

Incorporating stable isotope data from all four elements into a Canonical discriminant function analysis indicated reasonable separation among the different groups (Figure 10a). There was considerable overlap in the clusters for slimy sculpin caught in Kokanee spawning streams. Consequently, of the 124 sculpins sampled from streams where Kokanee spawn, 115 (93%) were assigned to their stream of capture or to a different Kokanee spawning stream indicating commonality of stable isotope signatures for slimy sculpin caught in the streams where Kokanee spawn. The assignment was lower (74%) for sculpins from non-Kokanee streams; 26% of the sculpins were assigned to Kokanee streams (Table 4). There was no temporal effect of sampling on the classification matrix; sculpin sampled in the fall did not differ from those sampled in the summer.

Kokanee from the Williston Reservoir clearly cluster together, but apart from all the sculpin sampled and also the Kokanee from Thutade Lake. Stable isotope signatures for Kokanee did not overlap with sculpin for discriminant function 1 (Figure 10a), however there was overlap for discriminant function 2 between Kokanee and sculpin. The second function was better at discriminating between Kokanee from Thutade Lake and Williston Reservoir Kokanee and also better separation between fish from tributaries where Kokanee spawned from the control streams. Overall classification by treatment (Slimy sculpin captured in non-Kokanee spawning systems, Slimy sculpin captured in systems where Kokanee spawn by major watershed,

spawning Kokanee captured in tributary streams to the Williston Reservoir, or Kokanee caught in Thutade Lake) was 74%.

Canonical discriminant function analysis for the stable isotope data from aquatic insect samples showed considerable overlap among the Kokanee and non-Kokanee streams (Figure 10b and c; Table 4, 5 and 6), however the overlap was less for samples collected in the summer than the fall. Discriminant function analysis also showed good separation for aquatic insects belonging to the order Ephemeroptera (Figure 10b), however, the differences for discriminant function 1 reflected geographic region more than treatment. The second discriminant function showed separation based on seasonal difference. Classification by treatment and location was lower than observed for fish at only 47% (Table 5).

Aquatic insects belonging to the Order Plecoptera did not show a clear pattern by Kokanee presence (Figure 10c) – although there were appreciable differences by sample date. There was considerable overlap among the streams by watershed and Kokanee presence for the discriminant function analysis (Table 6). The analysis for aquatic insects belonging to the order Plecoptera showed moderate separation among the different regions, but the control streams did not separate from the Kokanee streams (Figure 10c) and classification was only 53% (Table 6).

Trichoptera were less plentiful in the study streams and fewer samples were submitted for stable isotope analysis. No clear patterns were evident among the study streams for the discriminant function analysis and separation among the different treatments was poor; only 16% (Table 7).

Differences in stable isotopes were also found in lichen collected from trees within the riparian areas adjacent to our study streams in the Williston Reservoir. We targeted the species (*Parmelia squarrosa*) for the analysis as it was most consistently collected from vegetation adjacent to each of the streams where we sampled for fish and aquatic insects. Generally, lichen collected from streams where Kokanee spawn were depleted in ^2H , ^{13}C , ^{15}N , and ^{18}O compared to the streams sampled where Kokanee have not been observed to spawn (see also section “sample location” on geographic distribution of Kokanee). Consequently, differences in stable isotope signatures for each of the elements analyzed resulted in considerable separation of groups in the discriminant function analysis (Figure 10d) and also for the classification matrix; classification by treatment was 89% (Table 8).

Regression analysis for isotope signatures with latitude

There was a significant relationship between latitude and $\delta^{18}\text{O}$ for slimy sculpin sampled Williston Reservoir tributaries ($F_{1,9} = 38.54$; $P < 0.001$; Figure 11); there were no relationships between latitude and any of the other stable isotope relationships for slimy sculpin. Within the Parsnip River watershed, Analysis of Variance (ANOVA) revealed significant differences among $\delta^{13}\text{C}$ measurements ($F_{3,49} = 45.74$; $P < 0.001$), $\delta^{15}\text{N}$ measurements ($F_{3,49} = 19.03$; $P < 0.001$), $\delta^2\text{H}$ ($F_{3,49} = 13.09$; $P < 0.001$), but not for $\delta^{18}\text{O}$ measurements ($F_{3,49} = 0.25$; $P = 0.86$).

There was a significant relationship between latitude and $\delta^{18}\text{O}$ for mayflies sampled in Williston Reservoir tributaries during the summer ($F_{1,6} = 7.68$; $P < 0.05$); there were no relationships between latitude and any of the other stable isotope relationships for mayflies sampled in the

summer or fall (Figure 12). There were no relationships between latitude and any of the stable isotope ratios for stoneflies sampled in the summer or fall (Figure 13). There were also no relationships between latitude and any of the stable isotope ratios for caddisflies sampled in the summer or fall (data not shown).

There was a significant relationship between latitude and $\delta^2\text{H}$ for lichen sampled from riparian areas associated with Williston Reservoir tributaries ($F_{1,6} = 19.55$; $P < 0.005$; Figure 14). There was also a significant relationship between latitude and $\delta^{18}\text{O}$ for lichen sampled from riparian areas associated with Williston Reservoir tributaries ($F_{1,6} = 9.63$; $P < 0.05$). There was no relationship between latitude and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ for the lichen - *Parmelia squarrosa*.

Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among study streams – Parsnip Reach

Comparison of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for samples collected from streams spatially clustered within the Parsnip Reach of the Williston Reservoir watershed revealed some interesting patterns. Generally, average $\delta^{13}\text{C}$ was lower for all taxonomic groups in streams where Kokanee have been documented to spawn (Figure 15). Additionally, values for slimy sculpin captured in Kokanee streams overlapped with $\delta^{13}\text{C}$ measured for Kokanee spawners. Slimy sculpin from control streams, however, showed considerable variation and sculpin from Bill's and Lamonti Creeks were similar to those from the Kokanee streams with sculpin from Wooyadilka Creek having higher $\delta^{13}\text{C}$ values. $\delta^{15}\text{N}$ for slimy sculpin were consistently greater than all other taxa except for Kokanee, consistent with fish exhibiting a higher trophic state than the aquatic invertebrates.

Overlap in $\delta^{13}\text{C}$ values suggests that slimy sculpin may be feeding predominantly on stoneflies and caddisflies as consistently mayflies were depleted in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. The lower $\delta^{15}\text{N}$ values for mayflies suggests that the nymphs feed at a lower trophic level than the other aquatic invertebrates, but $\delta^{13}\text{C}$ values did not correspond well with values for periphyton – the putative base of the food web in each stream.

Differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among study streams – Williston Reservoir watershed

For slimy sculpin, streams where Kokanee spawned were generally depleted in ^{13}C and also had lower $\delta^{15}\text{N}$ than the control streams where no Kokanee spawned – although this pattern was not particularly strong (Figure 16). Kokanee captured in tributaries to the Williston and also Kokanee from Thutade Lake had $\delta^{13}\text{C}$ signatures that overlapped values for slimy sculpin caught in streams from the Ospika Reach of the Williston Reservoir; streams where densities of spawning Kokanee were high for 2016, 2017, and 2018. Streams with low escapement of Kokanee spawners were generally intermediate to the control and high spawner streams. Control streams where Kokanee do not spawn showed some of the highest $\delta^{13}\text{C}$, however, there was considerable variation among sites and some streams showed signatures that overlapped with values for Kokanee spawners. ANOVA comparing $\delta^{13}\text{C}$ values from Kokanee, slimy sculpin from Kokanee spawning streams, and slimy sculpin from non-Kokanee streams indicated a significant difference among treatments ($F = 14.957$; $P < .001$). ANOVA comparing $\delta^{13}\text{C}$ values between sculpins from control and experimental streams, however, did not differ significantly ($F = 0.671$; $P = 0.427$). Values for $\delta^{15}\text{N}$ were enriched for Kokanee compared to sculpins and there was a significant effect of treatment when Kokanee were included in the

model ($F = 499.032$; $P < 0.001$); however, there was no difference in $\delta^{15}\text{N}$ between sculpin from Kokanee spawning streams and control streams ($F = 0.127$; $P = 0.727$).

Stable isotope values showed considerable variation among the aquatic insect samples and no clear pattern was observed until we separated the data by Order. Ephemeroptera showed a pattern that was similar to the slimy sculpin. Mayfly nymphs collected from streams where Kokanee spawn tended to be depleted in ^{13}C and ^{15}N , although the absolute values for the samples differed temporally. Consequently, our analysis for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was restricted to samples collected in the summer months (Figure 17). We found no effect of treatment on $\delta^{13}\text{C}$ ($F = 0.007$; $P = 0.727$) or $\delta^{15}\text{N}$ ($F = 0.415$; $P < 0.533$).

Aquatic insects belonging to the Order Plecoptera did not show a clear pattern by Kokanee presence for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Figure 18) – although there were appreciable differences by sample date (Figure 13). There was considerable overlap among the streams by watershed and treatment and consequently, there was no effect of treatment on $\delta^{13}\text{C}$ ($F = 0.054$; $P = 0.821$) or $\delta^{15}\text{N}$ ($F = 1.359$; $P < 0.269$).

Few samples of Trichoptera were collected in the study streams. No clear patterns were evident among the study streams for the stable isotopes and there was no effect of treatment for $\delta^{13}\text{C}$ ($F = 0.232$; $P = 0.639$) or $\delta^{15}\text{N}$ ($F = 0.453$; $P = 0.519$).

We also compared the stable isotope signatures for the periphyton samples collected from the 3 control and 3 experimental streams in the Parsnip reach. Stable isotope ratios for $\delta^{13}\text{C}$ did not differ significantly between treatments, although the probability was lower than for the taxa examined ($F = 4.693$; $P = 0.096$). The ratio for $\delta^{15}\text{N}$ did not differ by treatment ($F = 0.116$; $P = 0.750$).

Differences in stable isotopes were also found in lichen collected from trees within the riparian areas adjacent to our study streams in the Williston Reservoir. Our analysis of the species *Parmelia squarrosa* for stable isotopes indicated that generally lichen collected from streams where Kokanee spawn were depleted in ^{13}C and ^{15}N compared to the streams sampled where Kokanee have not been observed to spawn (Figure 19b). Similar to our findings for periphyton, differences in $\delta^{13}\text{C}$ by treatment were not significant ($F = 4.124$; $P = 0.094$), but the probability was quite low. The ratio for $\delta^{15}\text{N}$ did not differ by treatment ($F = 0.943$; $P = 0.369$).

Table 2. Summary of sample dates, locations, species of fish caught, mean length (cm) with maximum, minimum, and number captured. Catch per unit effort (CPUE; number caught per 100 s) is given for slimy sculpin from each river and where multiple species were caught in a river, CPUE is provided for all species in parentheses. “K–” indicates rivers where Kokanee do not spawn and “K+” indicates rivers where Kokanee have been observed to spawn.

	Date	River	Site Type	Species	length	max	min	n	CPUE
Fall 2016	5-Oct-2016	Missinka	K–	Slimy sculpin	5.5	8	3.9	14	2.51
	5-Oct-2016	Parsnip	K–	Slimy sculpin	7.9	9.7	6.9	3	0.51
	5-Oct-2016	Parsnip	K–	Arctic grayling	6.4			2	(1.27)
	5-Oct-2016	Parsnip	K–	Mountain whitefish	6.1				
	5-Oct-2016	Parsnip	K–	Redside shiner	5.3	6.2	4.6	5	
	12-Oct-2016	Tenakihi	K+	Slimy sculpin	3.9	5	2.2	3	0.74
	12-Oct-2016	Tenakihi	K+	Burbot	11.1			1	
	12-Oct-2016	Osilinka	K+	Slimy sculpin	5.9	7.8	3.9	10	7.69
	12-Oct-2016	Osilinka	K+	Burbot	11.7	12.6	10.7	2	
	22-Sep-2016	Osilinka	K+	Kokanee	21.2	23.9	18.4	19	
	11-Oct-2016	Bruin	K+	Slimy sculpin	6.5	7.6	5.6	6	1.90
	11-Oct-2016	Bruin	K+	Bull trout	11.8	21.3	5.4	3	(2.99)
	11-Oct-2016	Bruin	K+	Rainbow trout	9.7			1	
	11-Oct-2016	Aley	K+	Slimy sculpin	7.7	8.7	6	4	0.45
	22-Sep-2016	Aley	K+	Kokanee	22.1	24.4	20	13	
	11-Oct-2016	Stevenson	K+	Slimy sculpin	4.8	8.2	2.2	9	1.57
	11-Oct-2016	Stevenson	K+	Bull trout	4.3			1	(1.88)
11-Oct-2016	Stevenson	K+	Rainbow trout	8.2			1		
22-Sep-2016	Stevenson	K+	Kokanee	22.6	24.4	19.2	10		
Summer 2017	24-Jul-2017	Wichcika	K–	Slimy sculpin	6.3	8.7	4.6	18	1.50
	24-Jul-2017	Wichcika	K–	Rainbow trout	13.9			1	(1.58)
	24-Jul-2017	Missinka	K–	Slimy sculpin	5.7	7.4	4.2	20	7.27
	31-Jul-2017	Cut Thumb	K+	Slimy sculpin	4.2	5.5	3.2	3	0.37
	31-Jul-2017	Mugaha	K+	Slimy sculpin	5.2	8.5	3.2	20	9.57
	26-Jul-2017	Tenakihi	K+	Slimy sculpin	6.4	9.3	4.4	10	3.31
	26-Jul-2017	Osilinka	K+	Slimy sculpin	5.6	8.5	4.6	20	3.75
	26-Jul-2017	Osilinka	K+	Burbot	15.0	16.0	14.3	3	(4.50)
	26-Jul-2017	Osilinka	K+	Rainbow trout	20.0			1	
	27-Jul-2017	Aley	K+	Slimy sculpin	5.5	8	3.3	11	1.23
	27-Jul-2017	Aley	K+	Bull trout	5.0	9.0	3.1	3	(1.57)
	27-Jul-2017	Stevenson	K+	Slimy sculpin	6.2	7.8	3.8	13	1.81
	27-Jul-2017	Stevenson	K+	Bull trout	9.4	13.2	7.2	4	(2.37)
	15-Jul-2017	Thutade Lk	K+	Kokanee	19.2	21.5	17.5	10	
Fall 2017	30-Oct-2017	Wichcika	K–	Slimy sculpin	5.5	8.9	2.6	8	1.02
	30-Oct-2017	Wooyadilinka	K–	Slimy sculpin	4.8	8.3	2.7	9	3.50
	15-Oct-2017	Cut Thumb	K+	Slimy sculpin	6.0	9.1	4.2	13	2.59
	15-Oct-2017	Cut Thumb	K+	Rainbow trout	5.7	5.7	5.7	2	(2.99)
	15-Oct-2017	Mugaha	K+	Slimy sculpin	5.1	8.4	3.7	9	7.44
	14-Oct-2017	Tenakihi	K+	Slimy sculpin	6.9	9	4.8	8	1.39
	14-Oct-2017	Osilinka	K+	Slimy sculpin	6.4	8.7	4	8	1.48
	02-Nov-2017	Stevenson	K+	Slimy sculpin	7.6	9	5.7	6	1.29
	12-Sep-2017	Stevenson	K+	Kokanee	22.2	22.9	20.6	10	
	02-Nov-2017	Aley	K+	Slimy sculpin	7.3	9.3	6.2	10	1.34
	02-Nov-2017	Aley	K+	Bull trout	11.0			1	(1.47)
	12-Sep-2017	Aley	K+	Kokanee	21.1	23	19.5	10	

	Date	River	Site Type	Species	length	max	min	n	CPUE
Fall 2018	30-Sep-2018	Bill's	K-	Slimy sculpin	5.2	7.4	4.2	12	9.45
	30-Sep-2018	Bill's	K-	Rainbow trout	10.6			1	(10.2)
	30-Sep-2018	Wooyadilinka	K-	Slimy sculpin	5.7	8.6	3.1	6	0.77
	30-Sep-2018	Wooyadilinka	K-	Bull trout	25.0			1	(0.90)
	30-Sep-2018	Lamonte	K-	Slimy sculpin	6.7	8.6	3.5	8	2.87
	30-Sep-2018	Patsuk	K+	Slimy sculpin	6.9	10.5	4.2	7	1.20
	30-Sep-2018	Patsuk	K+	Bull trout	6.9	7.0	6.8	2	(1.54)
	30-Sep-2018	Scott	K+	Slimy sculpin	8.0	11.3	4.2	10	1.69
	30-Sep-2018	Scott	K+	Bull trout	11.4	21.3	6.4	6	(2.70)

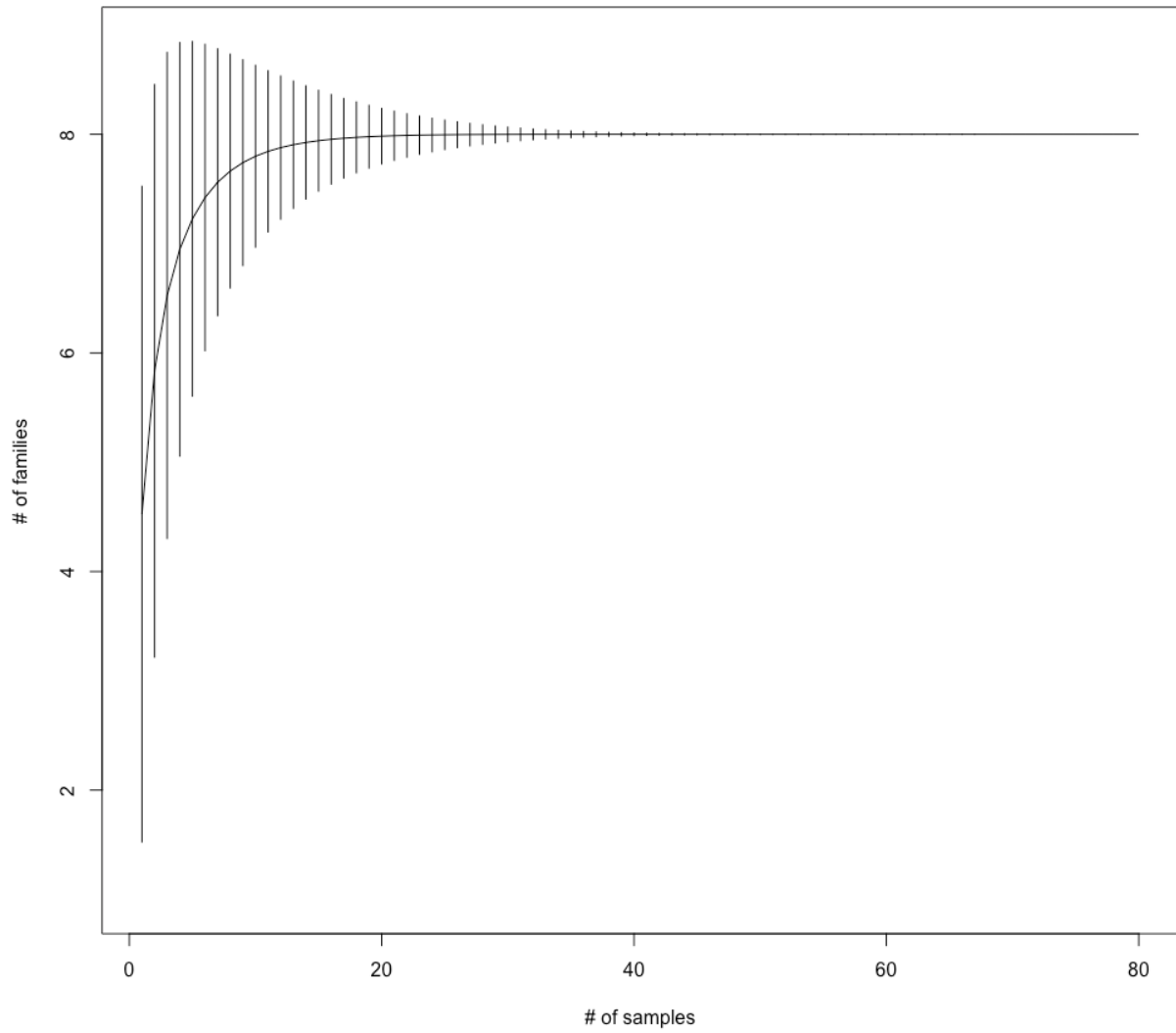


Figure 2. Accumulation curve for families of Plecoptera collected by Surber sample from tributary streams to the Williston Reservoir.

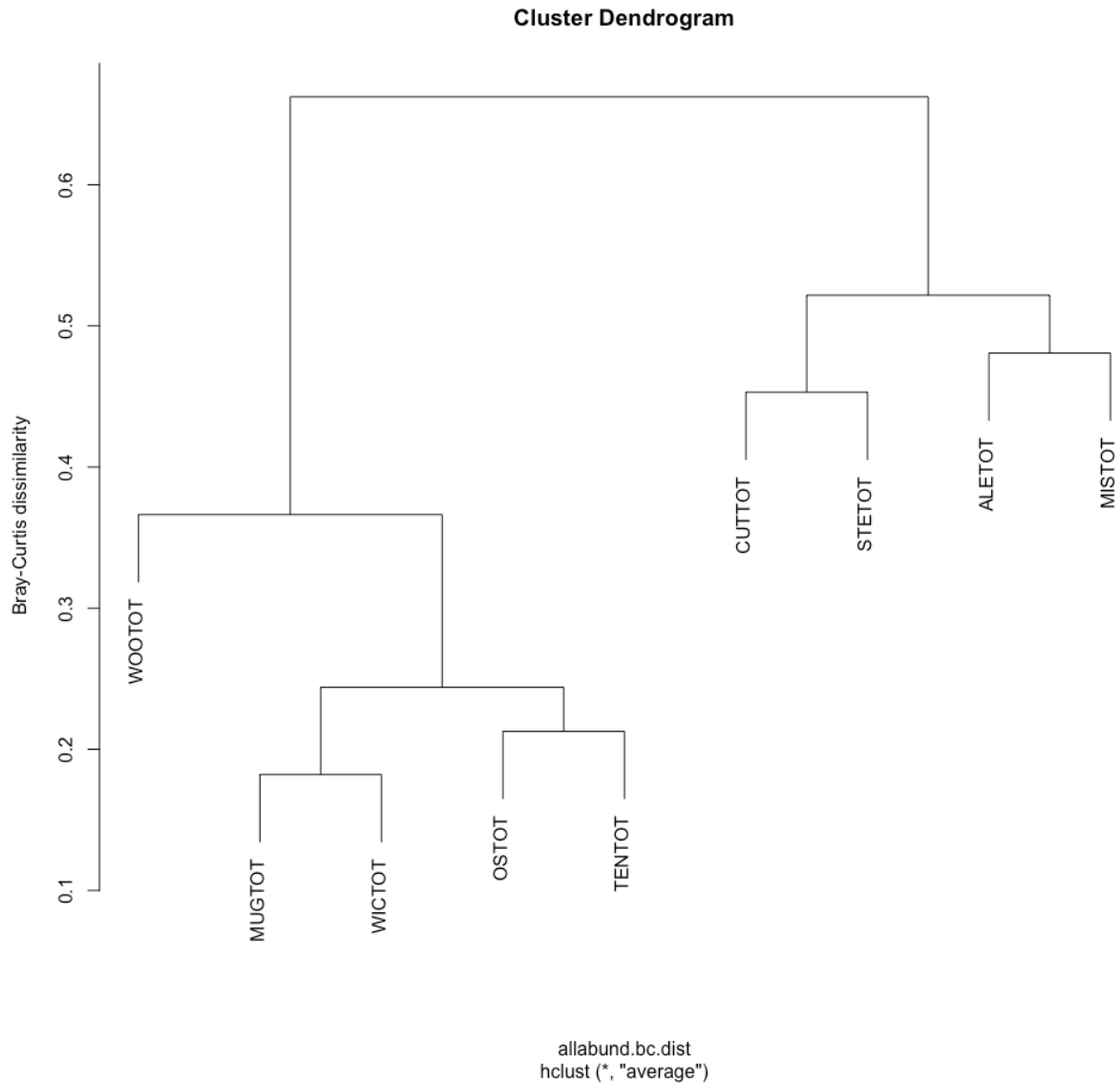


Figure 3. Cluster dendrograms for families belonging to the order Plecoptera with seasonal samples combined. Sample locations where Kokanee have not been observed to spawn are Missinka River (MIS), Wichcika Creek (WIC), and Wooyadilinka Creek (WOO). Sample locations where Kokanee have been observed to spawn are Cut Thumb Creek (CUT), Mugaha Creek (MUG), Osilnka River (OSI), Tenakihi Creek (TEN), Aley Creek (ALE), and Stevenson Creek (STE). TOT indicates that samples collected in the summer and autumn were combined.

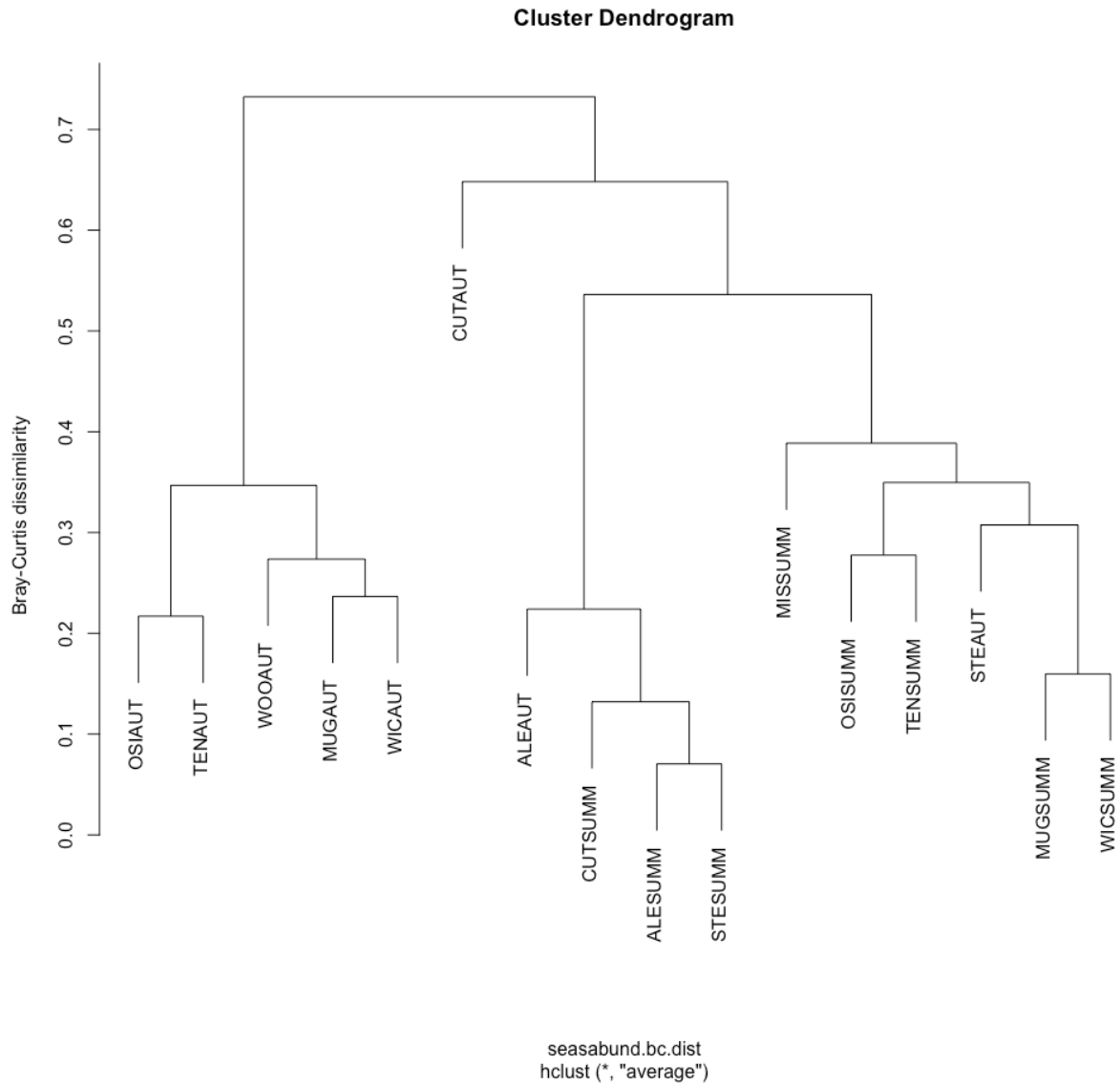


Figure 4. Cluster dendrograms for families belonging to the order Plecoptera with samples separated by season; samples collected in July are designated as “SUM”, samples collected in October and November are designated as “AUT”. Samples sites as indicated in Figure 3.

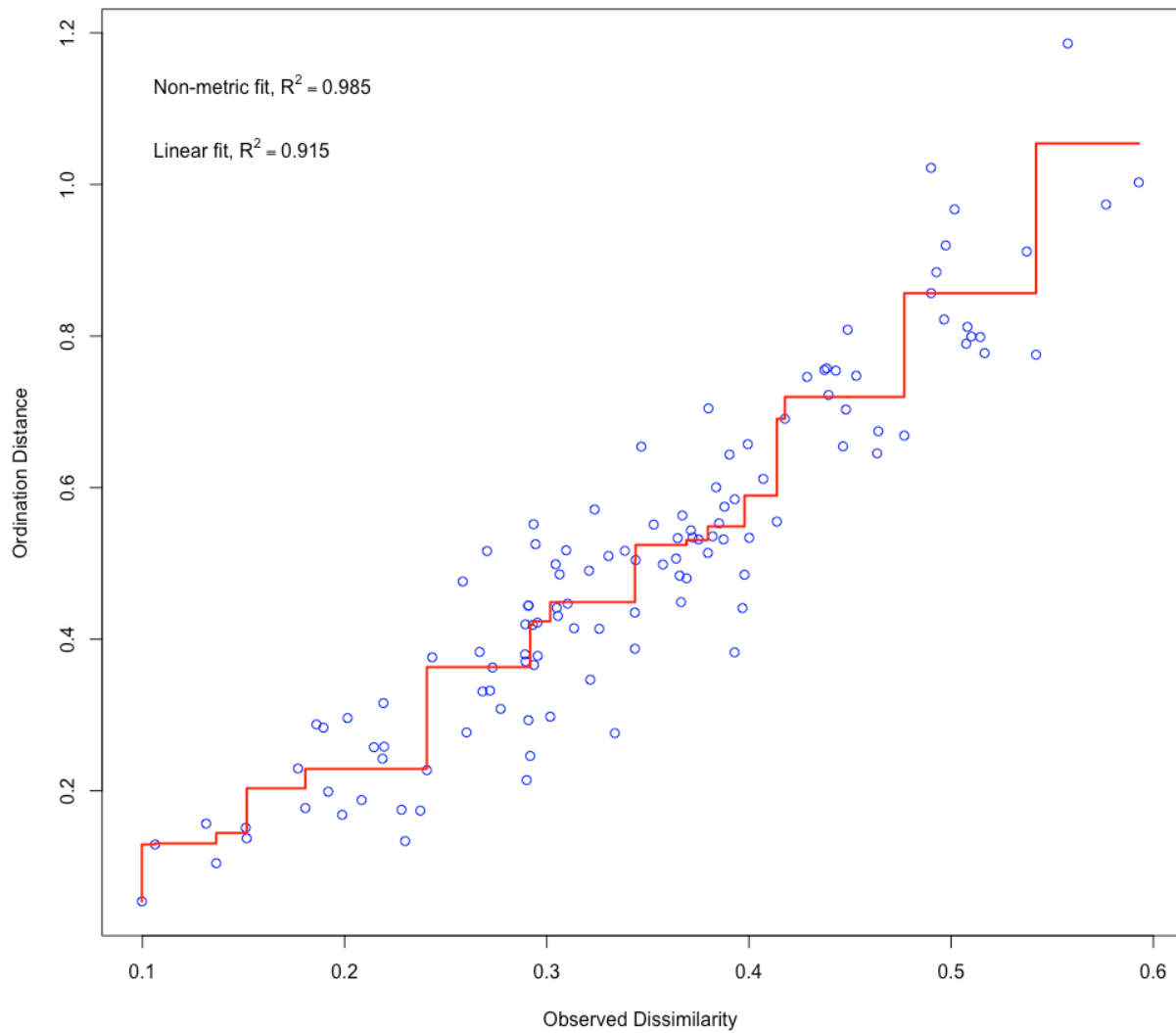


Figure 5. Stress plot for the non-metric multidimensional scaling analysis for Plecoptera.

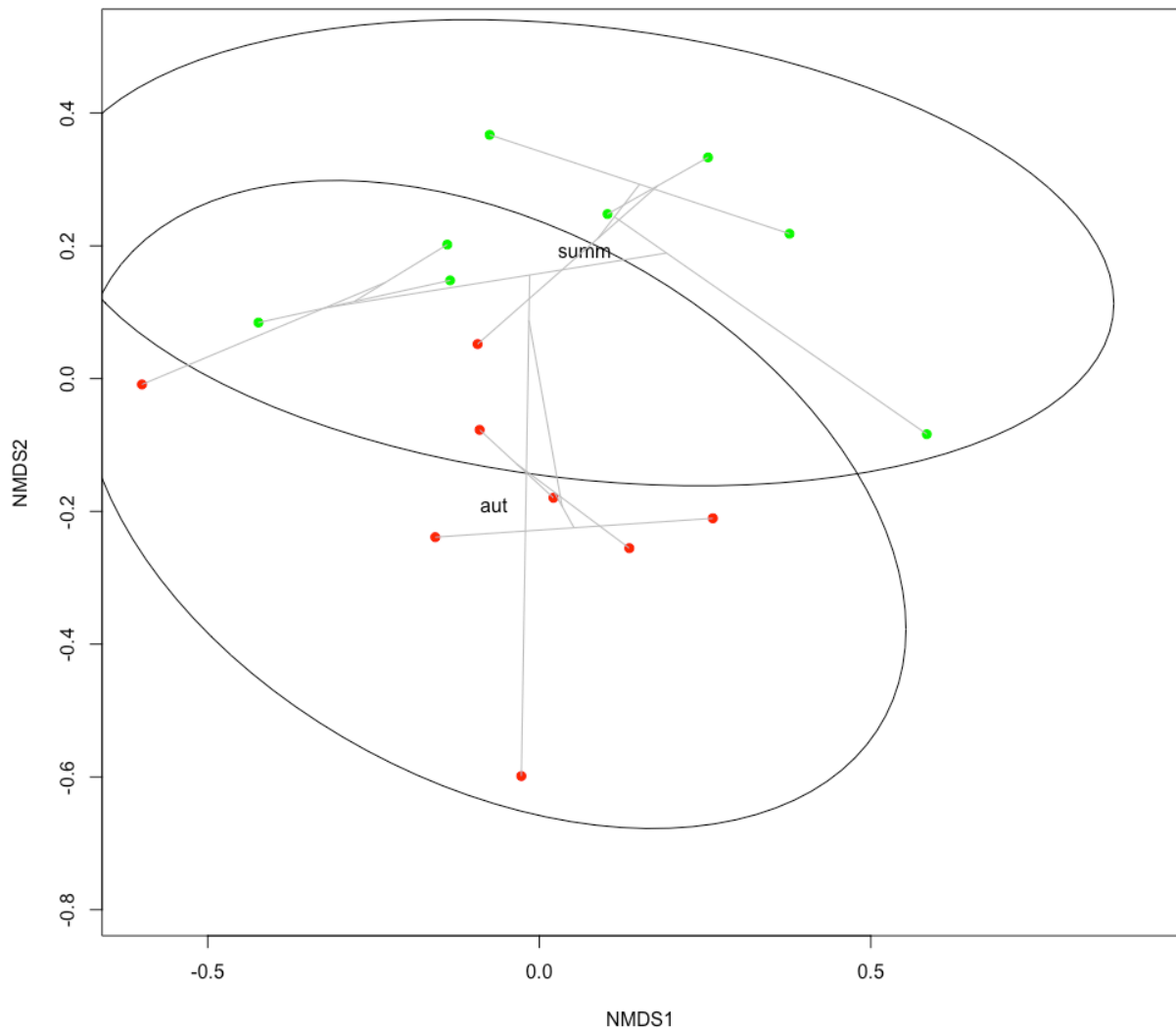


Figure 6. Non-metric multidimensional scaling ordination plot (Axis 1 versus Axis 2) for families of Plecoptera collected by Surber sample from tributary streams to the Williston Reservoir.

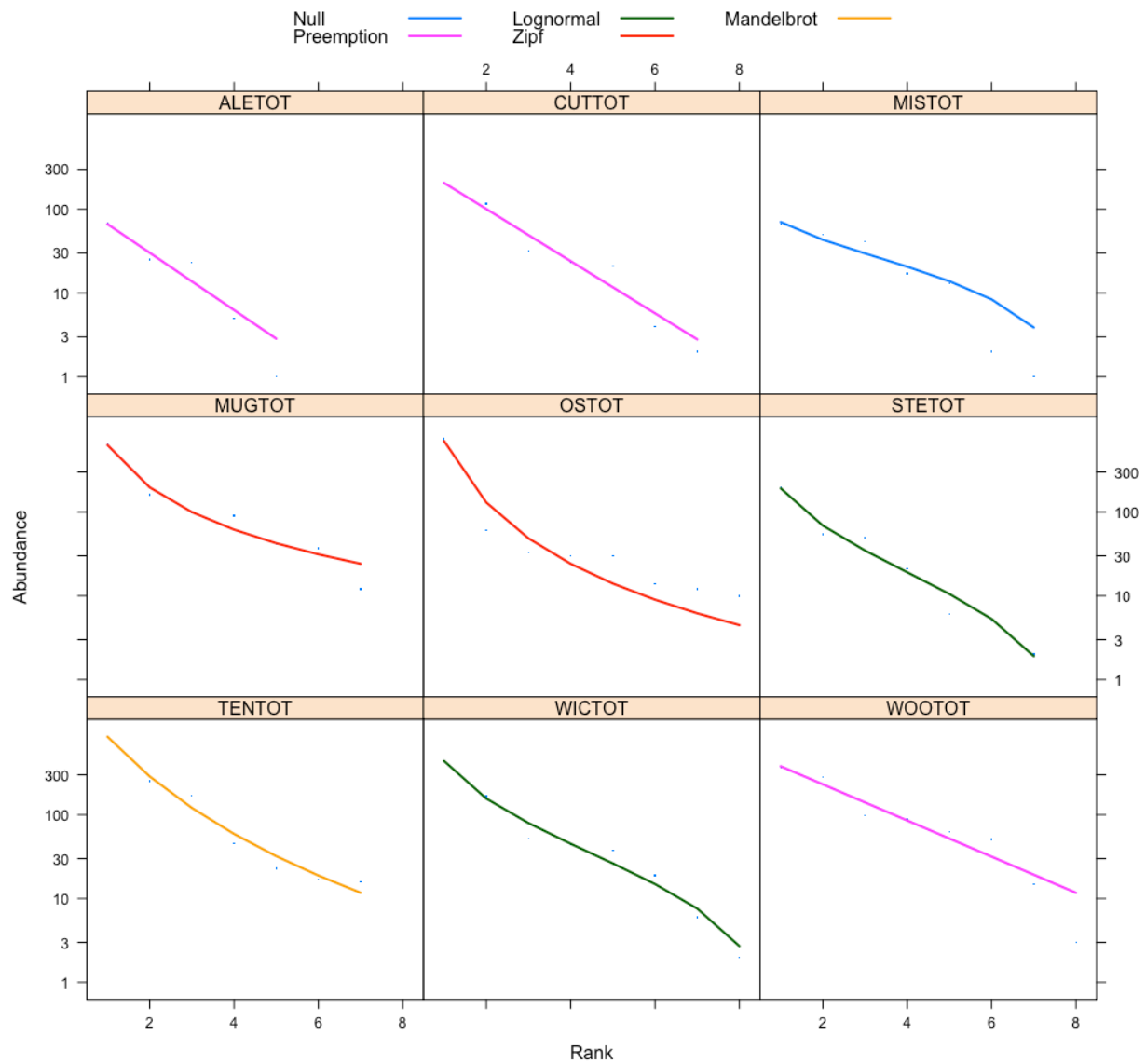


Figure 7. Rank-abundance plots for Plecoptera families from the different tributary streams to the Williston Reservoir collected by Surber sampler in total (summer + autumn samples combined). Samples sites as indicated in Figure 3.

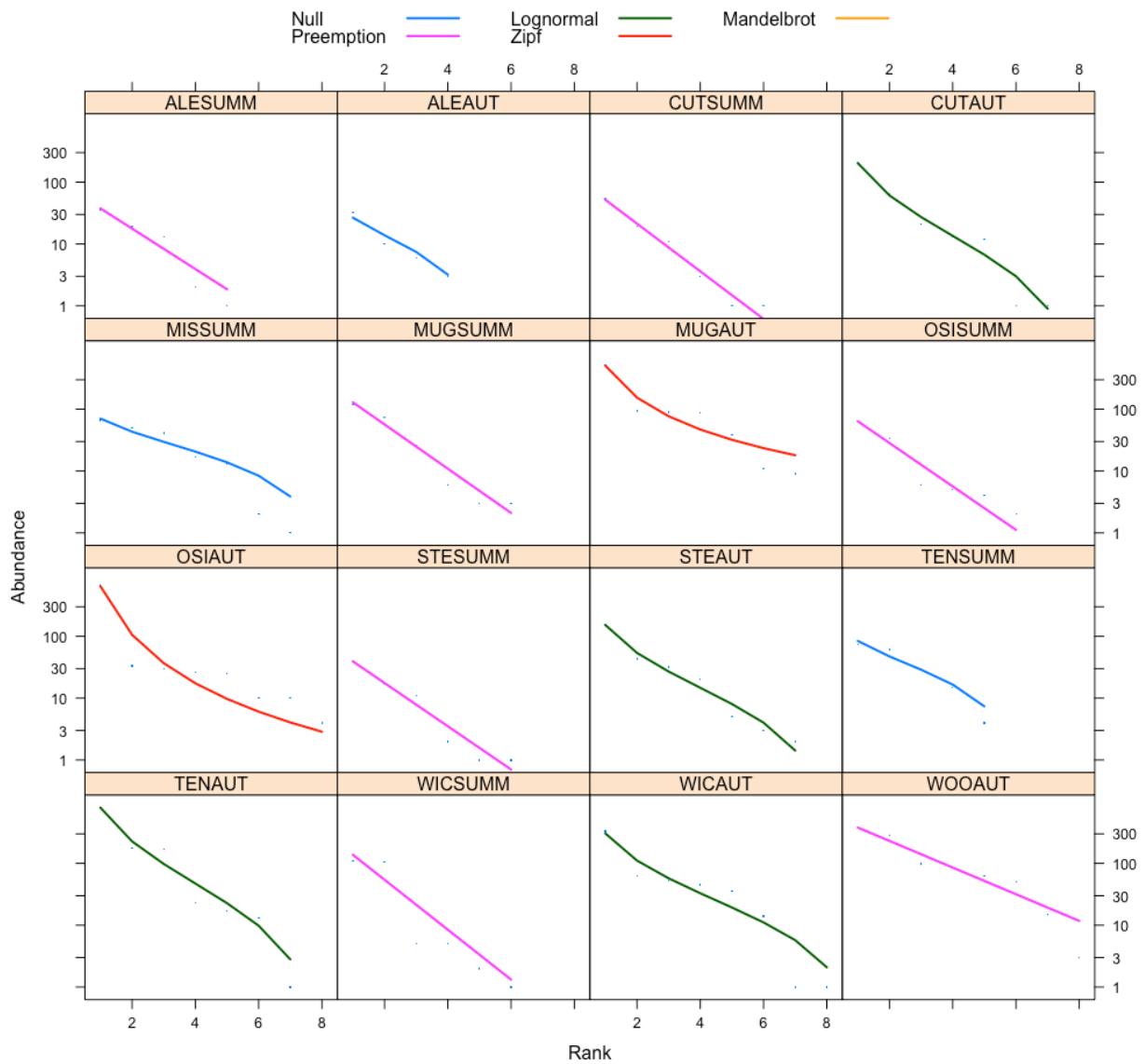


Figure 8. Rank-abundance plots for Plecoptera families collected from tributary streams to the Williston Reservoir collected by Surber sampler for each tributary stream in summer and autumn. Samples sites as indicated in Figure 3.

Table 3. Number of lichen species by functional group for Peace-Williston study sites.

Lichen group		Number of Lichen Species					
		Crooked n = 5	Hominka n = 5	Missinchinka n = 5	Pack n = 5	Upper Parsnip n = 3	Table n = 5
Macrolichen	Chloro	30	32	39	27	27	28
Macrolichen	Cyano	11	20	13	10	13	16
Microlichen	Calicioid	3	8	5	3	2	3
Microlichen	Trebouxioid	51	69	67	41	24	59
Microlichen	Trentepohlioid	1	3	4	1	0	1
Total		96	132	128	82	66	107
Macrolichen and Microlichen	Nutrient indicating Physciaceae & Teloschistaceae	8	17	18	19	14	15

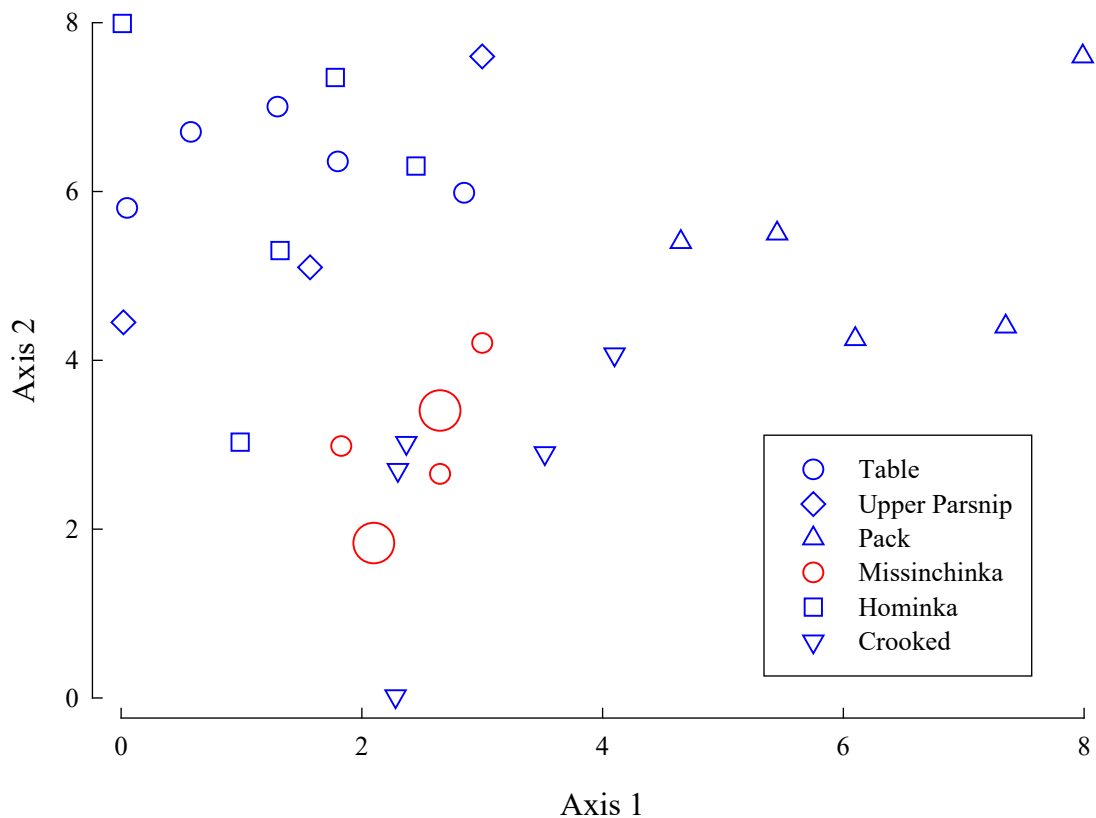


Figure 9. Non-metric multidimensional scaling ordination plot (Axis 1 versus Axis 2) for lichen plots on tributary streams to the Williston Reservoir.

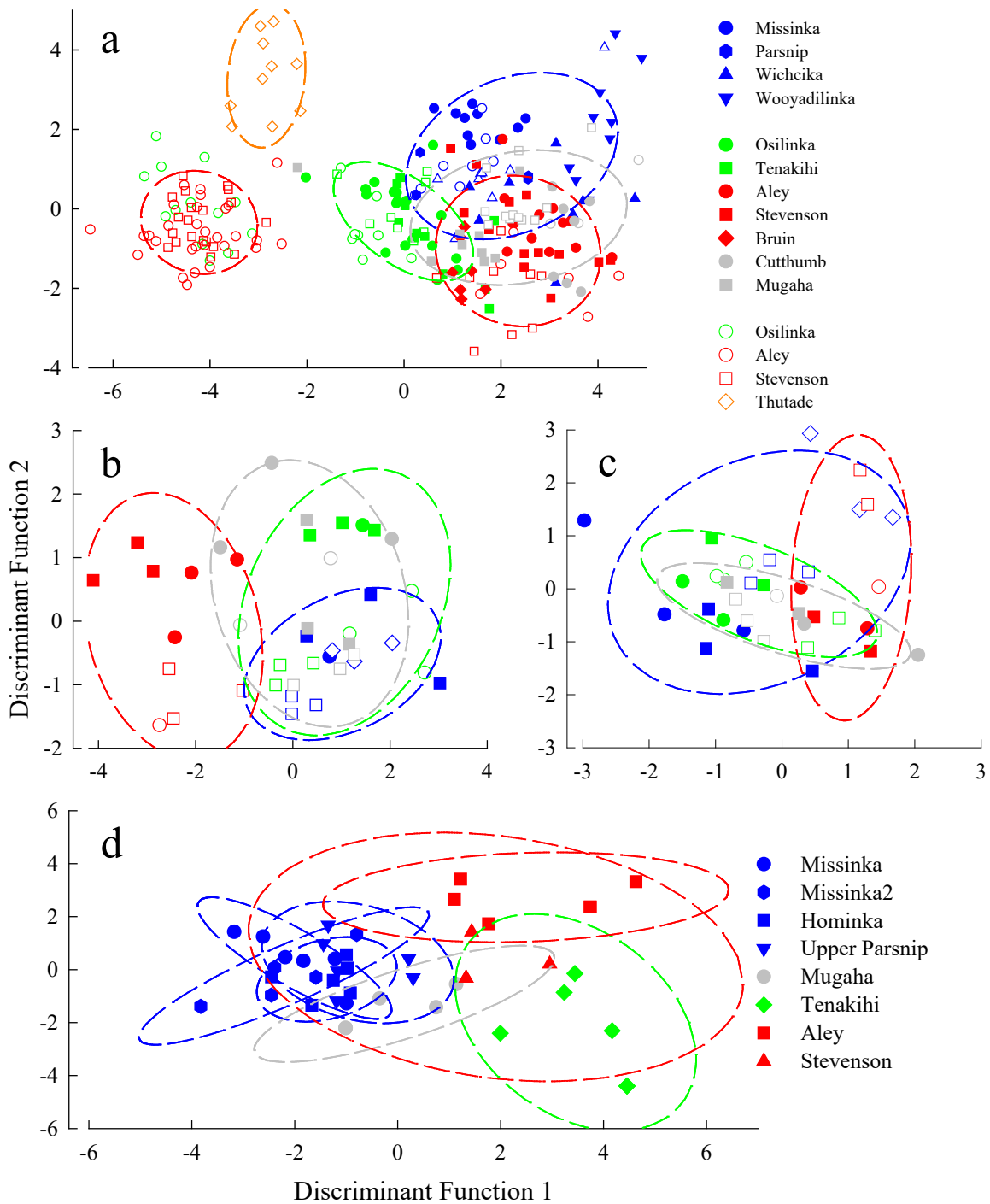


Figure 10. Canonical discriminant function analysis using jackknife resampling for $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{18}\text{O}$. (a) Fish caught in tributary streams; closed symbols are slimy sculpin; blue symbols are non-Kokanee streams and red, green and grey symbols are from systems where Kokanee spawn separated by major watershed. Open symbols in (a) are for spawning Kokanee captured in tributary streams to the Williston Reservoir or non-spawning Kokanee from Thutade Lake. Aquatic insects from the orders Ephemeroptera (b) and Plecoptera (c); symbols as in (a) except closed symbols are samples collected in July 2017 and open symbols are samples collected in October 2017. (d) Lichen (*Parmelia squarrosa*) samples collected from tributary streams to the Williston Reservoir.

Table 4. Classification matrix from discriminant function analysis for stable isotope analysis of $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ with jackknifed resampling for Slimy sculpin and Kokanee. Slimy sculpin samples were collected from streams where Kokanee have not been documented to spawn (Missinka, Upper Parsnip, Wichcika, and Wooyadilinka) and where Kokanee have been observed to spawn (Osilinka, Tenakihi, Aley, Stevenson, Bruin, Cut Thumb and Mugaha). % T is the % classification by treatment (Slimy sculpin captured in non-Kokanee spawning systems, Slimy sculpin captured in systems where Kokanee spawn by major watershed, spawning Kokanee captured in tributary streams to the Williston Reservoir, or Kokanee caught in Thutade Lake). Parsnip are tributaries that flow into the Parsnip River before it flows into the Williston Reservoir, and Mackenzie are tributary streams north of Mackenzie that flow into the Parsnip reach of the Williston Reservoir.

	Slimy sculpin											Kokanee				%T
	Non-Kokanee				Kokanee spawning systems							OSk	ALk	STk	THk	
	MI	Parship		WO	Osilinka		Ospika		Mackenzie							
	IP	WI		OS	TE	AL	ST	BR	CT	MU						
Missinka (MI)	13			1	1						2					
UParsnip (UP)		3				1										
Wichcika (WI)	1	1	7					2	1		4				74	
Wooyadilinka (WO)				9												
Osilinka (OS)	1				21	3					1					
Tenakihi (TE)					6	9		1	2		1				87	
Aley (AL)	1		2				8	3	2		3					
Stevenson (ST)			3		1		2	8	2	2	4				63	
Bruin (BR)									5		2					
Cut Thumb (CT)							1	1		8					52	
Mugaha (MU)	2				2		1	8			8					
Osilinka (OSk)												4	5	4		
Aley (ALk)												4	16	8	1	
Stevenson (STk)												9	10	1		
Thutade (THk)															10	
	18	4	12	10	31	14	14	22	12	10	24	17	31	13	11	
															74	

Table 5. Classification matrix from discriminant function analysis for stable isotope analysis of $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ with jackknifed resampling for aquatic insects belonging to the Order Ephemeroptera. Samples were collected from streams where Kokanee have not been documented to spawn (Missinka, Wichcika, and Wooyadilinka) and where Kokanee have been observed to spawn (Osilinka, Tenakihi, Aley, Stevenson, Cut Thumb and Mugaha). % T is the % classification by treatment and watershed (non-Kokanee spawning systems or systems where Kokanee spawn by major watershed). Parsnip are tributaries that flow into the Parsnip River before it flows into the Williston Reservoir, and Mackenzie are tributary streams north of Mackenzie that flow into the Parsnip reach of the Williston Reservoir.

	Non-Kokanee			Kokanee spawning systems						% T
	Parship			Osilinka		Ospika		Mackenzie		
	MI	WI	WO	OS	TE	AL	ST	CT	MU	
Missinka (MI)					1					
Wichcika (WI)		1			2				3	40
Wooyadilinka (WO)			3							
Osilinka (OS)				3				1		40
Tenakihi (TE)		3			1				2	
Aley (AL)						2	1	1		80
Stevenson (ST)	1					2	3			
Cut Thumb (CT)			1	1		1		2		27
Mugaha (MU)	1	2			2				1	
Total	2	6	4	4	6	5	4	4	6	47

Table 6. Classification matrix from discriminant function analysis for stable isotope analysis of $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ for aquatic insects belonging to the Order Plecoptera. Samples were collected from streams where Kokanee have not been documented to spawn (Missinka, Wichcika, and Wooyadilinka) and where Kokanee have been observed to spawn (Osilinka, Tenakihi, Aley, Stevenson, Cut Thumb and Mugaha). % T is the % classification by treatment and watershed (non-Kokanee spawning systems or systems where Kokanee spawn by major watershed). Parsnip are tributaries that flow into the Parsnip River before it flows into the Williston Reservoir, and Mackenzie are tributary streams north of Mackenzie that flow into the Parsnip reach of the Williston Reservoir.

	Non-Kokanee			Kokanee spawning systems						% T
	Parship			Osilinka		Ospika		Mackenzie		
	MI	WI	WO	OS	TE	AL	ST	CT	MU	
Missinka (MI)	2			1						
Wichcika (WI)	1			1	3				1	46
Wooyadilinka (WO)			3							
Osilinka (OS)	1			3	1					70
Tenakihi (TE)				1	2			1	1	
Aley (AL)						2		1		57
Stevenson (ST)							2	2		
Cut Thumb (CT)						1	1	1		38
Mugaha (MU)		2			1				2	
Total	3	2	3	6	7	3	3	5	5	53

Table 7. Classification matrix from discriminant function analysis for stable isotope analysis of $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ for aquatic insects belonging to the Order Trichoptera. Samples were collected from streams where Kokanee have not been documented to spawn (Missinka, Wichcika, and Wooyadilinka) and where Kokanee have been observed to spawn (Osilinka, Tenakihi, Aley, Stevenson, Cut Thumb and Mugaha). % T is the % classification by treatment and watershed (non-Kokanee spawning systems or systems where Kokanee spawn by major watershed). Parsnip are tributaries that flow into the Parsnip River before it flows into the Williston Reservoir, and Mackenzie are tributary streams north of Mackenzie that flow into the Parsnip reach of the Williston Reservoir.

	Non-Kokanee			Kokanee spawning systems						% T
	Parship			Osilinka		Ospika		Mackenzie		
	MI	WI	WO	OS	TE	AL	ST	CT	MU	
Missinka (MI)								1		
Wichcika (WI)	1			1						20
Wooyadilinka (WO)							1	1		
Osilinka (OS)		1		2						
Tenakihi (TE)		1			1			1	1	43
Aley (AL)						0				0
Stevenson (ST)		1	1							0
Cut Thumb (CT)	2									0
Mugaha (MU)	2			1						0
Total	5	3	1	4	1	0	1	3	1	16

Table 8. Classification matrix from discriminant function analysis for stable isotope analysis of $\delta^2\text{H}$, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ for lichen (*Parmelia squarrosa*) samples collected from riparian zones of streams where Kokanee have not been documented to spawn (Missinka, Hominka, and Upper Parsnip) and where Kokanee have been observed to spawn (Mugaha, Tenakihi, Aley and Stevenson). The Missinka River was sample in 2016 and 2017 and results shown separately. % R is the % classification by River system. % T is the % classification by treatment (non-Kokanee spawning systems or systems where Kokanee spawn).

	Non-Kokanee systems				Kokanee spawning systems				% R	% T
	M1	M2	HO	UP	MU	TE	AL	ST		
Missinka1 (M1)	5				1				83	
Missinka2 (M2)		5							100	90
Hominka (HO)			4	2					67	
UParsnip (UP)	1		2	2				1	33	
Mugaha (MU)		1	1		2				50	
Tenakihi (TE)						5			100	88
Aley (AL)							5		100	
Stevenson (ST)								3	100	
Total	6	6	7	4	3	5	5	4	50	

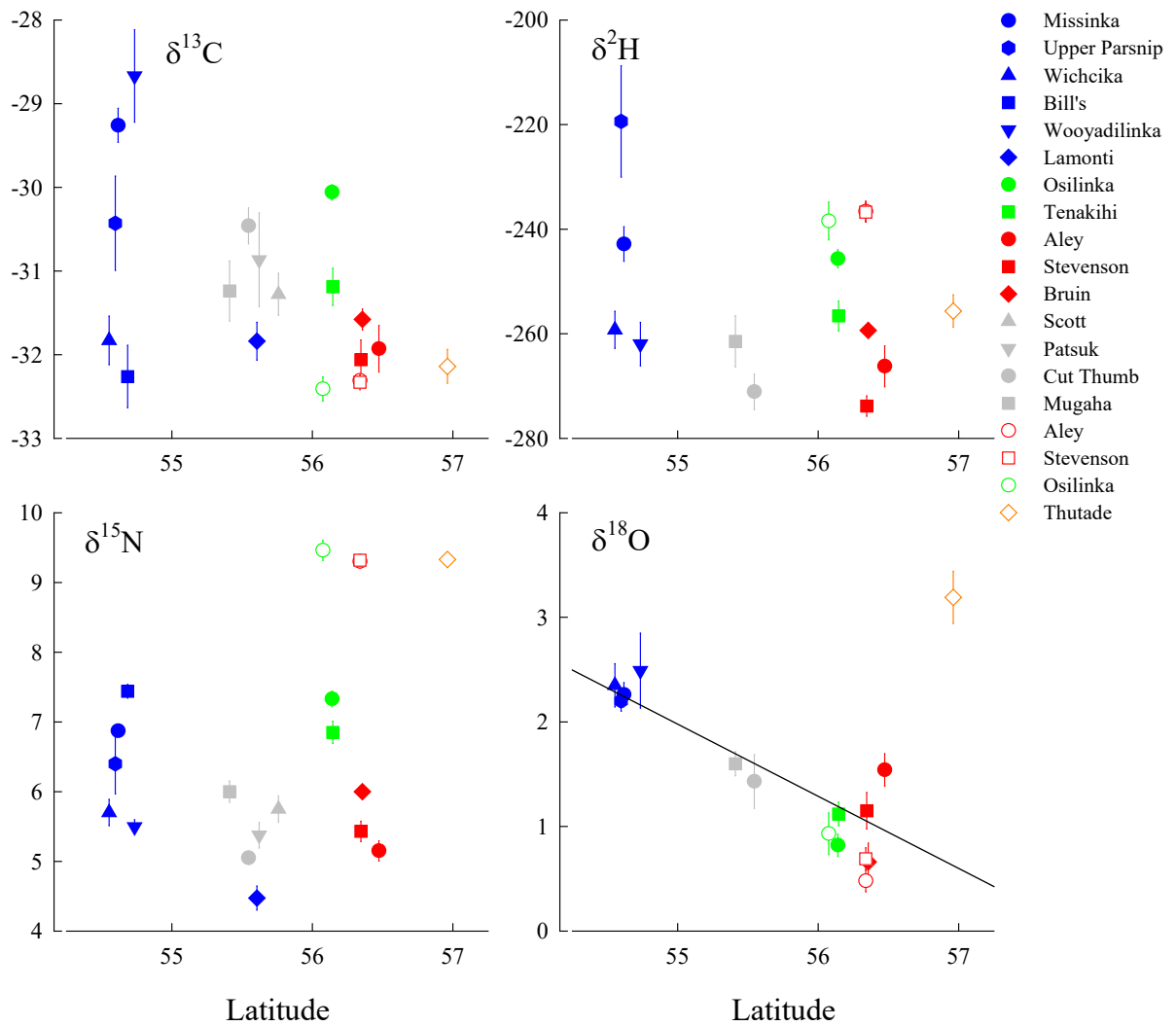


Figure 11. Stable isotope ratios for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ from fish caught in tributary streams to the Williston Reservoir. Closed symbols are slimy sculpin; blue symbols are non-Kokanee streams and red, green and grey symbols are from systems where Kokanee spawn separated by major watershed. Open symbols are for spawning Kokanee captured in tributary streams to the Williston Reservoir or non-spawning Kokanee from Thutade Lake. Data are presented as means ± 1 SE.

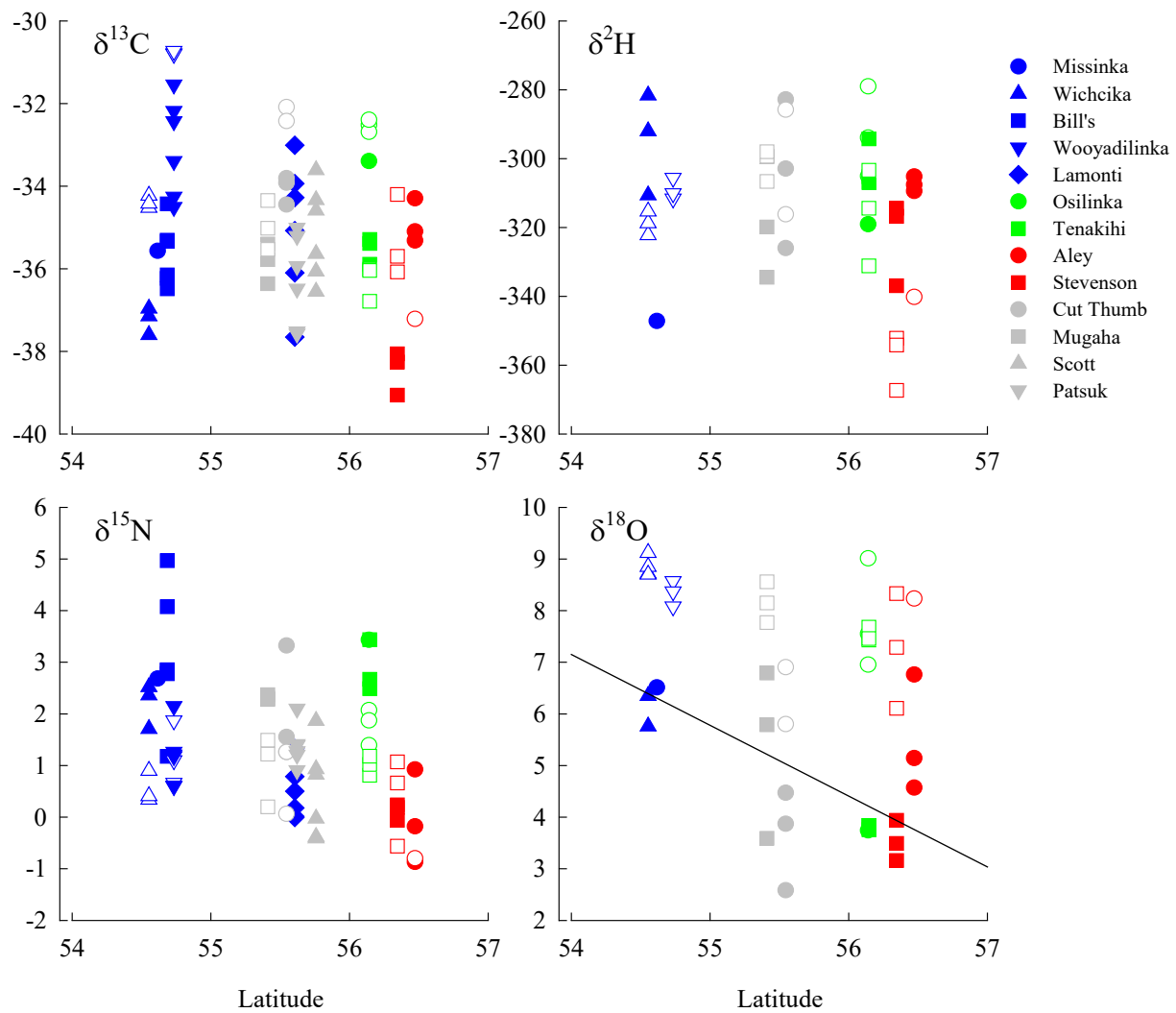


Figure 12. Stable isotope ratios for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ from aquatic insects, Order Ephemeroptera, caught in tributary streams to the Williston Reservoir. Blue symbols are non-Kokanee streams; red, green and grey symbols are from systems where Kokanee spawn separated by major watershed. Closed symbols are samples collected in July 2017 and open symbols are samples collected in October 2017.

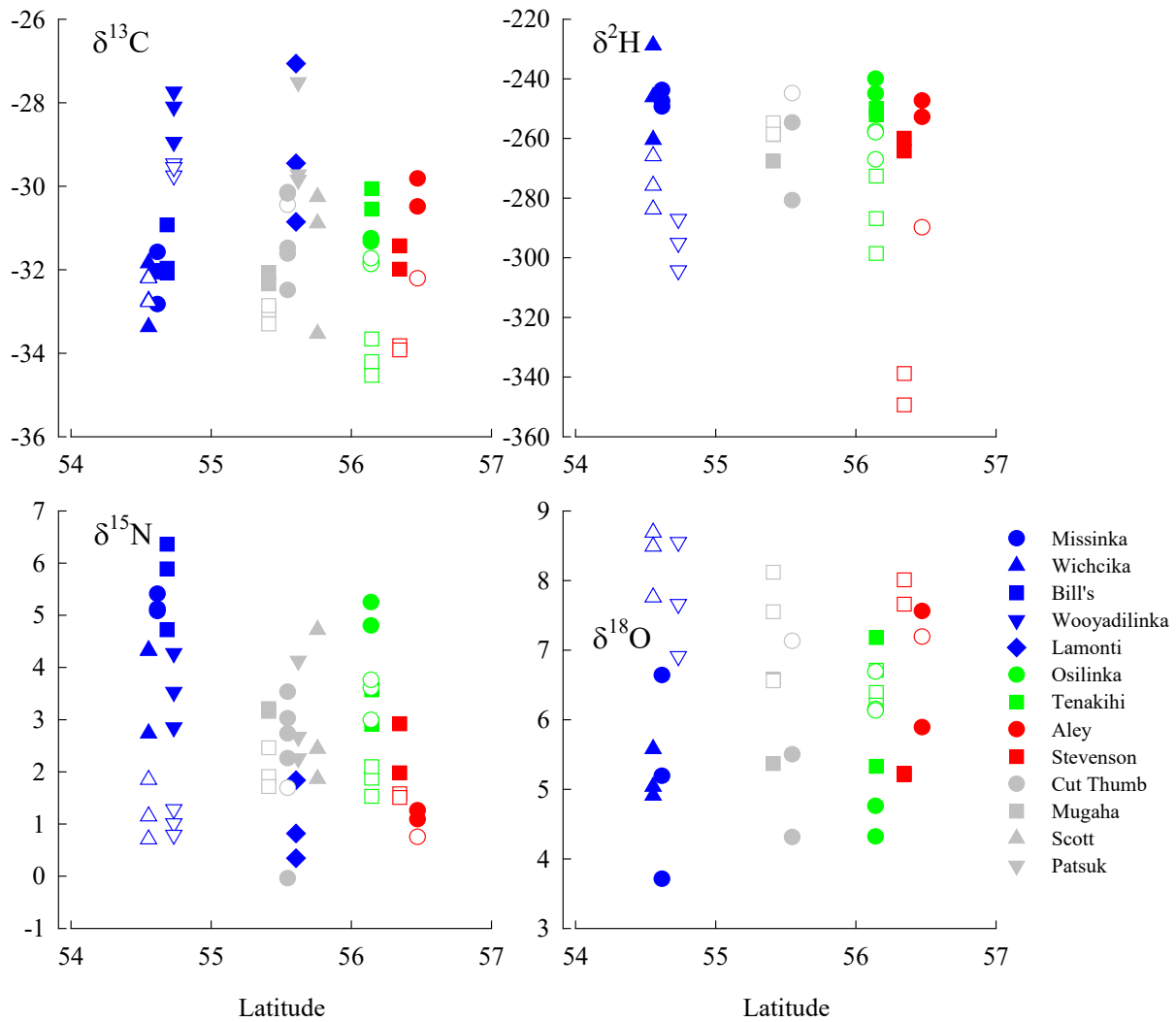


Figure 13. Stable isotope ratios for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ from aquatic insects, Order Plecoptera, caught in tributary streams to the Williston Reservoir. Blue symbols are non-Kokanee streams; red, green and grey symbols are from systems where Kokanee spawn separated by major watershed. Closed symbols are samples collected in July 2017 and open symbols are samples collected in October 2017.

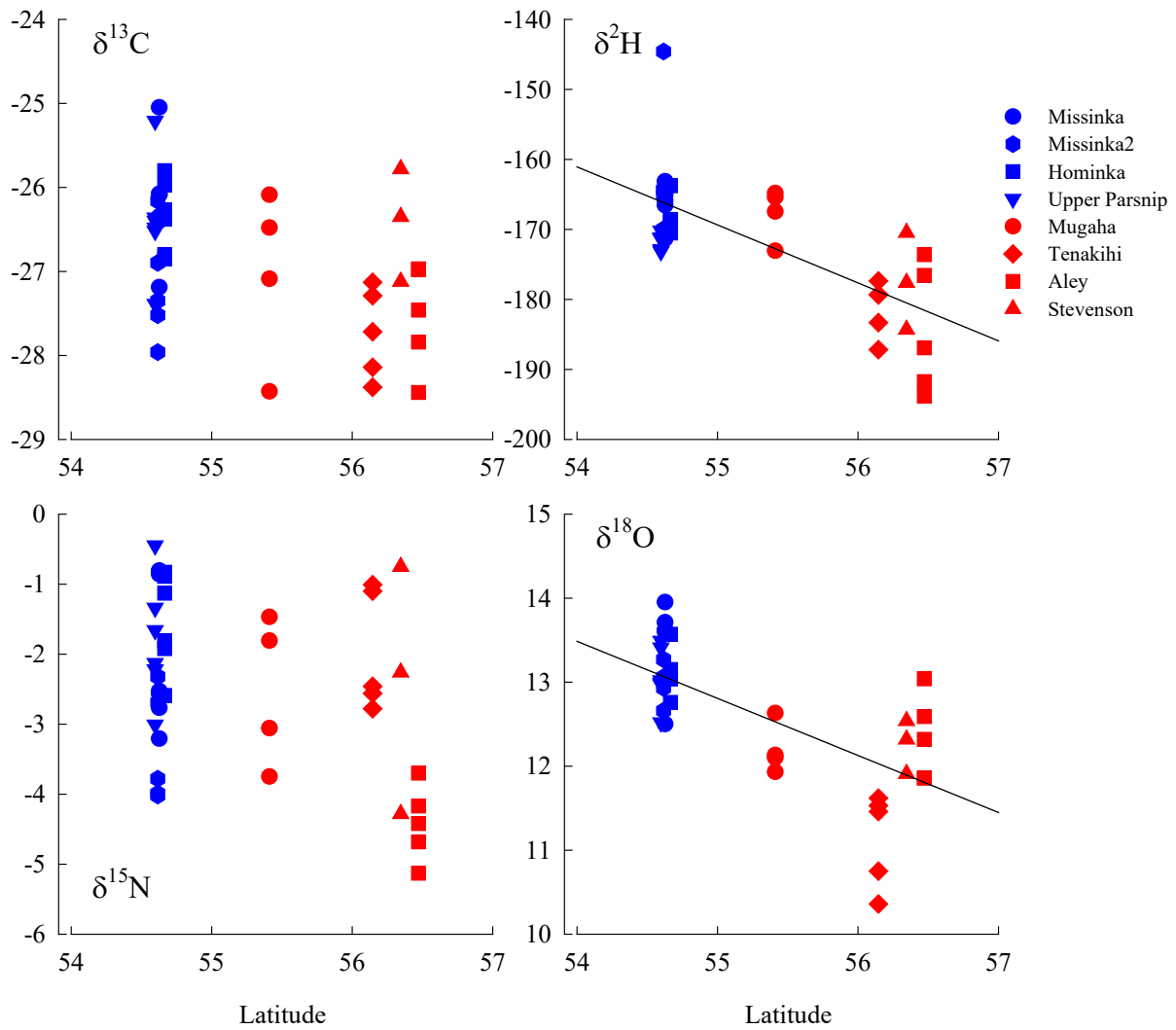


Figure 14. Stable isotope ratios for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^2\text{H}$, and $\delta^{18}\text{O}$ from lichen, *Parmelia squarrosa*, collected from riparian areas of tributary streams to the Williston Reservoir. Blue symbols are non-Kokanee streams; red symbols are from systems where Kokanee spawn.

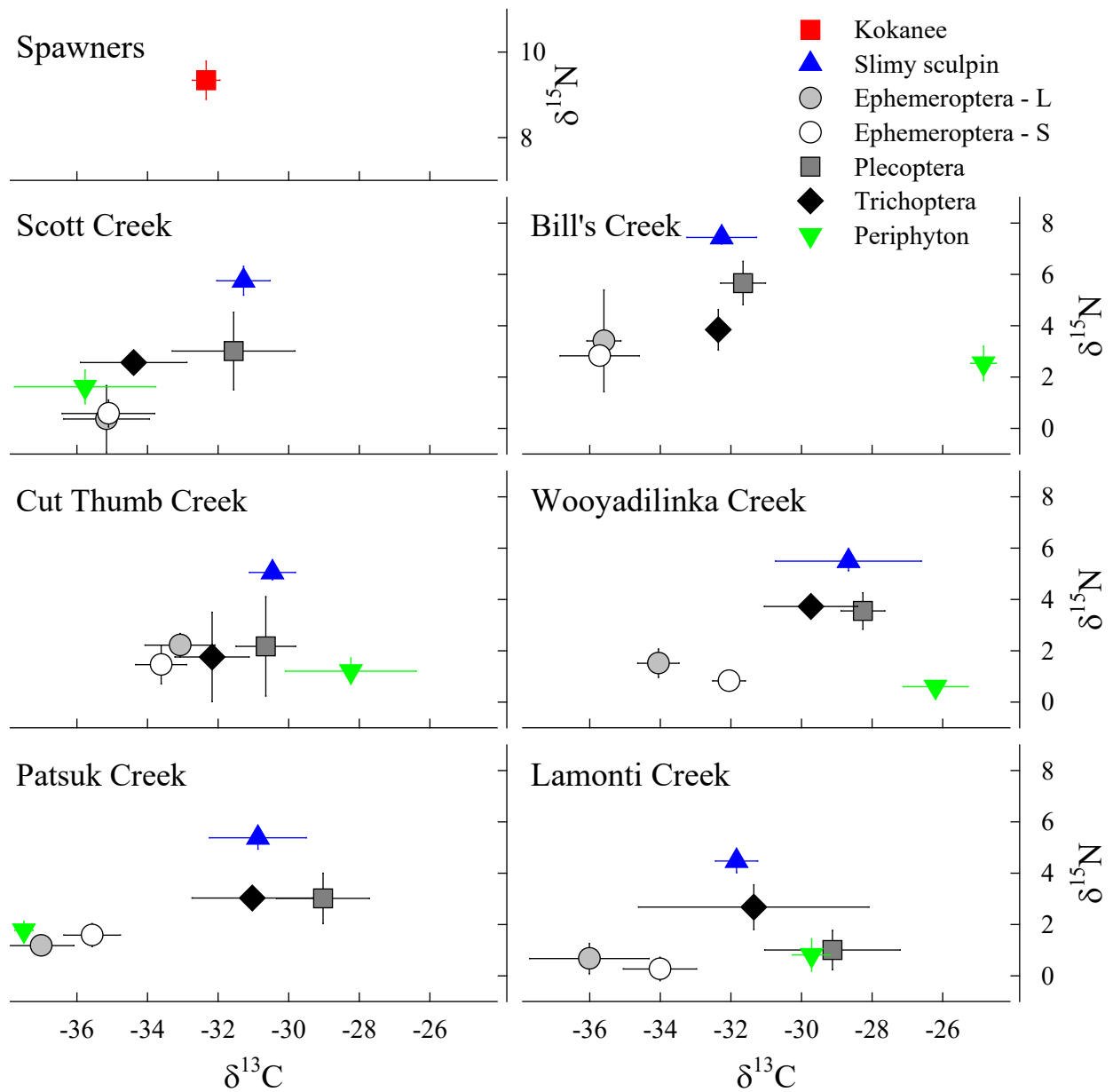


Figure 15. Stable isotope ratios for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for different taxonomic groups collected from three control streams where Kokanee do not spawn (Bill's, Wooyadilinka, and Lamonti Creeks) and from three experimental streams where Kokanee have been observed to spawn (Scott, Cut Thumb, and Patsuk Creeks). For comparison, values for Kokanee spawners are shown in the top panel. Data are presented as means ± 1 SE.

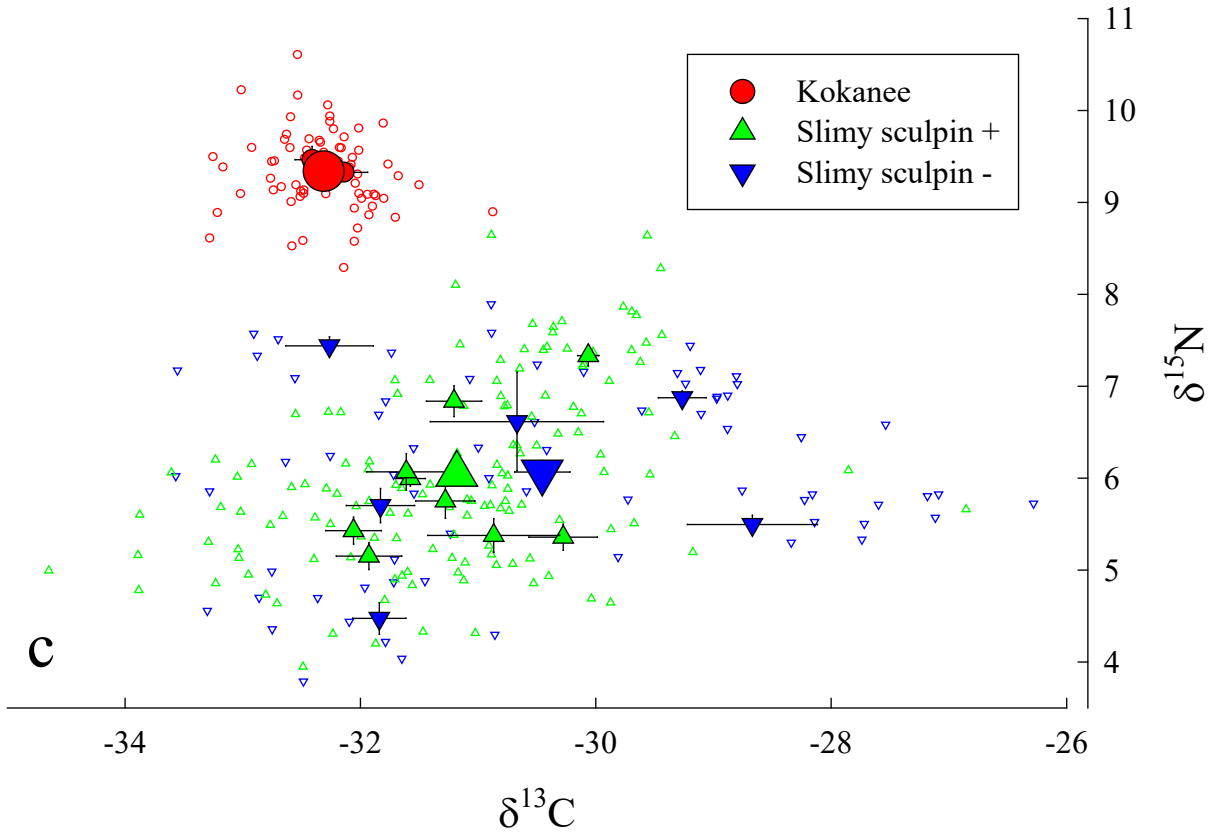


Figure 16. (a) Slimy sculpin from Aley Creek (M. Shrimpton). (b) Kokanee spawner from the Osilinka River (L. Anderson). Stable isotope, $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ (c) from fish caught in tributary streams to the Williston Reservoir. Slimy sculpin from non-Kokanee streams are blue symbols, green symbols are slimy sculpin from Kokanee spawning streams, and red symbols are Kokanee spawners. Open symbols are individual values, small closed symbols are means ± 1 SE for each tributary stream where fish were caught, and large symbols are treatment means ± 1 SE for each treatment group.

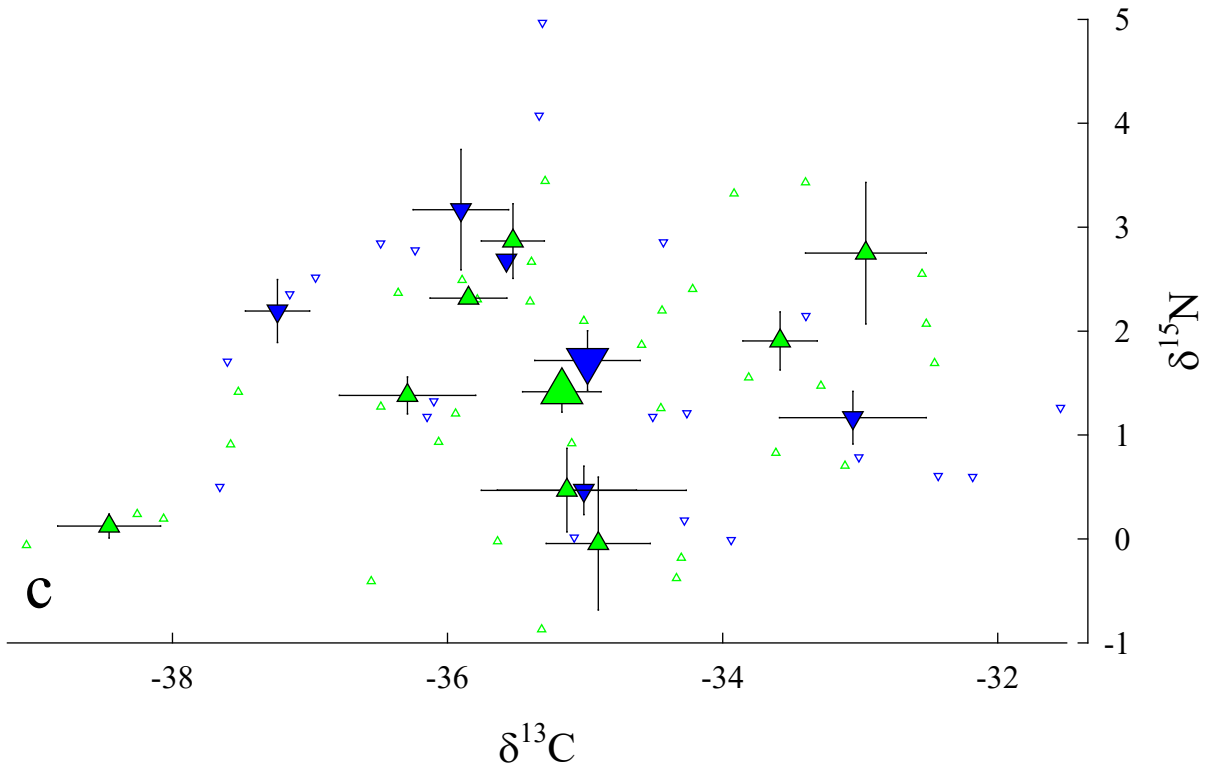
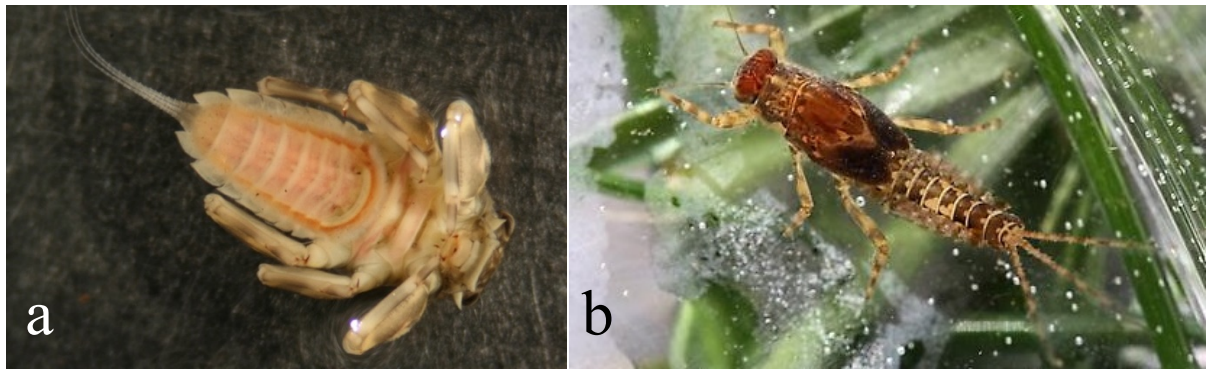


Figure 17. Representative aquatic insects belonging to the Order Ephemeroptera collected in tributaries to the Williston Reservoir; (a) *Drunella doddsii* (C. Shrimpton) and (b) *Ephemerella dorothea* (Barcode of Life Database (BOLD)). Stable isotope, $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ (c) from pooled samples of Ephemeroptera collected from tributary streams to the Williston Reservoir in the summer of 2017 and 2018. Mayflies from non-Kokanee streams are blue symbols and green symbols are mayflies from Kokanee spawning streams. Open symbols are individual values, small closed symbols are means ± 1 SE for each tributary stream where fish were caught, and large symbols are treatment means ± 1 SE for each treatment group.

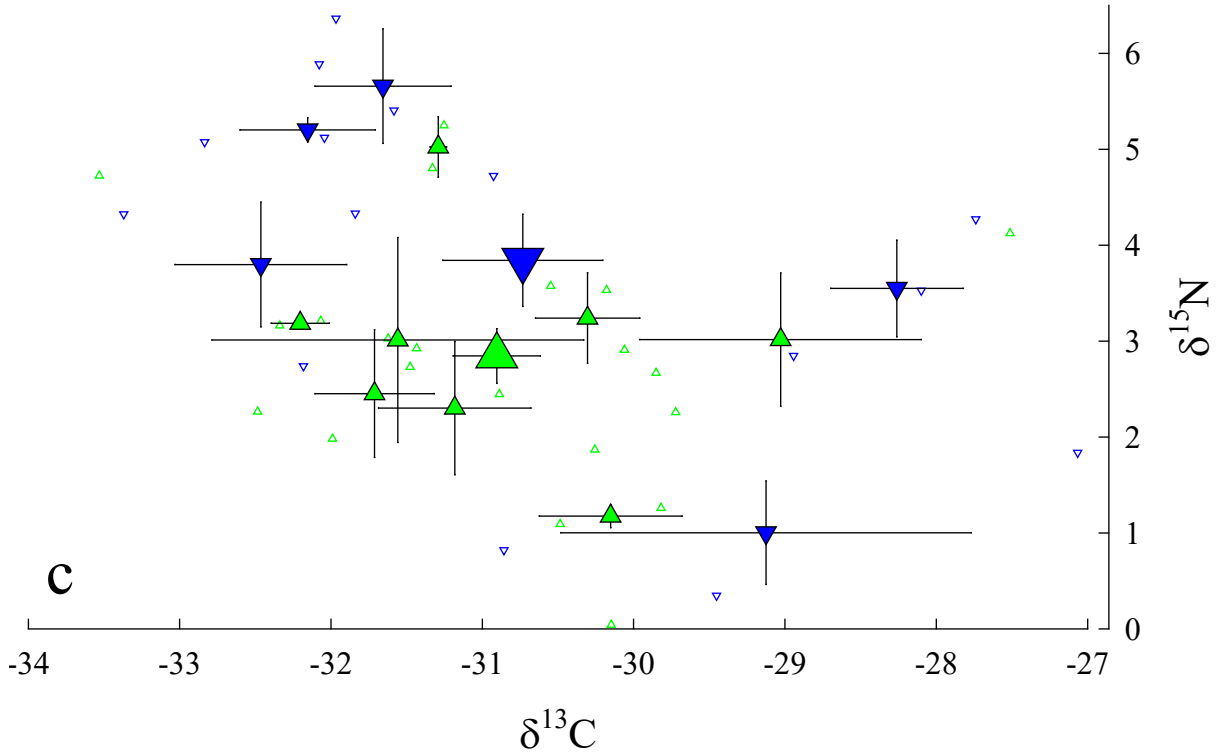
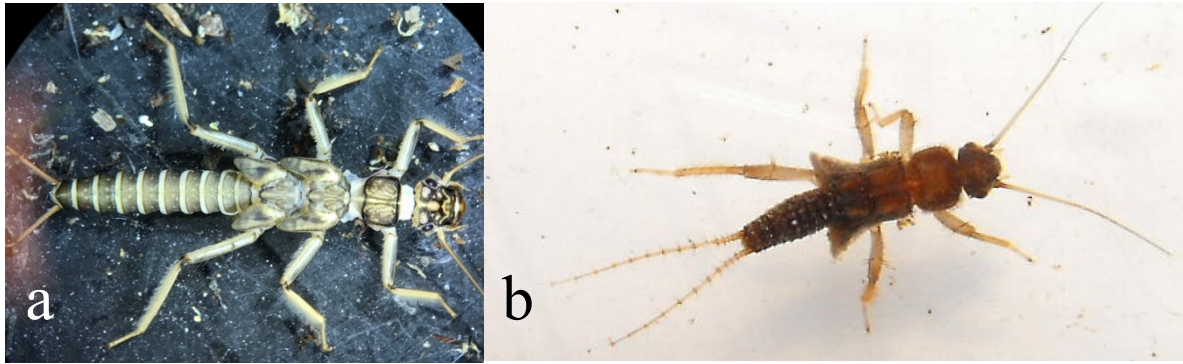


Figure 18. Representative aquatic insects belonging to the Order Plecoptera collected in tributaries to the Williston Reservoir; (a) *Hesperoperla pacifica* (A. Thielman) and (b) *Zapada cinctipes* (BOLD). Stable isotope, $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ (c) from pooled samples of Plecoptera collected from tributary streams to the Williston Reservoir in the summer of 2017 and 2018. Stoneflies from non-Kokanee streams are blue symbols and green symbols are stoneflies from Kokanee spawning streams. Open symbols are individual values, small closed symbols are means ± 1 SE for each tributary stream where fish were caught, and large symbols are treatment means ± 1 SE for each treatment group.

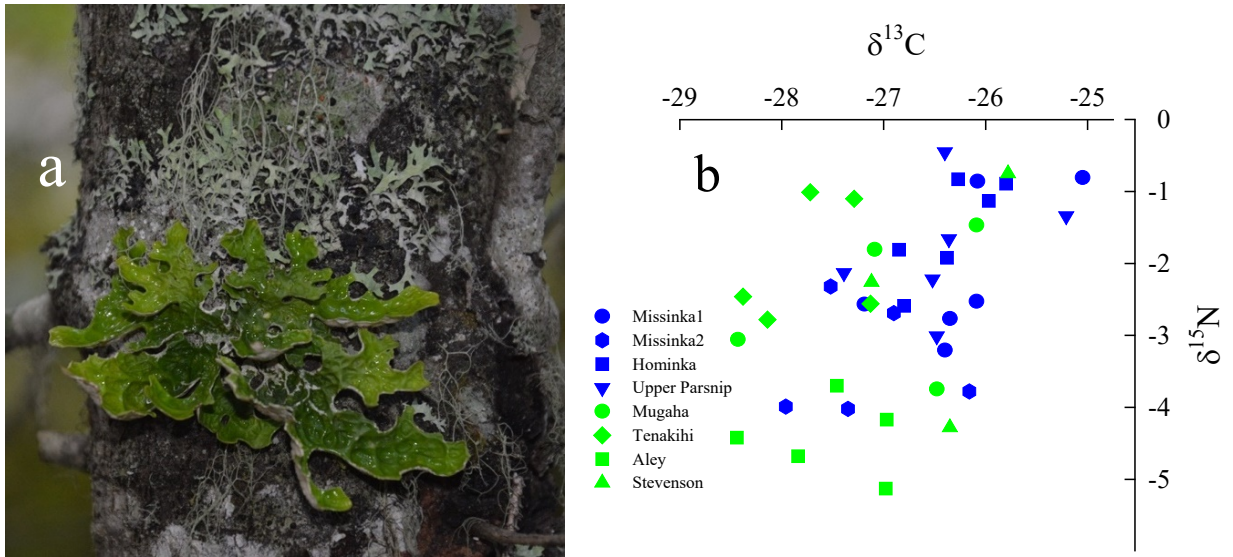


Figure 19. (a) Lichen growing on a deciduous tree in the riparian area of a tributary stream to the Williston Reservoir (M. Shrimpton); *Lobaria pulmonaria* (green, leafy), *Alectoria* sp. (hair lichen) and *Parmelia squarrosa* (light blue, square lobes). Stable isotope, $\delta^{13}\text{C}$ versus $\delta^{15}\text{N}$ (c) from lichen (*Parmelia squarrosa*) samples collected from tributary streams to the Williston Reservoir. Green symbols are from systems where Kokanee spawn and blue symbols are from systems where Kokanee do not spawn.

Extension and Community Engagement

Over the course of this 3-year project a number of activities were undertaken to communicate the results of this project to community members throughout the project area. During the first year of the project a brochure was produced which explained the proposed research and reported on initial survey results (Appendix III). The brochure was reviewed by First Nations project partners and distributed to project partners as well as other interested stakeholders and research colleagues.

On March 9th, 2018, one of the project leads, Dr. Mark Shrimpton, gave a presentation on the project as part of the Natural Resources and Environmental Studies Institute Friday colloquium series at the University of Northern British Columbia. These presentations are attended by graduate students, UNBC faculty members as well as interested members of the general public. The presentation was also broadcast on the internet via UNBC's LiveStream service for viewing by those unable to attend in person. The presentation was also recorded and stored on the UNBC video archive website for future viewing. The recording for this presentation can be found at

https://video.unbc.ca/media/NRESi+Colloquium+and+Annual+Awards+CeremonyB+Impact+of+stocked+kokanee+on+nutrient+enrichment+in+tributary+streams+to+the+Williston+Reservoir+-+Dr.+Mark+Shrimpton%2C+UNBC/0_nnrk0f4j/19801.

On September 20-22, 2018, members of the project team traveled to Tsay Keh Dene village on the shores of the Williston Reservoir to participate in the community's science week. The team met with and shared project objectives and results with the Elders from the community. Through the use of four posters (Appendix IV), prepared by Dr. Mark Shrimpton, the team discussed the research results for the fish (Kokanee and sculpins), invertebrate and lichen components of the projects, as well as the stable isotope data and results. In addition, the community members shared their perspectives on Kokanee and the changes that have occurred since the creation of the reservoir. Also, during this visit, the team met with school children from the community and provided an introduction to spawning Kokanee and benthic invertebrates. The team and the students traveled to Hydro Creek at the north end of the village where the team members provided hands-on training to the students on the use of Surber and kick nets to collect benthic invertebrates from the creek. Samples collected by the students were taken back to the classroom where they were examined under microscopes. The twelve students who attended, as well as their principal and educational assistant, learned about food web dynamics in fish-bearing streams and the flow of energy from the sun, to plants and other primary producers, to primary consumers (small invertebrates) and secondary consumers (large invertebrates and fish). The also learned about lichen and participated in a lichen-based activity involving the rehydration of dried lichen (grey) to watch them turn green again.

On November 27, 2018, project research team member Dr. Mark Shrimpton gave a presentation on the project to approximately 20-25 members of the public in Mackenzie, British Columbia. Attendees included representatives from the forest industry, the Mackenzie Community Forest, the Provincial government, academia, the consulting community, the BC

Trappers Association, the Mackenzie Nature Observatory, and the general public. Dr. Shrimpton provided a review of the methods, hypotheses, some of the techniques that were used in this research, including the stable isotope analysis and the project results to date. The talk was also broadcast over the internet through the UNBC Blue Jeans system and was also recorded. A recording of Mark's talk is available on-line at:

https://video.unbc.ca/media/Fish+and+Wildlife+Compensation+Program+-+November+27+2018/0_d756hw5a

DISCUSSION

Assemblage of study species

We sampled tributary streams in the Williston Reservoir watershed from a number of the major sub-watersheds; the Parsnip watershed, the Parsnip reach, the Osilinka watershed, and the Ospika watershed. We found considerable diversity of aquatic invertebrates within the stream, large diversity of lichen within the riparian areas adjacent to the streams, but relatively few fish.

From our DNA barcode survey of aquatic invertebrate biodiversity in 2016, we found a total of 115 species (Coxson et al. 2017). Diptera were the most diverse group, followed by Plecoptera and Ephemeroptera. Due to the sampling approach used in 2016, the number represents a conservative estimate for total biodiversity at each sampling location. Only three species of aquatic invertebrates, however, were found in all seven streams: *Zapada cinctipes* (Plecoptera), *Drunella grandis* (Ephemeroptera), and *Ephemerella* sp.3 (Ephemeroptera). Quite a few were found in only one or a small subset of streams even though we sampled in reasonably similar habitat in each stream (riffles or rapid flow and rocky bottoms). This is indicative of a high level of beta-diversity (i.e., differences in diversity between sites) within the region. Most of the species that were identified down to species via DNA barcode annotation have previously been collected in British Columbia. Although one species, *Apatania comosa*, had not been previously recorded in British Columbia and its range is currently considered so limited (portions of ID, MT, UT, WY) that it is ranked as “G2 - Imperiled” by NatureServe. If *A. comosa* exists in the Williston region, it would greatly expand that range extent and would perhaps indicate a more secure conservation rank.

In contrast to the DNA barcode survey, morphological identification of specimens to determine abundance revealed that the aquatic invertebrate samples were dominated by the Orders Ephemeroptera, Plecoptera and Trichoptera (EPT's; 78% of entire sample) – particularly Ephemeroptera. Samples were collected by kick netting, and are therefore less quantitative than other methods such as Surber or Hess sampling, but the findings reveal some interesting patterns. Relative abundance changed among the study streams, but there were also differences between the samples collected in the summer and the fall samples. Ephemeroptera comprised $60.7 \pm 4.6\%$ of the total number of aquatic insects in the summer; $45.7 \pm 3.0\%$ from the samples collected in the fall. Although specimens of Plecoptera were large, they were not particularly abundant in the summer ($10.9 \pm 1.1\%$); but were smaller and more abundant in the fall ($34.5 \pm 2.6\%$). Trichoptera were considerably less abundant in the samples; just $2.3 \pm 0.4\%$ of the specimens in all samples collected in summer and fall. All other aquatic invertebrates including Dipterans composed $21.8 \pm 2.6\%$ in the summer and fall samples.

Our 2017 stonefly data, while somewhat limited in terms of non-Kokanee systems, do not show a difference between Kokanee and non-Kokanee systems. There is a fair amount of stream-by-stream variation around the system in terms of rank-abundance, and in some cases the rank-abundance metric also changes within a stream between sampling episodes (summer and autumn). Clustering of biodiversity characteristics are also evident in relation to seasonality/sampling time, with autumn and summer sampling being somewhat distinct from

each other. In terms of total samples taken from both seasons, there are seem to be two overall groups of systems, and there is some evidence of geographical clustering of systems.

Lichen collected from tributary streams to the Parsnip River and streams flowing into the Parsnip Reach of the reservoir showed an impressive level of diversity including a number of rare species of conservation concern. Of the 229 lichen taxa observed in the 6 study sites, a previously undocumented federally listed rare species of cyano macrolichen, *Collema coniophilum* (listed under the Species at Risk Act, or SARA, ranked by the Committee on the Status of Endangered Wildlife in Canada) was found at all sites except for the Upper Parsnip location. Additionally, a species red-listed by the BC Conservation Data Centre (CDC), *Collema quadrifidum*, was found in the Hominka River watershed, a blue-listed lichen, *Nephroma isidiosum*, was found in the Upper Parsnip, and a conservation-ranked species of calicioid microlichen, *Phaeophyscia nigricans*, was found at the Missinchinka site. Consequently, our survey has greatly increased our understanding of lichen distribution in central BC.

All the study plots were rich in number of the Teloschistaceae and Physciaceae, except for the Crooked River which was the most species impoverished site for Teloschistaceae and Physciaceae. A pattern that may reflect either the lower precipitation at this site (683 mm) or past disturbance history. The one Kokanee-run stream (Missinchinka) fell among the richest set of sites. Interesting, there was a strong separation of the Pack River samples from all others along Axis 1 of the Non-metric multidimensional scaling (NMS) ordination. The Pack and Crooked River sites were the two most impoverished sites for total number of lichen species, and are the two driest sites in our data set, reinforcing the importance of precipitation availability as an ecological variate in the BC interior (Coxson et al. 2013). The NMS results show that the one Kokanee-run stream, the Missinchinka, shares strong ecological ties with all of the other high-precipitation sites (Figure 2).

The assemblage of fish species showed little diversity and relatively low abundance in our study streams. A single species from the family Cottidae, the slimy sculpin, was the most commonly captured fish and often the only species found while electrofishing the study streams. Sizes of slimy sculpin were similar among the experimental streams; however our sampling was designed to catch larger slimy sculpin as we have previously demonstrated that larger fish tend to be relatively sedentary (Clarke et al. 2015). Two species of Salmonidae were found in several streams, bull trout (*Salvelinus confluentus*) and rainbow trout (*Oncorhynchus mykiss*). There was considerable range of size for slimy sculpin caught in each system, indicating multiple year classes of fish, whereas bull trout and rainbow trout were typically small and likely young-of-the-year or one-year old fish. Occasionally, larger trout were captured – but often only a single fish per stream. Our findings are not surprising as many of the study streams are known systems for both bull trout and rainbow trout to spawn (Hagen and Pillipow 2014) and would, therefore, be restricted to younger age classes of fish outside the spawning seasons – the times when we surveyed each stream. Diversity of fish species was greater in the two largest systems surveyed; Parsnip River and Osilinka River. Fish belonging to other families were also caught in these rivers; Gadidae, Cyprinidae, and Thymallidae.

Stable isotope signatures

We used stable isotopes to track the potential delivery of nutrients by Kokanee from the reservoir to tributary streams and their riparian ecosystems using a benthic species of fish, stream dwelling aquatic insects, and a foliose green algal lichen. Intragravel flow of water carries nutrients to riparian vegetation, aquatic insects and stream resident fish feed on eggs and dead fish, which in turn feed numerous other organisms.

Slimy sculpin have the widest distribution among the family Cottidae in North America and have been increasingly used in impact assessment studies as they are relatively sedentary as adults with a small home range (Gray et al. 2018). Consequently, they are a useful model species for integrating environmental conditions of localized areas. Limited movement for older life stages of slimy sculpin (Clarke et al. 2015) make this species a good choice for looking at elemental signatures indicative of trophic influence and nutrient sources to the study streams. Although there was variation in the stable isotope signatures within each system, signatures from slimy sculpin collected in systems with high numbers of Kokanee spawners showed separation from signatures of fish collected from streams with no Kokanee for $\delta^2\text{H}$ and particularly $\delta^{18}\text{O}$. In contrast, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for slimy sculpin overlapped considerably among Kokanee and non-Kokanee streams and we found quite a large range of $\delta^{13}\text{C}$ values for the non-Kokanee streams – a finding that differed from results previously reported. Due to the spatial distribution of Kokanee spawners, there was little geographic overlap for control and experimental streams. Sampling in 2018 attempted to address the spatial distribution of sampling sites and we found little difference in stable isotope signatures for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for slimy sculpin or aquatic insects in streams located in the same region such as Lamonti Creek where Kokanee have never been documented to spawn and Patsuk Creek where Kokanee have been documented to spawn. The lack of a difference between these two streams may be limited escapement by Kokanee and little or no potential for nutrient transfer from the reservoir to tributary streams in this region of the watershed. Although we documented Kokanee spawning in Cut Thumb Creek in 2017, the number of spawners observed was less than 20. Additionally, DWB (2019) did not report any Kokanee spawners in the Parsnip Reach in 2018.

There was overlap in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ between the spawning Kokanee from the Williston Reservoir and sculpin caught in streams where Kokanee spawned – signatures quite distinct from the sculpin caught in tributary streams to the Parsnip River where Kokanee do not spawn. Carbon isotope measurements indicate dietary sources of nutrition (Peterson and Fry 1987). The considerable differences among sculpins sampled from the different systems suggests difference in source carbon, but sculpin sampled from streams where high numbers of Kokanee spawned had $\delta^{13}\text{C}$ values that were depleted and similar to values for Kokanee. Nitrogen isotopes function as trophic level indicators (Peterson and Fry 1987) and indicate that Kokanee are eating at a higher trophic level than the sculpins. In contrast $\delta^{18}\text{O}$ showed considerable difference across streams; Kokanee from the Williston Reservoir and slimy sculpin caught in Kokanee spawning streams were depleted in ^{18}O compared to slimy sculpin caught in Williston Reservoir streams where Kokanee do not spawn. Additionally, Kokanee from Thutade Lake were enriched in ^{18}O compared to all fish from the Williston Reservoir watershed.

Stable isotope analysis also included replicates of the major orders of aquatic insects, Ephemeroptera, Plecoptera and Trichoptera, to assess potential variation within each group, but also potential functional differences among groups. Ephemeroptera showed a pattern similar to the sculpin, but the pattern was less clear for Plecoptera and Trichoptera. For the aquatic invertebrate samples, there was also temporal variation in isotope signature for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$, but less so for $\delta^2\text{H}$. The isotopic composition of a specific animal tissue is controlled mainly by local dietary inputs, although various tissues integrate local diet over different temporal scales (Hobson 1999). Temporal differences among the aquatic invertebrates may represent stage of development as the nymphs collected represent different instars between the samples collected in July and October. July specimens were larger than October – likely indicating that the nymphs represented different feeding guilds and trophic levels – reflected in the shifts in stable isotope signatures among the seasons.

Perhaps most strikingly, the stable isotope data for the samples of the lichen - *Parmelia squarrosa* showed separation between streams where Kokanee spawn and do not spawn for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ suggesting transfer of reservoir nutrients beyond the stream and into the adjacent riparian areas. Our samples analyzed for stable isotopes were of stem dwelling Parmeliaceae, foliose green algal lichens that grow widely in the region (*Parmelia squarrosa*). This species represents a good model to assess potential nutrient transfer from the reservoir to riparian vegetation adjacent to streams where Kokanee spawn as they should be highly sensitive to nitrogen enrichment. Interestingly, lichen samples from streams where Kokanee spawn were depleted in ^{15}N – a pattern similar to that seen in slimy sculpin and mayflies.

As a group, lichens depend upon atmospheric sources (CO_2) for carbon supply. The fractionation of stable carbon isotopes is determined by the biochemical and physiological processes of the C3 photosynthetic pathway, with carbon isotope discrimination highest in young (rapidly growing) thalli, due to greater diffusive resistance for CO_2 uptake in old thalli (Maguas and Brugnoli 1996). As a result, lichens can show considerable variability in $\delta^{13}\text{C}$ data. Maguas and Brugnoli (1996) found $\delta^{13}\text{C}$ values between 11.3 and 14.8 for a range of green-algal bionts sampled in Portugal. Beck and Mayr (2012) found that lichen substrate was important to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Xanthoria*, with epiphytes showing $\delta^{13}\text{C}$ values between -18 to -22, reflecting their reliance on atmospheric carbon sources, whereas thalli growing on minerogenic substrates had $\delta^{13}\text{C}$ values fell between -22 to -26 $\delta^{13}\text{C}$.

Attempts to distinguish $\delta^{13}\text{C}$ values in lichens along climate and elevation gradients have generally shown weak or no correlations (Cuna et al. 2005). Marine derived carbon contributions from spawning salmon can be significant for many elements of riparian ecosystems, especially for invertebrates and resident fish populations, however, both Bilby et al. (1996) and Hicks et al. (2005) did not find evidence of carbon contributions in riparian vegetation from spawning salmon. This stands in marked contrast to the Williston Reservoir data, where $\delta^{13}\text{C}$ values were significantly different between tributaries ($P < 0.01$), where Kokanee spawned, versus those with no Kokanee (Figure 19b).

The evidence for $\delta^{15}\text{N}$ transfer from spawning salmon to riparian vegetation is more conclusive, with these same studies (Bilby et al. 1996; Hicks et al. 2005) finding enrichment of nitrogen from marine sources in riparian vegetation. Helfield and Naiman (2002) note that nitrogen

transfer from salmon may be less important where riparian vegetation is dominated by alders (as was the case for the Williston study sites), but this did not seem to be a major factor in our present data set (Figure 19; Table 2), where significant differences were seen in $\delta^{15}\text{N}$ ($p < 0.05$) with Kokanee spawning. We would therefore conclude that the stable isotope data for samples of *Parmelia squarrosa* shows a strong separation between streams where Kokanee spawn and do not spawn for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, suggesting transfer of reservoir nutrients beyond the stream and into the adjacent riparian areas, irrespective of geographic location.

As a foliose, green algal biont lichen, *Parmelia squarrosa* does not have access to internally fixed nitrogen (as in cyanolichens), and should therefore represent a good model to assess potential nutrient transfer from the reservoir to riparian vegetation adjacent to streams. Boltersdorf et al. (2014), notes that green-algal biont lichens are sensitive indicators to environmental N-uptake, with tissue $\delta^{15}\text{N}$ values commonly used to assess environmental deposition. Lakatos et al. (2007) similarly found that lichens represented a particularly sensitive system for evaluating stable isotopes, due to their unique dependence on ambient environment conditions for nutrients and moisture supply.

Although the $\delta^{18}\text{O}$ data for lichens also showed a strong (and significant; $p < 0.01$) difference between the Kokanee and non-Kokanee spawning streams, this data must be evaluated in context of known latitudinal gradients in $\delta^{18}\text{O}$ values (West et al. 2006). This is particularly relevant as the Kokanee and non-Kokanee bearing streams fall into two broad clusters (Figure 10d), approximately 1.9° latitude apart. The difference in mean $\delta^{18}\text{O}$ values per degree latitude in the lichen data, at 0.576‰ $\delta^{18}\text{O}$ per $^\circ$ latitude, is similar in pattern to that from studies on rainwater in northern Alberta (at 2.83‰ $\delta^{18}\text{O}$ per $^\circ$ latitude) (Waterisotopes 2019). Without concurrent studies on $\delta^{18}\text{O}$ composition of rainwater in the Williston region it will therefore be difficult to discriminate biological variation in $\delta^{18}\text{O}$ from atmospheric/geophysical variation.

Samples collected for fish, invertebrates and lichen from streams where Kokanee spawn were depleted in ^2H , ^{13}C , ^{18}O compared to streams where Kokanee did not spawn. There were considerable differences in locations around the Williston Reservoir where Kokanee spawn and the Parsnip River tributaries that we used as non-Kokanee control streams. Some stable isotope signatures have been shown to strongly vary by region, particularly for $\delta^{18}\text{O}$ and $\delta^2\text{H}$ which are influenced by environmental water (Soto et al. 2013). Recent work has indicated that $\delta^2\text{H}$ can improve the ability to track the source of organic material into aquatic food webs (Voigt et al. 2015) and is also strongly influenced by dietary sources of protein and lipids (Soto et al. 2013). Geographic location of the study streams likely played a role in the differences in stable isotope signatures among our study sites for $\delta^2\text{H}$, but particularly for $\delta^{18}\text{O}$. Stable oxygen and hydrogen isotope ratios in precipitation show a continent-wide pattern in North America with a general gradient of relatively enriched values in the south to more depleted values in the north (Hobson 1999). In fact, the greatest potential for applying hydrogen and oxygen isotope ratios as markers of animal movement exists in mid- to high-latitude continental regions, where strong spatial isotope gradients exist (Hobson 1999). The strong correlation between latitude and $\delta^{18}\text{O}$ indicates that location is driving the differences for $\delta^{18}\text{O}$ rather than diet. Wang et al. (2009) showed that oxygen isotopes from chironomid larvae reflect past habitat water isotopic signatures than hydrogen; $69.0 \pm 0.4\%$ of oxygen and $30.8 \pm 2.6\%$ of hydrogen in chironomid larvae was derived from habitat water. Measurements of $\delta^2\text{H}$ may be a complementary trophic

tracer in food web studies. While $\delta^{18}\text{O}$ measurements do not provide any trophic information, they can be useful for linking tissue to environmental water where was grown (Bowen et al. 2005). Although, there was a lack of significant relationships between $\delta^2\text{H}$ and latitude in our study for fish and aquatic invertebrates, the relationship between $\delta^2\text{H}$ and latitude for the foliose, green algal biont lichen, *Parmelia squarrosa*, weakens the link between nutrient source and $\delta^2\text{H}$.

Differences in $\delta^{13}\text{C}$ were appreciable for fish, aquatic invertebrates and lichen among the different streams examined in our study. Interestingly, the streams where Kokanee spawn showed less variation than non-Kokanee streams – particularly for samples from slimy sculpin. Streams where Kokanee spawn were sampled from a wide geographic range, streams from the Parsnip reach, Omineca watershed and Osilinka watershed, yet only varied in $\delta^{13}\text{C}$ by 2‰. In contrast, the non-Kokanee streams we sampled were exclusively from the Parsnip River watershed and varied in $\delta^{13}\text{C}$ by more than 4‰. Stable carbon isotope ratios of C3 terrestrial plant detritus in streams are relatively constant at about -28‰ (France 1995), similar to the values we found for Missinka River and Wooyadilinka Creek. Freshwater algae $\delta^{13}\text{C}$, however, can range between -47 and -12‰ (France 1995; Finlay et al. 1999). There was little variation in $\delta^{13}\text{C}$ for Kokanee spawners from both the Williston Reservoir and from Thutade Lake with an average value of -32.3‰ (Figure 16c) suggesting a fairly consistent carbon source for these systems. The overlap in $\delta^{13}\text{C}$ between Kokanee from the reservoir and for fish, aquatic invertebrates and even the depleted $\delta^{13}\text{C}$ values for lichen suggests Kokanee introduce carbon to streams where they spawn, but the variation in $\delta^{13}\text{C}$ for Parsnip River watershed streams is less clear. Freshwater benthic algae typically exhibit $\delta^{13}\text{C}$ values of approximately $-26 \pm 3\text{‰}$, whereas phytoplankton typically exhibit $\delta^{13}\text{C}$ of approximately $-32 \pm 3\text{‰}$ (France 1995). Depletion of ^{13}C for samples collected from the Parsnip River may represent autochthonous phytoplankton within the environment. Depletion of ^{13}C in samples from Wichcika and Bill's Creeks, however, are not as easy to explain. Wicheeda Lake flows into Wichcika Creek and may be a source of depleted ^{13}C , although the confluence for the stream is below where we sampled. The marshy area at the headwaters of Wichcika Creek, however, is the type of habitat with still waters where algae incorporate carbon depleted in ^{13}C (Finlay et al. 1999); a similar pattern may exist for Bill's Creek, but does not account for the depleted ^{13}C for samples collected from Lamonti Creek.

Kokanee like sockeye salmon may be enhancing the productivity of the river systems where they spawn (Quinn et al. 2018). Sculpin diets for two coastal species, Prickly sculpin (*Cottus asper*) and Coastrange sculpin (*C. aleuticus*) shifted from invertebrates and juvenile salmonids to salmon eggs when salmon arrived in autumn, with salmon-derived nutrient contributions to diets and sculpin condition increasing with increasing biomass of spawning salmon among streams (Swain et al. 2014). The overlap in $\delta^{13}\text{C}$ values for sculpin from streams where Kokanee spawn and Kokanee spawners, suggested that a potential source of nutrients in these streams is from the reservoir, but we found similar signatures from some of our control streams that do not appear to be linked to Kokanee spawning. Consequently, our findings suggest that Kokanee do not have an effect on stable isotope signatures and may not provide a significant source of nutrients to all tributary streams where they spawn. Escapement of spawning Kokanee to Williston Reservoir tributary streams is highly variable from year to year, however, potentially providing an inconsistent nutrient pulse from the reservoir.

A confounding effect on our study, however, is the variable escapement of Kokanee to spawning streams, both spatially and temporally. Following the introduction of Columbia River origin Kokanee to tributaries to the Williston Reservoir in the 1990s, there were relatively few Kokanee observed to spawn – only 81 000 spawners were observed in enumeration surveys in 2002 (Langston 2012). The number of spawners increased dramatically in subsequent years; over a million in 2006 and almost 1.7 million spawning Kokanee in 2010 were documented in 48 index streams. No census estimates were conducted again until 2018 when the same index streams were surveyed and a substantial (3.2 fold) decrease was observed between 2010 and 2018 (DWB 2019). The census estimates conducted by DWB also indicated a shift in the distribution of spawners to more northern regions and no spawners were counted in the Parsnip Reach. It is unclear what factors are driving spawning site selection for Kokanee in the Williston Reservoir watershed and the shift in spawner distribution may reflect a change in the environment or reflect the time required for Kokanee to naturally stray into novel spawning streams. It will require further investigation to determine whether Kokanee are responding to changes in the environment or straying to new locations.

RECOMMENDATIONS

This report is based on the findings from the three years of this project. For each of the locations, we sampled for aquatic invertebrates, benthic stream dwelling fish, and lichen within the riparian areas adjacent to the streams, although riparian lichen were not sampled in 2018. In our first year, we amassed a very large database extant of mayfly, caddisfly, and stonefly specimens stored in various locations in BC, Alberta, Saskatchewan, Ontario, and elsewhere. We focused our search on insect specimens that were collected in BC and Alberta, and our database contained geographic, taxonomic, and temporal data for each specimen. During the remainder of the time in this project, we have worked to improve the dataset by fixing ambiguous geographic references. More recently we have begun to work with a geographer to develop a tool using the database that will explore diversity measures throughout BC and neighbouring regions, and that will also provide guidance for past sampling sufficiency and point to areas – likely including the Williston region – that still require more sampling for good representation of local aquatic invertebrate taxa. To adequately assess the potential effects of anthropogenic disturbance on ecosystem integrity, it is vital that we understand the natural biodiversity of organisms. Kokanee are potentially supporting or modifying biodiversity across the system by nutrient transfer.

Our analysis revealed tremendous diversity of aquatic insects and riparian lichen among all sites and initially we found stable isotope signatures depleted in ^{13}C among study streams where Kokanee have been observed to spawn – suggestive of a nutrient influx from the reservoir by spawning Kokanee. The wide-spread geographic distribution of spawning Kokanee throughout tributaries flowing into the northern portions of the reservoir and absence of Kokanee in the southern-most watersheds has created a spatial bias to our study design – exemplified by the strong correlation between $\delta^{18}\text{O}$ measurements and latitude. For Year 3, we incorporated additional sites to compare Kokanee-spawning streams with non-Kokanee streams. Some of our control streams where Kokanee have not been documented to spawn, however, also exhibited stable isotope signatures depleted in ^{13}C indicating that other factors may be leading to the pattern observed.

The introduction of nutrients to the tributary streams to the Williston Reservoir from Kokanee does not appear to be having a substantial effect on diversity or abundance of the target groups of organisms we examined.

Recommendations based on the findings of the study and opportunities to improve study design

RECOMMENDATION 1: A decision is required whether control and management of Kokanee in the Williston Reservoir is a key priority for the Fish & Wildlife Compensation Program – Peace Region and the program partners – BC Hydro, the Province of BC, Fisheries and Oceans Canada, First Nations, and public stakeholders. Our survey conducted in 2016 indicated that knowledge of Kokanee and potential impacts to the ecosystem is a key priority. Consequently, there are a number of additional information gaps that should be addressed.

Our understanding of processes controlling nutrient flux in tributary streams to the Williston Reservoir is still incomplete and the spatial bias in spawning locations made it particularly difficult to address. We looked for sampling locations that presented a natural barrier to upstream migration for spawning, but such barriers were associated with reach breaks and substantive changes in stream morphology. If follow up studies to this work are considered, the following data gaps must be addressed:

1. A list of confirmed species in the region – particularly species that are suitable for assessing ecosystem integrity such as aquatic insects and foliose lichen – is required to allow for more effective assessments in future work.
2. Determine the spatial differences that exist in indicator species throughout the Williston Reservoir watershed.
3. Our work assessed effects of Kokanee introductions at trophic levels at the base of the food web, but it is not clear what the effects of Kokanee have been on other trophic levels such as piscivorous fishes. There are anecdotal reports of bull trout (*Salvelinus confluentus*) and lake trout (*S. namaycush*) feeding on Kokanee, but the effect on these species may be positive, but if so there may be increased abundance for both species and potentially greater interaction between the two species.
4. Our work focused on tributary streams dynamics, but Kokanee residence in streams is a relatively small component of their life cycle. Prior to the introduction of Columbia origin Kokanee, lake whitefish (*Coregonus clupeaformis*) were the most abundant pelagic species in the reservoir (Sebastian et al. 2003), but were displaced by Kokanee as the dominant pelagic species by 2008 (Sebastian et al. 2009). The impact of Kokanee on other species that fill the same trophic role in the reservoir should be determined.

RECOMMENDATION 2: We depended on historic information, our survey questionnaire, and field observations to define study streams – both for an understanding of where Kokanee spawn and locations where they do not spawn. Within the three years of our study, we observed considerable variation between years in the numbers of fish spawning within some of the study streams. Spawner abundance was low in some regions (Parsnip Reach), variable in other areas (Omineca Arm), and consistently high for some streams (Ospika Arm). Over the longer term, numbers of spawners have also shown considerable variation. Langston (2012) indicated that escapement of Kokanee spawners to tributary streams increased continually until 2006. Subsequent enumerations in 2018, however, indicated considerably fewer spawners (DWB 2019). The temporal variation in spawner abundance may also limit our ability to detect a difference between streams where Kokanee spawn and non-Kokanee streams.

Experimental manipulation of Kokanee density within replicated streams would control the spawner biomass and potential nutrient input. Temporary weirs or barriers could block upstream migration by Kokanee allowing for direct comparisons above and below the barrier, but also comparisons to adjacent streams. Alternatively, post-spawned Kokanee carcasses could be introduced to streams where Kokanee do not spawn to assess changes in the stream ecosystem productivity similar to the work done by Quinn et al. (2018). Both experimental designs would allow us to overcome the latitudinal variation that was observed in the present study. Such an approach would also enable the determination of turnover rate for nutrient signatures for different trophic levels. The duration of experimental treatment required to

achieve a response, however, is not known. The study by Quinn et al. (2018) was published after adding carcasses to one side of a stream for 20 years.

Recommendations related to the larger issue of Kokanee in the watershed

RECOMMENDATION 3: Our work has contributed to determining where Kokanee are known to spawn based on our survey questionnaire and field work, but the picture is still incomplete. We recommend aerial surveys be conducted annually to assess the number of spawners in key index streams. Such a project has been initiated by the FWCP – Peace Region and the annual monitoring of index streams will help to define changes in spawner escapement that occur from year to year. Aerial surveys will help us to understand the dynamics of Kokanee escapement within the Williston Reservoir watershed, but some runs are very small, and run timing is sporadic and often during poor weather making accurate assessment problematic. Targeting index streams may also miss potentially important habitat. Environmental DNA (eDNA) is a complementary method that could be used to identify rivers and streams used by Kokanee for spawning. A benefit to this approach is that it would be possible to completely survey almost every river and stream that flows into the reservoir for Kokanee, but also other fish, and invertebrates or biotic indices of productivity. The findings would allow us to better define biodiversity of species within the Williston Reservoir watershed. In the case of all of those, eDNA methods (using incidence data) would allow us to quickly classify the biodiversity characteristics of every system and to look in a more detailed way for a Kokanee signal in terms of effects on aquatic (vertebrate and invertebrate) biodiversity.

There is considerable variability in the number of spawning Kokanee within and among tributary streams. We do not know what factors are important for Columbia River-origin Kokanee to when selecting spawning locations and whether these are changing over time. Knowledge of physical characteristics that these fish are selecting for, or avoiding in the locations where they spawn is important for protecting or limiting a species ability to complete their life cycle.

RECOMMENDATION 4: Physical characteristics of spawning sites and the redd (spawning site) environment should be characterized for locations where Kokanee are known to spawn in high density and compared to areas where they were introduced and no longer spawn, and also where they have not been documented to spawn – but are not precluded from spawning. The Columbia River-origin Kokanee have strayed throughout large portions of the reservoir and it is not known whether they will continue to stray or have now selected specific locations where they show fidelity and homing.

Measures of productivity have been assessed for the Williston Reservoir in the past. The decline in number of spawning Kokanee for some locations within the three years of our study and the recent Kokanee spawning census (DWB 2019) suggest that the number of Kokanee may have declined in the reservoir. Such a decline could be linked to a change or decline in productivity and consequently carrying capacity. If Kokanee numbers are at carrying capacity and declining, potential effects on other species within the reservoir system that rely on Kokanee as a prey species may be profound.

RECOMMENDATION 5: Productivity within the Williston Reservoir should be assessed and compared to the previous measurements to determine how it has changed. Additionally, an assessment of Kokanee abundance, and other species, within the reservoir should be conducted to complement historic assessments. Changes in the number of fish in the reservoir – particularly Kokanee – will alter the potential for nutrient transfer to tributary streams.

In our discussions with First Nations, there is a concern about mercury (Hg) being brought from the reservoir into tributary streams. Testing for Hg was not part of our project, but could be done in conjunction with local First Nations collaborators – and work has been funded to examine Hg in Kokanee and other species in the Williston Reservoir. Contaminant testing, however, may also reveal important life history information for Kokanee in the Williston Reservoir. A project funded by the FWCP – Peace Region examining genetic population structure and demographics of Kokanee introduced to the Williston Reservoir has found a substantial decline in size at maturity from some of the first Columbia River-origin Kokanee that matured in 1994 to mature fish in 2016, 2017 and 2018 – potentially due to a change in productivity of the reservoir.

RECOMMENDATION 6: The decline in Kokanee escapement to tributary streams to spawn in recent years suggests changes in productivity within the reservoir. Work by Wilson, Withler, and Shrimpton (unpublished) has also shown a decline in size from the first Kokanee spawners that matured after Columbia origin fish were introduced to the Williston Reservoir watershed (Langston and Zemplak 1998). There does not appear to be a change in age of maturation, so other factor(s) may be driving the change in life history and size at maturation.

Candidate mechanisms that may drive life history differences such as size and age at maturation are metabolic and bioenergetic factors. Characterization of factors in wild populations has traditionally been challenging, however, new approaches now exist that use isotopic tracer such as Hg. Mass-balance models of the tracer body burden can be used to reveal intraspecific life-history variation. Kokanee spawners collected in 2006 (FWCP) and 2018 (DWB), and potentially even the scales from fish collected in 1994 (FWCP) may reveal temporal changes in energetics that has contributed to the reduction in size.

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 Aynsley Thielman, Lindi Anderson, Daemon Cline, Adam O’Dell, Claire Shrimpton, Ivy Strothers, Penny Raymond, Grace Osted, and Luc Turcotte. 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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APPENDIX I. Presence or absence of aquatic invertebrates collected by kick-net sampling for the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), Trichoptera (caddisflies), Diptera (true flies), and Coleoptera (beetles) detected by DNA barcoding for seven tributary streams in the Williston Reservoir watershed. Samples were collected from Missinka River (MI), Upper Parsnip River (UP), Osilinka River (OS), Tenakihi Creek (TE), Aley Creek (AL), Stevenson Creek (ST), and Bruin Creek (BR).

Order	Barcode-based ID	MI	UP	OS	TE	AL	ST	BR
Ephemeroptera	<i>Ameletus celer</i>					+	+	
Ephemeroptera	<i>Ameletus oregonensis</i>	+		+				
Ephemeroptera	<i>Ameletus velox</i>						+	
Ephemeroptera	<i>Ameletus</i> sp.1				+		+	
Ephemeroptera	<i>Baetis bicaudatus</i>				+	+	+	+
Ephemeroptera	<i>Baetis tricaudatus</i>	+	+	+		+		+
Ephemeroptera	<i>Baetis</i> sp.1							+
Ephemeroptera	<i>Baetis</i> sp.2						+	
Ephemeroptera	<i>Cinygmula</i> sp.1							+
Ephemeroptera	<i>Cinygmula</i> sp.2					+		+
Ephemeroptera	<i>Cinygmula</i> sp.3		+					
Ephemeroptera	<i>Cinygmula</i> sp.4			+	+	+	+	+
Ephemeroptera	<i>Drunella coloradensis</i>							+
Ephemeroptera	<i>Drunella doddsii</i>	+	+		+			+
Ephemeroptera	<i>Drunella grandis</i>	+	+	+	+	+	+	+
Ephemeroptera	<i>Drunella</i> sp.1				+			
Ephemeroptera	<i>Epeorus deceptivus</i>					+		
Ephemeroptera	<i>Epeorus grandis</i>						+	+
Ephemeroptera	<i>Epeorus longimanus</i>							+
Ephemeroptera	<i>Ephemerella</i> sp.1		+					
Ephemeroptera	<i>Ephemerella</i> sp.2	+	+					
Ephemeroptera	<i>Ephemerella</i> sp.3	+	+	+	+	+	+	+
Ephemeroptera	<i>Paraleptophlebia heteronea</i>			+				
Ephemeroptera	<i>Paraleptophlebia</i> sp.1			+				
Ephemeroptera	<i>Rhithrogena hageni</i>	+	+				+	+
Ephemeroptera	<i>Rhithrogena robusta</i>	+				+	+	
Ephemeroptera	<i>Rhithrogena</i> sp.1					+		
Ephemeroptera	<i>Rhithrogena</i> sp.2			+	+			+
Plecoptera	<i>Alloperla severa</i>	+	+					
Plecoptera	<i>Bolshecapnia milami</i>			+		+		
Plecoptera	<i>Capnia coloradensis</i>							+
Plecoptera	<i>Capnia nana</i>				+	+		
Plecoptera	<i>Capnia</i> sp.1							+
Plecoptera	<i>Capnia</i> sp.2					+		+
Plecoptera	<i>Capnia</i> sp.3	+						
Plecoptera	<i>Doddsia occidentalis</i>			+		+	+	
Plecoptera	<i>Doddsia</i> sp.1							+
Plecoptera	<i>Eucapnopsis brevicauda</i>					+		+
Plecoptera	<i>Hesperoperla pacifica</i>		+	+				
Plecoptera	<i>Isoperla petersoni</i>		+	+	+	+		
Plecoptera	<i>Isoperla sobria</i>					+	+	
Plecoptera	<i>Kogotus modestus</i>			+	+		+	

Order	Barcode-based ID	MI	UP	OS	TE	AL	ST	BR
Plecoptera	<i>Megarcys signata</i>					+	+	
Plecoptera	<i>Paraleuctra occidentalis</i>					+	+	+
Plecoptera	<i>Perlodidae</i> sp.1				+			
Plecoptera	<i>Plumiperla diversa</i>		+				+	+
Plecoptera	<i>Plumiperla</i> sp.1					+		
Plecoptera	<i>Prostoia besametsa</i>							+
Plecoptera	<i>Sweltsa borealis</i>					+	+	+
Plecoptera	<i>Sweltsa coloradensis</i>	+	+	+	+			
Plecoptera	<i>Sweltsa</i> sp.1				+			
Plecoptera	<i>Sweltsa</i> sp.2			+	+			
Plecoptera	<i>Taenionema pallidum</i>	+			+			
Plecoptera	<i>Utacapnia columbiana</i>	+						
Plecoptera	<i>Zapada cinctipes</i>	+	+	+	+	+	+	+
Plecoptera	<i>Zapada</i> sp.1							+
Plecoptera	<i>Zapada</i> sp.2						+	
Plecoptera	<i>Zapada</i> sp.3				+		+	
Plecoptera	<i>Zapada</i> sp.4					+	+	
Plecoptera	<i>Zapada</i> sp.5					+		
Trichoptera	<i>Apatania comosa</i>				+			
Trichoptera	<i>Arctopsyche grandis</i>			+	+	+	+	
Trichoptera	<i>Brachycentrus americanus</i>	+	+	+	+			
Trichoptera	<i>Ecclisomyia conspersa</i>				+			
Trichoptera	<i>Lepidostoma cascadenense</i>		+		+			
Trichoptera	<i>Lepidostoma pluviale</i>			+				
Trichoptera	<i>Micrasema bactro</i>	+						
Trichoptera	<i>Oligophlebodes sierra</i>							+
Trichoptera	<i>Onocosmoecus unicolor</i>		+					
Trichoptera	<i>Parapsyche elsis</i>				+	+	+	+
Trichoptera	<i>Rhyacophila brunnea</i>			+		+	+	+
Trichoptera	<i>Rhyacophila hyalinata</i>							+
Trichoptera	<i>Rhyacophila narvae</i>				+			
Trichoptera	<i>Rhyacophila pellisa</i>			+				+
Trichoptera	<i>Rhyacophila vaccua</i>					+		+
Trichoptera	Rhyacophilidae sp.1	+						
Diptera	<i>Agromyzidae</i> sp.1			+				
Diptera	<i>Ceratopogonidae</i> sp.1			+		+		+
Diptera	<i>Chelifera</i> sp.1	+						
Diptera	<i>Chironomidae</i> sp.1				+			
Diptera	<i>Chironomidae</i> sp.2	+						
Diptera	<i>Chironomidae</i> sp.3							+
Diptera	<i>Chironomidae</i> sp.4			+	+			
Diptera	<i>Chironomidae</i> sp.5			+				
Diptera	<i>Chironomidae</i> sp.6					+		
Diptera	<i>Chironomidae</i> sp.7			+				
Diptera	<i>Chironomidae</i> sp.8					+		
Diptera	<i>Chironomidae</i> sp.9							+
Diptera	<i>Chironomidae</i> sp.10						+	
Diptera	<i>Chironomidae</i> sp.11				+	+		
Diptera	<i>Chironomidae</i> sp.12			+				

Order	Barcode-based ID	MI	UP	OS	TE	AL	ST	BR
Diptera	<i>Chironomidae sp.13</i>					+	+	
Diptera	<i>Chloropidae sp.1</i>						+	
Diptera	<i>Conchapelopia pallens</i>	+				+		
Diptera	<i>Diamesa nr. hyperborea</i>					+		
Diptera	<i>Eukiefferiella claripennis</i>					+		
Diptera	<i>Helodon pleuralis</i>							+
Diptera	<i>Helodon onychodactylus</i>							+
Diptera	<i>Limoniidae sp.1</i>				+		+	+
Diptera	<i>Limoniidae sp.2</i>	+						
Diptera	<i>Limoniidae sp.3</i>				+			
Diptera	<i>Limoniidae sp.4</i>			+	+	+	+	+
Diptera	<i>Limoniidae sp.5</i>	+	+					
Diptera	<i>Microspectra sp.1</i>					+		
Diptera	<i>Microspectra sp.2</i>			+				
Diptera	<i>Neoplasta megorchis</i>		+					
Diptera	<i>Oreogeton scopifer</i>							+
Diptera	<i>Oreoleptidae sp.1</i>				+			
Diptera	<i>Oreoleptidae sp.2</i>							+
Diptera	<i>Pediciidae sp.1</i>			+				
Diptera	<i>Sphaeroceridae sp.1</i>					+		
Diptera	<i>Sphaeroceridae sp.2</i>				+			
Coleoptera	<i>Dytiscidae sp.1</i>	+						
Coleoptera	<i>Elmidae sp.1</i>	+	+					

APPENDIX II. Lichen species list by functional group for Peace-Williston study sites; Crooked River (CR), Hominka River (HR), Missinchinka River (MR), Pack River (PR), Upper Parsnip River (UP), and Table River (TR).

Group	Subgroup	Species	CR	HR	MR	PR	UP	TR
Macrolichen	Chloro	<i>Alectoria sarmentosa</i>	-	-	+	-	-	-
Macrolichen	Chloro	<i>Bryoria chalybeiformis (aurea form)</i>	-	-	-	-	+	-
Macrolichen	Chloro	<i>Bryoria fremontii</i>	-	-	-	+	-	-
Macrolichen	Chloro	<i>Bryoria fuscescens</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Bryoria glabra</i>	+	+	+	-	-	+
Macrolichen	Chloro	<i>Bryoria pikei</i>	+	+	+	-	+	+
Macrolichen	Chloro	<i>Bryoria pseudofuscescens</i>	+	-	-	-	-	-
Macrolichen	Chloro	<i>Bryoria yellow soralia</i>	-	-	-	-	+	-
Macrolichen	Chloro	<i>Cladonia carneola</i>	-	-	+	-	-	-
Macrolichen	Chloro	<i>Cladonia cenotea</i>	-	-	-	-	+	-
Macrolichen	Chloro	<i>Cladonia coniocraea</i>	+	+	+	+	+	-
Macrolichen	Chloro	<i>Cladonia fimbriata</i>	+	+	+	-	+	-
Macrolichen	Chloro	<i>Cladonia sulphurea</i>	-	-	-	-	+	-
Macrolichen	Chloro	<i>Hypogymnia recurva (coll)</i>	-	-	-	-	+	-
Macrolichen	Chloro	<i>Hypogymnia bitteri</i>	+	-	+	-	-	-
Macrolichen	Chloro	<i>Hypogymnia enteromorpha</i>	+	-	+	-	-	-
Macrolichen	Chloro	<i>Hypogymnia occidentalis</i>	+	+	+	-	+	+
Macrolichen	Chloro	<i>Hypogymnia physodes</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Hypogymnia protea</i>	-	-	+	-	+	-
Macrolichen	Chloro	<i>Hypogymnia rugosa</i>	-	-	-	-	+	+
Macrolichen	Chloro	<i>Hypogymnia tubulosa</i>	+	+	+	-	+	+
Macrolichen	Chloro	<i>Hypogymnia vittata</i>	-	-	-	-	+	-
Macrolichen	Chloro	<i>Hypogymnia wilfiana</i>	-	-	-	-	+	-
Macrolichen	Chloro	<i>Melanelixia glabratula</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Melanelixia subaurifera</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Melanohalea exasperatula</i>	+	-	+	-	+	-
Macrolichen	Chloro	<i>Melanohalea glabratula</i>	-	+	-	-	-	-
Macrolichen	Chloro	<i>Melanohalea multispora</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Melanohalea septentrionalis</i>	-	-	-	-	+	+
Macrolichen	Chloro	<i>Melanohalea subaurifera</i>	+	-	-	-	-	-
Macrolichen	Chloro	<i>Micarea denigrata</i>	-	-	+	+	-	-
Macrolichen	Chloro	<i>Parmelia hygrophila</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Parmelia sulcata</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Parmelia sulymae</i>	+	-	+	+	+	-
Macrolichen	Chloro	<i>Parmeliopsis ambigua</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Parmeliopsis hyperopta</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Physcia alnophila</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Physcia caesia</i>	-	+	-	-	-	-
Macrolichen	Chloro	<i>Physcia stellaris</i>	-	-	-	+	-	-
Macrolichen	Chloro	<i>Physconia enteroxantha</i>	-	-	-	+	-	-
Macrolichen	Chloro	<i>Physconia perisidiosa</i>	-	+	-	-	-	+
Macrolichen	Chloro	<i>Platismatia glauca</i>	+	+	+	-	+	+
Macrolichen	Chloro	<i>Ramalina ameriana</i>	-	-	-	+	-	-
Macrolichen	Chloro	<i>Ramalina dilacerata</i>	-	+	+	+	-	+
Macrolichen	Chloro	<i>Ramalina farinacea</i>	-	-	-	+	-	-
Macrolichen	Chloro	<i>Ramalina intermedia</i>	-	+	-	-	-	-
Macrolichen	Chloro	<i>Ramalina obtusata</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Ramalina thrausta</i>	+	+	+	+	+	+

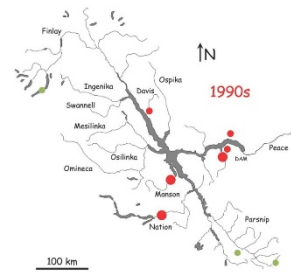
Group	Subgroup	Species	CR	HR	MR	PR	UP	TR
Macrolichen	Chloro	<i>Tuckermannopsis chlorophylla</i>	+	+	+	+	+	+
Macrolichen	Chloro	<i>Usnea barbara f. isidiomorpha</i>	-	+	-	-	+	+
Macrolichen	Chloro	<i>Usnea barbata f. scabrata</i>	-	+	-	-	+	+
Macrolichen	Chloro	<i>Usnea cavernosa</i>	-	-	+	-	-	-
Macrolichen	Chloro	<i>Usnea chaetophora</i>	+	-	+	-	-	-
Macrolichen	Chloro	<i>Usnea dasopoga</i>	-	+	-	-	-	-
Macrolichen	Chloro	<i>Usnea filipendula</i>	-	-	+	-	-	-
Macrolichen	Chloro	<i>Usnea glabrata</i>	-	-	-	-	-	+
Macrolichen	Chloro	<i>Usnea lapponica</i>	+	+	-	-	-	+
Macrolichen	Chloro	<i>Usnea scabrata</i>	+	-	+	+	-	-
Macrolichen	Chloro	<i>Usnea subfloridana</i>	-	-	+	-	-	-
Macrolichen	Chloro	<i>Usnea substerilis</i>	-	-	+	-	-	+
Macrolichen	Chloro	<i>Vulpicida pinastri</i>	+	+	+	+	-	+
Macrolichen	Chloro	<i>Xanthomendoza fallax</i>	-	-	-	+	+	-
Macrolichen	Chloro	<i>Xanthomendoza fulva</i>	-	+	+	+	-	-
Macrolichen	Chloro	<i>Xanthoria candelaria</i>	-	-	+	+	-	-
Macrolichen	Chloro	<i>Xanthoria fallax</i>	-	-	-	-	+	-
Macrolichen	Chloro	<i>Xanthoria kaernefeltii</i>	-	-	-	-	+	-
Macrolichen	Cyano	<i>Collema coniophilum</i>	+	+	+	+	-	+
Macrolichen	Cyano	<i>Collema quadrifidum</i>	-	+	-	-	-	-
Macrolichen	Cyano	<i>Collema striatum</i>	-	-	-	-	-	+
Macrolichen	Cyano	<i>Collema subflaccidum</i>	-	+	+	+	+	+
Macrolichen	Cyano	<i>Collema subfuscum</i>	-	-	-	-	-	+
Macrolichen	Cyano	<i>Leptogium cellulorum</i>	+	+	+	+	+	+
Macrolichen	Cyano	<i>Leptogium compactum</i>	-	-	-	-	-	+
Macrolichen	Cyano	<i>Leptogium cookii</i>	+	-	-	+	-	-
Macrolichen	Cyano	<i>Leptogium saturninum</i>	+	+	+	+	+	+
Macrolichen	Cyano	<i>Leptogium teretiusculum</i>	+	+	+	+	-	+
Macrolichen	Cyano	<i>Lobaria hallii</i>	-	+	+	+	+	+
Macrolichen	Cyano	<i>Lobaria pulmonaria</i>	+	+	+	+	+	+
Macrolichen	Cyano	<i>Lobaria scrobiculata</i>	+	+	+	-	+	-
Macrolichen	Cyano	<i>Nephroma bellum</i>	+	+	+	-	+	+
Macrolichen	Cyano	<i>Nephroma isidiosum</i>	-	-	-	-	+	-
Macrolichen	Cyano	<i>Nephroma parile</i>	+	+	+	+	+	+
Macrolichen	Cyano	<i>Nephroma resupinatum</i>	+	+	+	-	+	-
Macrolichen	Cyano	<i>Parmeliella triptophylla</i>	-	+	-	-	-	-
Macrolichen	Cyano	<i>Peltigera aphthosa</i>	-	+	-	-	-	-
Macrolichen	Cyano	<i>Peltigera collina</i>	+	+	+	+	+	+
Macrolichen	Cyano	<i>Peltigera membranacea</i>	-	+	-	-	+	+
Macrolichen	Cyano	<i>Pseudocyphellaria anomala</i>	-	+	-	-	+	+
Macrolichen	Cyano	<i>Santessoniella saximontana</i>	-	+	-	-	-	-
Macrolichen	Cyano	<i>Sticta fuliginosa</i>	-	+	-	-	-	+
Macrolichen	Cyano	<i>Sticta sylvatica</i>	-	+	+	-	-	-
Microlichen	Calicioid	<i>Calicium adaequatum</i>	-	-	-	-	+	-
Microlichen	Calicioid	<i>Calicium parvum</i>	-	-	+	+	+	-
Microlichen	Calicioid	<i>Calicium viride</i>	+	+	+	+	+	+
Microlichen	Calicioid	<i>Chaenotheca cinerea</i>	-	+	-	-	-	-
Microlichen	Calicioid	<i>Chaenotheca hispidula</i>	-	-	-	-	-	+
Microlichen	Calicioid	<i>Chaenotheca trichialis</i>	-	+	-	-	-	-
Microlichen	Calicioid	<i>Chaenothecopsis populicola</i>	-	+	-	-	-	-
Microlichen	Calicioid	<i>Chaenothecopsis sp. unknown</i>	-	+	-	-	-	-
Microlichen	Calicioid	<i>Mycocalicium subtile</i>	+	-	-	-	-	-
Microlichen	Calicioid	<i>Phaeocalicium compressulum</i>	-	+	+	-	-	+
Microlichen	Calicioid	<i>Phaeophyscia kairamoi</i>	-	+	-	-	-	-

Group	Subgroup	Species	CR	HR	MR	PR	UP	TR
Microlichen	Calicioid	<i>Phaeophyscia nigricans</i>	-	-	+	-	-	-
Microlichen	Calicioid	<i>Stenocybe pullatula</i>	+	+	+	+	-	-
Microlichen	Trebouxioid	<i>Arthonia apatetica</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Arthonia edgewoodensis</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Arthonia leptalea ined.</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Arthonia lignariella</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Arthonia muscigena</i>	+	-	-	-	-	-
Microlichen	Trebouxioid	<i>Bacidia beckhausii</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Bacidia circumspecta</i>	+	+	+	+	-	-
Microlichen	Trebouxioid	<i>Bacidia idahoensis</i>	+	-	+	-	-	-
Microlichen	Trebouxioid	<i>Bacidia laurocerasi s. str.</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Bacidia rosellizans</i>	-	-	+	-	-	+
Microlichen	Trebouxioid	<i>Bacidia subincompta</i>	-	+	-	-	-	+
Microlichen	Trebouxioid	<i>Bacidia subintricata</i>	-	-	-	+	-	-
Microlichen	Trebouxioid	<i>Bacidia vermifera</i>	-	+	-	-	-	+
Microlichen	Trebouxioid	<i>Biatora efflorescens</i>	-	-	+	-	+	+
Microlichen	Trebouxioid	<i>Biatora pallens</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Biatora rufidula</i>	+	-	+	-	-	-
Microlichen	Trebouxioid	<i>Biatora subduplex</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Biatora vacciniicola</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Biatoridium delitescens</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Biatoridium delitescens</i>	-	+	-	-	+	+
Microlichen	Trebouxioid	<i>Bilimbia sabuletorum</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Buellia arborea</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Buellia disciformis</i>	+	+	+	+	-	+
Microlichen	Trebouxioid	<i>Buellia griseovirens</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Buellia nicolaensis ined.</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Buellia penichra</i>	-	+	-	-	+	+
Microlichen	Trebouxioid	<i>Buellia punctata</i>	-	+	+	+	-	-
Microlichen	Trebouxioid	<i>Buellia triphragmioides</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Buellia unknown species (erumpent)</i>	+	+	-	-	-	-
Microlichen	Trebouxioid	<i>Caloplaca ahtii</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Caloplaca atosanguinea</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Caloplaca cf. oleicola</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Caloplaca flavorubescens</i>	-	+	+	+	-	-
Microlichen	Trebouxioid	<i>Caloplaca pyracea</i>	+	-	+	+	-	+
Microlichen	Trebouxioid	<i>Caloplaca sorocarpa</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Caloplaca tricolor</i>	-	-	-	+	+	-
Microlichen	Trebouxioid	<i>Caloplaca urceolata</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Candelariella efflorescens</i>	+	+	-	+	-	+
Microlichen	Trebouxioid	<i>Candelariella reflexa</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Catinarina atropurpurea</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Chaenotheca chrysocephala</i>	+	-	-	-	-	-
Microlichen	Trebouxioid	<i>Chaenotheca phaeocephala</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Chrysothrix candelaris</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Cliostomum griffithii</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Cliostomum pulchellum ined.</i>	+	-	+	+	-	-
Microlichen	Trebouxioid	<i>Cliostomum spribillei</i>	+	+	+	-	-	+
Microlichen	Trebouxioid	<i>Cyphelium inquinans</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Japewia subaurifera</i>	+	-	-	-	-	+
Microlichen	Trebouxioid	<i>Japewia tornoensis</i>	-	-	+	-	-	+
Microlichen	Trebouxioid	<i>Lambiella caeca</i>	-	-	-	+	-	-
Microlichen	Trebouxioid	<i>Lecania cyrtellina</i>	-	+	-	-	-	-

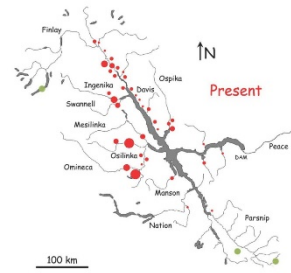
Group	Subgroup	Species	CR	HR	MR	PR	UP	TR
Microlichen	Trebouxioid	<i>Lecania madida</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Lecanora allophana f. allophana</i>	+	+	-	+	-	-
Microlichen	Trebouxioid	<i>Lecanora allophana f. soralifera</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Lecanora cadubriae</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Lecanora chlarotera</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Lecanora farinaria</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Lecanora farinaria cfr. (cottonwood form)</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Lecanora fuscescens</i>	-	-	-	-	+	-
Microlichen	Trebouxioid	<i>Lecanora hagenii</i>	-	-	-	+	-	-
Microlichen	Trebouxioid	<i>Lecanora hybocarpa</i>	+	+	+	+	-	+
Microlichen	Trebouxioid	<i>Lecanora impudens</i>	-	+	+	+	+	+
Microlichen	Trebouxioid	<i>Lecanora phaeostigma</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Lecanora pulicaris</i>	+	-	+	-	-	-
Microlichen	Trebouxioid	<i>Lecanora symmicta</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Lecidea albohyalina</i>	-	-	-	-	+	+
Microlichen	Trebouxioid	<i>Lecidea betulicola</i>	-	-	-	-	+	-
Microlichen	Trebouxioid	<i>Lecidea erythrophaea</i>	+	+	+	+	-	+
Microlichen	Trebouxioid	<i>Lecidea hypnorum</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Lecidea leprarioides</i>	+	-	+	-	+	+
Microlichen	Trebouxioid	<i>Lecidea nylanderii</i>	+	-	+	-	-	+
Microlichen	Trebouxioid	<i>Lecidea pullata</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Lecidella euphorea</i>	+	-	+	-	+	-
Microlichen	Trebouxioid	<i>Lepraria torii</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Leptorhaphis epidermidis</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Lopadium disciforme</i>	-	+	+	-	+	-
Microlichen	Trebouxioid	<i>Micarea paupercula ined.</i>	-	+	-	-	+	-
Microlichen	Trebouxioid	<i>Micarea prasina group</i>	-	+	+	-	+	+
Microlichen	Trebouxioid	<i>Mycobilimbia carneoalbida</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Mycobilimbia epixanthoides</i>	+	+	+	+	-	+
Microlichen	Trebouxioid	<i>Mycobilimbia tetramera</i>	+	+	+	+	-	-
Microlichen	Trebouxioid	<i>Mycoblastus affinis</i>	+	+	+	-	+	+
Microlichen	Trebouxioid	<i>Mycoblastus alpinus cfr.</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Mycoblastus sanguinarioides</i>	+	+	+	-	+	-
Microlichen	Trebouxioid	<i>Mycoblastus sanguinarius</i>	-	+	-	-	+	-
Microlichen	Trebouxioid	<i>Ochrolechia androgyna</i>	+	+	+	-	+	+
Microlichen	Trebouxioid	<i>Ochrolechia juvenalis</i>	+	-	+	-	-	-
Microlichen	Trebouxioid	<i>Ochrolechia oregonensis</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Ochrolechia szatalaensis</i>	+	+	+	-	+	+
Microlichen	Trebouxioid	<i>Pertusaria amara</i>	-	-	+	-	+	+
Microlichen	Trebouxioid	<i>Pertusaria carneopallida</i>	+	+	+	-	+	+
Microlichen	Trebouxioid	<i>Pertusaria multipuncta</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Pertusaria ophthalmiza</i>	+	+	+	-	+	+
Microlichen	Trebouxioid	<i>Pertusaria pupillaris</i>	+	-	-	-	-	-
Microlichen	Trebouxioid	<i>Pertusaria sommerfeltii</i>	-	+	-	-	-	-
Microlichen	Trebouxioid	<i>Pertusaria stenhammari</i>	+	+	+	-	+	-
Microlichen	Trebouxioid	<i>Phlyctis argena</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Phlyctis speirea</i>	-	-	+	-	-	+
Microlichen	Trebouxioid	<i>Physcia adscendens</i>	+	-	+	+	-	-
Microlichen	Trebouxioid	<i>Physcia aipolia</i>	+	-	+	-	-	-
Microlichen	Trebouxioid	<i>Pycnora sorophora</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Ramboldia cinnabarina</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Rinodina capensis</i>	+	+	+	+	+	+
Microlichen	Trebouxioid	<i>Rinodina colobina</i>	-	+	-	+	-	-

Group	Subgroup	Species	CR	HR	MR	PR	UP	TR
Microlichen	Trebouxioid	<i>Rinodina degeliana</i>	+	+	+	+	-	+
Microlichen	Trebouxioid	<i>Rinodina disjuncta</i>	-	-	+	+	+	+
Microlichen	Trebouxioid	<i>Rinodina efflorescens</i>	-	-	+	-	-	-
Microlichen	Trebouxioid	<i>Rinodina flavosoralifera</i>	-	+	+	+	+	-
Microlichen	Trebouxioid	<i>Rinodina griseosoralifera</i>	-	-	+	+	+	+
Microlichen	Trebouxioid	<i>Rinodina metaboliza (coll)</i>	-	-	-	+	+	-
Microlichen	Trebouxioid	<i>Rinodina orculata</i>	+	+	+	-	+	+
Microlichen	Trebouxioid	<i>Rinodina oregana</i>	-	+	+	+	+	-
Microlichen	Trebouxioid	<i>Rinodina pyrina</i>	-	-	-	-	-	+
Microlichen	Trebouxioid	<i>Rinodina stictica</i>	-	+	-	-	-	+
Microlichen	Trebouxioid	<i>Rinodina trevisanii</i>	-	+	-	-	-	+
Microlichen	Trebouxioid	<i>Schaereria corticola</i>	+	+	+	-	+	+
Microlichen	Trebouxioid	<i>Toensbergia leucococca</i>	-	+	+	+	-	+
Microlichen	Trebouxioid	<i>Varicellaria rhodocarpa</i>	+	-	-	-	-	-
Microlichen	Trentepohlioid	<i>Arthonia didyma</i>	-	+	-	-	-	+
Microlichen	Trentepohlioid	<i>Arthonia dispersa</i>	-	-	+	-	-	-
Microlichen	Trentepohlioid	<i>Arthonia radiata</i>	+	+	+	-	-	+
Microlichen	Trentepohlioid	<i>Arthonia vivida ined.</i>	-	-	+	-	-	-
Microlichen	Trentepohlioid	<i>Bacidina arceutina</i>	-	-	+	+	-	-
Microlichen	Trentepohlioid	<i>Opegrapha varia</i>	-	+	-	-	-	-

APPENDIX III. Project Brochure – March 2017



Locations where Kokanee were introduced (red circles) and of native Kokanee populations (green circles)



Kokanee spawning locations based on our 2016 survey questionnaire and compensation program reports

Spawning Kokanee have selected tributaries in the Williston watershed that flow into the north and western portions of the reservoir – not the regions originally stocked

For more information about the project or if you have information about Kokanee in the Williston watershed, please contact:
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We thank our Research Partners

Luke Gleeson



Shawna Case



Mike Tilson



We thank the Fish & Wildlife Compensation Program – Peace Region for funding the work



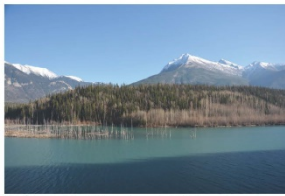
... and the technical expertise of:

Lindi Anderson
 Daemon Cline
 Adam O'Dell
 Claire Shrimpton

This project is an initiative of the Natural Resources and Environmental Studies Institute at the University of Northern British Columbia



Effect of Kokanee introductions to the Williston Reservoir on tributary streams



Osoyka Area of the Williston Reservoir (M Shrimpton)

BACKGROUND

The Williston Reservoir was created in 1968 following the construction of the WAC Bennett Dam and impounding the Peace River in the canyon near Hudson's Hope, BC. With a volume of 74 billion cubic meters, the Williston Reservoir is the largest lentic freshwater system in British Columbia. In an attempt to increase productivity in the watershed, Kokanee were stocked into the Williston Reservoir from 1990 to 1998 to create a Kokanee sport fishery and a prey source for larger fish. During this time, over three million juvenile Kokanee were stocked into rivers that flow into the Williston Reservoir.

The goal of our project is to understand the ecosystem impact of nutrient enrichment by Kokanee to streams flowing in to the Williston Reservoir and potential risks of Kokanee introductions.



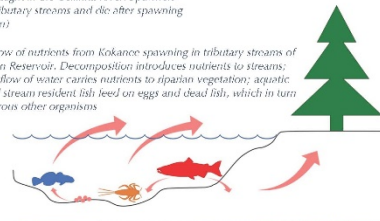
A. Aley Creek, abundant Kokanee spawners (M Shrimpton). B. Osoyinka River, low Kokanee abundance (L Anderson). C. Missinka River, no Kokanee observed in this system (M Shrimpton)

Interactions between fish, stream-living macroinvertebrates, and the surrounding riparian zone are complex and the introduction of Kokanee to the Williston watershed has the potential to dramatically alter this relationship. Spawning Kokanee were collected from the Osoyinka River, Stevenson Creek and Aley Creek. After the spawning period, we collected stream resident fish in the Missinka and upper Parsnip Rivers (where Kokanee are not found), Tenakichi Creek and upper Osoyinka River (where Kokanee spawned in low numbers), and Aley, Stevenson, and Bruin Creeks (where spawning Kokanee were abundant). Aquatic insects from these systems were collected to assess species diversity. We are also looking at the abundance of lichens that grow on streamside trees (epiphytes), many of which are sensitive indicators of site nutrient availability and air quality. To test if nutrient inputs from Kokanee can be traced through the stream and riparian ecosystems, stable isotopes, particularly carbon isotopes ($\delta^{13}C$), are useful because of the wide range of $\delta^{13}C$ in algae at the base of food webs. The other major source of energy in river food webs, terrestrial detritus, has a much more constrained $\delta^{13}C$ signature, so that algae and detritus are often isotopically distinct.

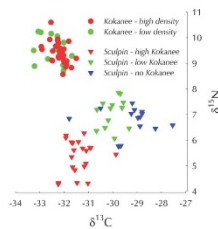


Kokanee caught in the Osoyinka River. Spawners return to tributary streams and die after spawning (L Anderson)

Potential flow of nutrients from Kokanee spawning in tributary streams of the Williston Reservoir. Decomposition introduces nutrients to streams; integral flow of water carries nutrients to riparian vegetation; aquatic insects and stream resident fish feed on eggs and dead fish, which in turn feed numerous other organisms

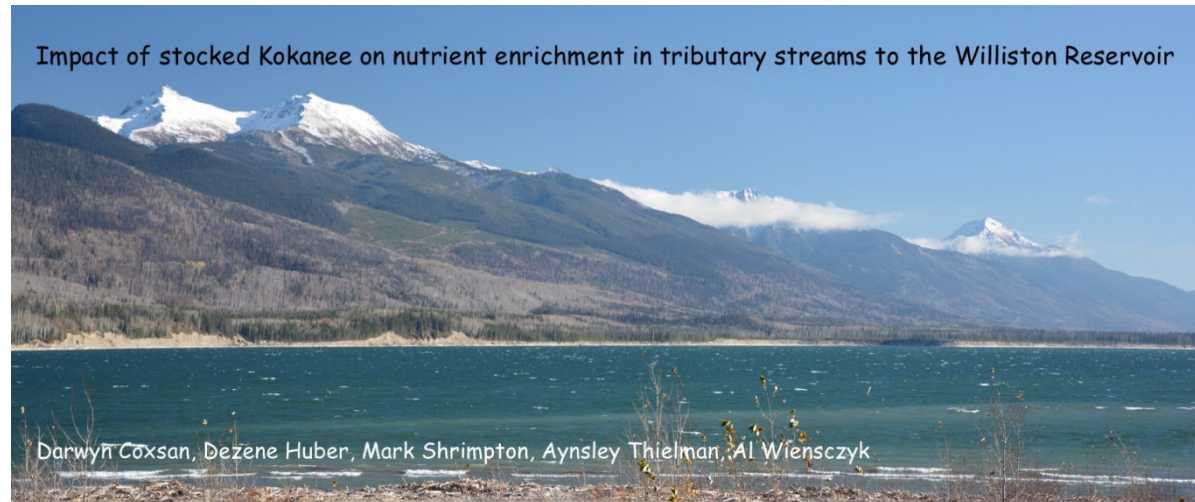


A. Slimy sculpin (*Cottus cognatus*) are an abundant stream resident fish (M Shrimpton). B. Mayfly nymphs such as *Drumella dookibi* are common throughout the Williston Watershed (C Shrimpton). C. Lignwort lichens are highly sensitive biological indicators. Shown are *Leptogium*, the dark lichen in the center, and *L. pulmonaria*, light green lichen on the lower right (D Coxson)



Stable isotope ratios for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) for spawning Kokanee and slimy sculpin caught in tributaries where Kokanee do or do not spawn

Values for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes do not differ among sampled Kokanee. Stable isotope signatures from slimy sculpin, however, disagree among streams. The overlap in $\delta^{13}C$ values for sculpin from streams where Kokanee spawn indicates that a potential source of nutrients in these streams is from the reservoir. We are expanding our study for 2017 to include more streams and more sampling times.

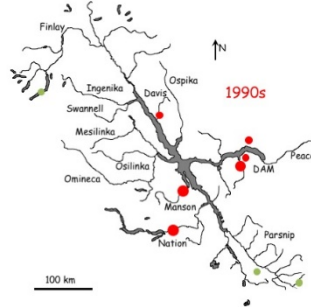


BACKGROUND

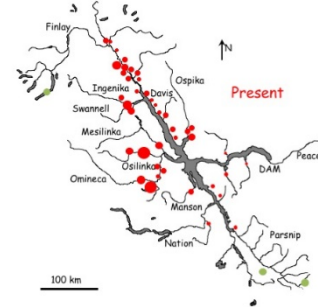
The Williston Reservoir was created in 1968 following the construction of the WAC Bennett Dam and impounding the Peace River in the canyon near Hudson's Hope, BC. With a volume of 74 billion cubic meters, the Williston Reservoir is the largest lentic freshwater system in British Columbia. In an attempt to increase productivity in the watershed, Kokanee were stocked into the Williston Reservoir from 1990 to 1998 to create a Kokanee sport fishery and a prey source for larger fish. During this time, over three million juvenile Kokanee were stocked into rivers that flow into the Williston Reservoir.

The goal of our project is to understand the ecosystem impact of nutrient enrichment by Kokanee to streams flowing in to the Williston Reservoir and potential risks of Kokanee introductions.

Locations where Kokanee were introduced (red circles) and of native Kokanee populations (green circles)



Kokanee spawning locations based on our 2016 survey questionnaire and compensation program reports



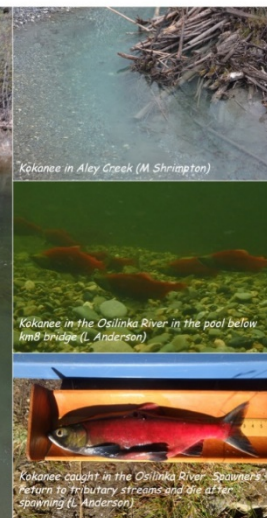
Spawning Kokanee have selected tributaries in the Williston watershed that flow into the north and western portions of the reservoir - not the regions originally stocked



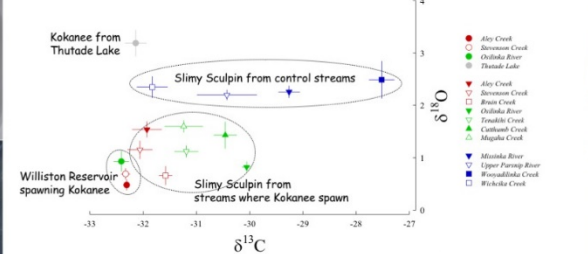
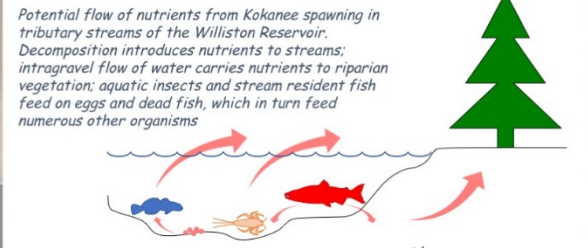
We thank the Fish & Wildlife Compensation Program - Peace Region for funding the work



We thank our Research Partners



For more information about the project or if you have information about Kokanee in the Williston watershed, please contact:
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Values for carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotopes do not differ among sampled Kokanee from tributaries to the Williston Reservoir. Stable isotope signatures from slimy sculpin, however, differ among streams. The similarity in values for sculpin from streams where Kokanee spawn to the values for Kokanee indicates that a potential source of nutrients in these streams is from the reservoir.



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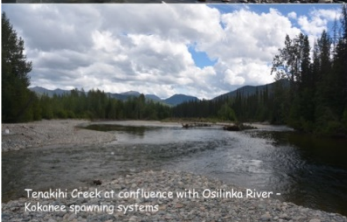
FWCP Fish & Wildlife COMPENSATION PROGRAM



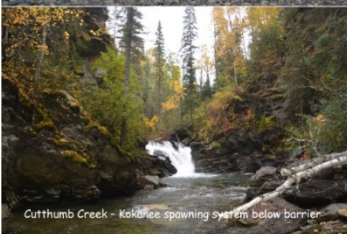
Impact of stocked Kokanee on nutrient enrichment in tributary streams to the Williston Reservoir



Stevenson Creek - Kokanee spawning system



Tenakhi Creek at confluence with Osilinka River - Kokanee spawning systems



Cutthumb Creek - Kokanee spawning system below barrier



Wichcika Creek - control system



Wooyadilinka Creek - control system

Interactions between fish, stream-living macroinvertebrates, and the surrounding riparian zone are complex and the introduction of Kokanee to the Williston watershed has the potential to dramatically alter this relationship. Spawning Kokanee were collected from the Osilinka River, Stevenson Creek and Aley Creek. After the spawning period, we collected stream resident fish in Missinka & upper Parsnip Rivers, Bill's, Lamonti, Wichcika & Wooyadilinka Creeks (where Kokanee are not found), Tenakhi Creek & Osilinka River, Aley, Stevenson, & Bruin Creeks, Mugaha, Weston, Patsuk & Cutthumb Creeks (where Kokanee spawn). Aquatic insects from these systems were collected to assess species diversity. We are also looking at the abundance of lichens that grow on streamside trees (epiphytes), many of which are sensitive indicators of site nutrient availability and air quality. To test if nutrient inputs from Kokanee can be traced through the stream and riparian ecosystems, stable isotopes, particularly carbon ($\delta^{13}C$) isotopes, are useful because of the wide range of $\delta^{13}C$ in algae at the base of food webs. The other major source of energy in river food webs, terrestrial detritus, has a much more constrained $\delta^{13}C$ signature, so that algae and detritus are often isotopically distinct.



Caddisfly *Phycophila pellisa*

Aquatic insects, particularly in their immature stages, provide food for fish. Aquatic insect feeding activities - often processing allochthonous organic matter - affect stream productivity and other characteristics. Emergent adult aquatic insects influence surrounding riparian areas through substantial deposition of nutrients. In addition to affecting surrounding forests, aquatic insect assemblages are also themselves affected by the riparian forest characteristics and the resulting allochthonous input into their streams. Examples of the diversity of aquatic insects are shown above and below.



Apatania comosa (Tricoptera - caddisfly) may be the first record for all of Canada

Only 3 species were found in all systems: *Zapada cinctipes* (Plecoptera, above right), *Drunella grandis* (Ephemeroptera, below left), and *Ephemerella* sp. 3 (Ephemeroptera, below right - *Ephemerella dorothaea*).

Riparian lichen communities strongly reflect the health and composition of streamside riparian forests, which provide a natural microclimate buffering for streams and rivers and transfer large amounts of organic matter into aquatic ecosystems.



DNA barcodes assisted rapid assessment of aquatic macroinvertebrate biodiversity and yielded a total of 115 OTUs (operational taxonomic units) of which we were able to provide species-level identifications for 54. Some are shown above.



Lobaria pulmonaria (green, leafy), *Alecatoridae* (hair lichen) and *Parmelia squarrosa* (light blue, square lobes)

Lichen

Lichen and bryophytes

Peltigera sp.

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