BENTHIC MACROINVERTEBRATE MONITORING PLAN FOR LARGE TRANSBOUNDARY RIVERS IN THE ALBERTA-NWT REGION

ASSESSMENT OF RESULTS FROM THE SECOND YEAR OF SAMPLING

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Executive Summary

Introduction

The Government of the Northwest Territories (GNWT) and the Government of Alberta (GOA) are working to establish a monitoring program for the bioassessment of large transboundary rivers. The establishment of this program is in recognition of the potential for future impacts to transboundary waters as a result of activities in the upstream catchment. Establishment of long-term monitoring and assessment supports the future detection of impacts that may arise from human development, but also supports the detection of ecological changes in response to a warming climate. The initial focus of the transboundary monitoring program is on benthic macroinvertebrate (BMI) assemblages, which are an important ecosystem component to monitor in northern rivers as an integrated measure of water quality and habitat condition. Within the Northwest Territories-Alberta transboundary river regions, there is relatively little information about the current composition and natural variation of benthic communities. Therefore, it is vital that routine monitoring be established to secure information about current conditions in these assemblages and to provide sufficient information to allow for future detection of change.

One approach to biomonitoring is the use of the normal range, which prescribes the collection of sufficient contemporary data from a region to allow estimation of the range of variability that is acceptable given current conditions in a system. In the case of the transboundary monitoring program, this approach provides an ideal method to characterize natural variability at the GNWT-GOA border in the absence of significant impacts and identify the magnitude of change in future conditions that would require additional monitoring/assessment and potentially management action. Quantifying the normal range for a system requires not only characterizing spatial variability but also assessing short-term temporal variability to allow for the detection of potentially subtle changes happening over a long time scale. The normal range also provides a measure of the level of change that would be deemed significant enough to be ecologically relevant, termed the critical effect size (CES). In ongoing monitoring, the CES identifies the magnitude of change that is required before management action is taken, but in the development of monitoring programs, CES can also be used to ensure sampling designs are sufficient to detect impairment. Initial establishment of CES to quantify spatial variability can be done with pilot-year monitoring data, but as more data are collected, it is important to refine the spatial CES to account for short-term temporal variability that is likely to be observed within systems. Once at least three years of data have been collected, the CES can begin to be refined to capture site-specific temporal variability and quantify confidence intervals that can be used in future years to detect deviations from normal range.

The objective of this report is to assess spatial variability within the Hay and Slave rivers from the second year of sampling in the GNWT and GOA large transboundary river BMI monitoring program, and to begin to examine temporal patterns through comparison of data from the first two years of the program. The Hay River and the Slave River in northern Alberta-southern Northwest Territories were sampled in August and September 2018 (respectively). Water chemistry, sediment chemistry, physical habitat, and BMI data were analyzed to characterize variability within the rivers, and to quantify the normal range of spatial variability for each river in the second year of sampling, adding to the quantification of CES for a number of biotic metrics. In this second year of sampling, the goal was to capture temporal variability in habitat and assemblage conditions to enhance the development of normal ranges for the rivers.

Methods

During the second year of sampling, the Hay River and Slave River were sampled August 28-31 and September 9-11, respectively (one additional reach of the Hay River sampled September 5). Both rivers were accessed via boat launches on the Alberta side of the border. Kick-sampling reaches of approximately 500 m in length were chosen within each river. Sampling took place in each reach on the river bank where rocky habitat was located. Sample reach KS4 in the Slave River was the only location where sampling was feasible on both banks. In both rivers, five sites were selected within each reach, spaced evenly along the reach when habitat availability allowed. Sites were generally of similar substrate composition, although some sites were more silt-dominated. Sample collection followed the Canadian Aquatic Biomonitoring Network (CABIN) sampling protocol modified for large rivers, as described in the monitoring plan (see Lento 2018). Water chemistry, physical habitat descriptions, and sediment chemistry samples were collected at kick-sites as supporting variables. Hester-Dendy samples were also collected in each river. Hester-Dendy samplers were deployed in four different reaches within each river, but given differences in method-specific sampling location requirements, Hester-Dendys could generally not be deployed at kick-sites.

Water chemistry, sediment chemistry, and biotic metrics were summarized by reach, and chemical parameter means were compared with CCME guidelines and river-specific long-term means or triggers, as available. Among-reach variation in water chemistry and BMI assemblage composition was assessed separately for Hay River and Slave River using a one-way analysis of variance (ANOVA) design, with an abiotic parameter or biotic metric as the response variable and the reach as the grouping factor. Multivariate analysis was used to fully characterize the biotic assemblage and abiotic environment of each river. Chemical and habitat parameters measured at all sites were used to assess variation and identify major gradients in the abiotic environment through Principal Components Analysis (PCA). BMI relative abundance data from kick samples and from Hester-Dendy samples were summarized at the family/subfamily level, and assessed using PCA. The relationship between the BMI data from kick samples and abiotic data was tested with Redundancy Analysis (RDA), with a subset of abiotic parameters selected for inclusion based on their importance in the abiotic PCA.

Although a full assessment of temporal trends was not possible with only two years of monitoring, results from 2018 were compared with 2017 monitoring results to assess variation between the two years. Multivariate analysis was used to compare similarities in chemical/physical habitat and BMI assemblage composition between years. Repeated measures ANOVA was used to compare some biotic metric values between years. Biotic metrics were compared with CESs developed using 2018 data, to evaluate possible outliers in that dataset, and with CESs developed using data from 2017 and 2018, to begin to estimate the normal range in these systems.

Results and Discussion

Kick Sample Sites

Hay River

Water chemistry concentrations in the Hay River did not exceed CCME guidelines for the protection of aquatic life, and reaches were classified as mesotrophic based on mean total phosphorus (TP) values. Management triggers for the Hay River, which are guidelines based on long-term data from the river, were exceeded for pH, alkalinity, conductivity, and two major ions: calcium and magnesium.

Exceedances should be interpreted with caution, as sampling through this program involved only spot measurements (conditions at time of sampling) that may not reflect longer-term patterns in water chemistry in the system.

For most water quality parameters, there was generally little variability among samples collected in a single reach, and as a result, standard deviations were low (< 1 for alkalinity, < 0.05 for pH, < 0.005 for phosphorus, and < 0.1 for nitrogen variables for most reaches). There was also fairly low variation in water quality among reaches in Hay River, although comparison of multivariate ordinations from 2017 and 2018 indicated that the relationships between sites based on their chemical/physical habitat changed significantly in 2018, which was due in part to some sites becoming more dissimilar. Dissolved and total metals displayed more variability among sites and reaches, but overall variability remained low. Interim management triggers for metals were exceeded for a small number of parameters, but these exceedances were minor, and the metals were well below CCME guideline levels.

Reach 6 was added to the monitoring plan in 2018, and though it was similar to the other reaches in terms of a number of chemical parameters, it had significantly lower levels of dissolved nitrogen (DN) and total nitrogen (TN) than all other reaches. However, this reach was sampled nearly one week after the other reaches, and as these represent spot measurements of water chemistry, more data will be required to confirm any differences in the chemical habitat of this downstream reach.

Sediment chemistry analysis found elevated levels of several metals, and these levels were higher than CCME guidelines, though these results should be interpreted with caution as they represent spot measurements. Arsenic and cadmium levels exceeded interim freshwater sediment quality guidelines (ISQGs) and chromium exceeded ISQGs and probable effect levels (PELs). The strongest differences in sediment chemistry values in the Hay River were between samples collected in the same reach, which made it difficult to characterize reaches.

Biotic metrics examined differences in overall abundance and taxonomic richness as well as the relative abundance and richness of Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) (EPT), which are generally considered to be sensitive to pollutants, and the relative abundance and richness of Chironomidae (midges), and of Diptera (true flies) + Oligochaeta (segmented worms), all of which are generally considered to be more tolerant of pollutants and tolerant of the cold temperatures and harsh environmental conditions characteristic of northern rivers. There were clear longitudinal differences in the Hay River based on abundance. In general, total abundance and the abundance of specific taxonomic groups were higher in the three upstream reaches (reaches 1-3) than in the three downstream reaches (reaches 4-6). The patterns in relative abundance and absolute abundance together indicated that there was a decline in abundance across all BMI, including EPT and Chironomidae, in the downstream reaches, but that the decline was somewhat higher in EPT taxa. Total abundance ranged from 199 to nearly 4000 individuals across all kick-sites. Taxonomic richness was more similar among reaches than abundance, although the richness of EPT taxa did appear to be higher in the upstream reaches than in Reach 4 or Reach 5. General spatial similarity among reaches suggested strong potential for defining the normal range in taxonomic richness across the Hay River.

Overall, multivariate analysis of assemblages in Hay River indicated differences among sites and gradients that separated reaches, but there were no strong outliers, which suggested that no sites were ecological outliers with respect to assemblage composition. Although site HR-KS1-2A appeared to be an outlier in 2017, the sampling location was shifted in 2018, and it no longer appeared to be an outlier in

multivariate analysis, instead plotting close to other sites in Reaches 1-3. Taxonomic associations suggested that primary separation of the kick-sites may have reflected small differences in velocity and substrate composition across reaches. The range of taxa across these reaches, which include taxa that prefer slow-flowing water with soft sediments as well as some taxa that tolerate fast flow, indicate diverse assemblages with clear upstream-downstream differences that could be useful for detecting change along the longitudinal extent of the river. However, it should be noted that some of the differences between upstream and downstream reaches may relate to the low water level conditions in 2018, which may have disproportionately affected the BMI assemblages in the shallower downstream reaches.

Temporal analysis found evidence of strong declines in total abundance and abundance of EPT and Chironomidae in several reaches compared with what was observed in 2017. In the upstream reaches (Reach 1 and Reach 2), there was a significant increase in abundance in 2018, whereas abundance decreased significantly in Reach 3, Reach 4, and Reach 5. Richness measures, however, remained similar between years.

Comparison of biotic and abiotic data through RDA indicated that some differences in assemblage structure along the first axis of the BMI PCA reflected variation in velocity and substrate size. Secondary separation was due to substrate size and concentrations of metals and TP. Most water chemistry parameters did not play a large role in describing assemblage patterns, which may have been due to the low variability in water chemistry within and among reaches.

Slave River

Water chemistry concentrations for ions, nutrients, and physicals in the Slave River did not exceed CCME guidelines for the protection of aquatic life, though TP was generally higher compared to the Hay River, and most reaches were classified as eutrophic to hyper-eutrophic based on mean TP values and the Canadian Guidance Framework. TP levels in 2018 did not relate to TSS levels, and dissolved and total phosphorus levels were similar in several reaches. There was relatively little variability between samples collected in a single reach, and standard deviations for reaches remained fairly low (< 2 for alkalinity and ions, < 0.04 for phosphorus variables and < 0.04 for nitrogen variables). However, the Slave River had more variation than the Hay River among reaches. A number of parameters, including alkalinity, calcium, conductivity, and dissolved nitrogen, were higher at Reaches 1 and 2 than in the downstream reaches. Concentrations of dissolved metals were generally low in Slave River reaches, with many metals at or below detection limits, and no CCME total metal guidelines for the protection of aquatic life were exceeded. Concentrations of dissolved aluminum, iron, and manganese displayed some variability among reaches. Concentrations of total metals exceeded CCME guidelines for some parameters, including total aluminum, copper, and iron; however, mean values of these metals were generally consistent with long-term means for the Slave River. Exceedances should be interpreted with caution, as sampling through this program involved only spot measurements (conditions at time of sampling) that may not reflect longer-term patterns in water chemistry in the system.

Multivariate analysis of abiotic data for Slave River indicated some correlations among sites that reflected differences in water chemistry and physical habitat among reaches, but there was stronger similarity within reaches than was evident for Hay River. In particular, Reach 2 and Reach 5 were separated from other reaches due to a positive correlation with ions, nutrients, sand, turbidity, and metals. Overall, the spread of kick-sites indicated strong similarity within reaches, but differences in

water chemistry among reaches, providing good characterization of spatial variation in chemistry. Physical habitat variables played a lesser role in distinguishing among reaches. Temporal assessment indicated that the relationships between sites based on chemical/physical habitat were similar in 2017 and 2018.

Analysis of sediment chemistry data indicated that PAHs were present at levels above detection limits in several reaches. In particular, 2-Methylnaphthalene exceeded the CCME interim freshwater sediment quality guideline. However, these results should be interpreted with caution as they represent spot measurements. Sites within the same reach were more similar in the Slave River than was found for the Hay River, indicating fairly high precision between samples.

Biotic metrics were variable within reaches in the Slave River, particularly when abundance-based metrics were considered. Total abundance ranged from 9 individuals to 3260 individuals across all sites. The abundance of Chironomidae was similar across all reaches, and mean abundance values were extremely low, ranging from only 19 to 63 individuals. As a result, the percentage composition of Chironomidae in Slave River reaches ranged from 2.6% to 10.2%, indicating that they made up only a minor portion of the samples in 2018, contrasting with 2017 when Chironomidae made up 12-63% of the total assemblage. This drop in abundance of Chironomidae may have reflected the sampled habitat in 2018, as a peak in flow late in the season likely resulted in the sampling of temporary habitat. Taxonomic richness was similar among the upstream reaches (Reaches 1-3), but more variable downstream. Across all sites, total taxonomic richness ranged from 4 taxa to 35 taxa per site. Reach 4A generally stood out as having lower abundance and richness of most groups of organisms, and notably included a site with no Chironomidae and only one individual of EPT. Strong differences between this reach and other reaches in 2018 and in 2017 suggest that it may not be an ideal reach for long-term monitoring of BMI assemblages.

Multivariate analysis indicated the presence of strong outliers, notably KS4-1A, KS4-2A, and KS2-1A. These sites had low abundance and low richness, and compositional differences between these sites and the other Slave River sites dominated the first axis of the PCA plot as a result. There were few taxa associated with the outlier sites, but overall, the composition of these kick-sites was suggestive of a lower-velocity environment with softer sediments.

One of the most obvious changes from 2017 to 2018 in the Slave River was a sharp decline in abundance, and in particular the abundance of Chironomidae, in Slave River sites. However, there was not a consistent gain or loss of individuals across all Slave River sites in 2018. In total, the abundance in 12 sites changed by more than 500 individuals, with 7 sites increasing in abundance and 5 sites decreasing in abundance. Over half of the Slave River sites saw a decline in percent composition of Chironomidae of greater than 30% from 2017 to 2018. Chironomidae relative abundance declined from 70-80% down to less than 10% of the total abundance in some samples. The decline in Chironomidae across many sites in 2018 was quite severe, contributing in particular to sharp declines in total abundance in Reach 2. In part, this decline may have reflected a sampling artifact due to changes in water level. With a surge in flow only 45 days before sampling occurred (compared to over 100 days in 2017), wadeable areas on the banks of the Slave River likely consisted of temporary habitat, i.e., habitats that were not underwater prior to the recent increase in water level. Organisms that are highly mobile and good colonizers benefit from this temporary increase in available habitat, but organisms that are less mobile are less likely to be encountered in these areas. The loss of Chironomidae in 2018 may also have reflected a response of some subfamilies to flow instability throughout the summer. Previous

studies have examined the response of Chironomidae to changes in flow, and found that diversity within this group is affected by flow stability. The decline in Chironomidae abundance has two important implications: (1) variation in flow throughout the year is important and should be considered when finalizing timing of sampling and when analyzing data resulting from monitoring activities, because the characteristic assemblage in unusually high flow conditions may differ from that in more stable flow conditions; and (2) Chironomidae (and particular subfamilies) could be explored as potential indicators of flow-related differences between years.

The RDA of Slave River samples confirmed that the gradient in kick-sites reflected a primary gradient in velocity, as well as nutrients. Velocity and ammonia separated sites along the first axis, while the second axis describes a gradient in metal concentrations and substrate size. The sites that appeared to be ecological outliers separated from other sites along the velocity gradient, and were associated with lower velocities.

Hester-Dendy Sample Sites, Hay River and Slave River

Hester-Dendy samplers in the Hay River collected between 120 and 290 individuals, on average. The first reach was the most variable, with the lowest average total abundance, whereas the three reaches from farther downstream were more consistent, with similar mean abundance and low standard deviations. All reaches were dominated by EPT taxa, which made up 66-85% of the total assemblage. Taxonomic richness in Hay River Hester-Dendy samples was lowest in Reach 1 (where abundance was also lowest), and more consistent across the other three reaches, ranging from 16 to 22 taxa on average. In the Slave River, abundance was much more variable among reaches, ranging from 91 individuals to 366 individuals on average, and the highest average abundance was found in Reaches 2 and 4. Similar to the Hay River, EPT dominated the Hester-Dendy samples collected in Slave River reaches, ranging from 64-87% of the sample on average. Taxonomic richness was variable within Slave River reaches, which made it difficult to detect differences among reaches. Total richness varied from 10 to 16 taxa on average.

PCA ordinations of Hester-Dendy samples indicated generally strong similarity within and among reaches, with only a few samples that differed from the rest. For example, Hay River sites were generally clustered by reach, and the reaches were primarily located close to the origin (indicating similarity in composition), with the exception of one sample in Reach 1. In the Slave River, Hester-Dendy samples were also quite similar within and among reaches, with the exception of a small number of samples. There was generally strong overlap among reaches near the origin of the plot (indicating strong similarity within and among reaches), and this was in contrast to 2017 when there was greater spread of samples in ordination space.

Rarefaction curves were used to compare family richness of Hester-Dendy samples and kick samples in the Hay and Slave Rivers. Kick samples consistently collected more families in both rivers, but the difference was only statistically significant for the Hay River.

Assessment of study design

CES was set to 2 standard deviations (SDs) from the mean for each biotic metric. CES was calculated for 2018 data alone, to assess spatial variation, and was calculated using data from both 2017 and 2018, to begin to establish normal range. Most Hay River sites fell within the CES range established based on 2018 data, which indicated that there were few outliers among the sample sites. There were more deviations from normal range evident in the Hay River when 2018 metric data were compared with CESs

developed based on data from both 2017-2018. The range of total abundance across Hay River reaches was larger in 2018 than in 2017 because of lower abundance in downstream reaches, and many downstream sites were below the lower CES limit. There were a number of sites in the Hay River that were outside the 2017-2018 CES ranges for relative abundance of EPT, Chironomidae, and Diptera + Oligochaeta, though the frequency of exceedance of CES was lower than for total abundance. For the relative abundance of EPT, the strongest deviations were in the downstream reaches. When total richness was considered, sites in Reach 2, Reach 3, Reach 4, and Reach 6 were at or above the upper CES limit, whereas sites in Reach 5 fell below the lower limit. These exceedances primarily reflected variation in richness of Chironomidae among sites.

Comparison of Slave River metric data with the 2018 CESs indicated that the strongest outliers were sites KS2-1A, KS4-1A, and KS4-2A, which had lower total abundance, total richness, and richness of EPT taxa than other sites. Comparison of Slave River data from 2018 with CESs developed using data from both 2017 and 2018 indicated the strength of the variation in biotic metrics between years. Variation in abundance among sites was strong in the Slave River in 2018, and both total abundance and relative abundance of taxonomic groups varied widely from what was observed in 2017. The result of the inter-annual differences in abundance was a wide normal range as defined by the combination of 2017 and 2018 data, and a large number of sites in 2018 that were outside of this normal range.

Exceedances of 2017-2018 CES limits in the Slave River also reflected the shift in dominance in the river in 2018, with lower relative abundance of Chironomidae and higher relative abundance of EPT taxa. The relative abundance of EPT was higher than the upper CES limit in 10 sites in the Slave River, including all sites in Reach 1. The relative abundance of Chironomidae was near the lower CES limit, and the normal range for this metric, as defined based on the combination of 2017 and 2018 data, was much wider than the range for EPT or for total abundance. If variability in this metric remains large after additional years of sampling, it may mask future variability that occurs due to impacts. Assessment of temporal trends using data from 2017-2019 will begin to indicate whether this metric can be used to develop management triggers for the river, or whether it is too variable. The strong variability in some metrics between the first two years of sampling for both the Hay and Slave rivers suggests that several years of data (likely more than three) may be necessary to accurately estimate the range of natural variability in abundance in these systems.

Sample size for water quality analysis changed from 2017 to 2018, as the first year of sampling began with one sample per site, and this was reduced to collect samples only in odd-numbered sites in 2018. This change is beneficial in terms of cost, as it requires less lab processing of samples. The variability among samples was tested by assessing the coefficient of variation among samples. Among all the water quality parameters calculated in both rivers in 2017 and 2018, there were 126 instances (16% of those assessed) where variability within the reach was higher than would be acceptable for duplicate samples. Exceedances were found in all reaches besides HR-KS6, though some reaches had more exceedances (i.e., more parameters with high variability) than others. Of the parameters and samples assessed, 84% had acceptable levels of precision, which suggests that fewer replicates may be acceptable in future sampling. However, there are other benefits to collecting multiple samples per reach when conducting sampling for this benthic monitoring program. Water chemistry sampling from this program is intended to support the detection of patterns and trends in the BMI data, and is not sufficient to act as a stand-alone measure of water quality trends at these locations. However, the water chemistry data collected through the BMI monitoring program could supplement existing water quality monitoring that is

ongoing in the area, adding to the spatial and temporal extent of that monitoring. Furthermore, samples at multiple sites in each reach would support the assessment of biotic-abiotic relationships within and among reaches. The collection of only three water quality samples per reach in 2018 represented a compromise between cost and data quality. If only one or two water chemistry samples were collected per reach, it would hinder the ability to detect biotic responses to changes in water quality, because biotic differences within a reach could not be related to variability in chemical parameters. For this reason, it is recommended that water chemistry samples continue to be collected at a minimum of three sites per reach, particularly as baseline data are collected and the normal range of variability is established.

Recommendations and Comments

A number of recommendations have come from analysis of the first two years of monitoring data. In summary, recommendations for future monitoring include:

- Continue to sample Hay River in mid-late August and sample Slave River in early/mid-September, but adjust sample timing annually depending on flow conditions in each river. Hay River samples appeared to be affected by low water levels, with a significant loss of abundance in downstream reaches, although richness measures were not strongly altered. Slave River samples appeared to have been collected in temporary habitat due to high water levels, resulting in a significant loss of abundance, particularly of Chironomidae. Where possible, allow for some flexibility in the timing of sampling to ensure it does not follow a surge in water levels (as this appeared to have a greater impact than low water levels).
- Continue sampling the sample sites and reaches in the Hay River (including the new reach, Reach 6), as these appeared to characterize the river. The addition of Reach 6 increased the sample size of reaches downstream of the boat launch, which will allow for more power in the assessment of longitudinal changes in the river. There was evidence of longitudinal patterns in the river in both 2017 and 2018, so these patterns should continue to be monitored.
- In the Slave River, sites KS2-1A, KS4-1A, and KS4-2A were clearly different from other sites, and Reach KS4A in general tended to stand out in the assessment. Conditions at these sites, particularly with respect to water velocity, may drive the differences relative to other sites. In the long term, these sites may not be ideal for monitoring, due to the low abundance and richness found there. Consider removing Reach 4A from future monitoring, and monitor conditions in site SR-KS2-1A.
- Efforts should be made to locate and sample another reach in the Slave River to ensure sufficient replication and characterization of variability, particularly if Reach 4A is removed.
- Although variability among water chemistry samples was fairly low, there were a number of parameters that varied among the three sites in each reach, generally with higher levels at only one site. Because of this variability and because of the need for site-scale supporting variables to assess biotic-abiotic relationships, continue collection of water chemistry samples at odd-numbered sites in each reach unless chemistry results in future years suggest that more sampling is necessary.
- Sediment-bound metals are not readily biologically available in oxygenated and pH stable environments, and thus shifts in these concentrations may not provide an estimate of the potential risk to biota. Furthermore, where bound metals may be biologically available, uptake of sediment-bound metals is dependent on the level of exposure from feeding habits and

habitat preferences of individual species. In the future, it is important to ensure continued collection of dissolved metal samples to estimate biotic response to metals, or to explore the use of regression to predict dissolved metals from total metals and TSS.

- Sediment chemistry was not strongly related to biota in the Hay River or Slave River. Although it may be desirable to continue collection of these samples to monitor changes in PAHs in the sediments, they may not need to be collected as regularly as water chemistry samples.

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1. Introduction

The Government of the Northwest Territories (GNWT) and the Government of Alberta (GOA) are working to establish a monitoring program for the bioassessment of large transboundary rivers (MacDonald Environmental Sciences Ltd. 1995, Lento 2017). Transboundary rivers provide unique challenges to assessment, as monitoring designs must meet the objectives of multiple jurisdictions that may differ with respect to economic and social goals as well as environmental management strategies (MacDonald Environmental Sciences Ltd. 1995). However, the potential for upstream development within one jurisdiction to cause downstream impacts within another jurisdiction emphasizes the need for cooperation in the monitoring of transboundary waters to ensure the detection of changes to ecosystem health (Flotemersch et al. 2011). While there are no concerns about particular stressors at the GNWT-AB border, the establishment of this program is in recognition of the potential for future impacts to transboundary waters as a result of activities in the upstream catchment. Establishment of long-term monitoring and assessment supports the future detection of impacts that may arise from human development, but also supports the detection of ecological changes in response to a warming climate.

1.1. General Approach of the Monitoring Program

Monitoring questions related to assessing ecosystem health may be focused on comparison of reference sites with test sites in the presence of a known stressor, or they may be focused on characterizing the contemporary status of biotic and abiotic ecosystem components and evaluating whether any temporal changes have occurred (e.g., see Environment Canada 2011, Culp et al. 2012b). One approach used in biological monitoring, particularly in the case of detecting future evidence of impairment, is to estimate the normal range of community composition based on natural variability in the system, and to detect any shifts in the diversity or abundance of organisms that occur over time (Munkittrick et al. 2009, Munkittrick and Arciszewski 2017). Where there is not a clear stressor in place, determining the range of "normal" variation in the data can be used to establish a baseline ecological condition, providing information that can be used in future years (with continued monitoring) to begin to address targeted questions as stressors increase (Munkittrick et al. 2009, Munkittrick and Arciszewski 2017). Quantification of variation that might be expected in the absence of impairment can support the development of "trigger" levels, or levels at which the magnitude of observed change is greater than expected, necessitating additional monitoring or management action (Arciszewski and Munkittrick 2015). Future assessments could focus on examining relationships of natural and anthropogenic drivers of change with ecosystem health, and detecting evidence of cumulative impacts (e.g., from a combination of climate change, development, resource exploration, or other stressors; Dubé 2003, Dubé et al. 2013, Somers et al. 2018). Establishing a strong baseline for comparison is a vital step in this process to allow for future detection of ecosystem responses to change (Culp et al. 2012b).

The initial focus of the GNWT and GOA transboundary monitoring program is on benthic macroinvertebrate (BMI) assemblages, which are an important ecosystem component to monitor in northern rivers as an integrated measure of water quality and habitat condition (Culp et al. 2012b, Buss et al. 2015, Lento et al. 2019). BMIs are commonly chosen for biomonitoring because they are widespread, easy to sample and identify, species-rich, have limited mobility, and have known tolerances and sensitivities to habitat conditions that can support the detection of anthropogenic impacts (Bonada et al. 2006, Resh 2008, Buss et al. 2015). Because they have generally low mobility, BMI responds to

local-scale changes in water chemistry and habitat quality and are an excellent indicator of the physical and chemical impacts of disturbance. Moreover, BMI diversity at northern latitudes is strongly linked with temperature as a result of taxon-specific thermal tolerances (Culp et al. 2018, Lento et al. 2019). With climate change, it is predicted that biodiversity in northern regions will begin to resemble more closely those of temperate systems through the northward movement of eurythermic and cold-intolerant species (Culp et al. 2012a, Lento et al. 2019). Thus, the long-term assessment of BMI assemblages has the potential to detect changes in response to a warming climate in addition to detecting future impacts from human development. Within the Alberta-Northwest Territories transboundary river regions, there is relatively little information about the current state and composition of benthic communities. Assessments of BMI assemblages in the large transboundary rivers of the Alberta-Northwest Territories region have been limited (but see Paterson et al. 1991, Paterson et al. 1992 for baseline assessment of Slave River BMIs, and Golder Associates 2010 for an overview of existing assessments), and Dagg (2016b) noted that this lack of background knowledge has made it difficult to identify water quality concerns and potential for impairment during local community discussions of potentially vulnerable ecosystem components. Therefore, it is vital that routine monitoring of large transboundary rivers be established to secure information about baseline conditions in these assemblages and to provide sufficient information to allow for future detection of trends.

1.2. Establishing Normal Ranges

In biomonitoring, the concept of the normal range is based on the idea that it is not always possible to access data from before any perturbation occurred in a region (Arciszewski and Munkittrick 2015), nor is it necessarily desirable to use such historical data if they do not accurately represent attainable water quality levels (Stoddard et al. 2006, Munkittrick and Arciszewski 2017). Instead, if sufficient contemporary data are collected to allow estimation of the range of variability that is acceptable given current conditions in a system, then this information can be used to detect any future deviations and pinpoint where targeted sampling should take place to identify causes of impacts (Kilgour et al. 2017, Munkittrick and Arciszewski 2017). However, quantifying the normal range for a system requires not only characterizing spatial variability but also assessing short-term temporal variability to allow for the detection of potentially subtle changes happening over a long time scale (e.g., 10+ years; Arciszewski and Munkittrick 2015). Baseline data must be collected for multiple reference sites over multiple years, with sampling taking place in a single season (e.g., fall), and subsequent monitoring activities must continue at multiple sites for many years to allow for effective detection of change (Arciszewski and Munkittrick 2015). In the first year of collecting baseline data, spatial variability among sites is captured, and in subsequent years the natural temporal variability is quantified, and measures of temporal and spatial variability are refined. At least three years of baseline data must be collected before the regional normal range can begin to be characterized, and additional sampling beyond three years is recommended to achieve accurate estimates of normal range and to detect any shifts in normal range due to climate change (Arciszewski and Munkittrick 2015).

1.3. Quantifying Meaningful Change: Critical Effect Sizes

The concept of the normal range applies well to the situation where a monitoring program is being established in anticipation of potential future impacts, because it allows for quantification of the current status in the system as well as the level of change that would be deemed significant enough to warrant concern, termed the critical effect size (CES; Munkittrick et al. 2009, Arciszewski et al. 2017, Munkittrick and Arciszewski 2017). The CES is the magnitude of difference between sites or change across time that

is considered to be meaningful and to have ecological implications (Munkittrick et al. 2009). It can act as a trigger point in adaptive monitoring plans to identify when additional sampling is necessary to investigate potential drivers of change (Somers et al. 2018).

In ongoing monitoring, the CES identifies the magnitude of change that is required before management action is taken, but in the development of monitoring programs, CES can also be used to ensure sampling designs are sufficient to detect impairment (Munkittrick et al. 2009). For example, as the normal range of variability across systems is quantified in pilot sampling years, the CES can be determined and used in power analysis to estimate the number of samples that would be required to detect a meaningful difference among sites. A number of different approaches have been used to determine CES for different groups of organisms (see review in Munkittrick et al. 2009); however, studies of BMI assemblages that assess natural variability within and among sites have generally relied on standard deviation (SD) units or similar approaches (e.g., confidence intervals or probability ellipses) to set CES. For example, the CES for invertebrate abundance might be set to 2 SDs above and below the mean abundance observed in baseline data. Exceedance of the CES by any site in future years would then act as a trigger to increase sampling efforts and determine if impairment has occurred. Initial establishment of CES to quantify spatial variability can be done with pilot-year monitoring data, but as more data are collected, it is important to refine the spatial CES to account for short-term temporal variability that is likely to be observed within systems (Arciszewski and Munkittrick 2015). Once at least three years of data have been collected, the CES can begin to be refined to capture site-specific temporal variability and quantify confidence intervals that can be used in future years to detect deviations from normal range. For example, river flow can be highly variable from one year to the next due to inter-annual climatological variation, and these changes in flow can have noticeable impacts on BMI assemblage composition, with higher flow years favouring taxa that prefer fast velocities and low flow years resulting in a dominance of taxa that prefer slower velocities (Monk et al. 2008). Such shifts in assemblage composition might appear indicative of impacts if there is no quantification of the natural flow regime in a system.

1.4. Purpose and Objectives

The objective of this report is to assess spatial variability within the Hay and Slave rivers from the second year of sampling in the GNWT and GOA large transboundary river BMI monitoring program, and to begin to examine temporal patterns through comparison of data from the first two years of the program. The Hay River and the Slave River in northern Alberta-southern Northwest Territories were sampled in August and September 2018 (respectively). Water chemistry, sediment chemistry, physical habitat, and BMI kick samples were collected using the methods described by Lento (2018b), and data were analyzed to characterize variability within the rivers, and to quantify the normal range of spatial variability for each river in the second year of sampling, adding to the quantification of CES for a number of biotic metrics. In this second year of sampling, the goal was to begin to capture temporal variability in habitat and assemblage conditions to enhance the development of normal ranges for the rivers. Although two years of data collection does not allow for extensive formal assessment of temporal trends, data from 2018 were briefly compared with data collected in 2017 to begin to examine the magnitude of inter-annual variation in these systems.

Artificial substrate samplers (Hester-Dendy) were also deployed in the two rivers for a second year over a one-month period to collect benthic invertebrates and to continue to compare methodologies and results with the kick sampling technique. Artificial substrate samplers are intended for use in situations

where safety is a concern or kick-sampling protocols are unsuitable due to the absence of appropriate substrate (e.g., clay-silt bottom substrates).

Analysis of the samples collected during this pilot project will be used to inform future sampling efforts in these rivers and will begin to build a baseline database for continued monitoring. With two years of data collected thus far, the primary focus is on evaluating spatial variability within each river to gauge how BMI assemblages differ across the area of interest, though some comparison with 2017 is useful for an initial look at inter-annual variability. In the third year of the program, this assessment will be expanded upon to include a more formal analysis of temporal variation in the study rivers.

2. Methods

2.1. Study area and sample timing

The pilot program of the GNWT and GOA large transboundary river monitoring program is focused on the Slave River and the Hay River. Both rivers originate in Alberta flowing north into the Northwest Territories and terminating in Great Slave Lake (Figure 1), but they differ with respect to size, flow, and upstream land use (see overview in Golder Associates 2010). The Slave River is a large, fast-flowing river, with a mean annual discharge rate of 3,400 m^3/s (Sanderson et al. 2012) and a drainage basin of over

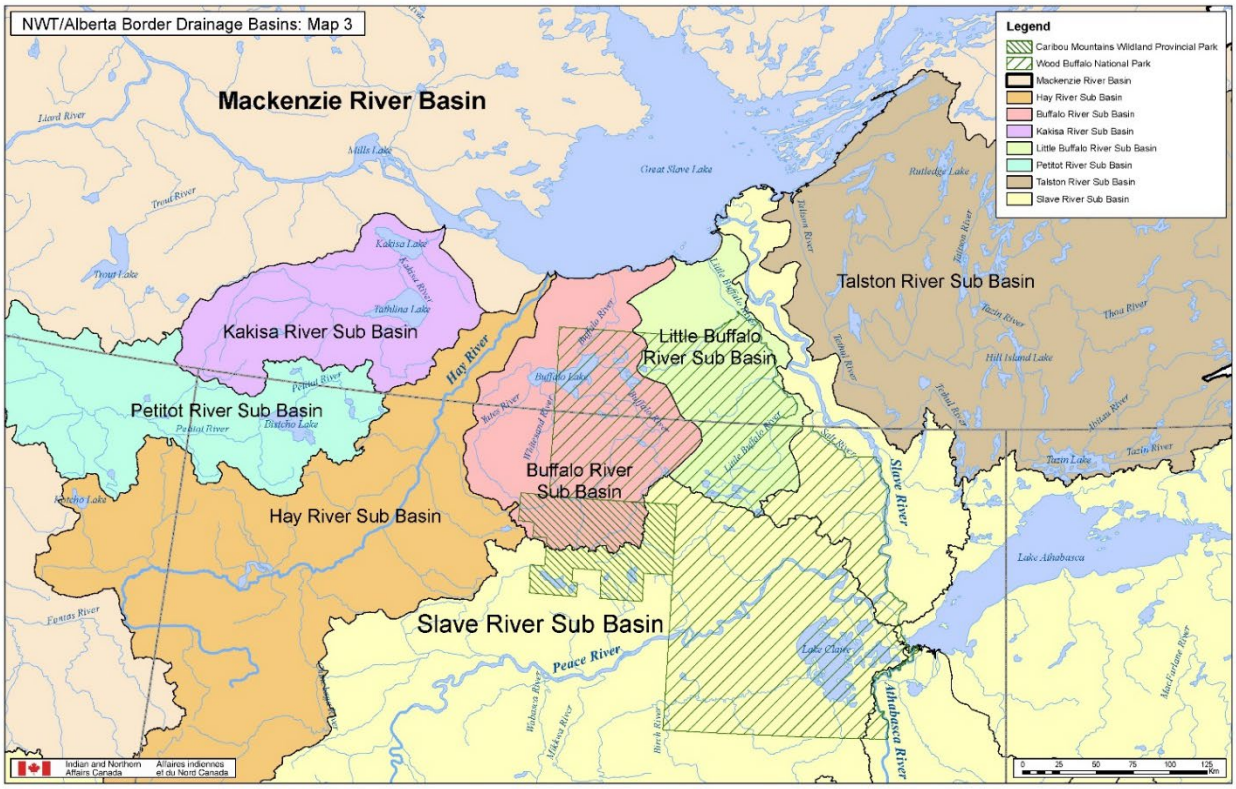

Figure 1. Drainage basins at the NWT/Alberta border, including the Hay River Sub Basin and Slave River Sub Basin. Map created by Indian and Northern Affairs Canada.

616,000 km^2 (Golder Associates 2010). The Hay River is narrower, more shallow, and slower-flowing, with a drainage basin of 48,100 km^2 (Golder Associates 2010). Details on the geology, climate, land cover, and land use history of both river catchments can be found in state of knowledge reports for the

Hay River (Stantec Consulting Ltd. 2016) and Slave River (Pembina Institute 2016). Both rivers have the potential to be impacted by a variety of human activities in the upstream basin, including oil and gas development and pulp and paper mills. Though change may have already occurred in these systems due to upstream activities, lack of historical baseline data precludes the assessment of such changes. The current program is aimed at characterizing the current ecological condition of these rivers as a baseline for future assessments.

The differences between these rivers with respect to size, depth, and flow lead to unique challenges that must be considered when planning and conducting BMI sampling. For example, although sampling is designed to occur in the fall to take advantage of increased access to the shoreline that is gained

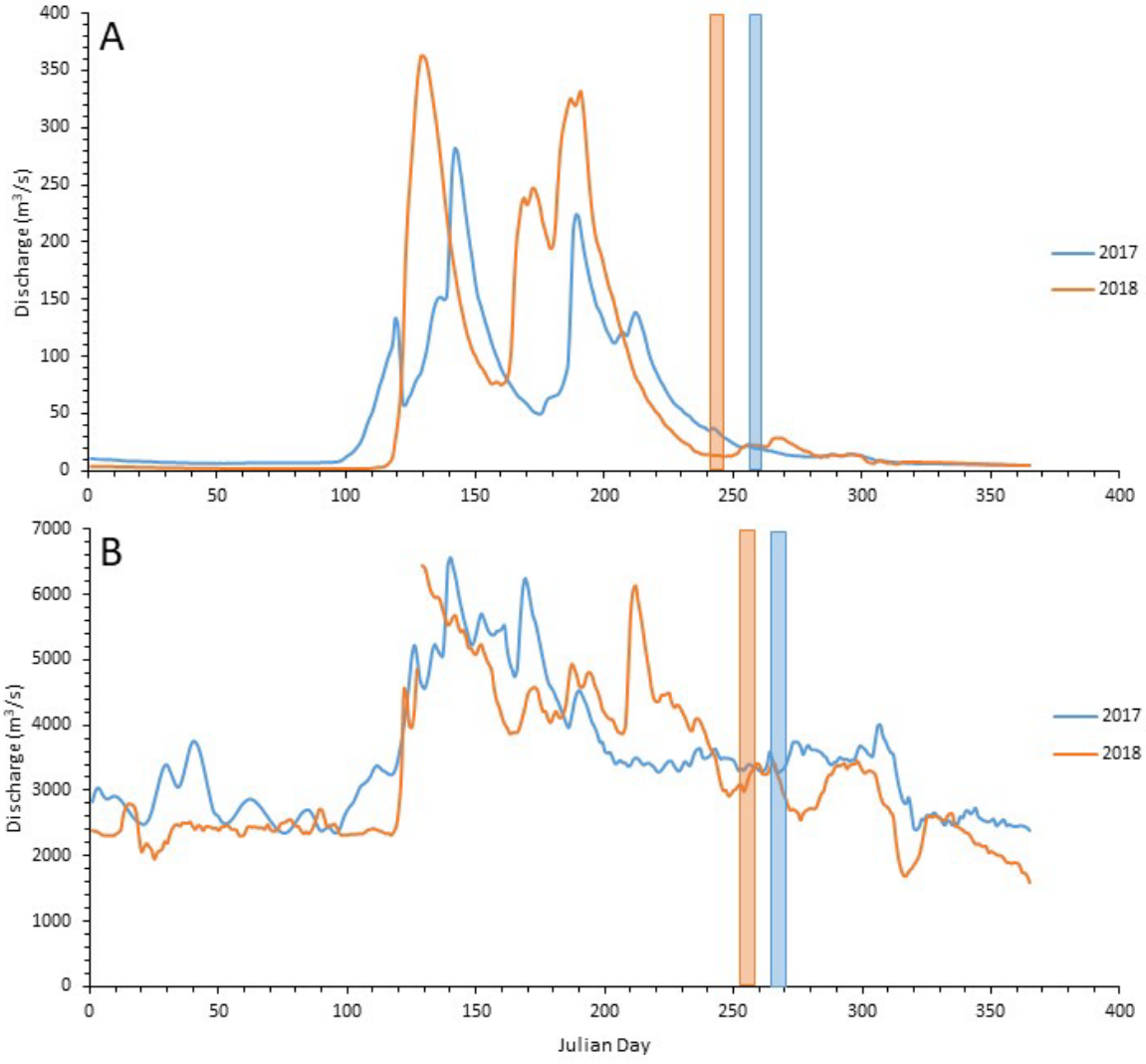

Figure 2. Hydrographs for (A) Hay River and (B) Slave River in 2017 (blue) and 2018 (orange), with vertical shaded bars indicating the timing of sampling in each year. Data for Hay River near ALTA/NWT boundary (station 07OB008) and Slave River near Fort Fitz (station 07NB001) from wateroffice.ec.gc.ca.

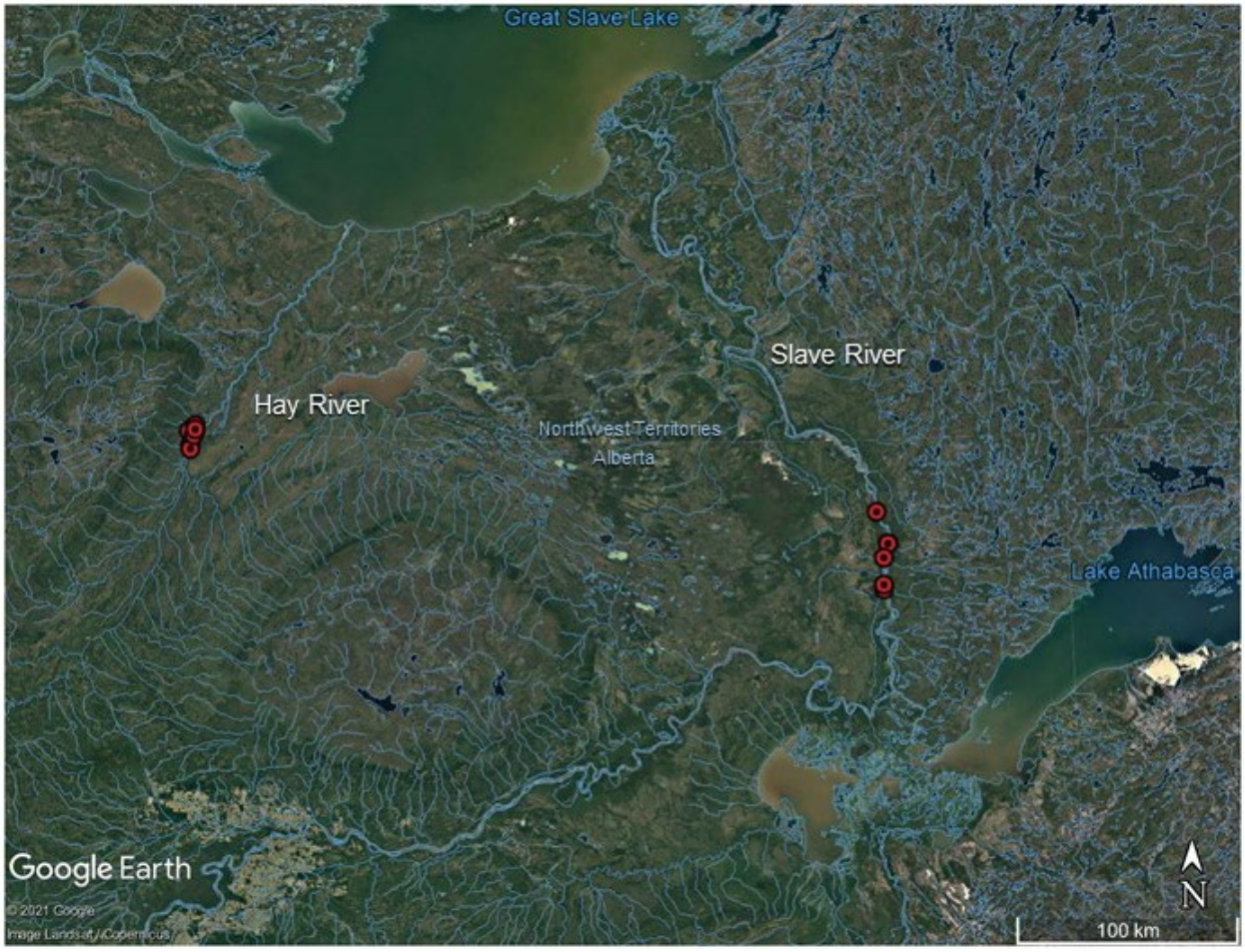

Figure 3. Map of Hay River and Slave River, showing kick-sampling reaches (red points) and an overlay of the stream network. Stream network layer from National Hydro Network (NHN) GeoBase Series (open.canada.ca).

when water levels recede, the exact timing required for sampling the Slave and Hay Rivers differs due to local conditions. The deep and fast-flowing Slave River has to be sampled late enough in the fall to allow safe access to shorelines for kick sampling, whereas the timing of sampling of the Hay River has to be early enough to ensure that the shallower river does not become inaccessible by boat. Furthermore, additional safety equipment is required to safely sample the deeper, faster-flowing Slave River, whereas the Hay River requires a lower-profile boat to maneuver through sand bars in low water level situations.

In 2017, both rivers were sampled in mid-September; however, this led to problems accessing some areas of the Hay River where water levels were low (Figure 2). As a result, sampling was shifted to August 28-31 for the Hay River in 2018 (with one additional reach sampled September 5) while the Slave River was sampled September 9-11, 2018. Water levels in the Hay River were at or below record minimum levels at the end of August 2018 (ECCC gauge Hay River near ALTA/ NWT boundary, station 07OB008; Figure 2), which resulted in lower water levels for sampling than observed the previous year. In contrast, there was a surge in water levels in the Slave River in late July 2018, and sampling in this year took place sooner after the peak flow than in 2017 (Figure 2). This variation in water level and its effects on habitat conditions and biotic assemblages represents natural inter-annual variability in the system that is important to capture while characterizing the normal range, as long as the habitat sampled within the river remains the same in these differing flow conditions.

2.2. Site selection

The BMI monitoring plan for large transboundary rivers (Lento 2018b) prescribes a sampling design with 5-10 approximately 500-m-long reaches sampled in a river (the number of reaches depending on what is required to characterize the river and achieve adequate power to detect biologically-meaningful differences among reaches, if they were to exist). Reaches are selected to have similar substrate composition throughout the reach (ideally rocky substrate, though soft sediments are acceptable if comparable substrates are sampled in additional reaches). Within each reach, five replicate kick-sites are sampled, approximately 50-125 m apart. If access to both banks of the river is possible, a total of 10 kick-sites are sampled within a reach (five on each river bank). This design allows for the application of multiple statistical analyses to characterize variability within a river. For example, sites can be compared directly along a longitudinal gradient, or sites can be treated as replicates in a statistical comparison of reaches. This design was applied during the first two years of sampling, though some adjustments were made to reflect local conditions.

Both rivers were accessed via boat launches on the Alberta side of the border (Figure 3). Five kick-sampling reaches were chosen within each river for the pilot year of sampling, and this number was increased to six in the Hay River in 2018 (Table 1; Figure 3). Sample reaches were selected to be approximately 500 m in length, though in some areas, the availability of suitable habitat limited the total

Table 1. Approximate coordinates in decimal degrees (DD) for each kick-sampling reach and Hester Dendy reach sampled in the Hay River and Slave River in August-September 2018. Reach codes are explained in text.

River	Sample Type	Reach	Latitude (DD)	Longitude (DD)
Hay River	Kick sample	HR-KS1	59.9321	-116.9524
		HR-KS2	59.9465	-116.9565
		HR-KS3	59.9885	-116.9304
		HR-KS4	60.0026	-116.9713
		HR-KS5	60.0113	-116.9218
		HR-KS6	60.0279	-116.9216
	Hester Dendy	Reach 1	59.9312	-116.9853
		Reach 2	59.9325	-116.9518
		Reach 3	59.9909	-116.9318
		Reach 4	60.0114	-116.9209
Slave River	Kick sample	SR-KS1	59.4085	-111.4620
		SR-KS2	59.4276	-111.4629
		SR-KS3	59.5350	-111.4577
		SR-KS4A	59.5912	-111.4195
		SR-KS4B	59.5903	-111.4225
		SR-KS5	59.7182	-111.5058
	Hester Dendy	Reach 1	59.6947	-111.5115
		Reach 2	59.7167	-111.5105
		Reach 3	59.8297	-111.5714
		Reach 4	59.8690	-111.5712

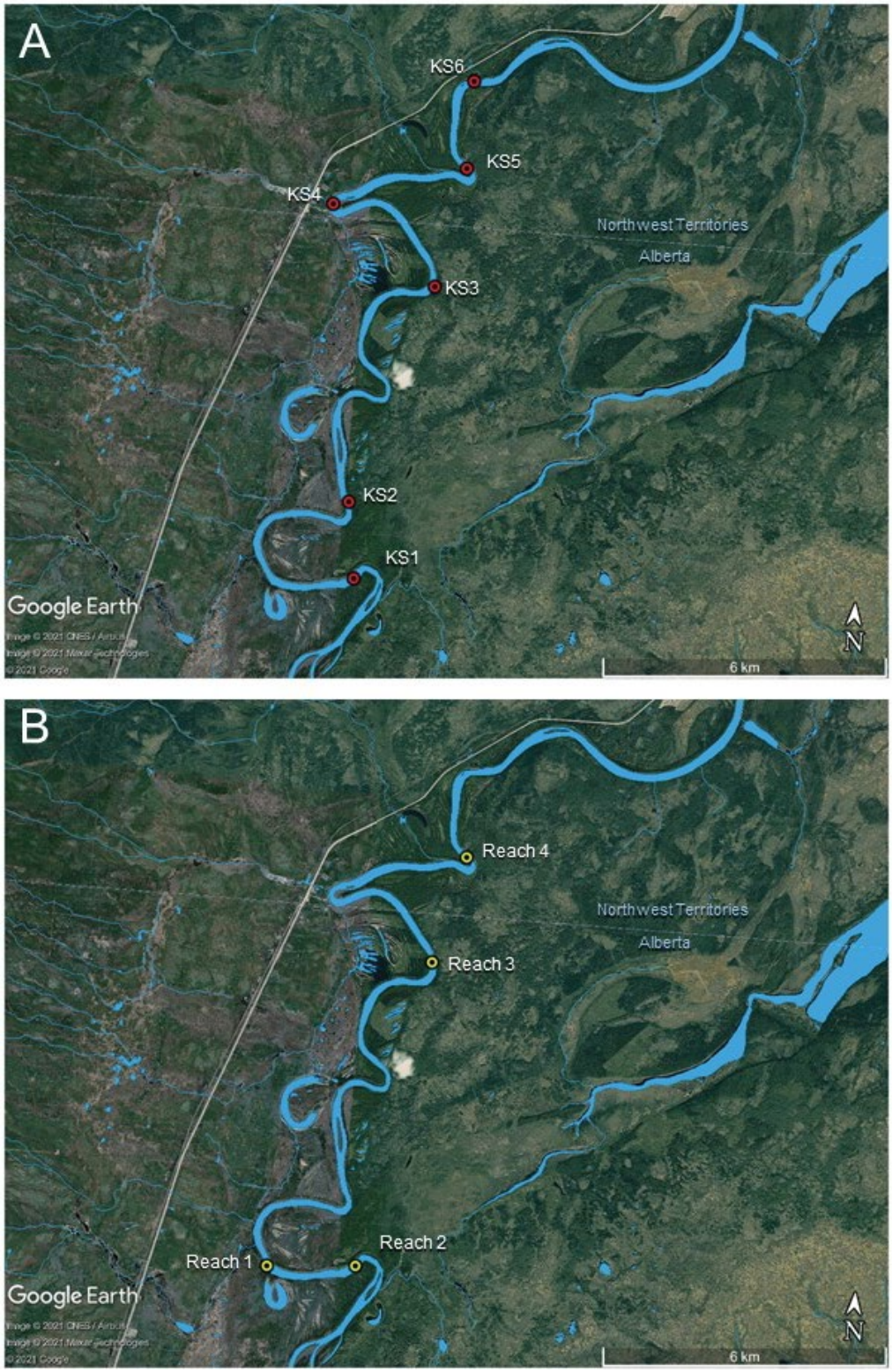

Figure 4. Hay River sample locations in 2018, including (A) kick-sample reaches (red points), and (B) Hester-Dendy reaches (yellow points). Reaches are labeled in white text. Water body and stream layers overlain on maps are from the National Hydro Network (NHN) GeoBase Series (open.canada.ca).

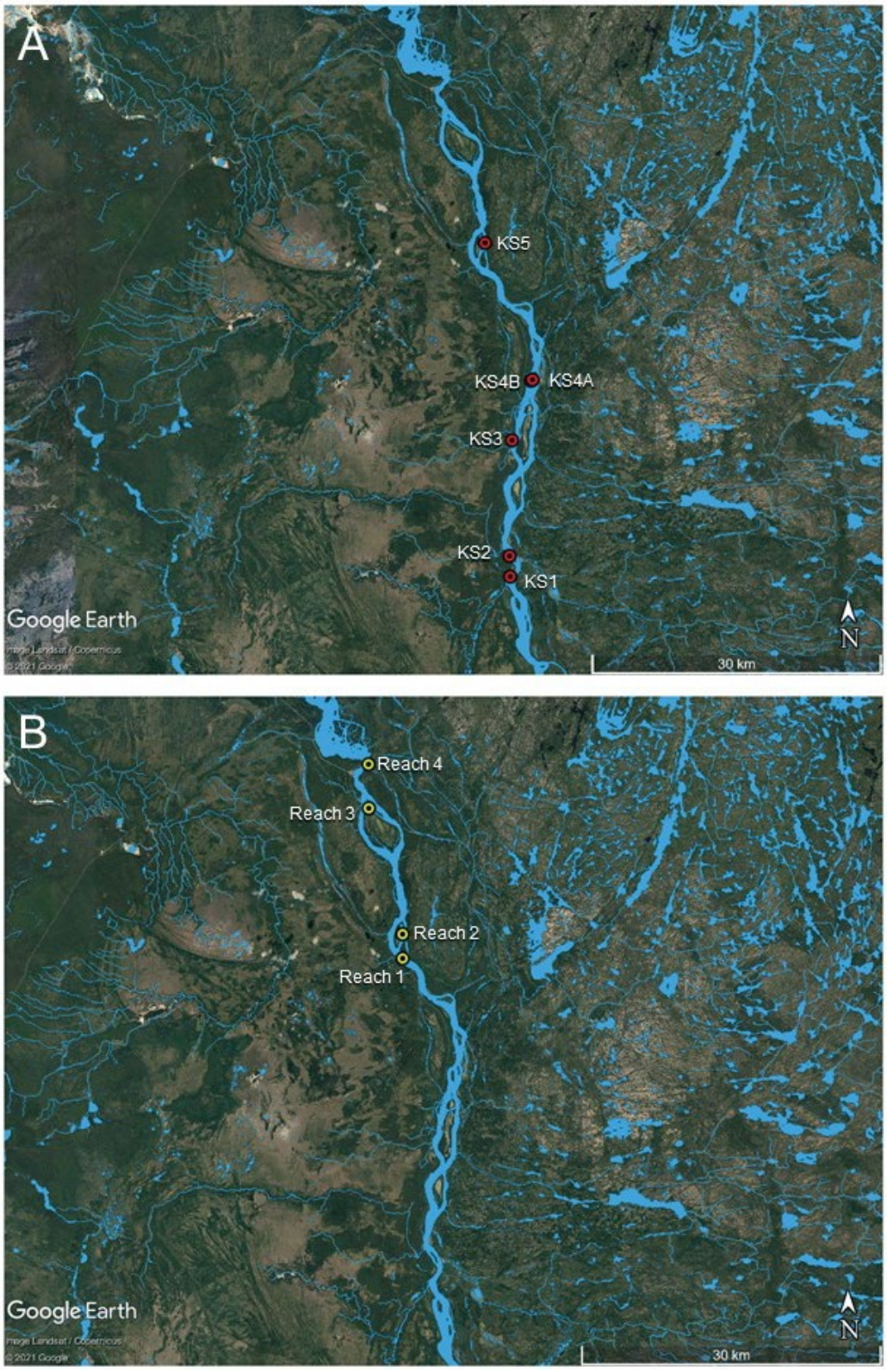

Figure 5. Slave River sample locations in 2018, including (A) kick-sample reaches (red points), and (B) Hester-Dendy reaches (yellow points). Reaches are labeled in white text. Water body and stream layers overlain on maps are from the National Hydro Network (NHN) GeoBase Series (open.canada.ca).

length of reaches (e.g., in the Hay River, reaches were 250 m to 500 m in length, whereas in the Slave River, reaches were 250 m to 600 m in length). Sample reaches were numbered KS1 to KS5 or KS6 in each river, with KS1 representing the farthest upstream sampling location and KS5 or KS6 representing the farthest downstream sampling location (Figure 4; Figure 5). Reach 4 of the Slave River was the only location where sampling took place on both banks of the river, resulting in two sets of sites (HR-KS4A and HR-KS4B) in the same reach (Table 1). In the Hay River, reaches were 2.5 to 6.7 km apart, whereas in the larger Slave River, reaches were 1.9 to 16 km apart.

The Hay River is sinuous with slow flow, and reaches with rocky habitat were generally found at the bends of the river, typically on the erosional banks (Figure 4; see appendix for photos of Reach 1). The depositional bank was generally a thick silty/muddy substrate that would not have allowed for access or for sampling (unlike sandy habitats, in which kick sampling can be conducted). Because of the shallow nature of some extents of the river, site selection was limited in some areas to reaches that could be accessed from the boat launch in a timely manner using a canoe with outboard motor. Analysis of reaches sampled in 2017 indicated that there were some differences between reaches upstream (HR-KS1-3) and downstream (HR-KS4-5) of the boat launch, and a recommendation was made to sample an additional reach downstream of the boat launch to ensure adequate replication downstream of this potential point-source impact. Reach HR-KS6 was added in 2018 in response to this recommendation (Table 1; Figure 4A).

The Slave River is wider than the Hay River with a straighter channel and faster flow (Figure 5; see appendix for photos of Reach 2). Rocky substrates were generally found in areas of rocky outcrops along the shoreline. In the analysis of data from 2017, substrate and flow appeared to play a large role in determining the BMI assemblage that was characteristic of a particular reach, and a recommendation was made to add another reach with rocky habitat and fast flow. Travel along the river to select reaches was through the most commonly-used channels, and further exploration of side channels or closer examination of the shorelines along the sampled length of the river could be used to identify additional reaches for future sampling. Though an additional reach was not sampled in 2018, a sixth reach was identified and sampled in 2019 and will be assessed as part of the next report.

Sampling took place in each reach on the bank where rocky habitat was located (e.g., see Figure 6 for an example of single-bank sampling design). Kick-sites within a reach were numbered 1-5, with site 1 as the farthest upstream site and site 5 as the farthest downstream site (consistent with the numbering of reaches); however, sampling was done at kick-site 5 first to avoid downstream contamination of samples. The right-hand bank while facing downstream (river right) was called the A bank and the left-hand bank (river left) was the B bank, and each site code was appended with A or B to indicate which side of the river was sampled. Reach KS4 in the Slave River was the only location (for either river) where sampling was feasible on both banks, and samples were collected from both the A and B banks in this reach to compare habitat conditions and BMI composition. Kick-sites were evenly spaced within reaches, when habitat availability allowed. Distance between kick-sites was generally 50-125 m, as allowed by reach length. Kick-sites within each reach were generally of similar substrate composition, and were chosen to minimize differences in substrate composition; however, there was some evidence of a higher silt concentration at some sites (apparent during field processing of BMI samples, which were muddy). Data from the first year of sampling indicated that there were some differences in BMI

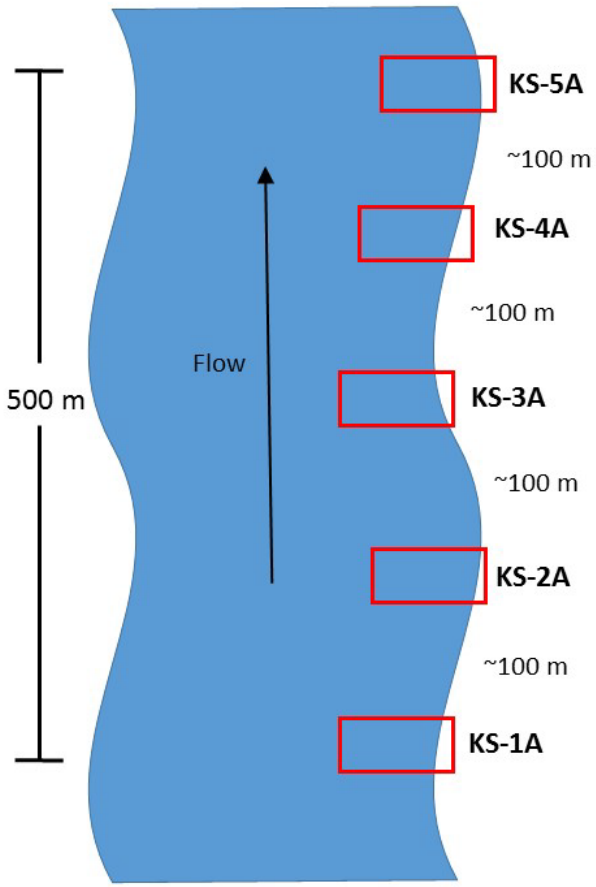

Figure 6. Example sampling design used for a single reach within the Hay River and Slave River, indicating the location of 5 sites within the 500 m reach and numbering of sites with respect to flow direction. Sampling of sites began downstream, at site KS-5A and worked upstream towards site KS-1A. Sites located on the opposite bank (left bank, when facing downstream) were numbered KS-1B through KS-5B. Sites were located approximately 100 m apart (50 m to 125 m) and sampling extended out into the river to a depth of approximately 1 m (maximum safe depth for kick sampling).

assemblages within reaches where substrate composition differed, and data from 2018 are evaluated in this report to assess whether similar differences were evident.

Hester-Dendy samples were also collected in each river in addition to carrying out the shoreline monitoring protocol. Hester-Dendy samplers were deployed in four reaches within each river (Figure 4; Figure 5). In the Hay River, three of the four Hester-Dendy reaches were located close to kick-sampling reaches. There were less overlap of sampling reaches in the Slave River, as samplers were not generally deployed near rocky shorelines, thus reducing comparability of these samples with kick samples.

2.3. Sample collection

Sample collection at kick-sampling locations followed the methods prescribed in the monitoring plan (Lento 2018b), including collection of water chemistry samples, use of handheld meters for field chemistry, a habitat survey (modified from the Canadian Aquatic Biomonitoring Network - CABIN), a modified three-minute CABIN kick sample, and a modified rock walk (see details in Lento 2018b). In addition, supplementary Hester-Dendy samples were collected in each river, though deployment locations for these passive samplers differed from reach and site locations for kick sampling.

2.3.1. Monitoring plan protocols

At three of the kick-sites in each reach (odd-numbered kick-sites), water samples were collected for analysis of a standard suite of parameters, including nutrients, ions, and suspended solids. This was reduced from the set of five samples (one per kick-site) that was collected in 2017 because of low variability in water quality parameter estimates among samples. Additional water samples were collected for the analysis of metals (including mercury) at the same three kick-sites. These samples represented spot measurements of water chemistry, and were intended to characterize the chemical habitat at the time of sampling to provide supporting information that could help in understanding the distribution of BMI assemblages. Water chemistry samples were kept cool and sent to Taiga Environmental Laboratory for analysis. A handheld meter was used to record air and water temperature, pH, specific conductivity, dissolved oxygen, and turbidity on-site.

Sediment samples were collected to analyze metals in soil, particle size, and polycyclic aromatic hydrocarbons (PAHs). Because BMI lives in contact with or burrow within the sediment, contaminant concentrations within the sediment may more accurately reflect their exposure levels. Sediment samples were taken from within the channel at two sites in each reach (sites 1 and 5) and placed into jars. Sediment samples were kept cool and sent to ALS Labs for analysis.

BMI kick samples were collected at each kick-site using a modified travelling kick method (Lento 2018b). The operator held a 400-μm-mesh kicknet with an attached collection cup downstream while standing in the river near the shore at a wadeable depth (approximately 1 m). The operator then kicked and disturbed the substrate upstream of the net for a period of three minutes while moving upstream in a slight zig-zag fashion (maintaining the same approximate depth). Because of the size of each river, sampling remained in the nearshore habitat rather than attempting to cross the channel as in a standard kick sample method. Samples were retrieved from the net and collection cup and stored in 95% ethanol for transport to the lab for sorting and identification. Samples were sorted and identified following standard CABIN protocols (Environment Canada 2014) by Biologica Environmental Services Ltd. In brief, samples were sorted using a Marchant box to randomly sub-sample until at least 300 individuals were counted. BMI were identified to the lowest practical taxonomic level. In addition, a large/rare sort was completed following the sub-sampling procedure, with an abbreviated survey of the remaining cells in the Marchant box to pick out any large or particularly rare taxa that might have been missed as part of the sub-sampling process.

CABIN field survey sheets (Environment Canada 2012) were completed at each site in order to characterize the in-stream and surrounding habitat. This survey included a description of riparian vegetation, surrounding land use, and % cover of macrophytes and % cover of periphyton in the river at each site. In addition, a modified rock walk was completed at each site. Operators selected substrate particles at random and measured the intermediate axis (b-axis) of each particle to the nearest mm to characterize substrate composition. This was completed for 20 substrate particles at each site. Rock walk data were summarized as percent composition in each particle size class.

2.3.2. Hester-Dendy samplers

Hester-Dendy samplers are a form of artificial substrate, meaning that they are deployed for a long period of time (on the order of weeks) and then collected to examine the invertebrates that have colonized the artificial substrate during the deployment period. This sampling method can be useful where it may be difficult to access sampling areas for more active sampling (Flotemersch et al. 2001),

and communities found on artificial samplers have been shown to reflect abiotic conditions in rivers (Blocksom and Flotemersch 2005). However, as with most passive sampling methods, artificial substrates have been found to be selective in the taxa that they collect, and they have been criticized for providing data that focus on the colonizing portion of the community and the portion prone to drift, which may not be representative of relative densities in natural substrates (Flotemersch et al. 2001, Jones and Davy-Bowker 2014). Moreover, artificial substrate replicates may be lost in high flow events, and their use may be cost-prohibitive as two site visits (separated by a span of weeks) are required for deployment and retrieval (Blocksom and Flotemersch 2005).

Hester-Dendy (HD) samplers were deployed in the Hay River and Slave River in the first two years of sampling to investigate whether representative assemblages were collected by this method. In 2018, six samplers were deployed in each of four reaches that were selected in the Hay River and Slave River (HD reaches differed from those selected for kick sampling; Figure 4; Figure 5), resulting in an initial *n* of 24 Hester-Dendy samplers per river. Hester-Dendy samplers were deployed August 8-9 in the Hay River and August 7 in the Slave River. Samplers were collected from the Hay River 21-28 days after deployment (August 30 or September 5-6), and from the Slave River 32-33 days after deployment (September 8-9). Samplers were retrieved, invertebrates were removed from the samplers, and BMI samples were preserved in 95% ethanol for identification in the lab. In some reaches, samplers were tangled by high flows (Slave River), or were presumably washed away due to high flow and/or debris, resulting in a final *n* of 23 samples in the Hay River and 17 samples in the Slave River.

2.4. Data Analysis

2.4.1. Characterization of reaches – monitoring protocols

2.4.1.1. Spatial variation in abiotic parameters and biotic metrics among reaches

Variability in water chemistry, sediment chemistry, physical habitat (e.g., substrate size, velocity, etc.), and BMI assemblage composition was summarized for the Hay River and Slave River in a series of tables showing the mean ± standard deviation for chemical parameters or biotic metrics. Biotic metrics included those commonly used in biomonitoring (total abundance; total taxonomic richness; abundance, relative abundance, and richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT; mayflies, stoneflies, and caddisflies), Chironomidae (midges), Diptera (true flies, including midges) + Oligochaeta (segmented worms), and Mollusca. Calculations of richness (total taxonomic richness, EPT richness, Chironomidae richness, Diptera + Oligochaeta richness, and Mollusca richness) were based on the number of unique taxa identified at the lowest practical taxonomic level. Water chemistry and sediment chemistry means were compared with CCME water and sediment quality guidelines, respectively (Canadian Council of Ministers of the Environment 2001b, a). Chemistry data for Hay River were further compared with interim water quality triggers developed for the river (Stantec Consulting Ltd. 2016). However, it should be noted that as chemistry samples represented only spot measurements, any exceedances of guidelines or triggers should be interpreted with caution, as they may not reflect long-term trends.

Among-reach variation in water chemistry and BMI assemblage composition was assessed separately for Hay River and Slave River using a one-way analysis of variance (ANOVA) design, with an abiotic parameter or biotic metric as the response variable and the reach as the grouping factor. This analysis was completed to identify any differences along the extent of the river, to characterize the degree of spatial variability among reaches. Because sediment chemistry samples were only taken at two sites per

reach, only summary statistics are provided. For water chemistry, the analysis focused on a selection of major ions, nutrients, physicals, and metals that displayed some variation among sites and where values were above detection limit for at least half of the sites (e.g., total suspended solids, total and dissolved nitrogen, total phosphorus, conductivity, pH, aluminum, iron). For the purpose of this analysis, values below the detection limit were changed to be half the detection limit. Analysis of BMI data used the previously-defined metrics as response variables. When a water chemistry or BMI ANOVA indicated a significant difference among reaches, a Tukey HSD post-hoc test was used to determine which reaches differed. Water chemistry parameters and metrics were log_{10}- or logit-transformed as needed to meet the assumptions of ANOVA. All ANOVAs were run in Systat12 (Version 12.02).

2.4.1.2. Multivariate assessment of spatial variation among reaches

Multivariate analysis was used to fully characterize the biotic assemblage and abiotic environment of each river using all measured parameters. This analysis was intended to estimate variability within and among reaches, and to identify any potential ecological outliers (e.g., sites with extremely high axis scores) or gaps in sample sites. Water chemistry and physical habitat parameters measured at all sites were used to assess variation and identify major gradients in the abiotic environment through Principal Component Analysis (PCA) with standardization of variable scores. Because water chemistry samples were only collected at odd-numbered sites, analysis was run first using only data from odd-numbered sites, and second using physical habitat data from all sites and average water chemistry data for even-numbered sites to fill gaps in data collection (e.g., site 2 used the average of sites 1 and 3, and site 4 used the average of sites 3 and 5 for each water chemistry parameter). PCA ordinations were visually similar, with little difference in site placement or axis scores depending on which method was used, and so only the results with three sites were presented to avoid drawing conclusions about sites where data were not collected. A separate PCA was run on sediment chemistry data (collected at two sites per reach) to characterize sediment chemistry differences among reaches. Prior to analysis, all abiotic parameters were log_{10}- or logit-transformed as appropriate.

BMI relative abundance data were summarized for multivariate analysis at the family/subfamily level, with Chironomidae at subfamily and all other taxa at family or higher (as this level has been shown to be sufficient to characterize Arctic river BMI data while reducing noise from more detailed taxonomy; Lento et al. 2013, Culp et al. 2019). Taxa identified to genus level were combined at the family/subfamily level, and those identified to a coarser level (e.g., order or higher) were retained if they were unique (i.e., not identified at family/subfamily or genus level in any sample from the river). Spatial variation in community structure among sites was assessed using PCA. The relationship between the BMI data and abiotic data was tested with Redundancy Analysis (RDA), with a subset of abiotic parameters (water chemistry and physical habitat) selected for inclusion based on their importance in the abiotic PCA. Because there were BMI data for all 5 sites in each reach, this analysis used average water chemistry values for site 2 and site 4 in each reach (e.g., site 2 used the average of sites 1 and 3, and site 4 used the average of sites 3 and 5 for each water chemistry parameter). A separate RDA was run for BMI data and sediment chemistry data collected from the same sites (two sites per reach), in order to assess the relationship between assemblage composition and sediment chemistry. Prior to analysis, all abiotic parameters were log_{10}- or logit-transformed as appropriate, and all BMI data were $log_{10}(x+1)$ transformed. Multivariate analyses were run in Canoco (version 4.05; ter Braak and Šmilauer 2002)

2.4.1.3. Preliminary assessment of temporal variation

Pie charts of the average relative abundance of major invertebrate groups across all reaches were used to compare composition between 2017 and 2018 samples for the Hay River and Slave River. These plots were used for a visual assessment of major changes that occurred between sampling years. Bar graphs were used to compare abundance at the site scale for taxonomic groups that showed a large change between 2017 and 2018.

Although formal tests of temporal trends cannot be completed with two years of data, it is possible to compare the chemical/physical habitat and biotic assemblages between years using Procrustes analysis. Procrustes analysis can be used to determine whether two ordinations (e.g., PCAs) are more similar than could occur by chance. One ordination (the rotational ordination) is rotated and stretched to best match the other ordination (the target ordination) and the fit of the two ordinations is assessed using the sum of squared residuals (m_{12}^2) for sample points (Jackson 1995). A randomization test is run with the analysis by comparing 999 random configurations of the sample points with the target ordination, and a significant result (at $\alpha = 0.05$) indicates that the target and rotational ordinations are more similar than could occur by chance. This analysis was completed for abiotic data (water chemistry and physical habitat) and biotic data using the vegan package (Oksanen et al. 2015) in R version 3.6 (R Development Core Team 2015).

Where possible and practical, biotic metric values were also compared between years using a repeated measures ANOVA, with reach as a factor and year as the repeated measure. If the analysis resulted in a significant interaction between reach and year (indicating that metric values increased in some reaches and decreased in others), reaches were grouped by positive or negative change over time and separate paired *t*-tests were used to compare years. Repeated measures ANOVA and paired *t*-tests were completed in Systat (Version 12.02).

2.4.2. Characterization of reaches – Hester-Dendy samples

Hester-Dendy samples were allocated to four reaches within each river, although these reaches were different from those that were used for the kick sampling protocol (though some were located in close proximity; Figure 5). A detailed comparison between Hester-Dendy samples and kick samples was completed using the 2017 sampling data (see details in Lento 2018a). Because that assessment clearly Hester-Dendy samplers collected a restricted portion of the BMI community compared to kick samples, analysis of 2018 Hester-Dendy data was focused on characterization of spatial differences among these passively-collected samples. Loss of samplers resulted in an unbalanced sample design for each river, with low numbers of samples in some reaches ($n < 3$). Biotic metrics were compared statistically among reaches using a one-way ANOVA design; however, due to low power in these analyses, the primary focus was on visual comparison of box plots of biotic metrics and multivariate assessment of Hester-Dendy samples, with relative abundance of BMI data at the family/subfamily level analyzed using PCA. Prior to analysis, all BMI relative abundance data were $\log_{10}(x+1)$ transformed.

2.4.3. Assessment of normal range and biomonitoring plan design

CES makes use of the variation among samples to determine if test samples are impaired (i.e., if they fall outside the normal range, or range of natural variability). Where sampling areas are at reference condition (unimpacted), samples above or below CES may have different habitat conditions (such as differences in substrate composition) that cause BMI assemblage differences. The CES is based on

variability in the data, and changes in habitat conditions that result from natural variability (i.e., due to shifts in flow, timing of the spring freshet, water temperature, etc.) may lead to different normal ranges from one year to the next.

During the initial two years of monitoring, CES was developed spatially using all sites sampled in the river. CES limits were determined for the Hay River and Slave River by calculating the mean and standard deviation of each BMI metric and setting bounds of CES equal to the mean ± 2 SD, following the approach of previous BMI monitoring programs (see Munkittrick et al. 2009). In 2017, the CES was calculated based on metric values from that year. For the 2018 data, BMI metric values were initially compared with CES limits calculated from the 2018 data alone, to identify any sites with extreme values for the metrics relative to the other samples from the same year. The 2018 BMI metrics were then compared with CES limits calculated from the 2017 and 2018 data combined, as an initial assessment of normal range across all reaches in the river.

After three years of data have been collected, temporal CESs can begin to be determined for each site or reach, representing the normal range of variability over time at that specific location (Arciszewski and Munkittrick 2015). For the BMI monitoring plan in the Hay and Slave rivers, where the end goal is to be able to detect impacts from upstream land use when they occur, reach-specific temporal CESs will allow for the determination of the magnitude of change required at that location to trigger additional sampling or investigation of possible impacts. These location-specific normal ranges will capture the natural inter-annual variability within the system, and can be adjusted with the addition of new data and with shifts in normal range that occur as a result of climate change. Normal range across all reaches in the river will continue to be assessed with CES limits based on the full set of data, but it is expected that these limits will have wider bounds than reach-specific CESs due to variation among reaches.

As an assessment of the monitoring plan design, the number of samples collected for water chemistry analysis was evaluated by determining the level of variability among sites in each reach. Sample size for water quality analysis was reduced from five samples per reach (one sample per site) to three samples per reach (one sample per odd-numbered site) in 2018. This change was made to reduce the costs of monitoring, as it allowed for less lab processing of samples. Further reduction of sample size, if considered, requires precision among samples (i.e., strong similarity in estimated parameter values among sites). The variability among samples was tested by assessing the coefficient of variation among samples. Following guidelines described by the Canadian Council of Ministers of the Environment (2011), the coefficient of variation (CV; the standard deviation divided by the mean) for each water quality parameter was calculated for each reach in 2017 and 2018. When the CV was less than 18%, this was taken to indicate low variability among samples in a reach (e.g., samples are essentially duplicates), as long as the mean value for the parameter was greater than 10 times the detection limit.

3. Results and Discussion

3.1. Characterization of reaches – monitoring protocols

3.1.1. Hay River

3.1.1.1. Water chemistry

Water chemistry samples were collected at three kick-sites to characterize BMI habitat and variability within and among reaches. These samples represented spot measurements of water chemistry conditions at the time of sampling, and were collected at three sites in each reach to account for local-

scale variability in BMI assemblages in response to the chemical environment. Although kick-sites were generally close together in a reach (50-100 m apart), some degree of variation in water chemistry might exist due to differences in velocity or groundwater seepage among sites, and this could impact the abundance and diversity of invertebrates collected at a site. Due to the meandering nature of the river channel, several reaches were on river bends, which led to variation in velocity between sites, and may have contributed to variability in water chemistry within reaches. However, the greatest level of variation in water chemistry was expected to be evident among reaches, which were farther separated geographically along the river. The longitudinal gradient of Hay River reaches extended from Reach 1 at the south (upstream) to Reach 6 at the north (downstream; Figure 4A). Reaches 4, 5, and 6 were located downstream of the boat launch (upstream sites on Reach 4 were located on the bank opposite the boat launch) and downstream of the inflow from two tributaries, which may have introduced some variability in water chemistry relative to reaches upstream of the launch. Analyses considered variation within and among reaches to account for differences due to reach location and location of sites within reaches.

3.1.1.1.1. Major ions, nutrients, and physicals

Three water samples were collected in each river reach (one sample per odd-numbered site) and analyzed for major ions, nutrients, and physicals. Mean levels of ions and a selection of nutrients (Table 2) were compared with Canadian guidelines for short-term and long-term exposure to identify any reaches where water chemistry was indicative of poor water quality (Canadian Council of Ministers of the Environment 2001b). Mean values for ions and nutrients did not exceed CCME guidelines for the protection of aquatic life for any reaches in the Hay River. Reaches were classified as mesotrophic based on mean total phosphorus (TP) values, which ranged from 0.022 to 0.031 mg/L (Canadian Council of Ministers of the Environment 2001b). This result is consistent with the trophic status of the Hay River reaches in 2017 and previous analyses (e.g., Stantec Consulting Ltd. 2016, where the Hay River was considered mesotrophic to eutrophic).

Water chemistry parameters were compared with interim management trigger levels for the Hay River, which were previously established based on water chemistry data collected from 1989 to 2014 (Stantec Consulting Ltd. 2016). These triggers were developed to identify values outside of the normal range (here quantified by the 50^{th} and 90^{th} percentiles) for the period of record. Management triggers are based on long-term data from the water body in question, and therefore represent site-specific guidelines. In 2018, spot measurements of water chemistry from the monitoring program exceeded management triggers for the Hay River for pH, alkalinity, conductivity, and two major ions: calcium and magnesium (Table 2). These values represent spot measurements of water chemistry, and higher levels of these parameters were generally evident across all reaches, which suggests that there were no

Table 2. Summary of ion, nutrient, and physical water chemistry parameters sampled in the Hay River at six sample reaches, indicating site mean ± standard deviation for each reach. When all sites in a reach were below detection limit, the detection limit is presented. When only a subset of sites in a reach was below detection limit, half the detection limit was used in calculations (number of sites below detection limit indicated in Parameter column). Values in red were greater than the 90th percentile interim trigger identified for the Hay River Border site (Stantec Consulting Ltd. 2016). Note that HR-KS6 was sampled nearly one week after the other reaches.

Parameter	HR-KS1	HR-KS2	HR-KS3	HR-KS4	HR-KS5	HR-KS6
Alkalinity (mg/L) (2 below DL)	137.7 ± 1.5	134.7 ± 0.6	135.7 ± 0.6	135.0 ± 0.0	137.0 ± 0.0	139.3 ± 0.6
Ammonia (mg/L)	0.034 ± 0.005	0.036 ± 0.002	0.026 ± 0.002	0.039 ± 0.013	0.019 ± 0.002	0.047 ± 0.078
Calcium (mg/L)	50.4 ± 3.7	49.5 ± 2.8	49.6 ± 2.5	50.1 ± 5.4	49.1 ± 2.6	54.8 ± 1.2
Chloride (mg/L)	3.93 ± 0.35	3.33 ± 0.06	3.40 ± 0.00	3.50 ± 0.00	4.03 ± 0.06	4.23 ± 0.06
Specific Conductivity (µS/cm)	443.7 ± 10.3	418.7 ± 1.5	430.7 ± 1.5	430.7 ± 0.6	439.3 ± 1.2	435.3 ± 1.2
Hardness (mg/L)	215.0 ± 24.5	200.3 ± 9.6	204.3 ± 11.6	209.0 ± 17.6	216.7 ± 16.8	218.0 ± 4.4
Magnesium (mg/L)	21.63 ± 5.35	18.57 ± 0.95	19.50 ± 1.51	20.33 ± 1.10	22.90 ± 2.60	19.73 ± 1.25
Dissolved N (mg/L)	0.770 ± 0.010	0.757 ± 0.006	0.753 ± 0.012	0.753 ± 0.023	0.747 ± 0.012	0.673 ± 0.012
Total N (mg/L)	0.813 ± 0.015	0.820 ± 0.017	0.817 ± 0.006	0.817 ± 0.012	0.810 ± 0.017	0.753 ± 0.006
DOC (mg/L)	24.90 ± 0.26	25.37 ± 0.06	25.23 ± 0.15	25.03 ± 0.32	25.73 ± 0.57	24.17 ± 0.12
TOC (mg/L)	25.63 ± 0.32	26.13 ± 0.12	26.03 ± 0.15	25.63 ± 0.12	26.60 ± 0.10	25.07 ± 0.12
pH	8.19 ± 0.01	8.20 ± 0.02	8.26 ± 0.04	8.24 ± 0.01	8.23 ± 0.00	8.27 ± 0.02
Total P (mg/L)	0.026 ± 0.002	0.024 ± 0.003	0.024 ± 0.003	0.031 ± 0.004	0.025 ± 0.001	0.022 ± 0.001
Potassium (mg/L)	1.60 ± 0.35	1.33 ± 0.49	1.70 ± 0.17	1.87 ± 0.15	1.57 ± 0.25	2.00 ± 0.10
Sodium (mg/L)	14.37 ± 2.34	12.80 ± 1.30	11.00 ± 1.08	13.37 ± 3.69	12.90 ± 1.51	12.90 ± 1.21
TDS (mg/L)	281.3 ± 9.0	269.3 ± 6.1	262.0 ± 8.0	267.3 ± 8.1	290.0 ± 14.0	302.0 ± 4.0
TSS (mg/L) (10 below DL)	5.0 ± 6.1	3.7 ± 3.8	3.8 ± 2.3	6.7 ± 3.1	1.5 ± 0.0	3.7 ± 3.8
Sulphate (mg/L)	80.0 ± 3.5	73.7 ± 0.6	75.0 ± 0.0	75.0 ± 0.0	77.0 ± 0.0	79.3 ± 0.6
Turbidity (NTU)	6.7 ± 0.5	6.2 ± 0.2	6.6 ± 1.1	9.8 ± 2.4	7.4 ± 0.6	7.1 ± 0.3

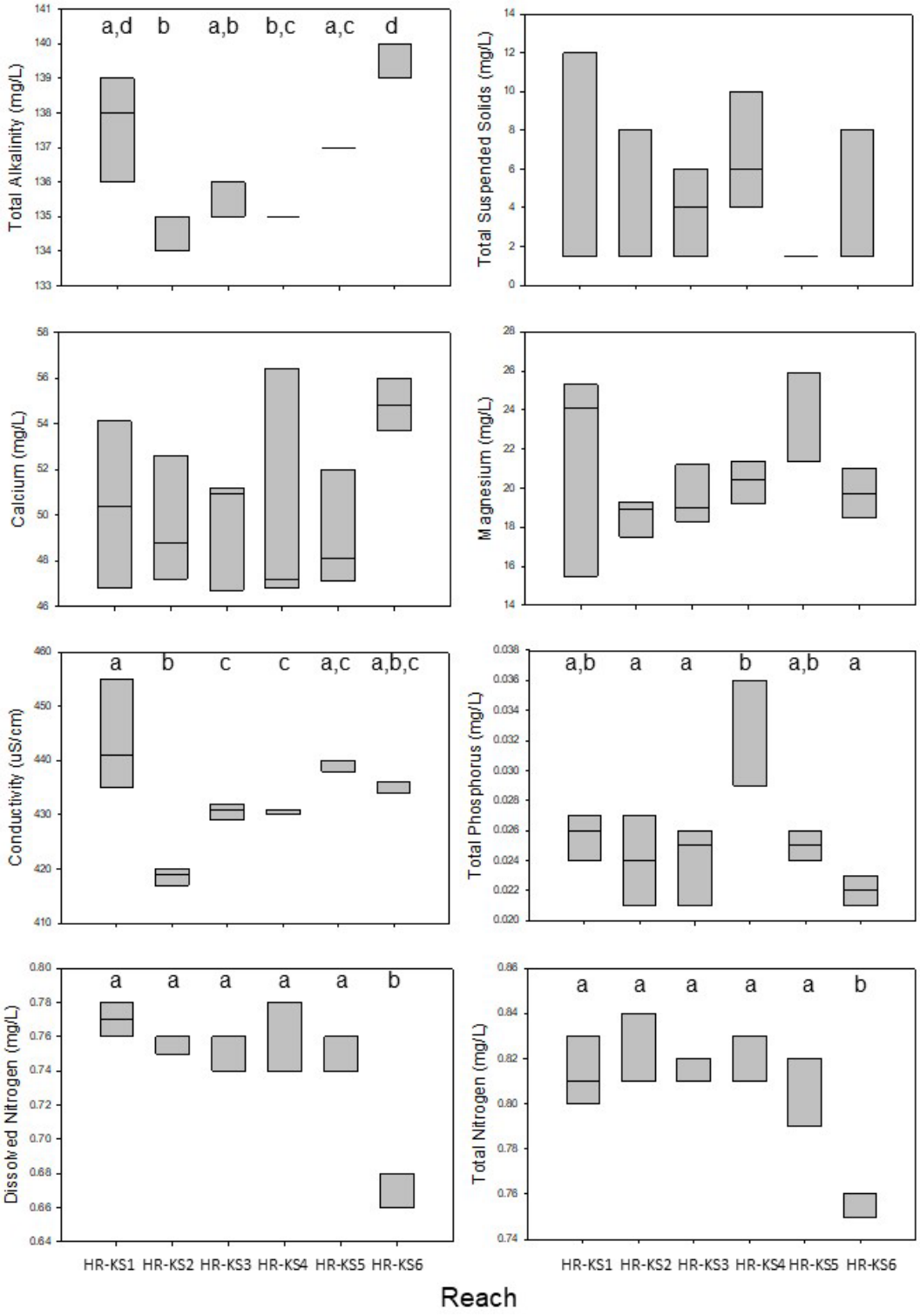

Figure 7. Box plots of ions, nutrients, and physicals water chemistry concentrations for all reaches sampled in Hay River. Plotted parameters include alkalinity, TSS, calcium, magnesium, conductivity, TP, DN, and TN. Lines through boxes indicate the median and lower and upper bounds plot the 25th and 75th percentiles, respectively. Letters on plots indicate significant differences ($\alpha = 0.05$) from one-way ANOVA and Tukey HSD tests. Note that HR-KS6 was sampled nearly one week after the other reaches.

specific localized sources within the sampling extent of the river.

The number of samples collected per reach decreased in 2018 (from five samples per reach to three), and as a result, variability was higher (e.g., standard deviations were higher) within reaches than was observed in 2017 (Lento 2018a). However, for most parameters, variability was fairly low among samples collected in a single reach, and as a result, standard deviations were low (Table 2). The greatest variability between sites was evident in Reach 1 for conductivity, hardness, magnesium, total suspended solids (TSS), and sulphate, and in Reach 4 for calcium, sodium, and total phosphorus (TP; Table 2, Figure 7).

Variation in ions, nutrients, and physicals was fairly low among reaches, but because within-reach variability was so low, there was high power to detect statistically significant differences among reaches (at α = 0.05) even if differences were not large. For example, Reach 1 differed with respect to conductivity, which was statistically significantly higher in that reach than in Reach 2, 3, or 4 (Figure 7). But the difference in mean conductivity between these reaches ranged from 13 to 25 µS/cm, which may not be large enough to be biologically meaningful. Mean TP was statistically significantly higher in Reach 4 than in several other reaches (Figure 7), but this reach also had higher TSS concentrations, suggesting a higher contribution of phosphorus bound to sediments (Sanderson et al. 2012).

Reach 6 was added to the monitoring plan in 2018, and one goal of this assessment was to evaluate suitability of the reach for long-term monitoring. The reach was similar to other reaches with respect to a number of parameters including conductivity, hardness, magnesium, and TSS (Table 2, Figure 7). However, Reach 6 had higher alkalinity and calcium than several other reaches (though not statistically significant in the case of calcium), and most notably, it had statistically significantly lower levels of dissolved nitrogen (DN) and total nitrogen (TN) than all other reaches (Figure 7). However, Reach 6 was sampled nearly one week after the other reaches, and as these represent spot measurements of water chemistry, more data will be required to confirm any differences in the chemical habitat of this downstream reach.

3.1.1.1.2. Metals

Dissolved and total metals were tested in water quality samples collected at three sites per reach (sites 1, 3, and 5) to further characterize the chemical habitat in the water column at the time of sampling. Levels of metals are related to the geology of a watershed, though concentrations of some metals may become elevated with upstream disturbance and have the potential to indicate anthropogenic impacts. Dissolved metals in water are more biologically available to BMI, whereas total metals include those bound to sediments, and may not represent relevant exposure levels for organisms. Some metals, such as aluminum and mercury, are known to be harmful to aquatic organisms at elevated levels, and mercury in particular can accumulate up the food web, impacting the fish that feed on BMI.

Dissolved metals had generally low variability within reaches (between kick-sites), though several metals including dissolved aluminum and dissolved iron had high variability in Reach 1 (Table 3, Figure 8). Dissolved manganese varied within reaches, particularly in Reach 1 and Reach 4, but also differed among reaches, as Reach 4 had a significantly higher mean than Reach 2 (Table 3, Figure 8). Though some dissolved metals varied among reaches, none were found to exceed CCME guidelines for the protection of aquatic life (guidelines for long-term exposure to total metals).

Interim triggers (50th and 90th percentile, based on long-term data) were identified for metals in water samples from the Hay River by Stantec Consulting Ltd. (2016), although it was noted that these trigger levels should be

Table 3. Summary of metal water chemistry parameters sampled in the Hay River at six sample reaches, indicating site mean ± standard deviation (for 2 or more samples) for each reach. When all sites in a reach were below detection limit, the detection limit is indicated. When only a subset of sites in a reach was below detection limit, half the detection limit was used in calculations (number of sites below detection limit indicated in Parameter column). Dissolved metal values were excluded when they exceeded total metals. Values in bold were greater than CCME long-term exposure guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment 2001b), and values in red were greater than the 90th percentile interim trigger identified for the Hay River Border site (Stantec Consulting Ltd. 2016). Note that HR-KS6 was sampled nearly one week after the other reaches.

Parameter	HR-KS1	HR-KS2	HR-KS3	HR-KS4	HR-KS5	HR-KS6
Aluminum Diss. (µg/L)	7.70 ± 7.97	3.27 ± 0.38	3.53 ± 0.31	3.40 ± 0.10	3.27 ± 0.12	3.67 ± 0.83
Aluminum Total (µg/L)	83.5 ± 32.7	**157.9 ± 67.5**	**113.8 ± 26.0**	**178.7 ± 68.0**	**112.7 ± 9.5**	86.4 ± 2.2
Antimony Diss. (µg/L)	0.100 ± 0.000	0.100 ± 0.000	0.100 ± 0.000	0.100 ± 0.000	0.100 ± 0.000	0.100 ± 0.000
Antimony Total (µg/L)	0.17 ± 0.06	0.13 ± 0.06	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Arsenic Diss. (µg/L)	0.600 ± 0.000	0.600 ± 0.000	0.533 ± 0.058	0.600 ± 0.000	0.600 ± 0.000	0.500 ± 0.000
Arsenic Total (µg/L)	0.93 ± 0.06	0.93 ± 0.06	0.93 ± 0.06	1.00 ± 0.00	0.90 ± 0.00	0.87 ± 0.06
Barium Diss. (µg/L)	50.6 ± 0.8	48.6 ± 0.3	49.0 ± 0.7	50.0 ± 1.1	48.9 ± 0.2	48.8 ± 0.7
Barium Total (µg/L)	52.3 ± 0.6	53.0 ± 1.2	54.3 ± 1.4	56.3 ± 2.0	53.6 ± 0.6	52.5 ± 2.5
Beryllium Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Bismuth Total (µg/L)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Boron Diss. (µg/L)	41.0 ± 2.0	38.8 ± 0.3	39.4 ± 0.3	38.8 ± 0.6	39.7 ± 0.1	40.4 ± 1.1
Boron Total (µg/L)	41.0 ± 1.0	39.5 ± 0.3	40.6 ± 0.8	40.2 ± 1.0	40.6 ± 0.7	41.0 ± 2.0
Cadmium Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cesium Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Chromium Diss. (µg/L) (9 below DL)	< 0.1	0.20	0.10 ± 0.00	< 0.1	< 0.1	0.07 ± 0.03
Chromium Total (µg/L)	0.20 ± 0.10	0.30 ± 0.10	0.23 ± 0.06	0.33 ± 0.06	0.23 ± 0.06	0.20 ± 0.00
Cobalt Diss. (µg/L)	0.20 ± 0.00	0.25 ± 0.01	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00
Cobalt Total (µg/L)	0.20 ± 0.00	0.27 ± 0.06	0.20 ± 0.00	0.33 ± 0.06	0.27 ± 0.06	0.23 ± 0.06
Copper Diss. (µg/L)	1.87 ± 0.06	2.07 ± 0.15	1.90 ± 0.00	1.90 ± 0.14	1.77 ± 0.06	1.77 ± 0.06
Copper Total (µg/L)	2.07 ± 0.06	2.23 ± 0.15	2.17 ± 0.06	2.23 ± 0.06	2.03 ± 0.06	2.00 ± 0.10
Iron Diss. (ug/L)	84.3 ± 58.6	57.3 ± 10.7	54.0 ± 7.2	59.3 ± 3.1	64.7 ± 11.7	49.0 ± 6.6
Iron Total (µg/L)	**769.3 ± 35.1**	**821.3 ± 97.1**	**809.7 ± 42.3**	**1024.0 ± 140.6**	**944.7 ± 12.7**	**911.7 ± 47.5**
Lead Diss. (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Lead Total (µg/L)	0.20 ± 0.00	0.23 ± 0.06	0.20 ± 0.00	0.23 ± 0.06	0.20 ± 0.00	0.13 ± 0.06
Lithium Diss. (µg/L)	N/A	14.1 ± 0.1	14.3 ± 0.2	14.1 ± 0.3	15.2 ± 0.1	15.2 ± 0.0
Lithium Total (µg/L)	14.5 ± 0.2	14.2 ± 0.1	14.6 ± 0.3	14.3 ± 0.4	15.3 ± 0.2	15.1 ± 0.8
Manganese Diss. (µg/L)	23.8 ± 6.9	19.2 ± 0.9	24.2 ± 3.2	32.7 ± 7.6	30.1 ± 0.2	29.3 ± 0.5
Manganese Total (µg/L)	47.4 ± 5.9	49.7 ± 3.8	49.7 ± 3.0	58.0 ± 9.2	52.2 ± 1.5	44.7 ± 2.8
Mercury Total (UL) (ng/L)	1.67 ± 0.12	1.80 ± 0.17	1.70 ± 0.10	1.97 ± 0.31	1.70 ± 0.10	1.43 ± 0.06
Molybdenum Diss. (µg/L)	N/A	1.40	1.43 ± 0.06	1.40	1.43 ± 0.06	1.47 ± 0.06
Molybdenum Total (µg/L)	1.40 ± 0.00	1.43 ± 0.06	1.50 ± 0.00	1.47 ± 0.06	1.47 ± 0.06	1.47 ± 0.06
Nickel Diss. (µg/L)	2.70 ± 0.00	N/A	2.80 ± 0.00	2.60	2.63 ± 0.06	2.77 ± 0.12
Nickel Total (µg/L)	2.70 ± 0.00	2.90 ± 0.10	2.90 ± 0.17	2.97 ± 0.12	2.90 ± 0.00	2.80 ± 0.17
Rubidium Diss. (µg/L)	1.10 ± 0.00	1.10 ± 0.00	1.17 ± 0.06	1.03 ± 0.06	1.10 ± 0.00	1.00 ± 0.00
Rubidium Total (µg/L)	1.17 ± 0.06	1.33 ± 0.15	1.30 ± 0.10	1.40 ± 0.17	1.30 ± 0.00	1.13 ± 0.12

Parameter	HR-KS1	HR-KS2	HR-KS3	HR-KS4	HR-KS5	HR-KS6
Selenium Diss. (µg/L) (10 below DL)	0.35 ± 0.18	0.23 ± 0.14	0.20 ± 0.09	0.27 ± 0.20	0.27 ± 0.20	0.32 ± 0.18
Selenium Total (µg/L) (14 below DL)	< 0.5	< 0.5	0.37 ± 0.20	< 0.5	0.40 ± 0.26	0.48 ± 0.20
Silver Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Strontium Diss. (µg/L)	N/A	157.7 ± 0.6	160.0 ± 2.0	158.3 ± 1.5	163.0 ± 0.0	170
Strontium Total (µg/L)	161.0 ± 2.6	157.7 ± 0.6	163.3 ± 3.1	160.7 ± 2.1	164.7 ± 1.5	167.7 ± 9.3
Thallium Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Tin Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Titanium Diss. (µg/L) (14 below DL)	0.33 ± 0.49	0.07 ± 0.03	0.08 ± 0.03	< 0.1	< 0.1	< 0.1
Titanium Total (µg/L)	3.47 ± 2.38	5.83 ± 1.33	4.87 ± 1.08	4.97 ± 0.83	4.53 ± 0.71	3.63 ± 0.35
Uranium Diss. (µg/L)	0.90 ± 0.00	0.87 ± 0.06	0.90 ± 0.00	0.90 ± 0.00	0.87 ± 0.06	0.93 ± 0.06
Uranium Total (µg/L)	0.90 ± 0.00	0.90 ± 0.00	0.90 ± 0.00	0.90 ± 0.00	0.90 ± 0.00	0.97 ± 0.06
Vanadium Diss. (µg/L) (4 below DL)	0.17 ± 0.06	< 0.1 ± 0.00	0.20 ± 0.00	0.15 ± 0.09	0.17 ± 0.06	0.17 ± 0.06
Vanadium Total (µg/L)	0.63 ± 0.15	0.97 ± 0.38	0.70 ± 0.10	0.93 ± 0.15	0.67 ± 0.06	0.60 ± 0.00
Zinc Diss. (µg/L) (17 below DL)	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	0.40 ± 0.35
Zinc Total (µg/L)	< 5	< 5	< 5	< 5	< 5	< 5

interpreted with caution (particularly for dissolved metals), due to a high number of readings below detection limits and low number of readings for some parameters. Mean values of dissolved and total metals from the 2018 Hay River water chemistry samples were compared with these interim triggers to identify any parameters that were outside the long-term normal range for the river. Molybdenum was the only dissolved metal to exceed the 90th percentile interim triggers for the Hay River. However, the exceedances remained minor, and dissolved molybdenum levels remained well below CCME guidelines for the protection of aquatic life (73 µg/L; Canadian Council of Ministers of the Environment 2001b). Furthermore, exceedances in spot measurements of water chemistry may not be indicative of ongoing trends in the system. Total metals in 2018 water chemistry samples were highest and most variable in Reach 4, which is across from the boat launch (Figure 8). Downstream of this reach, total metals (aluminum in particular) were less variable.

Despite some variability within reaches, mean values were similar across most reaches for a number of metals including total aluminum and total manganese (Table 3, Figure 8). However, iron appeared to be elevated in the three downstream reaches (Reach 4, 5, and 6), and Reach 4 was found to have statistically significantly higher levels of total iron than Reach 1 or Reach 3 (Figure 8). Reach 4 also had higher total mercury levels than other reaches, with a statistically significantly higher mean than Reach 6 (Table 3). However, total metal concentrations were generally lower in all reaches in 2018 compared to 2017 (Lento 2018a), indicating some inter-annual variability in these parameters that may have been related to differences in flow and water levels in 2018 (Figure 2A).

Despite the elevated levels of total mercury in Reach 4, concentrations across all reaches in 2018 were well below the CCME guideline for the protection of aquatic life (26 ng/L; Canadian Council of Ministers of the Environment 2001b). There were high levels of other total metals, however, that exceeded the long-term exposure guidelines. Total aluminum exceeded the guideline of 100 µg/L in Reaches 2, 3, 4, and 5, but was

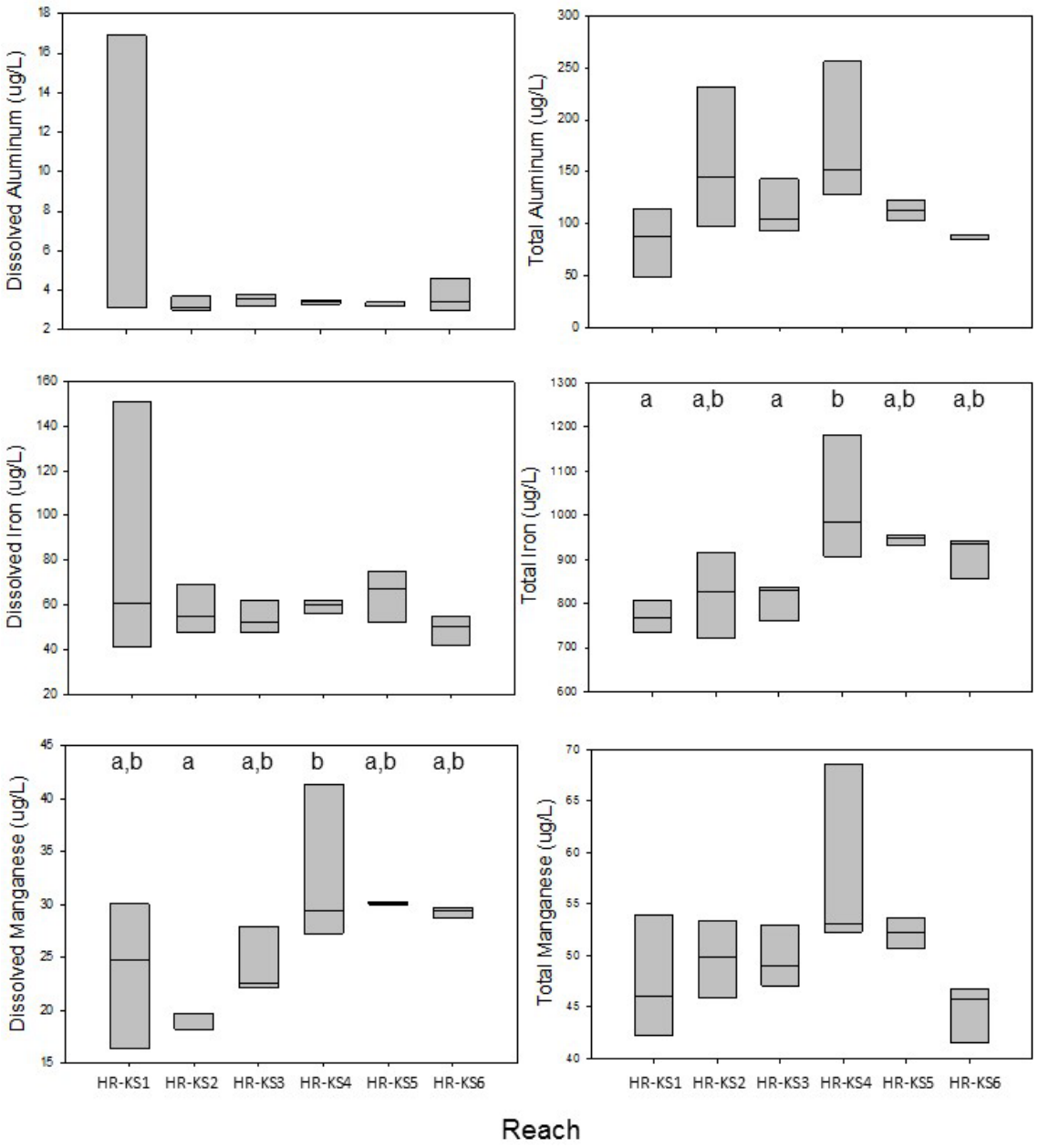

Figure 8. Box plots of dissolved and total metal concentrations for all reaches sampled in Hay River. Plotted is (left column, top to bottom) dissolved aluminum, dissolved iron, dissolved manganese, and (right column, top to bottom) total aluminum, total iron, and total manganese, all measured in ug/L. Lines through boxes indicate the median and lower and upper bounds plot the 25th and 75th percentiles, respectively. Letters on plots indicate significant differences ($\alpha = 0.05$) from one-way ANOVA and Tukey HSD tests. Note that HR-KS6 was sampled nearly one week later than other reaches.

below guidelines in the furthest upstream and downstream reaches (Table 3). Iron was higher than CCME guidelines (300 µg/L; Canadian Council of Ministers of the Environment 2001b) in all reaches. The river has naturally high levels of some metals including iron, and the observed levels (ranging on average from 769 to 1024 mg/L) were much lower than those described in long-term monitoring of the Hay River (90th percentile for iron in open water season = 6434 µg/L; Stantec Consulting Ltd. 2016). As with dissolved metals, the 90th

Table 4. Physical habitat variables measured in the Hay River in 2018, summarized by reach. Velocity (spot measurement), bankfull width, and wetted width are presented as mean ± standard deviation (calculated based on 5 sites per reach); dominant streamside vegetation and periphyton coverage presented as the most common category in each reach across 5 sites; substrate composition presented as the sum of rock counts for each reach (20 rocks measured per site). Note that HR-KS6 was sampled nearly one week after the other reaches.

Parameter	HR-KS1	HR-KS2	HR-KS3	HR-KS4	HR-KS5	HR-KS6
Velocity (m/s)	0.18 ± 0.08	0.21 ± 0.18	0.17 ± 0.10	0.17 ± 0.09	0.18 ± 0.09	0.14 ± 0.13
Bankful width (m)	134.0 ± 8.8	121.9 ± 12.4	139.5 ± 21.9	129.5 ± 9.7	104.3 ± 14.2	120.2 ± 9.0
Wetted width (m)	88.0 ± 20.0	100.0 ± 10.4	92.0 ± 36.0	100.7 ± 17.5	67.7 ± 5.2	107.1 ± 10.3
Dominant streamside vegetation	shrubs and deciduous trees	deciduous trees	coniferous trees	deciduous trees	deciduous trees	coniferous trees
Periphyton coverage	1-5 mm thick, slippery, green to brown patches	0.5-1 mm thick, slightly slippery	0.5-1 mm thick, slightly slippery	0.5-1 mm thick, slightly slippery	0.5-1 mm thick, slightly slippery	1-5 mm thick, slippery, green to brown patches
Substrate - sand (%)	1	0	5	6	2	3
Substrate - gravel (%)	20	17	27	35	44	24
Substrate - pebble (%)	75	66	58	55	56	66
Substrate - cobble (%)	4	17	15	10	0	10
Substrate - boulder (%)	0	0	0	0	0	0
Substrate - bedrock (%)	1	0	0	0	0	0

percentile management trigger for total molybdenum was exceeded in all Hay River reaches (Table 3), but these values remained well below CCME guidelines. Total strontium levels in all reaches also exceeded the 90th percentile management trigger for the river (156 mg/L), but the magnitude of exceedance was small.

3.1.1.2. Physical habitat

Measurements were taken at each site to characterize they physical habitat in BMI sampling locations, including variables such as velocity, river width, streamside vegetation, in-stream periphyton cover, and substrate composition (Table 4). Velocity at the time of sampling was similar across all reaches (ranging on average between 0.14 and 0.18 m/s; Table 4), and deciduous or coniferous trees were generally the dominant type of streamside vegetation. Periphyton coverage was low in most reaches, though Reach 1 and Reach 6 both had thicker algal coverage with green to brown patches. Substrate composition was generally dominated by pebble or gravel in all reaches, though Reach 2 and Reach 3 had slightly higher percent composition of cobble (Table 4).

3.1.1.3. Characterization of chemical and physical habitat

Principal Component Analysis (PCA) was used as an exploratory analysis to characterize water chemistry and physical habitat conditions within and among reaches. In this analysis, a large suite of water chemistry parameters and field-measured habitat parameters was included to characterize the abiotic habitat for all sites, and the spatial arrangement of sites in the resulting PCA plot was used to identify abiotic gradients in the data

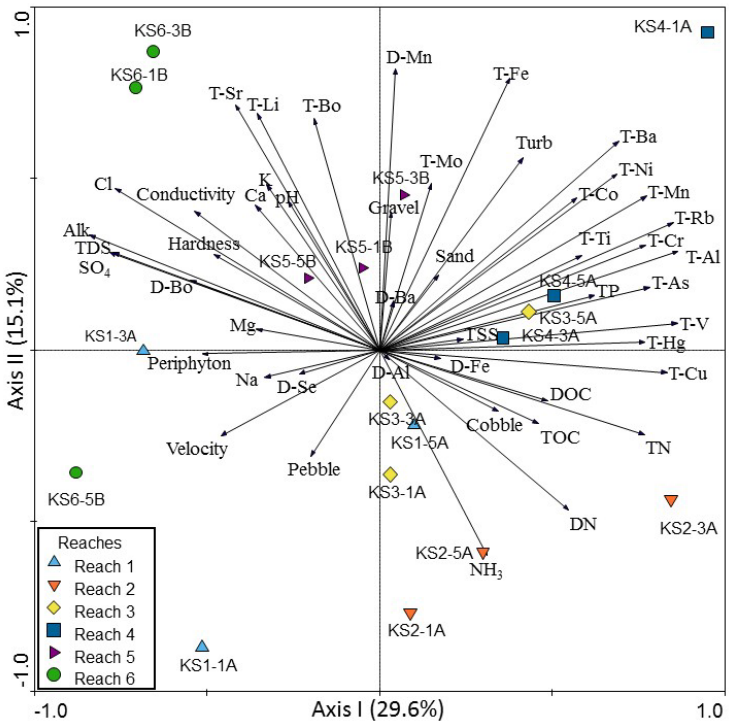

Figure 9. PCA ordination of water chemistry and habitat variables at Hay River kick-sites, with sites colour-coded based on reach number. Arrows point in the direction of increasing values of parameters, and correlations of sites with parameters are indicated by the location of kick-site points in proximity to arrows. Kick-site points located near the origin have similar correlations with all measured parameters." D- "in front of metals indicates dissolved form, and "T- "indicates total metals.

and important variables for biotic-abiotic analysis. In the ordination plot, sites located on the same end of gradient vectors had similar chemical and physical habitats, while those at opposite ends of gradient vectors were negatively correlated with respect to one or more abiotic parameters. The analysis is correlation-based, and thus the degree of separation among sites and parameters reflects differences in correlation, rather than differences in the magnitude of measured variables.

Though water chemistry values were generally quite similar across reaches of the Hay River (Table 2, Table 3), there was a degree of separation of sites in the PCA of water chemistry and physical habitat data that reflected correlations of sites with particular suites of parameters. The first axis of the PCA, which explained 29.6% of the variation among sites, separated sites that were positively correlated with metals and nutrients on the positive end of the axis from those that were positively correlated with velocity, periphyton, ions, and measures of ionic strength or buffering capacity (conductivity, hardness, and alkalinity; Figure 9). The second axis, which explained 15.1% of the variation among sites, was positively associated with gravel and sand, as well as turbidity and a selection of metals including dissolved manganese and total iron, and was negatively associated with pebble, cobble, and velocity, as well as ammonia and dissolved nitrogen (Figure 9). Sites in Reach 4 and sites KS3-5A and KS2-3A were positively associated with the first axis (metals and nutrients), though both KS4-1A (positively correlated with total iron and dissolved manganese, among other metals) and KS2-3A (positively correlated with nitrogen parameters) were intermediate to both axes I and II (Figure 9). Sites KS1-3A and KS6-5B were both found on the negative end of the first axis gradient, and were positively correlated with velocity, periphyton cover, pebbles, and ions and buffering capacity. The other sites in Reach 6 were also on this end of the first axis

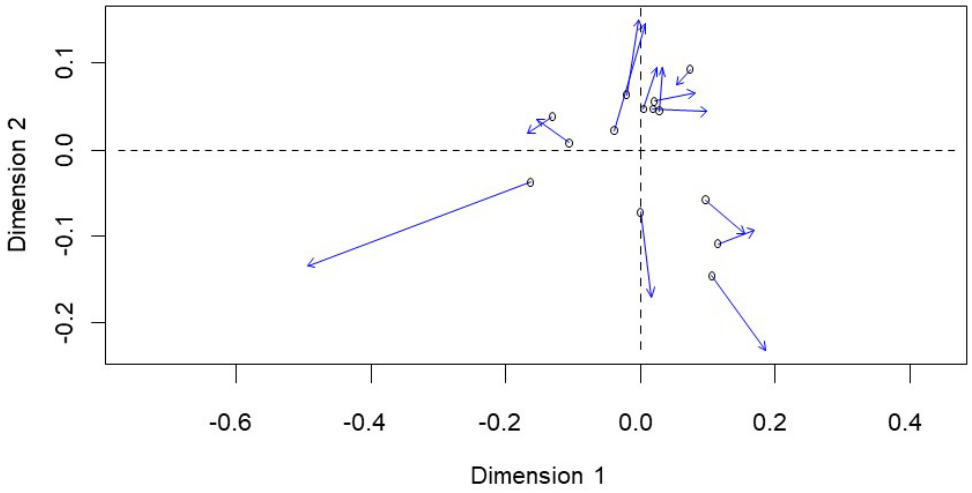

Figure 10 Residuals from Procrustes analysis of Hay River chemical/physical habitat ordinations from 2017 and 2018 using only odd-numbered sites in each reach, showing the position of sites in 2017 (circles) and the distance and direction moved in multivariate space in 2018 (arrows). The longer the arrow, the more a site moved between years, and the more different its chemical/physical habitat was from the sites it resembled in 2017.

gradient, but were more strongly correlated with conductivity, ions, and pH, and were also positively correlated with metals on the second axis (Figure 9). Other sites were more strongly associated with the second axis gradient. For example, all three sites in Reach 5 were positively correlated with the second axis, whereas remaining sites in Reach 1, Reach 2, and Reach 3 were positively correlated with nutrients on the negative end of the second axis gradient (Figure 9). Sites within a reach were generally not tightly clustered in the ordination plot, with the exception of Reach 5. However, overall variance explained by the first two axes was fairly low, reflecting the overall low variability within and among reaches with respect to water chemistry.

3.1.1.3.1. Temporal comparison

Robust temporal analysis of water chemistry trends requires many years of data, ideally with multiple samples taken per year, to ensure trends are not calculated based on spot measurements alone. With only two years of data and single measurements taken each year, there were not sufficient data for a comprehensive assessment of temporal trends in water chemistry within the Hay River. However, some comparison was made between chemical and physical habitat data collected in 2017 and 2018 to explore inter-annual variation and possible differences related to differing flow regimes at the time of sampling.

There were some similarities in the chemical environment of Hay River reaches between 2017 and 2018, including similar levels of TP and several ions (calcium, chloride, potassium, sodium). However, values of some water chemistry parameters were higher in 2018 than 2017, including specific conductivity, hardness, magnesium, and sulphate. In order to compare the chemical and physical habitat of the sample sites between 2017 and 2018, Procrustes analysis was used to test whether there were significant differences in the spatial arrangement of sites between ordinations of each year. This analysis used only sites sampled in both 2017 and 2018 (i.e., only the three odd-numbered sites in each reach), and only chemical/physical parameters that were available for both years (including ions, nutrients, physicals, total and dissolved metals, and physical habitat variables).

When the two ordinations were compared, with 2017 as the target matrix and 2018 as the rotational matrix, the results indicated that there were differences in the spatial arrangement of sites between the two ordinations. The sum of squared residuals (m_{12}^2) was 0.33, and the permutation test indicated that the two ordinations were not more similar than could occur by chance (p = 0.065). Therefore, correlations among sites based on the suite of abiotic variables differed between 2017 and 2018. Water levels in the Hay River were extremely low at the time of sampling, and flow patterns during the year differed from those in 2017, and thus evidence of differences in the abiotic habitat between years is not surprising. HR-KS2-1A changed the most between years, with a residual of 0.35 that reflected its shift away from other sites such as HR-KS4-1A. These two sites were extremely similar in 2017, but were found on opposite ends of the second axis gradient in 2018, as HR-KS2-1A was no longer positively correlated with TSS, TP, and turbidity. Residual vectors for the comparison between ordinations (Figure 10) pointed outward for several sites, which indicated that not only did sites change position between years, but they moved farther apart in the ordination, and thus became more strongly negatively correlated in 2018.

3.1.1.4. Sediment chemistry

Sediment chemistry samples were collected from two sites in each reach (sites 1 and 5) and analyzed for metals and polycyclic aromatic hydrocarbons (PAHs). PAHs have the potential to impact the environment and human health if present in sufficient quantities, and in particular, they have the potential to be carcinogenic (Canadian Council of Ministers of the Environment 2010). PAHs are persistent and have been detected not only in freshwater sediments (Sanderson et al. 2012), but also in a number of freshwater organisms, including invertebrates (Rabodonirina et al. 2015) and fish (Ohiozebau et al. 2016, Ohiozebau et al. 2017). Although there are natural sources for PAHs (e.g., forest fires and volcanos), they are prevalent in the environment primarily due to anthropogenic sources, including oil spills, refineries, waste disposal sites, and sewage (Canadian Council of Ministers of the Environment 2010). Concentrations in sediment samples from 2018 were compared with sediment quality guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment 2001a), which include interim freshwater sediment quality guidelines (ISQGs) and probably effect levels (PELs). In addition, Benzo[a]pyrene Total Potency Equivalents and the Index of Additive Cancer Risk (IACR) were compared with guideline levels to ensure protection of humans and drinking water, respectively (Canadian Council of Ministers of the Environment 2010). All sediment chemistry results are presented as dry weight.

Mean values of all PAHs were below detection limit in Hay River sediment samples, and both the Benzo[a]pyrene Total Potency Equivalents and the Index of Additive Cancer Risk (IACR) were below detection limit, which indicated low risk to human health and drinking water quality (Table 5). Some metal levels were above ISQGs and PELs; for example, arsenic was above the ISQG in all reaches, cadmium was above the ISQG in Reach 4 and Reach 6, and cadmium was above both the ISQG and PEL in Reaches 1, 3, and 4 (above ISQG only in Reaches 5 and 6; Table 5). In particular, chromium levels in sediment were much higher than guidelines in some reaches (ISQG = 37.3 mg/kg and PEL = 90 mg/kg; Canadian Council of Ministers of the Environment 2001a). Levels of other metals, including mercury, were lower than CCME guidelines (Table 5).

The proportion of clay in the samples from Reaches 1-3 was high (20-31%; Table 5), which may have contributed to elevated levels of some metals due to increased sorption of metals to clay particles (Foster and Charlesworth 1996). However, Reach 4 also had high concentrations of some metals (notably chromium and cadmium) and the clay fraction made up a small percentage of the sample (5%; Table 5). Standard deviations were high for many of the reaches with elevated levels of metals (e.g., chromium in Reach 1 was estimated at 210.0 ± 230.5

Table 5. Summary of sediment chemistry parameters sampled in the Hay River at six sample reaches, indicating site mean ± standard deviation for each reach. When both sites in a reach were below detection limit, the detection limit is indicated (note that detection limits differed among sites for some parameters). When only one site in a reach was below detection limit, half the detection limit was used in calculations (number of sites below DL indicated in Parameter column). Values were compared with CCME sediment quality guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment 2001a), and values in bold were greater than interim freshwater sediment quality guidelines (ISQGs) whereas values in red were greater than probable effect levels (PELs).

Parameter	HR-KS1	HR-KS2	HR-KS3	HR-KS4	HR-KS5	HR-KS6
Particle size/physicals						
% Clay (<2µm)	20.9 ± 17.1	30.4 ± 9.4	31.7 ± 41.2	5.0 ± 3.5	5.6 ± 7.1	8.0 ± 10.5
% Sand (2.0mm – 0.05mm)	39.1 ± 35.2	24.5 ± 19.9	50.2 ± 48.2	72.8 ± 6.7	83.9 ± 21.1	70.2 ± 39.2
% Silt (0.05mm – 2µm)	40.0 ± 18.1	45.2 ± 10.5	18.2 ± 6.9	22.3 ± 3.2	10.5 ± 14.1	21.7 ± 28.8
Moisture %	50.8 ± 9.1	36.4 ± 22.7	32.5 ± 12.4	30.7 ± 18.5	36.2 ± 24.1	31.2 ± 21.8
Metals (mg/kg)						
Antimony (Sb) (3 below DL)	0.390 ± 0.170	0.410 ± 0.170	0.345 ± 0.191	0.285 ± 0.262	0.240 ± 0.198	0.235 ± 0.191
Arsenic (As)	**10.63 ± 2.36**	**9.72 ± 4.22**	**8.24 ± 1.91**	**9.98 ± 2.86**	**10.90 ± 0.57**	**8.72 ± 1.22**
Barium (Ba)	245.0 ± 113.1	268.5 ± 101.1	338.5 ± 60.1	126.0 ± 2.8	102.9 ± 87.9	197.6 ± 231.1
Beryllium (Be)	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Cadmium (Cd) (5 below DL)	0.590 ± 0.085	0.480 ± 0.325	< 0.5	**0.705 ± 0.644**	0.465 ± 0.304	**0.620 ± 0.141**
Chromium (Cr)	**210.0 ± 230.5**	28.3 ± 10.7	**96.5 ± 55.9**	**273.0 ± 46.7**	**80.8 ± 110.5**	**60.7 ± 82.5**
Cobalt (Co)	9.7 ± 1.3	8.8 ± 3.0	7.8 ± 2.7	9.6 ± 1.7	6.7 ± 3.3	6.2 ± 4.1
Copper (Cu)	18.15 ± 2.62	17.80 ± 5.80	20.40 ± 18.95	14.05 ± 3.32	8.00 ± 8.06	10.10 ± 11.17
Lead (Pb) (6 below DL)	6.75 ± 6.01	10.40 ± 3.39	7.75 ± 7.42	< 5.0	4.70 ± 3.11	5.15 ± 3.75
Mercury (Hg)	0.0428 ± 0.0388	0.0689 ± 0.0107	0.0375 ± 0.0347	0.0181 ± 0.0151	0.0289 ± 0.0224	0.0281 ± 0.0318
Molybdenum (Mo) (2 below DL)	15.55 ± 15.77	3.05 ± 0.92	7.30 ± 5.37	24.60 ± 0.14	7.55 ± 9.97	5.35 ± 6.86
Nickel (Ni)	127.2 ± 121.4	30.2 ± 2.8	64.2 ± 22.1	163.0 ± 21.2	52.0 ± 64.3	40.6 ± 50.4
Selenium (Se) (5 below DL)	0.60 ± 0.50	0.81 ± 0.18	0.60 ± 0.49	0.81 ± 0.78	0.51 ± 0.37	0.43 ± 0.25
Silver (Ag) (10 below DL)	0.16 ± 0.08	0.19 ± 0.12	< 0.2	< 0.2	< 0.2	< 0.2
Thallium (Tl)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Tin (Sn)	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0	< 5.0
Uranium (U) (10 below DL)	< 2.0	< 2.0	< 2.0	2.0 ± 1.3	< 2.0	1.6 ± 0.8
Vanadium (V)	31.6 ± 6.8	29.5 ± 9.8	34.6 ± 19.4	19.3 ± 5.2	14.5 ± 10.8	17.5 ± 14.8
Zinc (Zn)	81.8 ± 38.5	82.1 ± 46.6	61.0 ± 30.6	81.7 ± 42.9	59.3 ± 28.8	75.4 ± 6.8
Polycyclic Aromatic Hydrocarbons (PAHs) (mg/kg)						
2-Methylnaphthalene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Acenaphthene	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Acenaphthylene	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Anthracene	< 0.004	< 0.004	< 0.004	< 0.004	< 0.004	< 0.004

Parameter	HR-KS1	HR-KS2	HR-KS3	HR-KS4	HR-KS5	HR-KS6
B(a)P Total Potency Equivalent	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Benz(a)anthracene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(a)pyrene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(b&j)fluoranthene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(g,h,i)perylene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(k)fluoranthene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Chrysene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Dibenz(a,h)anthracene	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005
Fluoranthene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Fluorene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
IACR (CCME)	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15	< 0.15
Indeno(1,2,3-c,d)pyrene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Naphthalene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Phenanthrene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pyrene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Quinoline	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

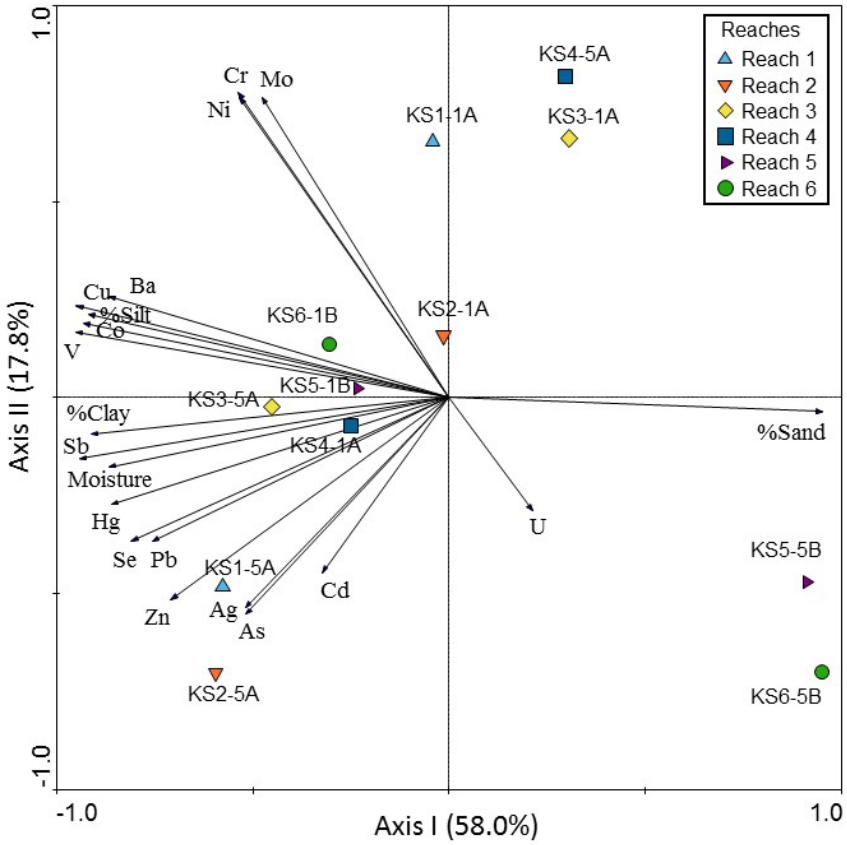

Figure 11 PCA ordination of sediment chemistry samples from Hay River kick sites, including two samples from each of six reaches (sample points coloured by reach). Ordination includes physical attributes of sediment sample and concentrations of metals (PAHs were excluded because they were all below detection limit). Arrows point in the direction of increasing values of parameters, and correlations of sites with parameters are indicated by the location of kick-site points in proximity to arrows. Kick-site points located near the origin have similar correlations with all measured parameters.

mg/kg, and cadmium in Reach 4 was estimated at 0.705 ± 0.644 mg/kg), indicating low precision between the two samples collected in each reach. Such high variability could have resulted from variation in sediment sample collection method (e.g., depth of sample), or could suggest that the distribution of metals may not be homogeneous throughout the reach, and the mean estimates may not accurately characterize the chemical habitat of the river sediments.

Ordination of sediment chemistry data for the Hay River provided further evidence of differences between sites sampled in the same reach, as none of the sites grouped by reach, and all reach pairs were separated along the first or second axis (Figure 11). For example, sites KS5-5B and KS6-5B were positively correlated with the % sand and concentrations of uranium along the first axis, whereas sites KS5-1B and KS6-1B were negatively correlated with this axis. These latter sites were positively correlated with clay, silt, and the majority of metals. Similarly, sites in Reaches 1-4 were separated along the second axis. Sites KS1-1A, KS2-1A, KS3-1A, and KS4-5A were positively correlated with metals such as nickel, chromium, and molybdenum, whereas sites KS1-5A, KS2-5A, KS3-5A, and KS4-1A were positively correlated with metals such as arsenic, cadmium, mercury, lead, and zinc (Figure 11).

Overall, particle size distributions and sediment chemistry were variable within and among reaches. The dominant gradients in the sediment chemistry samples indicated a distinction in metal concentrations that was strongly related to fine grain size. Sites with larger grain size (sand) were negatively or uncorrelated with most metals, whereas sites that were more dominated by clay and/or silt were positively correlated with most metals. This result is consistent with general dynamics of metal sorption to sediments, which predict higher sorption of metals in clays due to the higher surface area of clay particles (Foster and Charlesworth 1996). However, additional data is required to better understand the spatial variability of near-shore sediment quality throughout the study area, and more effort may be necessary to ensure samples are all collected in a similar manner.

3.1.1.5. Biotic assemblages

3.1.1.5.1. Summary metrics

Biotic metrics were calculated for kick sample data to summarize compositional differences among kick-sites and reaches. These metrics also provided a means to estimate normal range and CES for the BMI assemblage in each river (see section 3.3). Biotic metrics examined differences in total abundance and taxonomic richness. There were also metrics for specific taxonomic groups, including the relative abundance and richness of EPT, Chironomidae, Diptera + Oligochaeta, and Mollusca. EPT are generally considered to be sensitive to pollutants and disturbance, whereas Chironomidae are generally considered to be more tolerant of pollutants and disturbance and also tolerant of the cold temperatures and harsh environmental conditions characteristic of northern rivers. The Diptera + Oligochaeta metric also includes a number of taxa tolerant of cold, harsh environmental conditions, and pollutants, and were included as an additional measure of the abundance and richness of tolerant organisms. The richness and relative abundance of Mollusca was included as a summary metric due to a high contribution of this taxonomic group in some reaches, though it was primarily excluded from statistical analyses of metrics because abundance and richness remained low in most reaches.

There were clear longitudinal differences in metrics based on abundance. In general, total abundance and the abundance of specific taxonomic groups were higher in the three upstream reaches (reaches 1-3) than in the three downstream reaches (reaches 4-6; Table 6). In some cases, these differences were statistically significant. For example, total abundance was statistically significantly higher in Reach 1 and Reach 2 than in Reach 4 (Figure 12). In addition, the abundance of EPT was statistically significantly higher in Reach 1 and Reach 2 than it was in

Table 6 Summary of biotic metrics for kick-site reaches sampled in the Hay River in 2018, including the mean ± standard deviation for BMI abundance and taxonomic richness metrics. EPT is the sum of Ephemeroptera, Plecoptera, and Trichoptera orders, Chironomidae is a family of Diptera, and Diptera + Oligochaeta includes all true flies and segmented worms.

Biotic Metric	HR- KS1A	HR-KS2A	HR-KS3A	HR-KS4A	HR-KS5B	HR-KS6B
Total abundance	2836 ± 1207	2573 ± 904	1865 ± 966	430 ± 349	1053 ± 807	926 ± 464
EPT abundance	1352 ± 627	1550 ± 468	941 ± 558	230 ± 267	436 ± 340	385 ± 390
Chironomidae abundance	558 ± 314	567 ± 359	444 ± 141	126 ± 41	249 ± 144	302 ± 147
Diptera + Oligochaeta abundance	665 ± 358	774 ± 448	586 ± 220	176 ± 75	565 ± 487	409 ± 191
Mollusca abundance	696 ± 447	169 ± 63	244 ± 271	9 ± 18	0 ± 0	95 ± 63
Percent EPT	48.0 ± 8.2	61.4 ± 7.9	49.4 ± 10.5	44.7 ± 15.6	40.6 ± 15.7	35.9 ± 20.6
Percent Chironomidae	19.6 ± 5.3	21.0 ± 7.9	25.7 ± 7.0	36.0 ± 10.9	31.3 ± 22.8	35.4 ± 10.5
Percent Diptera + Oligochaeta	23.1 ± 4.7	28.6 ± 8.1	33.0 ± 8.5	48.2 ± 11.3	53.6 ± 16.1	48.8 ± 16.3
Percent Mollusca	24.4 ± 12.5	7.4 ± 3.5	12.4 ± 10.1	4.3 ± 8.9	0.0 ± 0.0	11.6 ± 5.7
Taxonomic richness	30.0 ± 2.8	32.2 ± 4.0	33.2 ± 1.1	30.0 ± 4.6	28.2 ± 4.4	33.0 ± 2.5
EPT richness	12.0 ± 1.9	12.0 ± 2.3	11.6 ± 2.1	10.6 ± 1.3	9.8 ± 1.5	11.2 ± 1.8
Chironomidae richness	8.4 ± 1.5	10.6 ± 3.0	11.8 ± 1.5	11.4 ± 3.5	11.0 ± 2.9	10.4 ± 1.9
Diptera + Oligochaeta richness	12.4 ± 1.8	16.4 ± 2.9	16.8 ± 2.3	15.2 ± 4.1	15.0 ± 3.1	16.6 ± 3.2
Mollusca richness	1.4 ± 0.5	1.0 ± 0.0	1.0 ± 0.0	0.8 ± 0.8	0.0 ± 0.0	1.0 ± 0.0

Reach 4, 5, or 6. Differences in the abundance of Chironomidae were less striking (Table 6), and the only significant difference was between Reach 2 and Reach 4. However, Chironomidae contributed less to the metric Diptera + Oligochaeta in reach 5; whereas 72-85% of the abundance of Diptera + Oligochaeata on average was due to Chironomidae in all other reaches, this family only contributed to 58% of the abundance of Diptera + Olgichaeta in Reach 5 (Table 6).

A shift in numerical dominance among EPT and Chironomidae was also evident between upstream and downstream reaches, as the relative abundance of Chironomidae increased downstream while there was a somewhat smaller decrease in the relative abundance of EPT (Table 6). Although differences among reaches were not significant (Figure 12), the shift in downstream reaches resulted in more similar proportions of these two major taxonomic groups compared to the upstream reaches where EPT were predominant (Figure 13). The patterns in relative abundance and absolute abundance together indicated that there was a decline in abundance across all BMI, including EPT and Chironomidae, in the downstream reaches, but that the decline was somewhat higher in EPT taxa.

There was some evidence of variability in abundance-based biotic metrics between kick-sites in each reach (Figure 12). The percent composition of EPT (which ranged from 19% to 72% of the sample across all kick-sites) had similar variability across all reaches, whereas the percent Chironomidae (which ranged from 11% to 69% of the sample across all kick-sites) appeared less variable in Reaches 1, 2, 3, and 6 (Figure 12). Both percent EPT and percent Chironomidae were more variable among sites in downstream reaches. In contrast, overall abundance was the most variable among sites in the upstream reaches (particularly in Reach 1; Figure 12). Total abundance ranged from 199 to nearly 4000 individuals across all kick-sites.

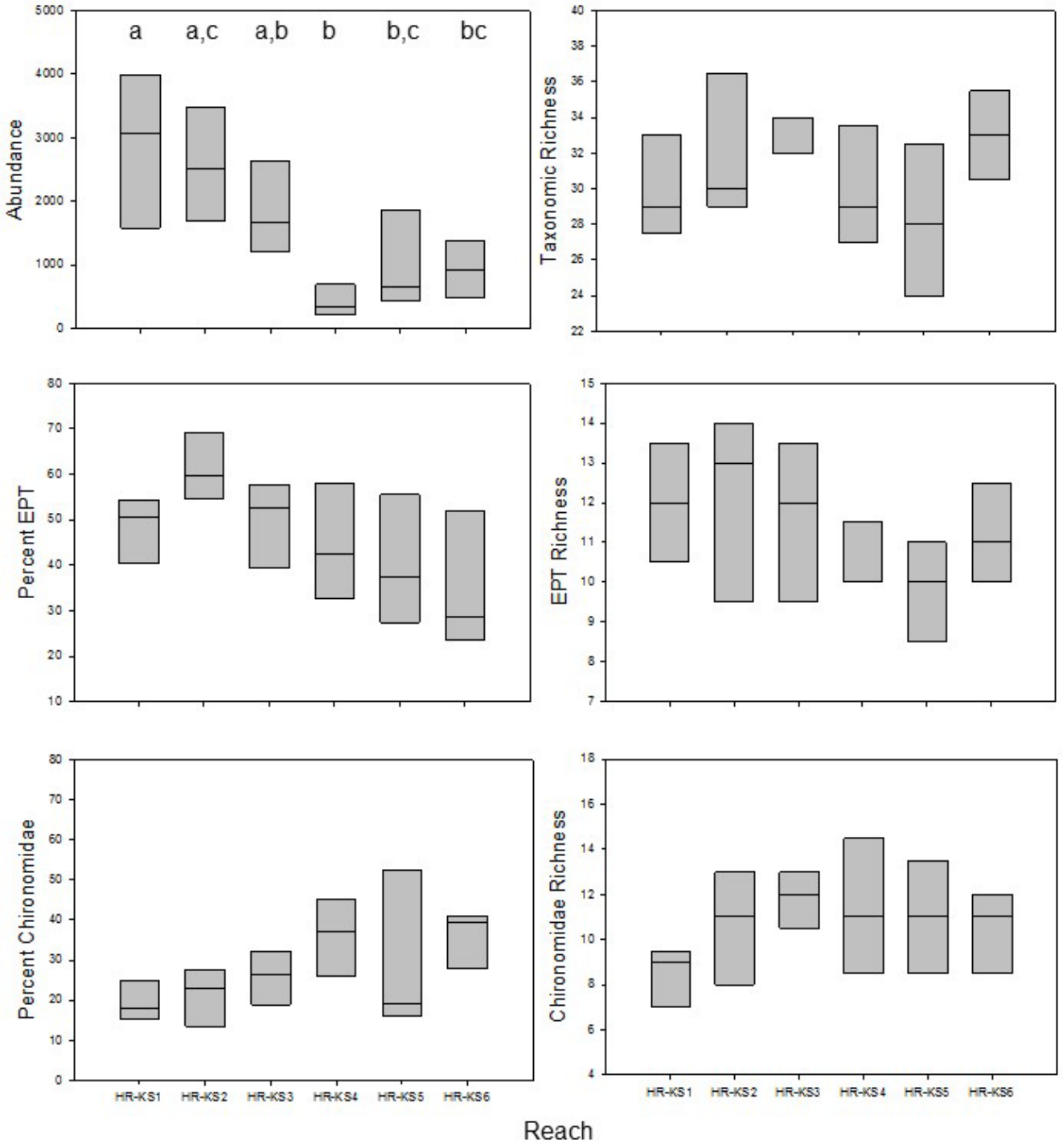

Figure 12. Box plots of BMI metrics for the six reaches in the Hay River that were sampled with kick sampling protocols. Metrics include overall abundance and richness, percent composition and richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT), and percent composition and richness of Chironomidae (midges). Lines through boxes indicate the median and lower and upper bounds plot the 25th and 75th percentiles, respectively. Letters on plots indicate significant differences (α = 0.05) from one-way ANOVA and Tukey HSD tests. Note that HR-KS6 was sampled nearly one week later than other reaches.

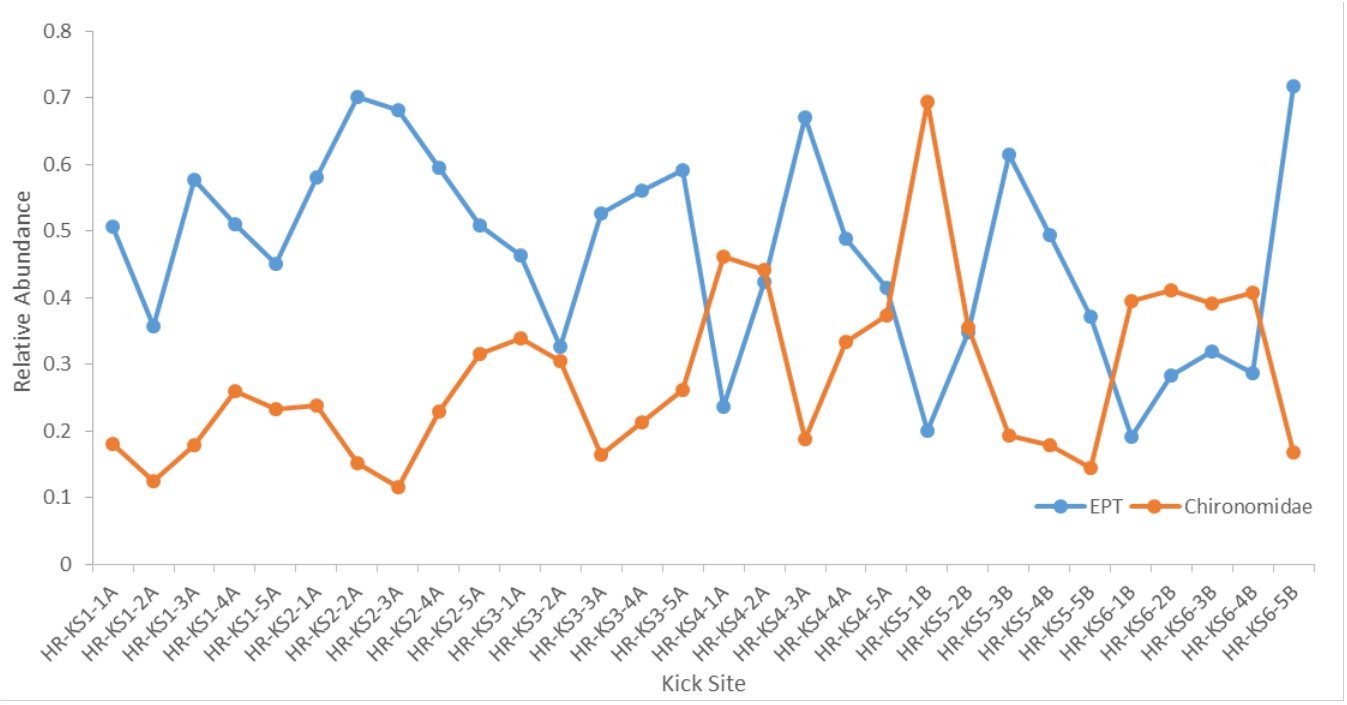

Figure 13. Relative abundance of Ephemeroptera, Plecoptera, and Trichoptera (EPT; blue points) and Chironomidae (orange points) across Hay River kick sites, with sites ordered from upstream (left) to downstream (right).

Taxonomic richness was more similar among reaches than abundance, although the richness of EPT taxa did appear to be higher in the upstream reaches than in Reach 4 or Reach 5 (though not statistically significant; Table 6, Figure 12). This is in contrast to 2017, when Reach 4 had elevated richness of EPT relative to other reaches. However, general similarity among reaches overall and similarity in richness levels compared to 2017 suggested strong potential for defining the normal range in taxonomic richness across the Hay River.

3.1.1.5.2. Assemblage composition and biotic-abiotic relationships

Multivariate analysis was used to characterize the biotic assemblage of the Hay River and evaluate similarities and differences in assemblage composition among reaches and sites. BMI relative abundance data were assessed at the family/subfamily level for this analysis (subfamily for Chironomidae and family or higher for all other taxa). This analysis was intended to assess correlations within and among reaches, and to identify any potential outliers or gaps in sample stations.

Overall, multivariate analysis of assemblages in Hay River indicated differences among sites and gradients that separated reaches, but there were no strong outliers (Figure 14A), which suggested that no sites were ecological outliers with respect to assemblage composition. This is in contrast to 2017, when multivariate assessment of BMI assemblages indicated that HR-KS1-2A differed strongly from other sites, suggesting that it was an ecological outlier. It was recommended that this site be moved upstream or downstream to avoid silty habitat. In 2018, the sampling location for HR-KS1-2A was shifted, and it no longer appeared to be an outlier in multivariate analysis, instead plotting close to other sites in Reaches 1-3 (Figure 14).

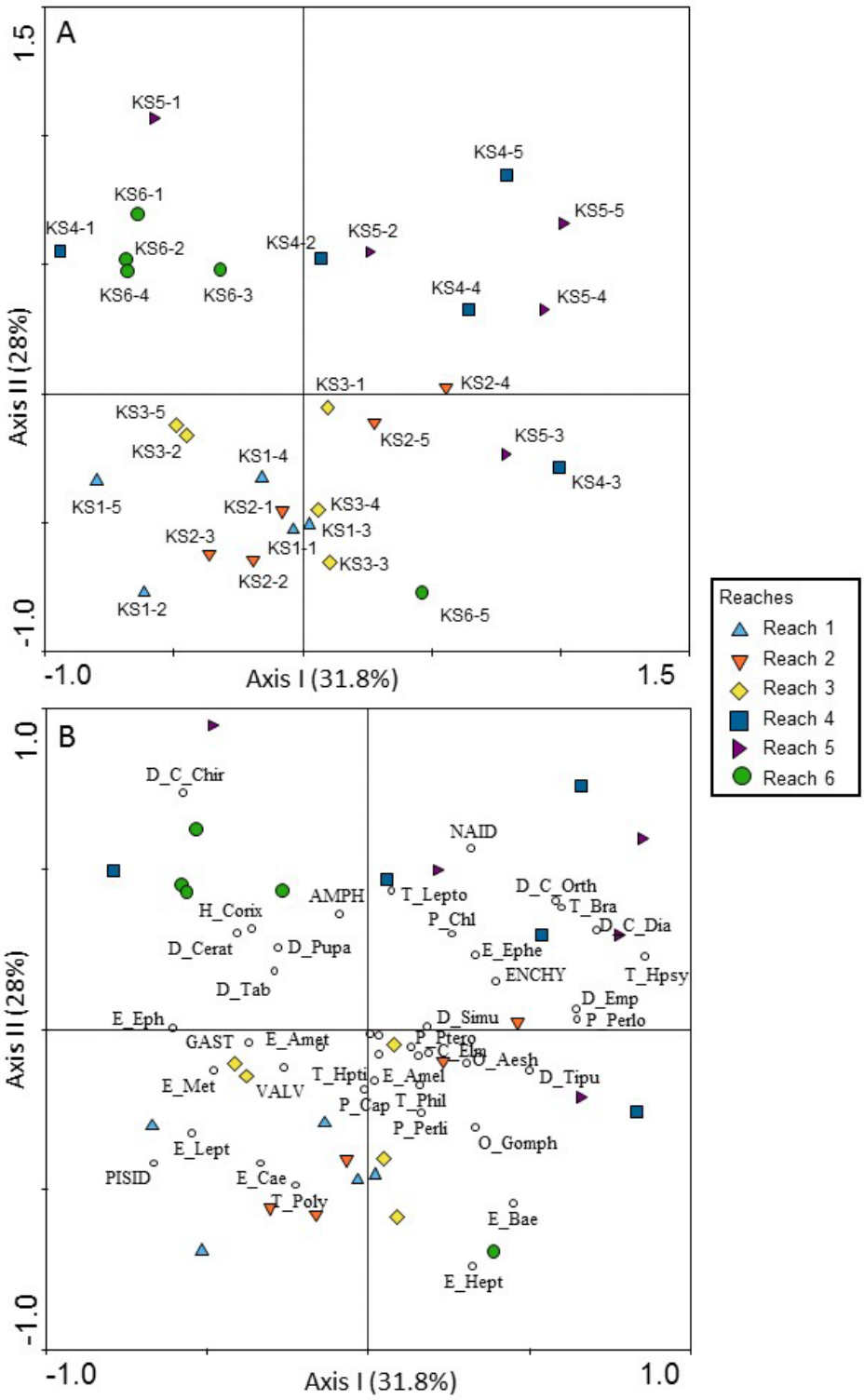

Figure 14 Multivariate analysis of BMI from kick samples in Hay River, including (A) PCA of BMI data showing labelled sample points, and (B) PCA biplot of BMI data showing sample points and labelled taxa, both with sample points coloured by reach. Kick-sites in close proximity have similar assemblages, whereas samples on opposite ends of gradients have differences in their assemblages. Samples at right angles through the origin are uncorrelated. Kick-sites are located close to taxa with which they are positively associated. Taxa near the origin are not labelled for ease of interpretation. Taxonomic abbreviations are listed in the appendices.

Sites in Reach 1, Reach 2, and Reach 3 (the upstream reaches) were generally fairly closely clustered in the ordination plot (Figure 14), which indicated strong similarity in assemblage composition within and among these reaches. Sites in the downstream reaches (Reaches 4-6) were more variable, plotting out along the full extent of the first axis (Figure 14). The second axis separated most sites in Reaches 1-3 from those in Reaches 4-6 (Figure 14), indicating differences in composition between upstream and downstream reaches. Reaches 1-3 were characterized by higher relative abundance of several molluscs (Pisidiidae, Gastropoda, Valvatidae), as well as several families of Ephemeroptera and Plecoptera, and were negatively associated with worms (Naididae and Enchytraeidae) and some Diptera families (including two subfamilies of Chironomidae: Orthocladiinae and Diamesinae; Figure 14). The lower left quadrant of the PCA biplot (negative first and second axes), which included most of Reach 1 and approximately half the sites from Reach 2 and Reach 3, was characterized by taxa that prefer slow-flowing waters or pools, often with silty substrate, such as Pisidiidae, Gastropoda, Valvatidae, Metretopodidae, Caenidae, and Leptophlebiidae (McCafferty 1998, Monk et al. 2018). In contrast, the lower right quadrant of the PCA biplot (positive first axis and negative second axis), which included the remaining sites from Reach 2 and Reach 3, as well as single sites from each of the downstream reaches (Figure 14), was associated with a number of taxa that are found in faster flow or riffles, including Heptageniidae, Baetidae, Perlidae, Philoptamidae, and Capniidae (Monk et al. 2018), and taxa that are associated with woody debris, detritus, or vegetation, including Aeshnidae, and Tipulidae (McCafferty 1998). On the positive end of the second axis, most sites in Reaches 4-6 were more strongly associated with a number of families of Diptera, as well as some caddisfly families, worms, and amphipods (Figure 14). Flow preferences of these taxa varied, and some taxa that prefer fast flow were associated with Reaches 4 and 5 in the upper right quadrant (positive first and second axis), including Chloroperlidae, Hydropsychidae, Perlodidae, and Ephemerellidae (McCafferty 1998, Monk et al. 2018). Most sites in Reach 6 were more strongly associated with taxa that prefer slow-flowing or pool-like conditions (including Amphipoda) (Monk et al. 2018). However, site KS6-5 in this reach was negatively correlated with the other reach sites, which suggested some variation in assemblage composition within the reach.

The range of taxa across these reaches, which include taxa that prefer slow-flowing water with soft sediments as well as some taxa that tolerate fast flow, indicate diverse assemblages with clear upstream-downstream differences that could be useful for detecting change along the longitudinal extent of the river. However, it should be noted that some of the differences between upstream and downstream reaches may relate to the low flow conditions in 2018, which may have disproportionately affected the BMI assemblages in the shallower downstream reaches. Water levels in the Hay River were at or below record minimum levels at the end of August 2018 (ECCC gauge Hay River near ALTA/ NWT boundary, station 07OB008; Figure 2A), which resulted in lower water levels for sampling than observed in the previous year. Continued monitoring in years with higher flow will make it possible to assess whether this strong distinction relates to flow impacts or characterizes general differences between upstream and downstream reaches.

3.1.1.5.3. Temporal comparison

Sampling of BMI has taken place over two years, and although such a short time-span does not allow for many formal assessments of temporal trends, some simple analyses were possible to compare the results of the two years of sampling. This included comparison of some metrics between years using repeated measures ANOVA and paired *t*-tests, as appropriate, and comparison of the full assemblage using Procrustes analysis of BMI ordinations.

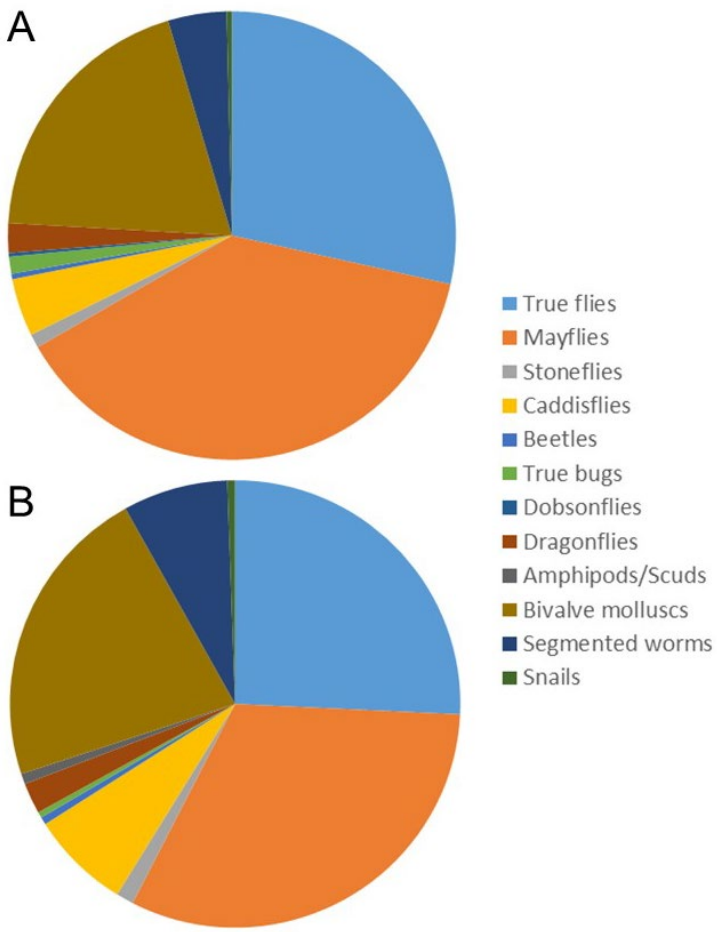

Figure 15. Average relative abundance of major BMI taxonomic groups in Hay River kick samples collected in (A) 2017 and (B) 2018. Taxa are grouped as true flies (Diptera), mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera), beetles (Coleoptera), true bugs (Hemiptera), dobsonflies (Megaloptera), dragonflies (Odonata), amphipods/scuds (Amphipoda), bivalve molluscs (Bivalvia), segmented worms (Oligochaeta), and snails (Gastropoda)

Compositional changes from 2017 to 2018 were summarized at the river level by assessing the average relative abundance or major taxonomic groups across all reaches in each year (Figure 15). Across all reaches, the relative abundance of Diptera (true flies) and Ephemeroptera (mayflies) decreased in 2018, whereas the relative abundance of Oligochaeta (segmented worms) and Bivalvia (bivalve molluscs, which combine with Gastropoda to make up the phylum Mollusca; Figure 15). The largest shift appeared to be the decline in Ephemeroptera in 2018.

When abundance was assessed at the site and reach level, there was evidence of strong declines in total abundance (Figure 16) and abundance of EPT and Chironomidae in several reaches compared with what was observed in 2017. Repeated measures ANOVA of total abundance as a function of reach and year (with sites included as replicates) indicated that patterns of change in abundance between years differed depending on which reach was considered (reach*year interaction $F_{4,20} = 7.56$, $p = 0.001$). Paired *t*-tests were therefore used to compare abundance across years separately for Reaches 1 and 2 (which appeared to increase in abundance in 2018) and Reaches 3-5 (which appeared to decrease in abundance in 2018; Reach 6 was not included because it

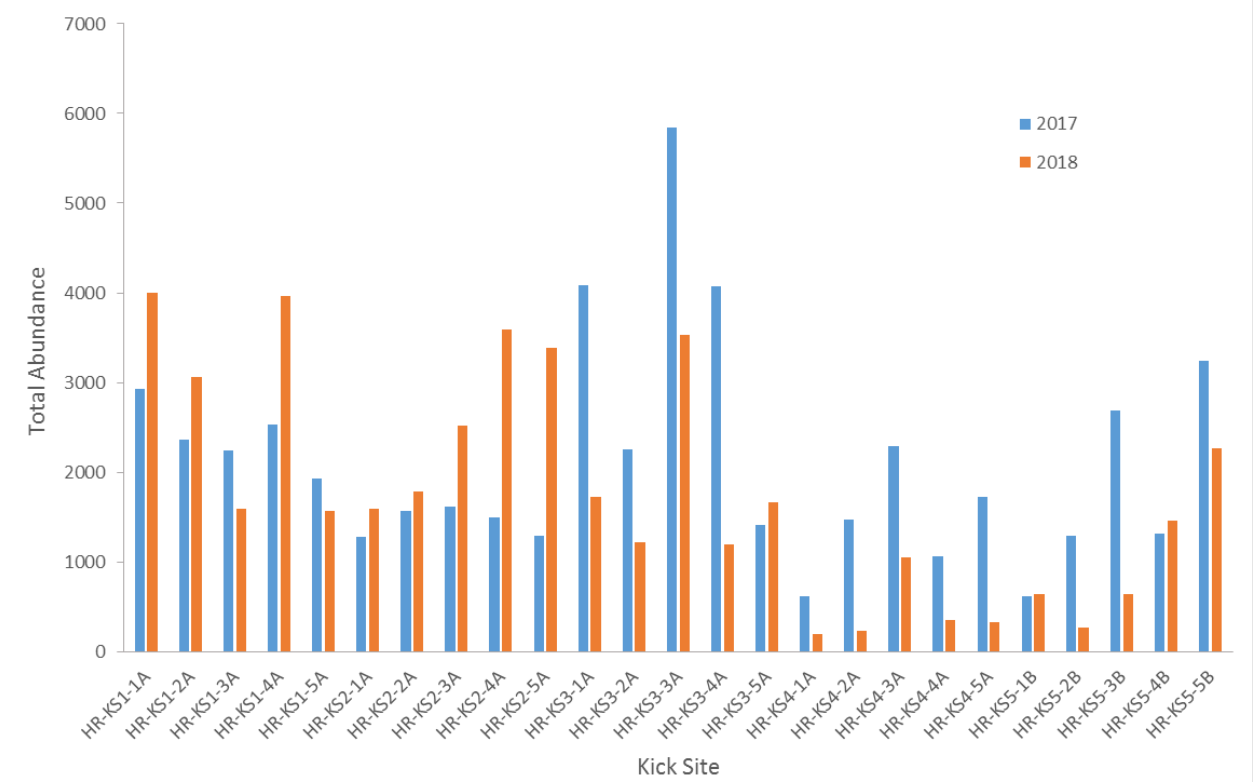

Figure 16 Total abundance of BMI in each Hay River kick-site sampled in 2017 (blue) and 2018 (orange).

was not sampled in 2017). Results of the paired *t*-test for Reaches 1 and 2 (with sites used as replicates) indicated a significant change in abundance between years ($t_{0.05(2),9}$ = -2.65, p = 0.026), with abundance increasing at most kick-sites, and sometimes doubling (Figure 16). In sharp contrast to this pattern in upstream reaches, abundance decreased in 2018 in Reaches 3-5 (Figure 16), and the difference, which was quite large in many sites, was significant ($t_{0.05(2),14}$ = 4.71, p < 0.001). Given the lower water levels in 2018, this decline in abundance in downstream reaches (which were shallower than Reach 1 and Reach 2) may have reflected a necessary lateral shift in sampling location further into the channel.

Similar results were found for the abundance of EPT and abundance of Chironomidae. For EPT, there was a significant interaction term in the repeated measures ANOVA, which indicated that the difference between years depended on the reach ($F_{4,20}$ = 3.35, p = 0.030). However, while there was a significant difference between years in Reaches 3-5 ($t_{0.05(2),14}$ = 3.15, p = 0.007), with lower abundance of EPT in 2018, the difference between years for Reaches 1-2 was not significant ($t_{0.05(2),9}$ = -1.40, p = 0.194). This appeared to be due to variable patterns among kick-sites, as all sites in Reach 2 had increased abundance of EPT in 2018, but only two sites in Reach 1 showed a similar pattern. Chironomidae results were very similar to those for EPT abundance. There was a significant interaction term in the Chironomidae abundance repeated measures ANOVA, which indicated that the difference between years depended on the reach ($F_{4,20}$ = 3.13, p = 0.038). Similar to EPT, there was a significant difference between years for reaches 3-5 ($t_{0.05(2),14}$ = 4.04, p = 0.001), with lower abundance of Chironomidae in 2018, but the difference between years for Reaches 1-2 was not significant ($t_{0.05(2),9}$ = -0.74, p = 0.478), which reflected strong similarity in Chironomidae abundance between years in these reaches.

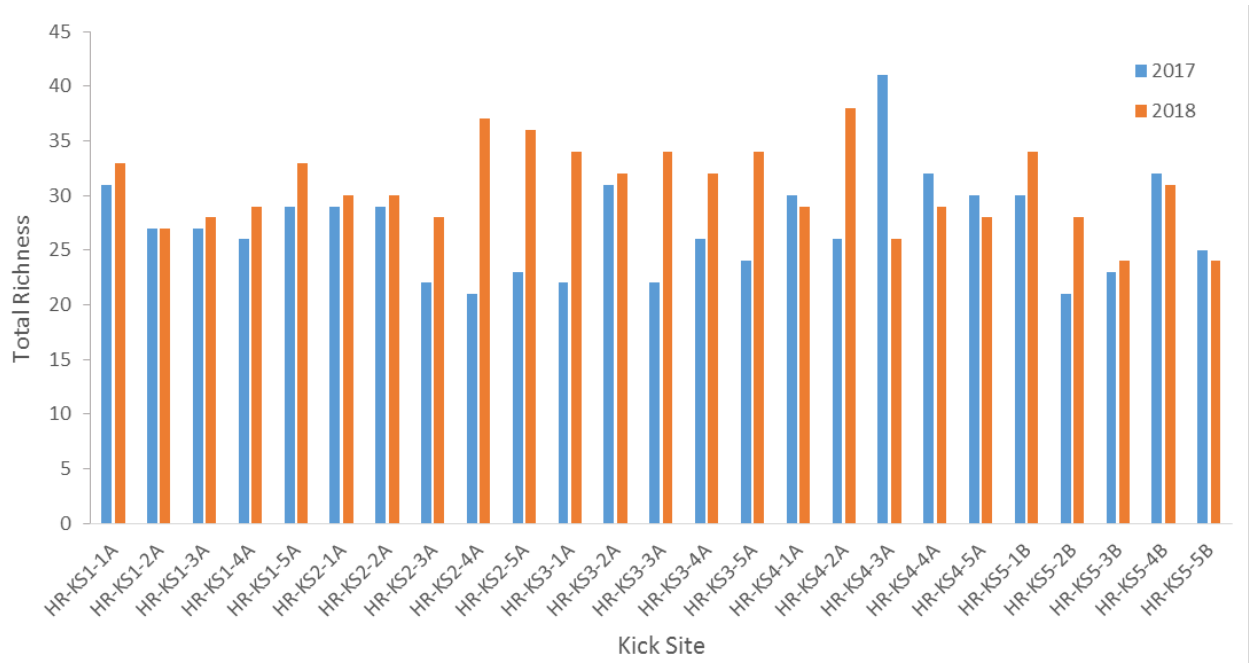

Figure 17 Total taxonomic richness at BMI kick-sites in the Hay River sampled in 2017 (blue) and 2018 (orange).

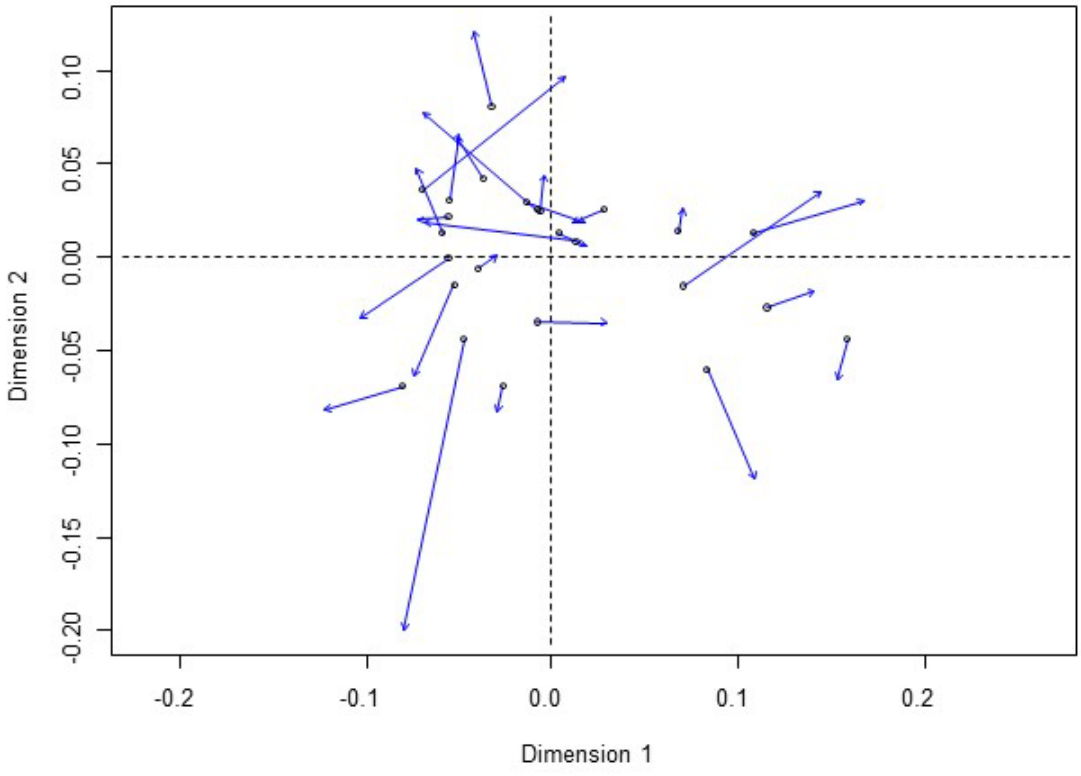

Figure 18 Procrustes residuals from a comparison of the ordination of 2017 Hay River BMI data (target matrix) with the ordination of 2018 Hay River BMI data (rotational matrix), with blue arrows indicating the movement of kick-sites in ordination space from 2017 to 2018. The longer the arrow, the more the assemblage composition at a site changed from one year to the next. Ordinations used the full compositional data in each year (not metrics)

Where it was necessary to sample further into the river channel (latitudinally) due to low water levels, a decline in EPT might be expected due to differences in the habitats being sampled. For example, EPT should be more predominant on the margins of a large river, where substrate size is larger and flow conditions support a diverse assemblage, whereas Chironomidae and other worm-like taxa may dominate habitats closer to the centre of the channel, where substrate size is smaller, water is generally deeper, and burrowing habits are supported. The effects of shifting the sample location closer towards the centre of the channel may have been more evident in the downstream reaches, where the water is generally more shallow. However, declines in abundance were evident in both EPT and Chironomidae in the downstream reaches, which suggests that the loss of abundance was non-selective. Furthermore, taxonomic richness was similar at a number of sites in both years, or was greater in 2018 in sites from KS2-4A to KS4-2A (Figure 17), which is contrary to the suggestion that sampling further into the channel would result in a less diverse assemblage. Additional sampling under different flow conditions will be necessary to further evaluate these temporal patterns in abundance and diversity.

Procrustes analysis of BMI ordinations from 2017 and 2018 for Hay River (Figure 18) indicated that the two ordinations were significantly more similar than could be obtained by chance (sum of squared residuals m_{12}^2 = 0.27, p = 0.001, comparison of ordinations with 25 sites). In contrast, comparison of ordinations based on the chemical/physical habitat between years (section 3.1.1.3.1) indicated a significant difference in years, reflecting a shift in water chemistry that may have been due to the low water levels, but this comparison was based on spot measurements of water chemistry. BMI assemblage structure integrates long-term responses to changes in abiotic variables, and thus provides a more reliable measure of the magnitude of change between years than single samples of water chemistry. Although a small number of sites appeared to differ in their assemblage composition from one year to the next (evidenced by large vectors in the Procrustes residuals diagram; Figure 18), the position of most sites changed little in the ordination diagram in 2018. This indicated that similarities and differences in composition among sites remained fairly constant from one year to the next. Thus, although abundance changed in many sites (increasing or decreasing, depending on the reach), and although there were some differences in richness between years, the relationship between sites (based on assemblage composition) remained similar.

3.1.1.6. Biotic-abiotic relationships

Biotic-abiotic relationships were assessed in Hay River kick samples using Redundancy Analysis (RDA), a multivariate approach that uses environmental variables to constrain the spatial arrangement of sites based on BMI relative abundance. RDA assesses the amount of variation in the unconstrained ordination (the PCA of BMI samples) that is explained by relating the data to chosen environmental variables and identifies major abiotic gradients in the data. This analysis was completed separately for water chemistry/physical habitat parameters and for sediment chemistry, due to differences in the sites sampled for each. Prior to analysis, correlations between environmental parameters were examined in combination with the abiotic PCAs to pick out important drivers of differences among sites that were uncorrelated with each other (low correlations between environmental parameters were chosen to avoid multicollinearity). This also worked to reduce the number of environmental parameters in the analysis and avoid over-fitting the data. The final RDA for water chemistry and physical habitat variables included velocity, periphyton cover, % sand, % pebble, % cobble, dissolved aluminum, total aluminum, ammonia (NH_3), conductivity, hardness, dissolved manganese (D-Mn), total molybdenum (T-Mo), dissolved nitrogen (DN), dissolved organic carbon (DOC), total phosphorus (TP), total selenium (T-Se), sodium (Na), and total suspended solids (TSS). Other ions, nutrients, physical measures, and total and dissolved

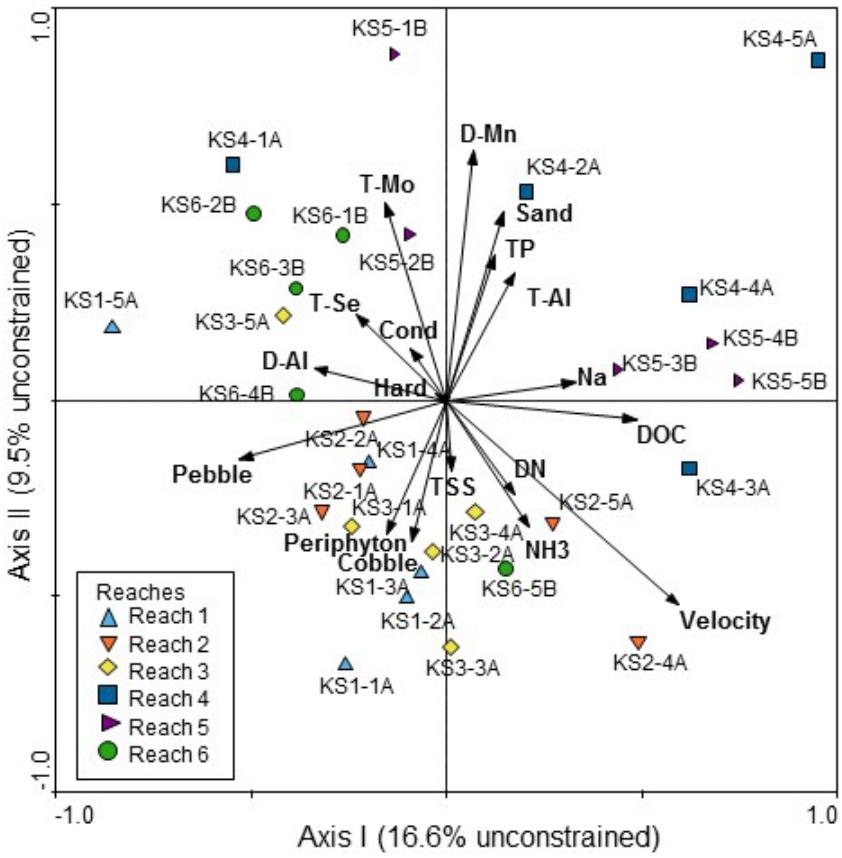

Figure 19 RDA ordination of BMI data constrained by physical and chemical habitat data at each site, with sites coloured by reach. Even-numbered sites use average values of chemical parameters from neighbouring odd-numbered sites. Kick-sites in close proximity have similar assemblages, whereas samples on opposite ends of gradients have differences in their assemblages. Samples at right angles through the origin are uncorrelated. Vectors indicate direction of change of physical and chemical parameters, and sites are ranked along these vectors based on the strength of their correlation with each parameter.

metals were highly correlated with the chosen variables. For example, total aluminum was strongly positively correlated with total arsenic, total barium, total chromium, total cobalt, total copper, total manganese, total mercury, total nickel, total rubidium, total titanium, and total vanadium. Thus, any patterns described for this parameter also apply to the correlated parameters.

The first axis and all axes of the RDA of BMI relative abundance and chemical and physical parameters were statistically significant (Monte Carlo permutation test: first axis $F = 2.195$, $p = 0002$; all axes $F = 1.537$, $p = 0.002$), and the first three axes explained 33.6% of the variation among samples. The spatial arrangement of sites in the ordination was similar to that observed in the PCA of BMI data (Figure 14, Figure 19), which indicated a good fit of the environmental parameters to the biotic data (i.e., constraining the biotic data to environmental variables did not lead to shifts in the relationships among sites). Velocity was the strongest driver in the RDA (greatest individual effect and conditional effect, in combination with other parameters) and its effect on the fit of the model was significant ($F = 2.9$, $p = 0.002$). This result was consistent with what appeared to be the dominant gradient underlying the BMI PCA, as there were differences in flow preferences of invertebrates among quadrants of the PCA. For example, sites in the lower right quadrant of the PCA (positive first axis, negative

second axis) were associated with a number of taxa that prefer fast-flowing water, whereas sites in the upper left quadrant of the PCA (negative first and second axis) were associated with taxa that prefer pool-like conditions (Figure 14). In the RDA, these sites varied along a gradient in velocity (Figure 19), consistent with the flow preferences of the taxa that were correlated with these sites. Although the velocity measurements included in this analysis were spot measurements, they appeared to accurately characterize general flow conditions at the sites, based on correlations of BMI taxa with those sites.

Substrate size and water chemistry also played an important role in the RDA. The % pebble, % sand, % cobble, DOC, dissolved manganese, and dissolved aluminum all had high individual effects, and most had significant conditional effects (with the exception of DOC and dissolved aluminum, though conditional effects of the latter were high). The % pebble in particular contributed to separation within groups of sites that were either positively or negatively correlated with velocity. Higher velocity sites with a higher % pebble were positively correlated with periphyton cover and % cobble, whereas higher velocity sites with lower % pebble were positively correlated with % sand, DOC, sodium, TP, and total aluminum (Figure 19). Lower velocity sites with higher % pebble were positively correlated with dissolved aluminum, total selenium, and total molybdenum, and were negatively correlated with ammonia and DN (Figure 19). Overall, the RDA indicated strong effects of velocity and substrate size, as well as some characterization of reaches based on water chemistry, though water chemistry appeared to be a less important driver, likely because water chemistry values were similar across sites.

Analysis of biotic-abiotic relationships was also completed using a subset of BMI kick-sites and sediment chemistry data. However, sediment chemistry parameters were generally quite highly correlated, which meant that there were few parameters that could be chosen. In an effort to retain only parameters that were uncorrelated with each other, the chosen subset was arsenic, copper, molybdenum, and uranium. But the resulting RDA indicated a lack of significance of the first axis and all axes (p = 0.566 and 0.464, respectively), and the ordination plot indicated that most sites were orthogonal to (i.e., uncorrelated with) the chosen parameters (results not shown). Therefore, the relationship of these sediment chemistry parameters with biotic assemblage composition was weak.

3.1.2. Slave River

3.1.2.1. Water chemistry

Water chemistry samples were collected in the Slave River to act as supporting variables for the BMI data. These samples represented spot measurements of water chemistry conditions at the time of sampling, and were collected at three sites per reach to account for local-scale variability in BMI assemblages in response to the chemical environment. The Slave River is large (wetted width ~300-600 m compared with ~50-70 m in the Hay River), and habitat conditions and assemblages were expected to differ among reaches, which were far apart geographically. Flow velocity in the channel was fast (velocity was 0.2-0.5 m/s across a number of kick-sites), and some degree of variation in water chemistry among sites was expected due to differences in flow. In their analysis of long-term trends in water quality of the transboundary waters of the Slave River, Sanderson et al. (2012) found that some temporal trends in water chemistry parameters actually reflected temporal change in flow (with summer/fall flows decreasing over time in the river), and correction for flow resulted in the removal of temporal trends in those parameters. Changes in flow and water chemistry patterns over time in this river are a reflection in part of the impacts of the construction of the William A. C. Bennett dam in the upstream Peace River basin in northern British Columbia (Glozier et al. 2009, Sanderson et al. 2012). In the short term, flow differences between 2017 and 2018 (Figure 2B) may also have contributed to variation in water chemistry

parameters between years. In 2018, water levels were high due to a surge in the month of August before sampling took place, resulting in different flow conditions in the period prior to sampling.

The longitudinal gradient of Slave River reaches extended from Reach 1 at the south (upstream) to Reach 5 at the north (downstream; Figure 5A). Reach 4 had sampling sites on both banks (Reach 4A and Reach 4B), which were determined in 2017 to have different habitat conditions (including different substrate composition). Analyses of all data from the Slave River considered variation within and among reaches to account for differences due to reach location and location of sites within reaches.

3.1.2.1.1. Major ions, nutrients, and physicals

Three water samples were collected in each river reach (one sample per odd-numbered site) and analyzed for major ions, nutrients, and physicals. Mean levels of ions and nutrients (Table 7) were compared with Canadian guidelines for short-term and long-term exposure to identify any reaches where water chemistry was indicative of poor water quality (Canadian Council of Ministers of the Environment 2001b).

Mean values for ions, nutrients, and physicals did not exceed CCME guidelines for the protection of aquatic life for any reaches in the Slave River (Table 7). Levels of total suspended solids (TSS) were higher in the upstream reaches in 2018 than was found in 2017, and exceeded the CCME guideline in Reach 2 (based on background annual median of 108 mg/L reported by Sanderson et al. 2012). However, the Slave River has a high sediment load (Dagg 2016a) and TSS levels have been shown to vary quite widely at the water chemistry monitoring station at Fort Smith (Sanderson et al. 2012). Although Sanderson et al. (2012) reported a long-term (1982-2010) mean TSS of less than 100 mg/L in August and September at Fort Smith, long-term means for June and July ranged between 400 and 550 mg/L, indicating the high variability that results from variable flow in this river. In 2018, flow increased in August, resulting in higher water levels at the time of sampling. This increase in flow may also have contributed to increased sediment transport in the system. Furthermore, high winds at the time of sampling or recent rain events may have also contributed to the high TSS noted in Reach 2 of the Slave River. Given that these were spot measurements of water chemistry, and thus highly reflective of conditions at the time of sampling, results should not be interpreted as an indication of longer-term trends.

Estimates of mean TP in the Slave River were generally higher than in the Hay River (Table 7), and reaches were classified as eutrophic or hyper-eutrophic (Reach 4B and Reach 5) based on the Canadian Guidance Framework. Analysis of long-term trends in phosphorus (total and dissolved) in the Slave River found elevated levels in the Slave River relative to the Athabasca and Peace Rivers that flow into the Slave (Glozier et al. 2009). The spot measurements of TP collected in the Slave River in 2018 (Figure 20) were below the median of 0.078 mg/L from long-term routine monitoring data (Glozier et al. 2009). There was only a weak correlation between TP and TSS in 2018 (r = -0.17), which suggested that higher nutrient levels at downstream reaches may not have been due to high levels of phosphorus bound to sediment, but rather associated with organic particles. Dissolved phosphorus was measured at four of the reaches in 2018, and accounted for 5 to 9% of TP among the reaches, which is consistent with long term monitoring data available for near the AB-NWT border.

Variability between samples collected in a single reach was somewhat higher than observed for the Hay River, though standard deviations for reaches remained fairly low (< 2 for alkalinity and ions, < 0.04 for phosphorus variables and < 0.04 for nitrogen variables; Table 7). Several water chemistry parameters were statistically significantly different among reaches, in part due to low variability within a number of reaches. For example, conductivity had low variability within reaches and was statistically significantly higher at Reach 1 and Reach 2 than all other reaches (Figure 20). Reach 4A also differed statistically from all other reaches with respect to

Table 7. Summary of water chemistry parameters sampled in the Slave River at all sample reaches, indicating site mean ± standard deviation for each reach. When all sites in a reach were below detection limit, the detection limit is indicated. When only a subset of sites in a reach was below detection limit, half the detection limit was used in calculations (number of sites below DL indicated in Parameter column). Values in bold are those that were above the CCME long-term exposure guidelines for the protection of aquatic life. N/A indicates parameters that weren't measured.

Parameter	SR-KS1	SR-KS2	SR-KS3	SR-KS4A	SR-KS4B	SR-KS5
Alkalinity (mg/L)	87.0 ± 1.1	85.4 ± 1.7	70.4 ± 0.2	78.3 ± 0.5	70.8 ± 0.6	71.4 ± 0.1
Ammonia (mg/L) (12 below DL)	0.003 ± 0.000	0.118 ± 0.187	0.045 ± 0.074	0.012 ± 0.011	0.007 ± 0.007	0.003 ± 0.000
Calcium (mg/L)	27.5 ± 1.9	25.8 ± 0.8	20.5 ± 1.1	23.5 ± 1.4	21.4 ± 0.9	24.1 ± 0.9
Chloride (mg/L)	7.27 ± 0.21	6.50 ± 0.20	5.50 ± 0.00	5.57 ± 0.15	5.57 ± 0.06	5.90 ± 0.00
Specific Conductivity (µS/cm)	244.7 ± 4.7	237.3 ± 4.0	197.7 ± 1.5	217.3 ± 1.5	198.3 ± 1.2	200.0 ± 2.0
Hardness (mg/L)	127.0 ± 4.4	120.0 ± 5.6	97.3 ± 3.5	110.3 ± 6.7	106.7 ± 9.9	126.7 ± 1.5
Magnesium (mg/L)	14.10 ± 1.54	13.47 ± 0.91	11.23 ± 1.01	12.53 ± 0.91	12.97 ± 2.04	16.13 ± 0.51
Nitrate (mg/L)	< 0.005	< 0.005	< 0.005	< 0.005	< 0.005	N/A
Nitrite (mg/L)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	N/A
Dissolved N (mg/L)	0.220 ± 0.000	0.203 ± 0.006	0.190 ± 0.010	0.190 ± 0.000	0.187 ± 0.006	0.193 ± 0.012
Total N (mg/L)	0.337 ± 0.012	0.357 ± 0.015	0.293 ± 0.012	0.330 ± 0.035	0.293 ± 0.006	0.333 ± 0.015
DOC (mg/L)	6.33 ± 0.12	6.27 ± 0.21	5.37 ± 0.06	5.80 ± 0.17	5.40 ± 0.10	5.83 ± 0.15
TOC (mg/L)	6.37 ± 0.15	6.20 ± 0.00	5.43 ± 0.06	5.77 ± 0.21	5.47 ± 0.06	6.00 ± 0.35
pH	8.21 ± 0.02	8.18 ± 0.01	8.15 ± 0.02	8.17 ± 0.01	8.14 ± 0.02	8.09 ± 0.01
Ortho-phosphate (mg/L)	0.0023 ± 0.0000	0.0024 ± 0.0003	0.0018 ± 0.0001	0.0028 ± 0.0004	0.0021 ± 0.0002	N/A
Dissolved P (mg/L)	0.0055 ± 0.0006	0.0056 ± 0.0003	0.0049 ± 0.0003	0.0057 ± 0.0004	0.0052 ± 0.0012	N/A
Total P (mg/L)	0.064 ± 0.012	0.073 ± 0.015	0.053 ± 0.032	0.084 ± 0.030	0.103 ± 0.038	0.107 ± 0.015
Potassium (mg/L)	1.30 ± 0.26	1.60 ± 0.17	1.17 ± 0.15	1.13 ± 0.06	1.23 ± 0.06	1.10 ± 0.17
Sodium (mg/L)	8.00 ± 1.76	7.77 ± 1.17	5.50 ± 1.05	5.43 ± 0.70	5.43 ± 0.47	5.30 ± 0.36
TDS (mg/L)	140.7 ± 1.2	135.3 ± 5.0	114.7 ± 9.0	124.0 ± 7.2	118.7 ± 7.6	130.0 ± 2.0
TSS (mg/L)	100.7 ± 27.6	148.7 ± 39.1	81.3 ± 8.1	96.0 ± 17.4	74.0 ± 7.2	96.0 ± 26.0
Sulphate (mg/L)	23.7 ± 0.6	22.7 ± 0.6	18.0 ± 0.0	20.7 ± 0.6	18.0 ± 0.0	18.0 ± 0.0
Turbidity (NTU)	45.5 ± 4.7	57.2 ± 8.1	39.0 ± 2.8	47.2 ± 5.9	38.8 ± 1.8	65.2 ± 8.4

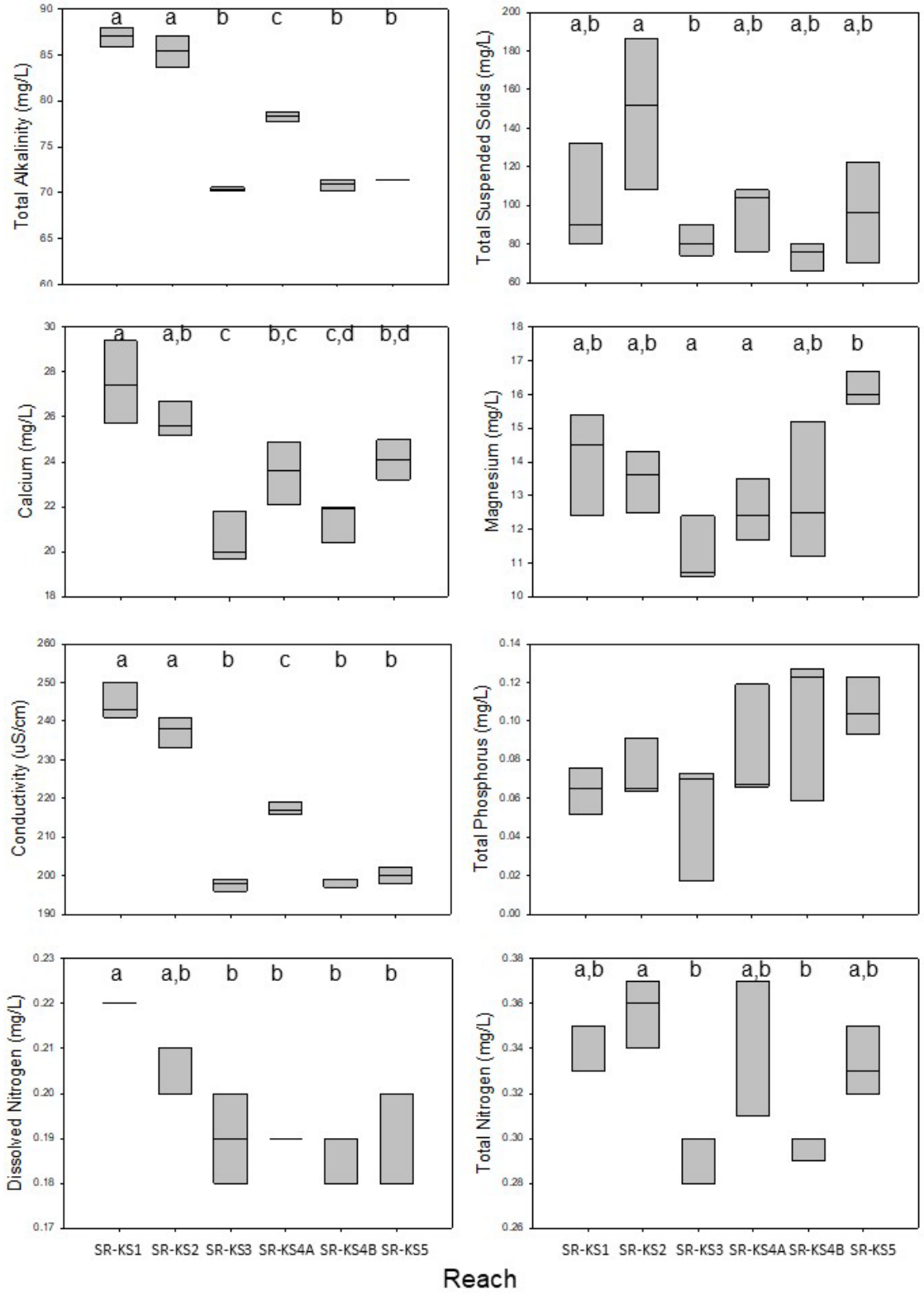

Figure 20. Box plots of ions, nutrients, and physicals water chemistry concentrations for all reaches sampled in Slave River. Plotted is alkalinity, TSS, calcium, magnesium, conductivity, TP, DN, and TN. Lines through boxes indicate the median and lower and upper bounds plot the 25th and 75th percentiles, respectively. Letters on plots indicate significant differences (α = 0.05) from one-way ANOVA and Tukey HSD tests.

conductivity, though it only differed by approximately 20-30 μS/cm, and thus may not have reflected a biologically-significant difference (Figure 20). Dissolved nitrogen, calcium, and alkalinity were also higher in Reaches 1 and 2 than a number of downstream reaches, though the difference was not always statistically significant (Figure 20). Reach 3 and Reach 4B had lower levels of a number of measured parameters than was observed in the other reaches, though the differences were not always statistically significant (Figure 20). All parameter estimates reflected water chemistry at the time of sampling, and should not be taken to suggest long-term patterns.

3.1.2.1.2. Metals

Dissolved metals provide a more accurate estimate of the relevant exposure of biota than total metals because they are generally more biologically available than the particulate forms, which are included in estimates of total metals (Sanderson et al. 2012). Dissolved metal concentrations were generally low in Slave River reaches, with many metals at or below detection limits (Table 8), and no CCME guidelines for the protection of aquatic life were exceeded. Metal concentrations from spot measurements represented conditions at time of sampling, but average values of dissolved metals were generally similar to long-term (1982-2010) median values for the river (Sanderson et al. 2012). Some variability was evident among reaches for dissolved aluminum, though most concentrations were extremely low (Figure 21). Dissolved iron appeared quite variable among reaches, but only Reach 5 was found to differ significantly from Reach 3 (Figure 21) and most mean values were in the range of 3-9 μg/L (Table 8). Dissolved manganese was elevated at Reach 2 and Reach 5, and statistically significantly higher at these reaches than at most other reaches (Figure 21).

Table 8. Summary of metal water chemistry parameters sampled in the Slave River at all sample reaches, indicating site mean ± standard deviation (for 2 or more samples) for each reach. When all sites in a reach were below detection limit, the detection limit is indicated. When only a subset of sites in a reach was below detection limit, half the detection limit was used in calculations (number of sites below DL indicated in Parameter column). Dissolved metal values were excluded when they exceeded total metals. Values in bold are those that were above the CCME long-term exposure guidelines for the protection of aquatic life.

Parameter	SR-KS1	SR-KS2	SR-KS3	SR-KS4A	SR-KS4B	SR-KS5
Aluminum Diss. (μg/L)	1.87 ± 0.15	1.83 ± 0.21	1.50 ± 0.10	1.87 ± 0.35	2.10 ± 0.95	2.73 ± 0.67
Aluminum Total (μg/L)	**993.7 ± 131.6**	**1543.3 ± 405.0**	**1131.7 ± 262.7**	**1108.7 ± 188.6**	**947.7 ± 90.1**	**2090.0 ± 409.3**
Antimony Diss. (μg/L) (15 below DL)	< 0.1	0.067 ± 0.029	< 0.1	0.067 ± 0.029	0.067 ± 0.029	< 0.1
Antimony Total (μg/L)	0.10 ± 0.00	0.13 ± 0.06	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Arsenic Diss. (μg/L)	0.367 ± 0.058	0.400 ± 0.000	0.367 ± 0.058	0.400 ± 0.000	0.400 ± 0.000	0.400 ± 0.000
Arsenic Total (μg/L)	1.23 ± 0.15	1.53 ± 0.15	1.30 ± 0.26	1.20 ± 0.10	1.17 ± 0.06	1.43 ± 0.15
Barium Diss. (μg/L)	45.8 ± 0.6	46.5 ± 0.3	38.3 ± 0.4	42.1 ± 0.4	38.7 ± 0.4	44.8 ± 0.2
Barium Total (μg/L)	73.3 ± 4.7	92.8 ± 15.6	73.9 ± 15.2	69.5 ± 5.6	65.8 ± 3.2	90.3 ± 5.1
Beryllium Diss. (μg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Beryllium Total (μg/L) (13 below DL)	< 0.1	0.07 ± 0.03	0.07 ± 0.03	< 0.1	< 0.1	0.10 ± 0.00
Bismuth Diss. (μg/L)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2
Bismuth Total (μg/L) (16 below DL)	< 0.2	< 0.2	0.13 ± 0.06	< 0.2	0.13 ± 0.06	< 0.2
Boron Diss. (μg/L)	17.7 ± 0.5	17.4 ± 0.4	15.8 ± 0.3	16.3 ± 0.2	15.7 ± 0.2	17.6 ± 0.1
Boron Total (μg/L)	20.0 ± 0.7	20.3 ± 0.2	18.5 ± 0.3	18.6 ± 0.5	18.6 ± 0.3	20.9 ± 0.2
Cadmium Diss. (μg/L)	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04

Parameter	SR-KS1	SR-KS2	SR-KS3	SR-KS4A	SR-KS4B	SR-KS5
Cadmium Total (µg/L) (17 below DL)	< 0.1	0.07 ± 0.03	< 0.1	< 0.1	< 0.1	< 0.1
Cesium Diss. (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cesium Total (µg/L)	0.30 ± 0.00	0.40 ± 0.10	0.33 ± 0.06	0.33 ± 0.06	0.27 ± 0.06	0.40 ± 0.00
Chromium Diss. (µg/L) (14 below DL)	< 0.1	0.13 ± 0.14	0.10 ± 0.09	0.12 ± 0.08	< 0.1	< 0.1
Chromium Total (µg/L)	1.60 ± 0.26	2.40 ± 0.60	1.83 ± 0.42	1.77 ± 0.31	1.57 ± 0.12	2.97 ± 0.25
Cobalt Diss. (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Cobalt Total (µg/L)	0.97 ± 0.12	1.37 ± 0.40	1.07 ± 0.38	0.97 ± 0.21	0.87 ± 0.06	1.23 ± 0.25
Copper Diss. (µg/L)	1.03 ± 0.06	0.93 ± 0.06	0.90 ± 0.00	0.97 ± 0.06	0.90 ± 0.00	1.00 ± 0.00
Copper Total (µg/L)	**3.00 ± 0.35**	**3.70 ± 0.75**	**3.17 ± 0.74**	**3.00 ± 0.46**	**2.87 ± 0.23**	**3.77 ± 0.45**
Iron Diss. (ug/L) (5 below DL)	7.0 ± 0.0	7.7 ± 0.6	3.3 ± 1.4	5.5 ± 2.8	3.7 ± 2.0	9.0 ± 0.0
Iron Total (µg/L)	**2260.0 ± 338.7**	**3276.7 ± 895.6**	**2486.7 ± 792.2**	**2333.3 ± 480.1**	**2063.3 ± 172.1**	**3153.3 ± 396.8**
Lead Diss. (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Lead Total (µg/L)	1.10 ± 0.17	1.53 ± 0.45	1.27 ± 0.38	1.13 ± 0.25	1.03 ± 0.12	1.57 ± 0.31
Lithium Diss. (µg/L)	5.2 ± 0.1	5.1 ± 0.1	4.5 ± 0.1	4.7 ± 0.1	4.5 ± 0.0	4.8 ± 0.1
Lithium Total (µg/L)	6.5 ± 0.1	7.1 ± 0.5	6.1 ± 0.5	6.0 ± 0.4	5.8 ± 0.2	7.0 ± 0.1
Manganese Diss. (µg/L)	0.4 ± 0.1	2.4 ± 1.3	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	6.0 ± 3.0
Manganese Total (µg/L)	71.8 ± 6.2	88.5 ± 17.6	67.2 ± 16.2	65.8 ± 9.1	59.8 ± 4.1	83.3 ± 12.8
Mercury Total (UL) (ng/L)	4.57 ± 0.21	9.97 ± 4.50	4.57 ± 1.16	4.87 ± 0.87	4.83 ± 0.40	7.07 ± 1.35
Molybdenum Diss. (µg/L)	0.80 ± 0.00	0.80	N/A	0.70 ± 0.00	0.70	0.70
Molybdenum Total (µg/L)	**0.77 ± 0.06**	0.73 ± 0.06	0.60 ± 0.00	0.70 ± 0.00	0.63 ± 0.06	0.60 ± 0.10
Nickel Diss. (µg/L)	0.97 ± 0.06	1.33 ± 0.49	0.87 ± 0.06	1.03 ± 0.15	0.80 ± 0.00	0.90 ± 0.00
Nickel Total (µg/L)	3.43 ± 0.40	4.53 ± 1.05	3.70 ± 1.06	3.37 ± 0.57	3.20 ± 0.30	4.27 ± 0.60
Rubidium Diss. (µg/L)	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00
Rubidium Total (µg/L)	3.20 ± 0.26	4.10 ± 0.75	3.43 ± 0.59	3.37 ± 0.40	3.07 ± 0.21	4.53 ± 0.21
Selenium Diss. (µg/L) (11 below DL)	0.33 ± 0.06	0.23 ± 0.14	0.20 ± 0.09	0.27 ± 0.20	< 0.3	0.23 ± 0.14
Selenium Total (µg/L) (16 below DL)	< 0.5 ± 0.00	< 0.5 ± 0.00	< 0.5 ± 0.00	0.37 ± 0.20	0.37 ± 0.20	< 0.5 ± 0.00
Silver Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Strontium Diss. (µg/L)	153.0 ± 2.6	148.3 ± 2.9	121.7 ± 1.5	135.7 ± 0.6	121.3 ± 2.1	127.7 ± 1.2
Strontium Total (µg/L)	161.3 ± 4.6	158.0 ± 0.0	131.7 ± 5.0	140.0 ± 1.7	130.0 ± 2.6	134.7 ± 1.5
Thallium Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Tin Total (µg/L)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Titanium Diss. (µg/L) (15 below DL)	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.10 ± 0.00
Titanium Total (µg/L)	14.93 ± 1.69	19.73 ± 3.90	15.87 ± 3.12	15.23 ± 2.00	13.80 ± 1.20	40.43 ± 25.45
Uranium Diss. (µg/L)	0.40 ± 0.00	0.40 ± 0.00	0.30 ± 0.00	0.40 ± 0.00	0.30 ± 0.00	0.30 ± 0.00
Uranium Total (µg/L)	0.50 ± 0.00	0.50 ± 0.00	0.43 ± 0.06	0.43 ± 0.06	0.40 ± 0.00	0.47 ± 0.06
Vanadium Diss. (µg/L)	0.20 ± 0.00	0.23 ± 0.06	0.20 ± 0.00	0.20 ± 0.00	0.20 ± 0.00	0.30 ± 0.00
Vanadium Total (µg/L)	3.50 ± 0.46	5.13 ± 1.15	4.00 ± 0.87	3.87 ± 0.60	3.43 ± 0.35	6.43 ± 0.76
Zinc Diss. (µg/L)	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4	< 0.4
Zinc Total (µg/L)	**8.50 ± 2.51**	**11.80 ± 3.35**	**9.20 ± 3.42**	**8.33 ± 2.02**	**7.67 ± 0.93**	**11.60 ± 1.87**

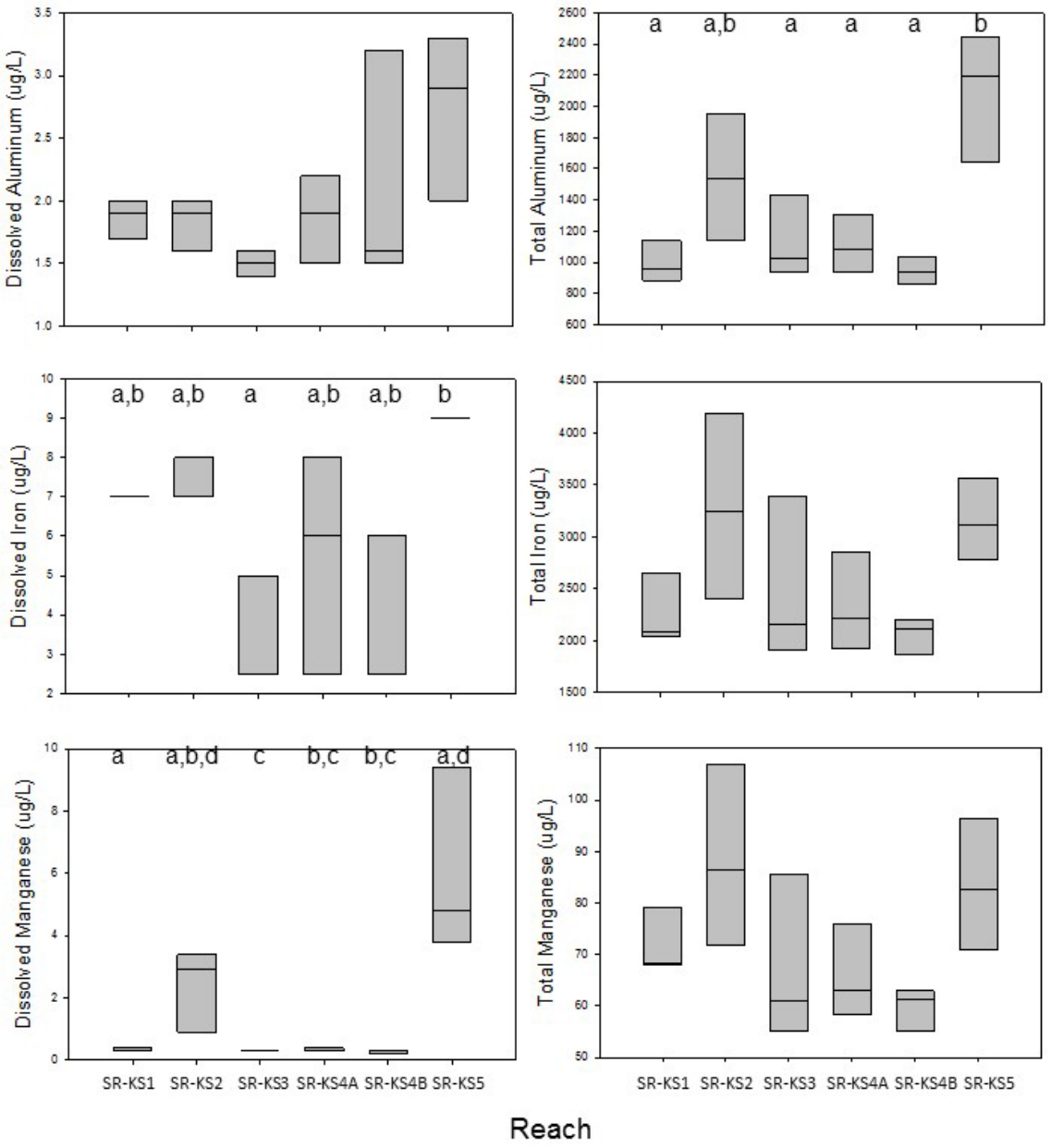

Figure 21. Box plots of dissolved and total metal concentrations for all reaches sampled in Slave River. Plotted is (left column, top to bottom) dissolved aluminum, dissolved iron, dissolved manganese, and (right column, top to bottom) total aluminum, total iron, and total manganese, all measured in ug/L. Lines through boxes indicate the median and lower and upper bounds plot the 25th and 75th percentiles, respectively. Letters on plots indicate significant differences ($\alpha = 0.05$) from one-way ANOVA and Tukey HSD tests.

Total metals appeared somewhat less variable among reaches, which suggested that the increased flow prior to sampling may have resulted in stronger homogeneity along the longitudinal sampling extent. For example, whereas total iron ranged from a mean of 962 to 4110 µg/L across reaches in 2017 (Lento 2018a), the range of means across reaches in 2018 was 2063 to 3076 µg/L (Table 8) and there were no statistically significant differences among reaches (Figure 21). Although mean values of total iron exceeded the CCME long-term exposure guidelines for the protection of aquatic life (Table 8), they were similar to or less than the long- term

Table 9. Physical habitat variables measured in the Slave River in 2018, summarized by reach. Velocity (spot measurement) and wetted width are presented as mean ± standard deviation (calculated based on 5 sites per reach); dominant streamside vegetation and periphyton coverage presented as the most common category in each reach across 5 sites; substrate composition presented as the sum of rock counts for each reach (20 rocks measured per site). Due to time constraints at the time of sampling, not all physical habitat variables could be measured for reach KS4A.

Variable	SR-KS1	SR-KS2	SR-KS3	SR-KS4A	SR-KS4B	SR-KS5
Velocity (m/s)	0.38 ± 0.12	0.18 ± 0.07	0.37 ± 0.13	NA	0.17 ± 0.08	0.26 ± 0.10
Wetted width (m)	431.0 ± 65.5	189.8 ± 3.3	593.6 ± 14.3	NA	178.8 ± 10.9	214.0 ± 27.9
Dominant streamside vegetation	deciduous and coniferous trees	shrubs	coniferous trees	NA	deciduous trees	deciduous trees
Periphyton coverage	< 0.5 mm thick	< 0.5 mm thick	< 0.5 mm thick	NA	< 0.5 mm thick	< 0.5 mm thick
Substrate - sand (%)	6	20	6	7	14	8
Substrate - gravel (%)	14	26	20	12	25	25
Substrate - pebble (%)	66	29	56	77	37	50
Substrate - cobble (%)	19	42	21	10	38	25
Substrate - boulder (%)	1	2	3	0	0	0
Substrate - bedrock (%)	0	1	0	0	0	0

median value of 3526 µg/L reported for the Slave River at Fort Smith (Sanderson et al. 2012), which indicated that they were not unusual for this system. Total aluminum was similar across most reaches, but statistically significantly higher in Reach 5 (Figure 21). Though total aluminum concentrations exceeded the CCME long-term exposure guidelines for the protection of aquatic life (Table 8), they were lower than the long-term median value of 4360 µg/L reported for the Slave River at Fort Smith (Sanderson et al. 2012). Total manganese showed no statistically significant difference among reaches, but was somewhat higher on average in Reaches 2 and 5 than the long-term median value for the river (67 µg/L; Sanderson et al. 2012).

3.1.2.2. Physical habitat

Measurements were taken at each site to characterize they physical habitat in BMI sampling locations, including variables such as velocity, river width, streamside vegetation, in-stream periphyton cover, and substrate composition (Table 9). Due to time constraints at the time of sampling, some variables were not measured at Reach 4A. Sites fell into two groups based on velocity at the time of sampling, with lower velocity at Reach 2 and

Reach 4B (and at Reach 4A, though this was not measured; average velocity 0.17-0.18 m/s in these reaches), and higher velocity at Reach 1, 3, and 5 (0.26-0.38 m/s on average; Table 9). The higher velocity reaches included those with the largest wetted width measurements. Deciduous or coniferous trees were generally the dominant type of streamside vegetation, though the streamside vegetation of Reach 2 was dominated by shrubs. Periphyton coverage was extremely low in most reaches, with little to no visible algal coverage on rocks. Substrate composition was generally dominated by a combination of pebble, gravel and cobble in all reaches, though Reach 2 and Reach 4B had slightly higher percent composition of sand than the other reaches (Table 9).

3.1.2.3. Chemical and physical habitat

Multivariate analysis of abiotic data was used as an exploratory analysis of patterns in water chemistry and physical habitat within and among reaches in the Slave River, and to identify parameters that might be used for analysis of biotic-abiotic relationships. The PCA for the Slave River indicated some negative correlations among reaches with respect to water chemistry and physical habitat, but stronger positive correlations within reaches than was evident for Hay River (Figure 22). The first axis of the PCA explained 41.5% of the variation among sites, and separated sites in Reach 2 (particularly KS2-1A and KS2-5A) and Reach 5 from sites in Reach 3, Reach 4A, and Reach 4B (Figure 22). On the positive end of this axis, sites in Reach 2 were positively correlated with ions, nutrients (primarily nitrogen and carbon), sand, turbidity, and metals, whereas sites in Reach 5 were primarily associated with metals, turbidity, gravel, and nutrients (primarily TP and ammonia; Figure 22). The sites on the negative end of the first axis gradient were negatively correlated with nearly all chemical parameters and were only positively correlated with substrate size (pebble and boulder). The second axis, which explained 22.2% of the variation in sites, separated higher pH sites on the positive end of the gradient (including sites in Reach 1), which were positively associated with ions, from lower pH sites on the negative end of the gradient, which were positively associated with metals (Figure 22). Sites in Reach 1 and Reach 2 were similarly positively correlated with pH when data were analyzed for 2017 (Lento 2018a), whereas Reach 5 was similarly negatively correlated with ions and buffering capacity in 2017, indicating some similarity among years.

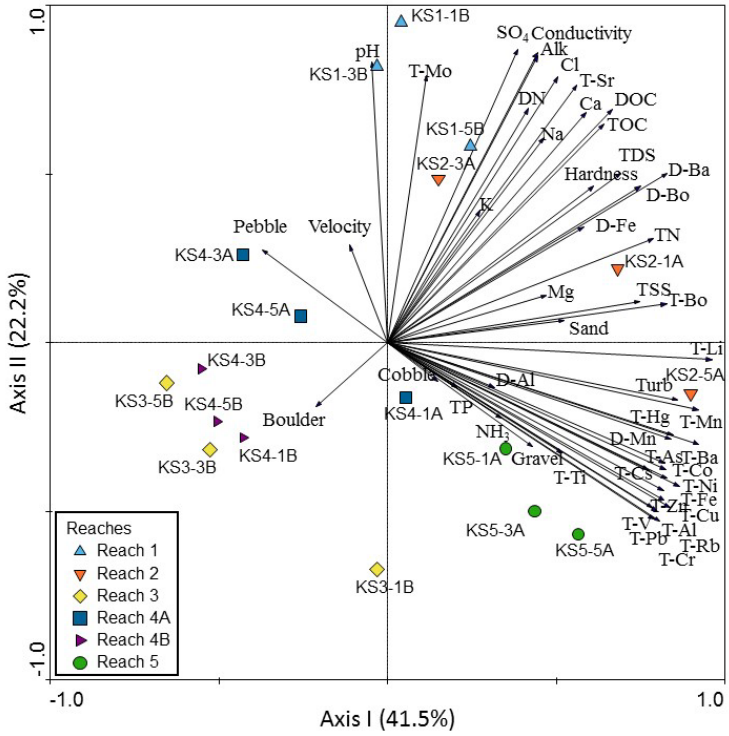

Figure 22. PCA ordination of water chemistry and habitat variables at Slave River sample kick-sites, with kick-sites colour-coded based on reach number. Arrows point in the direction of increasing values of parameters, and correlations of kick-sites with parameters are indicated by the location of kick-site points in proximity to arrows. Kick-site points located near the origin have similar correlations with all measured parameters." D- "in front of metals indicates dissolved form, and "T-"indicates total metals.

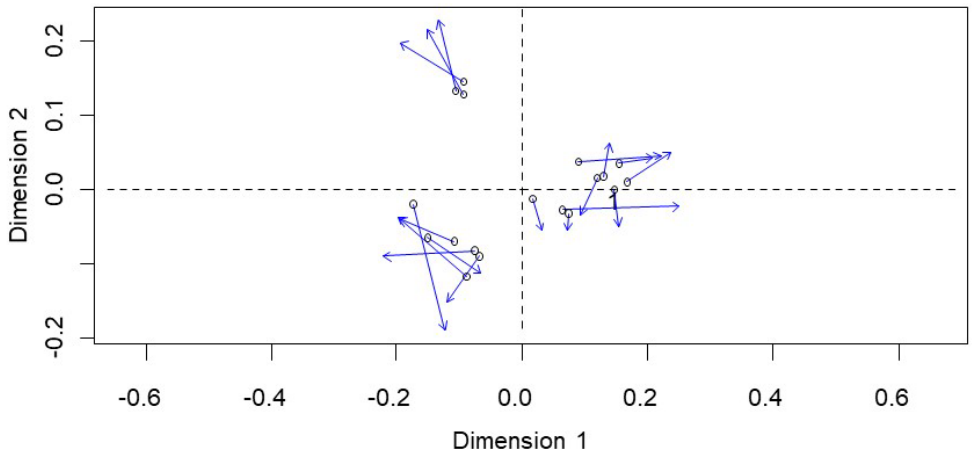

Figure 23. Residuals from Procrustes analysis of slave River chemical/physical habitat ordinations from 2017 and 2018 using only odd-numbered sites from each reach, showing the position of sites in 2017 (circles) and the distance and direction moved in multivariate space in 2018 (arrows). The longer the arrow, the more a site moved between years, and the more different its chemical/physical habitat was from the sites it resembled in 2017.

3.1.2.3.1. Temporal comparison

Comparisons of water chemistry data from spot measurements in 2017 and 2018 should be made with caution, as they are not indicative of long-term trends. However, comparison of ordinations was completed as an exploratory analysis of the differences at the time of sampling in each year. Procrustes analysis was used to compare the chemical and physical habitat of the sample sites between 2017 and 2018 (with 2017 as the target matrix and 2018 as the rotational matrix), and test whether there were significant differences in the spatial arrangement of sites between ordinations of each year. This analysis used only sites sampled in both 2017 and 2018 (i.e., only odd-numbered sites in each reach), and only chemical/physical parameters that were available and above detection limit for both years (chosen from the full suite of ions, nutrients, physicals, dissolved and total metals, and physical habitat variables).

The results of the Procrustes analysis for the Slave River indicated that the two ordinations were more similar than could be obtained by chance (p = 0.001). There were differences evident in the spatial arrangement of sites, and the sum of squared residuals (m_{12}^2) was 0.35, similar to what was found for Hay River chemical and physical variables; however, there were more sites compared between years for the Slave River (21 sites for the Slave River and 18 sites for the Slave River) and thus there was less variation per site between sampling years (similar sum of squared residuals with greater n). The notable difference between the Slave River residuals and those for the Hay River was that Slave River sites remained in fairly close proximity to each other in 2018, despite some shifting of relationships among sites (Figure 23). Clusters of sites in 2017 remained largely the same in 2018, but with some shifting of position among sites (Figure 23). This is in contrast to the Hay River, where sites moved farther apart in 2018.

3.1.2.4. Sediment chemistry

Sediment chemistry samples were collected from two sites in each Slave River reach (sites 1 and 5) and analyzed for metals and polycyclic aromatic hydrocarbons (PAHs). Samples from Reaches 1 through 4B were additionally analyzed for trace elements (not completed for Hay River samples or the sample from SR-KS5, which was analyzed with Hay River samples). To determine whether levels of metals or PAHs were elevated beyond

Table 10. Summary of sediment chemistry parameters sampled in the Slave River at all sample reaches, indicating site mean ± standard deviation for each reach. When both sites in a reach were below detection limit, the detection limit is indicated. When only one site in a reach was below detection limit, half the detection limit was used in calculations (number of sites below DL indicated in Parameter column). Note that detection limits differed among sites for some parameters. N/A is indicated when parameters were not measured at a site. Values were compared with CCME sediment quality guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment 2001a), and values in bold were greater than interim freshwater sediment quality guidelines (ISQGs) whereas values in red were greater than probable effect levels (PELs).

Parameter	SR-KS1	SR-KS2	SR-KS3	SR-KS4A	SR-KS4B	SR-KS5
Particle size/physicals						
% Clay (<2µm)	8.6 ± 1.8	3.8 ± 0.1	7.3 ± 2.1	3.4 ± 1.8	4.7 ± 1.2	10.5 ± 4.5
% Sand (2.0mm-0.05mm)	37.1 ± 10.0	49.1 ± 36.8	50.5 ± 19.2	74.7 ± 1.7	59.6 ± 1.1	35.2 ± 22.5
% Silt (0.05mm - 2µm)	54.4 ± 8.3	47.1 ± 37.0	42.3 ± 17.0	21.9 ± 0.1	35.8 ± 0.1	54.3 ± 18.0
Moisture %	31.4 ± 2.5	28.0 ± 4.0	32.1 ± 0.6	21.5 ± 4.3	31.3 ± 8.8	37.7 ± 0.8
Metals (mg/kg)						
Aluminum (Al)	6600.0 ± 99.0	6905.0 ± 3387.0	6590.0 ± 1159.7	4780.0 ± 664.7	7625.0 ± 629.3	N/A
Antimony (Sb)	0.330 ± 0.000	0.320 ± 0.085	0.360 ± 0.000	0.290 ± 0.028	0.425 ± 0.106	0.370 ± 0.042
Arsenic (As)	5.835 ± 0.049	**5.995 ± 1.011**	**6.365 ± 0.035**	5.305 ± 0.955	**7.115 ± 0.912**	**6.725 ± 0.417**
Barium (Ba)	287.0 ± 31.1	273.5 ± 20.5	276.0 ± 62.2	248.0 ± 60.8	266.5 ± 6.4	300.5 ± 2.1
Beryllium (Be) (2 below DL)	0.38 ± 0.02	0.41 ± 0.18	0.40 ± 0.04	0.28 ± 0.02	0.47 ± 0.06	< 1.0
Bismuth (Bi)	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	N/A
Boron (B) (4 below DL)	5.5 ± 0.3	5.1 ± 3.7	4.6 ± 3.0	< 5.0	6.7 ± 0.8	N/A
Cadmium (Cd) (2 below DL)	0.369 ± 0.023	0.366 ± 0.182	0.403 ± 0.028	0.258 ± 0.059	**0.612 ± 0.182**	0.250 ± 0.000
Calcium (Ca)	24600 ± 1838	19200 ± 141	20750 ± 1061	18550 ± 1202	19300 ± 566	N/A
Chromium (Cr)	13.7 ± 0.3	14.8 ± 4.0	13.8 ± 1.6	13.2 ± 0.2	15.6 ± 0.2	22.2 ± 9.2
Cobalt (Co)	6.6 ± 0.2	7.1 ± 1.2	7.3 ± 0.3	6.6 ± 1.3	7.7 ± 0.6	7.1 ± 0.4
Copper (Cu)	11.20 ± 0.71	10.95 ± 6.58	12.10 ± 0.14	9.91 ± 5.08	16.50 ± 3.96	14.45 ± 1.06
Iron (Fe)	15500 ± 141	16750 ± 3465	16350 ± 71	14500 ± 990	17300 ± 849	N/A
Lead (Pb)	6.31 ± 0.23	6.50 ± 2.50	6.77 ± 0.08	5.90 ± 1.87	7.91 ± 1.08	7.40 ± 0.99
Lithium (Li)	8.9 ± 0.3	9.6 ± 4.4	9.2 ± 0.8	7.0 ± 1.2	9.8 ± 0.6	N/A
Magnesium (Mg)	7045 ± 346	6775 ± 912	6800 ± 156	6365 ± 417	6865 ± 191	N/A
Manganese (Mn)	275 ± 6	280 ± 94	278 ± 8	222 ± 20	288 ± 21	N/A
Mercury (Hg)	0.0434 ± 0.0078	0.0451 ± 0.0092	0.0418 ± 0.0080	0.0270 ± 0.0012	0.0445 ± 0.0047	0.0512 ± 0.0078
Molybdenum (Mo) (1 below DL)	0.78 ± 0.01	0.69 ± 0.19	0.77 ± 0.00	0.56 ± 0.06	0.85 ± 0.13	1.15 ± 0.92
Nickel (Ni)	19.2 ± 0.4	20.3 ± 4.8	20.7 ± 0.5	17.4 ± 2.3	22.7 ± 2.0	24.8 ± 4.0
Phosphorus (P)	749 ± 13	738 ± 36	697 ± 8	731 ± 59	680 ± 1	N/A
Potassium (K)	865 ± 49	915 ± 488	885 ± 247	610 ± 99	1040 ± 99	N/A
Selenium (Se) (4 below DL)	0.39 ± 0.04	0.26 ± 0.22	0.37 ± 0.02	0.19 ± 0.12	0.44 ± 0.13	< 0.5
Silver (Ag) (6 below DL)	0.08 ± 0.04	0.10 ± 0.06	0.10 ± 0.00	0.05 ± 0.00	0.14 ± 0.04	< 0.2
Sodium (Na)	79 ± 4	80 ± 18	81 ± 4	62 ± 4	84 ± 6	N/A
Strontium (Sr)	51.7 ± 0.9	48.5 ± 6.3	52.5 ± 2.8	43.6 ± 0.3	53.5 ± 6.5	N/A
Sulfur (S)	< 1000	< 1000	< 1000	< 1000	< 1000	N/A

Parameter	SR-KS1	SR-KS2	SR-KS3	SR-KS4A	SR-KS4B	SR-KS5
Thallium (Tl) (2 below DL)	0.1115 ± 0.0021	0.1130 ± 0.0636	0.1155 ± 0.0106	0.0745 ± 0.0163	0.1450 ± 0.0240	< 0.5
Tin (Sn)	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 5.0
Titanium (Ti)	93.1 ± 11.2	109.5 ± 34.7	88.1 ± 26.7	137.0 ± 33.9	104.0 ± 5.7	N/A
Tungsten (W)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	N/A
Uranium (U) (2 below DL)	0.841 ± 0.045	0.807 ± 0.224	0.832 ± 0.016	0.736 ± 0.069	0.952 ± 0.042	< 2.0
Vanadium (V)	24.7 ± 1.3	27.2 ± 6.3	25.2 ± 4.1	23.5 ± 0.5	29.1 ± 2.0	24.8 ± 1.1
Zinc (Zn)	62.0 ± 2.3	63.4 ± 19.5	64.6 ± 1.3	51.6 ± 8.3	71.1 ± 5.2	66.0 ± 6.7
Zirconium (Zr)	3.3 ± 0.0	3.5 ± 0.9	3.6 ± 0.1	3.0 ± 0.2	4.6 ± 1.1	N/A
Polycyclic Aromatic Hydrocarbons (PAHs) (mg/kg)						
1-Methylnaphthalene	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	N/A
2-Methylnaphthalene	**0.024 ± 0.001**	**0.022 ± 0.012**	**0.029 ± 0.001**	0.017 ± 0.001	**0.046 ± 0.018**	0.018 ± 0.001
Acenaphthene	< 0.005	< 0.019	< 0.005	< 0.005	< 0.005	< 0.005
Acenaphthylene	< 0.005	< 0.019	< 0.005	< 0.005	< 0.005	< 0.005
Anthracene	< 0.004	< 0.019	< 0.004	< 0.004	< 0.004	< 0.004
B(a)P Total Potency Equivalent (11 below DL)	< 0.02	0.017 ± 0.009	< 0.02	< 0.02	< 0.02	< 0.02
Benz(a)anthracene	< 0.01	< 0.019	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(a)pyrene	< 0.01	< 0.019	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(b&j)fluoranthene (3 below DL)	0.014 ± 0.000	< 0.019	0.017 ± 0.002	0.011 ± 0.000	0.022 ± 0.004	0.008 ± 0.004
Benzo(b+j+k)fluoranthene (7 below DL)	< 0.015	< 0.027	0.013 ± 0.007	0.008 ± 0.000	0.022 ± 0.004	N/A
Benzo(g,h,i)perylene (3 below DL)	0.015 ± 0.001	0.012 ± 0.010	0.016 ± 0.000	0.011 ± 0.001	0.018 ± 0.003	< 0.01
Benzo(k)fluoranthene	< 0.01	< 0.019	< 0.01	< 0.01	< 0.01	< 0.01
Chrysene (10 below DL)	< 0.02	< 0.03	< 0.03	< 0.03	< 0.03	0.013 ± 0.004
Dibenz(a,h)anthracene	< 0.005	< 0.019	< 0.005	< 0.005	< 0.005	< 0.005
Fluoranthene (11 below DL)	< 0.01	< 0.019	< 0.01	< 0.01	0.0090 ± 0.0057	< 0.01
Fluorene	< 0.01	< 0.019	< 0.01	< 0.01	< 0.01	< 0.01
IACR (CCME) (6 below DL)	0.17 ± 0.00	< 0.023	0.18 ± 0.01	< 0.15	0.22 ± 0.03	< 0.15
Indeno(1,2,3-c,d)pyrene	< 0.01	< 0.019	< 0.01	< 0.01	< 0.01	< 0.01
Naphthalene (6 below DL)	0.0140 ± 0.0014	< 0.019	0.0170 ± 0.0014	0.0050 ± 0.0000	0.0260 ± 0.0127	< 0.01
Phenanthrene	0.0260 ± 0.0014	0.0240 ± 0.0156	0.0305 ± 0.0021	0.0180 ± 0.0014	0.0415 ± 0.0120	0.0180 ± 0.0028
Pyrene (5 below DL)	0.0115 ± 0.0007	< 0.019	0.0135 ± 0.0021	0.0050 ± 0.0000	0.0185 ± 0.0035	0.0075 ± 0.0035
Quinoline	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.01

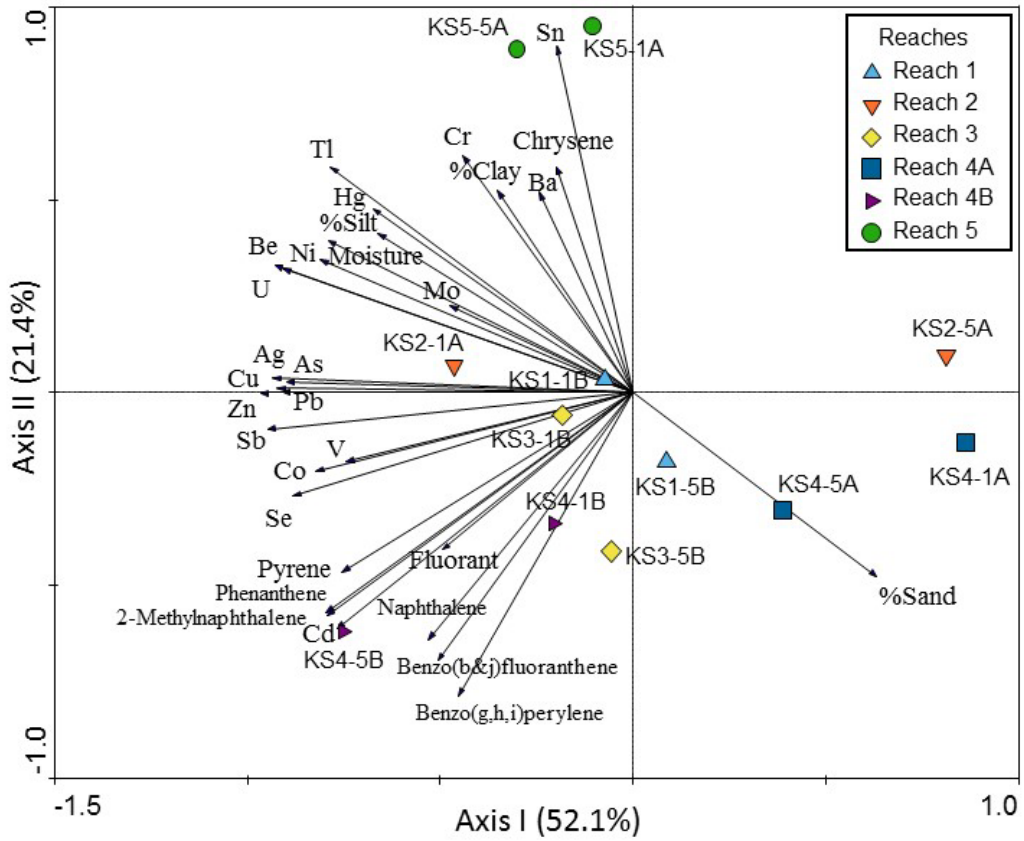

Figure 24. PCA ordination of sediment chemistry samples from Slave River kick sites, include two samples from each of six reaches (sample points coloured by reach). Ordination includes physical attributes of sediment sample and concentrations of metals and PAHs that were above detection limit and sampled in all reaches. Arrows point in the direction of increasing values of parameters, and correlations of sites with parameters are indicated by the location of kick-site points in proximity to arrows. Kick-site points located near the origin have similar correlations with all measured parameters.

recommended levels in Slave River samples, mean values for each site were compared with CCME sediment quality guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment 2001a), which include interim freshwater sediment quality guidelines (ISQGs) and probably effect levels (PELs). In addition, Benzo[a]pyrene Total Potency Equivalents and the Index of Additive Cancer Risk (IACR) were compared with guideline levels to ensure protection of humans and drinking water, respectively (Canadian Council of Ministers of the Environment 2010).

Average values for several PAHs were above detection limits in the Slave River reaches, though most PAHs were below guideline levels. 2-methylnaphthalene, which is a methyl polycyclic aromatic hydrocarbon (Me-PAH) was the only PAH species that was found at concentrations above the ISQG (Table 10). A study of PAH uptake by invertebrates indicated a low negative correlation between sediment concentrations and concentrations of 2-methylnaphthalene in tissues of amphipods and polychaetes, and suggested that uptake via sediment ingestion was not a primary route of exposure for this and similar species of PAHs (Meador et al. 1995). Furthermore, tests on exposure of fish to these compounds indicated high uptake of 2-methylnaphthalene from water (Melancon Jr. and Lech 1979). Therefore, high levels of 2-methylnaphthalene in sediment may not have strong implications for biota if concentrations in the water are not similarly high.

Some PAHs with potential carcinogenic effects (listed in Canadian Council of Ministers of the Environment 2010) were above detection limit in several reaches, including benzo(b+j+k)fluoranthene, benzo(g,h,i)perylene, and chrysene (Table 10). Levels of these PAHs remained fairly low, and were below guideline levels, but the B(a)P Total Potency Equivalent was elevated above the detection limit in Reach 2 (Table 10). In contrast, the IACR, which measures threat to drinking water, was below detection limit in all reaches.

There were fewer metals in Slave River sediment samples that were above the ISQG than was observed for the Hay River, and no metals exceeded PELs (Table 10). Arsenic was the only metal to exceed the ISQG, and these exceedances were minor (Table 10). The percent clay was low in all reaches (ranging on average from 3.4 to 10.5%; Table 10), which may have contributed to lower levels of metals in sediment samples. Variability was generally low for metals, indicating fairly high precision between samples collected in the same reach.

Low variability within reaches resulted in close proximity of sites from the same reach in a PCA ordination of sediment chemistry data for the Slave River (Figure 24). Most site pairs were close together in ordination space, indicating overall similar sediment chemistry within reaches, with the exception of KS2-1A and KS2-5A, which were located on opposite ends of the first axis (Figure 24). The remaining reaches had generally low variability in grain size and concentrations of metals and PAHs.

The first axis of the Slave River PCA, which explained 52.1% of variation among samples, was dominated by a gradient in fine grain size and associated concentrations of metals (Figure 24), similar to the patterns observed in Hay River. On the positive end of the first axis gradient, sites such as KS2-5A and the two sites in Reach 4A were positively correlated with % sand and negatively correlated with the majority of metals (Figure 24). Some sites had a positive correlation with % sand, but were also positively correlated with cadmium and a number of PAHs along the second axis; this included sites in Reach 4B and site KS3-5B (Figure 24). In contrast, sites in Reach 5 were positively correlated with % clay and silt, as well as several metals (including tin, barium, chromium, thallium, mercury) and the PAH chrysene (Figure 24). Although levels of metals did appear to relate to grain size in Slave River sediment samples, levels of PAHs were generally uncorrelated with grain size and were positively correlated with only a small number of sites.

3.1.2.5. Biotic assemblages

3.1.2.5.1. Summary metrics

Biotic metrics were used to compare abundance, relative abundance, and taxonomic richness of key organism groups among sites and reaches. Biotic metrics were quite variable among reaches, particularly when abundance-based metrics were considered (Table 11). Total abundance ranged from 9 individuals to 3260 individuals across all sites. On average, abundance was highest in Reach 1 and Reach 3, and was lowest in Reach 2 (Table 11). This is in contrast to 2017, when abundance in Reach 2 was higher than in Reach 3. Total abundance in 2018 was statistically significantly higher in Reach 1 and Reach 3 than in Reach 4A (Figure 25). Three of the sites in Reach 4A had extremely low

abundance (ranging from 9 to 23 individuals in a sample). EPT abundance followed similar patterns to total abundance because these three orders made up a large proportion of the total assemblage in several reaches (Reaches 1, 3, and 5, where % EPT ranged from 62-85%; Table 11, Figure 25). Total abundance of EPT ranged from 1 individual to 2170 individuals per site. Average EPT abundance was statistically significantly higher in Reach 1 and Reach 3 than in Reach 2 or Reach 4A, and Reach 3 also had statistically significantly higher EPT abundance than Reach 4B (Table 11). The abundance of Chironomidae, in contrast, was similar across all

Table 11. Summary of biotic metrics for kick-site reaches sampled in the Slave River in 2018, including the mean ± standard deviation for BMI abundance and taxonomic richness metrics. EPT is the sum of Ephemeroptera, Plecoptera, and Trichoptera orders, Chironomidae is a family of Diptera, and Diptera + Oligochaeta includes all true flies and segmented worms.

Biotic Metric	SR- KS1B	SR-KS2A	SR-KS3B	SR-KS4A	SR-KS4B	SR-KS5A
Total abundance	1192 ± 406	319 ± 290	1604 ± 1175	531 ± 887	784 ± 395	592 ± 353
EPT abundance	1037 ± 418	187 ± 265	1105 ± 736	127 ± 223	269 ± 83	414 ± 282
Chironomidae abundance	28 ± 18	28 ± 31	63 ± 42	31 ± 51	19 ± 10	55 ± 35
Diptera + Oligochaeta abundance	39 ± 20	45 ± 40	82 ± 51	35 ± 52	25 ± 13	72 ± 48
Mollusca abundance	6 ± 9	6 ± 5	4 ± 4	0 ± 0	0 ± 0	13 ± 16
Percent EPT	85.0 ± 8.0	39.2 ± 31.3	74.3 ± 16.8	16.5 ± 8.5	38.6 ± 15.9	62.9 ± 19.6
Percent Chironomidae	2.9 ± 2.5	10.2 ± 10.8	4.1 ± 1.7	6.7 ± 4.5	2.6 ± 1.3	10.2 ± 7.0
Percent Diptera + Oligochaeta	3.9 ± 2.8	23.3 ± 23.8	5.4 ± 1.9	18.1 ± 11.8	3.6 ± 2.0	13.9 ± 10.0
Percent Mollusca	0.6 ± 0.9	1.9 ± 1.0	0.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 1.7
Taxonomic richness	20.6 ± 7.0	20.6 ± 7.4	22.4 ± 3.0	11.8 ± 8.1	18.2 ± 4.3	27.0 ± 7.3
EPT richness	9.6 ± 1.9	7.0 ± 3.5	9.2 ± 0.8	3.8 ± 3.0	8.2 ± 0.8	10.6 ± 2.5
Chironomidae richness	4.8 ± 4.3	4.8 ± 2.2	5.4 ± 1.3	3.8 ± 4.1	5.2 ± 2.4	7.0 ± 2.0
Diptera + Oligochaeta richness	6.4 ± 4.6	8.8 ± 4.3	8.2 ± 1.5	5.8 ± 4.4	7.4 ± 3.6	11.4 ± 3.8
Mollusca richness	0.6 ± 0.9	1.4 ± 0.5	0.8 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 1.8

reaches, and mean abundance values were extremely low, ranging from only 19 to 63 individuals (Table 11). As a result, the percent composition of Chironomidae in Slave River reaches ranged from 2.6% to 10.2% (Table 11, Figure 25), indicating that they made up only a minor portion of the samples in 2018, contrasting with 2017 when Chironomidae made up 12-63% of the total assemblage. This drop in abundance of Chironomidae likely reflected flow conditions in 2018, as low water levels followed by increased flow late in the season (Figure 2B). Sampling in 2017 occurred approximately 100 days after a peak flow, whereas sampling in 2018 took place approximately 45 days after a peak in discharge (Figure 2B). Because water levels remained high so late in the season, many shoreline samples may have been collected in temporary habitat without established BMI assemblages. Generally, such sampling can be expected to result in low abundance and diversity of BMI samples, and samples may be dominated by more mobile taxa, such as Ephemeroptera, whereas burrowing taxa such as some Chironomidae and worms may not have colonized the temporary habitat, or may be buried deep within the substrate if that habitat had previously dried up. The change in Chironomidae abundance from 2017 to 2018 will be further discussed in section 3.1.2.5.1).

Taxonomic richness was similar among the upstream reaches (Reaches 1-3), but more variable downstream (Figure 25). Across all sites, total taxonomic richness ranged from 4 to 35 taxa per site. Average richness was lowest at Reach 4A and highest at Reach 5; these two reaches had statistically significantly different mean taxonomic richness (Figure 25). Reach 5 had higher richness of both EPT and Chironomidae than other reaches (though only EPT richness was significantly different than other reaches; Figure 25), whereas Reach 4A had lower EPT richness and Chironomidae richness than other reaches (Table 11, Figure 25). In other reaches, EPT richness and Chironomidae richness were similar. Reach 4A generally stood out as having lower abundance and richness of most groups of organisms, and notably included a site with no Chironomidae and only one individual

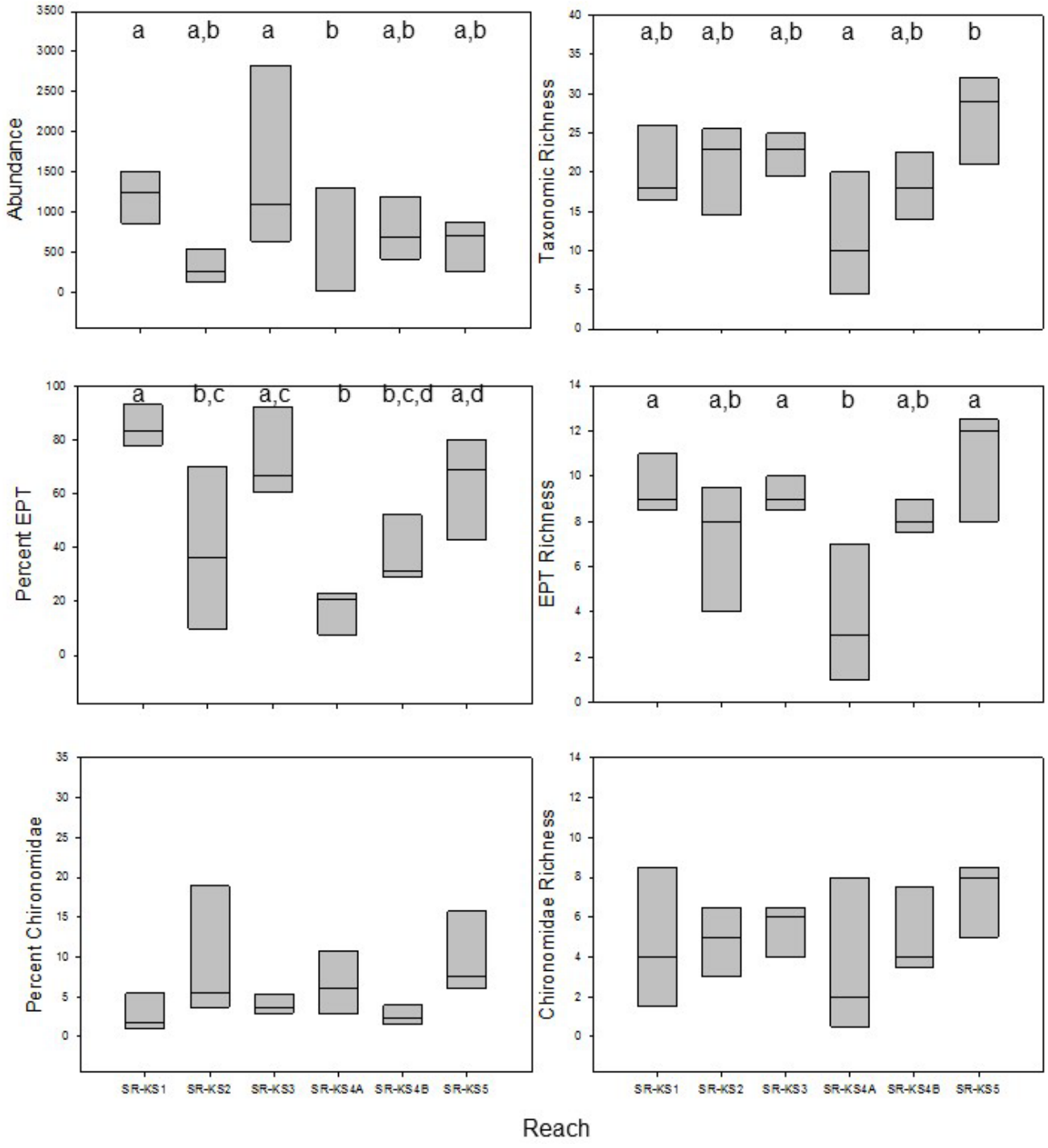

Figure 25. Box plots of BMI metrics for the reaches in the Slave River that were sampled with kick sampling protocols. Metrics include overall abundance and richness, percent composition and richness of Ephemeroptera, Plecoptera, and Trichoptera (EPT), and percent composition and richness of Chironomidae (midges). Lines through boxes indicate the median and lower and upper bounds plot the 25th and 75th percentiles, respectively. Letters on plots indicate significant differences ($\alpha = 0.05$) from one-way ANOVA and Tukey HSD tests.

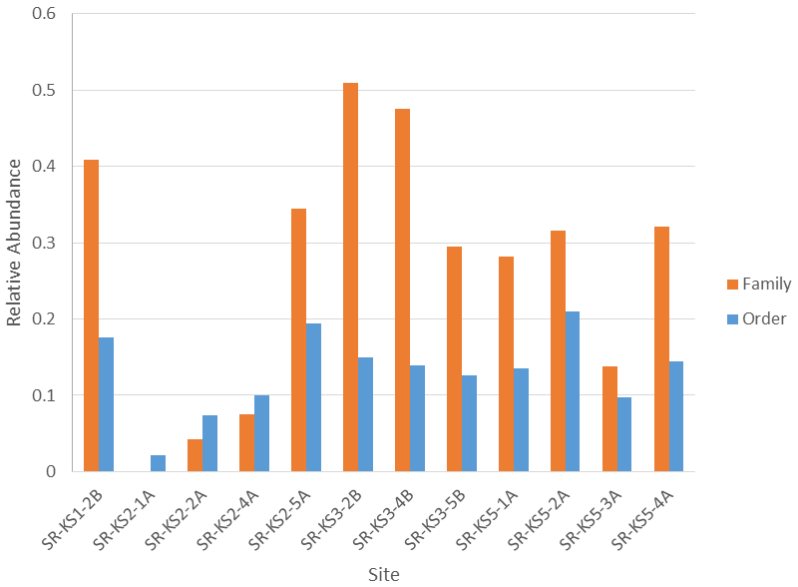

Figure 26. Relative abundance of Trichoptera in Slave River samples, showing the portion identified to family level (orange) or order level (blue) as a function of the total abundance of all organisms in the sample.

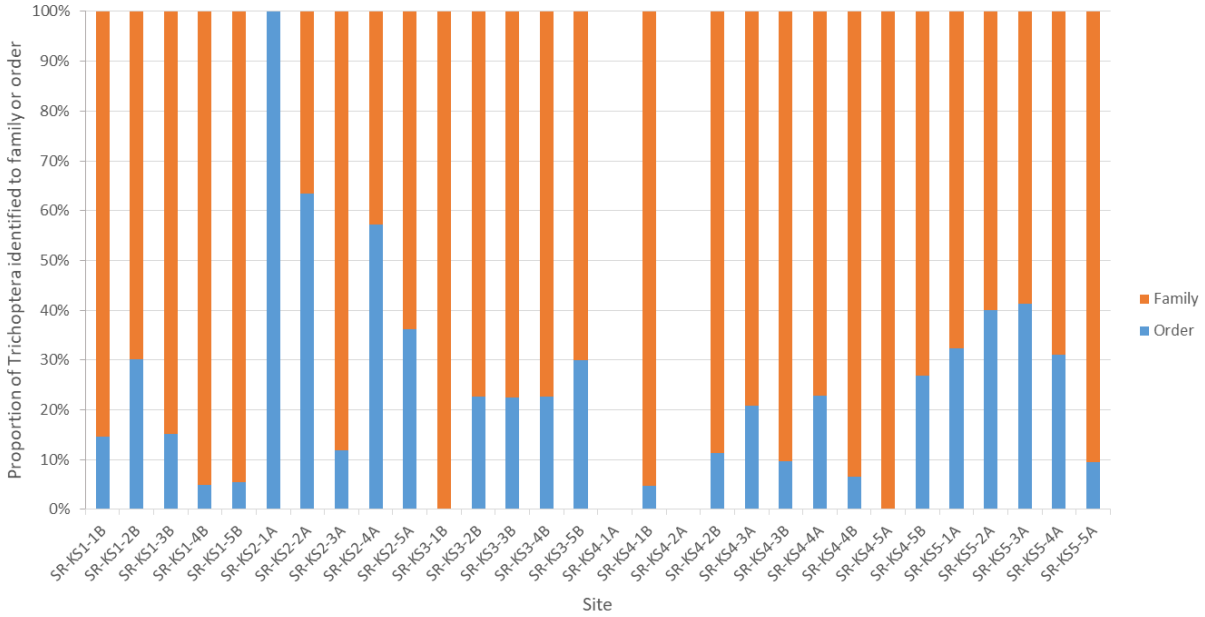

Figure 27. Proportion of Trichoptera in each Slave River sample that was identified to the level of family (orange) or order (blue). Values represent the proportion of all Trichoptera taxa, rather than the proportion of the full sample.

of EPT. Strong differences between this reach and other reaches in 2018 and in 2017 suggest that it may not be an ideal reach for long-term monitoring of BMI assemblages.

There was a large number of Trichoptera individuals in Slave River samples that were identified to order level because they were too small to distinguish identifying features (Biological personal communication). Order-level identification of Trichoptera occurred for all sites in the Slave River in which this group was found (all but two

sites), and order-level individuals accounted for as much as 21% of the entire sample (Figure 26). Among Trichoptera, individuals identified to order level accounted for greater than 50% of all Trichoptera in three sites in Reach 2, and greater than 30% of Trichoptera in four sites in Reach 5. Although this did not affect the calculation of abundance-based metrics that included Trichoptera, it may have led to underestimates of total richness and EPT richness across all sites, but particularly in Reach 2 and Reach 5.

3.1.2.5.2. Assemblage composition and biotic-abiotic relationships

Multivariate analysis was used to characterize the biotic assemblage of the Slave River and evaluate similarities and differences in assemblage composition among reaches and sites. This analysis was intended to assess correlations within and among reaches, and to identify any potential outliers or gaps in sample stations. BMI relative abundance data for all taxa were assessed at the family/subfamily level. Because of the large number of Trichoptera that were identified to order level, there was an issue of mixed-level taxonomy in the data. To avoid taxonomic redundancy in the analysis (i.e., finding differences between sites due to Trichoptera at order level or family level, when they could actually refer to the same taxon), individuals identified as Trichoptera were removed from the analysis. Analyses were run in this manner, and were also run with all Trichoptera grouped together at the order level to assess the impact of removing these individuals. Multivariate analysis results were similar for both datasets, so results for the analysis at family level (excluding individuals identified to order level for Trichoptera) were retained.

Multivariate analysis indicated the presence of strong outliers, notably KS4-1A and KS4-2A (Figure 28A). Sites in Reach 2 were also variable and spread across the first and second axes, with KS2-1 differing the most from other sites along the first axis. The difference between these sites and the remaining Slave River sites contributed to most of the spread along axis I of the PCA ordination, which explained 56.8% of the variation among samples. Remaining samples were primarily spread along the second axis, which explained 17.5% of the variation among samples. Given the results of the biotic metric comparison, it was not surprising to find that sites in Reach 2 and Reach 4 were outliers in the analysis of assemblage composition. These reaches had low abundance and low richness, and compositional differences between these sites and the other Slave River sites dominated the first axis of the PCA plot as a result.

Sites in some reaches were more similar than they were in 2017. For example, sites in Reach 3 clustered together in 2018, but were further separated in 2017. In addition, most of the sites in Reach 4B were more tightly clustered in 2018. However, there was one site in each of reaches 1, 4B, and 5 that did not group with the rest of the reach sites (Figure 28A), indicating some dissimilarity within reaches. There were some similarities among reaches, evidenced by site groupings in ordination space (Figure 28A). Most sites in Reach 1 were grouped with Reach 3, which is consistent with the biotic metric comparison, while most sites in Reach 4B were grouped with Reach 5. Reach 2 and Reach 4A were more variable due to outlier sites. These associations differ somewhat from patterns observed in 2017, when Reach 1 and Reach 4B were found to be similar and Reach 5 was more similar to Reach 2 and Reach 4A (Reach 3 was variable). Differences in similarity among reaches likely reflect the effects of temporal variability and flow differences between years. Additional monitoring data from 2019 will be used to further assess and confirm relationships among reaches.

There were few taxa associated with the outlier sites, which reflected the low taxonomic richness and abundance that was particularly evident in KS4-1A and KS4-2A (Figure 28B). These sites were associated with taxa such as Corixidae (true bug), which is not benthic, highly mobile, and generally prefers pool or wetland habitats, and two families of segmented worms (Enchytraeidae and Naididae) that also prefer slow velocity (Monk et al. 2018). KS2-1A, which was also widely separated from other sites, was additionally positively

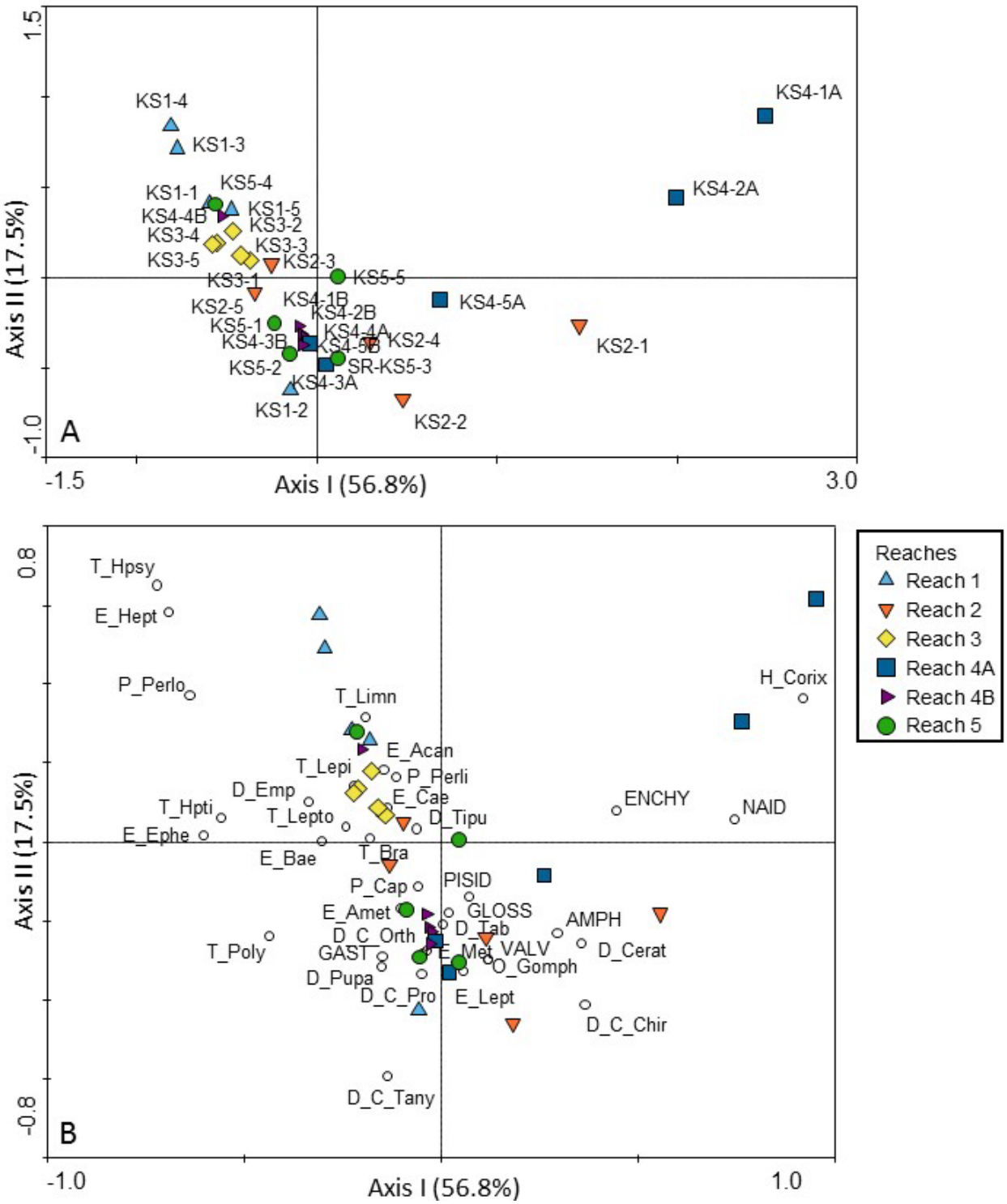

Figure 28. Multivariate analysis of BMI from kick samples in the Slave River, including (A) PCA of BMI data with sites labelled, and (B) PCA biplot of BMI data with labelled taxa and with sample points coloured by reach. Kick-sites in close proximity have similar assemblages, whereas samples on opposite ends of gradients have differences in their assemblages. Samples at right angles through the origin are uncorrelated. Kick-sites are located close to taxa with which they are positively associated. Taxonomic abbreviations are listed in the appendices.

associated with Amphipoda (another non-benthic taxon that prefers pools) and the Diptera Ceratopogonidae and Chironominae (Figure 28B). This site was strongly negatively associated with taxa more common in faster flows, including Hydropsychidae, Heptageniidae, and Perlodidae (McCafferty 1998, Monk et al. 2018). The taxon associations for these three sites are suggestive of slower flow and more sheltered pool-like habitats.

Because Reach 4A appeared to be such a strong outlier, multivariate analysis was also run while excluding sites from that reach. This allowed for evaluation of the spread of sites in multivariate space without the first axis being dominated by the strong outlier sites. The first axis of this reduced PCA was still driven by the spread of some outliers (here, sites in Reach 2) that were associated with taxa that prefer slow-flow conditions or pools, including some non-benthic taxa, and by sites that had low richness in general (thus low association with most taxa; Figure 29). The remaining sites were generally clustered by reach, with the exception of Reach 5, which was spread along the second axis gradient (Figure 29). On the positive end of the first axis gradient, sites were generally associated with indicators of slow flow, whereas sites on the negative end of the first axis gradient had a stronger association with a number of EPT taxa that are indicative of faster flows, including Hydropsychidae, Heptageniidae, Perlodidae, and Perlidae. Shifting kick sites laterally in the river channel to ensure more similar velocities across all reaches may reduce the dominance of taxa with a preference for slow flows, improving the similarity among reaches. However, the lower variance explained by the PCA that excluded Reach 4A (Figure 29) speaks to a degree of similarity among other reaches, with less predominant gradients in assemblage structure.

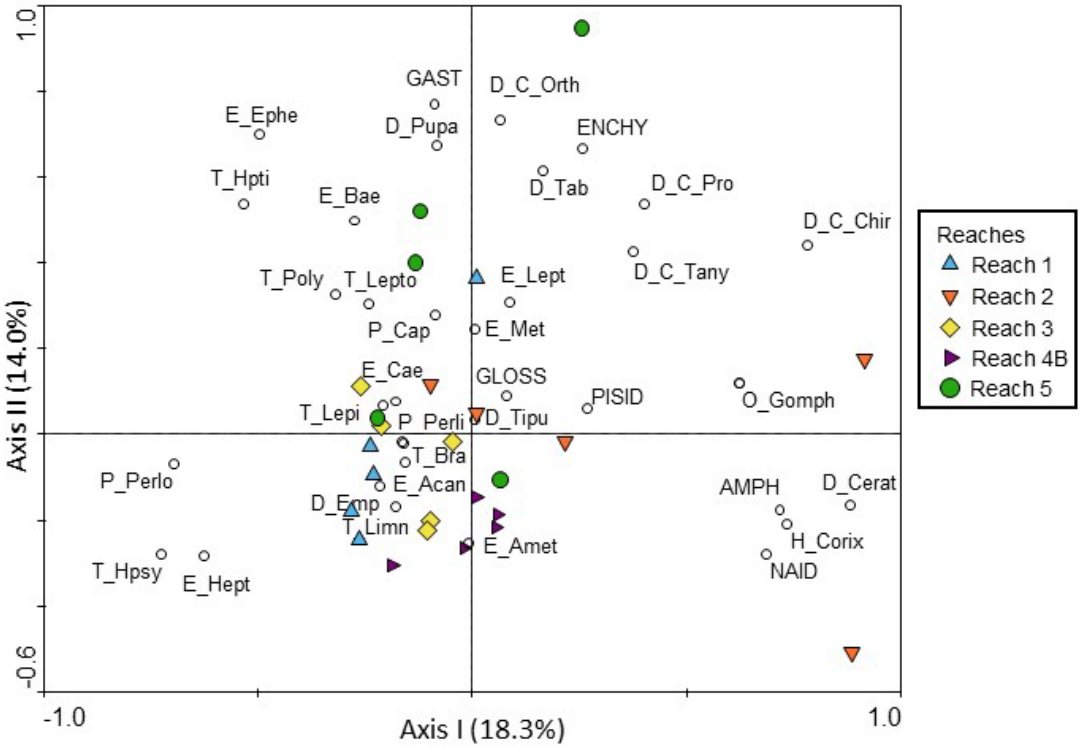

Figure 29. PCA ordination of BMI from kick samples in the Slave River, excluding sites in Reach 4A, with labelled taxa and with sample points coloured by reach. Kick-sites in close proximity have similar assemblages, whereas samples on opposite ends of gradients have differences in their assemblages. Samples at right angles through the origin are uncorrelated. Kick-sites are located close to taxa with which they are positively associated. Taxonomic abbreviations are listed in appendices.

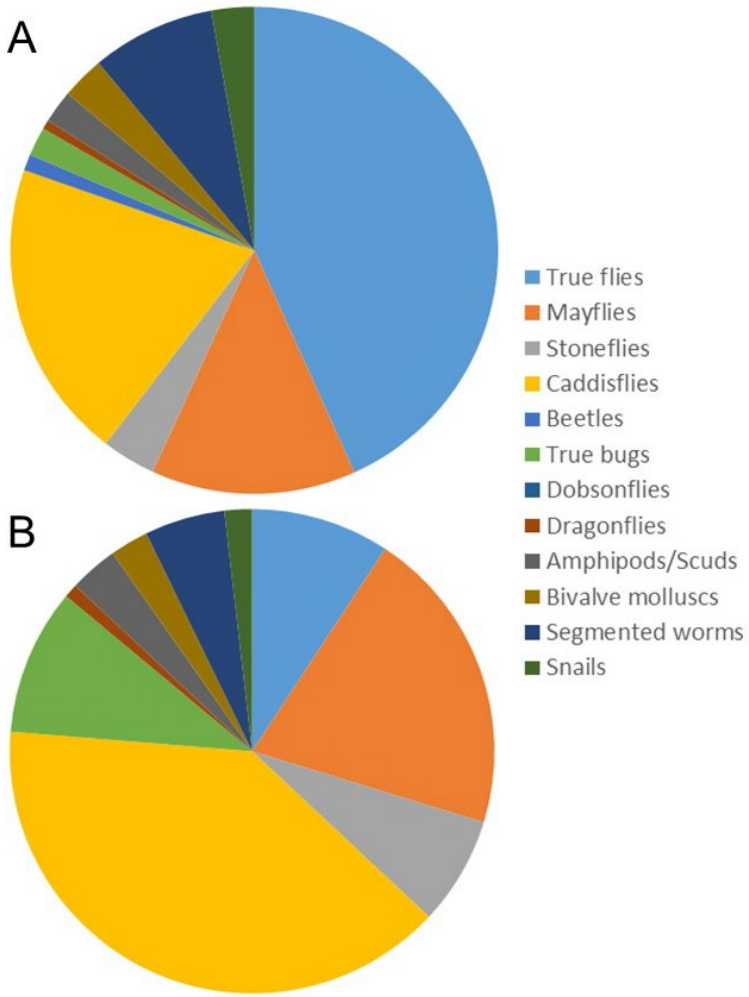

Figure 30. Average relative abundance of major BMI taxonomic groups in Slave River kick samples collected in (A) 2017 and (B) 2018. Taxa are grouped as true flies (Diptera), mayflies (Ephemeroptera), stoneflies (Plecoptera), caddisflies (Trichoptera), beetles (Coleoptera), true bugs (Hemiptera), dobsonflies (Megaloptera), dragonflies (Odonata), amphipods/scuds (Amphipoda), bivalve molluscs (Bivalvia), segmented worms (Oligochaeta), and snails (Gastropoda)

3.1.2.5.1. Temporal comparisons

Sampling of BMI has taken place over two years, and although such a short time-span does not allow for many formal assessments of temporal trends, some simple analyses were possible to compare the results of the two years of sampling in the Slave River. This included comparison of some metrics between years using repeated measures ANOVA and paired *t*-tests, as appropriate, and comparison of the full assemblage using Procrustes analysis of BMI ordinations.

Compositional changes from 2017 to 2018 were summarized at the river level by assessing the average relative abundance or major taxonomic groups across all reaches in each year (Figure 30). One of the most obvious changes between years was a sharp decline in the relative abundance of Diptera (true flies) and increase in relative abundance of Trichoptera (caddisflies; Figure 30). Increases in relative abundance of other mobile taxa such as Ephemeroptera (mayflies), Plecoptera (stoneflies), and Hemiptera (true bugs) were also evident.

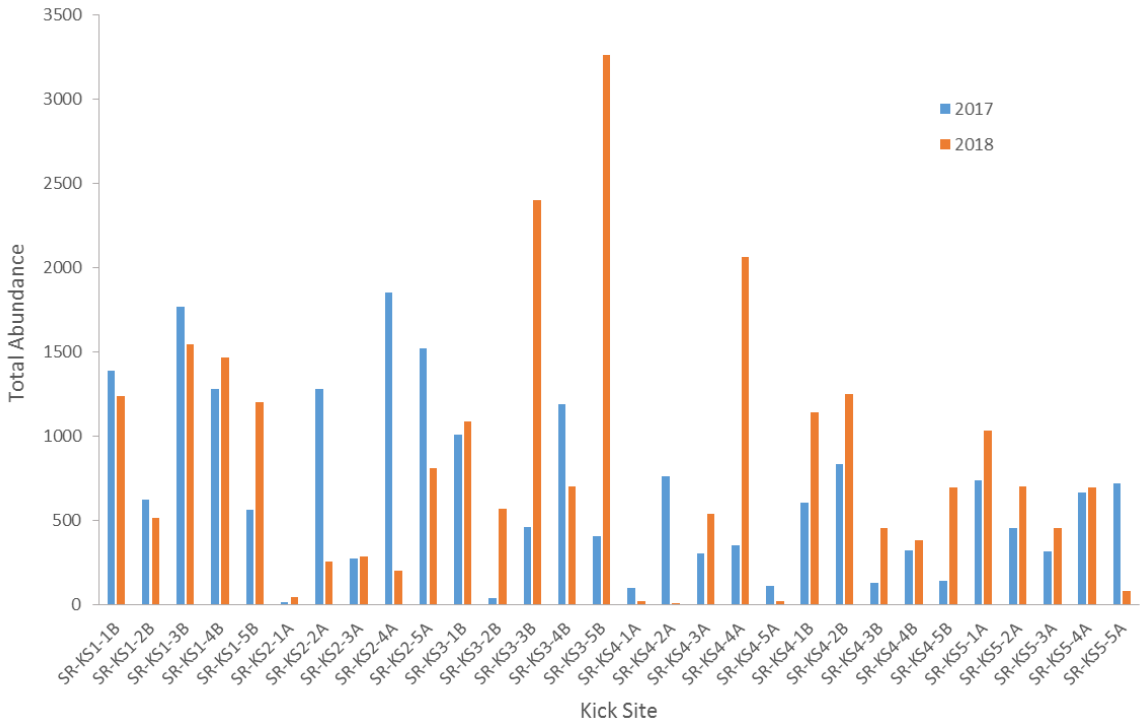

Figure 31. Total abundance of BMI in each Slave River kick-site sampled in 2017 (blue) and 2018 (orange).

At the site scale, dramatic changes in abundance were evident when total abundance was compared between 2017 and 2018. However, there was not a consistent gain or loss of individuals across all Slave River sites in 2018. For example, abundance declined sharply in Reach 2, increased sharply in Reach 3, and sites in Reach 1 maintained similar total abundance in 2018 compared to 2017 (Figure 31). Other sites in different reaches also had similar abundance across both years. However, in total, the abundance in 12 sites changed by more than 500 individuals per site, with 7 sites increasing in abundance and 5 sites decreasing in abundance. While some fluctuation of this magnitude does occur regularly in macroinvertebrate samples, the change was extreme in several cases. For example, KS4-2A went from 762 individuals in 2017 to only 9 individuals in 2018. Reach 2 also showed strong declines in 2018 across three sites, as did site KS5-5A (Figure 31). In contrast, several sites had strong increases in abundance in 2018, including sites KS3-2B, KS3-3B, KS3-5B, and KS4-4A (Figure 31). Site KS3-3B went from 460 individuals in 2017 to 2400 in 2018. Site KS3-5B changed from just over 400 individuals in 2017 to 3260 individual in 2018. But these patterns were rarely consistent across entire reaches. Because of the strong differences both within and among reaches, a repeated measures ANOVA of total abundance as a function of reach and year did not detect a significant interaction between year and reach ($F_{4,24} = 2.43$, $p = 0.064$), meaning that there were no consistent patterns over time across all reaches, and there was no evidence of an effect of year on total abundance ($F_{1,24} = 1.35$, $p = 0.257$).

Changes in abundance from 2017 to 2018 were far more consistent when the abundance of Chironomidae was considered (Figure 32). Over half of the Slave River sites saw a decline in percent composition of Chironomidae of greater than 30% from 2017 to 2018 (Figure 32). Chironomidae relative abundance declined from 70-80% down to less than 10% of the total abundance in some samples. In 2017, the maximum % Chironomidae was 85% and 18 sites had > 30% Chironomidae. In contrast, the maximum % Chironomidae in 2018 was 29%. Ten sites had a decline of > 200 Chironomidae individuals in 2018 compared to 2017 (Figure 32). The decline in

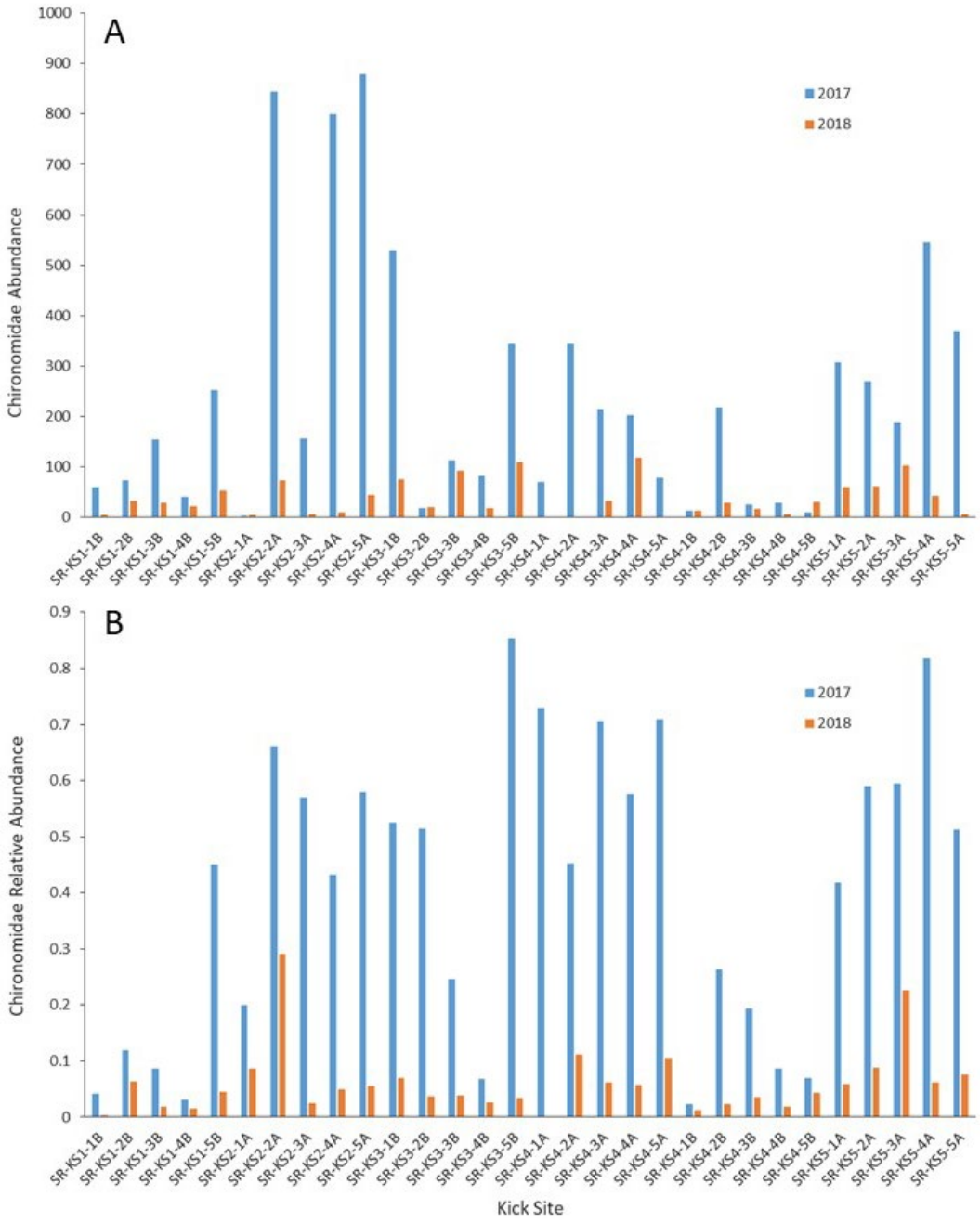

Figure 32. Comparison of Chironomidae (A) abundance and (B) relative abundance in each Slave River kick-site sampled in 2017 (blue) and 2018 (orange).

Chironomidae across many sites in 2018 was quite staggering, contributing in particular to sharp declines in total abundance in Reach 2 (Figure 32; Figure 31). In part, this decline may have reflected a sampling artifact due to changes in flow. With a surge in water level only 45 days before sampling occurred, wadeable areas on the banks of the Slave River likely consisted of temporary habitat, i.e., habitats that were not underwater prior to

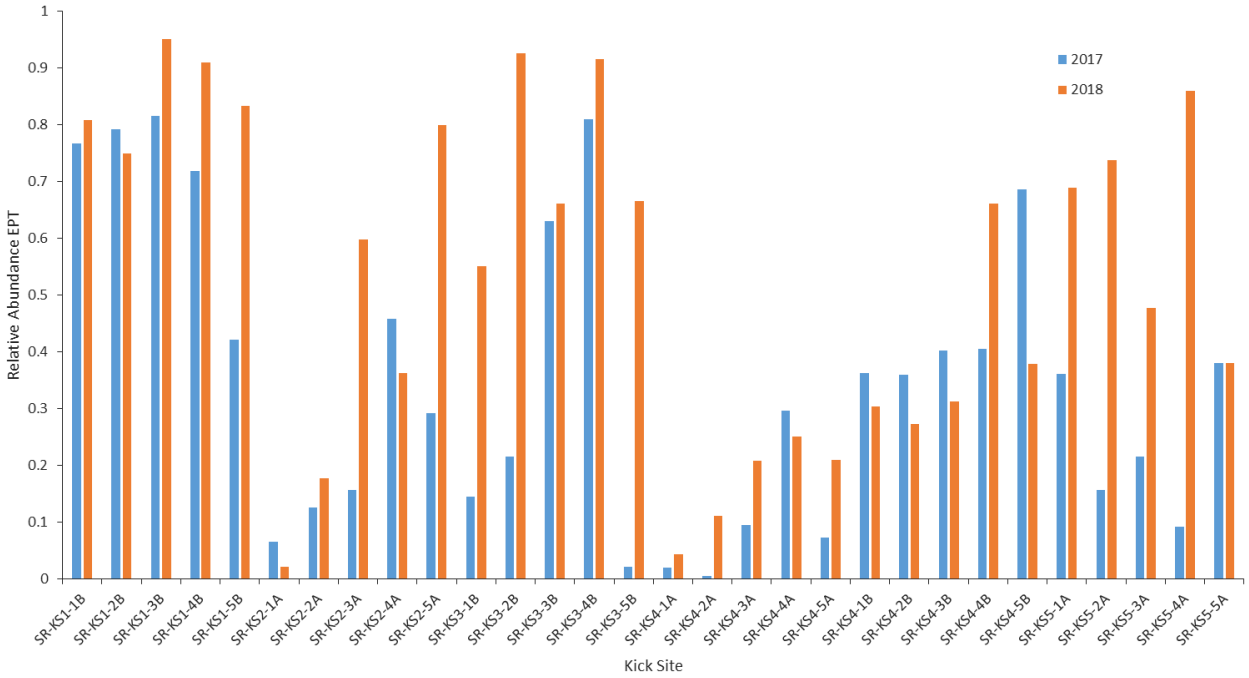

Figure 33. Relative abundance of EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera) in Slave River kick samples in 2017 (blue) and 2018 (orange).

the recent increase in flow. Organisms that are highly mobile and good colonizers (e.g., the dominant taxonomic groups collected on Hester-Dendy samplers) benefit from this temporary increase in available habitat, but organisms that are less mobile are less likely to be encountered in these areas. The relative abundance of EPT taxa did increase in 2018 in several sites that saw concurrent decreases in the relative abundance of Chironomidae (Figure 33). For example, Reach 2 and Reach 5, both of which had large declines in relative abundance of Chironomidae, saw large increases in the relative abundance of EPT (Figure 32; Figure 33).

The loss of Chironomidae in 2018 may also have reflected a response of some subfamilies to flow instability throughout the summer. The W.A.C. Bennett Dam on the Peace River in BC has been shown to have an impact on flows in the Slave River, leading to an earlier spring freshet (Sanderson et al. 2012). But there have also been more flow peaks throughout the year than were observed prior to construction of the dam, which may be related to impacts from the dam or may be a response to higher climatic variability (Sanderson et al. 2012). The hydrograph for 2018 showed lower water levels than 2017 following the spring freshet, followed by a later peak in the summer, only 45 days before the sampling event (Figure 2B). Whereas there was a long period of stable flows prior to sampling in 2017, flow was much more variable in the period leading up to sampling in 2018 (Figure 2B). Previous studies have examined the response of Chironomidae to changes in flow, and found that diversity within this group is affected by flow stability. For example, a comparison of regulated and unregulated rivers found assemblage composition differed between the rivers, and there was less inter-annual variability in composition in the regulated river where changes in flow were more gradual (and thus more stable; Armitage and Blackburn 1990). Furthermore, Collier (1993) found differences in depth and velocity preferences among genera of Chironomidae, noting that some genera prefer more stable flows, some genera have a preference for faster flows, while other taxa, particularly those in the subfamily Orthocladiinae, have a wider range of

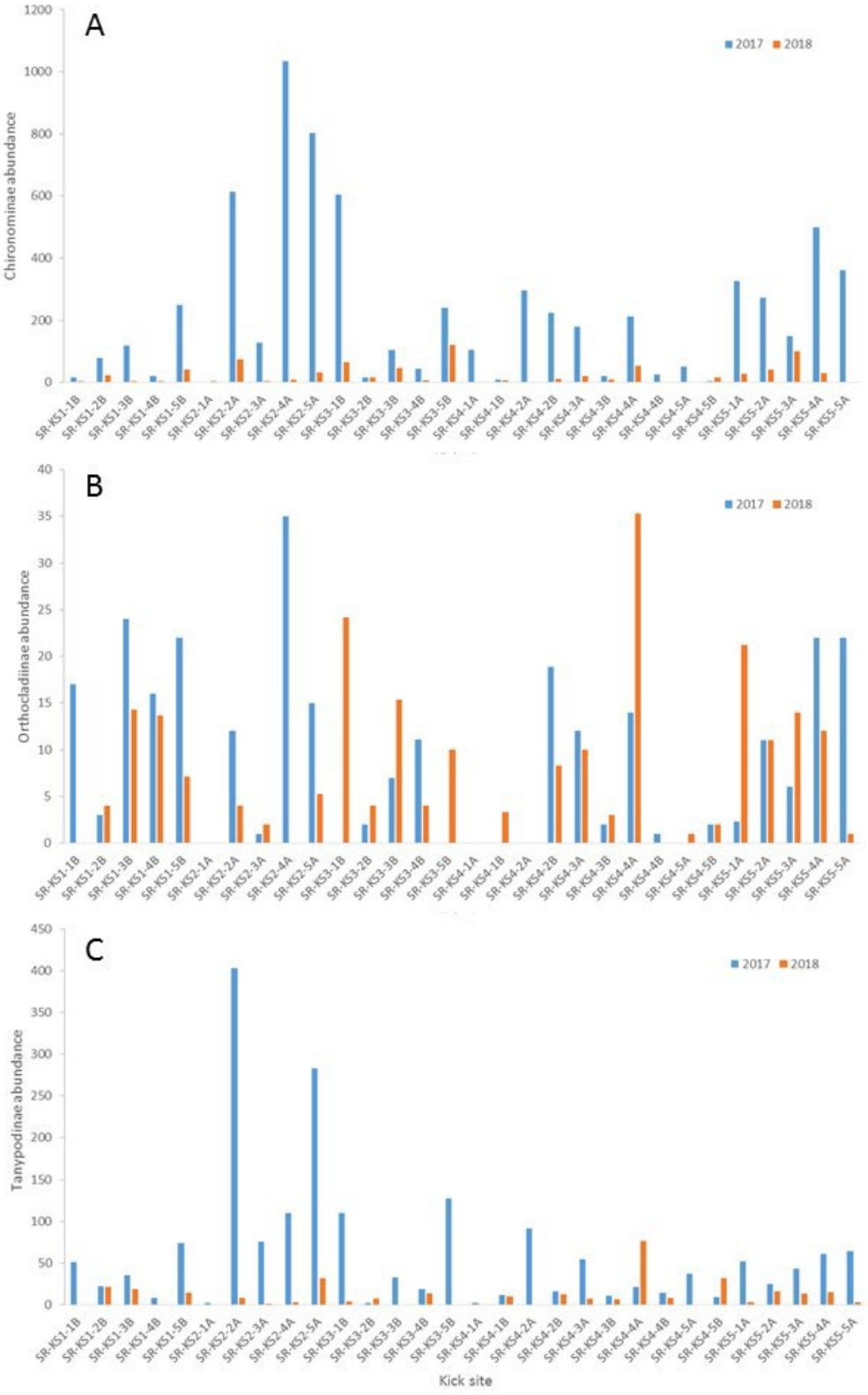

Figure 34 Abundance of Chironomidae subfamilies (A) Chironominae, (B) Orthocladiinae, and (c) Tanypodinae in each Slave River kick-site sampled in 2017 (blue) and 2018 (orange)

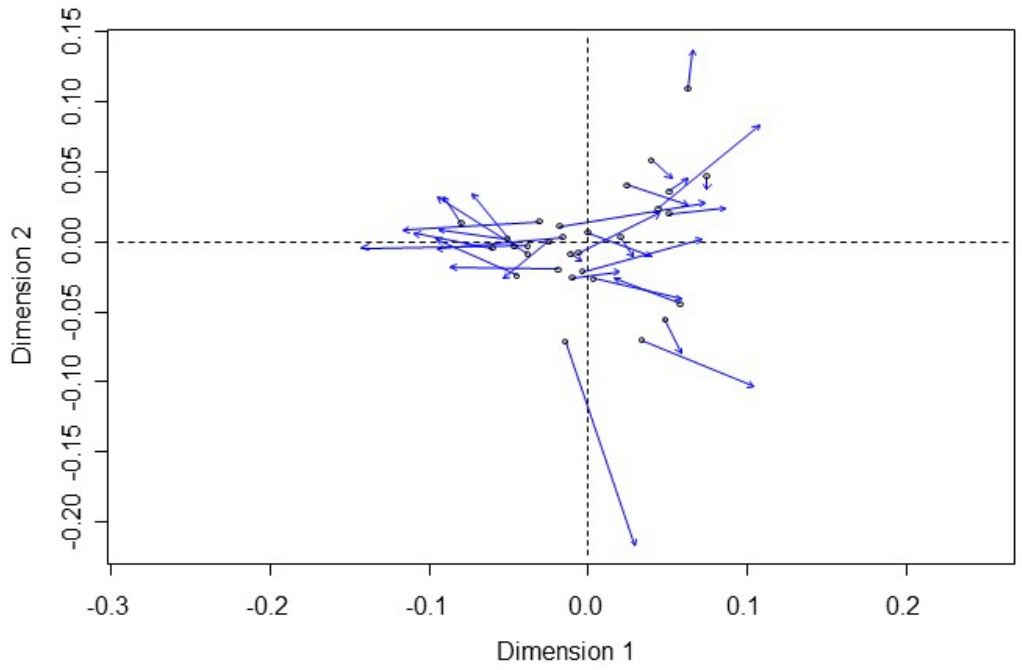

Figure 35 Procrustes residuals from a comparison of the ordination of 2017 Slave River BMI data (target matrix) with the ordination of 2018 Slave River BMI data (rotational matrix), with blue arrows indicating the movement of kick-sites in ordination space from 2017 to 2018. The longer the arrow, the more the assemblage composition at a site changed from one year to the next.

preferences. Accordingly, Orthocladiinae in the Slave River appeared to have the smallest decrease in abundance from 2017 to 2018 compared to the subfamilies Chironominae and Tanypodinae (Figure 34). Of these three common subfamilies of Chironomidae, Chironominae in particular appeared to show the strongest decline in abundance from 2017 to 2018.

The decline in Chironomidae abundance has two important implications: (1) variation in flow throughout the year is important and should be considered when finalizing timing of sampling and when analyzing data resulting from monitoring activities, because the characteristic assemblage in unusually high flow conditions (unusually high based on the previous months) may differ from that in more stable flow conditions; and (2) Chironomidae (and particular subfamilies) could be explored as potential indicators of flow-related differences between years. The latter point will continue to be explored as more data are collected.

Procrustes analysis of Slave River BMI ordinations from 2017 and 2018 indicated that the two ordinations were significantly more similar than could be obtained by chance ($m_{12}^2 = 0.37$, $p = 0.025$, 30 sites compared). Some sites did differ in assemblage composition from one year to the next (evidenced by large vectors in the Procrustes residuals diagram; Figure 35), particularly the sites in Reach 4A, which became stronger outliers. However, the position of most other sites changed little in the ordination diagram in 2018. This indicated that although strong declines in abundance were evident between years, similar declines were evident across all sites, and similarities and differences in composition among sites remained fairly constant from one year to the next.

3.1.2.6. *Biotic-abiotic relationships*

Biotic-abiotic relationships were assessed in Slave River kick samples using Redundancy Analysis (RDA), a multivariate approach that uses environmental variables to constrain the spatial arrangement of sites based on BMI relative abundance. RDA assesses the amount of variation in the unconstrained ordination (the PCA of BMI samples) that is explained by relating the data to chosen environmental variables, and identifies major abiotic gradients in the data. This analysis was completed separately for water chemistry/physical habitat parameters and for sediment chemistry, due to differences in the sites that were sampled for each. Prior to analysis, correlations between environmental parameters were examined in combination with the abiotic PCAs to pick out important drivers of differences among sites that were uncorrelated with each other (low correlations between environmental parameters were chosen to avoid multicollinearity). This also worked to reduce the number of environmental parameters in the analysis and avoid over-fitting the data. The final RDA for water chemistry and physical habitat variables included velocity, % sand, % pebble, % boulder, dissolved aluminum (D-Al), total aluminum (T-Al), ammonia (NH_3), dissolved iron (D-Fe), total nitrogen (TN), pH, total phosphorus (TP), potassium (K), dissolved selenium (D-Se), and total selenium (T-Se). Other ions, nutrients, physical measures, and total and dissolved metals were highly correlated with the chosen variables. For example, total aluminum was strongly correlated with most other total metals. Thus, any patterns described for this parameter also apply to the correlated parameters.

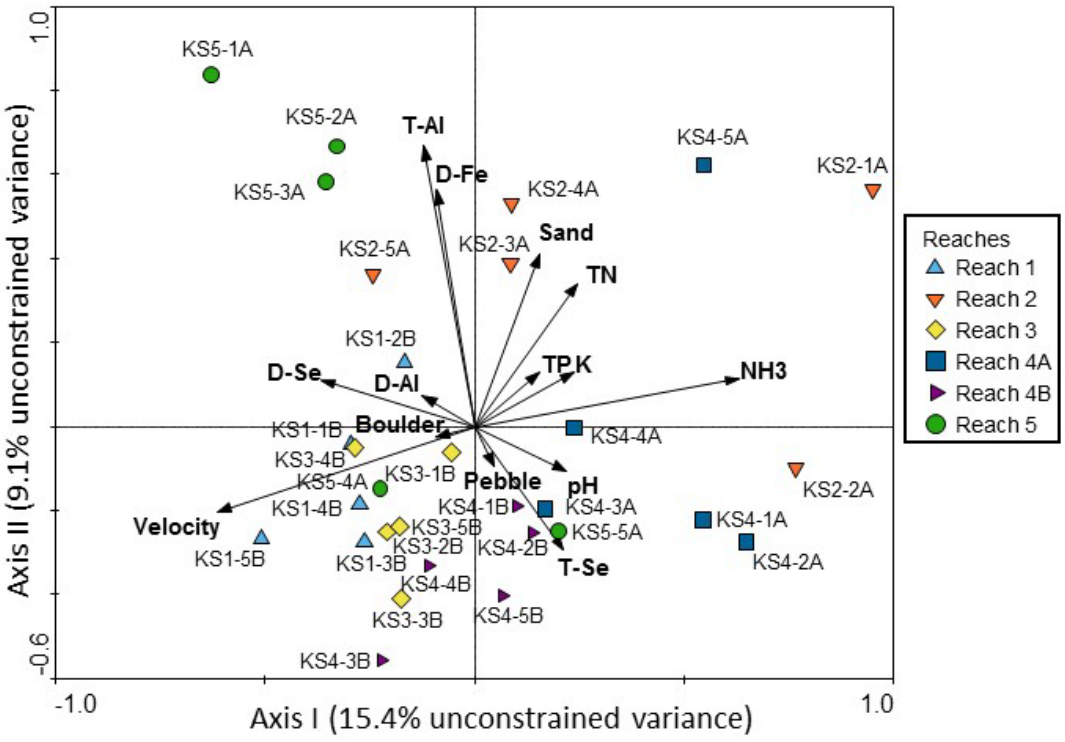

Figure 36 RDA ordination of Slave River BMI data constrained by physical and chemical habitat data at each site, with sites coloured by reach. Even-numbered sites use average values of chemical parameters from neighbouring odd-numbered sites. Kick-sites in close proximity have similar assemblages, whereas samples on opposite ends of gradients have differences in their assemblages. Samples at right angles through the origin are uncorrelated. Vectors indicate direction of change of physical and chemical parameters, and sites are ranked along these vectors based on the strength of their correlation with each parameter.

The first axis and all axes of the RDA of BMI relative abundance and chemical and physical parameters were statistically significant (Monte Carlo permutation test: first axis $F = 2.72$, $p = 0002$; all axes $F = 1.45$, $p = 0.004$), and the first three axes explained 31.1% of the variation among Slave River samples. Constraining the sites to environmental parameters lessened the dominance of sites that appeared to be outliers on the first axis, and increased the spread of sites in the ordination (Figure 36). Velocity was the strongest driver in the RDA (greatest individual effect and conditional effect, in combination with other parameters) and its effect on the fit of the model was significant ($F = 2.52$, $p = 0.002$). In the RDA plot, the outlier sites differed from other sites along a gradient in velocity, with lower velocity at the Reach 4A sites (Figure 36). This is consistent with the dominant gradient that appeared to drive the BMI PCA, as the taxa that were positively correlated with the outlier sites were those that have a preference for slower velocity or pools. Although the velocity measurements included in this analysis were spot measurements, they appeared to accurately characterize general flow conditions at the sites, based on correlations of BMI taxa with those sites.

Water chemistry parameters contributed strongly to spatial separation of sites in the Slave River RDA. Ammonia and total aluminum both had strong individual effects in the model and had significant conditional effects (p = 0.016 and 0.002, respectively). Ammonia was below detection limit in 12 of the 20 samples that were collected, so the importance of this variable was due to its detection in a small number of samples. Total aluminum and dissolved iron were both positively correlated with three site each from Reach 2 and Reach 5 along the second axis, which explained 9.1% of the unconstrained variance among sites (Figure 36). Substrate size played a lesser role in defining gradients among sites. Sites that were positively correlated with total aluminum and dissolved iron (and thus most other total metals) were also positively correlated with % sand (which includes sand and smaller particles, including clay), which is consistent with the affinity of metals to bind to fine particles. However, the % boulder and % pebbles did not contribute significantly to either axis. Overall, the RDA and permutation tests indicated that the strongest relationships were with velocity, followed by water chemistry, while substrate size was less important.

Analysis of biotic-abiotic relationships was also completed using a subset of BMI kick-sites and sediment chemistry data. However, sediment chemistry parameters were generally quite highly correlated, which meant that there were few parameters that could be chosen. In an effort to retain only parameters that were uncorrelated with each other, the chosen subset included % clay, % silt, 2-Methylnaphthalene (which was highly correlated with most other PAHs and metals), chromium, and chrysene (the latter two of which were only above detection limit in one site each). The resulting RDA indicated a lack of significance of the first axis, though all axes together were marginally significant (p = 0.248 and 0.036, respectively), but the ordination plot indicated that most sites were orthogonal to (i.e., uncorrelated with) the chosen parameters (results not shown). Only % clay was correlated with sites in the ordination. Therefore, the relationship of these sediment chemistry parameters with biotic assemblage composition was weak.

3.2. Characterization of reaches – Hester-Dendy samples (Hay and Slave Rivers)

Hester-Dendy samplers were deployed for approximately 3-4 weeks in the Hay River and Slave River. Samplers were grouped by reach (with reaches differing from kick sample reaches, though some were in close proximity; see Figure 5). Some samplers were lost, primarily in the Slave River, and samples were therefore not evenly distributed across reaches. However, data were summarized by biotic metrics to broadly compare across

reaches, and relative abundance data from each sampler were used to characterize reaches through multivariate analysis. Detailed comparison of kick samples and Hester-Dendy samples using 2017 data indicated

Table 12 Summary of biotic metrics calculated for Hester-Dendy samples collected from four reaches in the Hay River and Slave River, showing mean ± standard deviation of samples within each reach for BMI abundance and taxonomic richness metrics. EPT is the sum of Ephemeroptera, Plecoptera, and Trichoptera orders, and Chironomidae is a family of Diptera.

Biotic Metric	Hay River				Slave River			
	Reach 1	Reach 2	Reach 3	Reach 4	Reach 1	Reach 2	Reach 3	Reach 4
Total abundance	121 ± 107	290 ± 37	206 ± 23	254 ± 34	104 ± 53	205 ± 243	91 ± 66	366 ± 100
EPT abundance	70 ± 44	249 ± 36	136 ± 26	168 ± 33	74 ± 42	171 ± 228	70 ± 52	325 ± 108
Diptera abundance	33 ± 36	35 ± 12	51 ± 16	72 ± 14	16 ± 15	21 ± 23	6 ± 6	23 ± 15
Chironomidae abundance	31 ± 35	34 ± 12	51 ± 16	69 ± 17	16 ± 15	20 ± 23	5 ± 5	22 ± 14
Percent EPT	68.1 ± 15.4	85.9 ± 4.2	65.8 ± 9.5	66.2 ± 6.7	68.2 ± 11.6	69.9 ± 18.0	64.0 ± 26.1	87.6 ± 10.4
Percent Chironomidae	21.3 ± 7.9	11.8 ± 3.8	25.1 ± 8.9	27.5 ± 6.3	13.7 ± 15.2	13.6 ± 12.1	10.3 ± 10.4	6.5 ± 5.5
Taxonomic richness	16.0 ± 7.1	21.8 ± 1.2	21.2 ± 3.5	20.6 ± 4.2	13.7 ± 4.8	12.2 ± 5.9	10.3 ± 5.2	16.3 ± 4.3
EPT richness	9.8 ± 3.9	13.8 ± 1.0	10.2 ± 1.7	11.2 ± 2.5	5.3 ± 1.4	5.5 ± 2.2	5.3 ± 3.1	6.5 ± 1.2
Chironomidae richness	4.3 ± 3.4	5.7 ± 0.8	8.2 ± 2.6	6.6 ± 1.5	4.7 ± 2.9	4.5 ± 3.4	2.3 ± 1.3	5.5 ± 2.7

that Hester-Dendy samples were biased towards collecting more mobile taxa and colonizer taxa than kick samples (Lento 2018a), and thus a detailed comparison of the methods is not presented here.

Hester-Dendy samplers in the Hay River collected between 120 and 290 individuals, on average (Table 12). The first reach was the most variable, with the lowest average total abundance, whereas the three reaches from further downstream were more consistent with similar mean abundance and low standard deviations. All reaches were dominated by EPT taxa, which made up 66-85% of the total assemblage. Reaches 1, 3, and 4 all had similar percent EPT (65.8-68.1%), whereas Reach 2, which had the highest total abundance, had significantly higher percent EPT than the other reaches (85.9%; Table 12, Figure 37). Because EPT and Chironomidae were the dominant taxa in these samples, the opposite pattern was found in the % Chironomidae, with similar composition across Reaches 1, 3, and 4 (21.3-27.5%) and significantly lower % Chironomidae in Reach 2 (11.8%, significantly lower than Reach 3 and Reach 4; Table 12, Figure 37). Reach 1 and 2 of the Hester-Dendy sampling in the Hay River corresponded to kick site reaches 1 and 2, and the pattern of higher % EPT at Reach 2 was consistent with what was found at the kick-sites, although % EPT was elevated in the Hester-Dendy samples.

Taxonomic richness in Hay River Hester-Dendy samples was lowest in Reach 1 (where abundance was also lowest), and more consistent across the other three reaches (16 taxa on average in Reach 1, and 20.6-21.8 taxa on average in the other reaches; Table 12), though this pattern was not significant (Figure 38). Taxonomic richness in Reach 1 was generally quite variable among samples, whereas Reach 2 had low variability (Table 12,

Figure 38). Patterns of taxonomic richness of EPT and Chironomidae generally followed the pattern of total richness, though EPT richness was particularly elevated in Reach 2, where EPT were dominant numerically

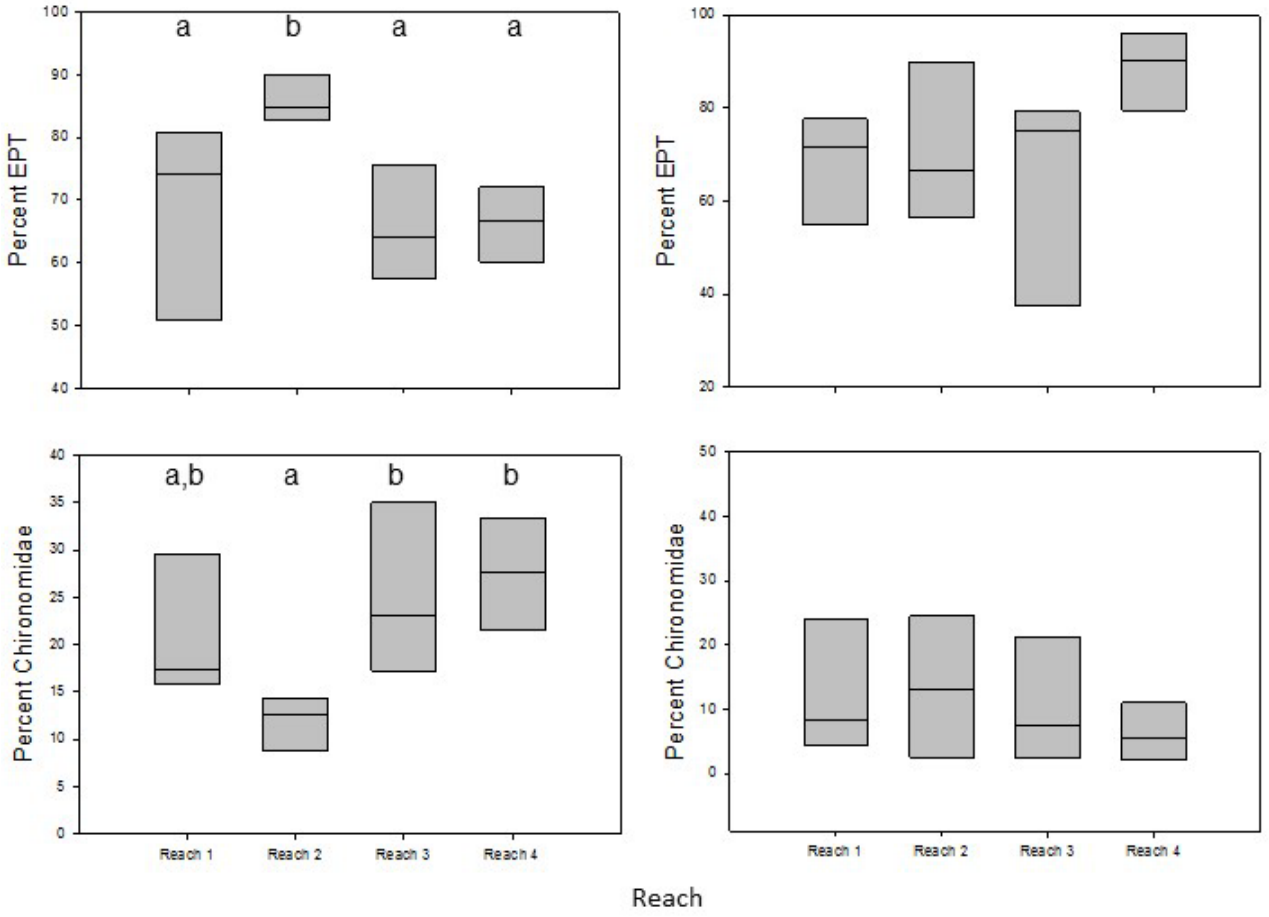

Figure 37 Box plots of percent composition of Ephemeroptera, Plecoptera, and Trichoptera (EPT), and percent composition of Chironomidae (midges) from Hester-Dendy samples in each of four reaches in the (left) Hay River and (right) Slave River. Lines through boxes indicate the median and lower and upper bounds plot the 25th and 75th percentiles, respectively. Letters on plots indicate significant differences (α = 0.05) from one-way ANOVA and Tukey HSD tests.

(Figure 38). Due to variability within reaches and low effect size (i.e., low magnitude of difference between reach means), no significant difference was found for EPT or Chironomidae richness (Figure 38).

In the Slave River, abundance was much more variable among reaches, ranging from 91 individuals to 366 individuals on average, and the highest average abundance was found in Reaches 2 and 4 (Table 12). Similar to the Hay River, EPT dominated the Hester-Dendy samples collected in Slave River reaches, ranging from 64-87% of the sample on average (Table 12). The percent EPT was elevated in Reach 4 (where average abundance was highest) compared to other reaches, but a significant difference was not detected due to high variability within reaches (Figure 37). The percent Chironomidae was somewhat lower in Reach 4, but all reaches had low values for this metric, which varied from 6.5-13.7% on average, and was not significantly different among reaches (Table 12, Figure 37).

Taxonomic richness was variable within Slave River reaches, which made it difficult to detect differences among reaches. Total richness varied from 10 to 16 taxa on average (Table 12). The total taxonomic richness and richness of Chironomidae were lower on average in Reach 3 than in the other reaches, but these differences

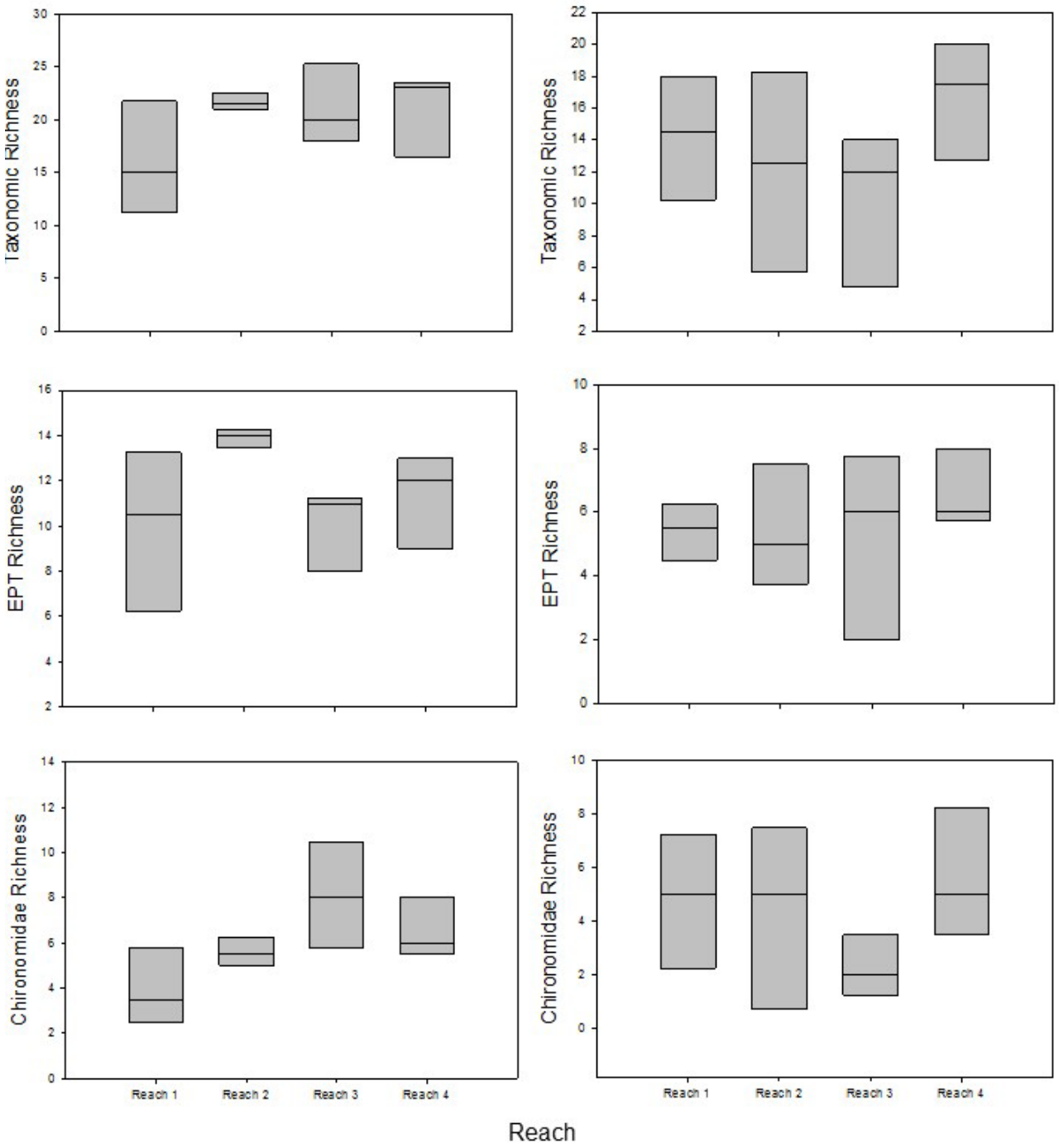

Figure 38 Box plots of total taxonomic richness, Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness and Chironomidae (midge) richness from Hester-Dendy samples in each of four reaches in the (left) Hay River and (right) Slave River. Lines through boxes indicate the median and lower and upper bounds plot the 25th and 75th percentiles, respectively.

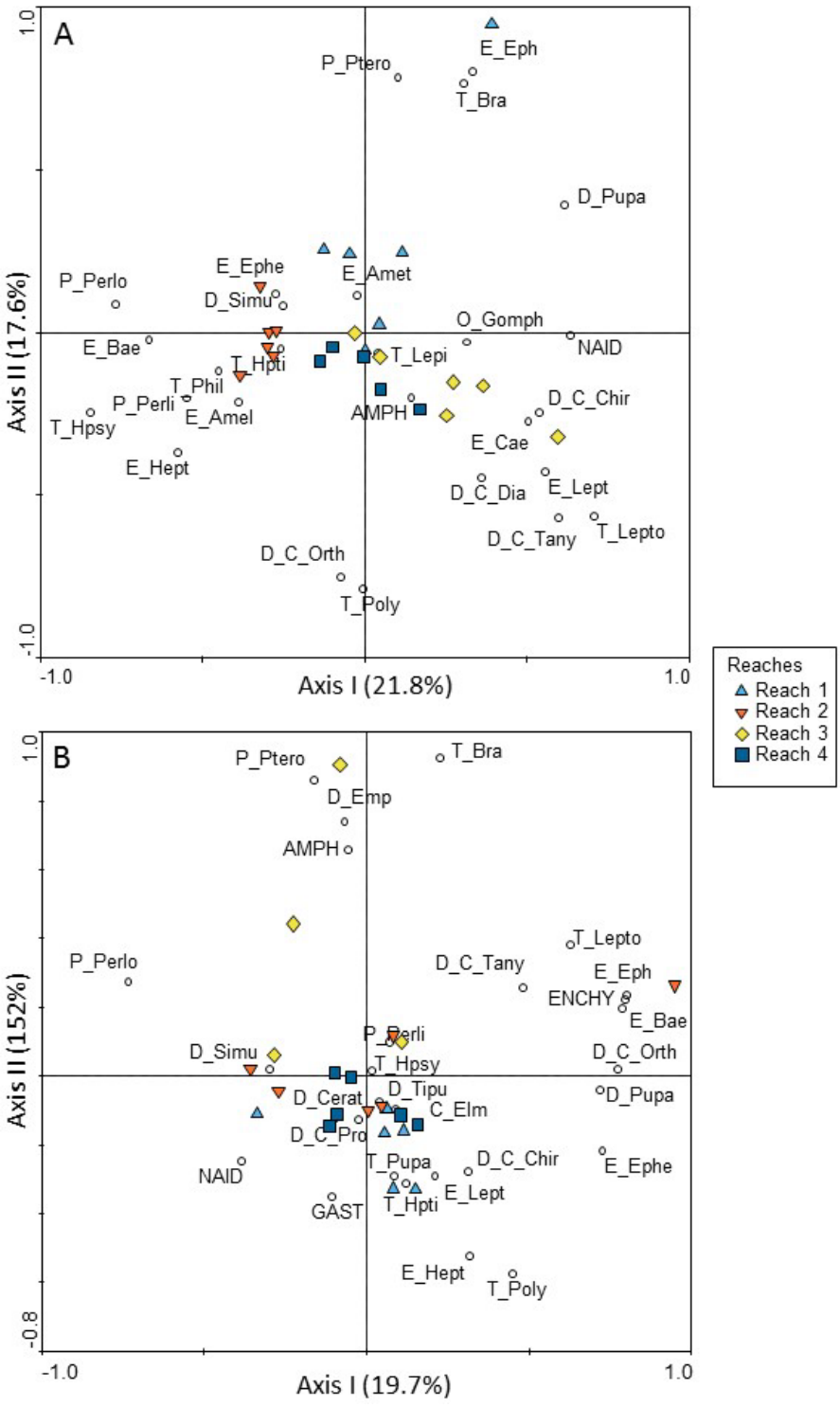

Figure 39 PCA ordinations of BMI from Hester-Dendy samples in (A) Hay River and (B) Slave River, with sample points coloured by reach. Sites in close proximity have similar assemblages, and sites are located close to taxa with which they are positively associated. Taxon abbreviations can be found in the appendices.

were not statistically significant (Figure 38). The number of EPT taxa and the number of Chironomidae taxa were generally fairly similar on average across reaches in the Slave River.

Multivariate analysis was used to assess how variable samples were within and among reaches. PCA ordinations of Hester-Dendy samples indicated generally strong similarity within and among reaches, with only a few samples that differed from the rest. For example, Hay River sites were generally clustered by reach, and the reaches were primarily located close to the origin (indicating similarity in composition), with the exception of one sample in Reach 1 (Figure 39A). That sample was strongly associated with Ephemeridae, Pteronarcidae, and Brachycentridae, families of Ephemeroptera, Plecoptera, and Trichoptera (respectively). Reach 3 did have some variation among samples compared to other reaches, as one sample was more strongly associated with three

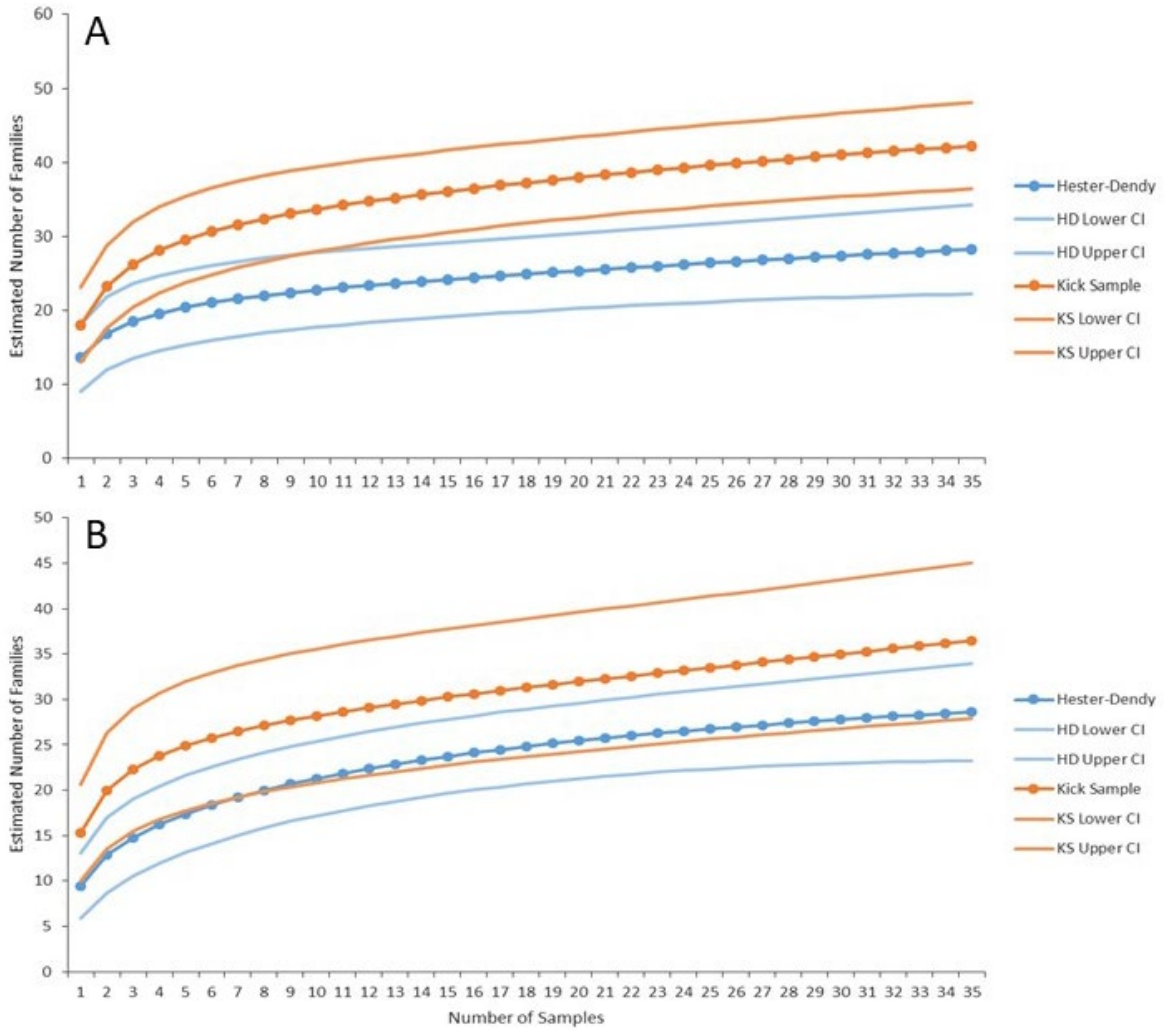

Figure 40. Rarefaction curves for (A) Hay River and (B) Slave River, comparing the estimated number of families found in different numbers of Hester-Dendy (blue) or kick samples (orange). 95% confidence intervals are shown around rarefaction curves. Rarefaction curves are extrapolated following Colwell et al. (2004) to estimate richness in as much as 35 samples based on data from the full number of samples collected by each method (23 and 22 HD samples from the Hay and Slave rivers, respectively, and 30 samples each from the Hay and Slave rivers).

subfamilies of Chironomidae and other taxa that prefer slow-flowing waters. But in general, Hay River Hester-Dendy samples were much more similar in 2018 than was found in 2017, when there was variation both within and among reaches. In the Slave River, Hester-Dendy samples were also quite similar within and among reaches, with the exception of a small number of samples. There was generally strong overlap among reaches near the origin of the plot (indicating strong similarity within and among reaches; Figure 39B), and this was in contrast to 2017 when there was greater spread of samples in ordination space. One sample from Reach 2 differed from the other samples along the first axis, as this sample was associated with several families of Ephemeroptera as well as Chironomidae subfamilies and worms (Figure 39B). Along the second axis, two sites from Reach 3 were separated from the remaining sites due to a strong positive association with amphipods, Pteronarcidae, Brachycentridae, and the dipteran family Empididae (Figure 39B). The Hester-Dendy samples that were outliers in the PCA plot were generally from different reaches than outlier samples observed in 2017, and the bulk of the samples were more tightly clustered, with stronger overlap in assemblage composition.

Family richness in Hester-Dendy samples was compared with family richness in kick samples for each river to explore whether richness was lower in Hester-Dendy samples, as was found in 2017 (Lento 2018a). Because different numbers of samples were collected with each method, rarefaction curves were created to compare family richness at a set number of samples (using the program EstimateS; Colwell 2013). Rarefaction curves were extrapolated following Colwell et al. (2004) to a total of 35 samples for each method (extrapolation from 23 and 22 Hester-Dendy samples from the Hay and Slave rivers, respectively, and from 30 kick samples in both rivers) and 95% confidence intervals were used to determine whether family richness estimates were significantly different between methods. For the Hay River, family richness of kick samples was consistently higher than that from Hester-Dendy samples, and the difference was statistically significant after approximately 12 samples (Figure 40A). The difference between the two methods was greater than that found in 2017, when there was more overlap of confidence intervals between methods (Lento 2018a). In contrast, though family richness estimates were consistently higher for kick samples in the Slave River than they were for Hester-Dendy samples, the different was not statistically significant (Figure 40B). This result is similar to 2017 (Lento 2018a), but with less support for eventual convergence of richness estimates in 2018 (Figure 40B).

3.3. Assessment of study design

3.3.1. Biotic metric CES

CES was used to identify samples that fell outside of the normal range of variability based on several BMI metrics: total abundance, relative abundance of EPT, relative abundance of Chironomidae, relative abundance of Diptera + Oligchaeta, total taxonomic richness, richness of EPT, richness of Chironomidae, and richness of Diptera + Oligochaeta. CES makes use of the variation among samples to determine a normal range and set bounds to trigger management action if test samples are impaired (i.e., if they fall outside the range of natural variability). Normal range is commonly defined as the range within which 95% of samples fall, equivalent to two standard deviations from the mean in a normal distribution (Munkittrick et al. 2009). While it is possible for samples to fall outside the CES, there is a low probability (5% chance) of this happening if the sample is representative of the normal range. Thus, where sites have been exposed to anthropogenic impacts, samples outside of the CES may be an indication of impairment in a system. Where sampling areas are at reference condition (unimpacted), samples above or below CES may have different habitat conditions (such as differences in substrate composition) that cause BMI assemblage differences, and thus, these bounds can be used to identify potential outlier sites that may need to be replaced as baseline data are established. However, the CES is based on variability in the data, and changes in habitat conditions that result from natural variability (i.e., due to shifts in flow, timing of the spring freshet, water temperature, etc.) may lead to different normal ranges from

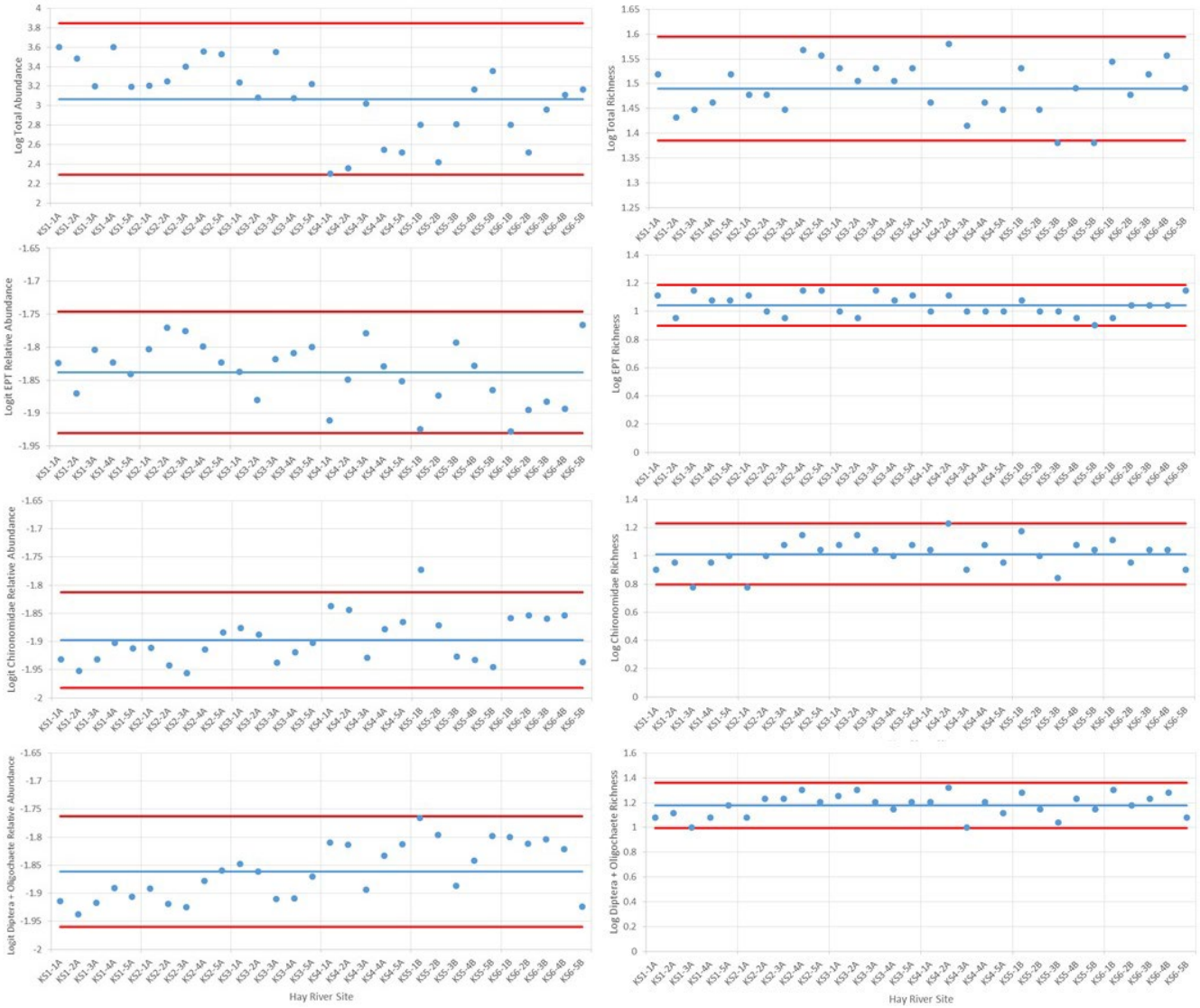

Figure 41. Biotic metrics plotted for each site in the Hay River with 2018 mean (blue line) and the upper and lower critical effect size (CES; red lines), calculated as mean ± 2SD (calculated based on 2018 data). Each point represents a kick-site, moving from reach 1, site 1 (far left) to reach 6, site 5 (far right) on each plot. Metrics include (left column) log_{10} abundance, logit EPT relative abundance, logit Chironomidae relative abundance, logit Diptera + Oligochaeta relative abundance, and (right column) log_{10} taxonomic richness, log_{10} EPT richness, log_{10} Chironomidae richness, and log_{10} Diptera + Oligochaeta richness.

one year to the next. Furthermore, sites that were within the normal range in one year may fall outside the normal range in the next year if they are strongly affected by natural variability in the system. Monitoring of assemblages over several years can therefore be used to get a better, more accurate estimate of the CES or normal range in a system that accounts for this natural variability.

For this analysis, mean values of each metric and standard deviations across all reaches in a river were calculated, and CES was set to 2 SDs from the mean. Two sets of CES were calculated: one based on only the 2018 data, to identify any sites that appeared to be outliers in this year, and a second set based on the mean

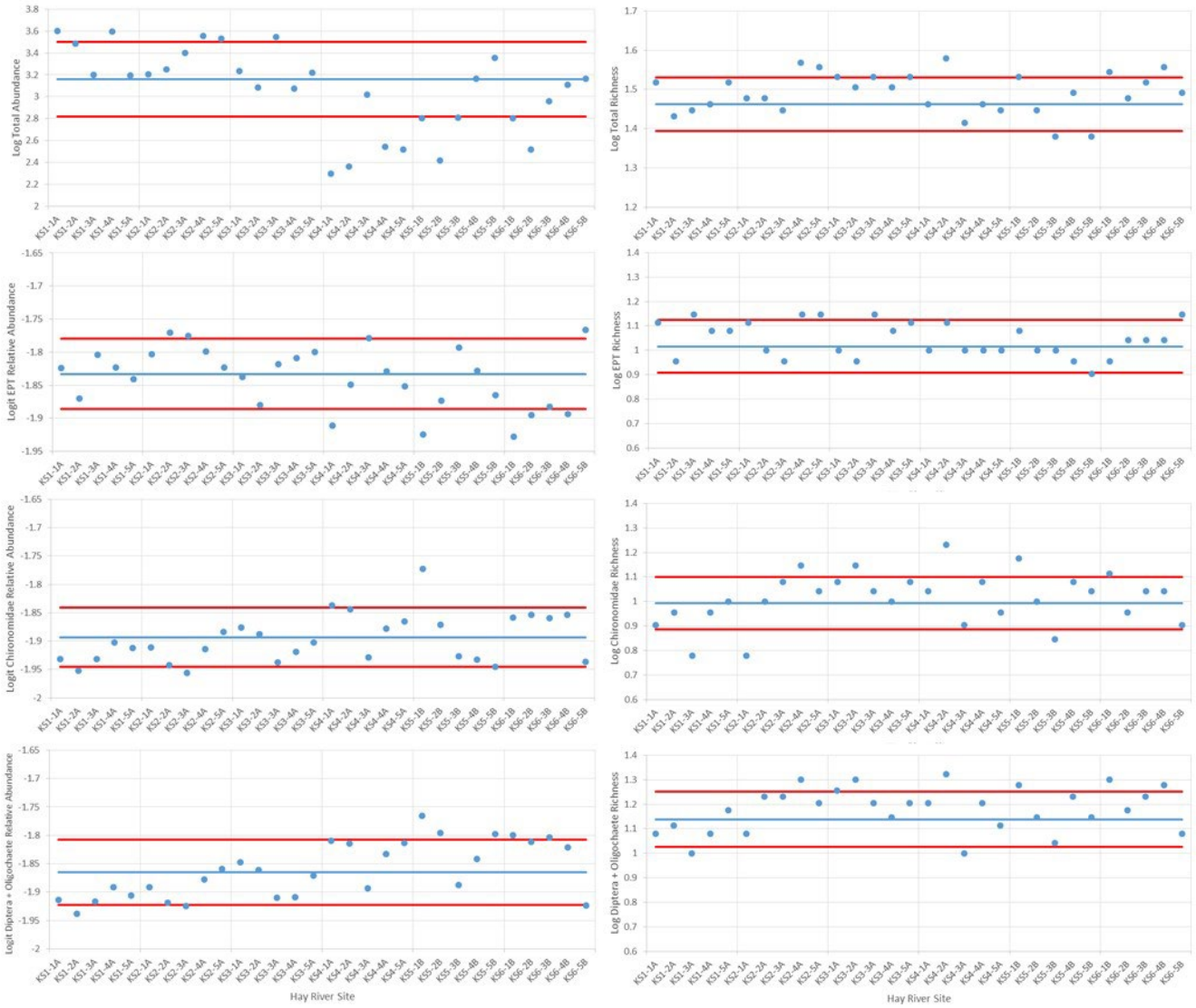

Figure 42. Biotic metrics plotted for each site in the Hay River with a grand mean (mean of 2017 and 2018 data; blue line) and the upper and lower critical effect size (CES; red lines), calculated as mean ± 2SD (calculated based on 2017 and 2018 data). Each point represents a kick-site, moving from reach 1, site 1 (far left) to reach 6, site 5 (far right) on each plot. Metrics include (left column) log_{10} abundance, logit EPT relative abundance, logit Chironomidae relative abundance, logit Diptera + Oligochaeta relative abundance, and (right column) log_{10} taxonomic richness, log_{10} EPT richness, log_{10} Chironomidae richness, and log_{10} Diptera + Oligochaeta richness.

and standard deviation of combined 2017-2018 data. The latter set of CES builds the foundation for temporal assessment in the following years of analysis. This analysis was completed with log_{10}- or logit-transformed data, as appropriate, to stabilize residual variance and ease visual interpretation of the plots.

Samples collected in 2018 generally fell within CES developed with only 2018 data (Figure 41), indicating that few sites appeared to be outliers. Site KS5-1B had a high relative abundance of Chironomidae that was more than 2 standard deviations from the mean across all sites for 2018 (Figure 41). There were also some sites with low richness that was slightly more than 2 standard deviations below the mean; for example, KS5-3B and KS5-5B had low total richness, while KS2-3A and KS2-1A had low richness of Chironomidae (Figure 41). No sites had EPT

relative abundance outside the normal range that was developed for 2018 data. The location of site HR-KS1-4A, which was outside the normal range for EPT relative abundance in 2017 and was determined to be an ecological outlier, was moved slightly to ensure sampling did not take place in an overly silty location. As a result, the relative abundance of EPT at this site was close to the average value for all sites, which indicated that HR-KS1-4A was no longer an ecological outlier. Overall, the majority of sites were within or at the bounds of the CES developed using 2018 data.

There were more deviations from normal range evident in the Hay River when 2018 metric data were compared with CESs developed based on data from both 2017-2018 (Figure 42). The range of total abundance across Hay River reaches was larger in 2018 than in 2017 because of lower abundance in downstream reaches, particularly Reach 4, and many downstream sites were below CES (Figure 42). Abundance in four sites in Reach 4 ranged from 199 to 350 individuals in 2018, whereas their totals in 2017 ranged from 613 to 1728 individuals. Reach 5 also had lower abundance in 2018, ranging from 262 to 643 individuals in three sites in 2018, and from 616 to 2688 individuals in 2017. This decline in abundance may have been due to annual variation in water levels, though these reaches are also located downstream of the boat launch and stream inflows. The most upstream sites of the newly-added Reach 6 had low abundance (329-634 individuals), but higher abundance was found at the three downstream sites in this reach (913-1466 individuals). In contrast to the downstream reaches, several upstream sites in Reaches 1-3 had total abundance above the two-year CES. The strong variability between the first two years of sampling suggests that several years of data (likely more than three) may be necessary to accurately estimate the range of natural variability in abundance in these systems.

There were a number of sites in the Hay River that were outside the 2017-2018 CES ranges for relative abundance of EPT, Chironomidae, and Diptera + Oligochaeta, though the frequency of exceedance of CES was lower than for total abundance (Figure 42). For the relative abundance of EPT, the strongest deviations were in the downstream reaches (Reaches 4, 5, and 6), where relative abundance was below CES in five sites (Figure 42). The relative abundance of Chironomidae and relative abundance of Diptera + Oligochaeta were elevated in these sites, though not all were above CES. Sites in Reaches 1, 2, and 3 were less variable with respect to the relative abundance of these taxonomic groups, and there were fewer sites outside CES (Figure 42).

Richness metrics for 2018 Hay River data fell outside the normal range that was developed using 2017 and 2018 data in a number of sites (Figure 42). When total richness was considered, sites in Reach 2, Reach 3, Reach 4, and Reach 6 were at or above the upper CES limit, whereas sites in Reach 5 fell below the lower limit. These exceedances primarily reflected variation in richness of Chironomidae among sites (Figure 42). For example, Chironomidae richness exceeded the upper CES in sites SK2-4A, KS3-2A, KS4-2A, KS5-1B, and KS6-1B, but was below the lower CES in sites KS1-3A, KS2-1A, and KS5-3B (Figure 42). In contrast, while EPT richness exceeded the upper CES in several sites, it was not at the same magnitude. Values of Diptera + Oligochaeta richness that were outside the normal range largely reflected the patterns of Chironomidae richness in most sites.

The frequency of values of biotic metrics in the Hay River that are outside the normal range that was developed using data from both 2017 and 2018 speaks to the degree of variation between years of sampling. If 2018 data had been similar to those collected in 2017, the CES would have been expected to encompass most of the metric values calculated using 2018 data. Collecting additional years of data will allow for the refinement of the CES range to better reflect inter-annual variability in the sampled metrics.

Biotic metric data for the Slave River from 2018 were initially compared with CESs determined using only 2018 data, to identify any sites that appeared to be outliers. When total abundance was considered, Reach 4A stood out for having three sites below the lower CES limit (Figure 43). The remaining sites fell within the bounds of the normal range for 2018, though that range was large due to high variability among sites. When relative

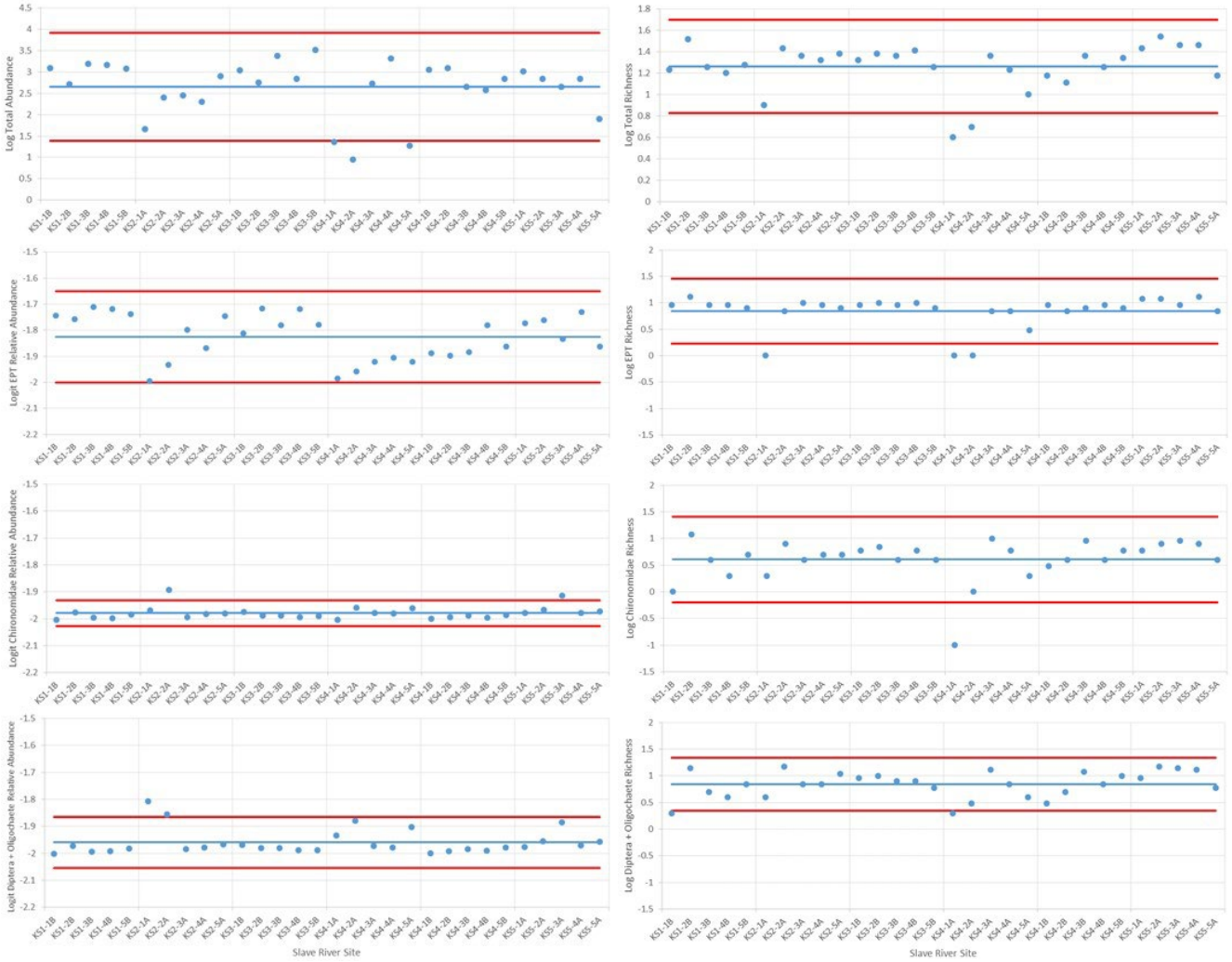

Figure 43. Biotic metrics plotted for each site in the Slave River with 2018 mean (blue line) and the upper and lower critical effect size (CES; red lines), calculated as mean ± 2SD (calculated based on 2018 data). Each point represents a kick-site, moving from reach 1, site 1 (far left) to reach 5, site 5 (far right) on each plot. Metrics include (left column) log_{10} abundance, logit EPT relative abundance, logit Chironomidae relative abundance, logit Diptera + Oligochaeta relative abundance, and (right column) log_{10} taxonomic richness, log_{10} EPT richness, log_{10} Chironomidae richness, and log_{10} Diptera + Oligochaeta richness.

abundance was considered, there were fewer sites that fell outside the normal range for 2018, and the observed exceedances were sites with relative abundance of Chironomidae or Diptera + Oligochaeta greater than the mean + 2SD (Figure 43). These exceedances were due in part to very low Chironomidae abundances across most sites, which contributed to a narrow CES range. Slave River sites fell below the lower CES limit for richness metrics in sites KS2-1A, KS4-1A, and KS4-2A (Figure 43), all of which stood out in the multivariate analysis of BMI data. All three sites had low EPT richness, and KS4-1A also had low Chironomidae richness (it was the only site at which no Chironomidae were found), contributing to total richness below CES in KS4-1A and KS4-2A.

Comparison of Slave River data from 2018 with CESs developed using data from both 2017 and 2018 indicated the strength of the variation in biotic metrics between years. Variation in abundance among sites was strong in the Slave River in 2018, and both total abundance and relative abundance of taxonomic groups varied widely from what was observed in 2017. The large changes in total abundance in Slave River sites from 2017 to 2018

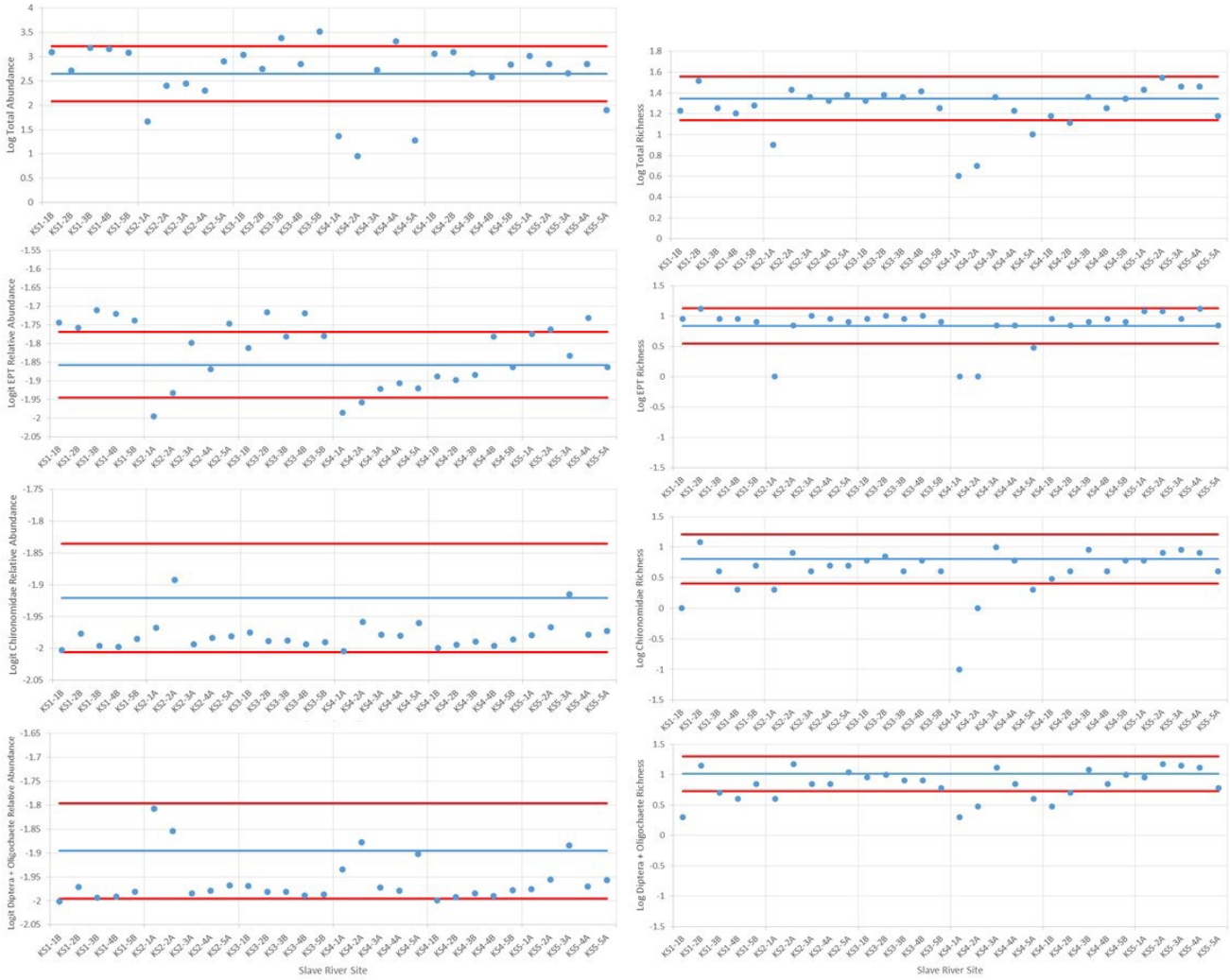

Figure 44. Biotic metrics plotted for each site in the Slave River with a grand mean (mean of 2017 and 2018 data; blue line) and the upper and lower critical effect size (CES; red lines), calculated as mean ± 2SD (calculated based on 2017 and 2018 data). Each point represents a kick-site, moving from reach 1, site 1 (far left) to reach 6, site 5 (far right) on each plot. Metrics include (left column) log_{10} abundance, logit EPT relative abundance, logit Chironomidae relative abundance, logit Diptera + Oligochaeta relative abundance, and (right column) log_{10} taxonomic richness, log_{10} EPT richness, log_{10} Chironomidae richness, and log_{10} Diptera + Oligochaeta richness.

were not characterized by a consistent gain or loss of individuals. In total, the abundance in 12 sites changed by more than 500 individuals, with 7 sites increasing in abundance and 5 sites decreasing in abundance. In some cases, there was a very large change in abundance, with a loss or gain of hundreds or thousands of individuals per site (for example, KS4-2A went from 762 individuals in 2017 to only 9 individuals in 2018, and KS3-3B went from 460 individuals in 2017 to 2400 in 2018). The result of these changes was a wide normal range as defined by the combination of 2017 and 2018 data, and a large number of sites in 2018 that were outside of this normal range (Figure 44). Total abundance was below the lower CES level in sites KS2-1A, KS4-1A, KS4-2A, KS4-5A, and KS6-5A; whereas exceedances of the upper CES were evident in sites KS3-3B, KS3-5B, and KS4-4A (Figure 44).

Exceedances of 2017-2018 CES limits in the Slave River also reflected the shift in dominance in the river in 2018, with lower relative abundance of Chironomidae and higher relative abundance of EPT taxa. The relative

abundance of EPT was higher than the upper CES limit in 10 sites in the Slave River, including all sites in Reach 1. The relative abundance of Chironomidae and Diptera + Oligochaeta was near the lower CES limit, and the normal range for these metrics as defined based on the combination of 2017 and 2018 data was much wider than the range for EPT or for total abundance (Figure 44). The single-year CES for Chironomidae relative abundance was vastly different in 2018 from the normal range determined for 2017. The CES in 2017 ranged from -2.03 to -1.69 (logit scale), whereas the range in 2018 was from -3.90 to -2.62 (logit scale), indicating that there was no overlap of the normal range for these two years. This strong difference results from the sharp declines in Chironomidae abundance that were described in section 3.1.2.5.1. Over half of the Slave River sites saw a decline in percent composition of Chironomidae of greater than 30% from 2017 to 2018, and Chironomidae relative abundance declined from 70-80% down to less than 10% of the total abundance in some samples. If variability in this metric continues to be large with additional years of sampling, it may mask future variability that occurs due to impacts. However, the shift in Chironomidae abundance from 2017 to 2018 could also have the potential to provide information on changing flow conditions and permanency of the sampled habitat, and such low abundances of this taxon could be used as a trigger to indicate that temporary habitats were sampled. Analysis of the power to detect differences in temporal data (within sites and reaches) will be necessary when additional years of data have been collected, to determine whether the normal range for these metrics is too large. Assessment of temporal trends using data from 2017-2019 will begin to indicate whether this metric can be used to develop management triggers for the river, or whether it is too variable.

Total taxonomic richness in the Slave River was lower on average in 2018 than it was in 2017, due in part to low taxonomic richness at a number of sites that fell below the lower CES limit for 2017-2018 data (sites KS2-1A, KS4-1A, KS4-2A, and KS4-5A; Figure 44). These patterns reflected low richness of EPT taxa at these sites, as well as low richness of Chironomidae and Diptera +Oligochaeta (below the lower CES limit in each site; Figure 44). Other sites in the Slave River generally had low variability in richness of these metrics, particularly EPT richness, which was similar across most sites. Overall, sites KS2-1A, KS4-1A, and KS4-2A stood out as having the lowest abundance and diversity compared to other sites, though Reach 4A in general appeared to differ from other reaches. The loss of high numbers of Chironomidae in 2018 contributed to large differences between years, and will need to be further investigated, particularly once 2019 data are available.

3.3.2. Sample size for water quality

Sample size for water quality analysis changed from 2017 to 2018, as the first year of sampling began with one sample per site, and this was reduced to collect samples only in odd-numbered sites in 2018. This change is beneficial in terms of cost, as it allows for less lab processing of samples. In addition, variability among water quality samples was generally low in 2017, which indicated that samples collected at one site were generally characteristic of the results at other sites in the reach. The variability among samples was tested by assessing the coefficient of variation (CV) among samples. Following guidelines described by the Canadian Council of Ministers of the Environment (2011), CV (the standard deviation divided by the mean) for each water quality parameter was calculated for each reach in 2017 and 2018. When the CV was less than 18%, this was taken to indicate low variability among samples in a reach (e.g., samples are essentially duplicates), as long as the mean value for the parameter was greater than 10 times the detection limit.

Over 2017 and 2018 in both the Hay River and Slave River, there were 779 instances when a parameter mean was greater than 10 times the detection limit in a reach, and of these, 126 exceeded a coefficient of variation of 18% within the reach. In other words, among all the water quality parameters calculated in both rivers in 2017 and 2018, there were 126 instances (16% of samples considered) where variability within the reach was higher than would be acceptable for duplicate samples. Such exceedances were found in all reaches besides HR-KS6, though some reaches had more exceedances (i.e., more parameters with high variability) than others (Table 13). For example, Reach HR-KS5B had only one parameter with high variability in 2017 and 2018, whereas SR-KS2A

Table 13 The number of water quality parameters exceeding a coefficient of variation of 18% across sites in each reach in 2017 and 2018, for those parameters that had a mean value >10 MDL.

Reach	Year	Number of parameters
HR-KS1A	2017	9
HR-KS1A	2018	7
HR-KS2A	2017	11
HR-KS2A	2018	4
HR-KS3A	2017	7
HR-KS3A	2018	2
HR-KS4A	2018	4
HR-KS5B	2017	1
HR-KS5B	2018	1
SR-KS1B	2017	2
SR-KS1B	2018	4
SR-KS2A	2017	3
SR-KS2A	2018	17
SR-KS3B	2017	4
SR-KS3B	2018	14
SR-KS4A	2017	15
SR-KS4A	2018	4
SR-KS4B	2017	7
SR-KS4B	2018	1
SR-KS5A	2017	2
SR-KS5A	2018	7

had high variability in 17 parameters in 2018. In some reaches there were more parameters with high variability in 2018 (when fewer samples of ions, nutrients, and physicals were collected), though this was not consistent across all reaches (Table 13). Furthermore, this high variability was not limited to ions, nutrients, and physicals, and also included metals. Ions, nutrients, and physicals that were variable within reaches included ammonia (2018), magnesium (2018), total nitrogen (2017), dissolved phosphorus (2017), total phosphorus (both years), potassium (2018), sodium (2018), total suspended solids (both years), and turbidity (both years). Metals that were variable within reaches included dissolved aluminum (2018), total aluminum (both years), total barium (both years), total chromium (both years), total cobalt (both years), total copper (both years), dissolved and total iron (both years), total lead (both years), total lithium (2017), dissolved and total manganese (both years), total mercury (both years), dissolved nickel (2018), total nickel (both years), total rubidium (both years), total titanium (both years), and total vanadium (both years).

Of the parameters and samples assessed, 84% had acceptable levels of precision, which suggests that fewer replicates may be acceptable in future sampling. However, there are other benefits to collecting multiple samples per reach when conducting sampling for this benthic monitoring program. Water chemistry sampling from this program is intended to support the detection of patterns and trends in the BMI data, and is not sufficient to act as a stand-alone measure of water quality trends at these locations. However, the water chemistry data collected through the BMI monitoring program could supplement existing water quality monitoring that is ongoing in the area, adding to the spatial and temporal extent of that monitoring.

Furthermore, samples at multiple sites in each reach support the assessment of biotic-abiotic relationships within and among reaches. Although sites may be considered as replicates for each reach, the assessment of biotic assemblages indicates that there is variability in assemblage composition among sites. Although many sites did group by reach in multivariate analysis, there were sites that were separated from other sites in the same reach in multivariate space, which indicates compositional differences. Furthermore, there was variability in metric values within reaches. In order to properly assess whether differences in biotic composition among sites are due to chemical or physical habitat conditions, it is necessary to have supporting data (chemical and physical habitat data) for each site. Otherwise, there is no way to associate biotic differences in one site with a particular driver.

The collection of only three water quality samples per reach in 2018 represented a compromise between cost and data quality. The processing of samples cost less, but when biotic patterns were related to chemical and physical drivers, it was necessary to create estimates for even-numbered sites based on averaging water chemistry values at neighbouring sites. For two sites per reach, this may be an acceptable compromise. But if only one or two water chemistry samples were collected per reach, it would greatly hinder the ability to detect biotic responses to changes in water quality, because biotic differences within a reach could not be related to variability in chemical parameters. For this reason, it is recommended that water chemistry samples continue to be collected at a minimum of three sites per reach, particularly as baseline data are collected and the normal range of variability is established.

4. Recommendations and Conclusions

There are a number of general conclusions from the analysis of the second year of monitoring data.

Hay River:

- Water chemistry conditions differed in the Hay River from 2017 to 2018, with stronger variability within and among reaches. These differences may have reflected variation in flow between years, but they should be interpreted with caution, as they represent spot measurements that should not be used to draw conclusions about temporal trends.
- Variation in BMI abundance among Hay River sites was stronger in 2018 than in 2017, with lower abundance evident in the three downstream reaches, particularly HR-KS4. Abundance in upstream reaches increased significantly in 2018, whereas abundance in downstream reaches decreased significantly in 2018. This shift in the normal range indicates annual variability that likely reflected lower water levels.
- Multivariate analysis indicated no strong outliers among the sites. Sites in Reaches 1-3 appeared similar, and were characterized by a higher relative abundance of EPT taxa. Sites in Reaches 4-6 were generally similar, and were characterized by a higher relative abundance of Chironomidae taxa (with the exception of site HR-KS6-5B, which more closely resembled Reaches 1-3).
- There were a number of sites in the Hay River that were outside the normal range (calculated based on 2017 and 2018 data), reflecting low total abundance and relative abundance of EPT in the downstream reaches, and high richness of EPT and Chironomidae in Reaches 2, 3, 4, and 5. The frequency of sites outside the limits of the CESs is suggestive of high inter-annual variability in some biotic metrics. More years of data will be required to refine these normal range estimates.

- The sites that were identified as outliers in 2017 (HR-KS1-4A and HR-KS1-2A) did not appear to differ from other sites after shifting their physical locations in 2018.
- Reach 6 in the Hay River was added in 2018 to characterize the system farther downstream of the boat launch. This reach was within the 2018 normal range, and had average to high abundance and richness at most sites. Sites in this reach are compositionally similar to sites in multiple reaches, and therefore the continued inclusion of this reach will improve replication of these compositional patterns and improve estimates of normal range.

Slave River:

- Individual water chemistry parameters differed among Slave River reaches in 2018, though multivariate analysis indicated that differences in the chemical and physical habitat among sites were similar in both 2017 and 2018. CCME guidelines for the protection of aquatic life were exceeded for several parameters, but the mean values of these parameters were consistent with long-term means and interim triggers for the river. Furthermore, as these represent spot measurements of water chemistry at the time of sampling, any exceedances should be interpreted with caution, as they may not reflect long-term trends.
- Variation in BMI abundance among sites was strong in the Slave River in 2018. Total abundance was dramatically different between 2017 and 2018 in many sites along the river, with 12 sites having 500 more or 500 fewer individuals in 2018. However, the direction of change was not consistent within or among reaches.
- Chironomidae richness and abundance was overall much lower in Slave River sites in 2018 than was observed in 2017. Over half of the Slave River sites saw a decline in percent composition of Chironomidae of greater than 30% from 2017 to 2018. In some cases, chironomid abundance declined from 70-80% down to less than 10% of the total abundance in the samples. The losses seem to be primarily in the subfamilies Chironominae and Tanypodinae, though declines in Chironominae were more consistent. A preference in this subfamily for stable flow conditions suggests that this large shift may be related to inter-annual differences in water level, but more data are required before this can be confirmed.
- Assessment of biotic metrics for Slave River sites indicated that sites SR-KS4-1A, SR-KS4-2A, and SR-KS4-5A in Reach 4A and site SR-KS2-1A in Reach 2 had low abundance and low diversity compared to the other sites. Multivariate analysis confirmed that sites KS2-1A, KS4-1A, and KS4-2A were outliers, with different assemblage composition than was found at other sites. These sites were associated with few taxa, and primarily with taxa suggestive of slow flows. Reach 4A in particular differed strongly from other reaches, which suggests that it might not be ideal for continued monitoring.
- Due to low total abundance and low abundance of Chironomidae in the Slave River, the normal ranges for total abundance and relative abundance of Chironomidae shifted compared to 2017. As a result, the normal range calculated using both 2017 and 2018 data was wide for both metrics, and there were many sites that were outside the CES limits. These shifts in the normal range indicate annual variability, likely related to differences in flow between years. In the case of Chironomidae, it may indicate that temporary habitats were sampled, and it may be an artifact of the impact of a recent surge in water levels on site access.
- In addition to lower abundance, there was lower richness in many sites in 2018. In particular, sites SR-KS201A, SR-KS4-1A, and SR-KS4-2A were below normal range for total richness and EPT richness, and the latter two sites were also below normal range for richness of Chironomidae and Diptera +

Oligochaeta. KS4-1A was below normal range for Chironomidae richness because no chironomids were found there in 2018.

Recommendations for future monitoring in these systems based on the first two years of data include:

- Continue to sample Hay River in mid-late August and sample Slave River in early/mid-September, but adjust sample timing annually depending on flow conditions in each river. Hay River samples appeared to be affected by low water levels, with a significant loss of abundance in downstream reaches, although richness measures were not strongly altered. Slave River samples appeared to have been collected in temporary habitat due to high water levels, resulting in a significant loss of abundance, particularly of Chironomidae. Where possible, allow for some flexibility in the timing of sampling to ensure it does not follow a surge in water levels (as this appeared to have a greater impact than low water levels). Water levels should be similar to those at the time of sampling in 2017.
- Continue sampling the sample sites and reaches in the Hay River (including the new reach, Reach 6), as these appeared to characterize the river. The addition of Reach 6 increased the sample size of reaches downstream of the boat launch, which will allow for more power in the assessment of longitudinal changes in the river. There was evidence of longitudinal patterns in the river in both 2017 and 2018, so these patterns should continue to be monitored.
- In the Slave River, sites KS2-1A, KS4-1A, and KS4-2A were clearly different from other sites, and Reach KS4A in general tended to stand out in the assessment. Conditions at these sites, particularly with respect to flow, may drive the differences relative to other sites. In the long term, these sites may not be ideal for monitoring, due to the low abundance and richness found there. Consider removing Reach 4A from future monitoring, and monitor conditions in site SR-KS2-1A.
- Efforts should be made to locate and sample another reach in the Slave River to ensure sufficient replication and characterization of variability, particularly if Reach 4A is removed.
- Although variability among water chemistry samples was fairly low, there were a number of parameters that varied among the three sites in each reach, generally with higher levels at only one site. Because of this variability and because of the need for site-scale supporting variables to assess biotic-abiotic relationships, continue collection of water chemistry samples at odd-numbered sites in each reach unless chemistry results in future years suggest that more sampling is necessary.
- Sediment-bound metals are not readily biologically available in oxygenated and pH stable environments, and thus shifts in these concentrations may not provide an estimate of the potential risk to biota. Furthermore, where bound metals may be biologically available, uptake of sediment-bound metals is dependent on the level of exposure from feeding habits and habitat preferences of individual species. In the future, it is important to ensure continued collection of dissolved metal samples to estimate biotic response to metals, or to explore the use of regression to predict dissolved metals from total metals and TSS.
- Sediment chemistry was not strongly related to biota in the Hay River or Slave River. Although it may be desirable to continue collection of these samples to monitor changes in PAHs in the sediments, they may not need to be collected as regularly as water chemistry samples.

Analysis of the second year of data from the GNWT and GOA monitoring program for large transboundary rivers has provided a characterization of the BMI assemblages and abiotic environment in the Hay and Slave rivers under differing flow conditions from the pilot year. In addition to assessing abundance, diversity, and composition, and evaluating which chemical and physical parameters drive differences within and among

reaches, this has also contributed to the characterization of inter-annual variability related to flow, and will support the refinement of normal range estimates for these rivers. As additional years of sampling data are collected, it will be possible to characterize not only the spatial normal range but also the normal range of temporal variability. These are important first steps towards monitoring long term trends and detecting future impacts, as the information about natural spatial and temporal variation allows for the detection of changes above and beyond what is expected in the system under normal conditions.

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6. Appendices

Table 14 Names and coordinates of kick-sampling sites sampled in the Hay River and Slave River in 2018.

River	Reach	Site	Latitude	Longitude	Date	River	Reach	Site	Latitude	Longitude	Date
HAY RIVER	REACH 1	HR-KS1-1A	59.93403	-116.95028	2018-08-29	SLAVE RIVER	REACH 1	SR-KS1-1B	59.40805	-111.46321	2018-09-11
		HR-KS1-2A	59.93591	-116.95175	2018-08-29			SR-KS1-2B	59.40805	-111.46321	2018-09-11
		HR-KS1-3A	59.93211	-116.95237	2018-08-29			SR-KS1-3B	59.40846	-111.46196	2018-09-11
		HR-KS1-4A	59.93135	-116.95506	2018-08-29			SR-KS1-4B	59.40879	-111.46082	2018-09-11
		HR-KS1-5A	59.93124	-116.95613	2018-08-29			SR-KS1-5B	59.40913	-111.45985	2018-09-11
	REACH 2	HR-KS2-1A	59.94548	-116.95565	2018-08-28		REACH 2	SR-KS2-1A	59.42627	-111.46072	2018-09-11
		HR-KS2-2A	59.94617	-116.95618	2018-08-28			SR-KS2-2A	59.42709	-111.46199	2018-09-11
		HR-KS2-3A	59.94654	-116.95647	2018-08-28			SR-KS2-3A	59.42761	-111.46294	2018-09-11
		HR-KS2-4A	59.94703	-116.95702	2018-08-28			SR-KS2-4A	59.42799	-111.46361	2018-09-11
		HR-KS2-5A	59.94759	-116.95744	2018-08-28			SR-KS2-5A	59.42858	-111.46458	2018-09-11
	REACH 3	HR-KS3-1A	59.98767	-116.93236	2018-08-29		REACH 3	SR-KS3-1B	59.53395	-111.45934	2018-09-10
		HR-KS3-2A	59.98827	-116.93060	2018-08-29			SR-KS3-2B	59.53451	-111.45864	2018-09-10
		HR-KS3-3A	59.98845	-116.93037	2018-08-29			SR-KS3-3B	59.53502	-111.45774	2018-09-10
		HR-KS3-4A	59.99023	-116.93049	2018-08-29			SR-KS3-4B	59.53538	-111.45703	2018-09-10
		HR-KS3-5A	59.99182	-116.93127	2018-08-29			SR-KS3-5B	59.53562	-111.45651	2018-09-10
	REACH 4	HR-KS4-1A	60.00158	-116.97036	2018-08-30		REACH 4A	SR-KS4-1A	59.58906	-111.41968	2018-09-11
		HR-KS4-2A	60.00205	-116.97145	2018-08-30			SR-KS4-2A	59.58947	-111.4196	2018-09-11
		HR-KS4-3A	60.00261	-116.97126	2018-08-30			SR-KS4-3A	59.59122	-111.41951	2018-09-11
		HR-KS4-4A	60.00308	-116.97089	2018-08-30			SR-KS4-4A	59.59178	-111.41949	2018-09-11
		HR-KS4-5A	60.00319	-116.97009	2018-08-30			SR-KS4-5A	59.59225	-111.41946	2018-09-11
	REACH 5	HR-KS5-1B	60.01064	-116.92032	2018-08-31		REACH 4B	SR-KS4-1B	59.58887	-111.42283	2018-09-10
		HR-KS5-2B	60.01096	-116.92088	2018-08-31			SR-KS4-2B	59.58975	-111.42273	2018-09-10
		HR-KS5-3B	60.01125	-116.92177	2018-08-31			SR-KS4-3B	59.59026	-111.42253	2018-09-10
		HR-KS5-4B	60.01138	-116.92274	2018-08-31			SR-KS4-4B	59.5909	-111.42261	2018-09-10
		HR-KS5-5B	60.01163	-116.92348	2018-08-31			SR-KS4-5B	59.59139	-111.42264	2018-09-10
	REACH 6	HR-KS6-1B	60.02772	-116.92342	2018-09-05		REACH 5	SR-KS5-1A	59.71284	-111.50644	2018-09-09
		HR-KS6-2B	60.02779	-116.92217	2018-09-05			SR-KS5-2A	59.71304	-111.50646	2018-09-09
		HR-KS6-3B	60.02785	-116.92155	2018-09-05			SR-KS5-3A	59.71823	-111.50577	2018-09-09
		HR-KS6-4B	60.02787	-116.92075	2018-09-05			SR-KS5-4A	59.71853	-111.50594	2018-09-09
		HR-KS6-5B	60.02802	-116.91985	2018-09-05			SR-KS5-5A	59.7187	-111.50603	2018-09-09

Table 15 Names and coordinates of Hester-Dendy sites sampled in the Hay River and Slave River in 2018, with indication of lost and tangled samples.

River	Reach	Site	Depth Deployed (m)	Depth Retrieved (m)	Lat (decimal degrees)	Long (decimal degrees)	Deployment	Retreival	Notes
HAY RIVER		HR-HD-01	1.3	0.7	59.93200804	-116.986127	2018-08-09	2018-08-30	
		HR-HD-02	1.3	0.9	59.93161602	-116.985675	2018-08-09	2018-08-30	
		HR-HD-03	1.6	0.8	59.93120598	-116.985234	2018-08-09	2018-08-30	
		HR-HD-04	1.2	0.7	59.93097397	-116.985052	2018-08-09	2018-08-30	
		HR-HD-05	1.5	0.9	59.93074103	-116.984941	2018-08-09	2018-08-30	
	REACH 1	HR-HD-06	1.5	1.1	59.930562	-116.984475	2018-08-09	2018-08-30	
		HR-HD-07	1.3	0.7	59.93209798	-116.952273	2018-08-09	2018-08-30	
		HR-HD-08	1.2	0.6	N/A	N/A	2018-08-09	2018-08-30	
		HR-HD-09	1.0	0.5	59.93228498	-116.951964	2018-08-09	2018-08-30	
		HR-HD-10	1.0	0.4	59.93249997	-116.951792	2018-08-09	2018-08-30	
		HR-HD-11	1.1	0.5	59.932705	-116.951525	2018-08-09	2018-08-30	
	REACH 2	HR-HD-12	1.2	0.5	59.93286802	-116.951334	2018-08-09	2018-08-30	
		HR-HD-13	1.7	1.6	59.99144697	-116.932205	2018-08-09	2018-09-06	
		HR-HD-14	1.3	1.9	59.991194	-116.931937	2018-08-09	2018-09-06	
		HR-HD-15	2.3	2.0	59.99100197	-116.931794	2018-08-09	2018-09-06	
		HR-HD-16	1.8	1.6	59.99081003	-116.931722	2018-08-09	2018-09-06	
		HR-HD-17	1.7	2.0	59.99058899	-116.931596	2018-08-09	2018-09-06	
	REACH 3	HR-HD-18	1.7	1.4	59.99040199	-116.931667	2018-08-09	2018-09-06	
		HR-HD-19	2.7	LOST	60.01144996	-116.922387	2018-08-08	2018-09-05	Unable to retrieve
		HR-HD-20	2.2	2.0	60.01143001	-116.922185	2018-08-08	2018-09-05	
		HR-HD-21	1.9	0.8	60.01145298	-116.922049	2018-08-08	2018-09-05	
		HR-HD-22	2.0	1.6	60.01135097	-116.921836	2018-08-08	2018-09-05	
		HR-HD-23	1.7	1.4	60.01128601	-116.921483	2018-08-08	2018-09-05	
	REACH 4	HR-HD-24	1.6	1.0	60.01117	-116.921169	2018-08-08	2018-09-05	
SLAVE RIVER		SR-HD-01	2.8	2.1	59.694668	-111.511846	2018-08-07	2018-09-09	HD-01 and 02 tangled
		SR-HD-02	4.3	4.6	59.69454898	-111.509975	2018-08-07	2018-09-09	HD-01 and 02 tangled
		SR-HD-03	4.8	3.3	59.69460304	-111.5103	2018-08-07	2018-09-09	HD-03,04,05 and 06 tangled
		SR-HD-04	4.5	3.3	59.69475199	-111.511401	2018-08-07	2018-09-09	HD-03,04,05 and 06 tangled
		SR-HD-05	4.4	3.3	59.69481803	-111.512498	2018-08-07	2018-09-09	HD-03,04,05 and 06 tangled
	REACH1	SR-HD-06	3.2	3.3	59.69497704	-111.512752	2018-08-07	2018-09-09	HD-03,04,05 and 06 tangled
		SR-HD-07	4.5	4.1	59.71633698	-111.511951	2018-08-07	2018-09-09	
		SR-HD-08	4.8	4.8	59.71645198	-111.511345	2018-08-07	2018-09-09	
		SR-HD-09	4.6	4.8	59.71662699	-111.510742	2018-08-07	2018-09-09	
		SR-HD-10	4.9	3.4	59.71667603	-111.510327	2018-08-07	2018-09-09	
		SR-HD-11	4.4	3.7	59.71687803	-111.509451	2018-08-07	2018-09-09	
	REACH 2	SR-HD-12	4.2	7.2	59.717175	-111.509242	2018-08-07	2018-09-09	
		SR-HD-13	5.0	5.2	59.82785996	-111.566965	2018-08-07	2018-09-08	
		SR-HD-14	5.0	5.0	59.82872799	-111.569296	2018-08-07	2018-09-08	
		SR-HD-15	5.3	4.5	59.82938396	-111.570875	2018-08-07	2018-09-08	Tangled with 16
		SR-HD-16	5.2	4.5	59.83022902	-111.572796	2018-08-07	2018-09-08	Tangled with 15
		SR-HD-17	5.0	LOST	59.83063797	-111.573844	2018-08-07	2018-09-08	Could not retrieve
	REACH 3	SR-HD-18	5.3	LOST	59.83109898	-111.574749	2018-08-07	2018-09-08	Could not retrieve
		SR-HD-19	4.0	4.4	59.86809804	-111.572089	2018-08-07	2018-09-08	
		SR-HD-20	3.8	3.9	59.86838403	-111.571558	2018-08-07	2018-09-08	
		SR-HD-21	3.5	3.4	59.868781	-111.571169	2018-08-07	2018-09-08	
		SR-HD-22	3.0	2.7	59.86910697	-111.570912	2018-08-07	2018-09-08	
		SR-HD-23	3.4	3.1	59.86948801	-111.570704	2018-08-07	2018-09-08	
	REACH 4	SR-HD-24	2.9	2.6	59.86994499	-111.570804	2018-08-07	2018-09-08	

Table 16 BMI names and abbreviations used in PCA ordinations, with indication of the river and sampling method in which each taxon was found.

				Hay River		Slave River	
Order/Group	**Family**	**Subfamily**	**Code**	**HD**	**Kick**	**HD**	**Kick**
Amphipoda			AMPH	0	1	1	1
Bivalvia	Pisidiidae		PISID	0	1	0	1
Coleoptera	Elmidae		C_Elm	0	1	1	0
Diptera	Ceratopogonidae		D_Cerat	0	1	1	1
Diptera	Chironomidae	Chironominae	D_C_Chir	1	1	1	1
Diptera	Chironomidae	Diamesinae	D_C_Dia	1	1	0	0
Diptera	Chironomidae	Orthocladiinae	D_C_Orth	1	1	1	1
Diptera	Chironomidae	Prodiamesinae	D_C_Pro	0	0	1	1
Diptera	Chironomidae	Tanypodinae	D_C_Tany	1	1	1	1
Diptera	Diptera Pupa		D_Pupa	1	1	1	1
Diptera	Empididae		D_Emp	0	1	1	1
Diptera	Simuliidae		D_Simu	1	1	1	0
Diptera	Tabanidae		D_Tab	0	1	0	1
Diptera	Tipulidae		D_Tipu	0	1	1	1
Ephemeroptera	Acanthametropodidae		E_Acan	0	0	0	1
Ephemeroptera	Ameletidae		E_Amel	1	1	0	0
Ephemeroptera	Ametropodidae		E_Amet	1	1	0	1
Ephemeroptera	Baetidae		E_Bae	1	1	1	1
Ephemeroptera	Caenidae		E_Cae	1	1	0	1
Ephemeroptera	Ephemerellidae		E_Ephe	1	1	1	1
Ephemeroptera	Ephemeridae		E_Eph	1	1	1	0
Ephemeroptera	Heptageniidae		E_Hept	1	1	1	1
Ephemeroptera	Isonychiidae		E_Iso	1	1	1	1
Ephemeroptera	Leptophlebiidae		E_Lept	1	1	1	1
Ephemeroptera	Metretopodidae		E_Met	0	1	0	1
Gastropoda			GAST	0	1	1	1
Hemiptera	Corixidae		H_Corix	0	1	0	1
Hirudinea	Glossiphoniidae		GLOSS	0	0	0	1
Odonata	Aeshnidae		O_Aesh	0	1	0	0
Odonata	Gomphidae		O_Gomph	1	1	0	1
Oligochaeta	Enchytraeidae		ENCHY	0	1	1	1
Oligochaeta	Lumbriculidae		LUMB	0	1	0	0
Oligochaeta	Naididae		NAID	1	1	1	1
Plecoptera	Capniidae		P_Cap	0	1	0	1
Plecoptera	Chloroperlidae		P_Chl	0	1	0	0
Plecoptera	Perlidae		P_Perli	1	1	1	1
Plecoptera	Perlodidae		P_Perlo	1	1	1	1
Plecoptera	Pteronarcyidae		P_Pter	1	1	1	0
Trichoptera	Brachycentridae		T_Bra	1	1	1	1
Trichoptera	Hydropsychidae		T_Hpsy	1	1	1	1
Trichoptera	Hydroptilidae		T_Hpti	1	1	1	1
Trichoptera	Lepidostomatidae		T_Lepi	1	1	0	1
Trichoptera	Leptoceridae		T_Lepto	1	1	1	1
Trichoptera	Limnephilidae		T_Limn	0	0	0	1
Trichoptera	Philopotamidae		T_Phil	1	1	0	0
Trichoptera	Polycentropodidae		T_Poly	1	1	1	1
Trichoptera	Trichoptera Pupa		T_Pupa	0	0	1	1

Figure 45. Pictures of sample locations, including (A) upstream view from Hay River Reach 1, (B) downstream view from Hay River Reach 1, (C) upstream view from Slave River Reach 1, and (D) downstream view from Slave River Reach 1. Photos taken in 2017.