Aquatic Invertebrate Collection Methods Comparison Study

Results from the ABMI 2018 Wetland Aquatic Invertebrate Protocol Assessment

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Executive Summary

The development of wetland habitat and species models using bioindicators is dependent on collecting standardized data from a variety of wetland types and disturbance levels that span large spatial and temporal scales. Establishing cost effective and standardized methods can accelerate the accumulation of data by allowing for more efficient data collection and the direct comparison of wetland sampling information across programs.

In 2007, the Alberta Biodiversity Monitoring Institute (ABMI) implemented a set of standardized aquatic invertebrate sampling protocols (Composite Transect Approach = CTA), with the Canadian Aquatic Biomonitoring Network (CABIN) following up in 2017 with the creation of their own national protocols (Traveling Sweep Approach = TSA). Also in 2017, the Environmental Monitoring and Science Division (EMSD) of Alberta Environment and Parks (AEP) conducted a study comparing the two aquatic invertebrate sampling methods and concluded that the TSA method collected similar data at a much lower cost. With the pressure for ABMI to transition to the TSA protocols, and with a few unanswered questions remaining from the EMSD study, ABMI collected additional field and lab processing data in 2018 to develop a better understanding of the implications in making such a change.

Results from this study indicate that similarities between CTA and TSA aquatic invertebrate communities examined at the family level were consistent with EMSD study findings. Further, those similarities extended to analysis at finer taxonomic resolutions, something not explored in the EMSD study. While there were significant differences between total and relative abundance for some individual taxa, the differences between aquatic invertebrate communities as a whole were small and the data comparable. Further, model results examining relationships with environmental variables were very similar, which attests the possibility of integrating data from the two protocols with appropriate statistical controls.

We also found that the time/cost difference between the two sampling methods, while not as great as indicated in the EMSD study, is substantial enough to consider transitioning to the use of the more efficient TSA aquatic invertebrate collection protocols and a closer alignment with CABIN lab processing methods.

The cost/time savings will allow for more wetlands to be sampled each year, or more data to be collected at each site. More importantly, a closer alignment with the TSA collection method and CABIN lab protocols will improve confidence in direct data comparison and allow both ABMI and CABIN to leverage data between programs, thus enabling the development of stronger

wetland habitat and species models. However, further work is needed, including methodological development to integrate CABIN data with historical ABMI data, in order to use the combined datasets for species distribution modelling and population trend assessment.

Introduction

Wetlands - consisting of bogs, fens, marshes, seasonal and ephemeral ponds, and shallow open water - cover about 20% of Alberta's surface area (ESRD 2013). Aside from being an important habitat for the plants and animals adapted to wetland environments, the ecological influence of wetlands extends well beyond their boundaries, forming an interconnected habitat network with the surrounding terrestrial landscape. Ecologically intact wetlands, and their associated biodiversity, act as a catalyst for the physical, chemical, and biological processes that:

- provide clean water,
- mitigate flood and drought events,

- recharge ground and surface water,
- capture and store carbon,
- recycle nutrients,
- support a high level of primary productivity, and
- provide critical wildlife habitat.

In addition to these ecosystem services, wetlands contribute significantly to habitat complexity at local and regional scales through the creation of microhabitats, edge effects, and localized microclimates. This habitat complexity supports a high level of biodiversity, both in the wetland and the surrounding landscape.

Aquatic invertebrates are key drivers of many biological processes that support and maintain the ecological integrity of wetlands (Mazor *et al.* 2019; Merkin and Wrubleski 1988). This very diverse group of organisms, which includes representatives at every trophic level, ranges from tiny aquatic worms (Oligochaeta) that are just a few millimeters long to top predators like the giant water bug (*Lethocerus americanus*), which grows to over 50 mm long. Forming a large part of the biomass, aquatic invertebrate communities significantly influence wetland nutrient cycling and serve as a critical food source for fish, amphibians, and birds (Batzer *et al.* 1999; Covich *et al.* 1999). It is the diversity and richness of the aquatic invertebrate community that allows wetlands to function as an integrated ecological system.

Biomonitoring projects frequently use aquatic invertebrates as indicators of wetland health and function (Mazor *et al.* 2019; Rosenberg and Resh 1993) because they are a cost-effective way to characterize the ecological intactness of aquatic habitats (Mazor *et al.* 2019). As a group, aquatic invertebrates are strong bioindicators because they can be easily sampled, are ecologically well understood, can be identified using available taxonomic keys, and are sensitive to a variety of natural and anthropogenic stressors. Additionally, aquatic invertebrate lifecycles allow them to integrate responses to both acute habitat disturbances as well as longer term, cumulative effects (Mazor *et al.* 2019).

The development of regional biodiversity models for wetlands and estimating their ecological integrity using intactness indices from aquatic invertebrate community data is still in its infancy. It has only been in the last twenty years that researchers have attempted to characterize biodiversity of wetlands and monitor their ecological integrity using invertebrates as bioindicators (Mazor *et al.* 2019). While many small-scale wetland studies have been completed in past decades, comparison of invertebrate data across such studies is often difficult due to variation in sample collection methods. Standardized data from a variety of wetland types and disturbance

levels is key to developing regional biodiversity models applicable to overall wetland assessment spanning large spatial and temporal scales.

To develop a strategy for monitoring province-wide changes in wetland biodiversity over time, the Alberta Biodiversity Monitoring Institute (ABMI) developed a set of standardized wetland sampling protocols in 2006. The aquatic invertebrate sampling component of these protocols was partly based on the protocols developed by the Ontario Benthos Biomonitoring Network (OBBN) for sampling lakes. Given the diversity of wetland types across Alberta, the "jab and sweep" method that samples at multiple locations in the wetland (i.e. the Composite Transect Approach = CTA) was seen as the most adaptable, systematic, and consistent method of collection. The intent was to consistently apply these protocols to wetland sample collection across the province for the purpose of developing regional biodiversity models suitable for long-term ecological monitoring of wetlands.

Between 2012 and 2016, Environment and Climate Change Canada (ECCC) started to develop standardized national aquatic invertebrate sampling protocols (i.e. the Traveling Sweep Approach = TSA) under the Canadian Aquatic Biomonitoring Network (CABIN) banner, which was an expansion of their successful rapid assessment stream monitoring program. Designed to allow independent researchers and organizations to contribute to, and access, online data platforms and analytical tools, CABIN is a mostly user-driven initiative. Table 1 places CTA and TSA aquatic invertebrate protocols in a historical context.

Table 1 - Historical comparison of CTA and TSA wetland sampling protocols.

CTA Protocol	TSA Protocol
• Developed by ABMI in 2006, implemented in 2007, with a few refinements made in 2009 and 2011.	• Developed by Environment and Climate Change Canada (ECCC) 2012-2016, implemented in 2017.
• 1836 wetlands sampled to date province wide (659 as repeat wetland visits).	• ~324 wetlands sampled to date, mostly in the oil sands and Lower Athabasca region.
• Designed to be an intensive survey in multiple habitat types at different depths.	• Designed to be a rapid assessment of the dominant near-shore habitat type.
• Requires a boat to collect samples.	• No boat required - samples collected by wading.

In 2017, the Environmental Monitoring and Science Division (EMSD) of Alberta Environment and Parks (AEP) began using TSA protocols to sample wetlands in the oil sands region as part of their water quality-monitoring program. Because ABMI was also actively monitoring wetlands in the oil sands region using CTA protocols, EMSD conducted a comparison study of the two sampling methods to determine whether they described similar aquatic invertebrate communities and if one method was more cost effective than the other. Based on the results of this study, Hanisch *et al.* (2020) concluded that although there were notable differences in the aquatic invertebrate communities described by each protocol, "the differences were generally small and the communities similar". However, they also found that TSA protocols required significantly less time and resources to collect the samples compared to CTA protocols. As a result, Hanisch *et al.* (2020) recommended the TSA protocol as the preferred monitoring approach.

Following the EMSD study (Hanisch *et al.* 2020), ABMI set out to evaluate the possibility of switching its aquatic invertebrate sampling protocols to the TSA method. Despite some compelling evidence presented by Hanisch *et al.* (2020) indicating that the TSA method may be more cost effective, several questions remain unanswered. For example, aside from differences in field sampling, Hanisch et al. (2020) did not assess variation in laboratory processing protocols between ABMI and CABIN, a difference which may influence direct data comparability. In addition, Hanisch *et al.* (2020) only presented family-level data analyses which could mask more subtle variation in community structure detectable only at lower taxonomic resolution. After 13 consecutive years of sampling, a methodological adjustment of this nature raises concern about the utility of previous data and a potential risk to ABMI's ability to monitor changes in the aquatic invertebrate community over time. Thus, more work is needed to fully evaluate the two methodologies for monitoring aquatic invertebrate communities.

We compared the CTA and TSA collection methods for monitoring aquatic invertebrates across 26 wetlands in Alberta using both field and laboratory protocols outlined by each method. Our objectives were to: (1) determine if the similarities in family-level aquatic invertebrate communities are consistent at lower taxonomic levels; (2) examine the influence of differing laboratory processing protocols between the CTA and TSA methods in addition to field sampling methods; and (3) evaluate cost savings such as those presented in Hanisch *et al.* (2020).

Methods

Study Area and Wetland Characteristics

In this study, we selected twenty-six wetlands located in northern Alberta, with twenty-four wetlands situated in the Boreal Natural Region and two wetlands in the Canadian Shield Natural Region (Figure 1). Study sites were selected from a pool of established ABMI wetlands scheduled to be sampled in 2018. Selected wetlands followed the selection criteria outlined in ABMI field protocols and only included sites with permanent open water >0.5 m deep (ABMI 2018). Twenty-two of the wetlands were natural in origin and four were constructed wetlands, ranging from small dugouts to construction borrow-pits. Aside from an additional block of wetlands near Peace River, study wetlands followed a similar distribution pattern and origin to those used in Hanisch *et al.* (2020).

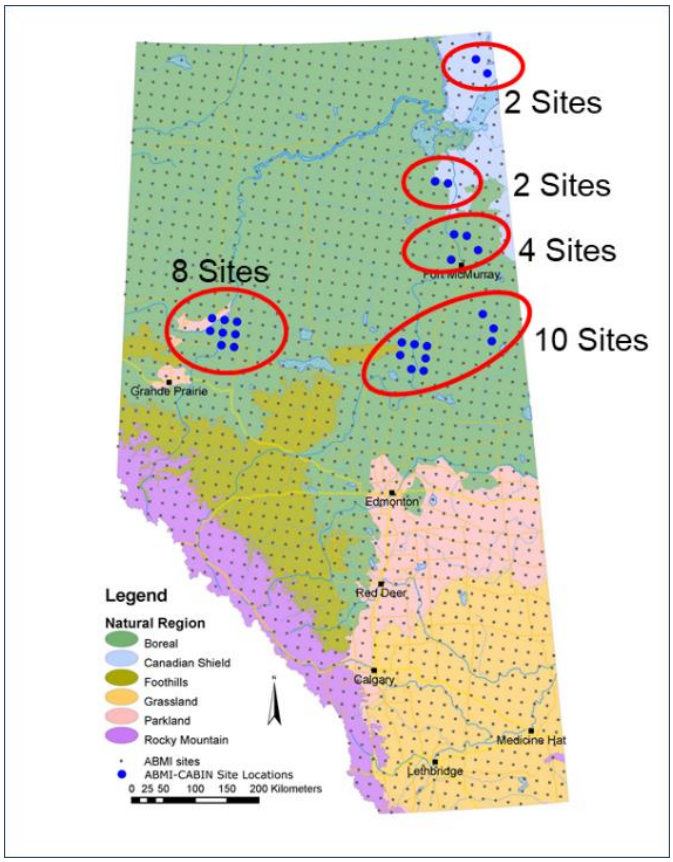

Figure 1 - Map of Alberta showing ABMI study wetland locations (blue dots, n = 26).

Habitat characteristics were assessed at each wetland using ABMI field protocols (ABMI 2018). A bathymetric profile for each wetland was produced based on depth measurements along three intersecting transects (ABMI rotation 1 data). Water temperature, dissolved oxygen, pH, salinity, and specific conductivity were measured at the three deepest open water points using a Hydrolab® multi-probe data sonde. Water samples were collected to test for total nitrogen, total phosphorus, and dissolved organic carbon. Total open water area and percent human disturbance within a 500 m buffer from the open water edge were delineated using 2018 SPOT satellite imagery and GIS datasets in ABMI's landcover and human footprint inventory. Wetland characteristics are summarized in Table 2.

Table 2 - List of wetlands sampled in this study, including public locations and general wetland characteristics (*indicates constructed wetland).

Site Code	Public Latitude	Public Longitude	Open Water Area (ha)	Average Depth (m)	Maximum Depth (m)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Specific Conductivity (µS/cm)	Human Footprint (%)
W19	59.60769	-110.41946	4.10	1.66	2.20	20.34	10.49	7.70	107.3	0.0
W39	59.39515	-110.22257	9.83	1.80	2.60	19.60	3.50	7.24	66.7	0.0
W298	57.97447	-111.85771	2.27	1.42	2.00	19.24	1.61	7.36	46.0	0.1
W299	57.91455	-111.57427	5.89	1.16	1.50	22.15	2.24	8.77	23.7	0.0
W418	57.19672	-111.56298	5.01	4.76	14.50	17.63	8.53	8.20	437.0	100.0
W419	57.15070	-111.22212	1.03	1.85	2.20	15.01	7.68	7.00	99.3	11.3
W450	56.90997	-110.96353	4.83	1.01	1.30	18.11	3.56	6.40	39.0	1.7
W478	56.84834	-111.66765	9.48	1.55	1.90	16.87	7.81	7.18	97.7	3.5
W605	56.00462	-110.94727	1.30	0.85	1.50	18.23	5.70	6.01	94.7	7.3
W638	55.78121	-110.72961	8.14	1.36	1.70	17.79	7.11	7.16	110.7	30.0
W670	55.62075	-110.72844	1.74	3.80	6.40	20.19	7.81	7.36	85.7	6.9
W679*	56.11992	-117.76007	1.56	5.00	7.90	14.83	7.91	7.55	192.3	95.8
W680	56.06201	-117.44726	0.53	1.85	3.90	17.07	4.02	8.07	2036.7	30.2
W681	56.05672	-117.07112	9.68	0.82	1.10	16.41	10.66	8.45	402.3	49.8
W694	55.67258	-113.04023	9.96	1.19	1.90	19.29	6.03	7.10	199.3	1.8
W695	55.67706	-112.73427	1.41	1.12	1.50	21.37	3.67	5.99	74.7	29.2
W696	55.65383	-112.42339	1.19	1.85	2.20	16.98	2.37	6.33	206.0	40.3
W710	55.93480	-117.79677	1.11	0.62	0.70	19.93	2.71	7.07	561.0	4.6
W711*	55.87299	-117.45109	0.09	3.06	4.90	18.74	8.94	8.32	230.7	96.5
W712	55.85571	-117.15543	21.22	0.87	1.00	21.71	2.71	7.95	921.7	40.1
W725	55.51790	-113.06996	1.94	1.20	1.70	19.00	4.67	6.48	133.0	2.0
W727*	55.44312	-112.50106	1.04	2.89	4.70	20.80	8.21	7.60	1289.3	30.7
W742	55.71991	-117.47964	0.62	1.47	2.40	15.75	0.25	7.09	646.0	4.7
W743*	55.69505	-117.22521	0.39	1.27	2.00	20.29	13.40	9.24	779.0	96.6
W757	55.32301	-112.86432	10.06	1.35	1.70	23.10	7.79	8.04	244.3	6.0
W758	55.27935	-112.54231	1.54	0.98	1.70	24.01	9.27	7.95	128.3	1.6

The average depth of wetlands used in this study ranged from 0.6 to 5.0 m, with maximum depths ranging from 0.7 to 14.5 m. The largest wetland had an open water area of 21.2 ha; all other wetlands were 10 ha or less in size (Table 2). Both the ABMI study and the EMSD study included wetlands with a similar mix of characteristics, and although the EMSD study included two larger wetlands (~59 ha each) and an outlier at 1434 ha, there was no significant difference in wetland size, depth, and water chemistry parameters between the two studies (Table 3).

Table 3 - Comparison of average wetland characteristics (± SE) between the current ABMI study (n=26) and the 2017 EMSD study (n=16, EMSD data from Hanisch *et al.* 2020).

	Open Water Area (ha)	Average Depth (m)	Maximum Depth (m)	Temperature (°C)	Dissolved Oxygen (mg/L)	pH	Specific Conductivity (µS/cm)	Human Footprint (%)
ABMI Study	4.46 ± 0.97	1.80 ± 0.23	2.97 ± 0.57	19.02 ± 0.47	6.10 ± 0.64	7.45 ± 0.16	355.9 ± 91.1	26.57 ± 6.70
EMSD Study	12.11 ± 5.23*	1.62 ± 0.29	3.04 ± 0.64	20.16 ± 0.56	6.53 ± 0.47	7.80 ± 0.25	284.4 ± 52.0	19.81 ± 5.02
p-value	0.171	0.636	0.928	0.124	0.593	0.240	0.500	0.425

** Outlier with open water area of 1434 ha removed from this calculation*

Field Methods

Aquatic invertebrate samples were collected in July as part of the regular 2018 ABMI field season. Field technicians received training on both CTA and TSA protocols prior to being deployed in the field. Each wetland was sampled using both CTA and TSA protocols on the same day, with two wetlands (W478 and W638) being sampled twice using each protocol on the same day, for a total of 28 paired samples. The CTA aquatic invertebrate sampling followed the layout as defined by the protocols, proceeding in a clockwise direction from the first transect (ABMI 2018). The TSA sampling layout was placed at least 50 m counterclockwise from the first CTA transect and continued in a counterclockwise direction (Figure 2). Additional layouts for wetlands that were sampled twice using each protocol were placed an additional 50 m clockwise or counterclockwise along the shoreline.

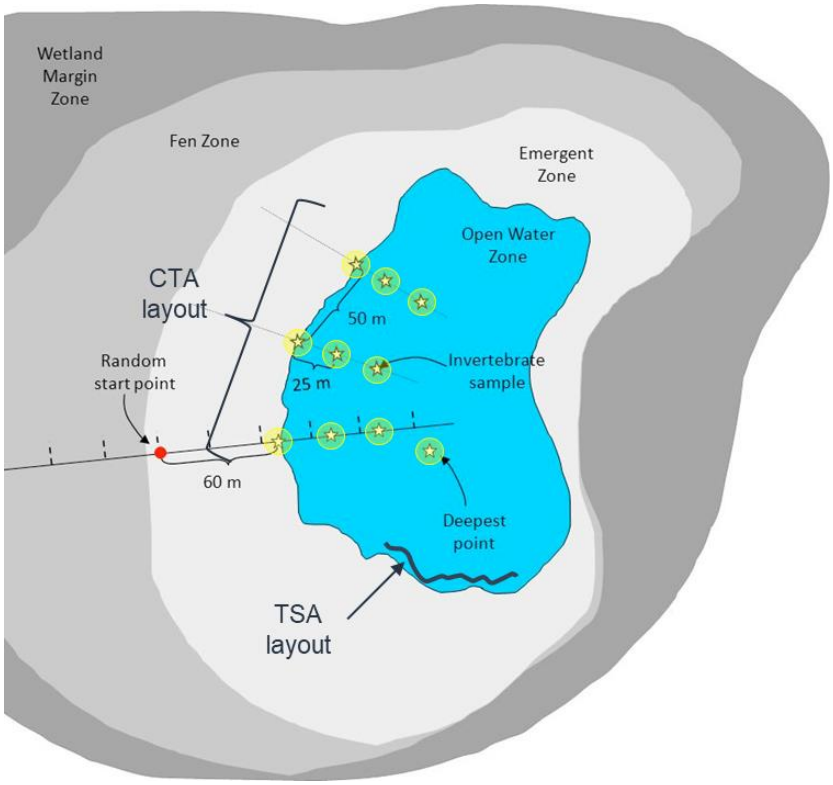

Figure 2 - Diagram of the CTA aquatic invertebrate sampling layout and the general placement of the TSA net sweep layout.

CTA Aquatic Invertebrate Sampling

Three transects were spaced every 50 m along the wetland edge extending perpendicular from the shoreline (Figure 2). Three sample points were established along each transect with one near the open water edge in at least 0.25 m of water, and two more at 25 m and 50 m intervals. A tenth point was located at the deepest point in the wetland. Each sample point was surveyed within a 10 m radius to find an area with at least 50% aquatic vegetation cover. If aquatic vegetation was sparse, the sample was collected in the area of highest vegetation cover. An inflatable boat was needed to navigate between sample points and access points in deeper water.

To collect the sample, a 500 µm net was quickly inserted into the water column at a 45-degree angle and rapidly drawn up through the aquatic vegetation three times in quick succession. If the water was less than 1 m deep, the entire water column, from just above the substrate to the water's surface, was sampled. If the water was greater than 1 m deep, only the top 1 m was sampled. Field technicians avoided digging the net into the mud covering the bottom, but stirring up unconsolidated organic debris resting on the bottom was permissible.

If the net contained large amounts of aquatic vegetation, larger pieces of vegetation were agitated in the net to dislodge any clinging organisms. The "washed" vegetation was inspected to make sure nothing was still attached before being discarded back into the wetland. Each sample was washed just enough so that the contents of the net would fit in a 1 L sample bottle. If the amount of aquatic vegetation was low, the contents of several net sweeps were combined into one bottle.

TSA Aquatic Invertebrate Sampling

Starting 50 m counterclockwise from the first CTA transect, the open water habitat along the shoreline was surveyed to find an area that was representative of the most common near-shore wetland habitat at least 0.25 m deep and having greater than 50% aquatic vegetation cover. If aquatic vegetation was sparse, the area of highest vegetation cover was selected. The technician then waded out to the start of the target area to begin collecting. If the wetland was deemed too dangerous to sample while wading (*e.g.* steep drop-off or very soft bottom), and sampling from the wetland edge was not possible due to shoreline structure (*e.g.* dense vegetation or floating mats), the sample was collected from a boat with one person paddling while the other technician collected the sample.

To collect the sample, the technician began a two minute timed net sweep using a 400 µm net. As the technician slowly moved forward, more or less parallel to the shoreline, the net was swept through the vegetation side to side and up and down to cover as much of the water column and area as possible. Field technicians avoided

digging the net into the mud covering the bottom, but tapping the net gently on the bottom and stirring up unconsolidated organic debris was permissible.

If the net became too full of aquatic vegetation before the timed net sweep was completed, the timer was paused while the technician transferred the net contents to a sample bottle. As with the CTA protocol, larger pieces of vegetation were agitated in the net to dislodge any clinging organisms. The "washed" vegetation was inspected to make sure nothing was still attached before being discarded back into the wetland. At each paused net cleaning, the technician washed and discarded just enough vegetation so that the contents of the net would fit in a 1 L sample bottle. If the amount of aquatic vegetation in the net was low, the contents of the entire timed net sweep could be collected in one bottle.

For both protocols, the amount of time needed to assess and establish sampling areas, collect the samples, and package the samples was recorded to the nearest minute for each protocol. Samples were not individually weighed since all sample bottles were filled to capacity to prevent sloshing and subsequent specimen damage during transport, meaning all sample bottles weighed roughly the same (~1.1 kg each). Table 4 summarizes the main differences between the two collection protocols.

Table 4 - Summary of the main differences between the CTA aquatic invertebrate collection protocol and the TSA protocol.

CTA Protocol	TSA Protocol
• Ten net sweeps collected on a grid pattern. • Samples collected along the shoreline and farther out into open (sometimes deeper) water. • Net sweeps target aquatic vegetation within a 10 m radius, habitat types may vary. • Samples collected from a boat. • Samples collected using 500 µm net.	• One continuous timed net sweep (2 minutes). • Sample collected along the shoreline in areas that can be accessed by wading. • Net sweep path targets aquatic vegetation that is considered representative of the wetland. • Sample collected while wading. • Samples collected using 400 µm net.

Once back at the vehicle, both CTA and TSA samples were preserved by straining off enough water to allow for the addition of at least 250 ml of 10% buffered formalin to each sample bottle. CTA samples were strained using 500 µm Nitex® mesh and TSA samples with 355 µm Nitex® mesh (400 µm mesh was not available from the supplier).

Lab Methods

Sample Processing

Aquatic invertebrate samples used for this study were processed by trained technicians in the ABMI sorting lab alongside, and interspersed with, regular 2018 ABMI field samples. Although the order of samples being sorted were not explicitly randomized, they were interspersed enough to minimize bias that can be introduced as technicians sort more samples and gain experience. We followed the ABMI (ABMI 2015) and CABIN (Environment Canada 2014) lab protocols to process and sort respective samples. Both CTA and TSA samples were subject to the same QA/QC procedures as outlined in the ABMI lab protocols.

Prior to being sorted, sample bottles from each wetland collected under each protocol were combined to form a composite sample for each protocol and site. Each composite sample was cleaned of excess vegetation using ABMI's elutriation process (ABMI 2015). CTA samples were cleaned to a final mesh size of 500 µm, TSA samples used a final mesh size of 355 µm (400 µm sieves were not available). Random subsamples of the excess vegetation were inspected for retained organisms prior to the cleaned sample moving on to the sorting phase. All samples met the QA/QC minimum 95% threshold for organisms passing through to the sorting phase on the first elutriation run.

The cleaned sample was placed in a 100-cell Marchant box. Samples were subsampled by randomly selecting a cell in the Marchant box and extracting the contents for sorting. Marchant box cells were sorted until a specific fixed count was reached, at which point the sorting was terminated upon completion of the current cell.

CTA samples were sorted until a fixed count of 350 primary organisms was reached (primary organisms exclude zooplankton). If the sample was likely to contain less than two times the fixed count (*i.e.* 700 organisms), the entire sample was sorted. For samples where organisms were prolific, a minimum of 3 cells were sorted regardless of the final count reached.

TSA samples were sorted to a fixed count of 300 primary organisms, with sparse samples containing less than 600 organisms being sorted in their entirety. Zooplankton was also sorted under CABIN protocols but because zooplankton can be very prolific in some samples, they are not included in the primary organism fixed count. If the count for any one taxonomic group reached 1000(usually zooplankton), sorting for that group was terminated at the completion of the current Marchant box cell, while the sorting of other groups continued. Prolific TSA samples were sorted to a minimum of 5 cells or maximum 2500 total organisms, whichever came first.

In-process QA/QC was used to monitor the efficacy of all samples sorted. The remaining residual from sorted cells, and the sorted organisms, were periodically inspected, both randomly during sorting and at the completion of each sample. Of the 56 samples sorted, only one failed to meet the <5% residual threshold. But in-process corrections, and resorting of cells not meeting the threshold, ensured that all sorted sites met the residual threshold upon completion. Organism sorting accuracy was high with an average of 0.24 errors per sample.

For both CTA and TSA samples, a 2-minute unique/mature organism search was completed on any unsorted material remaining in the Marchant box at the conclusion of the sort. The total time needed to process, sort, and label the samples was recorded to the nearest 15-minute interval.

Advanced Identification

Identification of sorted specimens was conducted at the ABMI Processing Centre at the Royal Alberta Museum. All primary organisms were identified to the lowest feasible taxonomic level (LFTL) by experienced taxonomists certified by the Society for Freshwater Science (SFS). Final identifications were reviewed and confirmed by at least one additional taxonomist.

Final specimen identifications were organized into a dataset that included all taxa regardless of the final taxonomic level reached. This complete ("all taxa") dataset was subsequently aggregated into four levels of identification which were the coarse group, family, genus, and species levels. Because some genus-only identifications had a high likelihood of including at least one unique species, genus-only identifications were included in the species matrix as species complexes. Each dataset included the original coarse sorting counts, original counts standardized to 100 Marchant box cells sorted (total abundance/adjusted-counts), original counts relative to the total sorting count in the sample (relative abundance), catch-per-unit-effort by time (CPUE-T), and catch-per-unit-effort by number of bottles collected (CPUE-B). Data were then reformatted as required for different analyses.

Statistical Methods

Diversity and Abundance

We examined differences in aquatic invertebrate species diversity and abundance between the CTA and TSA protocols using three complimentary methods. First, we compared the total number of distinct species (or other taxonomic units) and rate of species discovery with increased sampling effort by using species accumulation curves (SAC) using sites selected randomly from the sample pool (Ugland *et al.*,

2003). Second, we compared composite measures of species diversity (Shannon Index, Simpsons Index, species richness) and abundance (as defined below). For all three measures of diversity, we performed paired-sampled t-tests and a Pearson's correlation. We also used Wilcoxon Signed Rank tests to assess differences in abundance between protocols. Third, we examined whether difference of species abundance between the two protocols may cause differences in the relationship of abundance to putative habitat characteristics. For species with at least ten detections using either CTA or TSA protocols, we modeled log abundance as a function of the number of deep samples collected, habitat complexity, mean temperature, mean dissolve oxygen, mean salinity, mean pH, dissolved phosphorous, dissolved organic carbon, maximum depth, percent open water, and percent human footprint. All covariates and measures of abundance were *z*-transformed to standardize coefficient estimates for data based on each protocol.

Community Composition Structure

We used permutational multivariate ANOVA (PERMANOVA; Anderson, 2001) to assess for significant differences in invertebrate community composition between the CTA and TSA protocols. Community composition difference was represented by a Bray–Curtis dissimilarity matrix calculated using each type of abundance data as defined below. A significant result obtained with PERMANOVA could indicate that the centroid of the two protocols differ in their location in multivariate space. It is likely also the two protocols can differ in their dispersion from their respective group centroid. We tested for differences in within-group dispersion by means of a permutational analysis of multivariate homogeneity of group dispersions (PERMDISP; Anderson *et al.*, 2006). In addition to the overall composition differences between CTA and TSA protocols, we also assessed how communities vary among samples collected at different depths. Sampling events were categorized based on the number samples collected in the deep zone (>1 m deep) as follows: CTA_DEEP (>5 samples), CTA_MEDIUM (4-5 samples), CTA_SHALLOW (<4 samples), and TSA_SHALLOW (<4 samples).

Both PERMANOVA and PERMDISP assess the overall difference in composition structure between the two protocols (or depth classes) but are mute about how the composition differs for a given site differ between the two protocols. We compared the match between homologous sites (points) of the two dissimilarity matrices in multidimensional space Procrustes analysis. The pairwise composition dissimilarity matrices were first summarized in a lower dimensional space using non-metric multidimensional scaling (NMDS). For the NMDS, we selected the optimal number of dimensions as the one with stress value less than 0.2. Procrustes analysis uses a rotational-fit algorithm where one configuration is rotated, translated, and scaled to maximally fit (minimum distance between homologous sites) into another (target) configuration. The distance between homologous points can provide information of sites where difference of composition by the two protocols can be relatively higher. The overall pairwise distance between homologous points is summarized into a correlation-like statistic that measures the agreement between homologous points

in the two ordination, and its statistical significance is tested using a Procrustean randomization test (PROTEST, Jackson, 1995).

Each statistical test was performed at four levels of taxonomic resolution (coarse groups, family, genus, and species) using four types of abundance data (adjusted-counts, relative abundance, catch-per-unit-effort of time, and catch-per-unit-effort of bottles). Only adjusted-counts and relative abundance data was used for assessing communities at the species and genus level. All data cleaning, statistical analyses, and visualizations were performed using R v4.0.4 (R Core Team 2021) and the following packages: *vegan*, *usdm*, *grid*, *ggplot2*, and *abmi.themes*.

Results

Sample Collection Effort

Six of the 28 TSA samples required the use of a boat to collect, as the wetlands were deemed too dangerous to sample while wading (steep drop-off or very soft bottom), and shoreline sampling was not possible due to shoreline structure (dense vegetation or floating vegetation mats).

CTA samples required significantly more time to collect in the field than TSA samples, but there was no significant difference in resources required (Table 5). In collecting the CTA samples, a total of 65 bottles and 16.25 L of formalin were used; TSA samples used a total of 50 bottles and 12.5 L of formalin. The average cost of sample bottles used per site was similar (CTA = $9.86; TSA = $7.57), as was the average cost of preservative per site (CTA = $9.09; TSA = $6.98). There are notable differences in our results compared to the EMSD study (Table 5, EMSD data from Hanisch *et al.* 2020).

Table 5 - Comparison of average sample collecting time and sampling resources required (± SE) between the 2018 ABMI study and the 2017 EMSD study (EMSD data from Hanisch *et al.* 2020, significant differences shown in bold).

	Average Sampling Time/Site (minutes)			Average Number of Sample Bottles/Site			Total Cost of Bottles		Total Cost of Preservative	
	CTA	TSA	p-value	CTA	TSA	p-value	CTA	TSA	CTA	TSA
ABMI Study	**67.8 ± 4.4**	22.3 ± 1.5	**< 0.001**	2.32 ± 0.33	1.79 ± 0.28	0.130	$274.95	$211.58	$63.32	$48.71
EMSD Study	**103.8 ± 7.4**	86.3* ± 8.5	**< 0.05**	**5.3 ± 0.9**	0.8 ± 0.07	**< 0.05**	$901.38	$207.06	$387.67	$348.50

*Represents the total time to complete full CABIN wetland assessment

Sample Processing Effort

The average time required to process aquatic invertebrate samples in the lab (± SE) showed a significant difference between collection methods. On average, CTA samples were processed and sorted in 7.02 ± 0.81 hours while TSA samples required 13.02 ± 1.23 hours ($p = 0.001$). The time required to run the elutriation process was similar between collection methods (CTA = 0.85 ± 0.13 hours; TSA = 0.93 ± 0.17 hours; $p = 0.675$) indicating it was the actual sorting time where significant differences were seen. It is likely that this difference is due to the sorting of zooplankton from the TSA samples. These results are opposite of what was reported in the EMSD study where CTA samples required 11.1 ± 0.9 hours to sort while TSA samples took 5.9 ± 0.4 hours ($p < 0.05$)(Hanisch *et al.* 2020).

Advanced Identification and Abundance Measures

Advanced identification provided a complex set of data on multiple taxonomic levels. The number of taxa identified by each protocol and at each taxonomic level are summarized in Table 6. Also summarized are the number of rare taxa (those with less than three occurrences across all samples) and the number of taxa unique to a protocol. TSA protocols collected a significantly greater number of unique taxa per sample at the family level (CTA = 0.071 ± 0.050, TSA = 0.821 ± 0.186, $p < 0.001$) and genus levels (CTA = 0.607 ± 0.119, TSA = 1.429 ± 0.274, $p = 0.014$), but not at the species level (CTA = 2.571 ± 0.297, TSA = 3.464 ± 0.492, $p = 0.149$).

Table 6: A summary of the total taxa, rare taxa (< 3 occurrences), and unique taxa (detected by one method only) identified by each protocol at each taxonomic level.

	Total Taxa			Rare Taxa			Unique Taxa	
	CTA	TSA	Both	CTA	TSA	Both	CTA	TSA
All Taxa Combined	295	308	363	149	157	137	55	68
Species	257	267	316	137	144	122	49	59
Genus	124	139	150	51	63	46	11	26
Family	56	66	67	14	21	12	1	11
Coarse Group	18	19	19	1	2	1	0	1

There were significant differences in total number of individual organisms collected between the two protocols, both in terms of total abundance and catch-per-unit-effort. On average, TSA protocols collected a greater number of organisms overall (total abundance), per minute of collecting time (CPUE-T), and per sample bottle collected (CPUE-B) (Table 7). The same trend seen at the coarse and family levels carried through to genus and species (data not shown). While the EMSD study showed a similar result for CPUE, their results for total abundance are opposite of our results (Hanisch *et al.* 2020).

Table 7 - Summary comparing the average total abundance, catch-per-unit-effort by time in minutes, and catch-per-unit-effort by number of sample bottles used between CTA and TSA protocols (± SE), at the coarse group and family level, with a comparison to EMSD family data (EMSD data from Hanisch *et al.* 2020)

Taxonomic Level	Total Abundance (total number of specimens collected)			CPUE-T (specimens collected/collecting time)			CPUE-B/W (specimens collected /sample bottles or weight)		
	CTA	TSA	p value	CTA	TSA	p value	CTA	TSA	p value
Coarse Group	2100.97 ± 429.28	**4397.16 ± 848.78**	**< 0.001**	32.21 ± 5.96	**209.16 ± 43.27**	**< 0.001**	981.8 ± 211.22	**2518.46 ± 381.91**	**< 0.001**
Family (current study)	2006.18 ± 414.56	**4211.04 ± 826.94**	**< 0.001**	29.68 ± 5.62	**187.19 ± 37.56**	**< 0.001**	903.53 ± 197.62	**2259.39 ± 333.71**	**< 0.001**
Family (EMSD study)	**13,077.2 ± 4381.2**	5568.4 ± 978.2	**0.034**	NA	NA	NA	1952.2 ± 415.8	**5909.8 ± 1060.3**	**0.001**

** EMSD used specimens collected/sample weight*

Diversity and Abundance

Across the 28 sites, SACs by site established that 267 species were identified using the TSA protocol while 257 species were identified using the CTA protocol (Figure 3). The two SACs were highly correlated (0.999), with overlapping confidence intervals up until 24 sites were sampled. There were no significant differences in mean richness ($p = 0.56$; Pearson correlation = 0.67; Figure 4), Shannon diversity ($p = 0.18$; Pearson correlation = 0.69; Figure 5), or Simpson diversity ($p = 0.14$; Pearson correlation = 0.44; Figure 6) between protocols. Comparison of species abundance (using adjusted-counts) between protocols indicated that, 22 of the 315 species showed significant differences between protocols (Table 8; Appendix A).

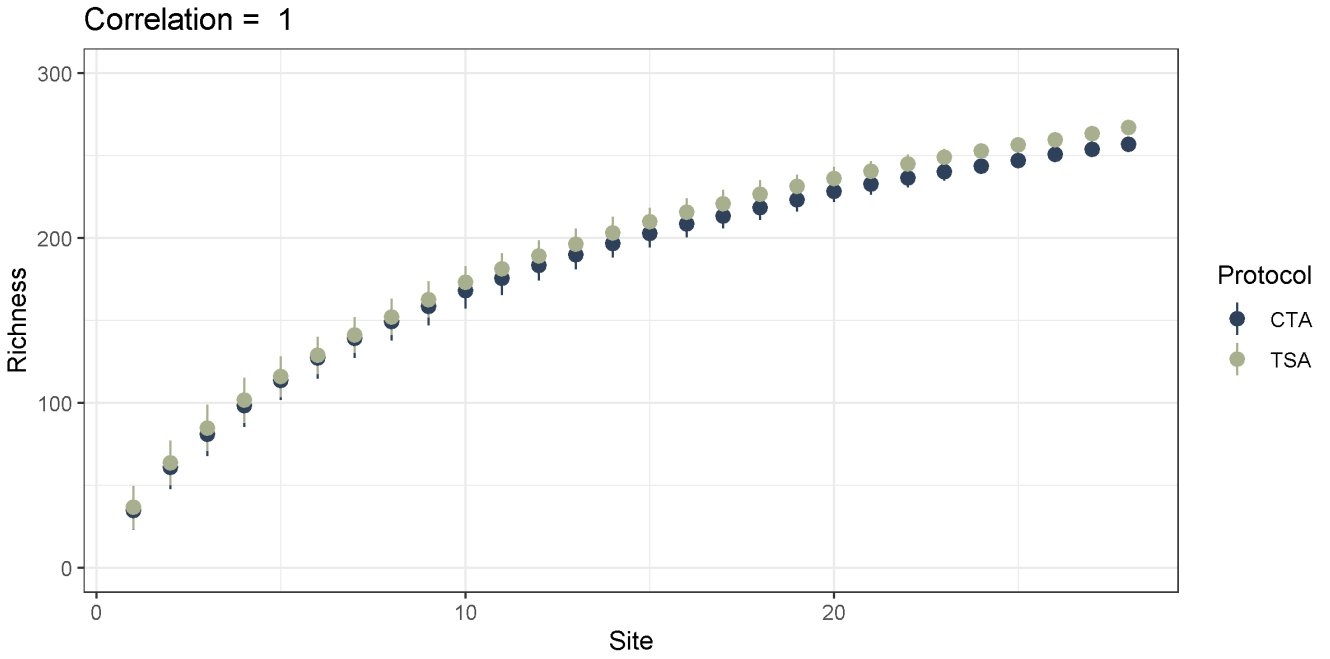

Figure 3 - Species accumulation curves for the CTA (dark) and TSA (light) protocols. Each curve was created using random site selection procedure, with the CTA protocol having a maximum richness value of 257 species and the TSA protocol having a maximum of 267 species.

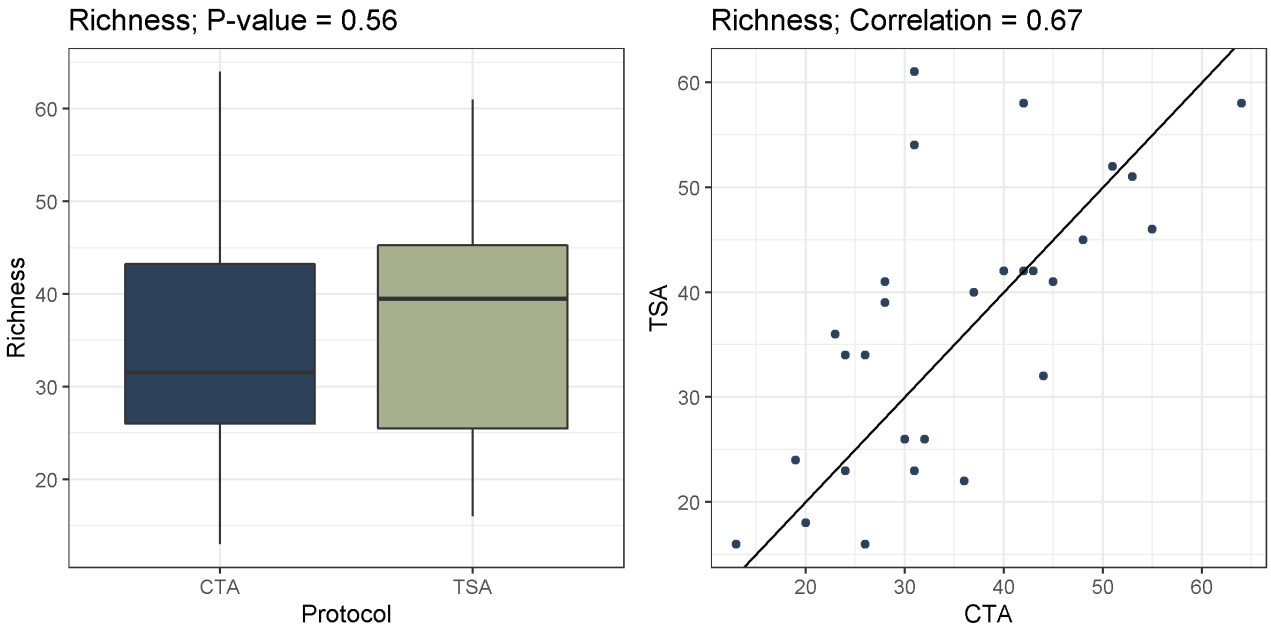

Figure 4 - Boxplot of total species richness illustrating no significant differences between protocols (paired t-test, p = 0.56; left). The Pearson's correlation coefficient (0.67) of total species richness (right).

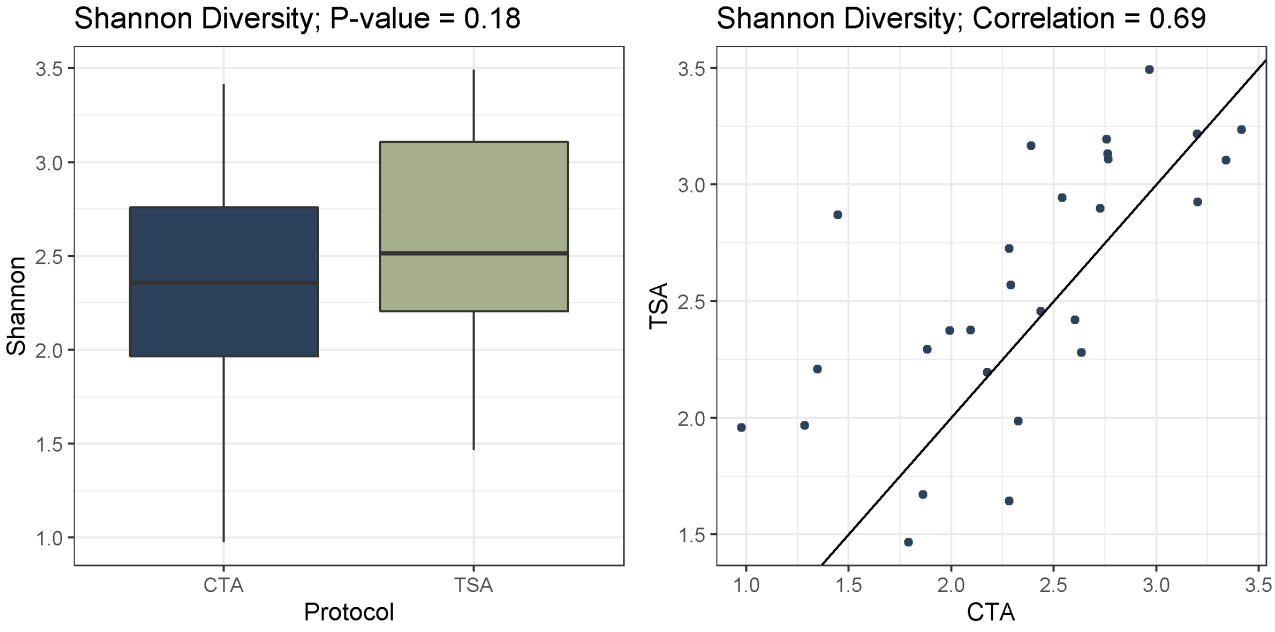

Figure 5 - Boxplot of Shannon diversity illustrating no significant differences between protocols (paired t-test, p = 0.18; left). The Pearson's correlation coefficient (0.69) of Shannon diversity (right).

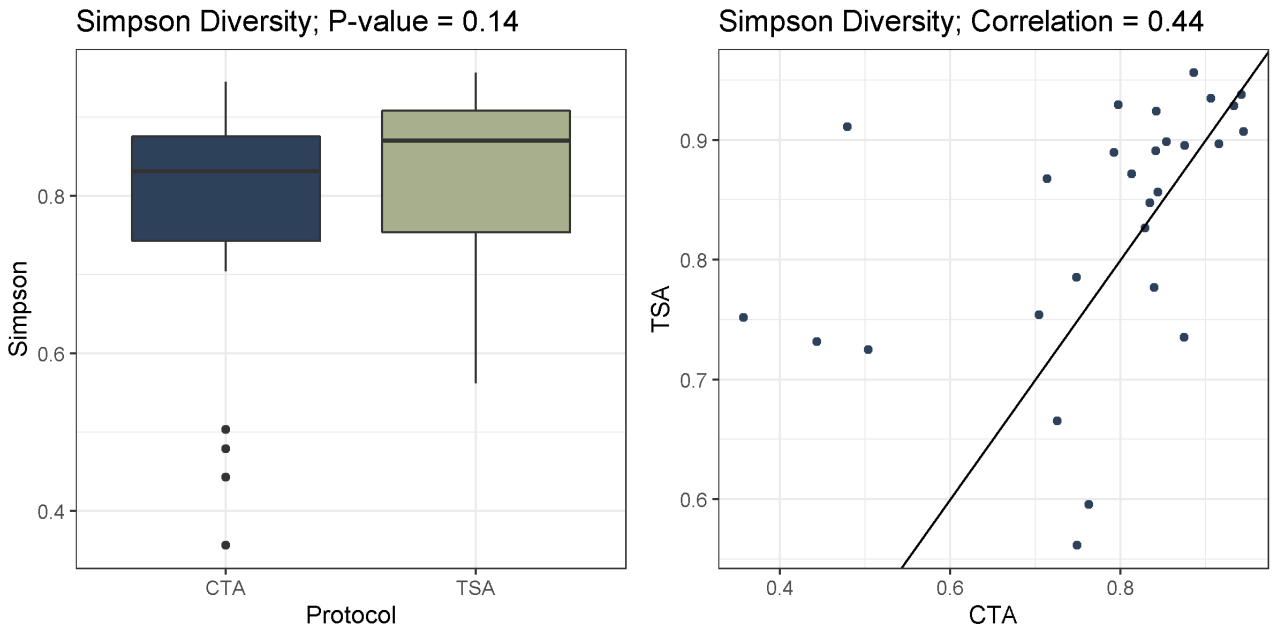

Figure 6 - Boxplot of Simpson diversity illustrating no significant differences between protocols (paired t-test, p = 0.14; left). The Pearson's correlation coefficient (0.44) of Simpson diversity (right).

Table 8 - Mean, standard deviation, and Wilcoxon Rank Sign tests using adjusted-counts for all species that exhibited significant differences (α = 0.05) between protocols.

Species	*TSA*	*CTA*	*P-value*
Ablabesmyia sp. D RPH	0.1(0.6)	1.8(4.2)	0.034
Bezzia/Palpomyia/Probezzia sp.	64.6(128.1)	10.0(30.5)	0.003
Chaetogaster cristallinus/diaphanus	70.1(122.7)	5.1(11.6)	0.004
Chaetogaster sp.	7.7(21.3)	0(0)	0.036
Corynoneura sp. C RPH	10.8(14.9)	1.9(4.7)	0.002
Cricotopus sp.	37.7(75.6)	9.9(24.0)	0.04
Dicrotendipes sp. B RPH	17.3(32.7)	1.4(3.8)	0.014
Graphoderus perplexus	6.4(12.1)	0.6(2.4)	0.029
Hydrozetes sp.	34.4(73.4)	0.9(4.7)	0.003
Labrundinia sp.	5.1(15.9)	0(0)	0.036
"Nais communis" complex	169.5(277.4)	28.8(41.2)	0.003
Nais simplex	137.5(412.4)	5.4(12.4)	0.012
Nanocladius sp. A RPH	21.4(68.8)	3.5(10.5)	0.042
Paratanytarsus sp. B RPH	48.3(73.7)	24.1(34.9)	0.049
Paratanytarsus sp.	19.7(35.0)	3.9(11.9)	0.029
Procladius sp.	5.9(11.6)	1.6(6.9)	0.044
Psectrocladius sp.	60.9(144.5)	5.1(11.9)	0.004
Psectrocladius sp. D RPH	51.4(115.7)	3.9(9.6)	0.014
Psectrocladius sp. I RPH	6.1(13.0)	0.7(2.2)	0.03
Tanytarsus sp. C RPH	32.1(120.6)	2.6(7.2)	0.033
Tanytarsus sp. D RPH	18.4(41.6)	2.8(6.2)	0.033
Tanytarsus sp.	22.2(37.8)	2.4(4.9)	0.009

We identified fourteen species with sufficient detections to use linear regressions to model the relationships between abundance and environmental variables using both the ABMI and CABIN protocols. For these species, Pearson correlation of standard coefficients of the adjusted-counts ranged between -0.06 - 0.89 with the majority of coefficient estimates overlapping between the two protocols (Table 9, Appendix B). In addition, species such as Bezzia/Palpomyia/Probezzia sp., which exhibited significant differences in abundances between protocols, did not have differences in model coefficients (Appendix B).

Table 9 - The number of species with overlapping coefficient estimates for each covariate used in the model.

Variable	*Species (14)*	*Genus (22)*	*Family (16)*	*Coarse (15)*
Human Footprint (%)	14	20	14	14
Open Water (%)	14	22	16	14
Maximum Depth	13	20	14	13
DOC	14	21	14	13
DP	13	20	13	12
pH	11	20	12	14
Salinity	14	19	14	13
DO	12	21	14	14
Temperature	13	21	14	14
Habitat Complexity	14	21	14	12
Deep Samples	12	19	13	13

Community Composition Structure

The original species community matrix was simplified to a 3-axis NMDS solution (stress = 0.189). The two protocols have overlapping community structures (Figure 7), with no significant differences in the centroid (PERMANOVA; F = 1.38, p-value = 0.076) and dispersion (PERMADISP; F = 0.24, p-value = 0.625). In addition, the shapes of the ordination space were significantly correlated (Procrustes; correlation = 0.84, p-value = 0.001). There were also no significant differences between protocols for along sampling depth (Figure 8; PERMANOVA; F = 1.258, p-value = 0.054).

All statistical tests performed at the four taxonomic resolutions and four types of abundance data show similar results to the species-level adjusted count data presented. A summary of results can be found in Table 10, while detailed results for each statistical test can be found in the supplementary data file (appendix-tables_2021-03-17). When assessing differences in abundance between protocols, we found the majority of taxonomic groups exhibited no significant differences. Therefore, we only created figures when we observed a significant result.

Table 10 - Summary table describing the significance difference (α = 0.01) between CTA and TSA samples results for each combination of taxonomy and abundance data (- = non-significant difference; + = significant difference).

Abundance	*Taxonomy*	*Richness*	*Shannon*	*Simpson*	*PERMANOVA*	*PROCRUSTES*	*PERMADISP*	*PERMANOVA (Depth)*
Adjusted	Coarse	-	-	-	-	-	-	-
	Family	-	-	-	-	-	-	-
	Genus	-	-	-	-	-	-	-
	Species	-	-	-	-	-	-	-
Relative	Coarse	NA	NA	NA	-	-	-	-
	Family	NA	NA	NA	-	-	-	-
	Genus	NA	NA	NA	-	-	-	-
	Species	NA	NA	NA	-	-	-	-
CPUE-T	Coarse	NA	NA	NA	+	-	-	+
	Family	NA	NA	NA	+	-	-	+
	Genus	NA	NA	NA	NA	NA	NA	NA
	Species	NA	NA	NA	NA	NA	NA	NA
CPUE-B	Coarse	NA	NA	NA	+	-	-	+
	Family	NA	NA	NA	+	-	-	+
	Genus	NA	NA	NA	NA	NA	NA	NA
	Species	NA	NA	NA	NA	NA	NA	NA

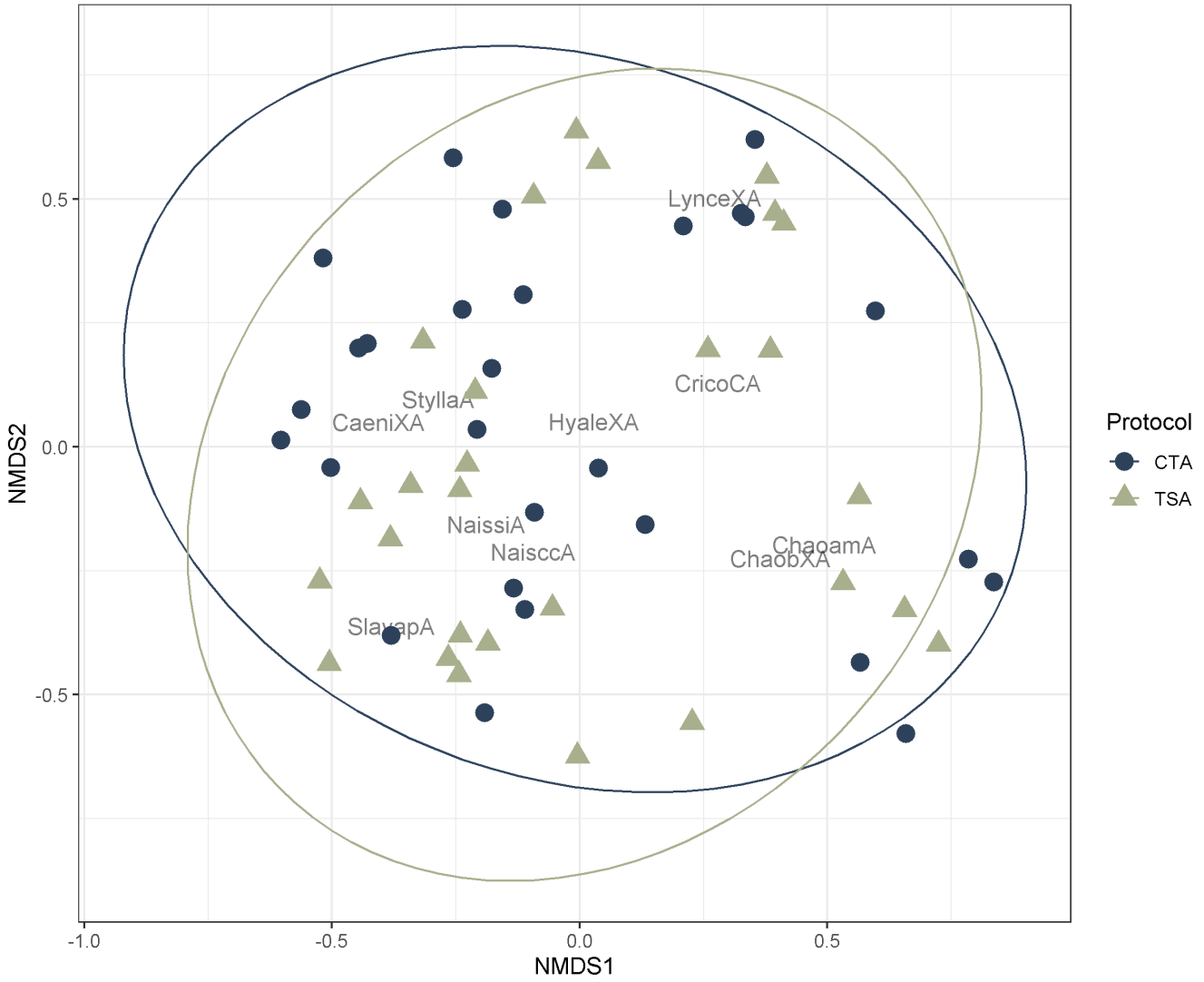

Figure 7 - First two axes for the NMDS ordination of the species community matrix using a Bray-Curtis transformation. Samples were categorized based on their sampling protocol. The ellipses indicate 90% confidence intervals.

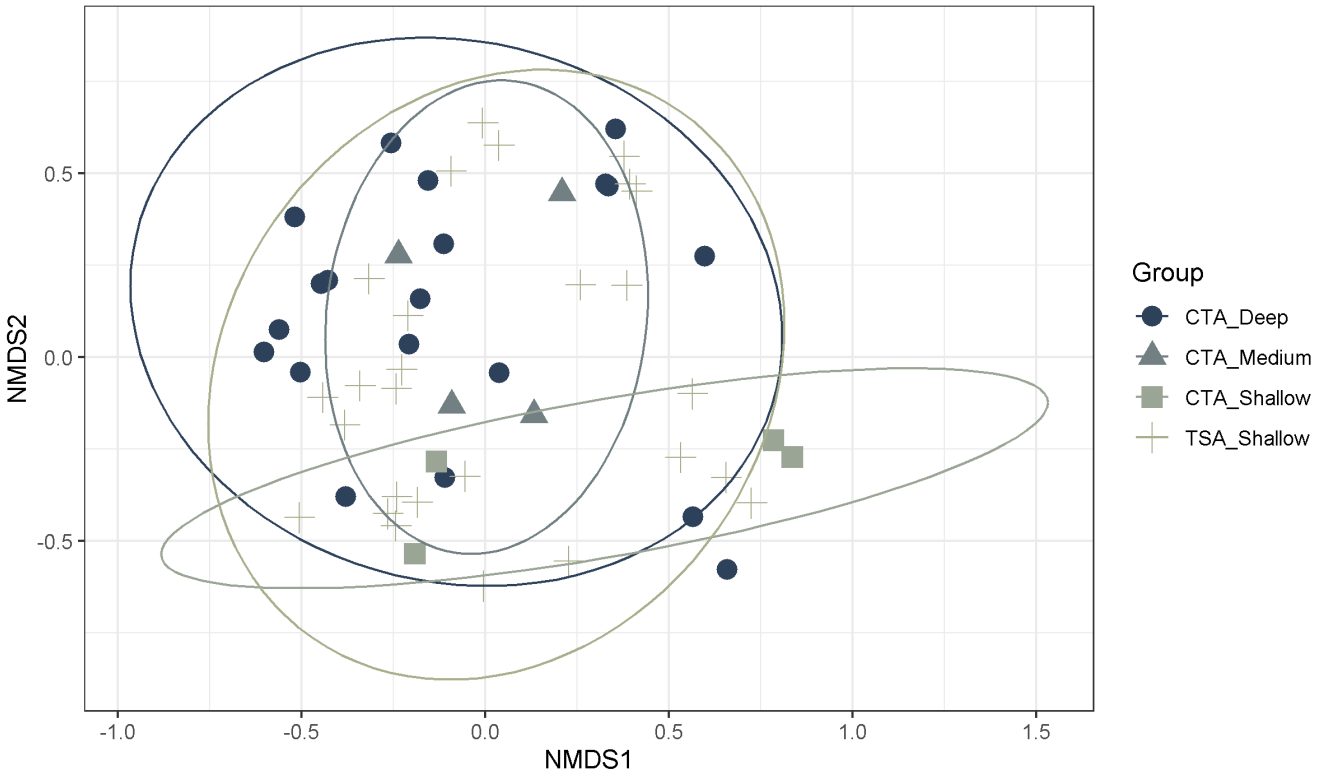

Figure 8 - First two axes for the NMDS ordination of the species community matrix using a Bray-Curtis transformation. Samples were categorized based on their sampling protocol and sample depth. The ellipses indicate 90% confidence intervals.

Discussion

Difference in Field Sample Collection Effort

Some of the cost savings associated with the TSA rapid sampling protocol are grounded on the assumption that wetlands can be sampled while wading or walking along the shoreline (*i.e.*, without the need for a boat). However, in practice, this may not always be practical. During training, field technicians waded into a variety of wetlands with different depths and bottom compositions. One particular wetland was 1.1-1.2 m (*i.e.*, chest) deep along the shoreline, with a moderately soft bottom. Technicians unanimously selected this wetland as possessing the maximum depth and sediment instability for safe collection on foot alone. In most instances, where a boat was used to collect the TSA sample, safety was the primary consideration.

In addition, the use of a boat to implement TSA sampling could be more efficient than wading along wetlands where dense vegetation can impede movement. At one of the repeat sample wetlands (W638), the first TSA sample was collected while wading through dense emergent vegetation along the shoreline, and the second TSA sample was collected using the boat. The first sample took ~40 minutes to collect while the second sample required the usual 20 minutes. With the variety of wetland types and profiles that ABMI samples, it is clear that sampling from a boat will be more efficient for some wetlands.

Investments in field equipment, especially when multiple field crews are deployed in a given season, can be substantial, and the price of inflatable boats represents a large part of that cost. Eliminating the boat from the field equipment list would save money. However, given that the boat is also used to execute other protocols during ABMI sampling, and to access the sampling area when the point of wetland access is far from the start pin, the boat will remain a necessary part of ABMI field gear regardless of the method of aquatic invertebrate sampling used.

Analyses presented in both the current study and Hanisch *et al.* (2020) support the conclusion that total sampling time was significantly higher for CTA collected samples compared to TSA samples. However, our study reported an average of 67.8 minutes to collect the CTA aquatic invertebrate samples, whereas Hanisch *et al.* (2020) reported an average of 103.8 minutes. The time needed to collect CTA samples with experienced field technicians in past years suggests that 60-75 minutes was typical. This is in line with the 67.8 minute average of this study. The higher value reported by Hanisch *et al.* (2020) may have been due to a lack of experience with maneuvering the boat under challenging conditions and establishing the CTA layout. Hanisch *et al.* (2020) reported an average of 86.3 minutes to collect the TSA samples, but this value includes the time needed to complete all CABIN wetland assessment protocols, not just the aquatic invertebrate component. In their

original 2018 presentation, EMSD reported an average of 20 minutes to complete the TSA invertebrate collecting, which is consistent with our finding of an average of 22.3 minutes. Using the same experienced technicians for both protocols, we found that the TSA layout and collection method is much quicker to implement in the field than the CTA, saving on average ~45 minutes per site.

In this study, we found no significant differences in resources required to implement either CTA or TSA protocols. However, historical long-term averages of CTA sampling suggests that over time, more field resources would be required for the CTA method than the TSA method. We reported an average (± SE) of 2.32 ± 0.33 bottles being collected per CTA site. ABMI historical records show an average of 2.80 ± 0.13 bottles per site for all samples collected in 2018 (n = 221), and 2.99 ± 0.05 bottles per site from 2008 to 2018 (n = 1658). In our comparison study, we found that the number of sample bottles were substantially lower for CTA samples than what was reported by Hanisch *et al.* (2020) (5.3 ± 0.9). We also found that the sample bottles required for the TSA method (1.79 ± 0.28) were higher than that reported by Hanisch *et al.* (2020) (0.8 ± 0.07). Regardless, historical ABMI data supports the conclusion that the collection of samples using the CTA protocol will likely require approximately 50-60% more sample bottles and preservative per site than the TSA protocol.

Difference in Lab Sample Processing Effort

We found that TSA samples required significantly more time to process in the lab than CTA samples, which was in contrast to findings reported by Hanisch *et al.* (2020). This difference is conceivably due to variance in lab protocols implemented by the two studies. In our study, we processed CTA samples using ABMI lab protocols (*i.e.* without sorting zooplankton), and processed TSA samples using CABIN lab protocols (*i.e.* zooplankton was sorted); Hanisch *et al.* (2020) used CABIN lab protocols for both CTA and TSA samples (*i.e.* zooplankton sorted from both samples).

Zooplankton are often the most abundant taxa in aquatic invertebrate sweep net samples. This, coupled with their small size and fragility, makes the sorting of zooplankton very time consuming. In 2007 and 2008, the ABMI also sorted zooplankton from wetland samples, which generally doubled the time it took to sort each sample (RPH pers. obs.). As a result, the time required to sort and identify zooplankton, even to just family level, was cost prohibitive leading ABMI to drop zooplankton as a bioindicator from its laboratory protocols in 2009.

Eliminating zooplankton from CABIN sorting would likely have dropped the average sorting time for TSA samples in our study from 13.02 hours to approximately 6.5 hours. This value is more similar to the average sorting time for CTA samples of 7.02 hours. The 0.5 hour difference can likely be attributed to the differing fixed

counts for the two protocols, with ABMI lab protocols sorting about 15% more organisms per sample (ABMI fixed count = 350 vs. CABIN fixed count = 300).

Hanisch *et al.* (2020) reported an average processing time of 11.1 hours for CTA samples and 5.3 hours for TSA samples. The reason for this difference remains unclear. If both samples were sorted to similar fixed counts, it is expected that sorting times would also be very similar. One possibility is that, although both ABMI and CABIN lab protocols call for cleaning the raw sample to remove excess vegetation, CABIN lab protocols do not have a defined, systematic approach to this cleaning step as in the ABMI lab protocols (*i.e.* the elutriation process). It is possible that lab technicians, inexperienced in handling samples with higher volumes of extraneous material, simply took longer to prepare the CTA samples for sorting, and/or failed to remove enough vegetation to make sorting efficient.

The average sample processing time for CTA samples in our study (7.02 hours) is comparable to historical ABMI sample processing data. The average sample processing time (± SE) for all ABMI sites sorted in 2018 was 7.69 ± 0.37 hours per sample (n = 217). ABMI historical data, from 2011 to 2018, shows an average sample processing time of 7.91 ± 0.14 hours per sample (n = 1381). This suggests that the sample processing times seen in our study more accurately reflect what can be expected from lab technicians specifically trained in processing higher volume samples using the ABMI sample cleaning process. While more data are needed to fully support similarities in processing times, the evidence suggests that CTA samples require an average of 0.5 more hours to sort compared to TSA samples, if zooplankton is not sorted.

While we did not track the time required to identify sorted organisms, it makes sense that, given the similar fixed counts between protocols, there would be no significant difference in the time required to identify primary organisms between the two collection and sorting methods. Hanisch et al. (2020) support this where they reported an average of 4.5 ± 0.5 hours for CTA samples and 4.1 ± 0.2 hours for TSA samples, with no significant difference between the two methodologies.

Differences in Diversity, Abundance, and Community Composition

Comparison of the invertebrate community structure (abundance, diversity, richness, and composition) using data collected by different protocol types can provide indication of differences that data from respective protocols can have on regional biodiversity model development and estimating ecological integrity of wetlands. For most tests that we conducted, by considering data organized by various scales of taxonomic resolution and abundance treatments, we found little difference in community indices between protocols. For example, total richness, Shannon diversity, and Simpson diversity were all highly correlated and no significant

differences between protocols were observed among the four taxonomic scales analyzed. SACs were also highly correlated between protocols, though the total number of families and genera identified was marginally higher for the TSA protocol (Appendix C). Moreover, we did not find notable difference in species composition between the two protocols (PERMANOVA, PERMDISP) as well as in PROCRUSTES results that tested for similarity between homologous sites. The only significant differences observed were found in the PERMANOVA analyses (protocol, depth) using the CPUE-T and CPUE-B data. Therefore, the overall patterns of diversity, and community structure are highly similar between these two protocols.

We found differences between protocols when comparing abundances across the different taxonomic resolution. We found that TSA protocol detected a higher adjusted count than CTA for 7% of species (22/316), 8% of genus (12/150), 10% of family (7/67), and 37% of coarse groups (7/19). However, many of the significant differences between protocols at the species taxonomic resolution were driven by the small number of detections.

A fundamental question for wetland biodiversity monitoring is how data generated by the two protocols may differ in making inferences and predictions about biological response to natural and anthropogenic causes. Our assessment of linear models assessing single species responses to environmental characteristics (Table 9) and NMDS ordinations assessing community composition found little differences between the CTA and TSA protocols (Figure 7). The magnitude and direction of coefficients from the linear models were similar even if the correlation of adjusted count abundance between protocols was low (appendix B; table 9). For example, the correlation in abundance *Stylaria lacustris* between the two protocols was very low (r=0.12); however, the derived coefficients were remarkably similar. While our current work is still preliminary, these results suggest, first, we may get similar inference about response of these species to ecological drivers. Secondly, it may be possible to combine datasets collected through each protocol to derive integrated species models by implementing statistical controls for potential effects of protocol changes.

However, we detected some significant differences in model coefficients for certain analyzed taxonomic groups. For example, we found contrasting model coefficients for *Gastropoda UID*, where CTA data showed a negative relationship with pH, DOC, and positive relationship with DP and mean Temp, which were in opposite direction to that obtained using TSA data. On the other hand, while TSA data showed negative effect of human footprint on abundance of *Gastropoda UID*, CTA data showed no difference. The reason for these differences remains unclear at this time and will require further investigation.

Efficiency and Cost Implications

TSA sampling protocols consistently outperformed CTA protocols in every metric used to measure collection efficiency. TSA sampling collected more specimens overall, more specimens per unit effort, and more unique organisms than CTA protocols. The EMSD study showed similar results for CPUE and unique organisms but found that total abundance was significantly higher in CTA samples. In looking at EMSD family level data (Hanisch *et al.* 2020, Table 2), the difference in total abundances was primarily driven by Naididae, Chironomidae, Ceratopogonidae, and Pisidiidae, groups that are more prolific in the sub-benthic sediment layers. It is possible that EMSD field technicians plunged the sweep net too deep when collecting some CTA samples and skewed the results in favor of more numerous, sub-benthic dwelling taxa.

The average difference in cost between CTA and TSA field resources (bottles plus preservative) amounts to just a few dollars per site ($4.39). The difference in labor cost for sample collection (2 technicians at ~$27.00/hr. each X 0.75 hours) is ~$40.50/site. The difference in sample processing, if zooplankton are not sorted, would be ~$13.50 (1 lab technician at ~$27.00/hr. X 0.5 hours), and the difference in specimen identification would be ~$18.00 (1 taxonomist X 0.4 hours, from EMSD data). This makes the average additional cost when collecting and processing samples using CTA protocols ~$76/site. When sampling 120 sites, Hanisch *et al.* (2020) calculated a difference of $48,000 between protocols. Our data support these findings although not to the same extent as we found a difference of ~$9,120.

Conclusions

We conclude that although there are differences in aquatic invertebrate communities sampled using CTA and TSA methods, those differences are small. Further, the similarities in community composition seen at the family level are consistent when analyzing data at finer taxonomic scales. We found no significant difference in richness and diversity measures at all taxonomic levels, and, with the exception of the family level adjusted counts, NMDS dispersion showed no significant difference. Family and species level adjusted counts indicated no significant difference in the centroid between groups, with the remaining taxonomic and abundance categories showing a significant difference but with overlapping ellipses and small p-values. The Procrustes tests showed a significant correlation across all taxonomic and abundance measures.

Our data also suggest that aquatic invertebrate samples collected and processed using CTA-ABMI protocols (including ABMI historical data) are generally comparable to samples collected and processed using TSA-CABIN protocols. NMDS ordinations of TSA shallow samples are interspersed with CTA deep and medium samples, with significant overlap between ellipses and no discernable patterns. CTA shallow ordinations seem to cluster with less overlap, but this category is limited by only

four sample events. Although sample collection depth does not appear to have a significant effect on aquatic invertebrate community composition, a recent study by Bush *et al.* (2020) suggests that random processes of aquatic invertebrate community assembly may mask differences. We acknowledge that more research is needed here to confirm historical data comparability and assess methods for reconciling data using statistical controls during analysis.

Finally, we conclude that the TSA method of aquatic invertebrate sample collection is more efficient and cost effective than the CTA method. While differences between the two methods were more subtle than those reported by Hanisch *et al.* (2020) our data generally agree with their findings particularly with respect to time and cost for collecting samples. Although these savings may not enable ABMI field crews to sample more than one wetland per day, a change to the TSA method may allow greater time for additional collection of habitat data at each site.

It is clear from the results of this study that there may be advantages to the ABMI to shift to the TSA protocol. Cost and time savings will allow for either more wetlands to be sampled each year, or more data to be collected at each site. A closer alignment with TSA protocols will improve confidence in direct data comparison and allow organizations using either CTA or TSA methods to leverage data between programs, thus enabling the development of stronger wetland and species models. Having a single approach to wetland monitoring and aquatic invertebrate collection across the province will strengthen ABMI's position in monitoring wetland biodiversity moving forward. However, because certain elements of the CABIN lab protocols actually lead to an increase in time/cost, each element will need to be considered carefully. In particular, unknowns surrounding historical data comparability need to be explored further to ensure the first 13 years of ABMI aquatic invertebrate data remain valuable for monitoring changes in wetland biodiversity over time.

Acknowledgements

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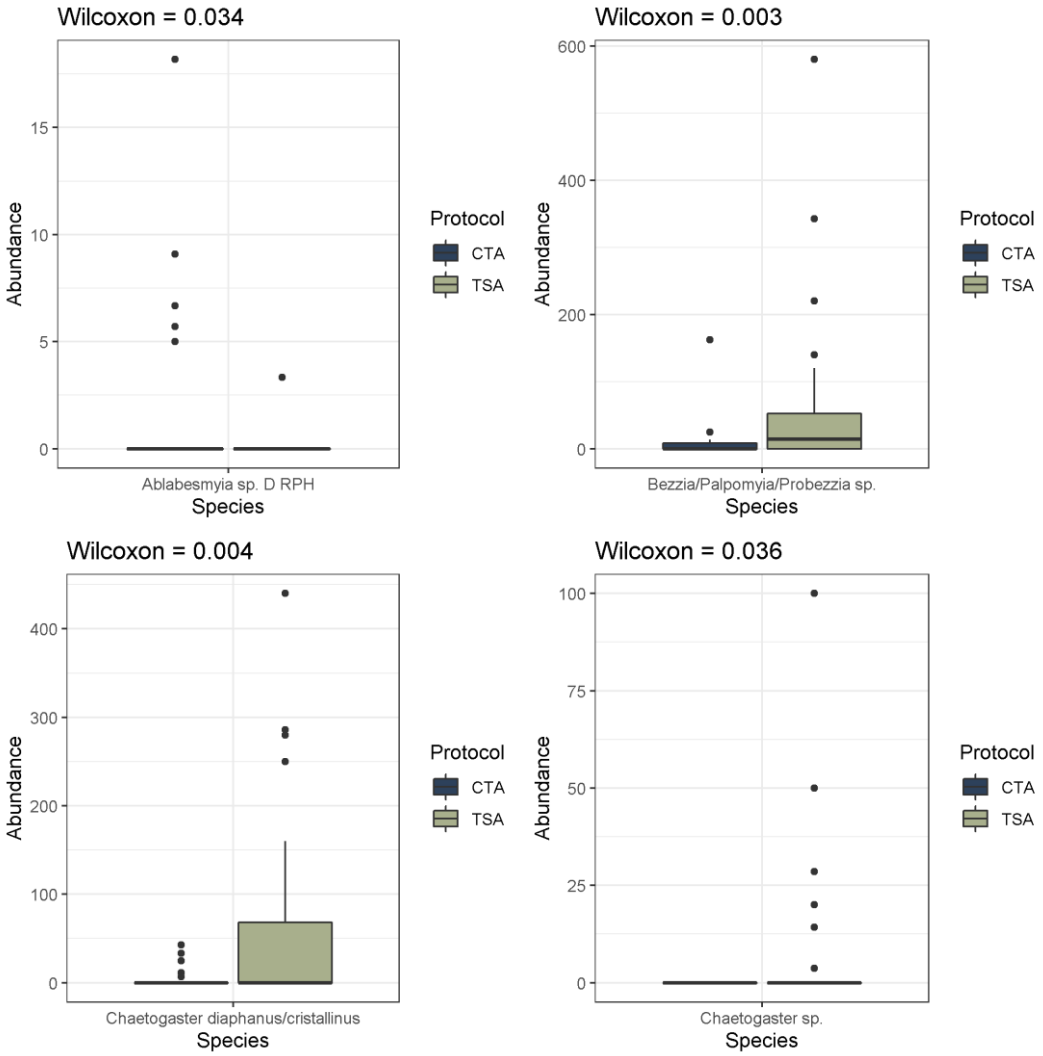
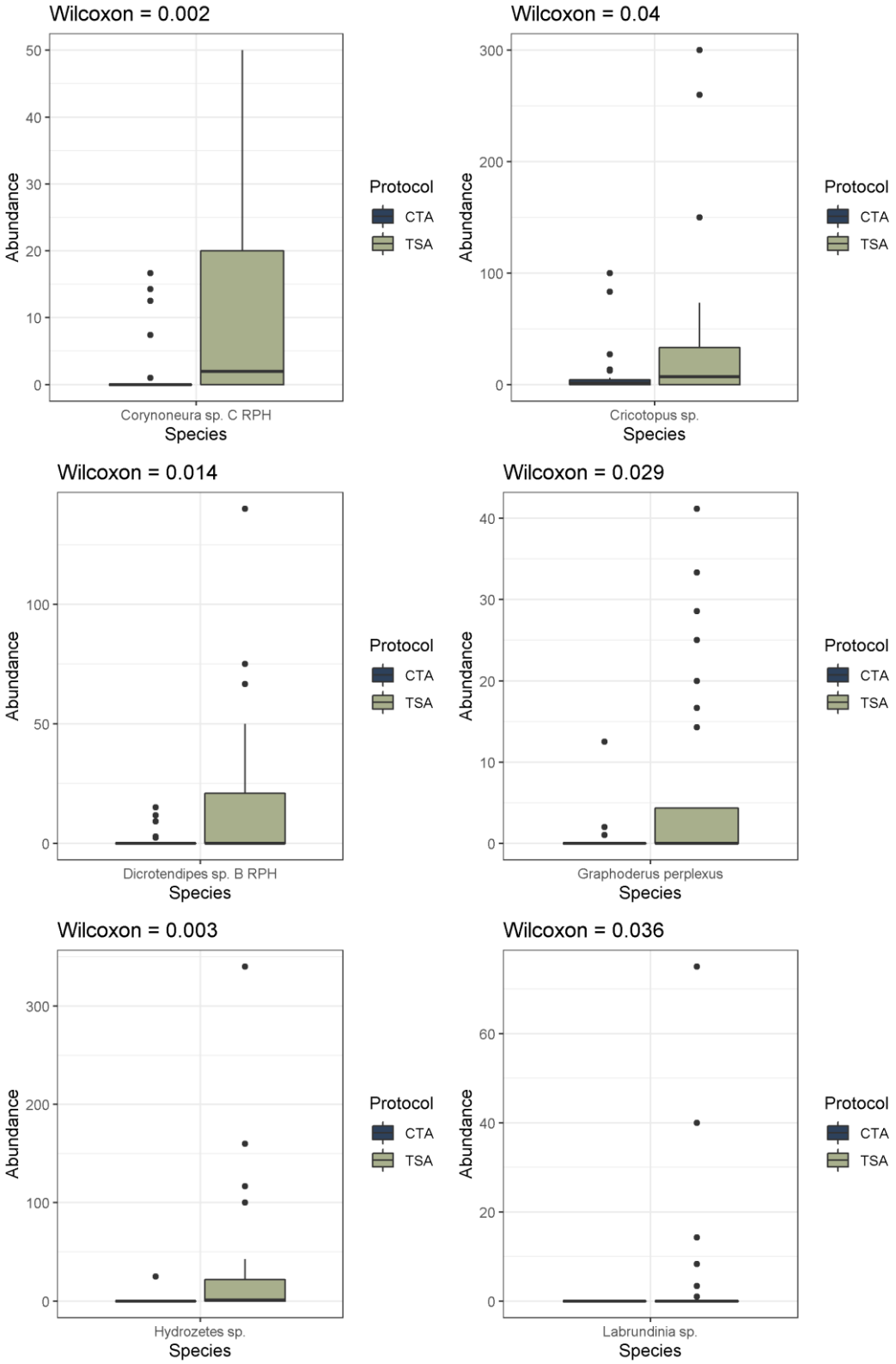
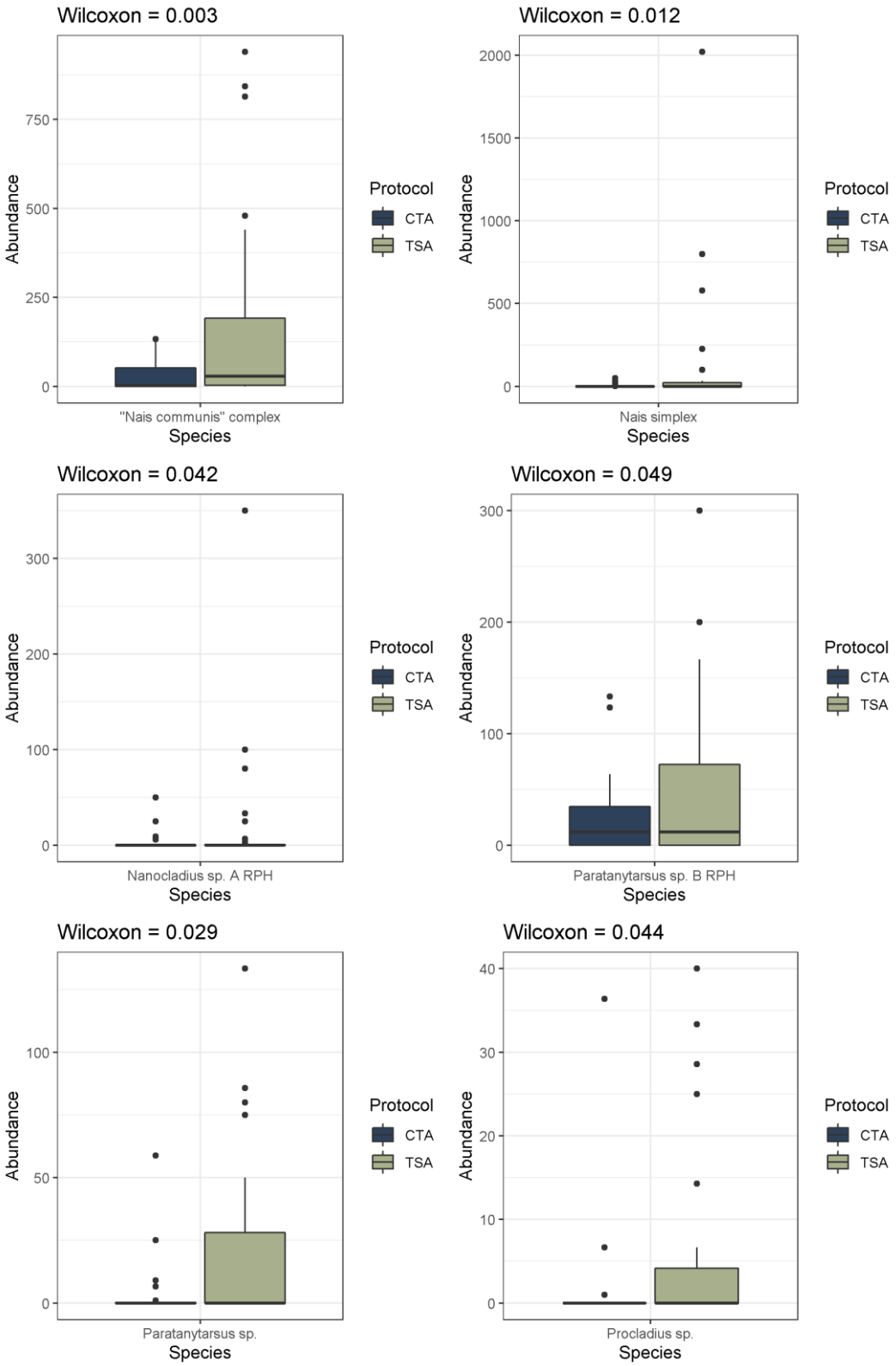
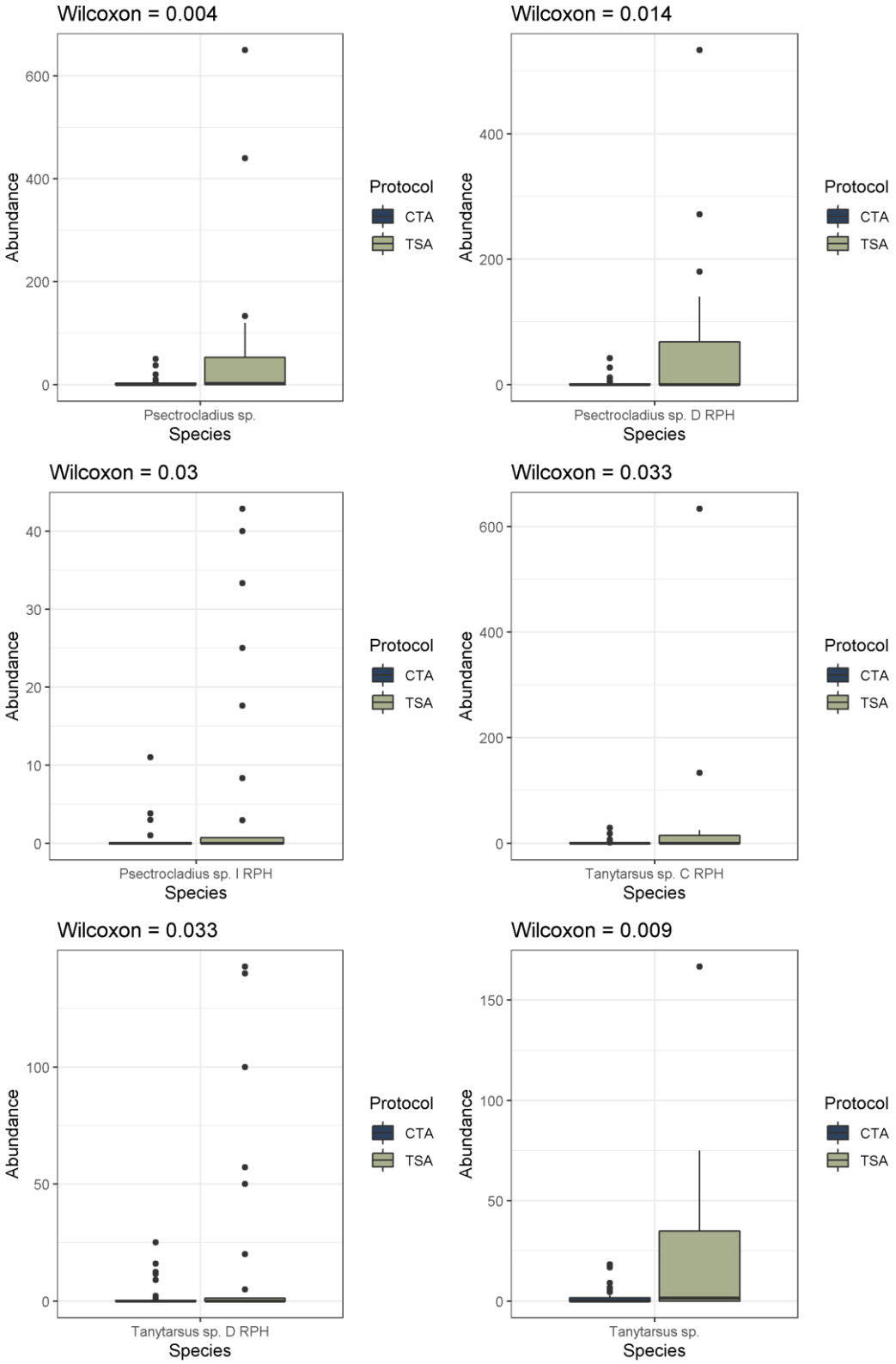
Appendix A

Boxplots and Wilcoxon Signed Ranked test results of species abundance (using adjusted-counts) for the 22 species that showed significant differences between protocols.



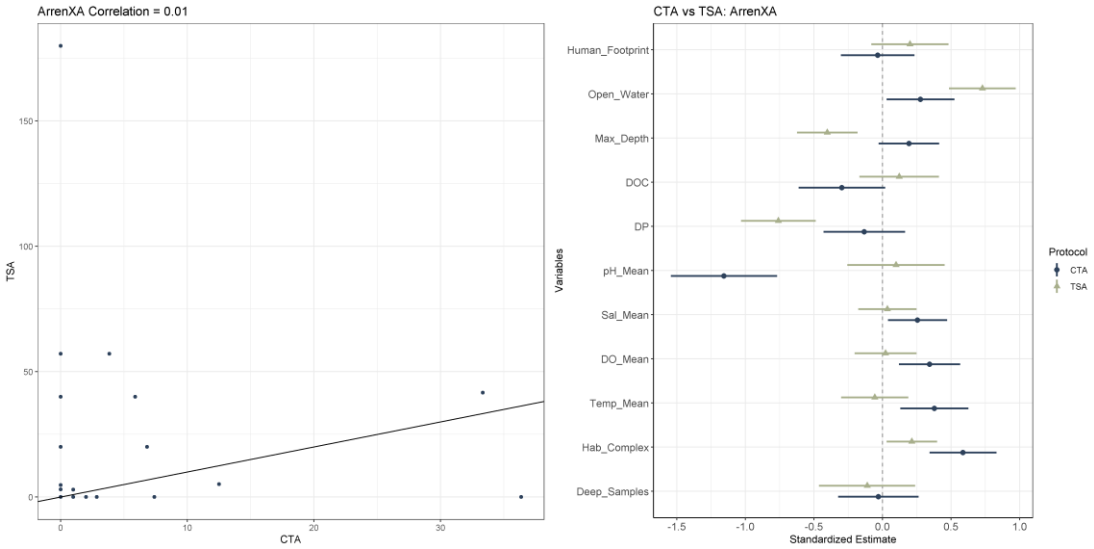

Wilcoxon = 0.002
Abundance
Corynoneura sp. C RPH
Species
Protocol
CTA
TSA
Wilcoxon = 0.04
Abundance
Cricotopus sp.
Species
Protocol
CTA
TSA
Wilcoxon = 0.014
Abundance
Dicrotendipes sp. B RPH
Species
Protocol
CTA
TSA
Wilcoxon = 0.029
Abundance
Graphoderus perplexus
Species
Protocol
CTA
TSA
Wilcoxon = 0.003
Abundance
Hydrozetes sp.
Species
Protocol
CTA
TSA
Wilcoxon = 0.036
Abundance
Labrundinia sp.
Species
Protocol
CTA
TSA

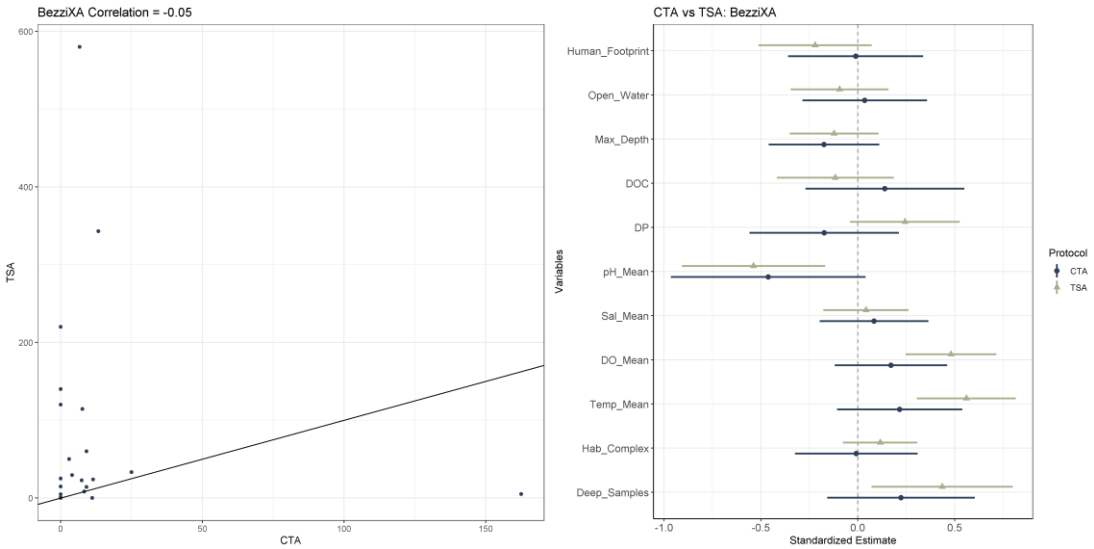

Wilcoxon = 0.003
Abundance
750
500
250
0
"Nais communis" complex
Species
Protocol
CTA
TSA
Wilcoxon = 0.012
2000
1500
1000
500
0
Nais simplex
Species
Protocol
CTA
TSA
Wilcoxon = 0.042
300
200
100
0
Nanocladius sp. A RPH
Species
Protocol
CTA
TSA
Wilcoxon = 0.049
300
200
100
0
Paratanytarsus sp. B RPH
Species
Protocol
CTA
TSA
Wilcoxon = 0.029
100
50
0
Paratanytarsus sp.
Species
Protocol
CTA
TSA
Wilcoxon = 0.044
40
30
20
10
0
Procladius sp.
Species
Protocol
CTA
TSA

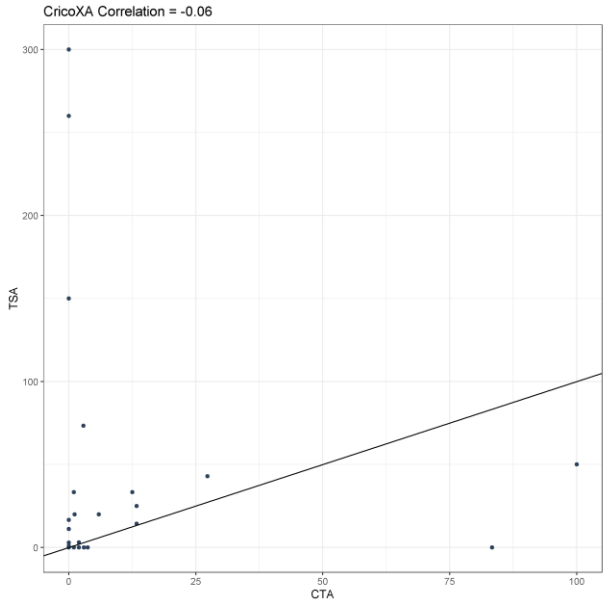

Wilcoxon = 0.004
Abundance
Species
Psectrocladius sp.
Protocol
CTA
TSA
Wilcoxon = 0.014
Psectrocladius sp. D RPH
Wilcoxon = 0.03
Psectrocladius sp. I RPH
Wilcoxon = 0.033
Tanytarsus sp. C RPH
Wilcoxon = 0.033
Tanytarsus sp. D RPH
Wilcoxon = 0.009
Tanytarsus sp.

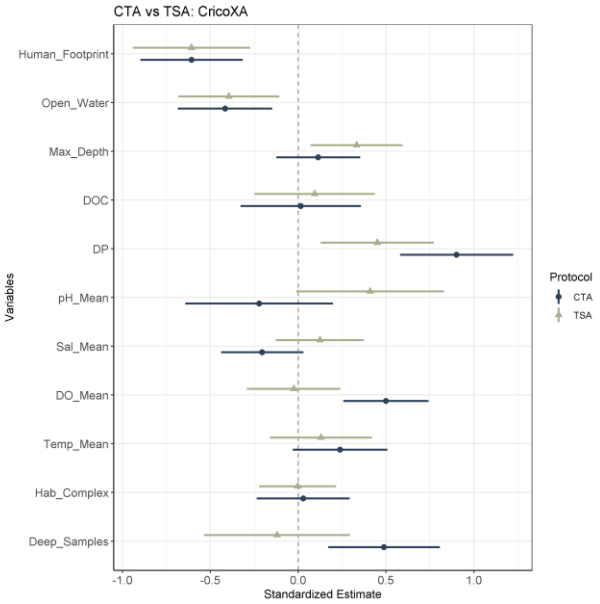
Appendix B

Linear regressions models and Pearson correlation of standard coefficients of the relationships between abundance and environmental variables using both the ABMI and CABIN protocols. ArrenXA = *Arrenurus sp.*, BezziXA = *Bezzia/Palpomyia/Probezzia* complex, CricoXA = *Cricotopus sp.*, EndocXA = *Endochironomus sp.*, EndoniA = *Endochironomus nigricans*, ParacDA = *Parachironomus sp.* D RPH, ParatBA = *Paratanytarsus sp.* B RPH, PionaXA = *Piona sp.*, SlavapA = *Slavina appendiculata*, StyllaA = *Stylaria lacustris*, TanytBA = *Tanytarsus sp.* B RPH.






CricoXA Correlation = -0.06
TSA
CTA
0
100
200
300
0
25
50
75
100


CTA vs TSA: CricoXA
Human_Footprint
Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
Variables
Standardized Estimate
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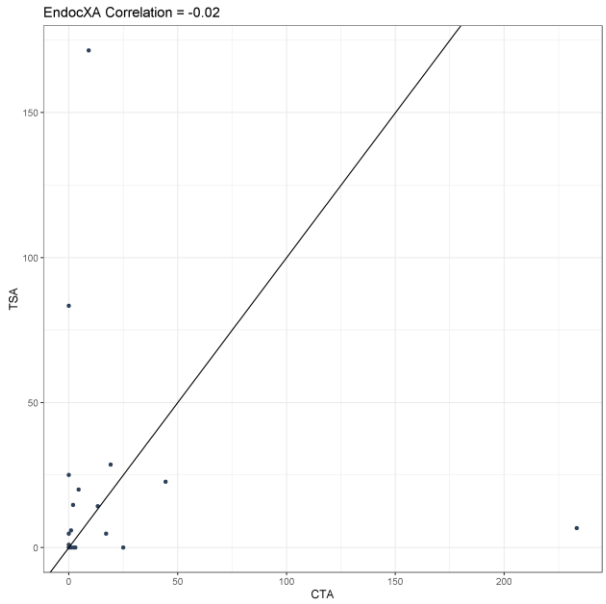
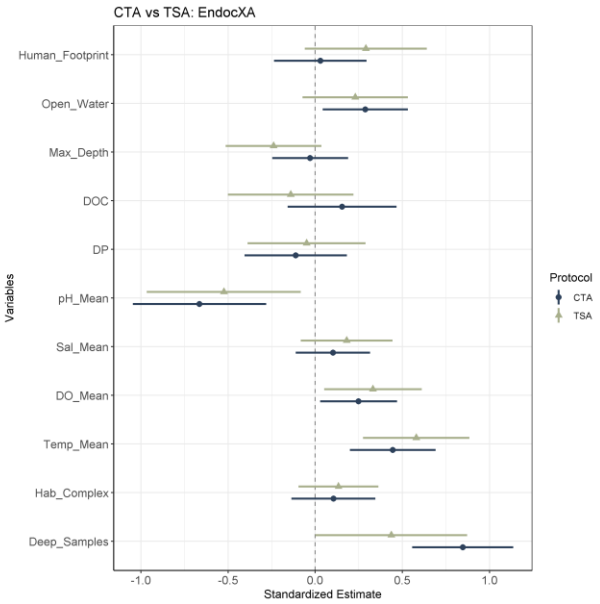
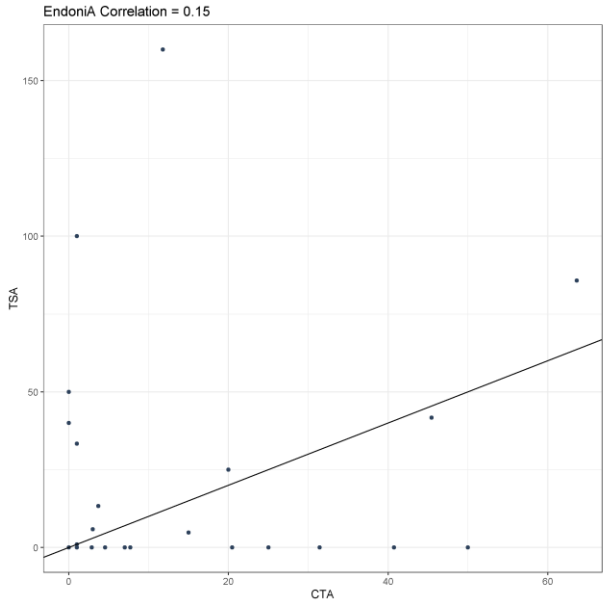
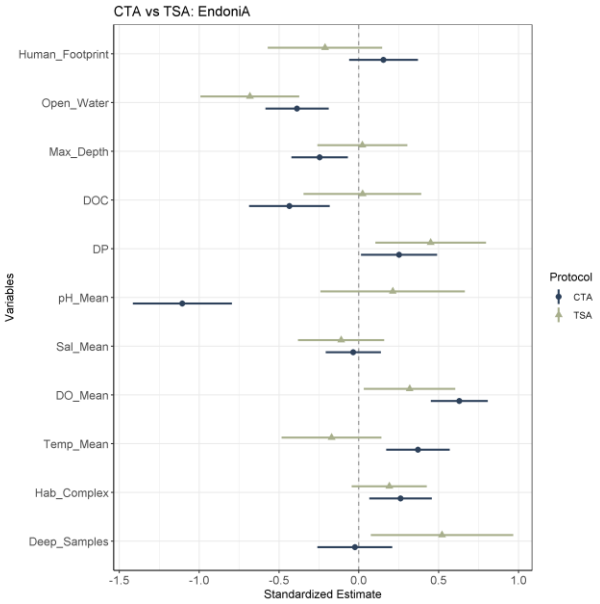
-0.5
0.0
0.5
1.0
Protocol
CTA
TSA


EndocXA Correlation = -0.02
TSA
CTA
0
50
100
150
0
50
100
150
200


CTA vs TSA: EndocXA
Human_Footprint
Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
Variables
Standardized Estimate
-1.0
-0.5
0.0
0.5
1.0
Protocol
CTA
TSA


EndoniA Correlation = 0.15
TSA
CTA
0
50
100
150
0
20
40
60


CTA vs TSA: EndoniA
Human_Footprint
Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
Variables
Standardized Estimate
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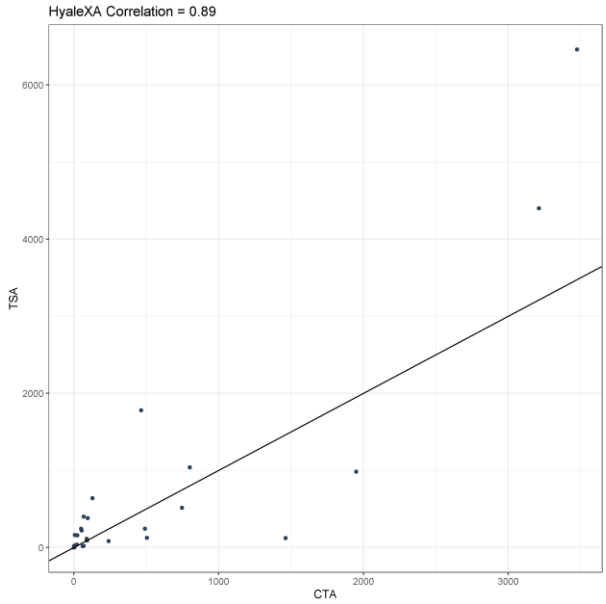
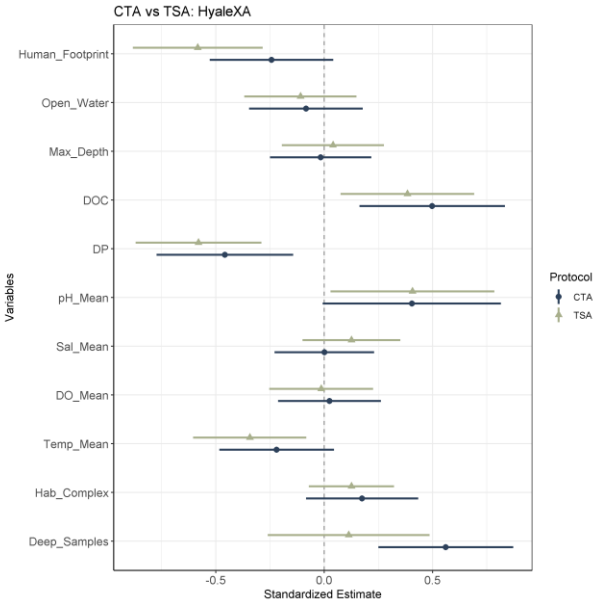
-1.0
-0.5
0.0
0.5
1.0
Protocol
CTA
TSA


HyaleXA Correlation = 0.89
TSA
CTA
0
2000
4000
6000
0
1000
2000
3000


CTA vs TSA: HyaleXA
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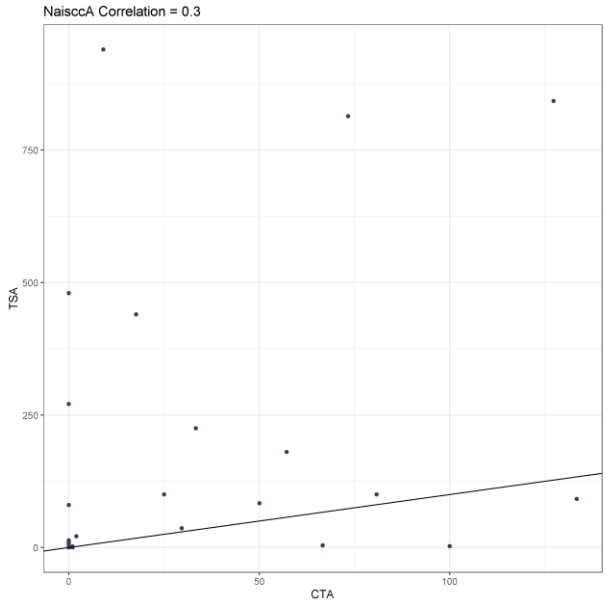
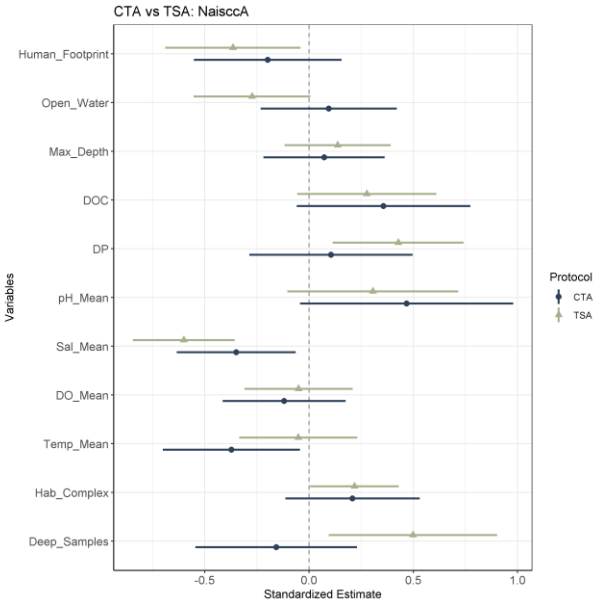
Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
Variables
Standardized Estimate
-0.5
0.0
0.5
Protocol
CTA
TSA


NaisccA Correlation = 0.3
TSA
CTA
0
250
500
750
0
50
100


CTA vs TSA: NaisccA
Variables
Human_Footprint
Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
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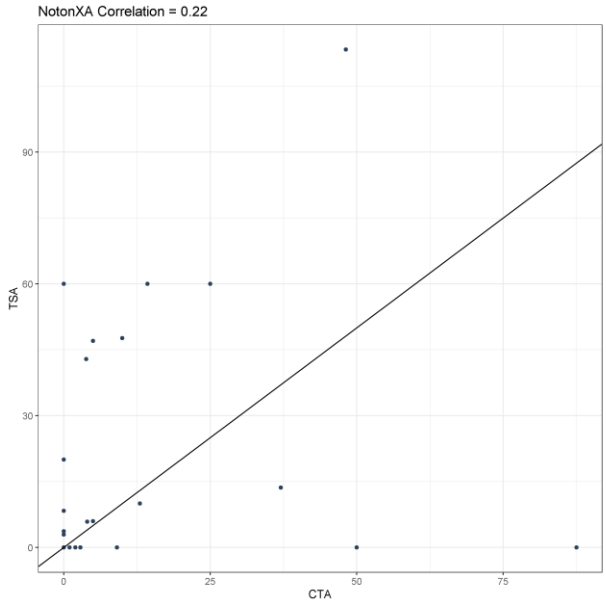
0.0
0.5
1.0
Standardized Estimate
Protocol
CTA
TSA


NotonXA Correlation = 0.22
TSA
CTA
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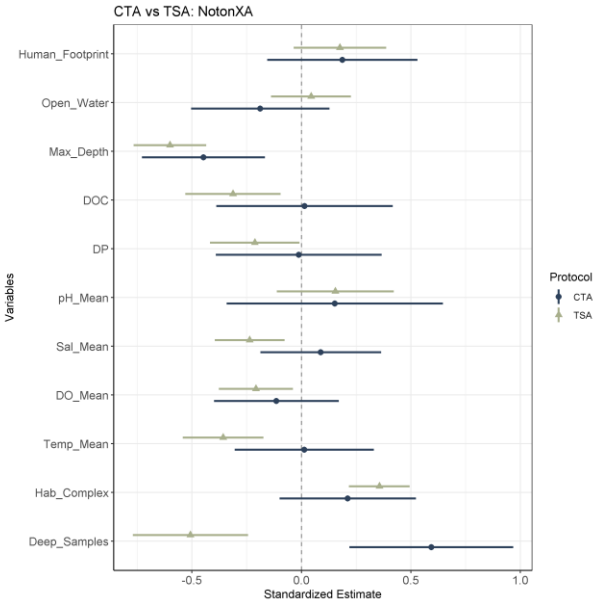
30
60
90
0
25
50
75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Max_Depth
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DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
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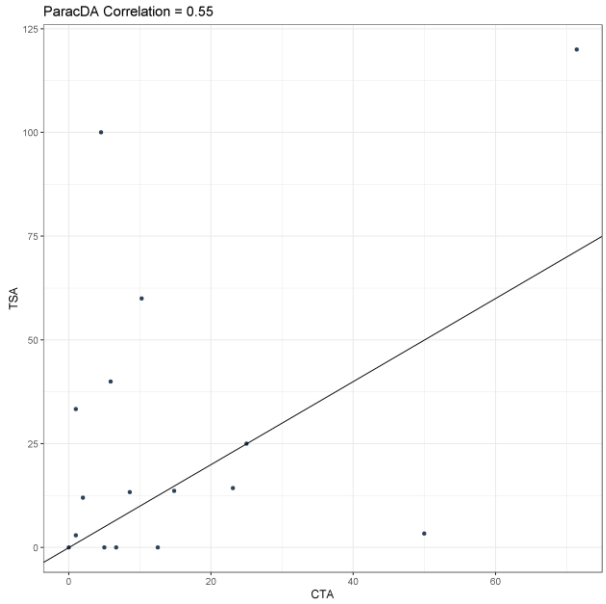
0.5
1.0
Standardized Estimate
Protocol
CTA
TSA


ParacDA Correlation = 0.55
TSA
CTA
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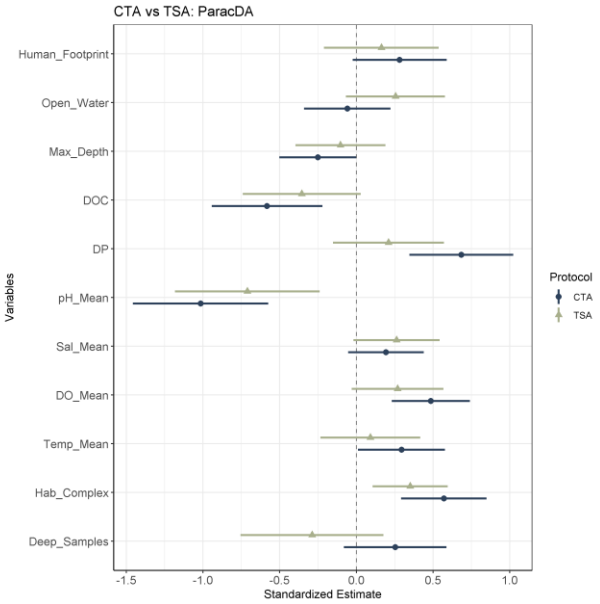
25
50
75
100
125
0
20
40
60


CTA vs TSA: ParacDA
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Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
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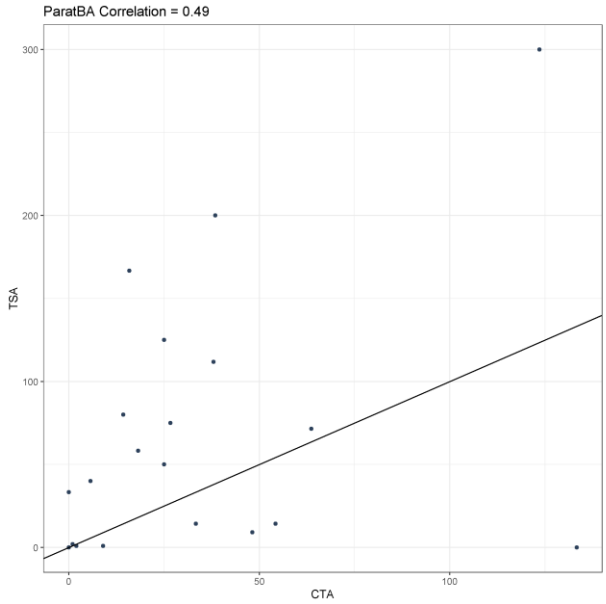
0.5
1.0
Protocol
CTA
TSA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TSA
CTA
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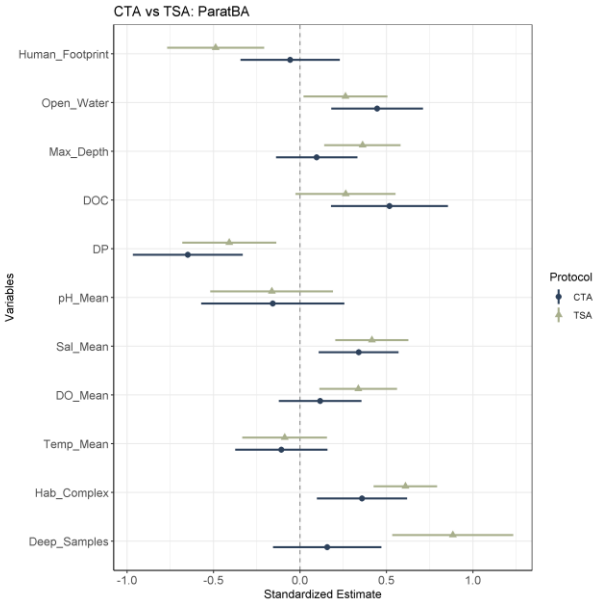
100
200
300
0
50
100


CTA vs TSA: ParatBA
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DP
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Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
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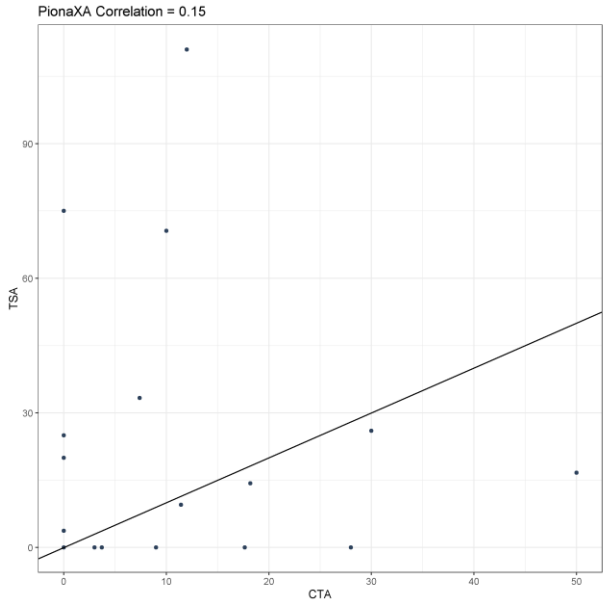
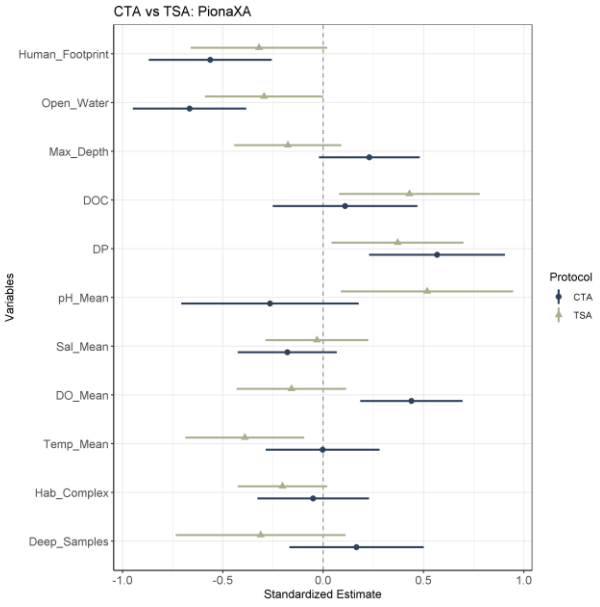
CTA
TSA


PionaXA Correlation = 0.15
TSA
CTA
0
30
60
90
0
10
20
30
40
50


CTA vs TSA: PionaXA
Human_Footprint
Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
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Variables
Standardized Estimate
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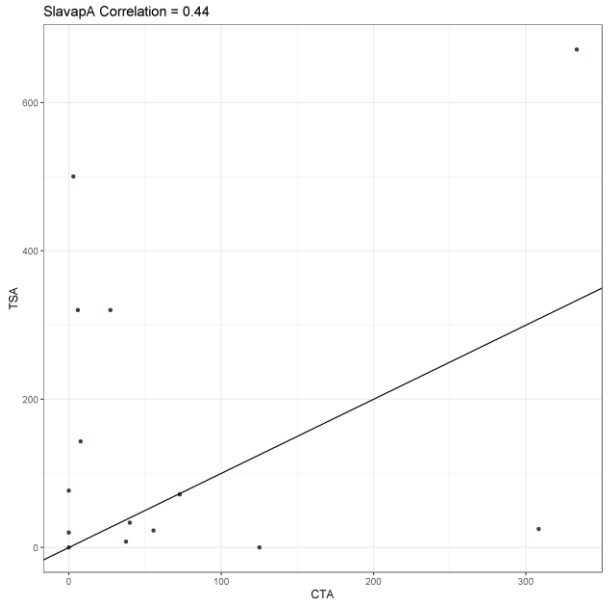
1.0
Protocol
CTA
TSA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TSA
CTA
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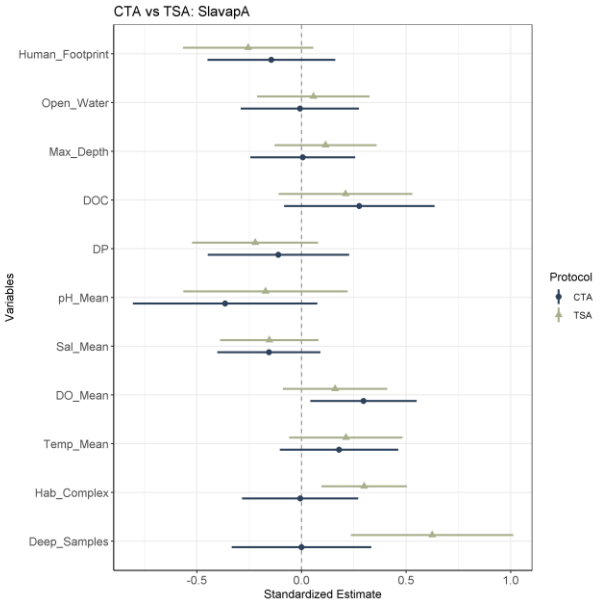
200
400
600
0
100
200
300


CTA vs TSA: SlavapA
Human_Footprint
Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
Variables
Standardized Estimate
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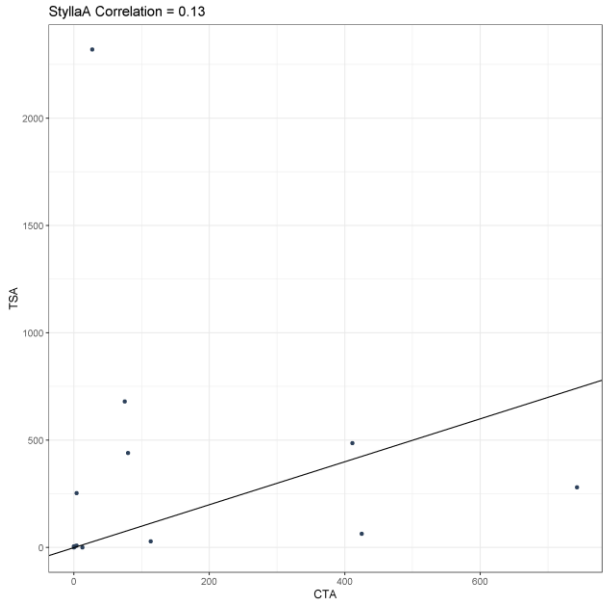
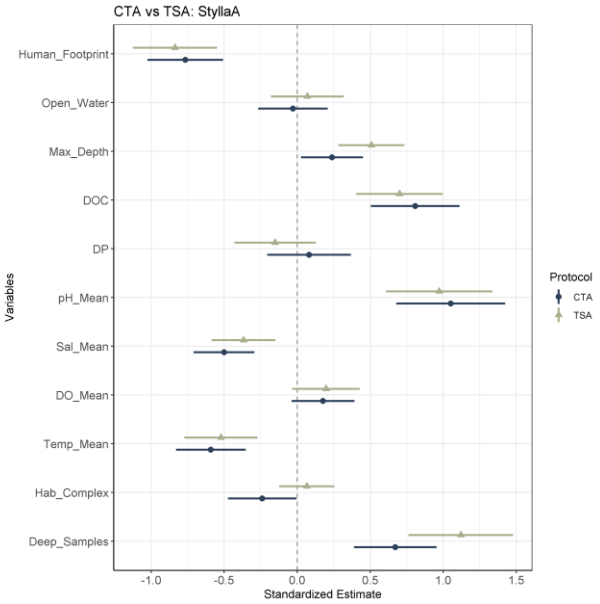
0.0
0.5
1.0
Protocol
CTA
TSA


StyllaA Correlation = 0.13
TSA
CTA
0
500
1000
1500
2000
0
200
400
600


CTA vs TSA: StyllaA
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Open_Water
Max_Depth
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DP
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Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
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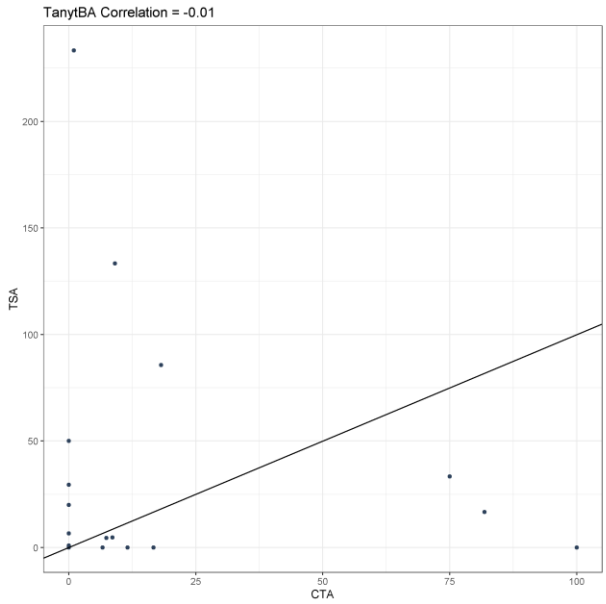
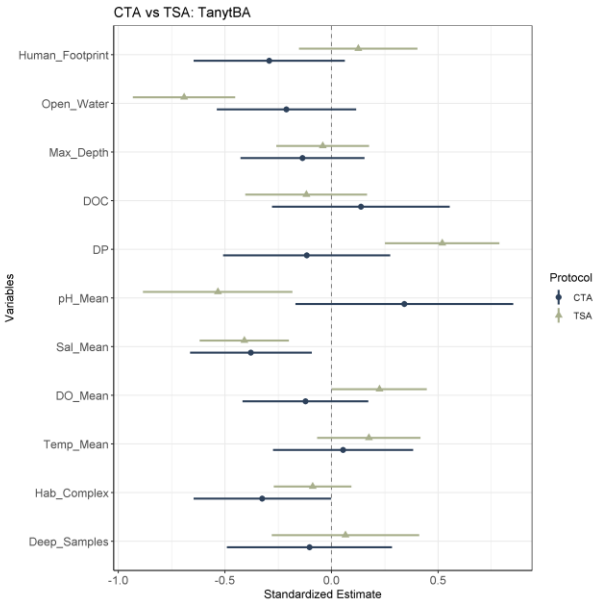
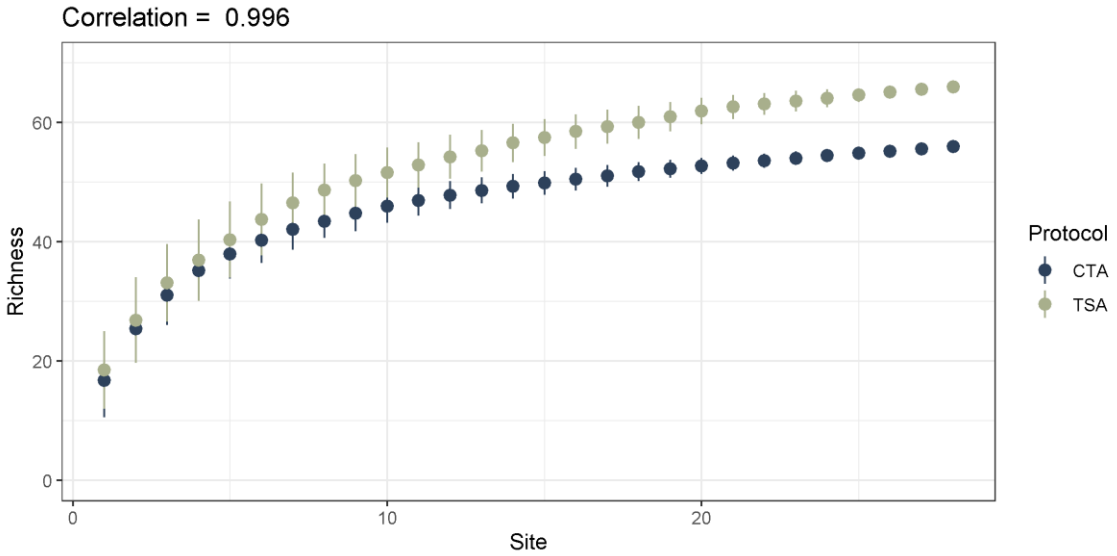
1.0
1.5
Standardized Estimate
Protocol
CTA
TSA

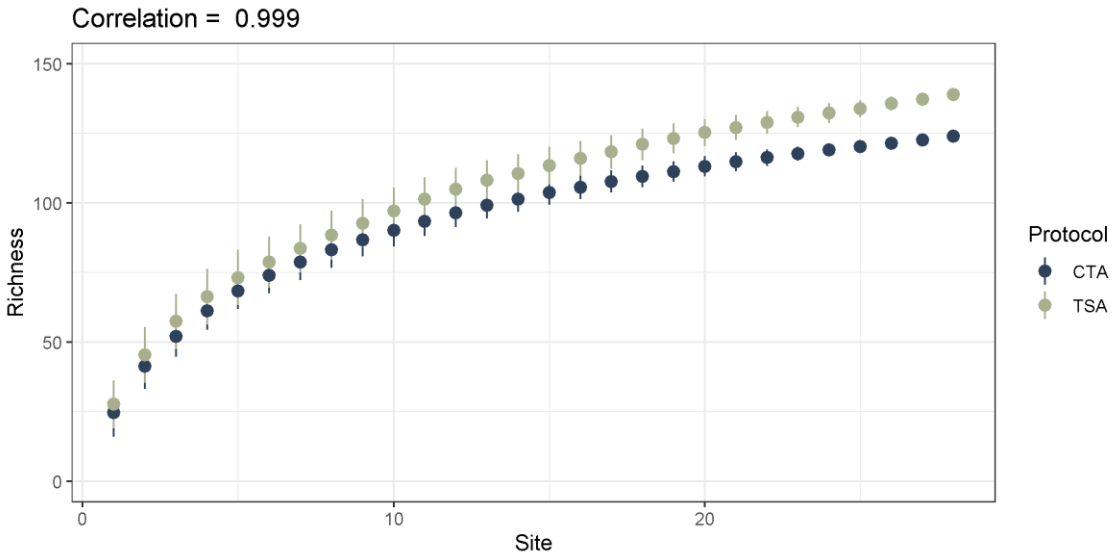

TanytBA Correlation = -0.01
TSA
CTA
0
50
100
150
200
0
25
50
75
100


CTA vs TSA: TanytBA
Human_Footprint
Open_Water
Max_Depth
DOC
DP
pH_Mean
Sal_Mean
DO_Mean
Temp_Mean
Hab_Complex
Deep_Samples
Variables
-1.0
-0.5
0.0
0.5
Standardized Estimate
Protocol
CTA
TSA

Appendix C



Family accumulation curves for the CTA (dark) and TSA (light) protocols. Each curve was created using random site selection procedure, with the CTA protocol having a maximum richness value of 56 families and the TSA protocol having a maximum of 66 families.



Genus accumulation curves for the CTA (dark) and TSA (light) protocols. Each curve was created using random site selection procedure, with the CTA protocol having a maximum richness value of 124 genera and the TSA protocol having a maximum of 139 genera.