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Assessment Of Stream Water Quality Comparing The Use Of Whole Count To Fixed-Count Subsampling Of Macroinvertebrates Collected Using Kick-Net Techniques

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Abstract

Assessment of water quality of streams frequently employs sampling of benthic macroinvertebrate assemblages. Many different sampling techniques and assessment measures have been used to achieve this goal. Quality sampling and methods are crucial to obtain optimum data at minimal expense in order to effectively assess water quality. The accuracy of the data collected is crucial to determining the need for intervention in a local aquatic ecosystem. Without accurate data collection agencies could use funds when intervention is not needed, or conversely it could appear a system in danger is without need of assistance. It is important for agencies involved to use a method that ensures accurate assessment of the quality in an ecosystem effectively while also limiting expenses during data collection. Using broad taxa categorization (family, order) of Illinois RiverWatch protocol (a citizen science assessment program), this study compared results obtained through using whole count samples from kick net collection of macroinvertebrates at two sites (upstream and downstream) in Lily Cache Creek in Illinois, to subsampling of 100+ specimens from the collection. When the subsample represented only 19.3% of the total number of macroinvertebrates collected, results varied more significantly than when the subsample represented 80.1% of the total count; however both methods of sampling resulted in similar conclusions in regard to the stream's water quality.

Keywords: Water Quality, Benthic Macroinvertebrate, Illinois

1. Introduction

The quality of local water sources is very important for wildlife and people alike. For instance, rivers are a large contributing source of our drinking water in addition to being the primary support for fish and many other aquatic species. There are over 3.5 million miles of rivers and streams in the United States.[1] The quality of river water can be impacted by many things including chemical pollutants, sewage, residential development, and farmland run-off. Pollution such as phosphorus and nitrogen, which can come from fertilizers and wastewater, can cause low oxygen levels and algae blooms in a river.[1,2] Changes in the flow of the water by creating reservoirs can create problems within the river by changing water levels and eliminating habitats. One way of determining the quality of local rivers is through biological monitoring or biosurvey. This involves the collection and analysis of aquatic organisms to help determine the health of the biological community within that river. The data drawn from this monitoring effort helps to determine the need for intervention and restoration of the river ecosystem.[2]

The most commonly studied organisms are fish, algae, and macroinvertebrates.[3,4] Macroinvertebrates include adult and larval aquatic insects, small crustaceans, snails, leeches and worms. The diversity and specific types of macroinvertebrates, known as taxa, greatly influence the food web within the river and can be a good indicator of the health of the river due to their life cycle. They remain in a relatively stationary position within their aquatic environment. Because of this, their presence or lack thereof can indicate short or long term changes in the quality of the water they are in. Several groups of these macroinvertebrates are extensively used for monitoring aquatic ecosystems. In comparison, performing a study of animals that are more mobile during their lifecycle only provide a small window of time for when the study is being done for analysis.[5] Many of these studies using freshwater invertebrates are conducted in developed countries worldwide. In these studies, certain taxa, particularly the insect orders Ephemeroptera, Plecoptera and Trichoptera are more abundant in areas of higher volume and rapidly flowing water and are considered better predictors of water quality because these taxa are also highly sensitive to the impact of lower quality water in their habitat.[6]

The methods of collecting and studying these macroinvertebrates within the United States are determined by local state agencies. These agencies issue protocol and methods for water sampling, macroinvertebrate identification and counting, and data analysis. The accuracy of the data collected is crucial to determining the need for intervention in a local aquatic ecosystem. Without accurate data, funds could be used when intervention is not needed, or it could appear a system in danger was without need of assistance. The expense of acquiring the data is also a consideration. It is important for agencies involved to use a method that ensures accurate assessment of the quality in an ecosystem effectively while also limiting expenses in the pursuit of the required data.[2,4]

There are many different and yet similar methods of collecting the samples used for water quality assessment. Even the methods of counting macroinvertebrates can vary from region to region. In order to keep expenses to a minimum while obtaining perceivably accurate assessment of a water body, many counts are done by use of fixed-count subsampling. A specified number of macroinvertebrates is randomly collected from a larger sample and then identified to represent the diversity of the whole. In addition to the fixed-count method, the whole count area-based method is also used using the entire sample instead of just a predetermined quantity of specimens. These two methods are the most commonly used.

This experiment was conducted to compare the analysis of the data collected from these samples to see if different methods of data sampling would result in different status of water quality in a water sample. Water samples were obtained from the Lily Cache Creek, part of the DuPage River watershed in Illinois. The data collection methods were conducted according to the Illinois RiverWatch protocol.[3] Illinois RiverWatch is a citizen scientist program overseen by the National Great Rivers Research and Education Center in East Alton, Illinois. The hypothesis in this experiment was that the data from the Illinois RiverWatch subsampling count method would result in a slightly lower taxa richness, EPT taxa richness (Ephemeroptera, Plecoptera,Trichoptera) and MBI (Macroinvertebrate Bioassessment Index) in comparison with the results of the full count in each sample recorded. If the subsampling count shows lower numbers in the results, it will appear that the quality of the water tested is not as good as the results would show using the full count method in the same water sample.

2. Methods

This study included two sites measured in 200 foot (61 m) stretches of the Will County Lily Cache Creek in Plainfield, Illinois. The downstream site was positioned within a forested area, containing a mixture of living trees and snag regions that provided a canopy cover of just less than 50% during the season the study was conducted. This site was in Van Horn Woods East, which was accessed by the US 55 West Frontage Road. The second upstream site was located approximately 3 miles (5 km) to the northeast of the Van Horn site at Four Seasons West Baseball Fields on Lockport Street. The Four Seasons site was characterized by an open canopy that was predominately populated with reed canary grass (*Phalaris arundinacea*). The sampling was conducted at both sites between 4 June and 25 June 2015. The sampling protocol followed was provided by Illinois RiverWatch.[3]

At each site, kick net sampling was performed by using D-Frame 500-micron kick nets to collect macroinvertebrates in a riffle area. Each net was used at a location upstream in a riffle and the other at a downstream riffle and collection was performed simultaneously. Samples were collected by kicking to a depth of 3 inches (7.5 cm) into the substrate approximately a foot (30 cm) in front of the net for three minutes. Both upstream and downstream samples were then condensed into one 5-gallon (18.93 L) bucket. A separate collection was taken at each site by scraping snags. At the Van Horn site, several small snags and branches that were located in the water were scraped for ten minutes. An approximately 4 foot (1-1.5 m) section of a submerged log was scraped for ten minutes in a similar fashion at the Four Seasons site. These samples were then combined with the riffle samples. After collection was complete, the samples were examined for macroinvertebrates. All collected rocks, twigs or plant life were examined, rinsed, and returned to the water. Any specimens located were placed into a rectangular sampling pan containing soda water in order to slow the movement of the organisms.

After all of the organisms were located, they were removed from the sampling pan and placed into sample jars containing 90% isopropyl alcohol until sorting and counting could take place. Each sample jar was labeled with the location and week of the collection. Macroinvertebrates that were collected were first subsampled randomly according to RiverWatch procedure.[3] Specimens from each sample were removed and placed into numbered-grid sampling trays. The tray was moved back and forth in a side-to-side motion to try to spread the organisms out evenly. Numbers were then selected randomly, and the invertebrates located within the numbered section of the gridded tray were counted. This process was repeated until a total sample size of 100 organisms was counted. In the instance the count exceeded 100 in the final numbered section; the additional organisms in that section were added to the count. All remaining macroinvertebrates in the tray were returned to the sample jar. The numbers in each square were recorded onto a data sheet. The counted macroinvertebrates were then examined under stereo microscope to identify taxa, and then double counted and recorded.

Results were recorded as number of total organisms, taxa richness (number of different organisms), EPT taxa richness (Ephemeroptera, Plecoptera, Trichoptera), and the Macroinvertebrate Biotic Indices (MBI) were calculated as defined by Illinois RiverWatch to determine quality ratings.[3] This process was repeated for both samples. After counting was completed for the subsample method, the remaining macroinvertebrates from each sample were also identified under stereo microscope and the total count for each site was recorded for comparison with the subsample results according to Illinois RiverWatch protocol.[3] Percentages of each macroinvertebrate were calculated for the subsamples and whole count method by sample location. Percentages were calculated for the occurrence of species that are pollution intolerant (tolerance indicator value $\leq$ 5.5) and tolerant (tolerance indicator value >5.5). Shannon Diversity Indices (SDI) were calculated for each sampling method and totaled for each site. Statistical analysis was calculated using Chi Square for each sampling method in the comparison of EPT and non-EPT specimens, pollution tolerant and intolerant taxa, and total EPT taxa varieties with non-EPT taxa varieties. Stream quality was determined by using the RiverWatch stream quality rating system (Table 1).

3. Results

Samples were first evaluated according to the RiverWatch quality calculations (Table 1). Using these calculations, further comparison was done by counting method. The Area-Based (Whole Count) method and the Fixed-Count (Subsample) method showed higher percentages of pollution tolerant specimens, a lower percentage of pollution intolerant specimens, a higher percentage of EPT taxa and a lower percentage of EPT specimens in the Whole Count (Table 2, Figure 1) at the 4 Seasons site, while the percentages showed minor differences between counting methods at the Van Horn site (Table 2, Figure 2). Individual macroinvertebrate frequencies also showed a greater difference between counting methods at the 4 Seasons site, while at the Van Horn site the differences were minimal for most specimen types (Figures 3, 4).

Table 1. Illinois River Watch Stream Quality Ratings.

	Taxa Richness	**EPT Taxa Richness**	**MBI**
Excellent	$\geq$ 14	$\geq$ 5	$\leq$ 4.35
Good	12-13	4	$\geq$ 4.36 - $\leq$ 5.00
Fair	9-11	3	$\geq$ 5.01 - $\leq$ 5.70
Poor	7-8	2	$\geq$ 5.71 - $\leq$ 6.25
Very Poor	$\leq$ 6	0-1	$\geq$ 6.26

Table 2. Results of area-based whole count sampling compared to subsampling of Lily Cache Creek, Will Co., IL at Van Horn Woods and Four Seasons West Baseball Fields, 2015.

	4 Seasons Whole Count	4 Seasons Subsample	Van Horn Whole Count	Van Horn Subsample
Total Specimens Sampled	741	145	161	125
Taxa Richness	16	13	16	16
EPT Taxa Richness	5	3	6	6
MBI	5.51 (fair)	5.28 (fair)	4.72 (good)	4.63 (good)
SDI	2.065	2.196	2.402	2.38
# EPT Specimens	88	43	70	59
# Pollution Tolerant Specimens	480	57	65	46
# Pollution Intolerant Specimens	261	88	96	79

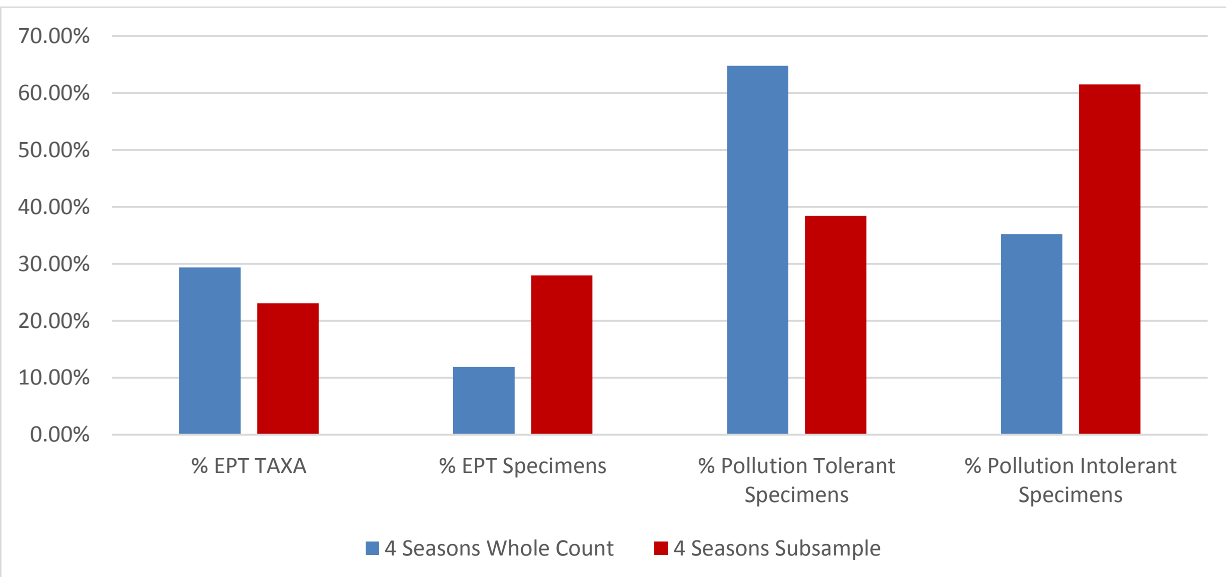

Figure 1. Percentage comparisons of EPT values and specimen tolerance by counting method at 4 Seasons site showing differences in results between counting methods.

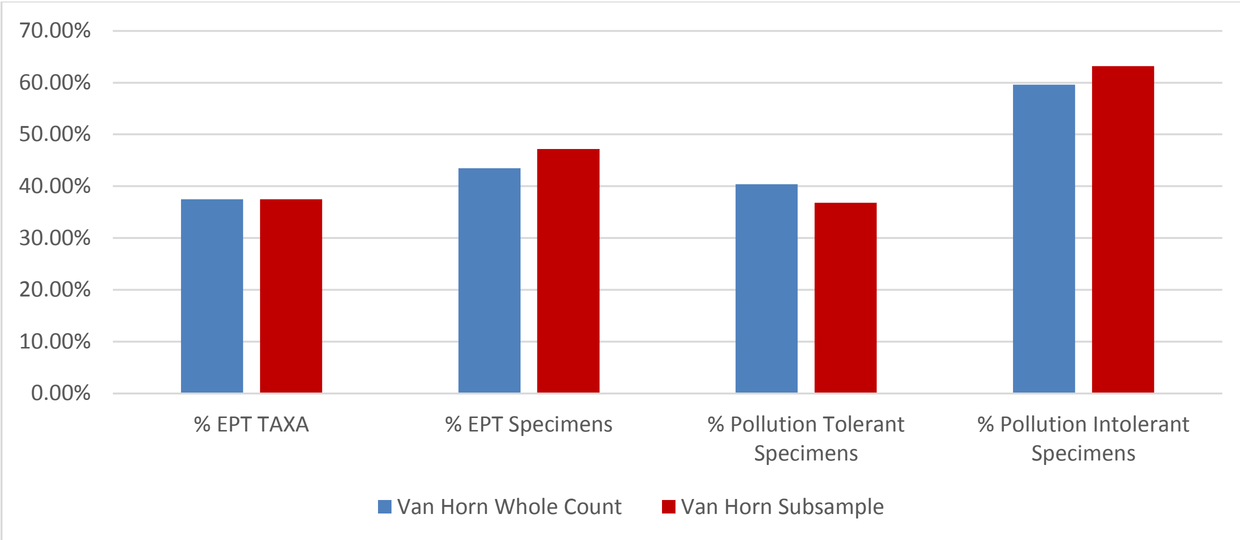

Figure 2. Percentage comparisons of EPT values and specimen tolerance by counting method at Van Horn site showing similar results between methods.

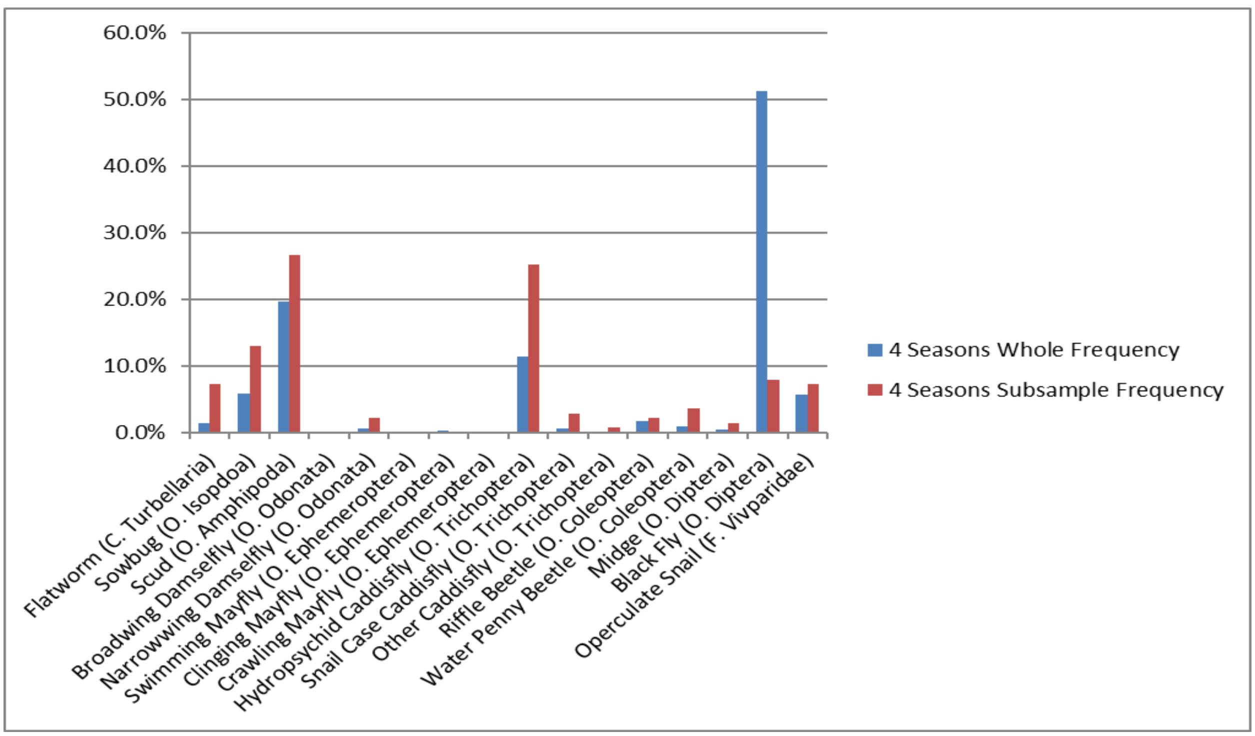

Figure 3. Comparison of individual macroinvertebrate frequency at 4 Seasons site by counting method showing differences between counting methods.

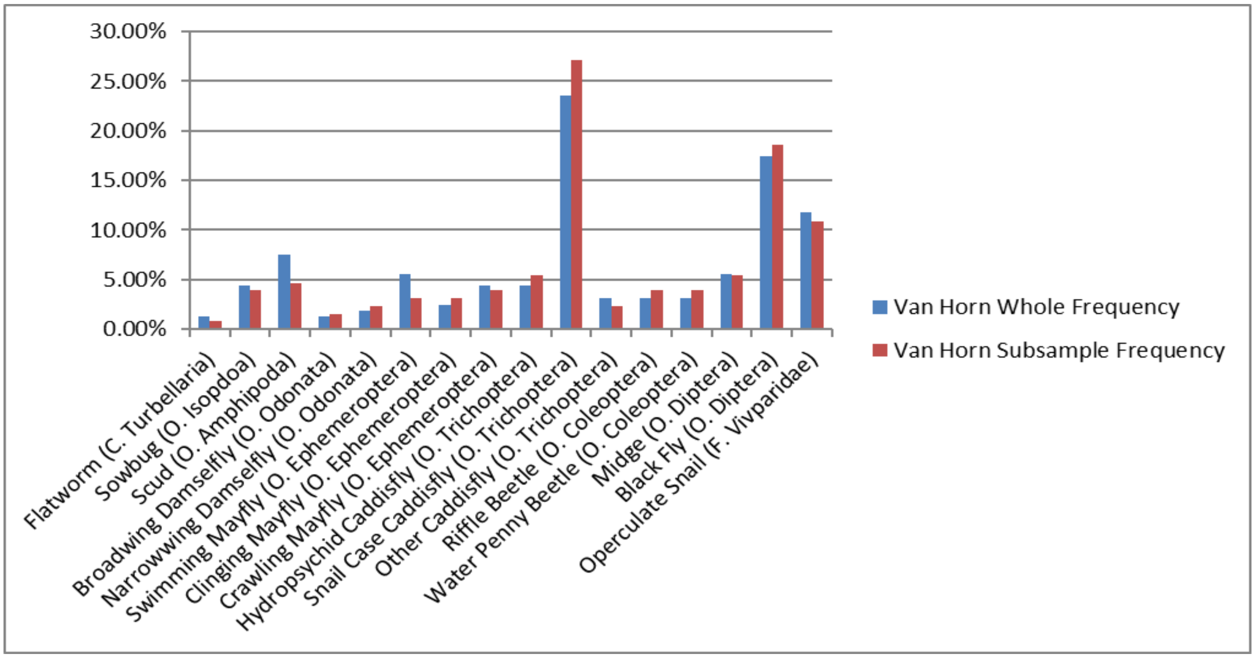

Figure 4. Comparison of individual macroinvertebrate frequency at Van Horn site by counting method showing little difference between methods.

Further analysis using the Shannon Diversity Index (SDI) showed the Van Horn site reflecting a similar diversity for both counting methods. In the 4 Seasons results, the different counting methods reflected different diversity. The predominant macroinvertebrates reflected by the SDI results were mainly pollution tolerant, except for hydropsychid caddisflies and snail case caddisflies. The total SDI values for each site and sample method showed good diversity in all of the samples, well within the 1.5 to 3.5 typical ecosystem range (Figure 5).

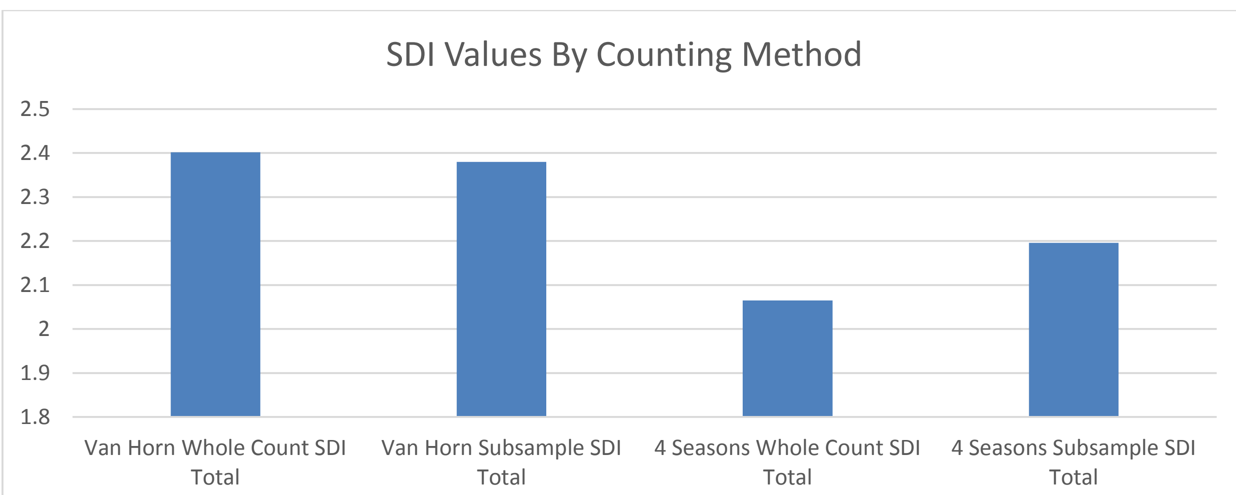

Figure 5. Comparison of total sample SDI values by site and count method showing good diversity.

All indicators of water quality in numbers of EPT and non-EPT, pollution tolerance, and species totals that were Chi-square analyzed for significance between count method showed no significance in the Van Horn comparisons ($p=0.25$ for EPT/non-EPT specimens; $p=0.29$ for pollution tolerant/intolerant species), but a significant difference was noted in the 4 Seasons comparison of the EPT and non-EPT taxa counts ($p< 0.001$), as well as the pollution tolerant and intolerant counts ($p< 0.001$).

4. Discussion

Many factors influence a sample count no matter the method employed: error, sorting location, recent changes in weather and the timeframe the sample count was taken. Each of these can impact the numbers contributing to the data. In order to reduce cost, many states employ fixed-count subsampling methods of varying amounts.[7,8] In some instances, a fixed-count could easily represent the whole as in the instance of the Van Horn results in this experiment. However, as certain taxa increase in a sample, the impact reflects in greater change and different results, as in the 4 Seasons sample results. The increased number of black fly specimens in the full count definitively impacted the results in comparison to the subsample count. The Van Horn results showed identical taxa richness as excellent ($\geq$ 14), excellent EPT taxa richness ($\geq$ 5) and good MBI ($\geq$4.36 - $\leq$5.00) between the two counting methods. The 4 Seasons results changed from good taxa richness (12-13) in the subsample to excellent in the whole count, from fair EPT taxa richness (3) to excellent comparing subsample to whole count, and held a fair MBI ($\geq$5.01 - $\leq$5.70) through both. The MBI quality rating reflecting the proportion of pollution tolerance stayed the same even when the numbers of pollution tolerant macroinvertebrates greatly increased because the numbers of multiple pollution intolerant macroinvertebrates also increased.

The significance reflected by the Chi-square calculations was also impacted by the large increase in these same macroinvertebrates. Some papers indicate that the methods of counting be taken on a case-by-case basis. The quantity of certain specimens in a sample could help in the final decision, but in that instance it would possibly be considered bias due to the amount of work involved in a whole count method with sample sizes that large. It has also been suggested that limiting individuals to 10 of any given taxa in order to prevent domination in sampling would help eliminate bias as well.[5,8] In sampling large amounts of macroinvertebrates, the size of the complete sample may be an indicator of how large a fixed-count subsample should be.

More significant conclusion in this experiment could possibly have been drawn with results obtained over multiple sampling instances. Having limited the results to one sampling at each location, it is difficult to gauge the true value of the comparison in counting methods. Had the results maintained equal measure over multiple sampling events, it would appear that fixed-count methods are at least adequate accurate indices of quality in comparison to whole count methods. Comparing multiple fixed-count methods based on fractions of the whole, for example using a 25%, 50% and higher fixed-count compared to a full count of a large amount such as the 4 Seasons sample might also be another means of comparing the outcome of the two method's results. Using fixed-count sampling has advantages to area-based sampling especially in relation to effort, time and expense resulting from the time it consumes. Finding a standard in all water sampling studies to base fixed-counts on would help to eliminate extra expenses and save time and effort in water quality studies nationwide.

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