Biological Monitoring of Aquatic Ecosystems- Comparison of Biotic and Diversity Indices

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ABSTRACT

Biological monitoring of aquatic ecosystems is a field in ecology that uses stream organisms to indicate the health of a given system. There are multiple methods and indices that have been established to be used as a basis for monitoring levels of pollution in waterways. Macroinvertebrates are most commonly used in biological monitoring for a multitude of reasons. The main reason they are used is because they are susceptible to degradation of water, sediment, and habitat, and therefore serve as good indicators of localized environmental conditions (USEPA). This study examines how adequate the Water Action Volunteers citizen monitoring biotic index compares to more quantified ecological measures such as Shannon's index of diversity. The Root River in Racine and the Pike River in Kenosha were used as sampling sites to compare the two indices being used. Using the macroinvertebrate community, this study showed that both indices were effective in monitoring the health of the rivers. Overall, it was shown that the WAV index, even though an abbreviated method when compared to other diversity indices is an adequate index that should continue to be used in the monitoring of Wisconsin's Rivers.

Introduction

As the human population continues to grow, it has become increasingly necessary for us to monitor and manage our natural resources wisely. Waterways are in constant jeopardy of degradation due to anthropogenic caused problems such as increased pollution levels due to point and non-point source pollutants. There are numerous methods that can be used to assess the health of a river at any given point in time and any type of monitoring can be useful when applied correctly (Ravera 2000). One common approach is to use the macroinvertebrate community within the river or stream to evaluate the environmental condition of the ecosystem. Though there are many biotic and diversity indices that can be used, the recent development of citizen based monitoring efforts has created valuable opportunities for adequate monitoring methods to be conducted with relative ease and convenience. This study examines how adequate the Water Action Volunteers citizen monitoring biotic index compares to more quantified ecological measures such as Shannon's index of diversity.

Literature Review/ Background

Biomonitoring and macroinvertebrates

The traditional water quality monitoring approach has been to collect stream water samples and analyze them in a laboratory for suspected physical and chemical pollutants (Feminella 1999). Unfortunately, because concentrations of pollutants vary greatly with time and location, physical and chemical monitoring alone often cannot detect all pollution related problems within watersheds (Feminella 1999). Additionally, because many of the physical and chemical sampling procedures and analysis are expensive and often labor intensive, biological assessment methods of aquatic environments offer an alternative, often more feasible approach. Biomonitoring, practiced since the early 1900's (Kolkwitz and Marson 1909), incorporates the use of stream organisms themselves, such as macroinvertebrates, as a basis for pollution detection (Feminella 1999).

Of all the freshwater organisms that have been considered for use in biological monitoring, benthic macroinvertebrates are most often recommended (Carter et al. 2007) partly because they are virtually ubiquitous (Hauer and Resh 2007) in the streams and rivers of the world. Benthic macroinvertebrates are small animals without backbones, which are visible to the naked eye and live on the bottom of a lake, stream, or pond for at least part of their lives (Feminella 1999). These organisms inhabit many different habitats throughout streams, such as in spaces between stones, within debris, on logs, or in the sediment (Feminella 1999). The macroinvertebrate community consists of species from numerous phyla including arthropods (insects, mites, crustaceans), mollusks (snails, clams, mussels), annelids (segmented worms and leeches), nematodes (roundworms), and turbellarians (flatworms) (Hauer and Resh 2007). Macroinvertebrates are used for stream monitoring purposes for a multitude of reasons: they are relatively immobile so they can't escape short or long-term pollution exposure (WI DNR), they constitute the majority of species present in streams, responses of many common species to different types of pollution has been established (Carter et al. 2007), and they are relatively easy to sample and identify (Essman and Zarpas 1990). By utilizing macroinvertebrate communities, state, local, and federal agencies have been able to implement biotic protocols as an effective means of sampling stream sites.

Benthic macroinvertebrates are susceptible to degradation of water, sediment, and habitat, and therefore serve as good indicators of localized environmental conditions (USEPA). Certain macroinvertebrates respond differently to the physical, chemical, and biological conditions within a stream, therefore their presence or absence can reflect a stream's general condition (WI DNR). Some organisms are highly pollution tolerant and can thrive under conditions with very low oxygen, while other species of organisms can only thrive under conditions which contain very low levels of pollution. Because of this, these organisms have frequently been coined with the term bioindicators (Feminella 1999). In fact according to the USEPA 2002, 49 of the 50 states in the United States use macroinvertebrates in water quality monitoring (and the 50th is developing a program), whereas only two-thirds of the programs use fish, and only one-third use algae (Carter et al. 2007).

To monitor the macroinvertebrate community both the number of different species and their relative abundance in the community are important (Smith et al. 2006). The simplest biotic measure of a community is a raw count of the number of species that occur within the community, referred to as species richness (Smith et al. 2006) which quantifies the amount of species within a community, but does not give a full representation of the sample community. A more quantified representation can be calculated by finding the relative abundance of each species in the sample. Relative abundance (also known as species evenness) is calculated by counting all of the individuals of each species in a sample within a community and determining what percentage each contributes to the total number of individuals of all species (Smith et al. 2006). This information can then be combined to compute an index of species diversity. Diversity is a valuable tool in assessment of communities because it does incorporate both richness and abundance to give an overall representation of the structure of the community. When conducting a survey of any community it is important to take species diversity (richness + evenness) into account to calculate a credible, representative sample of the community. Because diversity is such an important component of ecology, numerous indices have been created to calculate and assess the diversity of a community.

The many diversity indices are all based on two assumptions: a) stable communities have a high diversity value and unstable ones a low diversity, and b) stability, and thus diversity, is an index of environmental integrity (Ravera 2000). As a consequence, the diversity values usually decrease with environmental degradation and serve as a valuable indicator of pollution evidence (Ravera 2000).

Rapid Bioasessment Protocols, Diversity Indices, and Biotic Indices

Biological indices which incorporate concepts such as biological diversity and integrity provide important and ecologically dynamic measures of ecosystem health (Karr 1991). Ecologists, therefore, have developed many indices of species diversity (Molles Jr. 1999). One of the simplest and most widely used indices of diversity is the Simpson's index (Smith et al 2006). Simpson's index measures the probability that two individuals randomly selected from a sample will belong to the same species. Ravera (2000) found that to small differences between communities could be demonstrated by a diversity index with high discriminant ability, such as the Simpson's index. Another commonly applied measure of species diversity is the Shannon index of diversity which is possibly the most widely used diversity index in community ecology and is sometimes referred to as the Shannon-Wiener Information theory index. The Shannon Index is a composite index that takes both species richness and evenness into account in order to calculate an effective evaluation of the community being studied. This index gives an ecosystem a score for diversity and is most applicable to use when comparing multiple systems of similar composition. Indices based on diversity, such as Shannon's or Simpson's indices, may require more extensive lab quantifications and organism identification, but very often supply the researcher with more information on the overall community structure of the ecosystem when compared to a biotic index that focuses solely on species richness.

The concept of using the indicator species to evaluate the water quality of streams is one of the oldest assessment methods, having been used by Kolkowitz and Marsson (1909), Richardson (1928) and many others more recently (Hilsenhoff 1977). However, Chutter (1972) is one of the earliest to develop a biotic index that made use of the indicator species concept (Hilsenhoff 1977), where species were assigned a value based on pollution tolerance. Chutter (1972) assigned values to species based on where they were found (0 to those found in only the cleanest streams and a value of 10 to those species that could inhabit extremely polluted waters. In 1977, Hilsenhoff examined that the collection of macroinvertebrates provided excellent opportunities to evaluate diversity index and biotic index values as a means for evaluating water quality. Since then, large strides have been made in the world of biological monitoring.

The development of more cost-effective biological monitoring strategies has come to be known as “Rapid Bioassessment” (Lenat and Barbour 1994). Specifically, the goal of a “Rapid Bioassessment Protocol” is to expend the minimum amount of effort required to obtain reproducible, scientifically valid results (Lenat and Barbour 1994). In a 1998 USGS study, Kennen found that the introduction of Rapid Bioassessment Protocols by the EPA has provided local agencies with a cost effective framework for assessing the biological communities found in aquatic ecosystems. Biotic indices, which evaluate macroinvertebrate community structure, are widely used (Wallace 1996) and more than 90 different methods based on classification of lotic communities for the assessment of water quality are used (Ghetti and Ravera 1994). Many indices are exclusively adapted for use in specific regions and areas of the world. Examples of these indices include the North Carolina Biotic Index, the Trent Biotic Index, and the Family Biotic Index to name a few. A group of Wisconsin scientists from the DNR, the University of Wisconsin-Extension, and the University of Wisconsin-Madison designed the Citizen Monitoring Biotic Index specifically for use in streams and rivers in the state of Wisconsin. Chutter (1972) and Hilsenhoff (1977) found biotic indices did an excellent job of ranking streams according to water quality and helped illustrate that these indices can be very reliable ecological tools. It is now understood that biological organisms are diagnostic in determining the health of aquatic ecosystems and they can be measured quantitatively (Loeb 1994). To this extent, the presence (or absence) of some taxa is used to calculate species richness within various tolerance classes, that can be combined into a single indicator of the pollution level of the water body (Ravera 2000). Methods based on species richness work relatively well without needing an extensive amount of lab analysis because it is not required to count raw numbers of the organisms collected.

One widely used biotic index is the EPT index which focuses on the taxonomic richness of three intolerant taxa (Ephemeroptera, Plecoptera, and Trichoptera) to conduct a survey of a stream. These organisms: mayfly (Ephemeroptera), stonefly (Plecoptera), and caddisfly (Trichoptera) are insect orders that are widely used because of there known sensitivity to pollutants. Because the EPT index is a sensitive indicator of stream perturbations it is widely used by many agencies as part of their environmental monitoring programs (Wallace 1996). The EPT effectively uses these orders of insects as indicators of pollution and gives a relatively accurate evaluation of the stream or river being tested. Another index that is used by many ecologists to sample streams and rivers is the Hilsenhoff Biotic Index (HBI). This index identifies organisms down to the genus or species level, so only experts were able to use this index (WI DNR). Hilsenhoff emphasized that species identification enhances the sensitivity of a biotic index and is essential in the detection of minor degrees of pollution or disturbance, but in the case of detecting significant disturbances of pollution such a precise level of identification is unnecessary. Although very accurate, this index was very tough to use outside the lab setting, so less complex indices have been derived from this index to allow the involvement of a broader group of water monitors, such as the Citizen Monitoring Biotic Index in Wisconsin. The selection of an appropriate sampling strategy will depend on many factors including the purpose of the survey, the methods used by nearby monitoring groups, the expertise of collectors, preference for qualitative versus quantitative data, and the characteristics of streams in a particular geographic area (Lenat and Barbour 1994). An experimental study done by Wallace et al. (1996), demonstrated the potential of biotic indices such as the EPT index to detect and monitor stream ecosystem changes during and following disturbance. In their study, Wallace et al. (1996), experimentally manipulated the invertebrate community in a stream and investigated the ability of numerous indices to track these manipulations and alterations. They found that the EPT index was the easiest to use in relation to time spent and actual application of sample processing. The study strongly supported the inclusion of biotic indices into their regions stream monitoring programs to be used as indicators of environmental degradation.

Water Action Volunteers

One example of a frequently used biotic index in Wisconsin is the Water Action Volunteers (WAV) citizen monitoring biotic index which is a cooperative program between the University of Wisconsin-Extension and the Wisconsin Department of Natural Resources. This program encourages volunteers to become “citizen scientists” by helping to monitor their local waterways by taking an active role in their community. WAV focuses on five parameters to conduct an assessment of water quality, one of which is a biotic index. Once volunteers have a sample site, they collect macroinvertebrates from as many habitat types as possible (riffles, pools, undercut banks, snags, and leaf packs) by using dip nets and kick seine nets (if available). They then identify and classify the organisms on site using the citizen monitoring biotic index visual key that is provided by the WAV. This index was developed from the Family Biotic index (FBI) which is a less specific index that classifies organisms down to the Family level and was designed specifically for use with Wisconsin rivers. This index focuses solely on presence or absence of organisms when conducting and tallying the score for the stream. The index is an assessment of species richness and does not take into account abundance and therefore does not calculate a score for species diversity. When using the WAV protocol, all identification is done on site and then all organisms are promptly returned to the river or stream. WAV emphasizes the importance of quality control while conducting an adequate assessment of a stream or river. In the data sheet series, WAV defines quality control as: assurance that data are accurate by using a series of checkpoints to review procedures before the information is entered into a database. It is important to take this component of the protocol into consideration to ensure that the data being collected will be beneficial to the WAV program. Data is then sent in to the Water Action Volunteers and compiled on a database. Hilsenhoff (1977) found that biotic indices have great potential for quantitatively evaluating invertebrate fauna of streams in relation to water quality. More recently Ravera (2000) found that small differences between species associations are better highlighted if diversity indices that incorporate species abundance distribution are used rather than biotic indices.

This study tests whether citizen based monitoring methods (biotic indices) give an adequate assessment of waterways when compared to more quantifiable ecological methods (diversity indices). Ravera (2000) exemplifies that there is a very rich literature on the subject matter of biotic and diversity indices, but very few comparisons of these methods have been made. It becomes difficult to choose a correct index because there is no permanent agreement on the adequacy of any one universal index to be used as the standard model for biological monitoring. This brings up the question of comparing biotic and diversity indices by using the same set of data. By using two local river systems in Southeastern Wisconsin, this study will examine whether more detailed indices such as Shannon's index of diversity are required for an adequate evaluation of a stream's health, or will a biotic index such as the WAV Citizen Monitoring Biotic Index provide essentially similar results in relation to pollution levels.

Methods

Study Area

The Root River and the Pike River were used as sampling sites to obtain all of the data for this study. The Root River is a river system in Racine, Wisconsin that drains into Lake Michigan in Racine County. The Pike River is a river system that runs through Kenosha County, Wisconsin and drains into Lake Michigan south of Carthage College campus (maps attached in Appendix). The sampling site used on each river was chosen in accordance with the Water Action Volunteers (WAV) parameters for choosing a proper monitoring site. This means that the sites were 300 foot stretches of river that were easily accessible and wadable and had areas with examples of pools, riffles, and undercut banks. According to WAV, it is ideal to choose a site that seems to be a good representation of the entire stream being sampled and that was the goal of this study. The site on the Root River was located at Colonial Park in Racine and the site on the Pike River was located behind the Carthage Smeds Tennis Center. The Root River site was along a country club and the Pike River was near a commuter parking lot for Carthage students. Both areas had examples of riparian vegetation along the stream banks.

Water Action Volunteers

Between the fall of 2005 and the spring of 2007 weekly field trips were coordinated with Racine Montessori School and Kenosha Armitage Academy to the Root and Pike River, respectively. The students varied in age from 5th to 7th grade and groups on field trips ranged in size from seven to twenty students at a time. On these river trips, all of the parameters of the WAV series were carried out and sampled, but here, only the biotic index is the focus. To conduct the biotic index three samples within the 300' section were taken from as many various habitat types as possible. Mainly samples were taken from riffles, undercut banks, and snags/leaf packs. All samples taken from a riffle were combined to create one samples and the same was done for undercut banks and snags/leaf packs. This left us with three samples from three different habitats. Kick-seine and dip-nets were used for the collection of the macroinvertebrates and then they were sorted in trays at the sampling site. The students were supervised while sorting invertebrates to ensure that all of the identifications were correct. Afterwards, all organisms were returned to the streams.

All macroinvertebrates that were found in the samples were combined and then circled on the biotic index recording form in reference to four pollution tolerance groups. Group one contains organisms that are sensitive to pollutants, including stonefly larva, dobsonfly larva, alderfly larva, water snipe fly larvae. Group two contains organisms that are semi-sensitive to pollutants, including caddisfly larva, dragonfly larva, water penny beetle, crayfish, crane fly lava, freshwater mussels/clams, mayfly larva, damselfly larva, and riffle beetles. Group three contains organisms that are semi-tolerant of pollutants, including black fly larva, non-red midge larva, orb or gilled snails, and amphipods. Group four contains organisms that are tolerant of pollutants, including pouch snail, isopod, bloodworm midge larva, leech, and tubifex worms. The WAV protocol focuses only on the presence or absence of the types of individuals within these groups of invertebrates and does not tally any organisms that are not on the list. The biotic index gives us a score of species richness to calculate a score for the river or stream. The calculations for the index go as follows:

$$\text{WAV Index score} = \frac{4A + 3B + 2C + D}{A + B + C + D}$$

Where A is the number of animals from group one (sensitive), B is the number of animals from group two (semi-sensitive), C is the number of animals from group three (semi-tolerant), and D is the number of animals from group four (tolerant).

The point total is then used to classify the rivers quality by this scale: excellent - 3.6+, good – 2.6-3.5, fair - 2.1-2.5, poor – 1.0-2.0, (Data Sheet Attached in Appendix)

Independent Survey

An additional survey was performed at these same sites on September 29, 2007. Samples were collected using kick-seine and dip-nets as well. Multiple areas were sampled within the river stretch which included riffles, undercut banks, and leaf packs. Three kick-seine samples were taken from each river and 6-8 dip-net scoops were taken from each river site. The same collection methods that were used for the WAV methods were used for this phase of sampling. For sorting purposes, all samples were collected in buckets and taken back to the ecology lab at Carthage for analysis. This set of data was also used to calculate the WAV biotic index to keep a consistency in the data used to compare the two indices. The data from the collections with the kids was taken into account to look for trends but was not used in the comparative analysis of the diversity and biotic index values.

Independent Survey Laboratory Analysis

All of the macroinvertebrates were sorted down to the lowest practical taxon and the number of each macroinvertebrate was counted and tallied on a data sheet. Dissecting scopes and field guides were used to aid in the identification of all macroinvertebrates. For this analysis, there were no limitations on the macroinvertebrates included in the quantification and therefore all organisms found were factored into the index. To calculate the diversity index for these samples the Shannon Diversity index was used. The Shannon index is calculated as: $\mathbf{H` = \Sigma\, p_i(lnp_i)}$**,** where p_i = proportion of species i, and (lnp_i) = natural log of the proportion of species i.

Results

The Shannon diversity index scores were 2.16 for the Pike River and 2.20 for the Root River. Scores for Shannon’s index of diversity usually range between 1 and 3.5. The Water Action Volunteers citizen monitoring biotic index score was 2.2 for the Pike River and 2.1 for the Root River. See Table 1. The score for each river in the WAV biotic index indicates a fair quality river or stream. The Pike River had seventeen observed species in its samples and the Root River had twelve observed species in its samples giving the Pike River a higher count of species richness. The most abundant taxa found in the Pike River were Asellus (sowbug) and Elmidae (riffle beetle) while the most abundant taxa in the Root River were Asellus (sowbug) and Odonata (damselfly). Refer to Table 2 for a partial list of abundant species in each river. Taxons used in Table 2 were chosen because of their abundance and their known tolerance levels to pollution. The Shannon diversity scores for each river were very similar values and the scores from the WAV index were almost the exact same for each river. We are able to see that there is a very similar level of diversity in each river as well as an equivalent measure of stream health according to the WAV biotic index standards. A proportion of the taxon collected based on pollution tolerance levels is shown in Figure 1, which was compiled using mostly pollution tolerance levels from the WAV factsheet series, but some organisms not included in the WAV index were given tolerance values based on other parameters. (Raw data in appendix).

TABLE 1. Index Scores

	Pike River	Root River
WAV Index	2.2 (FAIR)	2.1 (FAIR)
Shannon Index	2.16	2.20

Taxon collected	Pollution Tolerance (WAV standards)	Pike River # (%)	Root River # (%)
Mayfly (Ephemeroptera)	Semi-sensitive	3 (1.4)	4 (4.6)
Damselfly (Odonata)	Semi-sensitive	13 (6.4)	14 (16.1)
Caddisfly (Trichoptera)	Semi-sensitive	18 (8.9)	9 (10.3)
Riffle Beetle (Elmidae)	Semi-sensitive	41 (20.3)	8 (9.2)
Aquatic Sowbug (Asselus)	Tolerant	63 (31.2)	18 (20.7)

Tubifex Worm (Tubificidae)	Tolerant	16 (7.9)	4 (4.6)
Bloodworm Midge Larvae (Chironomidae)	Tolerant	16 (7.9)	11 (12.6)

TABLE 2. Partial list of abundant taxon found in samples of independent survey

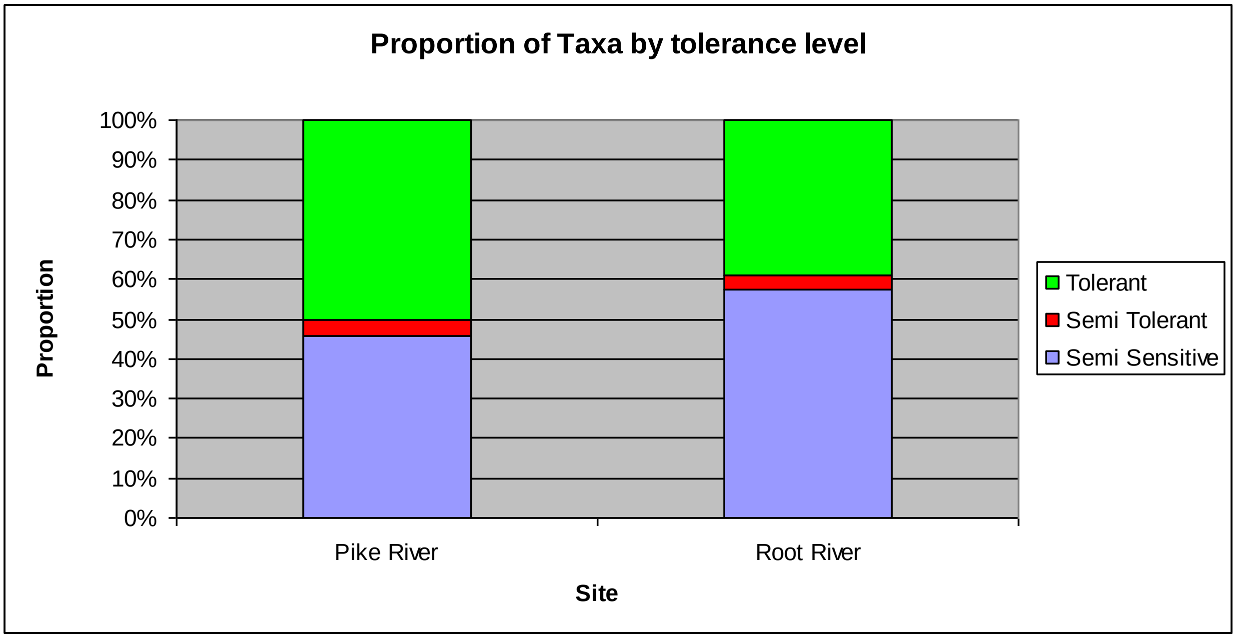

Figure 1. Proportion of taxon collected by pollution tolerance levels (Tolerance levels based on WAV standards)

Discussion

This study demonstrated that both of the indices used for this study were adequate methods of evaluating a river or stream. The results of this study suggest there are many adequate methods and indices that can be used to assess and evaluate the health of rivers and streams and methods that use rapid assessment not only save time, but offer a quick, easy and effective means of stream monitoring. The results showed us that there were very similar levels of diversity in both rivers and the same "health" score for each river as delineated by the WAV protocols tally score. Because the rivers are so geographically close to one another it is not surprising that the spectrum of invertebrates found in the two rivers were very similar, but the Pike River did have more species richness with seventeen species found compared to twelve in the Root River. We would assume that since the Pike River had a higher number for species richness that it would most likely have a higher value in the WAV index because the WAV index is based solely on species richness. However, the results from the WAV index gave the two rivers the same score because of the slightly different proportions of species based on tolerance levels and because the index only focuses on the 23 indicator species on its data tally sheet. This is one problem that was encountered when conducting the WAV biotic index- there are so many species present in streams and the exclusion of any organisms found might end up hindering the overall assessment of the river. In this study it did not reflect a lower score on the WAV biotic index, but this is definitely a drawback to consider when using the WAV index to evaluate the health of a river.

It is important to note that the exact same set of data was used to calculate both indices Therefore the collection methods for the WAV index and the independent survey were the same to ensure that the comparison was focused on the indices and not the collection methods themselves. Active sampling methods (kick-seine and dip nets) were used instead of passive methods, such as Hester-Dendy samplers, because the WAV index uses these methods and the use of passive sampling methods in the independent survey would have altered the basis for comparison between the indices. The Hester-Dendy sampler is a substrate sampler that is sits on the bottom of a river over a period of a few weeks and organisms will inhabit this sampler because it provides a suitable habitat. This type of sampler was not included in the experimental design because this study was looking at the adequacy of rapid testing protocols and the use of a Hester-Dendy sampler could skew any of the results because it is a passive sampling method that takes a longer period of time. The use of two separate rivers in this study also helped with the comparison of the methods because this way a comparison was built on the values being calculated in the different indices. Since the Shannon Diversity index and the WAV biotic index both give numerical scores for the community being sampled, having more than one site to compare helps to quantify the results. In this sense, we would expect a stream with a better "health" score to also have a higher value for diversity. We did see a similar trend in this study because the scores from both indices were very similar for both rivers.

Only data from the fall season was used in this study which might not be the most representative sample of the river. There is a large amount of seasonal variation in the macroinvertebrate community (Carter et al. 2007) which may complicate interpretations and/or comparisons. This being said a larger more in-depth analysis that sampled over a much longer period of time would be beneficial because it could take into account seasonal changes and life cycles of macroinvertebrates. The short life cycles of many species in the river is an advantageous aspect of macroinvertebrates as bioindicators because they may provide reliable and rapid evidence of water quality before or after a pollution event (Feminella 1999). If there were more time to develop this study, it would be desired to sample over a period of a few years and to include passive sampling methods to account for seasonal changes in community structure at the macroinvertebrate level.

Carter et al. (2007) illustrate many difficulties to consider when using macroinvertebrates for biological monitoring: seasonal variation may complicate interpretations or comparisons, there may be too many methods for analysis available (how does one pick the "right" procedure). Furthermore, certain groups of invertebrates are not well known taxonomically, some benthic macroinvertebrates may not be sensitive to some perturbations such as trace amounts of certain pollutants, and there are poorly established relationships between many specific stressors and commonly used metrics. This study specifically focused on the aspect of how one picks the “right” procedure to monitor a river. Choosing an appropriate sampling strategy will depend on many factors that must be considered to select a suitable method of evaluation. Lenat and Barbour (1994) highlight some of the most important factors to consider: purpose of survey, techniques used by local monitoring groups, location of streams, and the experience of the collectors. The WAV index and Shannon’s index of diversity were both logical to use in this region and could be completed with relative ease while still supplying discernable scores for each river. Also the WAV index is what is implemented by agencies in Wisconsin, so it was chosen to see how well it worked in comparison with Shannon’s index of diversity.

Hilsenhoff (1977) also brings up the point that many small cold streams have a naturally low diversity that is entirely unrelated to pollution. Small streams typically have lower diversity values than larger streams in similar habitats with similar substrates, which may lead to erroneous conclusions about water quality if evaluated with diversity indexes (Hilsenhoff 1977). This is an important point because we can see that sometimes biotic or diversity indices may not be appropriate to evaluate pollution, no matter which one is chosen. There are other environmental factors to always be considered when evaluating the condition of a river, stream, or any ecosystem for that matter.

Another problem that arises in assessing communities based on sampling sites is the problem of sample representativeness (Cao et al. 2002) when compared to the actual makeup of the larger ecological community. Cao et al. (2002) found that obtaining an adequate, representative sample of ecological communities to make compositional comparisons among sites is a continuing challenge. Furthermore, Cao et al (2002) points out that although randomization in the collection of sample units is often used to assure that sampling is representative, randomization does not convey the concept of how well samples represent the community or the site from which they are drawn, especially in an aquatic system. This brings up the question: does a sample taken from a random location in the stream necessarily represent the health or diversity of the entire river system? This study was not able to measure or explore this concept because of time constraints, but this is definitely a topic in science that many ecologists must address in order to evaluate a community accurately and effectively. One possibility would be to accumulate numbers over a much longer period of time and from many more sites along the river. With information from a longer period of time and from a larger pool of sampling sites, the problem of representativeness could be addressed more properly. Another aspect of sample representativeness to consider is how much a river system is constantly changing based on its order (first order, second order, etc) and its geographic location in relation to run-off and other components that will affect the waterway. Stream order, urbanization, and surrounding land use are factors that must be considered to create an accurate evaluation of the pollution level in any river.

Another point to consider is that monitoring of an aquatic system is an ongoing integrative process that cannot be fully exhausted by focusing on one aspect of a community, regardless of how important in the trophic level that community is. Paulsen and Linthurst (1994) point out that just as human health is not assessed by a single measurement, the status of each environmental value of concern for surface waters must be also assessed by a multitude of indicators. Karr (1994) points out that biological monitoring does need a firm foundation that must always incorporate the most up to date ecological principles, but monitoring approaches based on ecological principles should never be so rigorous that knowledge of a special circumstance at a site cannot be applied to provide a more effective biological evaluation. The problem of quality control is also an ongoing challenge that must always be addressed in biological monitoring. Karr (1994) states that only highly trained and skilled professionals should collect and interpret data for monitoring purposes to ensure quality assessment calculations. However, Carter et al. (2007), state that the quality of data obtained by volunteers using good equipment (proper nets and microscopes) and adhering to accepted protocols can be very similar to data being collected by professionals when the same techniques are followed. This being said, the WAV address this challenge very effectively by defining quality control in their data sheet series and providing lots of opportunities for stream monitors to become educated by attending training programs to learn the quality assurance procedures of the program. By following these steps WAV and the citizen monitor address a very relevant problem in the field of biological monitoring and allows the spectrum of water monitors to be much larger providing a much larger database of information on the health of Wisconsin's rivers and streams.

Conclusions and Future Direction

Overall, this study exemplified that both indices used were credible monitoring methods that provided valuable information on the macroinvertebrate communities in the river.

The Pike and Root Rivers both had intermediate levels of diversity based on Shannon's index of diversity and health scores of *fair* based on the WAV scoring parameters. This was an important study because it illustrated that Rapid Bioassessment protocols set up by local agencies provide efficient, easily conducted evaluations of rivers. We were able to see in this study that in many cases it is beneficial to uses a rapid protocol, such as the WAV Citizen Monitoring Biotic Index, because it takes less time and provides us with very similar results when compared to more quantified ecological measures, such as Shannon's index of diversity. By following the protocol closely and following the WAV's design of quality control this biotic index provides great opportunities in the field of biological monitoring. The WAV citizen monitoring biotic index may not be as in-depth of an assessment criteria when compared to an index such as Shannon's index of diversity, but when implemented correctly, it is a very effective biological monitoring tool.

Because the fields of biological monitoring and aquatic ecology are constantly evolving, it is important that the methods and indices in these fields are also evolving with the ongoing changes that we are seeing in the world's river systems. Karr (1994) states that biological monitoring should be based on the most up to date ecological principles, but it is important that no protocol is so strict as to deny the input of any external knowledge that may benefit an overall ecological evaluation of an ecosystem. That being said, this study leaves the door open for many future considerations to explore in the field of biological monitoring. To create a more elaborate comparison of biotic and diversity indices there are certain improvements that could have been applied to this study. It was observed in this study that biotic indices and diversity indices were both valuable tools for describing the macroinvertebrate communities in the river, but it would be very interesting to look at a study like this that lasted over a period of a few years. By doing this, the seasonal variation and life cycles of the macroinvertebrate community could be used to supplement a stronger analysis of the communities in the river and possibly draw deeper conclusions on the adequacy of the indices being evaluated. It would have also been beneficial to possibly use other sampling types besides those described in the WAV protocol. The inclusion of passive sampling methods, such as Hester-Dendy samplers, would provide another layer to the analysis of the rivers being studied and would possibly alter the results of the indices. The inclusion of new sampling methods or other indices to be compared would definitely be worthy supplements to the study and progression of biological monitoring.

There will always be room for improvement and modification of methods in the field of ecology and it is important to create and implement biological monitoring protocols that meet this criteria. The WAV Citizen Monitoring Biotic Index is an adequate tool in the field of biological monitoring that is implemented effectively and it should continue to be used in the monitoring of Wisconsin's Rivers.

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