Development of a Benthic Index of Biotic Integrity

Development of a Benthic Index of Biotic Integrity for Freshwater Systems in San Juan, Puerto Rico

Sponsored by The Conservation Trust of Puerto Rico (Fideicomiso)

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Chapter 1: Introduction

Populous islands place increased demands on natural resources, leading to the destruction of many natural habitats that protect valuable aquatic systems. This is especially true of freshwater sources, which suffer from urban encroachment, contamination from industrial and agricultural chemical run-off, and drought from overuse. Often one considers pollution on a local scale, but one rarely remembers to consider the impact of pollution on watersheds in extended zones. The limited size and availability of island watersheds, essential for sustaining natural habitats and providing potable water, amplifies the stresses imposed by human activity. Hence, severe pollution is of even more concern to an island watershed than to a watershed on a larger landmass because the effects of pollutants are magnified through smaller watershed systems.

Puerto Rico is a small, densely populated island with significant concerns for watershed health. Recent research indicates that Puerto Rico has considerably more damaged estuary systems than any other major region of the United States, leading to general concerns of watershed health (Borja et al., 2008). The poor ecosystem health in Puerto Rico is largely a result of limited conservation on the island and low public awareness of environmental health. Currently, a mere 7.2% of the land and water of Puerto Rico is actively protected. This is minimal in comparison to other Caribbean countries such as Costa Rica with 34% of the land protected, the Dominican Republic with 42% of the land protected, and the U.S. Virgin Islands with 54% of the land protected (The Conservation Trust of Puerto Rico, 2007). Therefore, it is critical that efforts be made to conserve and restore water sources in Puerto Rico, and to implement adequate monitoring.

The Rio Piedras watershed, located near the capital city of San Juan, suffers from the negative impacts of urban human activity. The First Aqueduct in Puerto Rico is located on the Rio Piedras and previously provided fresh drinking water to the area. Unfortunately, increasing urbanization in the 1960s polluted the river, which has since been surrounded by urban features and significantly eroded. These damages ultimately lead to closure of the aqueduct to the public for any use (The Conservation Trust of Puerto Rico, 2007).

The Conservation Trust of Puerto Rico, also known as Fideicomiso or simply “The Trust”, is an organization that has obtained and protects over 18,000 acres of land that has historical, cultural, or geological significance to Puerto Rico. Fideicomiso works to save and restore the splendor of the island and since its creation in 1970 has begun the restoration of vital environments, including areas of the Rio Piedras watershed such as the First Aqueduct of the Rio Piedras. Once reopened, the First Aqueduct will serve as a source of potable water again and operate as a park to educate the public about environmental conservation (The Conservation Trust of Puerto Rico, 2007). Currently, the First Aqueduct restoration is a model for future restorations The Trust intends to initiate on other properties it manages.

The Conservation Trust currently lacks metrics specific to Puerto Rico that will accurately describe the health of watersheds in their possession or that Fideicomiso seeks to acquire in the future. Past assessments of watershed health have studied the prominence of flora in the vicinity of the First Aqueduct and have also carried out basic chemical testing of water at a small number of sites. These studies ultimately resulted in a conservation management plan for the First Aqueduct of the Rio Piedras and were a significant step towards the restoration of that property (Zabinski et al., 2009). However, Fideicomiso has not been able to describe the quality of the water and aquatic species in any area that it currently owns. Therefore, The Trust is currently unable to properly evaluate the effects of the organization’s restorations on watershed health.

The goal of this project is to develop a multi-metric index that will measure the integrity of water sources in Puerto Rico and accurately reflect anthropogenic stresses on watersheds. This investigation will develop an Index to Biotic Integrity (IBI) that the research team will create through data analysis of biological surveys performed on streams in the San Juan area. IBIs have been popular methods for assessing the health of bodies of water since their conception in the 1980s by Dr. James Karr, Professor of Biology at the University of Washington (J. R. Karr, 1981). The underlying concept of this index is that all parts within a given ecosystem are interconnected, so the presence of certain species serves as a useful indicator for the overall health of the region. These species include fish, algae, plankton, and invertebrates. The abundance, diversity, and condition of these indicator species are used to classify the state of the watershed (U.S. Environmental Protection Agency, 2009).

Data that the team will collect, as well as additional data that the team will acquire from the University of Puerto Rico (UPR), will be used to formulate the Benthic Index to Biotic Integrity (B-IBI) for Fideicomiso. The technique of modifying an IBI is very common, but it is unacceptable to use when the ecosystem of study differs even slightly from the original ecosystem used to develop the IBI (Bedoya, Novotny, & Manolakos, 2009; Chainho, Costa, Chaves, Dauer, & Costa, 2007; Kerans & Karr, 1994; Seegert, 2000). To date, no IBI has been developed for Puerto Rico; thus, we will select our indices directly from the data we accumulate. If the development of the B-IBI is successful, the tool will accurately correlate the state of the benthic invertebrate system of a given river with the amount and severity of anthropogenic disturbances upstream and in the surrounding region. Ultimately, this tool will allow The Conservation Trust to determine the areas of highest priority for conservation and restoration in Puerto Rico. With an Index to Biotic Integrity, The Trust will be in a better position to understand the health of watersheds and to protect the land and water of Puerto Rico.

Chapter 2: Literature Review

In the following section, the background for this study will be detailed, including aspects of the ecosystem and fauna that the Interactive Qualifying Project team will study in Puerto Rico. In addition to this, this section details other relevant research performed by biologists, ecologists, and consultants in order to more firmly establish the scientific principles and commonly accepted techniques in designing an IBI. This section pays special attention to the manner in which the studies herein are conducted in order to build a more comprehensive picture of the steps that the team will need to take in order to correctly develop an IBI.

2.1 Introduction

In conjunction with spreading urbanization, the lack of effective water conservation plans in Puerto Rico has led to necessary updates in water preservation. Since the 1940s, Puerto Rico has developed rapidly through industrialization, mostly due to a combination of urban migrations and the downfall of the once thriving agricultural-based economy. At one point, 90% of the 8,900 square kilometers of Puerto Rico had been deforested (Ramirez, De Jesus-Crespo, Martino-Cardona, Martinez-Rivera, & Burgos-Caraballo, 2009). Conservation efforts have since furthered appreciation of natural environments, though a deeper understanding by the public and scientific community is imperative for their protection.

2.1.1 Trust History

The environmental degradation listed above as well as pollution resulting from urbanization and industrialization led to the creation of The Conservation Trust of Puerto Rico (also known as Fideicomiso) in 1970, in an effort to effectively conserve important areas of Puerto Rico. It has acquired twenty different sites totaling approximately 18,000 acres. One of these areas includes the Rio Piedras, which is located in San Juan (The Conservation Trust of Puerto Rico, 2007).

The health of the Rio Piedras is shaped by both natural and anthropogenic factors as it runs North through the city of San Juan. Negative urban impacts such as poor sewage removal and point-source pollutants prove harmful to water systems (Walton, Salling, Wyles, & Wolin, 2007). The influences of urban environments surrounding the watersheds explain to a certain extent the degradation of these rivers, but these areas are also affected by natural influences. Variations in rainfall, elevation, and temperature throughout Puerto Rico are examples of naturally occurring environmental interactions that affect the river. For example, higher elevation combined with lower average temperatures throughout the year tends to result in more rainfall, as in the Luquillo Mountains of Puerto Rico. San Juan, however, is located on the coast, with higher average temperatures and at an elevation close to sea level (Daly, Helmer, & Quinones, 2003). The effect of these features is less rainfall than in much of Puerto Rico as well as very little variance in the rainfall throughout the year.

So it can be shown that several factors influence the health of rivers, both metropolitan and natural. Unfortunately, the continuous urbanization of San Juan has surpassed management efforts of freshwater sources, causing recurrent problems with wastewater, drinking water, and the overall ecology of the environment. To better understand the health of the river, an overall understanding of ecology in the region is essential.

2.1.2 Index of Biotic Integrity: A Background

Dr. James Karr, a professor of biology at the University of Washington, invented a method to help advance the conservation and safeguard these valuable places. This method is the Index of Biotic Integrity (IBI), which is defined by the Watershed Science Institute to be an "indexing procedure commonly used…to assess watershed condition…[and] the effect of human disturbance on streams and watersheds" (United States Department of Agriculture Natural Resources Conservation Service, 2007). The IBI was invented in 1981 by Dr. Karr as a means for quantitatively assessing the health of ecosystems by using specific indicator species as samples. Karr used fish as the indicator species of the health of the system (J. R. Karr, 1981). Those not affected by humans contained the most diverse amounts of fish species, including those which are very susceptible to harm from their environments. Originally, twelve aspects of the fish community were observed. These twelve were given scores ranging from one to five. Five was considered the least affected by humans, while one was the most affected (U.S. Environmental Protection Agency, 2009). The low scoring regions contained few and abnormal fish, most of which were not especially vulnerable to the environment (J. R. Karr, 1981). This determination of water system health has since been updated and conformed to different locations around the world. Fish species are no longer the sole indicators of health, but others, such as benthic macroinvertebrates and algae, have also been included (U.S. Environmental Protection Agency, 2009).

For our study, we will be using strictly benthic macroinvertebrates as indicators to the health of freshwater systems. Benthic is defined simply as the lowest possible ecological region in a body of water. For streams and rivers, this includes the bottom sediment layer as well as the habitat underneath the surface of the sediment layer. Benthic macroinvertebrates are excellent health indicators in freshwater systems because of their crucial role in the food chain, as macroinvertebrates are a necessary source of nourishment for other organisms to survive. Certain indicators of macroinvertebrate populations, such as abundance, diversity, and overall health, correlate to the health of water source in which the macroinvertebrates live. These indicators can then be measured comparatively against similar freshwater streams with the same ecological parameters to determine the overall health of one system in relation to the other. The symposium of several comparisons as described above is commonly termed a multi-metric index. A biological multi-metric index, incorporating comparisons between various aspects of the macroinvertebrate population of streams in Puerto Rico could help The Trust evaluate the physical condition of each of its sites.

2.1.3 Data Collection

To a large extent, the results of macroinvertebrate samplings depend significantly upon how researchers collect, store, and analyze samples. The final outcome of any biological study is influenced heavily by the manner in which data is collected. Greg Seegert states that "with regard to data collection, a degree of standardization is necessary. Procedures should be developed that tell an investigator when, where, and how to sample. A suite of standard methods that covers the range of conditions and stream sizes likely to be encountered in the area being investigated should be established. Round-robin testing should then be used to establish the variability and reproducibility of each method. The applicability of each metric should be established for the geographic area in question and each metric should be carefully calibrated (Seegert, 2002)."

There are several sampling methods that have been developed for use in collecting macroinvertebrates. One common method for collecting measurements in freshwater streams involves a process commonly referred to as "kick sampling". In kick sampling, the recorder enters the stream with a mesh hand net and uses his/her feet to kick up debris from the streambed to collect the samples. It is important for the person recording to move around, reaching the different micro-habitats that exist within the stream system itself. To acquire the best sample possible, the collection net is held downstream from where the kicking occurs. The current of the river will allow the samples to flow into the net. When near grasses and roots, the data collector should run the net through the plant matter to ensure maximum collection. It may be necessary to break down larger samples into subsets of smaller samples in order to remove foreign debris from the samples. In order to make the process more standardized, collection time intervals should be set for each separate sample. Field workers should also wear protective gear, as it is very common for glass and metal pieces to be floating in the streams due to pollution (Freshwater Biological Association, 2008). This method provides the degree of standardization suggested by Seegert.

2.1.4 Data Analysis

Once the data is collected and catalogued in the correct manner, analysis is necessary to quantitatively score the overall health of the different streams and regions. The team is opting to use statistical analysis to simplify the handling of the large amounts of data collected. In addition, quantitative backing for qualitative observations facilitates the elimination of outliers and identification of the most relevant information. Because the data have inherent units attached to them (counts, percentages, etc.), normalizations enable comparison between different types of data. Ultimately, data analysis techniques allow the researchers to select indices for the final IBI that show strong correlations to environmental conditions. The statistical models chosen by the research team are mostly adapted from those presented in the case studies that we will discuss later in Section 2.2, particularly from a study in Florida.

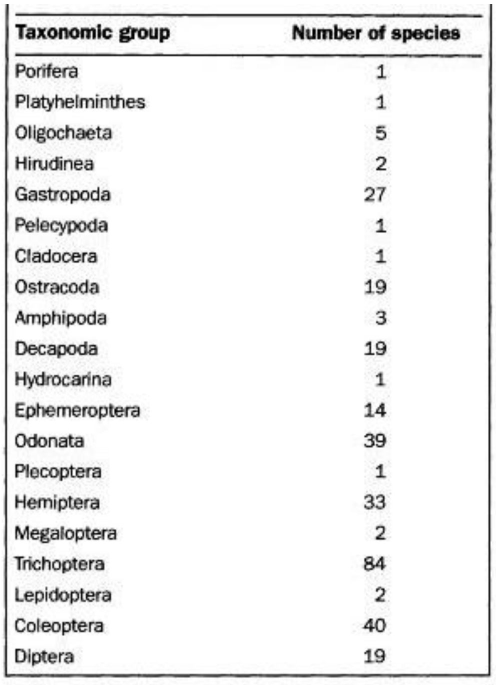
2.1.5 IBI Critics

There are various challenges associated with the development of an IBI. Strict guidelines are in place, including the use of indicator species that reflect the ecosystem of the specific geographical area studied. Data that researchers have previously collected, however, may not have followed the same guidelines that are currently used when acquiring data for an IBI. When researchers use past information, there may also be a disparity between current environmental conditions and the conditions at the time in which data was collected. One IBI proponent, Greg Seegert, considers it better to have small sets of complete, accurate data, rather than having large quantities of low quality data. Another developmental problem is borrowing metrics and grading procedures created from preexisting indices to generate a completely different IBI, without accounting for variations within each specific environment.

It is important to develop the proper metrics according to the geographical location (Seegert, 2000). Conclusions should not be based upon supposed accepted values, but rather upon site-specific data “because of differences in factors such as invertebrate taxa, climate, water chemistry, soil types, and management regimes” (Davis & Bidwell, 2008). As Seegert states, “Biological expectations should be reasonable and attainable, and therefore the limitations of each system must be taken into account. Expectations are sometimes set unreasonably high by resource/regulatory agencies, particularly in urban settings” (Seegert, 2000).

Multi-metric techniques such as the IBI are excellent for identifying impaired sites and for comparing environmental health among sites. However, the IBI is of limited use in establishing the causes for impairment. When environmental degradation is identified, follow-up studies of the impaired area will likely be necessary to determine the cause of the impairment. Thus an IBI should not be used to determine relationships of causality, but simply as a tool for monitoring environmental health.

Benthic IBI’s developed in other Caribbean nations provide helpful building blocks to the development of a Puerto Rican IBI. These indices have tracked all of the different taxonomic groups living within the Caribbean islands. With this knowledge, researchers may prescreen common indices for practicality with the expected population. The known Caribbean macroinvertebrates, for example, are shown in the table below.



Taxonomic group	Number of species
Porifera	1
Platyhelminthes	1
Oligochaeta	5
Hirudinea	2
Gastropoda	27
Pelecypoda	1
Cladocera	1
Ostracoda	19
Amphipoda	3
Decapoda	19
Hydrocarina	1
Ephemeroptera	14
Odonata	39
Plecoptera	1
Hemiptera	33
Megaloptera	2
Trichoptera	84
Lepidoptera	2
Coleoptera	40
Diptera	19

Table 1: Caribbean Macroinvertebrate Species (Bass, 2003)

In order to further examine the techniques described to develop an IBI, we will review the design of similar multi-metric indices. Specifically, the following section describes several case studies involving the use of benthic invertebrates. The techniques of data collection and analysis as well as the final results of each study are presented in order to exhibit techniques that have led to successful formation IBIs.

2.2 IBI Case Studies

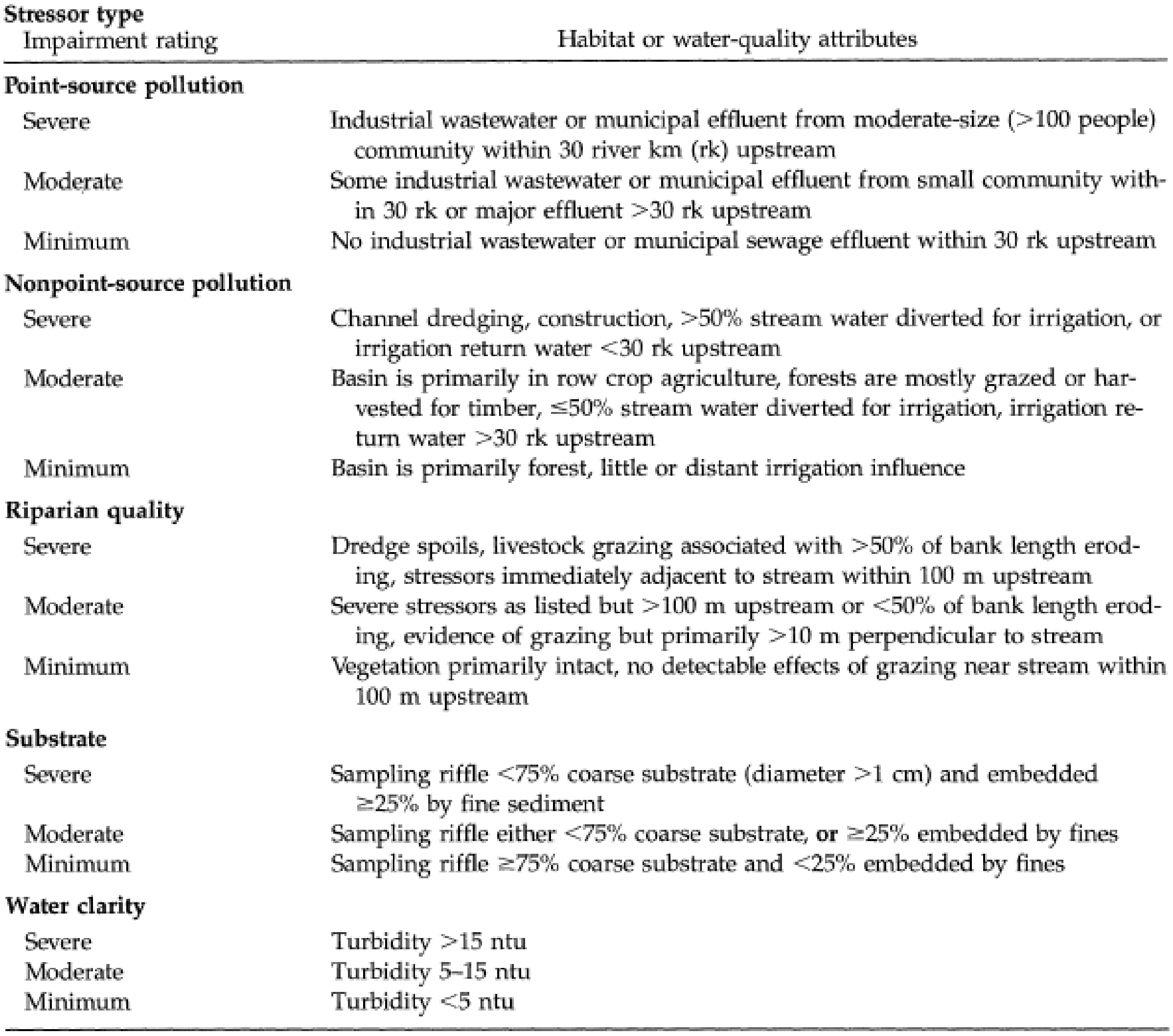
Three case studies are explored here to illustrate methods that are helpful in the determination of Indices of Biotic Integrity. The first case is an IBI performed for Watersheds in West-central Mexico. These watersheds face many of the same problems as The Trust has encountered in Puerto Rico and have similar climates and geographic features. The Sierra de Manantlán Biosphere Reserve is aware of environmental degradation, but lacks methods to determine the extent and perpetuation of environmental destruction. The lack of these standards motivated a study to develop a macroinvertebrate IBI (M-IBI) to better understand ecological integrity in the area. In designing the M-IBI, the researchers sought to select indices that accurately reflected environmental conditions observed in initial site surveys (Weigel, Henne, & Martinez-Rivera, 2002). The second case study is an IBI performed for the rivers of the

Tennessee Valley. This area has experienced severe anthropogenic stress through the works of the Tennessee Valley Authority, which has also monitored the water quality for many consecutive years in numerous sites. The research team validated the IBI performed through use of this extensive set of measurements. The final IBI reviewed was performed fresh water sources of Florida. This IBI highlights how statistical analyses should be used to eliminate researcher bias in the selection of final indices for a macroinvertebrate index of biotic integrity. Through these three case studies, a picture is built of the foundations for developing an IBI.

2.2.1 A Macroinvertebrate IBI in West-central Mexico

Researchers investigated initial metrics that would accurately assess the Sierra de Manantlán Biosphere Reserve ecosystem by measuring several key functions of watershed health. The initial selection included macroinvertebrate trophic structure, breadth of species, and function of population. In order to more accurately capture environmental variation in the study, a large number of sites were sampled both inside and out of the Sierra de Manantlán Biosphere Reserve, totaling 33 sites on 21 streams within 6 major river basins in the area at which data was accumulated (Weigel et al., 2002). Though this is a significant number and included a good variety of sampling sites, it falls slightly short of the range of 35-40 sampling sites suggested by Seegert. In addition to this, streams of similar sizes that go through similar climate zones were selected as the targets of the sampling, a practice that is necessary for developing an accurate IBI. Research indicates that selection methods based on similarities in slope of the river and type of river section also ensure consistency of results (J. R. Karr, 1981; Seegert, 2000; U.S. Environmental Protection Agency, 2009).

The researchers quantified ecosystem health prior to macroinvertebrate sampling in order to better select indices for the M-IBI. Providing initial data on ecosystem health in this way provided the researchers with comparison data to determine whether the final IBI accurately reflected known environmental conditions. The environmental pollution at each site was graded as severe, moderate, or minimum, corresponding to values of 0, .5, and 1 for each evaluation category (Weigel et al., 2002). Prior mapping was beneficial because it allowed verification of the IBI developed through the initial conditions observed. However, this specific technique also exhibits a notable drawback: the researchers introduce a large degree of bias upon initial quantification of ecosystem health (J. R. Karr, 1981; Seegert, 2000; Soldner et al., 2004). Five different anthropogenic stressors were quantified in the initial evaluation, including point-source pollution, nonpoint-source pollution, riparian quality, substrate, and water clarity resulting for a maximum total environmental grade rating of five and a minimum grade of zero. The highest quality sites were used as the reference sites: those sites exhibiting environmental stress can clearly not be used as a reference (Soldner et al., 2004; Weigel et al., 2002). These five categories accurately evaluate all types of pollution encountered in watersheds. The evaluation categories may be seen in the table below.

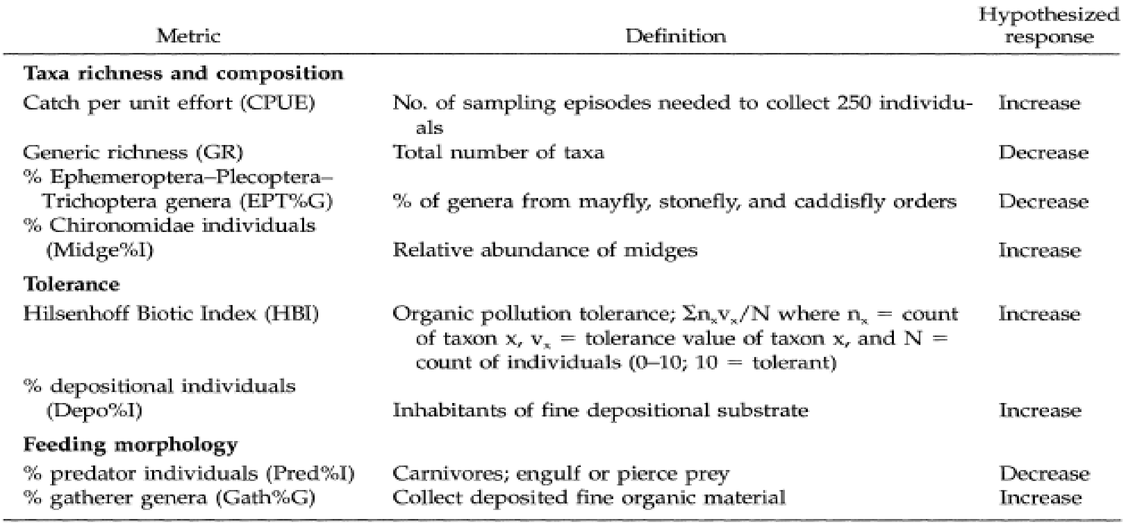


Stressor type Impairment rating	Habitat or water-quality attributes
Point-source pollution	
Severe	Industrial wastewater or municipal effluent from moderate-size (>100 people) community within 30 river km (rk) upstream
Moderate	Some industrial wastewater or municipal effluent from small community within 30 rk or major effluent >30 rk upstream
Minimum	No industrial wastewater or municipal sewage effluent within 30 rk upstream
Nonpoint-source pollution	
Severe	Channel dredging, construction, >50% stream water diverted for irrigation, or irrigation return water <30 rk upstream
Moderate	Basin is primarily in row crop agriculture, forests are mostly grazed or harvested for timber, ≤50% stream water diverted for irrigation, irrigation return water >30 rk upstream
Minimum	Basin is primarily forest, little or distant irrigation influence
Riparian quality	
Severe	Dredge spoils, livestock grazing associated with >50% of bank length eroding, stressors immediately adjacent to stream within 100 m upstream
Moderate	Severe stressors as listed but >100 m upstream or <50% of bank length eroding, evidence of grazing but primarily >10 m perpendicular to stream
Minimum	Vegetation primarily intact, no detectable effects of grazing near stream within 100 m upstream
Substrate	
Severe	Sampling riffle <75% coarse substrate (diameter >1 cm) and embedded ≥25% by fine sediment
Moderate	Sampling riffle either <75% coarse substrate, **or** ≥25% embedded by fines
Minimum	Sampling riffle ≥75% coarse substrate and <25% embedded by fines
Water clarity	
Severe	Turbidity >15 ntu
Moderate	Turbidity 5–15 ntu
Minimum	Turbidity <5 ntu

Table 2: Ratings of Environmental Pollution (Weigel et al., 2002)

For sampling, researchers used D-frame nets and kick-sampling to obtain macroinvertebrates. The kick-sampling method used by the researchers is an established method that is valid for macroinvertebrate samplings (Nerbonne, Ward, Ollila, Williams, & Vondracek, 2008; Soldner et al., 2004). In this case, the number of times the process was performed was used as a Catch Per Unit Effort (CPUE) index, which researchers decided was strongly correlated to abundance. At sites of intense environmental degradation, chronomids were omitted from the CPUE due to their abundance (Weigel et al., 2002). Seegert and Karr have both suggested that CPUE and other measures of abundance are not preferable to use in IBIs. However, under these circumstances, only chronomids could be considered an irruptive or overly abundant species, the primary factors that skew the accuracy of the CPUE index (irruptive species are defined as accounting for >50% of the average catch per sample). Since chronomids were omitted from the CPUE index, this process follows acceptable standards (J. R. Karr, 1981; Seegert, 2000).

After sampling, individuals were put in a sorting pan in the field. Researchers scanned the collection pan for rare taxa and then tabulated individuals starting from the same side of the sampling pan each time to avoid optical bias. The researcher counted the first 250 individuals encountered for the total of that specific sample (Weigel et al., 2002). Researcher bias is eliminated by the tabulating methodology, and rare individuals are not discounted due to chance. The indices for which data was tabulated can be seen in the table below.



Metric	Definition	Hypothesized response
Taxa richness and composition		
Catch per unit effort (CPUE)	No. of sampling episodes needed to collect 250 individuals	Increase
Generic richness (GR)	Total number of taxa	Decrease
% Ephemeroptera–Plecoptera–Trichoptera genera (EPT%G)	% of genera from mayfly, stonefly, and caddisfly orders	Decrease
% Chironomidae individuals (Midge%I)	Relative abundance of midges	Increase
Tolerance		
Hilsenhoff Biotic Index (HBI)	Organic pollution tolerance; $\Sigma n_x v_x / N$ where n_x = count of taxon x, v_x = tolerance value of taxon x, and N = count of individuals (0–10; 10 = tolerant)	Increase
% depositional individuals (Depo%I)	Inhabitants of fine depositional substrate	Increase
Feeding morphology		
% predator individuals (Pred%I)	Carnivores; engulf or pierce prey	Decrease
% gatherer genera (Gath%G)	Collect deposited fine organic material	Increase

Table 3: Final Indices Selected for the M-IBI (Weigel et al., 2002)

Only one index in this study is of concern. The researchers choose to use percentage Ephemeroptera-Plecoptera-Trichoptera (EPT) as an index because it is has been used historically. In this case, the EPT index correlated to environmental condition, but was not selected to describe a unique aspect of the ecosystem (Weigel et al., 2002). For that reason, further research would be necessary to determine legitimacy of the EPT index (J. R. Karr, 1981;

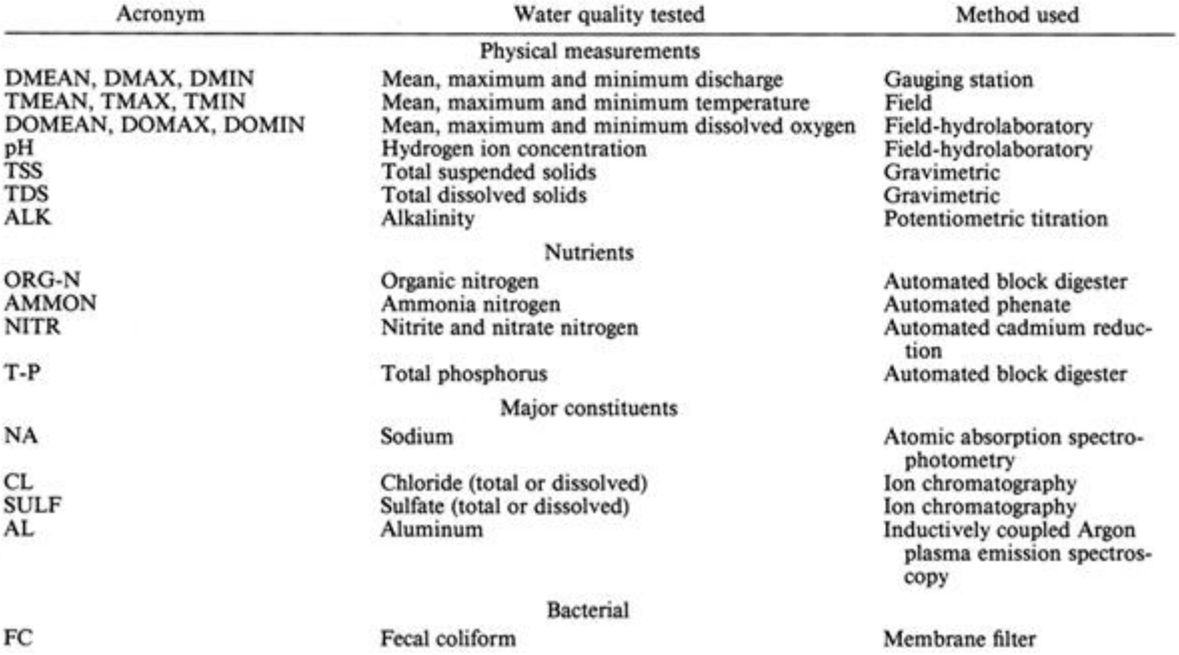
Seegert, 2000; U.S. Environmental Protection Agency, 2009). In addition to the indices used, the researchers performed statistical analyses on all data collected. Many of the analyses required data transformations, but resulted in isolation of outliers and yielded correlations for final indices (Weigel et al., 2002). Abnormalities in the natural world are often captured with relatively small sampling distributions, thus the use of statistical analysis tools insures the integrity of the data (Seegert, 2000; Soldner et al., 2004). The use of statistical tools helps eliminate the total degree of bias in this study.

2.2.2 A Benthic IBI for Rivers of the Tennessee Valley

Industry and human settlements in the Tennessee Valley of the United States subject the environment to various stresses. In 1986, the Tennessee Valley Authority (TVA) implemented a fixed station-monitoring program to observe the effect of human interference on the environment. Recently, researchers used the abundance of data collected to determine indices for a benthic IBI for the area in order to more accurately describe the effects of human stresses on the environment (Kerans & Karr, 1994). The climates and geographies of Tennessee and Puerto Rico are decidedly different. In turn, the landmasses can be expected to have vastly different ecosystems. The watersheds of the Tennessee Valley and Puerto Rico are distant enough that differences in topography prohibit the transference of results and indices (J. R. Karr, 1981; Seegert, 2000). Additionally, the rocky substrates of the Tennessee valley necessitate different sampling methodologies from those that are required on tropical islands, thus sampling methodologies will not be considered in this review (Bass, 2003; Nerbonne et al., 2008; Seegert, 2000). However, this study presents interesting index selection techniques, statistical tools, and validation methods that merit consideration.

As in most watersheds, the streams of the Tennessee Valley originate in the highlands and grow in size as they merge and head downhill. This standard geography of streams generates a relatively consistent set of aquatic features that house different assemblages of benthic invertebrates (Miller et al., 2008; Soldner et al., 2004). Inevitably, the problem occurs of how to determine the significance and weightings of different sub-habitats within a stream's ecosystem. In this study, the researchers opted to use methods of statistical analyses to reduce bias in visual selection techniques of indices. The use of statistics was favorable for the analysis of the large amount of data available to the researchers in advance of their work (Kerans & Karr, 1994).

The TVA amassed a significant dataset of both physical attributes of the water in the Tennessee Valley and of invertebrate populations over a large span of years. The data samplings were performed simultaneously and at regular intervals, providing a piecewise picture of the ecosystem health over time. The large collection of measurements of the physical properties of the water at each site assisted the researchers in evaluating the relevancy of each specific index (Kerans & Karr, 1994). It has been previously determined that accurate measurements of numerous parameters of water quality are essential in determining the correlation between individual indices and ecosystem health, resulting in a well supported index selection. In addition to extensive water quality sampling, these samples must be conducted at the same time as fauna sampling (Bedoya et al., 2009; J. R. Karr, 1981; Novotny, Bartošová, O'Reilly, & Ehlinger, 2005). The water quality parameters tested may be seen in the table below.



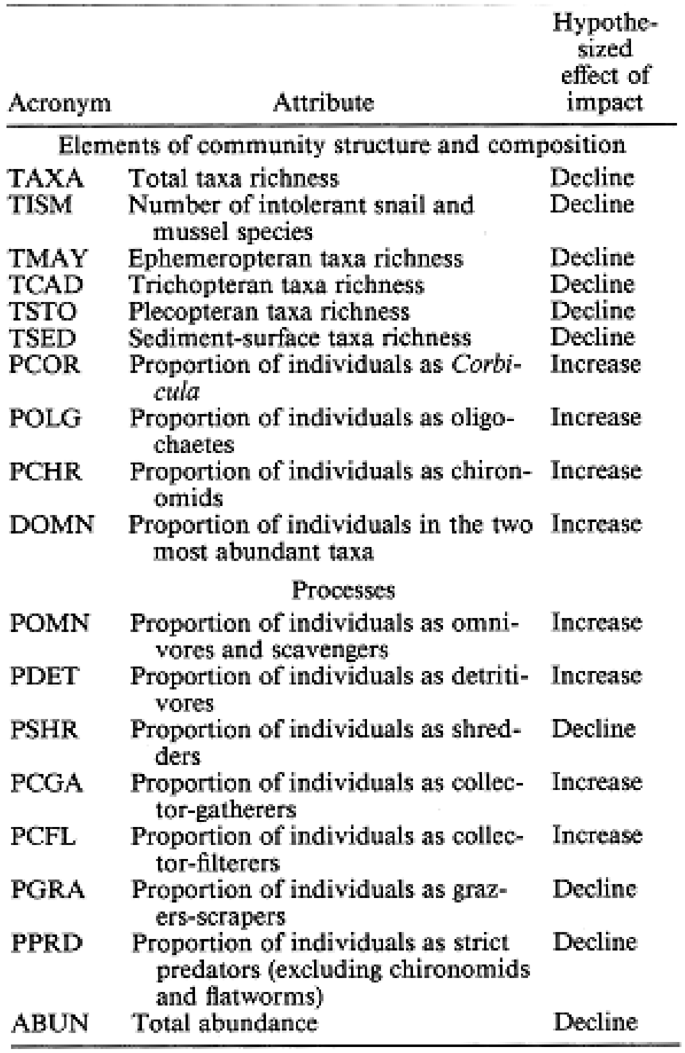
Acronym	Water quality tested	Method used
	Physical measurements	
DMEAN, DMAX, DMIN	Mean, maximum and minimum discharge	Gauging station
TMEAN, TMAX, TMIN	Mean, maximum and minimum temperature	Field
DOMEAN, DOMAX, DOMIN	Mean, maximum and minimum dissolved oxygen	Field-hydrolaboratory
pH	Hydrogen ion concentration	Field-hydrolaboratory
TSS	Total suspended solids	Gravimetric
TDS	Total dissolved solids	Gravimetric
ALK	Alkalinity	Potentiometric titration
	Nutrients	
ORG-N	Organic nitrogen	Automated block digester
AMMON	Ammonia nitrogen	Automated phenate
NITR	Nitrite and nitrate nitrogen	Automated cadmium reduction
T-P	Total phosphorus	Automated block digester
	Major constituents	
NA	Sodium	Atomic absorption spectrophotometry
CL	Chloride (total or dissolved)	Ion chromatography
SULF	Sulfate (total or dissolved)	Ion chromatography
AL	Aluminum	Inductively coupled Argon plasma emission spectroscopy
	Bacterial	
FC	Fecal coliform	Membrane filter

Table 4: Water Quality Parameters (Kerans & Karr, 1994)

Before statistical tests were performed on data, researchers checked the distribution of each data set. This was necessary because desirable indices are often proportional in nature (percent grazers, percent predators, percent scavengers, etc.), but unfortunately proportional indices do not generally follow a normal distribution and must be normalized. In this case, data transformations were chosen based on the mean and variance of each individual sample needing to be normalized, ultimately resulting in logarithmic transformations of all indices involving proportions as well as the abundance indices (Kerans & Karr, 1994). Statistical transformations of data of this type are necessary in order to achieve reasonable results. Without the data transformation, few significant comparisons could be drawn (Bedoya et al., 2009; Seegert, 2000; Soldner et al., 2004). Several other systems of data transformations and statistical methods for validating transformations of data to normality are mentioned, but are of a more technical nature than necessitates in depth discussion.

After data normalization, the correlations between attributes and sites were tested using two-way analyses of variance (ANOVA). ANOVA outliers were used to check homogeneity of variance and normality. A small number of ANOVAs displayed small tendencies away from normal distributions, as well as one displaying obvious heterogeneity. Use of ANOVAs allowed for unbiased selection of statistically supported indices (Kerans & Karr, 1994). The use of ANOVAs in determination of IBIs is a new technique. No relevant data supporting the use of ANOVAs in bio-criteria has been found, though statistical analysis has become prevalent, as is evidenced above. However, without the considerations of statistical techniques, researchers have observed that the selection of indices and determinations of selection criteria are highly subjective. By having researchers select these criteria using personal observations as opposed to mathematical models an enormous amount of researcher bias is introduced in a critical phase of development for the IBI (J. R. Karr, 1981; Kerans, Karr, & Ahlstedt, 1992; Seegert, 2000; Soldner et al., 2004).

After performing statistical analyses, the resulting indices were evaluated for scientific validity. The final selection of indices was described by the researchers, who stated, “We focus on taxa richness, taxa composition, and surrogates of biological processes (trophic and functional guild structure and total abundance…” (Kerans & Karr, 1994). The final list of indices used in the IBI may be seen in Table 5 below. The ANOVA statistical correlations to sites are also presented in the study.



Acronym	Attribute	Hypothesized effect of impact
	Elements of community structure and composition	
TAXA	Total taxa richness	Decline
TISM	Number of intolerant snail and mussel species	Decline
TMAY	Ephemeropteran taxa richness	Decline
TCAD	Trichopteran taxa richness	Decline
TSTO	Plecopteran taxa richness	Decline
TSED	Sediment-surface taxa richness	Decline
PCOR	Proportion of individuals as *Corbicula*	Increase
POLG	Proportion of individuals as oligochaetes	Increase
PCHR	Proportion of individuals as chironomids	Increase
DOMN	Proportion of individuals in the two most abundant taxa	Increase
	Processes	
POMN	Proportion of individuals as omnivores and scavengers	Increase
PDET	Proportion of individuals as detritivores	Increase
PSHR	Proportion of individuals as shredders	Decline
PCGA	Proportion of individuals as collector-gatherers	Increase
PCFL	Proportion of individuals as collector-filterers	Increase
PGRA	Proportion of individuals as grazers-scrapers	Decline
PPRD	Proportion of individuals as strict predators (excluding chironomids and flatworms)	Decline
ABUN	Total abundance	Decline

Table 5: Final Indices in the B-IBI (Kerans & Karr, 1994)

Researchers initially used 28 attributes previously determined to be useful indices of benthic macroinvertebrates as the baseline set of attributes to evaluate (Kerans et al., 1992). Through the statistical techniques presented above, 18 indices were selected as the final components of the B-IBI (Kerans & Karr, 1994).

2.2.3 A Benthic Macroinvertebrate IBI for Florida Streams

Terming their metrics by the general term of biocriteria, M. T. Barbour, et al. (1996) outlined a framework for the development of an index of biotic integrity in Florida. In their process, the team of researchers chose benthic macroinvertebrates to be the basis for their biological assessments. With goals similar to those of Fideicomiso in Puerto Rico, the Florida Department of Environmental Protection sought to reduce water pollution in Florida; to do so techniques must be developed to evaluate the current state of the region of interest (Barbour et al., 1996).

As a common theme in many articles, motivation for biological monitoring derives from the inadequacy of traditional chemical testing to assess the complete impact of nonpoint sources of pollution (Novotny et al., 2005; Zhu & Chang, 2008). In addition, the effect of physical disturbances may not be revealed by chemical tests, and as Bedoya points out, healthy biota is not necessarily indicated by "non exceedance for one chemical." Aquatic systems are multidimensional, with physical, chemical, and biological attributes (Bedoya et al., 2009). Thus, to reveal the effects of transient pollution as well as accumulated legacy pollution, "biological monitoring may be the most appropriate…" because "resident biota in a water body are natural monitors of environmental quality (Barbour et al., 1996)."

A biological index track changes in the integrity of a given eco-region relative to a well-defined set of reference sites should represent the best conditions possible. Thus, one must select reference sites that are very similar to the region being studied. The index can then be calibrated to the specific environment and geography. Many authors agree upon the high sensitivity of an index to context (Bedoya et al., 2009; Chainho et al., 2007; Miller et al., 2008; Novotny et al., 2005; Seegert, 2000; Zhu & Chang, 2008), so it is crucial that one choose "minimally disturbed streams with small catchments that were representative of and completely within subecoregions (Barbour et al., 1996)." In this study, eighty reference sites were sampled over three years, from 1992 to 1994.

Using a sampling technique analogous to that in Mexico, (Weigel et al., 2002), the researchers used a D-frame dip net to sweep the substrate and take a composite sample from multiple habitats. Preserved in formalin, the samples were processed in the laboratory and organisms were "identified to the lowest taxon possible, usually species (Barbour et al., 1996)." Because candidate metrics vary over regions, streams were classified in small groups based on geographic, hydrologic, and chemical data. Several statistical analysis methods then placed these streams into highly similar ecoregions. Dissimilarity measures between sites was determined by chord distance, but more recent authors with access to more powerful computer software have utilized Euclidean and Mahalanobis distances (Bedoya et al., 2009; Chainho et al., 2007). To evaluate robustness and variations from year to year, the researchers analyzed data from each year separately and data from all three years during the summer. They classified the sites based on their similarity or dissimilarity and created a map detailing the similar ecoregions.

The investigators selected 32 structural or functional biological metrics, mostly based on relative abundance or counts of taxa within the same grouping. Several measures indicate richness and diversity of benthic assemblages, which relate to the health of the biota within the stream ecosystem. The populations of a variety of indicator species change when perturbed due to differing tolerance to pollution. Intolerant taxa tend to disappear at higher levels of pollution, and hence a greater relative abundance of tolerant species correlates to greater pollution (Novotny et al., 2005). Trophic functions, given as relative abundance of specific sensitive organisms, indicate the effects of pollution on the availability and production of food sources (J. R. Karr, Fausch, Angermeier, Yant, & Schlosser, 1986).

The researchers screened each metric and eliminated it if it was highly correlated with another in a linear scatterplot, as it would provide redundant information. To account for variance, the sites were the treatments in an ANOVA. The authors tested the sensitivity of each metric, or "its ability to discriminate between reference and impaired sites (Barbour et al., 1996)," by the overlap in box-and-whisker plots. Finally, the researchers normalized the metrics responsive to perturbation in order to eliminate their dimensions (J. R. Karr et al., 1986; Kerans & Karr, 1994). The sum of these normalized scores gave the value of an index that the authors name a Stream Condition Index (SCI). This SCI gives a score to each metric by relative comparison of its value to expectation values based on the reference data set. A high score of 5 is given to a metric within the range of reference values, while a metric outside this range received a low score of 3; a metric was assigned a score of 1 if it highly deviated from the expected value.

In this study of streams in Florida, several issues that could be improved upon in future studies include investigator bias, assumptions of equal weighting in the calculation of the index, and calibration. Reference sites in each must be selected to have the least impairment in each region through analysis of land use, pollution sources, and past problems. Without exhaustive investigation, one must rely on one's own judgment to select sites representative of the healthiest streams. When the normalized metrics are aggregated into the index, there is an implicit assumption that each metric has the same weight. This single index is a simplified value used to determine if action is necessary, but the type of action necessary should only be evaluated by analysis of the component metrics. Finally, calibration of the methods in this report could be enhanced through collection of samples from a greater number of reference sites. Samples from as many sites as possible within each ecoregion should be collected in order to better define the site classifications. In addition, samples should be collected from sites suffering from a variety of stressors and from sites with unknown conditions to further test the ability of the index to predict the health of the sites. These new data could further reveal correlations for the tolerance of the benthic assemblages to their stressors (Barbour et al., 1996).

2.2.4 Insight from Case Studies

The case studies above present several useful methods in the development of an index to biotic integrity. Each study involved the identification of reference sites, sampling at impaired sites and reference sites, selection of metrics to be used in the index, and statistical analysis. Each of these steps is critical in the creation of an IBI, and bias or error in any one can impact the final result. The case studies above all demonstrate tactics that lead to the creation of useful IBIs that seemed to accurately reflect the health of the watershed of consideration. This is indubitably a result of the incorporation of several valid and successful techniques.

We have found that in the design of our procedures, we will need to minimize researcher bias by carefully designing our selection and sampling procedures. We have learned that kick-sampling is the preferred technique among researchers for macroinvertebrate in-stream sampling. Experts rely heavily on methods of statistical analysis to select the most relevant metrics while simultaneously reducing researcher bias. Statistical analysis also reduces redundancy among indices, allowing a more concise and appropriate IBI to be created. A large number of samples from a wide variety of sites in a relatively broad geographic area must be collected to strengthen the correlations.

Chapter 3: Methodology

In the interest of conserving natural areas, the Conservation Trust of Puerto Rico is taking initial steps toward the development of an Index of Biotic Integrity (IBI) for stream ecosystems. The overall goal of this project is to perform the initial steps in the development of an IBI. The Trust will select specific locations in watersheds in the San Juan area as the sampling sites that provide data for the IBI. The IQP team will collect data from water quality tests as well as macroinvertebrate samples. The team will observe the surroundings of each site in order to establish the presence of physical stressors in the area. Finally, to determine the characteristics of the macroinvertebrate that reflect environmental conditions, team members will compile, map, and compare the gathered information using various statistical measures. The project will analyze these comparisons critically in a scientific manner so as to avoid biases that could otherwise appear.

3.1 Materials

In order to complete all necessary fieldwork, proper materials should first be obtained. Bringing a digital camera to each site facilitates documentation of fieldwork and sampling locations; documentation of this sort is imperative in later reviews of the sample collection process. To acquire samples safely and efficiently, each team member will wear personal protective equipment in the field. The personal items include:

- Long sleeved button shirts
- Interchangeable field pant/shorts
- Light cotton or acrylic socks.
- Non-waterproof hiking boots
- Chest waders
- High quality plastic poncho

The long sleeved button shirts should be square cut on the bottom hem to shield skin from direct water contact and exposure to sunlight. For relief in the warm climate, pants with detachable zipper legs allow the wearer to convert one’s pants to shorts. Cotton and acrylic socks enable the feet to breath while providing comfort. It is essential that the field workers wear hiking boots that are not waterproof. Waterproof boots do not allow water that enters the inside of the boot to leave or evaporate, soaking the field worker’s feet and posing health concerns. Because some of the streambeds are rocky, the hiking boots support the ankles. Chest Waders offer protection for sampling in respect to urban stream work, since these streams may have higher levels of pollutants. A plastic poncho of high quality material also defends against the elements when necessary. Requiring all these personal items ensures maximum comfort and protection for the field worker.

3.2 IBI Design Method

In the first step, the research team selected the individual processes to perform site samplings to establish a reliable IBI. The site sampling methods were developed without tailoring to any of the individual sites, since the researchers will apply the sampling method to every site. The development of the sampling method also included the design of an initial site survey (see Section 3.3) to evaluate environmental conditions prior to macroinvertebrate samplings to be used as a reference. The sampling sites were selected by Fideicomiso ecologist Brick Fevold in order to reflect a wide variety of conditions in the San Juan area. Once the materials listed in Section 3.1 (above) are acquired, the team will begin to travel to the sampling sites selected with transportation provided by the Trust. After arrival on site, the team members will perform the initial site survey as well as the site samplings, which include macroinvertebrate collection and water quality testing. The macroinvertebrates collected will be stored in plastic containers for analysis by Fideicomiso experts. The procedure to perform all necessary tasks at each site is thoroughly described in Section 3.5. Subsequent to the collection of macroinvertebrate samples, the bins containing the macroinvertebrates will be analyzed individually and catalogued by knowledgeable Fideicomiso employees, resulting in lists of macroinvertebrate distributions by taxa as well as trophic groups.

The research team will then review the data produced and consult with Fideicomiso experts to determine if there are significant abnormalities in the data set that reflect an exterior influence or distribution atypical to that expected to be found in an ecosystem of any health in Puerto Rico. If it is found that the data set is abnormal, the team will return to the site and repeat the sampling process. However, if no abnormalities are found, the data will be tabulated and indices will be normalized to allow comparison. The primary analysis method used will be a two-way analysis of variance comparison (two-way ANOVA) to determine which indices accurately reflect site conditions documented in the initial site survey and found through water testing. Finally, indices will be selected for the IBI based on results of the statistical analyses.

After the final IBI is developed, the team will review the entire process leading up to the creation of the IBI, documenting its benefits, shortcomings, and limitations. The overall process may be seen in Figure 1, below.

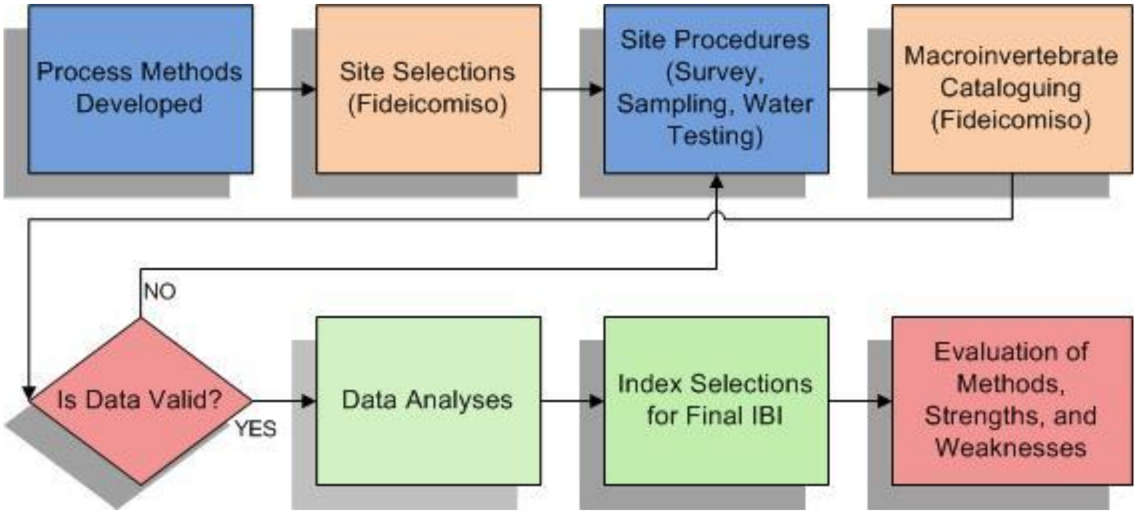


Figure 1: Overall Process Flowchart

3.3 Initial Site Survey

The initial site survey is a key component in all IBIs that do not have a recorded history of environmental conditions for all testing sites. The initial survey is designed to give an accurate, but brief, analysis of environmental pollution that affects macroinvertebrate life at each individual site. The research team proposes a survey that is adapted largely from the survey used by the research team in an IBI performed in West-central Mexico detailed in Section 2.2.1, though it also draws components from measures used in the IBI performed on the Tennessee River Valley detailed in Section 2.2.2. The survey consists of five measures that assess multiple characteristics of the environment that are known to reflect various forms of environmental pollution (Barbour et al., 1996; Kerans & Karr, 1994; Weigel et al., 2002). The origins and inherent flaws of each measure will be detailed in full in this section.

Each environmental characteristic is graded on a scale from 1-5, an adaptation from the scoring system used by Weigel, Henne, & Martinez-Rivera (Weigel et al., 2002). While the initial survey used in West-central Mexico scored measures on a three-point scale, the method detailed herein involves an expansion of the scoring range to a five-point scale for Puerto Rico. Variations in environmental conditions detailed by The Trust as well as variations found in a previous project suggest that an extended ranking system is necessary to accurately cover all environmental conditions (The Conservation Trust of Puerto Rico, 2007; Zabinski et al., 2009). The addition of two scoring points accounts for a larger variation in conditions as well as variations between the two sides of the river. The score given to each site is an average of the score for each bank rounded down. The adapted scoring system accounts for significant variations in riparian zone and river-bank quality between each side of the river, a condition that was found in multiple sites by previous research in the San Juan area (Zabinski et al., 2009). In each measure, a score of five ranks the least severe environmental degradation and a score of one ranks the most severe environmental pollution. The highest score a site can obtain is a score of 25, whereas the lowest score a site can achieve is a score of five. The values for each characteristic are chosen for simplicity in evaluation and applicability to macroinvertebrate health (Barbour et al., 1996; Kerans & Karr, 1994; Weigel et al., 2002). The five questions of the initial site survey are designed to monitor environmental factors in terms of distance from the site. The first items listed involve distances farthest from the macroinvertebrate habitat, progressing to factors geographically near to the habitat and finally to the substrate that defines the habitat. A copy of each statement from the initial site survey is presented below. (The whole survey may be found in Appendix A.)

1. **Agricultural Pollution**

1	2	3	4	5
Surrounding area is primarily used for agriculture	Area is developed and also supports agriculture	Area is not used for agriculture, but is not forested	Area is forested but has human development in some areas	Surrounding area is primarily forested

Agriculture has been shown to significantly affect multiple aspects of aquatic habitats, especially macroinvertebrate populations. This measure was adapted from the West-central Mexico IBI with expanded scoring categories to account for varying conditions. Near area agriculture was also determined to affect macroinvertebrate populations by other studies on macroinvertebrates (Cascorbi, 2002; Kerans & Karr, 1994). Bias is introduced through evaluation of this environmental condition by determination of extent of agricultural use of surrounding land. Researchers will use observations from satellite imagery such as Google Earth and observations in the field to evaluate an area within a radius of five miles.

2. Urbanization in Area

1	2	3	4	5
Urban areas overrun riparian zone	Urban area bordering riparian zone	Urban area within ½ mile of stream	Urban area within 1 mile of stream	Urban area 2 miles or greater away

Urbanization is a pseudo-measure of anthropogenic stresses adapted from multiple sources. Previous research sponsored by the trust as well as research performed in IBIs and IBI analyses suggests that urbanization within the immediate area of a water source significantly affects water quality due to runoff water circumventing natural ground filtrations. This leads to introduction of both natural and human produced pollutants into the water source without filtration (Kerans & Karr, 1994; Seegert, 2000; Weigel et al., 2002; Zabinski et al., 2009). The adaptation of this measure is defined by the characterizations of distance. Satellite imagery will be used to determine nearest distances to the sampling site.

3. Riparian Zone Quality (thickness and diversity, stream bank condition)

1	2	3	4	5
Very little or no shade, very few species, damaged banks with slides, zone 0-40’	Some shade, a small number of species, 50% or more of bank area is damaged, zone 40-80’	Moderate shade, some species diversity, few uncovered banks, zone 80-120’	Good shade cover, many species, very little bank damage, zone 120-160’	Abundant shade cover, many species evident, intact stream banks, zone 160’+

The use of measures of riparian zone quality is adapted directly from the West-central Mexico IBI. However, the classification of riparian zone health was expanded based upon classifications of the riparian zone of the Rio Piedras performed in previous work (Weigel et al., 2002; Zabinski et al., 2009). The only bias in assessment of a value is in assessment of distances and areas.

4. Immediate Area Pollution

1	2	3	4	5
Large pollutants (8ft^3 or greater) or toxic chemicals	Mid-sized pollutants (roughly 1ft^3)	Abundant small pollutants	Some small pollutants, little human evidence	No pollutants

Immediate area pollution is a symposium of two categories used by IBIs performed in the Tennessee River Valley and West-central Mexico. This characterization was evolved to detail immediate physical pollutants in order to increase ease of measurement as opposed to measured chemical sources and other near area pollutants used in other IBIs (Davis &

Bidwell, 2008; Kerans & Karr, 1994; Weigel et al., 2002). In this case, pollutants are referred to as any human wastes that are not naturally occurring. Toxic pollutants reference those used in the U.S. Clean Water Act found in §307(a)(1). In this measure, researchers can introduce bias through qualifications of abundance of pollutants and differentiation between sizes and severities of pollutants.

5. **Substrate Quality**

1	2	3	4	5
Predominantly silt and mud	Mostly silt or coarse substrate	Some silt and 1-2cm substrate	Finer gravel, good depth	No sediments, deep gravel

Substrate quality is a meter adapted from the IBIs performed in both West-central Mexico and the Tennessee River Valley. Substrate quality is a measure of the health of the habitat of benthic invertebrates (Cascorbi, 2002; Kerans & Karr, 1994; Weigel et al., 2002). Coincidentally, the substrate is also the breeding ground of many aquatic species and thus determines the health of a water sources to a significant extent: finer substrates with little silt have been seen to support a more diverse habitat. The substrate quality is a primary determinant of the benthic macroinvertebrate feeding structure and thus of significant concern to this study (Cascorbi, 2002).

The initial quantification of site quality is extremely important in determination of final IBI values. In this study, very little data exists to determine environmental health of sampling sites over the previous years; as a result, the entire validation and establishment of IBI indices must be based off of water quality tests and the initial site survey. The survey detailed above aims to reduce researcher bias through quantifications using measures of distance. Distance measures reduce differences in estimates between researchers, where other qualitative measures introduce the possibility of large differences between estimates performed by large teams. The initial survey also seeks to maximize the quality of environmental evaluations by evaluating a breadth of characteristics. The survey specifically targets far distant areas, urbanization, the riparian zone, immediate area pollutants, and substrate quality to build a comprehensive environmental picture of the area surrounding each sampling site. This survey will serve as a solid, though brief baseline of watershed health.

3.4 IBI Project Time

In order to perform precise, repeatable sampling, a systematic collection process that accomplishes all tasks in the same fashion each repetition is necessary. In the Site Sampling

Procedure, the process can be seen in a step-by-step manner in Figure 2. The process is broken down into a specific distribution with responsibilities for each team member (labeled as Team Member 1: TM1 though Team Member 4: TM4) in the overall process of the sampling. At each site, the team members will rotate positions. In this way, each team member will fill every role in four samplings, thus minimizing the bias introduced from having one team member repeat the same role every time. Consequently, this same technique may increase the variability of the results between sites due to different motions and techniques of each member of the four-person team.

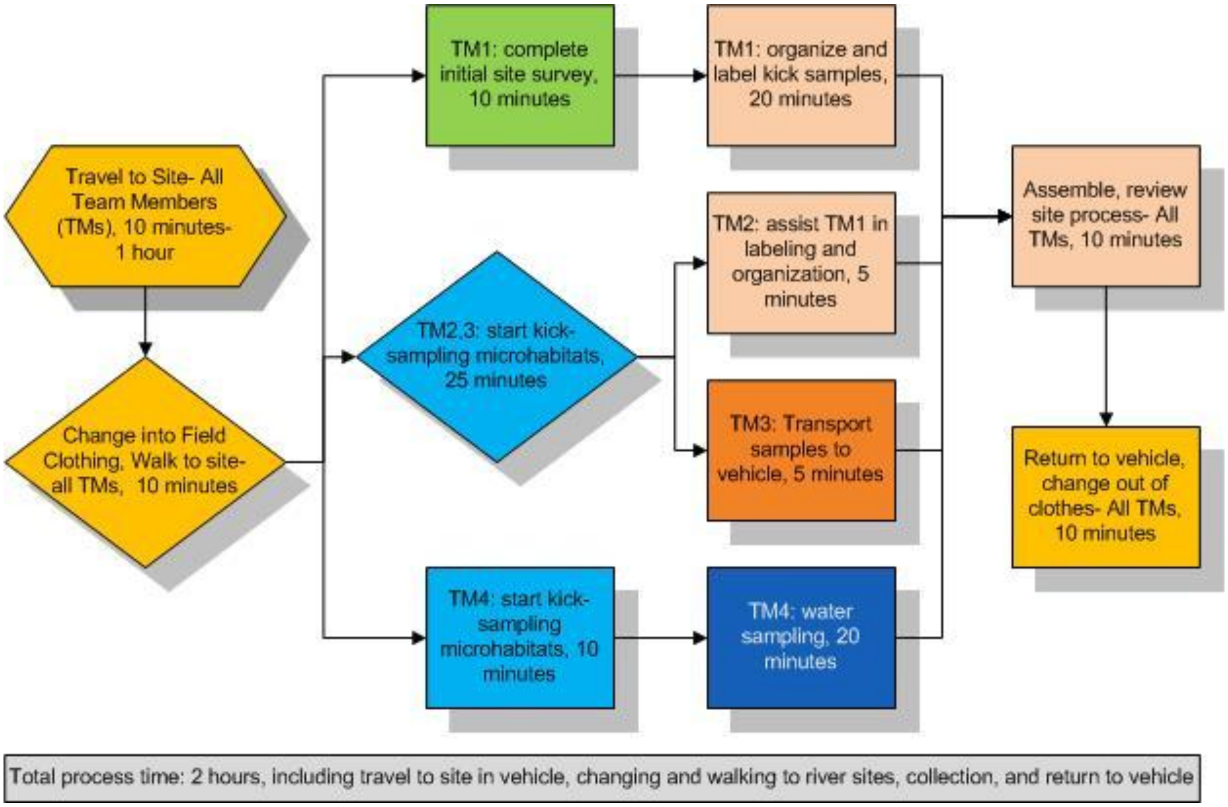


Figure 2: Site Procedure

Figure 2 above describes overall estimates of the amount of time the procedures will take at each site. This time estimate is critical because the number of sites at which the team could effectively collect samples in each day of fieldwork was based on this estimate, and thus the total number of days needed to collect samples for all sites is heavily dependent upon this initial calculation. Due to the importance of this time estimate, ranges are used to account for variability that will naturally occur; consequently, the team developed all estimates to be conservative in order to assure that the project will be completed. The details of these steps are described below.

At the outset, all team members will travel to the site to be examined. In addition to the four-person team, a Fideicomiso ecologist will travel to all sites providing transportation and oversight. Transportation is expected to take between ten minutes and one hour, depending on the distance of the site as well as traffic conditions. After arrival on site, all team members will change into the appropriate field clothing and walk to the actual sampling location, which could take up to 10 minutes depending on the distance of the site from available parking.

Each team member has a single role in the collection of data at each site, with team members rotating positions each time as was previously described in this section. TM1 will complete the initial survey of the site, while simultaneously TMs 2, 3, and 4 will be gathering samples via kick sampling. It will be important to gather samples from within each micro-habitat that exists in each stream, such as riffles, shallow waterbeds, algae beds, and other habitats encountered. It will be necessary to remove debris from each kick sample collected prior to performing analysis.

After ten minutes of kick sampling, TM4 will then complete the different water quality tests, which is assumed to take roughly 20 minutes. The water quality testing kit provided by The Trust is capable of testing the temperature, pH, dissolved oxygen, phosphate, and clarity levels, water qualities that have been tested and used as indicators in the IBI performed on rivers of the Tennessee Valley (Kerans & Karr, 1994).

Equipment such as nets and labeled plastic bins will be used to capture and separate the macroinvertebrates. The labeling convention TM1 uses will be a number for each sampling site followed by a dash, then an abbreviation for the microhabitat in which the sample was taken. Each label will also be marked with a time and date to help determine conditions on that day. Nets are essential in kick sampling in a freshwater stream because water currents tend to displace sample matter quickly. Each team member must hold the net downstream of one's own body positioning, providing the maximum amount of sample to be collected within one attempt. Placing the samples in plastic bins enables transportation to experts at The Trust for identification.

After performing the initial survey, TM1 will spend the remainder of the time at the site organizing and labeling the kick samples accumulated by the other team members. TMs 2 and 3 will then help with labeling after they complete the kick sampling procedure, subsequently transport the samples to the vehicle. Before leaving the site, the team members will assemble and review the process to ensure that all microhabitats were sampled, all processes were completed, and all samples were successfully collected. The team will then take all samples and gear to the vehicle and change out of field clothing.

As can be seen in Figure 2, the team estimates that on average it will take two hours to travel to a site, collect samples, and perform all other tests and analyses. Assuming that the sites are near each other and all procedures are carried out without interruption, the time required to travel to each site and collect samples could be as low as one hour. In adverse conditions, the whole process could take as long as three hours due to traffic, low sample turnouts, and unanticipated weather conditions.

With the time estimates above, it is reasonable to collect data from 2-4 sites per day spent in the field. Completion of 30 site samplings with these conditions would then take anywhere from 8-15 days of field work. Assuming 2-3 days of field work per work week, the entire sampling procedure would then last anywhere from 3-4 weeks. To meet the demands of the relatively short project timeline, expert cataloguing of macroinvertebrates must then take at most one week for each sampling site in order for the team to have adequate time to perform statistical analyses. Assuming that the last samples are collected at the end of the fourth week of the project, experts will then finish analyzing samples at the end of the fifth week. The team will have the remaining three weeks to finish data analyses and create final deliverables. The overall process timeline can be seen below in Figure 3.

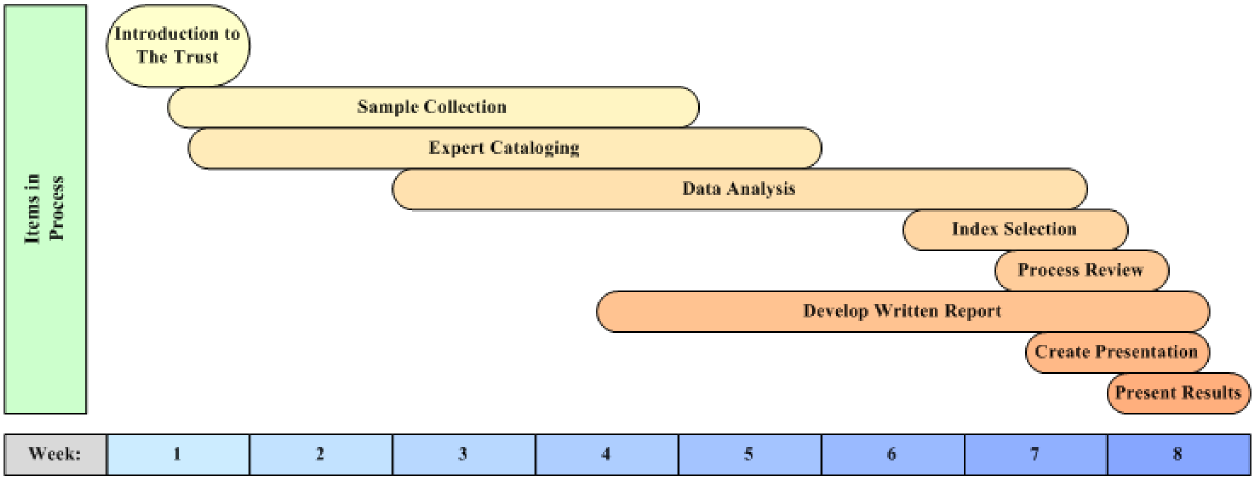


Figure 3: Project Timeline

The timeline above allots reasonable amounts of time for the completion of each step. In addition, it anticipates some of the lag time that naturally occurs in the first few days of work at a new location. It is apparent from this timeline that the project will have two phases that are time intensive. In the first 2-3 weeks, the team will need to conduct a large portion of the sample collections, meaning that the project will be partially front-loaded. After this point, there will be a slight lull in time commitment as the sample collections are completed, experts fully catalog all samples, and the initial steps of data analyses are performed. This period of lesser work will likely last from week 3-4, at which point the workload will then begin to increase. The second time intensive phase will start sometime in week six when data analysis will be in its final stages and index selection and development of project deliverables are all in progress. With this timeline, the team can plan in advance for the stages of work and develop strategies to work at alternate times in order to compensate for the time intensive weeks.

3.5 Statistical Analyses of Data

Once the experts at Fideicomiso identify the species, count, and characteristics of the samples collected, the team needs to compile and analyze the results. Based on numerous studies in the literature, species abundance, composition, and trophic function are the most useful attributes of the samples to be characterized. The data collected at each site are measured on different scales (species counts, percentages, distinct units, etc.); so, the data must be normalized before comparisons can be made. Additionally, from what we have learned in the literature, the IBI should integrate only the parameters that are the most relevant predictors of ecosystem health. Several authors recommend setting aside a portion of the data to be used for validation purposes (Bedoya et al., 2009; Weigel et al., 2002). Due to the limited duration of the project, the team will adopt a hybrid of several of the simplest techniques presented in the literature. The team will perform all data analysis using the Analysis ToolPak in Microsoft Office Excel.

3.5.1 Normalization of Indices

For indices measured on separate scales, normalization enables elimination of outliers and standardization of data. The data collected from one site will be collected into one data set; many microhabitats are sampled at each site in order to identify a large variety of the species that prefer specific microhabitats at each site. Because of this, the project team will investigate the distribution of each data set and normalize indices that are proportional to each other. As done by Kerans and Karr in their 1994 study of the Tennessee Valley, the team will accomplish this normalization through logarithmic transformations of some indices. As mentioned above, data transformation is necessary in order to bring the data into alignment for statistical analysis. Some indices are presented in percentage units while others are counts of species; this process normalizes them, allowing unitless scores to be assigned.

3.5.2 Selection of Indices

The index developed should show variations due to differences among site conditions rather than measurement errors. The IQP team will utilize a two-way analysis of variance (ANOVA) to indicate index responses to area and site condition and find the indices that correlate to the survey site conditions. The ANOVA technique makes comparisons between the variability of the index of interest within groups to the variability between groups. Consequently, higher variability between sites is preferable to variability within sites. The ANOVA enables detection of the effects of basin area and site conditions on the final IBI values. This in turn supports the selection of relevant indices.

3.5.3 Scoring of Indices and Calculation of IBI Value

Once the indices are selected, the team will use a technique modified after the original methods used by Karr and his colleagues (Barbour et al., 1996; J. R. Karr, 1981; J. R. Karr et al., 1986). Based on a consensus in the literature, several authors assign to each index a score ranging from 1 to 5. A score of 5 represents the values closest to reference conditions, while a score of 1 represents the greatest deviation from these conditions. In order to obtain a value for the index of biotic integrity, these scores must be aggregated. Based on the simplest technique, the team will sum the scores assigned to each relevant index in order to calculate the final IBI value for the developmental data set.

3.5.4 Validation

From the trends discovered in the developmental data set, the team will calculate IBI values for the validation data set, and then evaluate the strength of the relationship between the calculated values and the environmental conditions assessed in the initial site survey. As a standard analysis that quantifies the strength of the linear trend of two variables, Pearson pairwise correlations (critical $\alpha \leq 0.05$) test the power of the IBI value to predict the habitat and water quality (Weigel et al., 2002). When Pearson's $r > 0.80$ for correlation, the IBI will be considered valid.

3.6 Summary

In sum, the team has designed a methodology that will develop the IBI that the Conservation Trust of Puerto Rico needs to assess stream health in the San Juan area. Through the testing and sampling, the design and development of an IBI will be completed for Puerto Rico. The results will determine which indicators should be used for the creation of an accurate IBI as well as the water quality of the sites. From these studies, a better understanding of the areas can be realized and further efforts can be made for their conservation.

In this section, the team has identified the key concepts necessary in completing the IBI design process in Puerto Rico. We have identified important personal protective equipment in addition to the field equipment needed for sample collection. The team detailed a precise sampling method based on standard techniques found in the literature. From the sampling times associated with them, the project team created a timeline to identify the scope of the work. This timeline allows the team to coordinate and manage the efforts between the project and the Trust over the eight-week period.

Following sampling processes and the project timeline, this section describes statistical analyses that will be used to calculate the final IBI value. The team chose the simplest and most straight-forward procedures due to the short amount of time available for project completion and the team's lack of experience in the field statistical analysis. However, these methods are still commonly accepted in the literature. The integration of all the techniques presented here will result in an IBI value that accurately reflects environmental conditions with a low degree of bias.

Chapter 4: Conclusion

The Conservation Trust of Puerto Rico exists to protect elements of the Puerto Rican geography that have cultural and environmental importance to the island's inhabitants. A great part of The Trust's goals includes the procurement and maintenance of land reserves. Puerto Rico contains deserts and rain forests that protect the diversity in species that exists in the tropical climate as well regions that have cultural significance due to the heritage of the island. However, one unifying element of conservation in Puerto Rico is the island's freshwater sources. Important uses of freshwater sources include drinking water and water for agriculture, both of which are encountered daily in human life. Consequently, the quality of freshwater sources is integral in the sustainability of life on the island due to the limited size and number of watersheds available.

The Trust is currently seeking a metric specifically tailored to the ecology of Puerto Rico that can accurately assess the health of fresh water ecosystems on the island. Per The Trust's request, the steps proposed in this document will lead to the formulation of a Benthic Index of Biotic Integrity (B-IBI). The B-IBI will provide an accurate and reliable indication of ecosystem health based on the feeding structure, quantity, and species of benthic macroinvertebrates found in the streams and rivers near San Juan. Multi-metric indices based on aquatic organisms such as the B-IBI more accurately reflect ecosystem health than water quality measurements due to the sensitivity of invertebrates to complex environmental interactions. The Trust can use the B-IBI developed to assess the health of watersheds it currently owns as well as to evaluate other watersheds it intends to acquire in the future.

The formulation of a Benthic Index of Biotic Integrity is a scientific and systematic process. In its basic components, a B-IBI is designed through a straightforward scientific technique. Using professionals in the field, an accurate analysis of the data collected provides for a precise B-IBI that reflects many environmental conditions. Our team has designed the process for development of the B-IBI based on successful IBIs performed in multiple ecosystems by experts in the field, as well as based on several critical reviews of the IBI process by prominent ecologists and biologists. Since no prior data exists and no other IBIs have been performed in Puerto Rico to date, the B-IBI the team develops will be entirely original work not based off of other studies. The B-IBI will be the first IBI ever to be designed and used in Puerto Rico, and will be a significant step into conservation and environmental restoration efforts.

References

Barbour, M. T., Gerritsen, J., Griffith, G. E., Frydenborg, R., McCarron, E., White, J. S., & Bastian, M. L. (1996). A Framework for Biological Criteria for Florida Streams Using Benthic Macroinvertebrates. *Journal of the North American Benthological Society, 15*(2), 185-211.

Bass, D. (2003). A Comparison of Freshwater Macroinvertebrate Communities on Small Caribbean Islands. *Bioscience, 53*(11), 1094-1100.

Bedoya, D., Novotny, V., & Manolakos, E. S. (2009). Instream and offstream environmental conditions and stream biotic integrity: Importance of scale and site similarities for learning and prediction. *Ecological Modelling, 220*(19), 2393-2406.

Borja, A., Bricker, S. B., Dauer, D. M., Demetriades, N. T., Ferreira, J. G., Forbes, A. T., Hutchings, P., Jia, X., Kenchington, R., Marques, J. C., & Zhu, C. (2008). Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. *Marine Pollution Bulletin, 56*(9), 1519-1537.

Cascorbi, U. (2002). The influence of land use systems on substrate patterns in brooks and their benthic macroinvertebrate microdistribution. *Geoderma, 105*(3-4), 179-200.

Chainho, P., Costa, J. L., Chaves, M. L., Dauer, D. M., & Costa, M. J. (2007). Influence of seasonal variability in benthic invertebrate community structure on the use of biotic indices to assess the ecological status of a Portuguese estuary. *Marine Pollution Bulletin, 54*(10), 1586-1597.

Daly, C., Helmer, E. H., & Quinones, M. (2003). Mapping the climate of Puerto Rico, Vieques and Culebra. *International Journal of Climatology, 23*(11), 1359.

Davis, C. A., & Bidwell, J. R. (2008). Response of Aquatic Invertebrates to Vegetation Management and Agriculture. *Wetlands, 28*(3), 793-805.

Freshwater Biological Association. (2008). Collecting freshwater macroinvertebrate samples.

Karr, J. R. (1981). Assessment of Biotic Integrity Using Fish Communities. *Fisheries, 6*(6), 21.

Karr, J. R., Fausch, K. D., Angermeier, P. L., Yant, P. R., & Schlosser, I. J. (1986). *Assessing Biological Integrity in Running Waters: A Method and Its Rationale*. Urbana: Illinois Natural History Survey.

Kerans, B. L., & Karr, J. R. (1994). A Benthic Index of Biotic Integrity (B-IBI) for Rivers of the Tennessee Valley. *Ecological Applications, 4*(4), 768-785.

Kerans, B. L., Karr, J. R., & Ahlstedt, S. A. (1992). Aquatic Invertebrate Assemblages: Spatial and Temporal Differences among Sampling Protocols. *Journal of the North American Benthological Society, 11*(4), 377-390.

Miller, A. T., Hanson, M. A., Church, J. O., Palik, B., Bowe, S. E., & Butler, M. G. (2008). Invertebrate Community Variation in Seasonal Forest Wetlands: Implications for Sampling and Analyses. *Wetlands, 28*(3), 874-881.

Nerbonne, J. F., Ward, B., Ollila, A., Williams, M., & Vondracek, B. (2008). Effect of Sampling Protocol and Volunteer Bias When Sampling for Macroinvertebrates. *Journal of the North American Benthological Society, 27*(3), 640-646.

Novotny, V., Bartošová, A., O'Reilly, N., & Ehlinger, T. (2005). Unlocking the relationship of biotic integrity of impaired waters to anthropogenic stresses. *Water Research, 39*(1), 184-198.

Ramirez, A., De Jesus-Crespo, R., Martino-Cardona, D. M., Martinez-Rivera, N., & Burgos-Caraballo, S. (2009). Urban streams in Puerto Rico: what can we learn from the tropics? *Journal of the North American Benthological Society, 28*(4), 1070-1079.

Seegert, G. (2000). The development, use, and misuse of biocriteria with an emphasis on the index of biotic integrity. *Environmental Science & Policy, 3*(Supplement 1), 51-58.

Soldner, M., Stephen, I., Ramos, L., Angus, R., Wells, N. C., Grosso, A., & Crane, M. (2004). Relationship between macroinvertebrate fauna and environmental variables in small streams of the Dominican Republic. *Water Research, 38*(4), 863-874.

The Conservation Trust of Puerto Rico. (2007). *Fideicomiso.*

U.S. Environmental Protection Agency. (2009). *EPA Bioindicators- An Introduction to the Index of Biotic Integrity.*

United States Department of Agriculture Natural Resources Conservation Service. (2007). Watershed Science Institute: Watershed Condition Series Technical Note 2 Index of Biotic Integrity (IBI).

Walton, B. M., Salling, M., Wyles, J., & Wolin, J. (2007). Biological integrity in urban streams: Toward resolving multiple dimensions of urbanization. *Landscape and Urban Planning, 79*(1), 110-123.

Weigel, B. M., Henne, L. J., & Martinez-Rivera, L. M. (2002). Macroinvertebrate-Based Index of Biotic Integrity for Protection of Streams in West-Central Mexico. *Journal of the North American Benthological Society, 21*(4), 686-700.

Zabinski, K. B., Pollard, J. R., Kowaleski, M. C., Kennedy, C. L., Lemone, K. A., & Shockey, I. K. (2009). *Rio Piedras Conservation Management Plan*. Worcester, MA: Worcester Polytechnic Institute. URN: E-project-050509-105059

Zhu, D., & Chang, J. (2008). Annual variations of biotic integrity in the upper Yangtze River using an adapted index of biotic integrity (IBI). *Ecological Indicators, 8*(5), 564-572.

Appendix A

WPI IQP D-term 2010, The Conservation Trust of Puerto Rico (Fideicomiso)

Initial Site Survey

Performed by (circle): Andrew Black Mike Ford Krysta Keough Brad Richards

Date: ________________ **Site ID:** ________________

1. **Agricultural Pollution**

1	2	3	4	5
Surrounding area is primarily used for agriculture	Area is developed and also supports agriculture	Area is not used for agriculture, but is not forested	Area is forested but has human development in some areas	Surrounding area is primarily forested

2. **Urbanization in Area**

1	2	3	4	5
Urban areas overrun riparian zone	Urban area bordering riparian zone	Urban area within ½ mile of stream	Urban area within 1 mile of stream	Urban area 2 miles or greater away

3. **Riparian Zone Quality (thickness and diversity, stream bank condition)**

1	2	3	4	5
Very little or no shade, very few species, damaged banks with slides, zone 0-40’	Some shade, a small number of species, 50% or more of bank area is damaged, zone 40-80’	Moderate shade, some species diversity, few uncovered banks, zone 80-120’	Good shade cover, many species, very little bank damage, zone 120-160’	Abundant shade cover, many species evident, intact stream banks, zone 160’+

4. **Immediate Area Pollution**

1	2	3	4	5
Large pollutants (8ft^3 or greater) or toxic chemicals	Mid-sized pollutants (roughly 1ft^3)	Abundant small pollutants	Some small pollutants, little human evidence	No pollutants

5. **Substrate Quality**

1	2	3	4	5
Predominantly silt and mud	Mostly silt or coarse substrate	Some silt and 1-2cm substrate	Finer gravel, good depth	No sediments, deep gravel