DEVELOPMENT AND APPLICATION OF AN ASSESSMENT PROTOCOL FOR WATERSHED BASED BIOMONITORING

THESIS

Presented to the Graduate Council of the

University of North Texas in Partial

Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Joseph Howard Schwartz B.S.

Denton, Texas

May 1998

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With numerous bioassessment methodologies available, a regional protocol needs to be developed to ensure that results are comparable. A regional assessment protocol was developed that includes collecting five benthic macroinvertebrate samples, identifying organisms to genus, and calculating the following metrics: Number of Taxa, Total Number of Individuals, Simpson's Diversity Index, Shannon's Diversity Index, Percent Contribution of Dominant Taxa, Hilsenhoff's Biotic Index, and Percent Contribution of Dipterans.

Once the protocol was developed, it was used to assess the Bayou Chico tributaries and watershed. All three tributaries had been significantly impacted by human activity as had the watershed as a whole. This study indicates that a regional protocol could be developed and is appropriate for biomonitoring at the watershed scale.

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CHAPTER ONE

INTRODUCTION

The Clean Water Act directs the Environmental Protection Agency (EPA) to develop programs that will evaluate, restore, and maintain the chemical, physical, and biological integrity of our Nation's waters. In response to this mandate, States and EPA have implemented chemical based water quality standards. However, meeting these standards provides no assurance that aquatic biota are protected from the effects of undescribed chemical mixtures nor the effects of chemicals' interactions with environmental stresses. Due to this realization, many researchers have suggested the use of biological community attributes as indicators of water quality, under the assumption that particular biological community characteristics can provide information on water quality (Reice and Wohlenberg 1993). This approach has received considerable attention in the past 40 years and is a common strategy in water quality assessments.

Although biological communities are often used as indicators of water quality, the specific communities and endpoints to be evaluated have not been generally agreed upon. A host of community types have been suggested as the best indicators of pollution stress such as algae, fish, and macroinvertebrates, as well as aquatic macrophytes and zooplankton. In addition, how to extract the most information from these communities has also been debated. Structural attributes such as species composition and diversity (Patrick 1949, Simpson 1949, Brinkhurst 1966), and functional attributes such as trophic structure (Cummins 1973) have been suggested as effective methods of evaluating water quality. Others recommend an integration of both functional and structural aspects of the community be used to assess water quality (Hilsenhoff 1977, Plafkin et al 1989). The lack of consensus as to which communities be investigated and what aspects of those communities should be considered, requires resolution if EPA's directive to "evaluate, restore, and maintain the...integrity of our Nation's waters" is to be realized. Standardized protocols must be established to ensure that reliable and comparable evaluations will be accomplished. An overview of the most commonly used assessment strategies may help elucidate the reasons why a standard protocol has not been achieved.

Biological Monitoring

Biological monitoring can be defined as "surveillance using the responses of living organisms to determine whether the environment is favorable to living material" (Cairns and Pratt 1993). This concept has been used for centuries. Kings employing wine and food tasters and canaries in coal mines attests to the historic usage of biological monitors. However, the first rigorously scientific use of biological material to assess environmental conditions can be traced back to the work of Kolkowitz and Marsson in the early 1900's who developed the idea of saprobity in rivers to measure the degree of organic enrichment and the resulting decreases in dissolved oxygen (Cairns and Pratt, 1993). Realizing that certain taxa were restricted to particular ranges of organic contamination, a list of indicator organisms was constructed. With this list, it was theorized, one could determine the degree of contamination by comparing the taxa present at a site with the saprobian system's list of indicator organisms. While this system promises a quick indication of the degree of impact, in reality, it is less than ideal. The Saprobian system was developed to evaluate the impact of organic pollution exclusively, therefore it's use in North America is limited since many pollution problems on this continent are due to toxics or organics tainted with heavy metals (Washington 1984). Others have also criticized this system on theoretical grounds (Cairns and Pratt 1993, Johnson et al. 1993).

The next major advance in the use of biological material as indicators of water quality was the seminal work of Ruth Patrick in the late 1940's. Her work on diatoms made direct use of numbers and kinds of organisms to indicate stream condition. She realized that although individual species vary over time with no corresponding change in water quality, the unimpacted algal flora was typically represented by a high number of species, most of which had relatively small populations. In addition, at unimpacted sites, most algae were diatoms, with few green and blue-green species present. The algal community shifted away from the diatom-dominated community in lotic systems that had been exposed to anthropogenic stress (Patrick 1949).

Water quality assessments based on biological community attributes has become standard procedure since Patrick's pivotal work. Many biologists have researched this concept using aquatic macroinvertebrates and fish. From this conceptual trend came a plethora of indices having been developed to relate biological communities to environmental condition.

Other indices have been developed that have been used with varying success. There are essentially three types of indices that have been utilized to describe water quality based on biological communities, diversity, biotic, and similarity indices. Diversity indices use the number of species present and the abundance of individuals in each species to formulate a single number which can then be used to describe the community in question. Biotic indices are quite different in that they make use of the indicator organism concept. Often these indices use ratios of pollution tolerant to pollution intolerant taxa to describe the extent of environmental degradation. Similarity indices compare two samples with one usually being the control or reference condition. Numerous indices have been proposed to assess water quality using the aforementioned concepts, but none have been universally accepted. The following is a brief description of some of the more important and commonly used indices along with comments and criticisms they have received.

Simpson's Diversity Index "D" (Simpson, 1949) was one of the first and simplest indices formulated. This algorithm described the probability that two organisms, randomly and independently chosen, will belong to the same group. This function is based on the theory that a stressed system will have fewer species numbers, therefore the probability of obtaining two organisms belonging to the same group will increase as the degree of impact increases (Pontasch et al 1989). Fewer species at impacted sites was also one of Patrick's basic assumptions although she didn't assign probabilities in her investigations. Simpson's index has received much criticism over the years. For instance, Whilm (1967) states that Simpson's D is dependent on sample size and therefore is unreliable. Barton and Metcalf-Smith (1992) found that Simpson's D was temporally unstable and could not detect differences between control and disturbed sites. Conversely, in a study evaluating different endpoints in multi-species toxicity tests, Pontasch et al (1989) discovered that

Simpson's D was the most sensitive indicator of stress. However, these results are tenuous in that reduction of D was primarily due to an increase in one species of chironomid. When they calculated D without that chironomid, the index could not detect differences between control and contaminated experimental units. Another reservation that researches have asserted is that since Simpson's D is a function of both species richness and evenness, anthropogenic stresses that cause the number of individuals of all species to be reduced to low numbers resulting in increased evenness, can cause this index to increase, leading to erroneous conclusions (Pontasch and Brusven 1988). Although the use of Simpson's diversity index has been discouraged by several investigators, it's use in water quality assessments continues.

Another commonly used yet highly criticized diversity index is Shannon's H'. This index is based on information theory and was never applied to ecological systems by it's author (Washington 1984). It was originally developed to describe the probability of correctly predicting the next character in a message based on previous information (Fausch et al 1990). The measure of uncertainty in this prediction can be regarded as a measure of diversity.

Shannon's index has been widely used and has been considered a "magic bullet" in water quality assessments. Despite it's frequent use, it has continually come under scrutiny on both practical and theoretical grounds. Hurlbert (1971) called H' a "dubious" index due to the analogy between letters in a message and individuals in a community. Further, he states that H' has no more biological relevance than do the infinite number of other indices which have a minimum when S=1 and a maximum when S=N. Others have criticized this approach for more pragmatic reasons. Pontasch et al (1989) state that one drawback of using Shannon's index in pollution studies is that it doesn't consider the kinds of species or their absolute abundance, therefore, replacement of sensitive species by tolerant species after a toxic insult will not be detected. Similar problems have been discovered by Barton and Metcalf-Smith (1992). While investigating water quality effects in several watersheds exposed to agricultural, industrial, and municipal discharges, they found that Shannon's index could not detect changes in water quality. Conversely, Hughs (1978) found H' to be too sensitive and was effected by several other factors other than pollution including sampling method, sample size, and time of year.

Other diversity indices based on information theory exist, but they too are plagued with similar deficiencies. Despite their publicized weaknesses, competent biologists familiar with the systems they are investigating can make practical use of them in combination with other water quality assessment endpoints.

Another commonly used type of index is the biotic index. Employing the indicator species approach, ratio's of tolerant to intolerant taxa are calculated and the values ciphered are a measure of water quality. The indicator organisms are typically chosen for their specificity to a type of pollution (i.e., heavy metals, organic enrichment) which can make them very useful. However, this specificity may also be a hindrance since it may not be sensitive to other types of insults.

The indicator organism concept employs the idea that in clean streams there is generally a diverse fauna with low percentages of total abundance in each species while in impacted systems, the fauna will be restricted to a few tolerant species (Chandler 1970).

It is important to note that the mere presence of tolerant taxa does not in itself imply poor water quality. It is the dominance of the system by tolerant organisms which provides evidence of degraded conditions.

Kolkowitz and Marsson developed the Saprobian system in 1908, which can be considered the first biotic index. This index defined zones of organic enrichment and the organisms that were found there. By sampling a site and comparing the organisms present to the taxa in the Saprobian system's list, the site could be placed into one of the Saprobic zones. Because the index was based on organic contamination, it's use may be limited in systems which have a toxic contamination problem rather than organic inputs. Other drawbacks of this approach have also been mentioned. Hynes (1960) as cited in Washington (1984) criticizes that the Saprobian system takes no account of local factors making it unreliable. Chandler (1970) comments that the system only works in slow flowing rivers and that systems which have slow areas separated by riffle zones can not be assessed using this technique. Despite these criticisms, the development of this classification scheme was an important step towards effective water quality assessments using the indicator organism approach.

Another biotic index which has been developed is Wright and Tidds index (Wright and Tidd 1933). While investigating limnological parameters in Lake Erie, they employed the density of tubificid worms in benthic samples as a criteria of pollution. Similar to the Saprobian system, they established classifications depending on the density of these Oligochaetes. This index has been modified several times. For instance, Goodnight and Whitley (1960) suggested using the relative abundance of tubificids to all benthic organisms be used as an index. Brinkhurst (1966) brought the index one step further by adding the proportion of a particularly tolerant species, *Limnodrilus hoffmeisteri*, into the equation. Like the Saprobian system, these indices can only be used on a limited basis due to their insensitivity to pollutants other than organic enrichment. In addition, seasonal variability and physical factors may influence index values.

A slightly more sophisticated index was devised by an investigator studying the Trent River in England. The Trent Biotic index (Woodiwiss 1960) incorporates six key taxa and the number of species representing them to describe water quality. Plecopterans, Trichopterans, and Ephemeropterans, as well as *Gammarus*, *Asellus*, and tubificids/red chironomids are separated and the number of species in each group are counted. Woodiwiss devised a scoring system based on the above procedure and sites could be ranked accordingly. Although an improvement over some earlier indices, some major flaws with this technique have been identified. Physical parameters such as dissolved oxygen and flow can alter the index regardless of chemical factors (Balloch et al 1976). Geographic variability (Chutter 1972) and insensitivity (Washington 1984) have also been mentioned.

Probably the most commonly used biotic index is Hilsenhoff's Biotic Index "HBI" (Hilsenhoff 1977). Using the riffle ecotype, Hilsenhoff developed a list of organisms and assigned a tolerance score to each. When a site is sampled, each organism is identified and given it's corresponding score. The scores are summed and divided by the total number of organisms to produce an average score. If the average score is high, than poor water quality is implied. He also established a list which defines what degree of impact produces which scores. As with most biotic indices, the HBI was produced for organic contamination. However, describing tolerance values for other pollutant types could render this index useful for other classes of contaminants.

Similarity indices attempt to compare the biological communities of two separate sites. Typically, one of these sites is a control or reference stream while the other is the system which is being assessed. Their use in pollution surveys rests on the assumption that local ecosystems that receive similar stresses support similar biological communities (Pinkham and Pearson 1976). If an unimpacted site can be identified, community analysis at the site of interest can be compared to this reference site and relative water quality can be determined.

In 1908, Jaccard introduced his index, the ratio of the number of taxa common to both sites to the total number of taxa collected at both sites. Still in use today, Jaccard's similarity index was the first and simplest index that utilized these comparisons. Because it uses only presence-absence data and does not incorporate more quantitative data, some researchers feel that it is of limited use. Pinkham and Pearson (1976) felt that this lack of quantitative data usage tended to produce misleading results and attempted to compensate for it's shortcomings by developing their own index. The Pinkham-Pearson index incorporates both qualitative and quantitative data which they felt produced more reliable results. However, other biologist aren't as enamored with this index. Pontasch et al, (1989) concluded that this index was insensitive to increases in density and did not reflect the presence of dominant species. Similarly, Brock (1977) as cited in Washington (1984) claims that the Pinkham-Pearson index overemphasizes rare species and is very sensitive to regular sampling errors. Despite these criticisms, this index is commonly used and recommended by EPA in their Rapid Bioassessment Protocol (Plafkin et al 1989).

Since the development of the Pinkham-Pearson index, several other similarity indices have been suggested. Quantitative similarity indices for taxa and functional attributes have been devised, as well as percent dominants in common to two sites. Though comparisons of an experimental site to a reference site seems to be an effective method of determining relative water quality, the assumption of similar biological communities reflecting only similar chemical conditions seems tenuous. Physical conditions also effect communities which may render the assumption invalid. Nevertheless, with careful attention to physical parameters, similarity indices can be successfully used in pollution assessments.

Individual biotic and diversity indices have been extensively used in water quality assessments. A recent trend, however, has been the incorporation of several indices to generate a single number that reflects water quality. This approach attempts to accentuate the positive aspects of the individual indices (metrics) while compensating for their individual deficiencies. At the forefront of this conceptual leap are the metrics used in the EPA's Rapid Bioassessment Protocol (RBP) (Plafkin et al 1989). This protocol integrates several structural and functional parameters into a single evaluation of biotic condition. This approach has several advantages to the single index approach. Insensitivity of a metric to one particular type or class of contaminants can be compensated for by other metrics. In the calculation of the final score, effects due to non-water quality parameters on individual metrics may counteract each other so an erroneous result will not be produced. On the other hand, if water quality effects one metric in one direction and another metric in the opposite direction, they may cancel each other out and a conclusion of good water quality may mistakenly be made. Criticisms similar to those of the other biotic indices have been levied against the RBP metrics, for instance, insensitivity to non-organic pollution (Resh and Jackson 1993). Barbour et al (1992) evaluated the RBP metrics and found several of them to be too variable to be useful. In addition, they discovered that by using all the metrics simultaneously, redundancy of information was inevitable.

Integrative biotic indices for fish have also been developed. Karr (1981) established the Index of Biotic Integrity "IBI". Using both structural and functional parameters of fish communities, he devised a method to assess water quality. Because fish are easy to identify, long-lived, and meaningful to the general public, he felt that his index was the most appropriate method to describe biotic conditions in streams. Unfortunately, geographically specific metrics made his system difficult to interpret outside of it's area of development. However, Fausch et al (1984) was able to modify the IBI for several different watersheds in the mid-west and found it to accurately reflect stream conditions. Other biologists also give Karr's index accolades. Plafkin et al (1989) states that the IBI is "firmly grounded in fisheries community ecology" and that it can incorporate geographic perspectives. Although, some of the metrics in the IBI can easily be modified for different ecoregions, some metrics call for the enumeration of particular species which may or may not be present in the region. To modify these metrics, species having similar roles in the ecosystem would have to be substituted. Another disadvantage to this approach is the assumption that fish are exposed to all environmental stresses. This may not be the case. Due to their mobility, fish may be able to avoid periodic insults, thus not incorporating episodic events into the community structure. Unlike macroinvertebrates which have limited mobility, fish may travel several kilometers to avoid adverse conditions (Charles Gowan, unpublished data). Nevertheless, incorporating the IBI in water quality assessments is a potentially useful means of characterizing biological conditions in lotic systems.

Multi-metric approaches have been generally accepted and expanded upon. The EPA has suggested developing Biological Criteria based on this approach. Biological Criteria are developed from expected fish and benthic macroinvertebrate communities for the region or watershed (USEPA 1996). Biosurveys of least impacted systems are conducted using the Rapid Bioassessment Protocol, and biocriteria are established. When an assessment of a stream or watershed is conducted, the results can be compared to the biocriteria and a determination can be made as to whether that system meets the criteria. In this way, streams can be listed as exceeding or not exceeding their criteria and States can act accordingly.

Despite the drawbacks of the multi-metric assessment approach, this concept is attractive. By integrating various attributes of the biological communities, reliable determination of water quality can be accomplished. Although modification and standardization of the metrics utilized needs to be established, effective bioassessments can be conducted using these schemes. By establishing standard protocols on a regional basis, water quality trends within a system can be detected, and comparisons among watersheds can be performed. It was for this reason, establishment of a standard protocol, that the current research was undertaken.

CHAPTER II

OBJECTIVES

In 1990, the EPA implemented a program requiring states to establish biological criteria which will be used, in addition to chemical and physical parameters, to assess the quality of surface waters (USEPA 1990). For this policy to be meaningful, a protocol must be developed which will ensure that these assessments are reliable and comparable. Sampling methodology and data analysis techniques must be regionally standardized to ensure results can be compared from one system to another. To understand effects of water quality enhancement programs, biologists and policy makers must be able to understand the results of these assessments to defend their policy recommendations.

With the multitude of sampling techniques and assessment indices which have been described to measure water quality, biologists commissioned to conduct these assessments may be overwhelmed with the procedural diversity at their disposal. For this reason the primary objective of this study was to develop an efficient bioassessment protocol to be used in water quality monitoring programs. The protocol developed includes recommendations for assessment endpoints, sampling strategies, and level of taxonomic identification. The second objective of this study was to use the Bayou Chico watershed as a demonstration system and apply the newly developed assessment protocol to assess the health of the Bayou Chico tributaries. In addition to the emphasis on biological criteria recently put forth by the EPA, water quality management at the watershed level has also been suggested. For this reason, the third objective of this study was to assess the biological integrity of the Bayou Chico watershed.

An important consideration in the development of an efficient bioassessment protocol is the variability present in the endpoints that will be utilized for the assessment program. Using statistical analyses outlined in a subsequent section of this document, assessment indices were tested to determine the amount of variance associated with them. Indices with large amounts of variability are not useful in a monitoring program and were therefore rejected as an assessment endpoint. In addition to variability of the indices, the degree of redundancy of information contributed by the individual indices was elucidated. To provide useful information in an efficient manner, all endpoints used to describe the condition of an aquatic system should convey unique information. To this end, metrics were analyzed, using a procedure outlined in a subsequent section of this document, to evaluate the amount of unique information each endpoint provides. Indices that did not provide unique information were excluded from the assessment protocol. By performing the two previous procedures, from the numerous indices which exist, a more modest list of potentially useful assessment endpoints was constructed.

To further reduce the list of acceptable indices, they were tested to see which metrics can provide valuable information with the limited number of samples that scarce resources often dictate. Using power analysis described in the data analysis section of this document, the number of samples needed for each metric, based on the variability found in the systems in question, was calculated and indices that required few samples were incorporated into the assessment protocol.

Once a suite of indices had been identified, taxonomic sufficiency was addressed. Because identification of collected organisms is time consuming, the level of identification may have a large impact on the cost of analysis. If it could be shown that identifying organisms to the family level provides as much information as identifying them to genus, an efficient protocol would stipulate identification to the family level. Therefore, all samples were analyzed to address this issue.

Once the assessment protocol was established, it was applied to the streams draining the Bayou Chico watershed. The three streams were assessed using this protocol and the relative health of each stream was determined. In addition, the condition of the Bayou Chico watershed as a whole was assessed using this newly developed protocol.

CHAPTER III

DESCRIPTION OF STUDY AREA

The Bayou Chico watershed, located in southern Escambia County, Florida, comprises slightly more than 6500 acres (Figure 3.1). Land surface elevations range from sea level to about 90 feet above sea level. The watershed is divided into three subwatersheds, each drained by an individual stream. Surrounding land use is mixed, typically commercial/light industrial, institutional, open land, and medium-density residential (Figure 3.2). The smallest of the three sub-basins is drained by a small depositional stream known only as the Northeast tributary. With a drained surface area of 240 acres, this watershed drains approximately 4% of the total Bayou Chico watershed. A typical urban stream, the Northeast Tributary showed evidence of human impact. Trails leading to the water's edge, man-made objects in the channel, and other evidence suggested that this stream may be subject to anthropogenic stress. Land use in this watershed is predominantly medium density residential but some commercial areas are included. A sampling station was established approximately 500 yards upstream of Bayou Chico. Designated S1, this station had an average width of five meters, an average depth of 0.16 meters, and a flow of 0.1 m^3/sec. This site had trees right up to the banks and had almost complete overhead cover.

Largest of the three sub-basins is Jones' Creek watershed in the southwest region of the main watershed. At 2500 acres, this watershed drains 38% of the total watershed area and contains a large area of wetland habitat, approximately 400 acres. Known as Jones' swamp, this wetland is a major feature of the sub-watershed and has resulted in a large area of undeveloped land. Another main feature in this sub-basin is a military compound, Corry field. Jones' Creek is a relatively wide, deep, depositional habitat that also had signs of human impact. In the lower reaches, several shopping carts could be seen in the channel and many trails were evident along its banks. The water was dark and had a considerable amount of emergent and submerged vegetation present. A sampling station was set up approximately 850 yards upstream from the bayou. Designated S3, this station was relatively deep and wide with no discernable flow. Depth ranged from 0.64 to 0.76 meters and width ranged from 7.7 to 10.3 meters. This station was quite open with relatively little overhead cover.

Lying in the northwestern section of the Bayou Chico watershed is Jackson's Branch sub-watershed. Intermediate in size, Jackson's branch drains approximately 1400 acres or slightly more than 21% of the total area of the main watershed. A narrow, erosional stream, Jackson's Branch drains commercial, residential, and institutional land use areas and has the shortest above-ground drainage distance. Although some litter was present, this stream had the least evidence of human impact of the three. One station was established approximately 850 yards upstream of the bayou. Designated S2, this station was relatively shaded and cool. Depths ranged from 0.07 to 0.11 meters with an average width of 1.3 meters. Flow averaged 2 m^3/sec. The remaining 37% of the watershed drains directly into the bayou via overland flow and drainage canals.

Figure 3.1
Location of Bayou Chico Watershed

Figure 3.2
Map of Bayou Chico Watershed Showing Land Uses

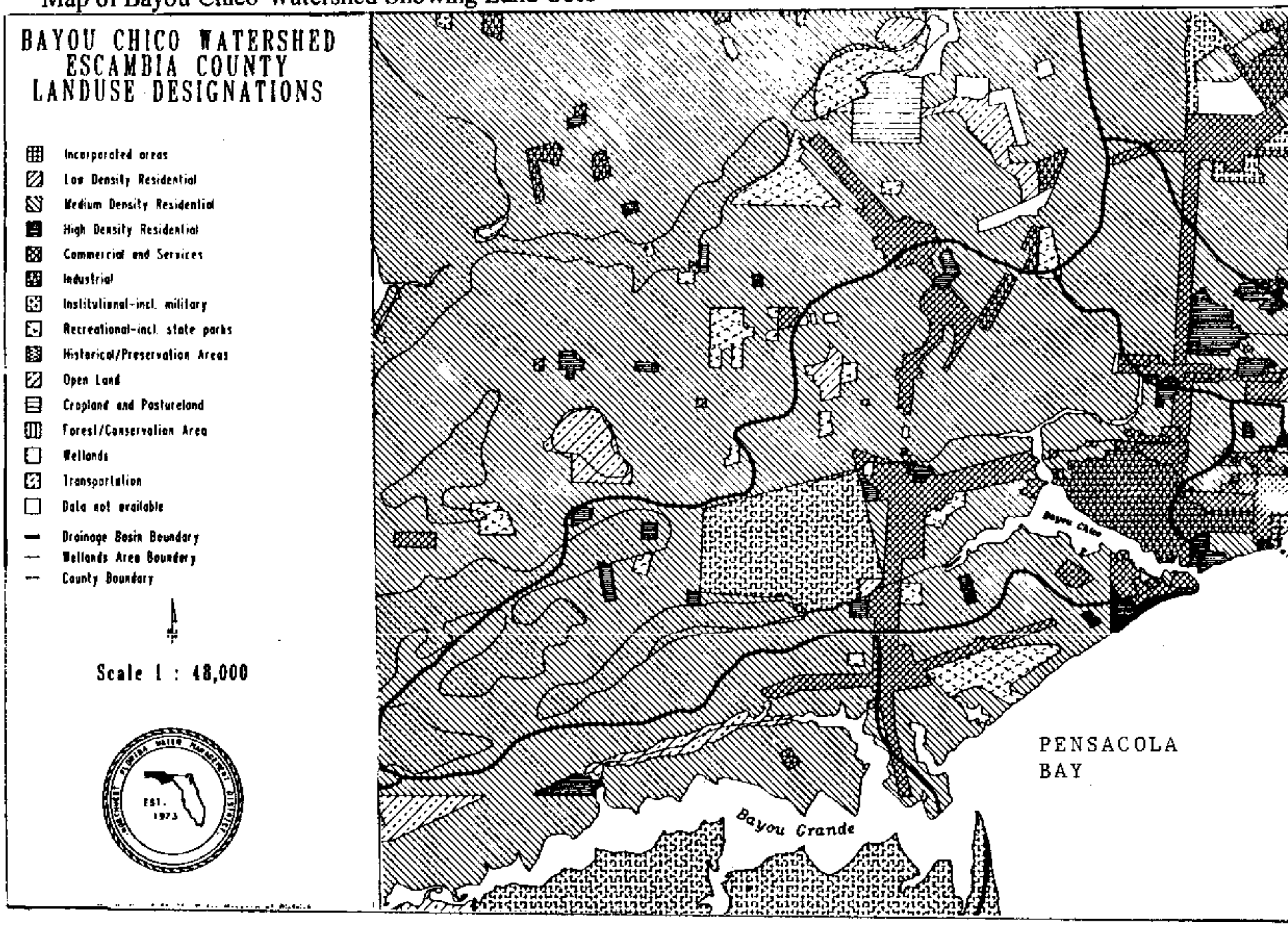
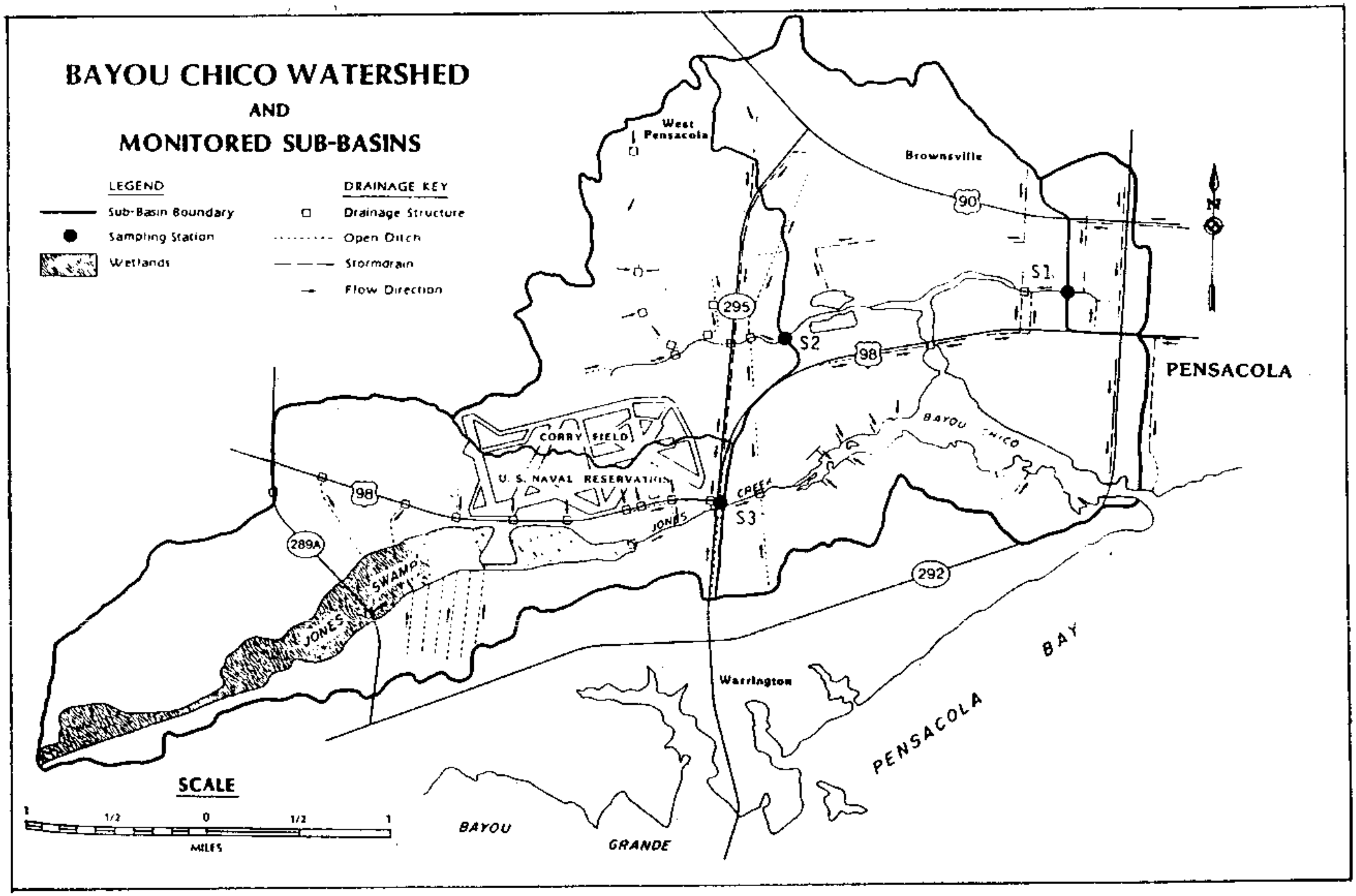


Figure 3.3
Map of Bayou Chico Showing Sampling Locations



CHAPTER IV

MATERIALS AND METHODS

One sampling station was established on each study stream. Each station was sampled during mid May 1994. Macroinvertebrate and fish community analysis and water chemistry analysis was conducted. In addition, water and sediment toxicity tests were employed to assess the water quality of the watershed. Benthic reference data were collected from Black Creek in mid August 1995. Additional data were provided by Florida Department of Environmental Protection. Fish reference data were provided by Florida Game and Freshwater Fish Commission. Water chemistry data for Black Creek and another reference stream, Dean Creek was obtained from FDEP (1996a).

Black Creek is a State designated reference stream and is located approximately 50 miles west of the Bayou Chico watershed. It is a slow, depositional type system that drains predominantly silvicultural land uses and is considered a least impacted site in the panhandle of Florida (FDEP 1996b). Water chemistry data were obtained from the State of Florida's 305b report (FDEP 1996a). Fish data were obtained from a large scale survey conducted by the Florida Game and Fresh Water Fish Commission (Bass 1993).

Toxicity Tests

Eight liters of stream water at each site were collected in two, 4 liter acid washed cubitainers and sent via Greyhound Express back to the aquatic toxicology laboratory at the University of North Texas on the day of collection. Short-term chronic (7-day) water column toxicity tests were conducted on *Ceriodaphnia dubia* and fat head minnows (*Pimephales promelas*) using standard EPA protocol. In addition, sediment samples were collected in acid washed tupperware® containers and shipped, along with the water samples, to the University of North Texas toxicology laboratory. Short-term chronic (7-day) sediment toxicity tests were conducted on *Ceriodaphnia dubia* using a static renewal procedure whereby the water is removed every 24 hours while the sediment and organisms remain in the test chamber.

Chemical Analysis

Routine chemical analyses such as pH, dissolved oxygen, conductivity, and temperature were conducted in the field using YSI field instruments. Hardness and alkalinity was measured in the field using titration methods in Standard Methods (APHA et al. 1992). In addition, water samples were analyzed for nutrients and toxics. Sediment samples were also evaluated.

Macroinvertebrate Community Analysis

Field Collection

Benthic macroinvertebrates were sampled at 6 randomly selected locations at each study site using either a petite ponar grab sampler or a Guzzler® hand pump sampler, depending on substrate. The grab sampler was employed at station S3 on Jones' Creek where substrate was silty and a high organic content was present. One grab was considered a sample. At stations S1 and S2, where the substrate was predominantly sand, the pump sampler was utilized. This method involved setting a bottomless 5 gallon bucket into the substrate until a seal was formed between the bucket edge and the stream bottom. While agitating the substrate, the hand pump was used to pump the substrate, via a 2" diameter flexible hose, out of the sampling bucket into a collection bucket. When two collection buckets were full, the sample was complete. Regardless of sampling method, six replicate samples were collected at each station.

Once a sample had been collected, it was sieved in the field using a #80 U.S. standard sieve and placed into a two liter Nalgene® container. Samples were preserved in Kahle's solution. Benthic macroinvertebrate samples were analyzed at the University of North Texas.

Laboratory Procedure

Macroinvertebrate samples were stained with Rose Bengal and allowed to sit for at least 48 hours. They were then rinsed and sorted using a dissecting microscope under at least 16x magnification. Organisms were then identified to the lowest taxonomic level possible with voucher specimens being prepared by making permanent slides using CMC mounting media when necessary. Voucher slides have been archived at the University of North Texas and will remain there for at least two years. Voucher specimens not requiring mounting were also archived.

Fish Community Analysis

Field Procedure

Fish were collected using a Smith-Root model 12 POW variable wave function electro-fisher. Approximately 50 meters at each sampling location were sampled making sure all habitats were included. Fish were removed from the stream and preserved in 70% ethanol. Large specimens were identified in the field and returned to the stream after sampling was concluded.

Laboratory Procedure

Fish were identified to species, counted, weighed, and measured. Voucher specimens have been retained at the University of North Texas.

Data Analysis

Benthic Macroinvertebrates

Statistical analyses of 17 bioassessment metrics for macroinvertebrates (Table 4.1) were conducted to determine the efficacy of these endpoints in the study area. Using data collected from a reference location, Black Creek, along with reference data provided by Florida Department of Environmental Protection (FDEPb) the metrics were evaluated for variation, power, and redundancy of information. In addition, the taxonomic level of identification needed to produce acceptable results was investigated.

Using the coefficient of variation (CV), the amount of variation associated with the individual metrics was determined. A high CV indicates large variation relative to the mean. This would suggest that the metric was insensitive to small changes which would preclude its inclusion in the protocol.

Redundancy of information, when using more than one metric, is also an important concern. An efficient protocol is of paramount importance when resources to accomplish monitoring studies are limited. Pairwise correlation analysis was performed on all metrics under consideration to ascertain the interdependence, or degree of redundancy among the endpoints. A high degree of correlation indicates a large amount of overlap of information from the metrics. Alternatively, a low amount of correlation demonstrates that unique information is being obtained through those measurements.

The number of samples required to detect a predetermined magnitude of change in a dependent variable is critical when choosing assessment metrics. Metrics that require large numbers of samples to detect a reasonable degree of change would not be useful in a monitoring program due to the cost of such an analysis. Endpoints which require few samples are required. To calculate the number of samples that are required to detect a specified difference between stations using the individual metrics, power analysis was conducted using the following equation:

$$n = s^2 / \delta^2 (t_{\alpha,v} + t_{\beta,v})^2$$

where n = the number of samples required, α can be $\alpha(1)$ or $\alpha(2)$ depending on whether a one tailed or two tailed test is used and where δ = detectable amount of change in metric value . However, since v is dependent on sample size, n cannot be calculated directly but must be obtained by iteration (Zar 1984).

To address the issue of taxonomic sufficiency, all organisms were identified to the lowest level possible, typically genus, and the metrics that had been deemed appropriate by the above procedure were calculated. These same metrics were then calculated at the family and then the order taxonomic level. A one way ANOVA was then employed to determine if the values for the metrics at different taxonomic levels were different. If the calculated metrics were similar at all taxonomic levels, the protocol would include the stipulation that organisms only be identified to order. If, on the other hand, the information obtained by identifying organisms down to genus resulted in a greater sensitivity of the metrics, than the protocol would reflect that fact.

Using the above mentioned statistical procedures, a suite of metrics was selected and the level at which organisms need to be identified was determined. Once the biological endpoints were selected, they were calculated for the three Bayou Chico tributaries. A Stream Condition Index (SCI) was then calculated by normalizing all metrics to obtain a Index value. Finally, the metrics and SCIs calculated for the tributaries were calculated for the Bayou Chico watershed and a watershed based assessment was accomplished.

Fish

In addition to benthic macroinvertebrates, fish communities were utilized in the assessment of the study site. Fish community structure of the Bayou Chico watershed was compared to reference data, provided by Florida Game and Freshwater Fish Commission using a modified IBI (Karr 1991) as modified by Bass (1993). These data were from a regional survey of first order streams in northern Florida. Individual metrics for Bayou Chico streams were calculated and assigned a score based on expected values calculated from reference streams in the region. These scores were then summed to establish an IBI for the sites.

The IBI was calculated by using 13 metrics that considered various aspects of the fish community present. Ranges and scores were taken from Bass (1993) whose survey of first order streams in northern Florida yielded 17,686 fish, representing 59 species from 75 streams. Minimum and maximum values for these metrics come from observations from this survey of all streams, so these values are actual observed values from this study.

Table 4.1
Benthic Metrics to be Evaluated Along with Rational for Use.

Metric	**Rational for Use**
Number of taxa	Decreases with disturbance
Number of individuals	Decreases with disturbance
Simpson's Diversity Index D	Decreases with disturbance
Number of EPT taxa	Decreases with disturbance
Shannon's Diversity Index H'	Decreases with disturbance
Number of Coleoptera taxa	Decreases with disturbance
Percent Dominant taxa	Increases with disturbance
Hilsenhoff's Biotic Index HBI	Increases with disturbance
Cricotopus+*Chironomus*/total Chironomidae	Increases with disturbance
Percent Chironomidae	Increases with disturbance
Scrapers/Filterers	variable
Percent Filterers	variable
Florida Index	Increases with disturbance
Percent Scrapers	variable
Percent Shredders	variable
Number of Chironomidae taxa	Decreases with disturbance
Percent Diptera	Increases with disturbance

CHAPTER V

RESULTS

Protocol Development

Benthic Macroinvertebrates

Coefficients of Variation

Using data from the reference stream, coefficients of variation (CV) were calculated for all metrics being considered for inclusion in the assessment protocol. Figure 5.1 shows the CV values calculated for each metric. Metrics that had high coefficients of variation were excluded from further consideration while those with a CV of less than one were not excluded from the protocol.

Simpson's Diversity Index and Hilsenhoff's Biotic Index had the least amount of variation relative to their means with a CV of 0.04 and 0.07 respectively. Conversely, the metric *Cricotopus*+*Chironomus*/total chironomids was the most variable with a CV of 1.7 which excluded it from further consideration. Scrapers/filterers had a CV of 1 which made it too variable to be included in the protocol. Percent Scrapers and # of Coleoptera Taxa were also quite variable with CVs of 0.96 and 0.9 respectively, but were retained for further analysis. All other metrics had low variability represented by CVs of 0.5 or less except for percent Filterers that had a CV of 0.7.

Of the original 17 metrics, two were excluded based on the large variability of these measurements as reflected in the coefficient of variation. Both *Cricotopus*

+*Chironomus*/ total chironomids and scrapers/filterers had CV of 1 or larger and were therefore excluded from the protocol. All other metrics were retained for further analysis.

Power Analysis

The remaining 15 metrics were subjected to power analysis to determine the number of samples required to detect a 50% change in either direction of the metric with a β of 0.1 and an α of 0.05. The criteria of five samples was established. Metrics that required five or fewer samples to detect a 50% change were not excluded from the protocol while metrics that required more than five samples were excluded. Figure 5.2 shows the number of samples required to detect a 50% change for each metric that passed the CV analysis.

Several of the metrics were shown to have insufficient power, with the variability of the reference data, to detect a 50% change using five or fewer samples. Percent Scrapers was the least powerful metric needing 45 samples. Number of Coleoptera taxa also had very little power requiring 34 samples.

The most statistically powerful metric was Simpson's Diversity Index D, needing only two samples to detect a 50% change. Shannon's H', Hilsenhoff's Biotic Index, and percent Diptera all had good power, needing only three samples to detect the required change. Other metrics with sufficient power were total number of taxa present, needing four, total number of organisms present, and percent dominant taxa, both needing five.

Fifteen metrics were analyzed to determine the number of samples required to detect a 50% change in the metric value using a β of 0.1 and an α of 0.05. Seven of these metrics were deemed powerful enough to be included in the protocol while the remaining eight metrics needed greater than five samples to detect a 50% change.

Correlation Analysis

Pairwise Correlation Analysis on the remaining metrics was conducted to determine the amount of overlap or redundancy that these metrics have. A large coefficient of correlation between two metrics would indicate the metrics are not contributing unique information, therefore the use of both metrics would not be an efficient use of resources. Table 5.1 shows that most metrics were highly correlated with each other. HBI was the only metric that was not highly correlated with the others, having correlation coefficients all under 0.6. Percent Diptera was the only other endpoint that was not highly correlated with all other metrics. This measure was only moderately correlated with the total number of individuals and percent dominance. All other metrics were highly correlated with one another with values ranging from -0.83 for HBI and percent Diptera, up to 0.999 for Shannon's H' and number of taxa.

Principal Components Analysis

A Principal Components Analysis was conducted to help determine a suite of metrics that would best serve to describe the biological condition of the study sites. Each time the analysis was run, the first two variables accounted for 100% of the variation in the data. For this reason, Principal Components Analysis was not deemed useful and the metrics that remained after the power analysis were used to assess the tributaries of Bayou Chico.

Of the original 17 benthic community metrics, seven were retained to be included in the assessment protocol (Table 5.2). These metrics had relatively small natural variability, as evidenced by the coefficients of variation, and sufficient power to detect differences between sites using a reasonable number of samples.

Taxonomic Sufficiency

Taxonomic sufficiency is an important issue when one is defining an assessment protocol. Identifications of benthic organisms is time consuming and can therefore play a large role in determining the total cost of an assessment. For this reason, an investigation into the appropriate level to which organisms should be identified was conducted. Metrics were calculated using data derived from identifying organisms to Family and to Genus to determine whether level of identification affected the results. Metrics that were included in the protocol were calculated, and a one-way ANOVA was conducted to determine if the values were significantly different. If there was a significant difference, the lower taxonomic level of identification would be used in the protocol since additional information could be obtained by the added resolution.

Family and genus level identifications did result in significant differences for number of taxa, Simpson's D, and Shannon's H' (Table 5.3). Total number, percent dominance, HBI, and percent Diptera had no significant differences between the two levels of identification. Because additional information could be obtained by using genus level identifications, all site assessments were conducted using this level of resolution. By analyzing the variance, power, and taxonomic sufficiency of the benthic metrics and by incorporating a previously established fish community index, a protocol for watershed based biological assessments had been developed. This protocol was then used to assess the Bayou Chico tributaries and watershed.

Site Assessments

Northeast Tributary (S1)

Water Column Toxicity Tests

A short-term chronic (7-day) toxicity test was performed on fathead minnows (*Pimephales promelas*). Organisms in Northeast Tributary samples exhibited 100% survival as did controls (Table 5.4). Therefore no toxicity is indicated.

In addition to fathead minnow tests, Short-term chronic (7-day) *Ceriodaphnia dubia* toxicity tests were conducted. Mean number of neonates per adult along with standard deviations and whether the tests show significance are shown in Table 5.5. A total of 189 neonates were produced by organisms tested in reconstituted soft water (control) with a mean of 19 neonates per adult. Organisms tested in water from the Northeast Tributary averaged 31 neonates per adult for a total of 310. A one-way ANOVA indicated a significant difference between Northeast Tributary test organisms and Controls. However, since Northeast Tributary values are greater than control values, no toxicity is indicated.

Sediment Toxicity Tests

The sediment survival and reproduction toxicity tests on *C. dubia* using Northeast Tributary sediment resulted in a total of 163 neonates being produced with a mean of 16.3 neonates per adult (Table 5.6). Although no control was used as a comparison, toxicity may be indicated since four of the original 10 organisms used in these tests died before they produced any offspring.

Chemical, Physical, and Microbiological analysis

Water quality analysis using chemical, physical, and microbiological sampling procedures was conducted. Table 5.7 lists the results of these analyses along with mean values for reference streams in the study region. Due to incomplete data, not all parameters were assessed.

A TOC of 3.05 mg/l was present in the Northeast Tributary. This value is similar to values observed by Pratt and his coworkers in their storm water assessment of the Bayou Chico watershed (Pratt et al. 1993). Unlike previous observations, both ammonia and nitrate + nitrite were quite high with values of 0.507 and 1.018 mg/l respectively. Relative to reference stream conditions, the Northeast Tributary was quite high in biological oxygen demand. With a value of 3 mg/l, BOD was 5 times that of the reference condition. Not surprisingly, DO was quite low with a value of 3.6 mg/l as compared with a mean of 8.4 for Dean and Black creeks. Other water quality parameters of note are fecal coliform and conductivity. An observed fecal coliform count of greater than 6000 cfu/100ml was several orders of magnitude greater than the reference condition.

Conductivity, too, was much higher than the reference stream.

Fish

A total of 234 individuals consisting of four fish species were collected from the Northeast Tributary (Table 5.8). Two species, *Gambusia Sp.* (148) and *Dormitator maculatus* (82) accounted for over 95% of the total individuals collected at this site. Two each of *Lepomis macrochirus* and *Poecilia lattipinna* were also collected. A modified IBI was calculated for the Northeast Tributary using the procedure of Karr (1981) as modified by Bass (1993). The scoring for this methodology is summarized in Table 5.9. Individual metric scores varied from a high of 5 out of a possible 5 for total number of individuals to a low of 0 for many metrics (Table 5.10). The metrics number of Darter species, number of intolerant species, number of positive species, percent insectivorous cyprinids, percent top carnivores, percent minnows, and percent Madtoms received the lowest possible score of zero because these organisms were absent from the sample. The metric percent omnivores received a score of zero due to the high number of these functional generalists. Other metrics received very low scores. An overall IBI score of 18 was calculated for this stream, which translates to a condition index of "very poor" (Table 5.11).

Benthic Macroinvertebrates

Individual metrics included in the protocol were calculated using five replicate samples of Northeast Tributary benthic organisms and compared to those of the reference stream (Table 5.14). With a total of 15 taxa present at S1, this station had a larger number of taxa than the reference stream. Similarly, total number of organisms present at this station, 2662, far outnumbered that of the reference condition. Another metric that exceeded that of the reference condition was percent Diptera. S1 had 21% of all organisms present comprised of dipterans, whereas the reference condition was composed of 49% dipterans. The four other metrics all fell short of the reference stream values. In addition, an overall score for S1 was calculated by aggregating individual metric values into a Stream Condition Index (SCI) using the procedure outlined in FDEP (1996b). For aggregation into an index, metrics were normalized since they have different numerical scales. Using the mean and standard deviation for metrics calculated for the reference stream, scores were assigned. The highest score, 5, was assigned to metric values greater than or equal to the mean minus 1 standard deviation for metrics that decrease in response to perturbation. For metrics that increase in response to impact, a high score was given to values less than or equal to the mean plus one standard deviation. Development of scoring criteria using this rational is illustrated in Table 5.12.

Four condition ratings, very poor, poor, good, and very good were assigned to SCI values based on quartile groupings. Since the highest possible SCI is 35 (seven metrics multiplied by the high score 5), the ratings were broken into groups of nine. An SCI of 1-9 was assigned a very poor rating, 10-18 a poor rating, 19-27 a good rating, and 28-35 a very good rating.

Three metrics calculated for the Northeast Tributary received the highest stream condition index score possible, number of taxa present, total number of individuals, and percent Diptera (Table 5.13). However, the other four metrics received the lowest score possible. With 2662 total organisms collected, this site had many more individuals than did the reference site and therefore received a 5 for this measure. Also, at this site, dipterans were far less numerous relative to other taxa as compared to the reference site. Dipterans comprised only 21% of the total number of organisms in the Northeast Tributary as compared to over 49% in the reference stream, Black Creek. The other metric to receive a high score was number of taxa present.

Conversely, Simpson's D, Shannon's H', percent Dominance, and Hilsenhoff's Biotic Index all received a low score of one. In all cases, the values calculated for the metrics were more than 3 standard deviations away from the reference condition indicating severe impairment.

Using the individual metrics, a Stream Condition Index (SCI) was calculated. A score of 19 placed this site in the "good" category despite the fact that four of the seven metrics received the lowest score possible.

Jackson's Branch (S2)

Water Column Toxicity Tests

Values for survival tests of the short-term chronic (7-day) toxicity test for fathead minnows (*Pimephales promelas*) are shown in Table 5.4. Organisms in Jackson's Branch samples exhibited 100% survival indicating no toxicity.

However, unlike the Northeast Tributary, Jackson's Branch's *C. dubia* tests showed significant toxicity (Table 5.5). There was 100% mortality by day 4 and no individual produced more than one brood. Average brood size was 3.7 neonates as compared to 19 for controls and one individual did not produce a single brood. The results of this test suggest significant reproductive and survival impairment as compared to controls.

Sediment Toxicity Tests

In addition to the basic sediment toxicity tests that were run on all the study sites, three replicate samples of sediment from Jackson's Branch were used to determine within-site variability of toxicity (Table 5.6). Replicate 1 had a mean of 20.7 neonates per adult with a total of 163 offspring produced. Three organisms did not survive to produce any progeny. Replicate 2 produced a total of 140 neonates with a mean of 14 offspring per adult. Similar to Replicate 1, this replicate had 3 organisms die before reproducing. Replicate 3 had a total of 209 neonates produced with a mean of 20.9 neonates per adult. This replicate had only 2 organisms die before producing its first brood. Although there was no control to compare these results to, some toxicity may be indicated due to the mortality of at least two adult organisms in each replicate.

To address the question of within-site variability of toxicity, an ANOVA was conducted to determine if location of the sediment taken within the site effected the results of the test. For the sediment collected from Jackson's Branch, no difference was detected ($p=0.39$) between replicate samples for mean number of neonates produced per organism.

Chemical Physical, and Microbiological Analysis

Results of chemical physical, and microbiological analysis of Jackson's Branch are summarized in Table 5.7. These results are substantially different from previous investigations and reference conditions. Biological Oxygen Demand, TOC, and NO_3 were all considerably higher than previous investigations (Pratt et al. 1993, FDEP 1996a) and BOD was 5 times higher than the reference values. On our sampling date, BOD was 3 mg/l as compared to 0.6 mg/l for the reference and 0.6 and 1 mg/l in previous studies. Nitrate concentration in Jackson's Branch was 5 times the reference stream's concentration. Conversely, other constituents were much lower in concentration such as TKN, total Phosphorous, dissolved oxygen, and pH. Dissolved oxygen was less than half the reference values, 3.8 mg/l as compared to 8.4 mg/l. Fecal coliform counts were lower in this study than in other studies and comparable to that of reference streams. On this sampling date, total fecal coliform were 204 cfu/100ml as compared to a mean of 975 cfu/100ml from samples taken from 1990-1995 (FDEP 1996a).

Fish

A total of 121 individuals consisting of two species were collected from Jackson's Branch (Table 5.8). Of these two species, *Gambusia Sp.* made up over 98 % of the sample with 119 individuals. Two *Anguilla rostrata* were also collected.

Individual metrics for this tributary typically scored exceedingly low except for the metrics number of negative species, receiving the highest score possible, percent top carnivores and total number of individuals which both received medium scores (Table 5.10). Due to the extremely low individual metric scores, this stream received a very poor rating with an overall IBI of 11.

Benthic Macroinvertebrates

Individual metrics and the SCI for Jackson's Branch were calculated as they were for the Northeast Tributary. In addition, the condition ratings, based on the same quartile groupings, were used to assess this site (Table 5.12).

As illustrated in Figure 5.3, S2 metrics mostly indicated a degraded condition. Only number of taxa and total number of organisms scored well. With 29 taxa present and 2023 individuals, these metrics would indicate a system with good biotic integrity. However, the other five metrics indicate otherwise. A percent dominance of 61.6% and a percent Diptera of 87.2%, indicates a severely degraded system, as does the other three metrics.

Similar to the Northeast Tributary, Jackson's Branch received the highest score possible for both number of taxa and total number of individuals (Table 5.13). With 29 taxa collected, this site had approximately two standard deviations more taxa than the reference stream. Moreover, the number of individuals collected was greater than one order of magnitude more than was collected at the reference site. However, all other metrics scored very poorly, receiving the lowest possible score for each. Shannon's H' was slightly under 3 standard deviations away from the reference condition and Simpson's D, percent Dominance, HBI, and percent Diptera were all greater than three standard deviations away from the mean of the reference condition indicating severe impairment.

Using the individual metric scores, an SCI was calculated. Due to five of the seven metrics obtaining the lowest possible score of 1, the overall SCI was 15 which corresponds to a condition rating of poor.

Jones Creek (S3)

Water Column Toxicity Tests

As was the case for the other two streams, Jones' Creek fathead minnow survival test showed no toxicity with 100% survival (Table 5.4).

Results similar to those observed in the *C. dubia* survival and reproduction test for the Northeast Tributary were observed in Jones' Creek samples (Table 5.5). The mean number of neonates per adult in this test was significantly greater than that of the controls. Organisms in Jones' Creek samples produced a total of 335 neonates with a mean of 34 per adult as compared to a mean of 19 for the controls. Therefore no toxicity is indicated.

Sediment Toxicity Tests

Unlike the other two study streams, S3 sediment produced no mortalities of adult *C. dubia*. All adults produced 3 broods for a total of 244 neonates with a mean of 24.4 per adult (Table 5.6). Therefore no toxicity is indicated.

Chemical, Physical, and Microbiological Analysis

Jones' Creek chemical analysis yielded results similar to previous investigations of this stream but not similar to reference conditions (Table 5.7). A fecal coliform count of 1070 cfu/100ml is slightly higher than the five year mean reported by Florida DEP (FDEP, 1996a) but probably not significantly different, though no measure of variance was reported in that report. Although possibly similar to previous conditions, fecal coliform were almost an order of magnitude greater than that reported for Black and Dean Creeks.

Other constituents such as BOD and NO_3 were in the normal range for this tributary as reported by Pratt and his coworkers (Pratt, et. al., 1993). Dissolved oxygen in Jones' Creek was significantly lower than in the reference streams while pH was higher.

Fish

A total of six species of fish were collected from Jones' Creek. Like the other two tributaries, the most numerous species present was *Gambusia Sp.*, comprising over 58% of the total number of individuals (Table 5.8). *Dormitator maculatus* was the second most numerous species with four individuals. The four other species present consisted of two each of *Micropterus salmoides* and *Eleotris pisonis* and 1 each of *Lepomis megalotis* and *Lepomis macrochirus*, for a total sample size of 25 individuals.

Jones' Creek had the highest IBI score of the three streams with a value of 25. This was primarily due to a high score in two metrics (Table 5.10). A score of 10 was calculated for the number of sunfish species metric due to the presence of two sunfish species, *L. macrochirus* and *L. megalotis*. One other top scoring metric was percent top carnivores. Due to the presence of two individuals of the top carnivore *M. salmoides*, this metric received a score of 5 out of a possible 5. Most other metrics received a score of 0 with the exception of number of species and number of intolerant species which received a 2, total number which received a 1, and percent sunfish which received a 5.

Benthic Macroinvertebrates

Individual metric values, SCI, and the condition ratings for Jones' Creek were calculated as previously described. As illustrated in Figure 5.3 all metric values except total number of individuals were significantly lower than those of the reference stream. Similarly, only total number of individuals, received a high SCI score of 5 (Table 5.13). Unlike the other two streams in this study, S3 only received a 3 for number of taxa, with slightly more than one standard deviation less taxa than the reference stream. Similar to the other creeks, however, all other metrics received a low score of 1. For this reason, Jones' Creek received the lowest SCI of the study, 13, corresponding to a condition rating of "poor".

Watershed Assessment

In addition to the assessments of the individual tributaries, a watershed level assessment was conducted. By combining SCI and IBI values for the each sub-basin, an overall assessment of the biological integrity for the Bayou Chico watershed was accomplished.

The Bayou Chico watershed received the highest score possible for number of taxa and total number of organisms with the SCI, as did the individual sub-basins (Table 5.14). With an average of 30 taxa present, this metric received the top score of a 5. Similarly, the metric total number of organisms received a 5 with an average of 1635 organisms. Unlike the individual sub-basins, percent Diptera received a score of 3. An average of 55.5 % of all individuals collected from the watershed were members of the order Diptera corresponding to the medium score. Although Jackson's Branch and Jones' Creek both had high percentages of dipterans, 87.2 and 58.2 respectively, the low percentage of this taxa in the Northeast Tributary (21%) compensated for the other two streams, resulting in a percentage that was slightly less than two standard deviations greater than the reference value. All other metrics received a low score of 1 for the watershed, similar to the individual streams.

A total of eight species of fish were collected from the Bayou Chico watershed with no more than six being collected from any one drainage. These fish tended to be pollution tolerant and functional generalists. There were no metrics that received a high score for the watershed and most scored either 0 or the next to lowest score (Table 5.15). This produced an IBI of 18, corresponding to a rank of "very poor".

Table 5.1
Results of Correlation analysis

Metrics	No. of taxa	total number	Simpson's D	Shannon's H'	% Dominance	HBI	% Diptera
No. of taxa	1						
total number	0.8613	1					
Simpson's D	0.9973	0.8961	1				
Shannon's H'	0.9998	0.8522	0.9958	1			
% Dominance	-0.9549	-0.9733	-0.9740	-0.9494	1		
HBI	-0.5544	-0.0547	-0.4921	-0.5691	0.2823	1	
% Diptera	0.9244	0.6026	0.8941	0.9310	-0.7696	-0.8298	1

Table 5.2
Benthic Metrics Included in Protocol

Metric	Rational for Use
Number of taxa	Decreases with disturbance
Number of individuals	Decreases with disturbance
Simpson's Diversity Index D	Decreases with disturbance
Shannon's Diversity Index H'	Decreases with disturbance
Percent Dominant taxa	Increases with disturbance
Hilsenhoff's Biotic Index (HBI)	Increases with disturbance
Percent Diptera	Increases with disturbance

Table 5.3
Metric Values for Family and Genus Level Identifications Including P-Values and Significance (α=0.05).

Metric	Family-level	Genus-level	p-value	Significant
no. taxa	10.7	19	0.033	yes
total number	184.7	184.7	1	no
Simpson's D	0.7794	0.8606	0.021	yes
Shannon's H'	2.4386	3.2977	0.018	yes
Percent dominance	31	24.7	0.150	no
HBI	6.33	6.63	0.371	no
Percent Diptera	49.6	49.3	1	no

Table 5.4
Results of 96 hour acute toxicity test for fathead minnows.

Site	% Survival	Significant Difference (α=0.05)
Control	100	n/a
S1	100	no
S2	100	no
S3	100	no

Table 5.5
Results of 7-day *Ceriodaphnia dubia* Water Column Toxicity Tests.

Site	**Number Neonates/Female mean ± sd**	**Significance (α=0.05)**
Control	19 ± 3.2	n/a
S1	31 ± 2.5	yes
S2	4 ± 1.5	yes
S3	34 ± 3.8	yes

Table 5.6
Results of 7-day *Ceriodaphnia dubia* Sediment Toxicity Test

Site	**Mean ± Standard Deviation**
S1	16.3 ± 14.2
S2 (1)	20.7 ± 14.6
S2 (2)	14 ± 11.53
S2 (3)	20.9 ± 11.46
S3	24.4 ± 2.17

Table 5.7
Results of Chemical, Physical, and Microbiological Analysis of Water Samples

Station	S1	S2	S3	Reference
Date	5/19/94	5/20/94	5/19/94	1993-1994*
TKN	n/a	0.22	0.43	n/a
P-total	n/a	0.02	0.29	0.01
DOC	2.4	2.2	7.01	n/a
TOC	3.05	6.08	8.04	n/a
NH_3	0.507	0.055	0.211	n/a
NO_3	1	2.305	0.752	0.11
NO_2	0.0182	0.0098	0.0098	n/a
Ortho-phosphate	0.0222	0.02	0.121	n/a
BOD	3	3	n/d	0.6
Fecal Coliform	>6000	204	1070	186.5
DO	3.6	3.8	2.0	8.4
pH	6.6	7.0	6.5	4.3
Temperature	24.0	23.0	19.0	n/a
Conductivity	300	160	200	39
Hardness	48	60	228	n/a
Alkalinity	40	40	265	1
Flow (M^3/S)	0.09	2	n/a	n/a

* indicates mean values for Black and Dean Creeks using several observations during the years 1993 and 1994. (FDEP, 1996a)

Table 5.8
List of Fish Collected From Bayou Chico Tributaries Including Numbers of Individuals.

Species	**(S1)**	**(S2)**	**(S3)**
Anguilla rostrata	0	2	0
Dormitator maculatus	82	0	4
Eleotris pisonis	0	0	2
Gambusia Sp.	148	119	14
Lepomis macrochirus	2	0	2
Lepomis megalotis	0	0	1
Micropterus salmoides	0	0	2
Poecilia lattipinna	2	0	0
Total	**234**	**121**	**25**

Table 5.10
Calculated Metric Values and IBI Scores For Bayou Chico Tributaries

Metric	(S1)	(S2)	(S3)
No. Species	2	2	2
Total Number	5	3	1
No. Darter Species	0	0	0
No. Sunfish Species	5	0	10
No. Intolerant Species[1]	0	0	2
No. Positive Species[1]	0	0	0
No. Negative Species[1]	1	3	0
% Omnivores	0	0	0
% Insectivorous cyprinids	0	0	0
% Top Carnivores	0	3	5
% Sunfish	5	0	5
% Minnows	0	0	0
% Madtoms	0	0	0
IBI Score	**18**	**11**	**25**

1 As defined by Florida Game and Fresh Water Fish Commission (Bass, 1993)

Table 5.11
Ranking of Stream Fish Assemblage[1]

IBI Score	Ranking	Characteristics of Site
80-100	Excellent	Pristine condition
60-80	Good	Least impacted. As good as can be expected in the region
40-60	Fair	Evidence of disturbance. Increased frequency of tolerant and negative indicator species.
20-40	Poor	Dominated by few generalist species.
1-20	Very Poor	Few fish and species. Typically tolerant forms.
0	No Fish	No fish present.

[1] Taken from Bass (1993)

Table 5.12
Descriptive Statistics and Scoring Criteria for Metrics Used in Protocol Based on Reference Stream Data.

Metric	**Mean ± 1 Standard Deviation**	**Score**		
		5	**3**	**1**
No. Taxa	19 ± 4	≥ 15	14 - 11	<11
Total No.	185 ± 54	≥ 131	130 - 77	<77
Simpson's D	0.8606 ± 0.0360	≥ 0.8246	0.8245 - 0.7886	<0.7886
Shannon's H'	3.2977 ± 0.3610	≥ 2.9367	2.9366 - 2.5757	<2.5757
% Dominance	24.7 ± 6.8	≤ 31.5	31.6 - 38.3	>38.3
HBI	6.63 ± 0.45	≤ 7.08	7.09 - 7.53	>7.53
% Diptera	49.3 ± 3.79	≤ 53.1	53.2 - 56.9	>56.9

Table 5.13
Calculated Metrics and SCI for Study Streams.

	S1		S2		S3	
Metric	**Value**	**Score**	**Value**	**Score**	**Value**	**Score**
No. Taxa	15	5	29	5	13	3
Total No.	2662	5	2023	5	219	5
Simpson's D	0.6874	1	0.5735	1	0.743	1
Shannon's H'	2.108	1	2.1961	1	2.5099	1
% Dominance	45.8	1	61.6	1	38.6	1
HBI	9.66	1	8.8	1	7.6	1
% Diptera	21	5	87.2	1	58.2	1
SCI	**19**		**15**		**13**	

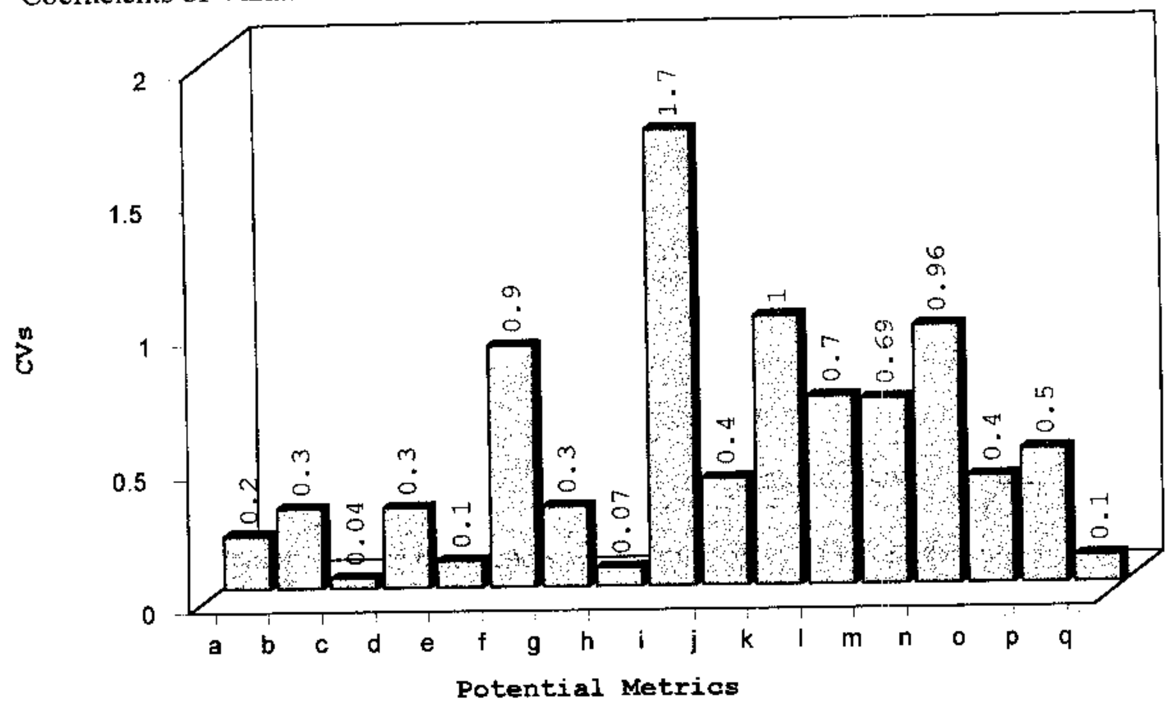
Table 5.14
SCI Scores and for Individual Sub-Basins and Bayou Chico Watershed

Metric	**S1**	**S2**	**S3**	**Bayou Chico Watershed**
No. of Taxa	5	5	5	5
Total No. of Individuals	5	5	5	5
Simpson's D	1	1	1	1
Shannon's H'	1	1	1	1
% Dominance	1	1	1	1
HBI	1	1	1	1
% Diptera	5	1	1	3
SCI	**19**	**15**	**13**	**17**

Table 5.15
Individual IBI Metric Scores for Sub-Basins and Bayou Chico Watershed

Metric	S1	S2	S3	Bayou Chico Watershed
No. of Species	2	2	2	2
Total No. of individuals	5	3	1	3
No. Darter sp.	0	0	0	0
No. Sunfish sp.	5	0	10	5
No. Intolerant sp.	0	0	2	1
No. Positive sp.	0	0	0	0
No. Negative sp.	1	3	0	1
% Omnivores	0	0	0	0
% Insectivorous cyprinids	0	0	0	0
% Top Carnivores	0	3	5	3
% Sunfish	5	0	5	3
% Minnows	0	0	0	0
% Madtoms	0	0	0	0
IBI Score	**18**	**11**	**25**	**18**

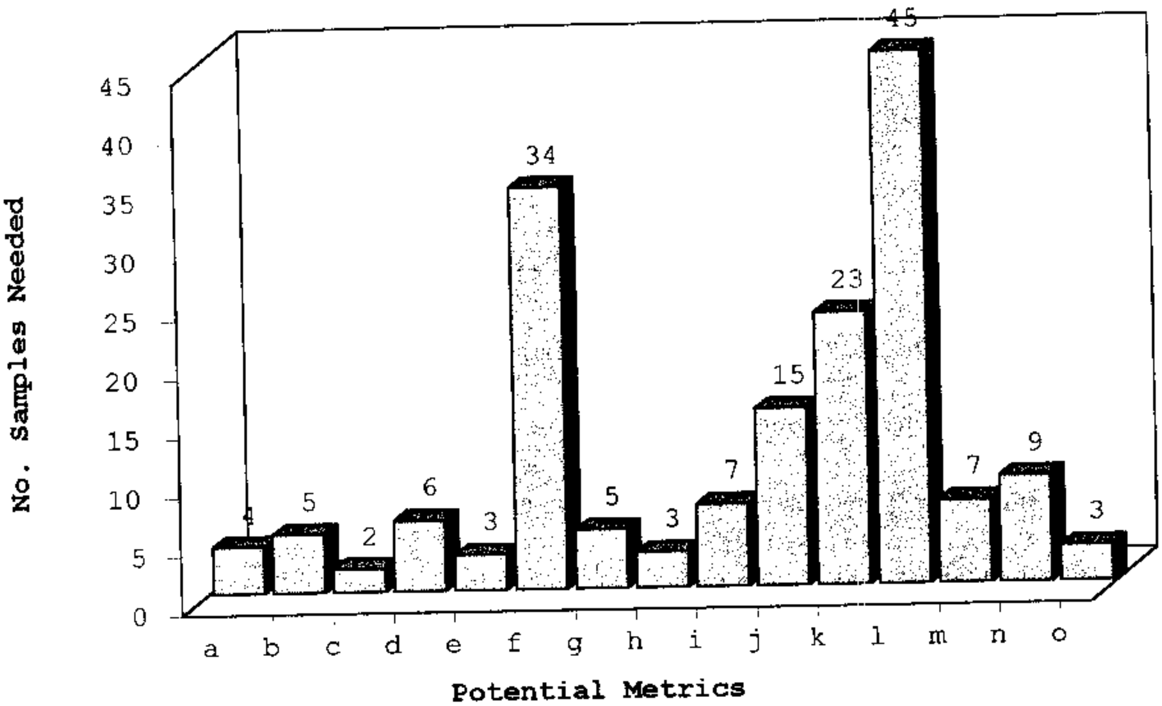
Figure 5.1
Coefficients of Variation for Metrics to be Considered in Assessment Protocol.



LEGEND

a = Number of taxa present
b = Total number of individuals
c = Simpson's Diversity Index
d =Number of EPT taxa (Ephemeroptera, Plecoptera, Trichoptera)
e = Shannon's Diversity Index
f = Number of Coleoptera taxa present
g = Percent composition of dominant taxa
h = Hilsenhoff's Biotic Index
I = *Cricotopus* + *Chironomus*/total Chironomidae
j = Percent Chironomidae
k = Ratio of Scrapers to Filterers
l = Percent Filterers
m= Florida Index
n = Percent Scrapers
o = Percent Shredders
p = Number of Chironomidae taxa
q = Percent Diptera

Figure 5.2
Number of Samples Required to Detect a 50% Change in Metric Value.
($\beta = 0.1$, $\alpha = 0.05$)



LEGEND

a = Number of taxa present
b = Total number of individuals
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f = Number of Coleoptera taxa present
g = Percent composition of dominant taxa
h = Hilsenhoff's Biotic Index
I = Percent Chironomidae
j = Percent Filterers
k = Florida Index
l = Percent Scrapers
m= Percent Shredders
n = Number of Chironomidae taxa
o = Percent Diptera

CHAPTER VI

DISCUSSION

Protocol Development

Coefficient of Variation

Of the original 17 metrics, two were rejected for inclusion in the protocol based on their variability as evidenced by the coefficient of variation. Both *Cricotopus* + *Chironomus* / total Chironomidae and Scrapers/ Filterers had CVs greater than one, indicating large variability relative to their means. The high variability in the *Cricotopus* + *Chironomus* / total Chironomidae metric was primarily due to the low numbers of Chironomidae in one of the replicate samples, relative to the others, causing a high metric value for that replicate. In addition, one sample contained no *Cricotopus* or *Chironomus*, further increasing the variability among samples. Other studies have found similar results. Barbour and his coworkers (1992) found that this metric was highly variable with a CV of greater than 1.5 and deemed it not an acceptable metric to be included in their suite of endpoints. Similarly, Barbour and another group of researchers found that two metrics that used ratios of groups within Chironomidae were too variable to be used in a Condition Index for Florida (FDEP 1996).

The high CV of the scrapers/filterers metric was mainly due to a high number of filterers in one sample as compared with that of the other replicates, while the number of scrapers remained relatively constant between samples. A total of 16 sphaeriids were collected in one replicate while one was collected in a second replicate and zero in a third. This filterer was the fifth most abundant organism in the first sample while being absent or virtually so in the other samples causing the metric to be relatively low in the sample with many filterers. Others have found similarly high CVs for this metric in studies. Hannaford and Resh (1995) found a CV of almost 2 in a study conducted in Berkeley, California, while Barbour and coworkers (1992) found a CV of over 2 in their evaluation of benthic metrics.

All other metrics in this study had CVs of less than one. Several of these metrics have been evaluated by other researchers with similar results. Taxa richness, HBI, and EPT, were found to have CVs of less than one as have other researchers (Barbour et al. 1992). Unlike other studies, percent scrapers had a CV of 0.96 in this study while others have found this metric to have a large CV (Hannaford and Resh 1995). Similarly, Barbour et al. (1992) found percent shredders to be quite variable while in our study it was relatively stable. Most other metrics were found to be similar in variability as other studies (Barbour et al. 1992, FDEP 1996, Hannaford and Resh 1995).

Power Analysis

Statistical power is defined as 1-β, where β is the probability of making a type II statistical error. In other words, β is the probability of not rejecting the null hypothesis when it is, in fact, false. Therefore, power (1-β) is defined as the probability of correctly rejecting a false null hypothesis (Zar 1984). So, when we say that the results showed no significant difference, we can know the probability that our assertion is correct by calculating β. This value is dependent on the variability of the system being studied and on number of samples, n. As variability decreases and n increases, the power of the test increases. Therefore, if we can estimate the variability of the data and know n, we can estimate the power of our test. Conversely, we can estimate the number of samples that are needed (n) to detect a specified amount of change in a monitoring endpoint by specifying the power, if we have an estimate of the variability. By so doing, we can have an estimate of the probability that our conclusion of no effect is correct.

For this study, a β of 0.1, α of 0.05, and a detectable difference of 50% was chosen as an acceptable resolution. Metrics that had CVs less than 1 were evaluated to determine the number of samples required to detect a 50% change in metric value using 5 or fewer samples. If a metric required 5 or fewer samples to detect a 50 % change, than it was deemed powerful enough to be included in the protocol; all others were excluded.

Eight of the remaining metrics required greater than 5 samples to detect the necessary change. Functional feeding group metrics were all excluded from the protocol by this analysis as were all but one taxa specific and two ratio endpoints. Low power in the percent scrapers metric was primarily due to the relative difference in abundance of the Chironomid *Phaenosectra sp.* and the elmid *Heterelmis sp.* between replicate samples causing the variance for this metric to be relatively high. Due to the high variance, this was the least powerful metric, requiring 45 samples to detect a 50 % change in value. Percent filterers was the next least powerful of the functional feeding group metrics, requiring 15 samples to detect the requisite change. This metric's lack of power was due to the large number of one taxa in one replicate sample. The 16 sphaeriids in the first sample and its absence or near absence in the other two, produced a large variance resulting in relatively low power.

Similar to the other two functional metrics, the low power of the percent shredders metric was due to the variability in relatively few taxa. High numbers of individuals in the genera *Polypedilum* and *Endochironomus* in one replicate and few individuals of these genera in the others, caused this metric to require 7 samples to detect a 50 % change in value. Percent Chironomidae also required 7 samples to detect a 50% change and was therefore excluded from the protocol. This was primarily due to the large variance caused by the high numbers of individuals of the genera *Polypedilum* and *Endochironomus* in the first sample and their low numbers in the other two samples. Similar to the metric percent shredders these two moderately pollution tolerant taxa were responsible for much of the variance for this metric.

Two other taxa specific metrics were found to have insufficient power to be included in the protocol. Number of Coleoptera taxa and number of Chironomidae taxa both required greater than 5 samples to detect a 50 % change in the metric value. Number of Coleoptera taxa required 34 samples and was the second least powerful metric of the 15. This was primarily due to the presence of only one kind of elmid beetle in each of two samples and it's absence in the other. Number of Chironomidae taxa required 9 samples to detect the required change. This was due to the relatively few Chironomidae taxa in the second sample (3) as compared with seven and nine for the other two. The only other metric with insufficient power to detect a 50 % change using as few as 5 samples was the number of EPT taxa metric which required 7.

Correlation Analysis

Correlation analysis was conducted to determine the degree of redundancy between the endpoints. Although the original criteria for inclusion in the protocol was a correlation coefficient of 0.80, almost all metrics' correlation coefficients exceeded this value. This should have been expected since most metrics were calculated using one or more of the other metrics. For instance, the number of taxa and total abundance are used to calculate both Simpson's D and Shannon's H'. These four metrics were all highly correlated. The only metric who's correlation coefficient did not exceed the established criteria of 0.8 was HBI. This was because an additional variable, pollution tolerance, is used to calculate this metric.

Although most metrics were highly correlated, the inclusion in the protocol of all metrics that had a low CV and acceptable power to assess the streams is appropriate. Community attributes such as percent dominance and percent Diptera may be highly correlated with other metrics, but they represent other important factors in the determination of the degree of stress put on a system (Plafkin et al. 1989, Resh and Jackson 1993).

Principal Component Analysis

Similar to the Correlation Analysis, PCA was not beneficial in the reduction of the number of metrics to be included in the assessment protocol. Due to the correlations between metrics, this analysis indicated that any one metric accounted for the majority of the total variance in the data. If all metrics are correlated to one another, any endpoint could describe the community and therefore represent most of the variability in the data.

Taxonomic Sufficiency

As expected the level to which organisms are identified had a significant effect on some of the metrics calculated. Because several families were represented by two or more genera, number of taxa values increased as the resolution of taxonomy increased. And since Simpson's D and Shannon's H' are calculated using the number of taxa, these metrics reacted accordingly. Also not unexpectedly, HBI was not significantly effected by taxonomic resolution. Since tolerance values for family level identifications are discerned by using those known for individual genera and species, the fact that taxonomic resolution had no effect on the outcome of the analysis is no surprise. Percent Diptera, too, was not effected by the level of taxonomy.

Conversely, one might expect that the percent dominance of a sample might be effected by the level of identification. However, this may not be the case. Because in two out of three samples of the reference stream the most numerous taxa could not be identified past the family level, whether the increased resolution would have an effect could not be determined.

Site Assessments

Northeast Tributary

The Northeast Tributary has been significantly impacted by human activity. With the exception of the water column toxicity test and possibly the sediment toxicity test, all assessment endpoints indicated severe impairment. Both water chemistry data analysis and the biological assessment imply human activities have degraded the condition of this drainage system.

Approximately 100 yards upstream from the sample site, a sanitary sewer line had ruptured and had been discharging untreated sewage onto the stream bank for an indeterminate length of time. In response to this discharge, a small rill had developed draining the waste into the creek and a colony of *Sphaerotilus* had developed indicating the discharge had been occurring for a significant amount of time. The high ammonia, nitrate and nitrite, BOD, conductivity, and fecal coliform count, as well as the low dissolved oxygen concentration, suggests this input is having a deleterious effect on the system. The absence of short-term chronic (7-day) water column toxicity suggests a stressor of an insidious nature such as a small but persistent influx of untreated sanitary waste. And although the sediment toxicity test did result in mortality, it is probably due to the methodology of the test rather than the toxic properties of the sediment.

The biological assessment also lends credence to this argument. Both species of fish that were present in significant numbers, *Dormitator maculatus* and *Gambusia Sp.*, are quite tolerant to organic pollution (Bass 1993). In addition, the dominant benthic organisms collected at this site are also highly tolerant of organic pollution (Hilsenhoff 1977) implying that the sewage is a significant stressor to the system.

In addition to water quality impairment, habitat degradation has also occurred. Eroding banks and significant siltation has occurred in this stream. Although a quantitative habitat assessment was not conducted, bank failure and the resultant deposition of sediment in the stream channel was observed. This reduction in habitat quality in conjunction with the chemical stresses, has resulted in a system with low biotic integrity.

Jackson's Branch

Similar to the Northeast Tributary, Jackson's Branch appears to have been significantly impacted by human activity. Basic water chemistry, sediment toxicity tests, water column toxicity tests, and biological community analysis all indicate anthropogenic degradation. Unlike the Northeast Tributary, this creek had no obvious point source impacting the system.

The biological assessments showed significant impairment in this system. Individual benthic metrics as well as the SCI indicates very poor water quality. This system had the lowest values of the three tributaries for Simpson's D and was greater than three standard deviations away from the reference condition. In addition, this tributary had the highest percent dominance and percent Diptera of the three streams studied in this project. Both percent dominance and percent Diptera metrics had values greater than 3 standard deviations larger than the reference condition. Percent dominance is an indication of community balance and increases with increased environmental stress (Plafkin et al. 1989). The large difference between the reference stream and Jackson's branch indicate a severely stressed system. Similarly, the percent of dipteran taxa in the community, which increases with increased perturbation (Plafkin et al. 1989, Resh and Jackson 1993), further demonstrate the impaired condition of the site. However, since percent Diptera may be affected by physical conditions such as water temperature and substrate and the percent composition of the dominant taxa may be affected by other factors (Resh and Jackson 1993) these measures must be used in conjunction with the other endpoints to accurately determine the condition of the site. The SCI, which uses all 7 of the metrics, also suggests severe impairment, as does the fish data. The overwhelming dominance of the very tolerant *Gambusia Sp.* (Bass 1993) to the exclusion of almost all other fish, as well as a very poor ranking in the IBI, corroborates the conclusion of a highly impacted system. The cause of this impact may be water quality or habitat quality induced.

Water chemistry analysis and toxicity data indicate the biological community effects are probably due to water quality impairment rather than habitat degradation. Short-term chronic water column toxicity tests on *C. dubia* showed reduced reproduction and high mortality. One hundred percent mortality by day 4 of *C. dubia* indicates this water was highly toxic. In addition, high Nitrate and BOD levels and the resultant low DO concentrations has produced a system that is severely water quality limited.

Jones' Creek

Fish data for this tributary suggested a system that has been impacted by anthropogenic stressors. An IBI score of 25, while highest among Bayou Chico tributaries, corresponds to a ranking of poor. While there were a few more taxa present than in the other creeks, this drainage has been impaired. In addition to the fish data, benthic macroinvertebrate analysis yielded similar results.

With 13 taxa collected, Jones' Creek had the least number of taxa present of the three tributaries studied and greater than one standard deviation less than that collected in the reference stream. Total abundance was also less in this creek than the other two Bayou Chico tributaries but greater than that of Black Creek. Based on these two endpoints, Jones' Creek is only moderately impacted. However, Simpson's D, Shannon's H', HBI, and percent dominance and percent Diptera indicate a more severely impacted system. While all these metrics except percent Diptera indicate a less impaired state than the other two streams, significant impacts have occurred. All five of these metrics received an SCI score of 1, which corresponds to degraded conditions. The fact that the richness metrics indicate only a moderately impacted system while the metrics that looked at the types of organisms present suggested more profound impairment, lends credence to the multi-metric approach.

While the toxicity test data showed no toxicity, the water chemistry data did indicate some impacts. High fecal coliform counts and low DO may suggest an input of animal waste. However, this may be coming from domestic or wild animals, or an anthropogenic source such as a broken or cracked sanitary sewer line. Since the coliform counts were significantly less than the Northeast Tributary's, a direct input such as the one found at the Northeast Tributary is unlikely.

Watershed Assessment

The biological integrity of the Bayou Chico watershed was assessed by combining the results of the benthic macroinvertebrate and fish community analysis. Averaging the metric scores for the individual tributaries, an IBI and an SCI was calculated for the entire drainage basin.

The Bayou Chico watershed is a highly impacted system suffering from point and non-point source pollution including raw sewage, severe bank erosion, and sedimentation. With an average of four fish species present, the Bayou Chico watershed received an IBI of 2. This indicates a very low species richness. Also, species present in the Bayou Chico watershed were typical of disturbed sites (Bass 1993). In the same study, Bass found the average number of fish species present per stream was 15.2 with a mean of 235.8 individuals being present. The low species richness and low total abundance in the Bayou Chico watershed is probably due to degraded physical habitat as well as poor water quality.

Similarly, SCI values for macroinvertebrates indicate severe impact. An average SCI of 17 corresponds to a rank of poor. Four of the seven metrics received the lowest score possible and only number of taxa and total number of individuals received a high score. By utilizing metrics that had been evaluated using regional variance estimates, the protocol developed was able to assess the condition of the watershed in an efficient manner.

CHAPTER VII

CONCLUSION

One of the main objectives of this study was to develop a bioassessment protocol using regional data. This protocol was developed and included calculating seven benthic macroinvertebrate metrics that provided efficient information to assess the integrity of the aquatic system. The newly developed protocol was determined to be an effective method of assessing the Bayou Chico tributaries and watershed and could be used to monitor other watersheds in the region. However, the reference stream used to evaluate the potential metrics is more impacted than was originally thought. This fact has probably affected the results of the metric evaluation. The degree of impact the Bayou Chico tributaries have received may be considerably greater than indicated due to the metric values being lower than what they might have been had a more pristine reference stream been utilized.

A second objective of this study was to assess the three tributaries draining into Bayou Chico. All three streams have been severely impacted by human activity. Point and non-point source pollution along with increased flood velocities caused by a high degree of impermeable surfaces has degraded the streams. The tributaries have very low chemical, physical, and biological integrity.

The third objective of this study was to look at the watershed as a whole and assess the condition of the entire drainage basin of Bayou Chico. This has been accomplished and the results are similar to the individual streams. The watershed has been impacted by residential, commercial, and institutional land uses and the pollutants that come with them. Raw sewage, habitat degradation, bank erosion, and sedimentation have all negatively impacted the Bayou Chico watershed.

APPENDIX

TAXA	Family	Order	S1
			REP 1
Unidentified Chrysomelidae	Chrysomelidae	Coleoptera	0
Heterelmis sp.	Elmidae	Coleoptera	0
Unidentified Hydrophilidae	Hydrophilidae	Coleoptera	0
unidentified Staphylinidae	Staphalynidae	Coleoptera	0
Tomocerus sp.	Entomobryidae	Collembolla	0
Unidentified Hypogastruridae	Hypogastruridae	Collembolla	0
Semicerura sp.	Isotomidae	Collembolla	0
Glutops sp.	Pelecorhynchidae	Dipera	0
unidentified Canaceidae	Canaceidae	Diptera	0
unidentified Ceratopogonidae	Ceratopogonidae	Diptera	0
unidentified Chaoboridae	Chaoboridae	Diptera	0
Ablabesmyia sp.	Chironomidae	Diptera	0
Chironomus sp.	Chironomidae	Diptera	270
Cladopelma sp.	Chironomidae	Diptera	0
Cladotanytarsus sp.	Chironomidae	Diptera	0
Cricotopus sp.	Chironomidae	Diptera	0
Cryptochironomus sp.	Chironomidae	Diptera	0
Dicrotendipes sp.	Chironomidae	Diptera	0
Endochironomus sp.	Chironomidae	Diptera	0
Hudsoimyia sp.	Chironomidae	Diptera	0
Labrundinia sp.	Chironomidae	Diptera	0
Monopelopia sp.	Chironomidae	Diptera	0
Paramerina sp.	Chironomidae	Diptera	0
Paratanytarsus sp.	Chironomidae	Diptera	0
Phaenosectra sp.	Chironomidae	Diptera	0
Polypedilum sp.	Chironomidae	Diptera	0
Procladius sp.	Chironomidae	Diptera	0
Psectrotanypus sp.	Chironomidae	Diptera	0
Rheotanytarsus sp.	Chironomidae	Diptera	0
Stenochironomus sp.	Chironomidae	Diptera	0
Tanypus sp.	Chironomidae	Diptera	8
Tanytarsus sp.	Chironomidae	Diptera	0
Thienemannimyia sp. group	Chironomidae	Diptera	0
unidentified Chironominae	Chironomidae	Diptera	0
unidentified Tanypodinae	Chironomidae	Diptera	0
Cryptotendipes sp.	Chrionomidae	Diptera	0
Lopescladius sp.	Chrionomidae	Diptera	0
Rheocricotopus sp.	Chrionomidae	Diptera	0
Culex sp.	Culicidae	Diptera	0
unidentifiable Culicidae	Culicidae	Diptera	1
unidentified Culicidae (pupae)	Culicidae	Diptera	0
Uranotaenia sp.	Culicidae	Diptera	7
Wyeomyia sp.	Culicidae	Diptera	0
unidentified Dolichipodidae	Dolichopodidae	Diptera	0
Unidentified Ephydridae	Ephydridae	Diptera	0

Unidentified Muscidae	Muscidae	Diptera	0
Pericoma sp. (Walker)	Psychodidae	Diptera	0
Psychoda sp. (Latreille)	Psychodidae	Diptera	0
unidentified Psychodidae	Psychodidae	Diptera	0
Tipula sp.	Tipulidae	Diptera	0
unidentified Tipulidae	Tipulidae	Diptera	0
Ephoron sp.	Polymitarcyidae	Ephemeroptera	0
Gyralus sp.	Planorbidae	Gastrapoda	1
Ferrissia sp.	Ancylidae	Gastropoda	2
Lymnaea sp.	Lymnaeidae	Gastropoda	0
Physa sp.	Physidae	Gastropoda	9
Hydrometra sp.	Hydrometridae	Hemiptera	0
Paraplea sp.	Pleidae	Hemiptera	0
Helobdella sp.	Glossiphoniidae	Hirudinea	0
unidentified Hydracarina #1		Hydracarina	0
Piona sp.	Hydracidae	Hydracarina	1
Estigmene sp.	Arctiidae	Lepidoptera	0
Simyra sp.	Noctuidae	Lepidoptera	0
Argiallagma sp.	Coenagrionidae	Odonata	0
unidentified Coenagrionidae	Coenagrionidae	Odonata	0
Gomphus sp.	Gomphidae	Odonata	0
Libellula sp.	Libelluidae	Odonata	0
Macrodiplax sp.	Libellulidae	Odonata	0
Pachydiplax sp.	Libellulidae	Odonata	0
Lumbriculus sp.	Lumbriculidae	Oligochaeta	1
Dero sp.	Naididae	Oligochaeta	158
Nais sp.	Naididae	Oligochaeta	7
Pristinella sp.	Naididae	Oligochaeta	14
unidentified Naididae	Naididae	Oligochaeta	14
Aulodrilus sp.	Tubificidae	Oligochaeta	14
Limnodrilus hoffmeisteri	Tubificidae	Oligochaeta	10
Tubifex sp.	Tubificidae	Oligochaeta	0
Tubificidae w/o cappiliform chaetae	Tubificidae	Oligochaeta	432
Unidentified Sphaeriidae	Sphaeriidae	Pelecypoda	0
Helicopsyche sp.	Helicopshchidae	Tricoptera	0
Neureclipsis sp.	Polycentropodidae	Tricoptera	0

S1	S1	S1	S1	S2	S2	S2	S2	S2
REP 2	REP 3	REP 4	REP 5	REP 1	REP 2	REP 3	REP 4	REP 5
0	0	0	0	0	0	0	1	0
1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0
0	0	0	1	0	0	0	0	0
0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	1	0	2
0	0	0	0	1	0	6	0	2
0	0	0	0	1	0	0	0	1
0	0	1	0	1	0	0	0	0
0	0	4	0	3	0	0	3	4
0	0	2	0	0	0	0	0	0
0	0	0	0	11	6	3	17	14
1295	141	540	35	883	1574	738	1881	1264
0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	1
0	0	0	0	0	1	18	29	12
0	0	0	0	0	0	0	9	0
19	3	8	1	46	32	108	63	57
0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	1	1
0	0	0	0	0	0	0	9	0
0	0	0	0	0	0	0	0	0
0	0	0	1	3	7	0	0	0
0	0	0	0	367	0	300	180	274
0	0	0	0	0	0	0	0	1
0	0	0	0	67	27	72	94	81
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	11	0	0	2
0	0	0	0	0	0	0	0	0
0	3	4	6	0	3	0	12	4
0	0	0	0	0	0	0	0	0
0	0	0	0	53	0	27	124	97
0	0	0	0	36	0	18	27	9
0	0	0	0	17	16	16	34	23
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	1	7	0	0	3	1	0	2
0	0	0	0	0	2	0	0	1
0	1	12	0	1	0	0	0	2
0	0	0	0	4	0	0	4	3

0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	2	0
0	0	0	0	0	0	0	2	0
0	2	0	0	0	0	0	0	0
0	1	0	0	0	0	5	5	2
0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	1	2	1	3	1
13	0	6	0	6	5	8	24	10
0	0	0	0	0	0	0	0	0
0	0	2	0	10	2	115	87	32
0	0	0	1	0	0	0	0	0
1	0	0	0	0	1	2	5	0
2	0	0	0	26	10	18	19	14
0	0	0	1	0	0	0	0	0
6	4	17	4	7	36	21	26	11
0	0	0	0	15	0	0	3	0
0	0	0	0	0	0	3	2	1
0	0	0	0	0	0	1	0	0
0	0	0	0	14	2	4	19	10
0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0
0	0	0	0	40	0	22	36	9
2922	208	3191	95	11	29	120	17	27
414	0	546	0	0	4	0	0	0
31	12	28	27	0	3	0	0	0
4	12	0	6	0	0	0	0	0
50	0	194	0	0	0	2	0	1
140	28	19	11	0	11	2	0	6
0	0	0	0	0	0	0	0	0
962	97	1055	120	7	109	70	26	63
4	0	3	0	0	23	0	21	0
0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0

S3	S3	S3	S3	S3	BLACK CREEK	BLACK CREEK
REP 1	REP 2	REP 3	REP 4	REP 5	REP 1	REP 2
0	0	0	0	0	0	0
0	0	0	0	0	1	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	1	0	1	0	36	47
0	0	0	0	0	0	0
5	30	1	23	14	10	0
5	10	3	8	9	0	0
0	0	1	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	2	0
0	0	0	0	0	0	0
5	15	0	47	13	0	0
0	0	0	0	0	24	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
1	1	0	2	0	0	0
0	0	0	0	0	4	2
19	18	2	12	12	4	0
0	0	2	2	0	41	19
0	0	4	4	0	2	0
0	0	3	0	0	0	0
0	0	0	0	0	0	0
0	1	0	0	0	0	0
0	0	1	0	1	0	2
0	0	0	0	0	4	0
0	0	0	0	0	0	0
0	3	1	6	2	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	2	0
0	0	0	0	0	0	0

0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	1	0
0	0	0	0	0	1	0
0	0	0	0	0	0	0
0	0	4	2	0	0	0
0	0	0	0	6	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	19	0	42	4
0	0	18	67	0	6	20
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	1	0	0	1	0	4
0	0	0	0	0	1	0
0	0	0	0	1	0	0
0	1	0	0	0	0	0
0	1	1	0	0	0	1
0	0	0	0	0	10	0
0	9	11	2	4	3	10
0	0	0	0	0	0	0
0	0	75	4	1	0	0
0	0	0	0	0	0	0
0	0	0	0	0	0	0
0	0	4	4	0	12	2
0	0	0	0	0	4	6
0	0	397	48	4	20	33
0	0	56	0	8	16	1
0	1	0	0	0	1	2
0	0	0	0	0	0	1

BLACK CREEK
REP 3
0
5
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1
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30
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