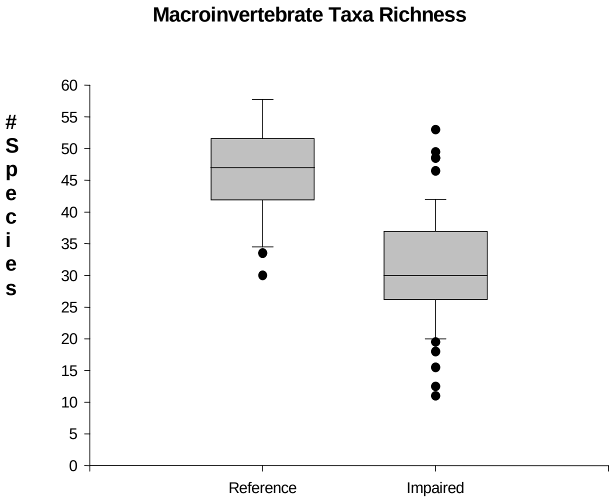
Biocriteria for Fish and Macroinvertebrate Assemblages in Vermont Wadeable Streams and Rivers

-Development Phase-



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INTRODUCTION

Purpose: The purpose of this document is to present the rationale, methodology, and results of analyses conducted by the Vermont Department of Environmental conservation to:

> 1) develop a scientifically based biological classification, based on macroinvertebrate and fish community characteristics, of wadeable streams in Vermont;
>
> 2) establish the range of community reference conditions for both macroinvertebrate and fish populations for each wadeable stream biological classification; and
>
> 3) develop a means of distinguishing a series of deviations from the reference condition that represent an heirarchy of increasing impact.

Information from this analysis will be used to develop, in a separate document, methods for implementing numerical biological criteria into aquatic life use support decisions based on the narrative biological criteria in the Vermont Water Quality Standards.

Theoretical Considerations for Biocriteria: The essential theory behind biocriteria includes the presumption that there exists a biological condition of, in this case, wadeable streams that represents conditions least impacted by human activities. This condition can be described by measuring the range of biological characteristics existing at sites which are minimally impacted by human activities. This range of values is known as the “reference” condition and describes the biological expectations for similar wadeable streams. The degree of deviation from this reference condition is an indication of the degree to which a stream is disturbed by anthropogenic activities. The degree of disturbance is presumed to produce a fairly quantifiable response within the biological community. The development and implementation of biological criteria requires 1) the selection of sites judged to be minimally impacted to serve as representatives of the reference condition, 2) the classification of those sites into categories of similar physical and biological characteristics, 3) the description of the essential biological characteristics of the reference waters, and 4) the development of methods for comparing the biological characteristics of impacted waters to the reference condition for the purpose of determining degree of impact.

The History of Biomonitoring and Biocriteria Implementation in Vermont: The VTDEC has been assessing the biological integrity of rivers and streams in Vermont since the early 1980's. These earlier assessments emphasized the use of ambient aquatic macroinvertebrate communities for assessing biological integrity. Since 1985, fish community assessments have also become a significant component of VTDEC bioassessment activities in streams and rivers. Standardized protocols for sampling fish and benthic macroinvertebrates in wadeable[1] streams were formalized in 1985 by biologists from the Biomonitoring and Aquatic Studies Section of VTDEC. These protocols have been used continuously since then without significant change. Since 1984, VTDEC biologists have conducted in excess of 1500 macroinvertebrate and 700 fish sampling events at over 1000 river and stream sites throughout the State. During that time period, the core staff of the Biomonitoring and Aquatic Studies Section has remained unchanged. This data base is the source of all data used in the analyses reported here.

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[1] The term “wadeable” is somewhat imprecise but refers to any stream or river that at some time during the year can be sampled by an individual wading into the thalweg of the stream channel; “wadeable” is a function of depth, velocity, and, to a lesser extent, investigator size and strength. The population of wadeable streams in Vermont is somewhat variable depending upon hydrological characteristics during the sampling period, and the robustness of field personnel at any given time (which may be declining over time with successive recruitment failures within an aging core staff of biologists).

Sampling of biological communities in wadeable streams has been conducted by VT DEC for a variety of reasons, including point and non-point source impact assessment (urban runoff, agriculture, hazardous waste sites and landfills, general construction and development, silviculture); permit evaluation (NPDES, indirect discharge, stormwater) compliance determination; evaluation of non-target impacts resulting from aquatic nuisance control activities; determination of biological condition status and trends; evaluation of the impacts of acid rain, and the determination of water quality classification status[2].

The Vermont Water Quality Standards (VWQS) in effect for the majority of that time period (1985 - 1999) address biological condition through narrative descriptors of designated values for aquatic condition. These standards have historically identified two classes of water:

> 1) Class A waters with designated values as *"high quality waters that have significant ecological value...*" and
>
> 2) Class B waters with designated values as "*...provides high quality habitat for aquatic biota, fish, and wildlife*."

The operative language relative to general biological protection has been in the form of a narrative standard applicable to both Class A and Class B waters:

> *"Aquatic Habitat - no change from background conditions that would have an undue adverse effect on the composition of the aquatic biota, the physical or chemical nature of the substrate or the species composition or propagation of fish."*

Additional language related to biological protection was provided in a section of the standards related to the regulation of large land-based waste disposal systems, and states that such waste disposal systems shall not "significantly alter the aquatic biota". Detailed sampling and analytical procedures are provided in the Indirect Discharge Regulations describing how compliance with this narrative is to be determined[3].

VTDEC biologists utilized macroinvertebrate and fish community monitoring data from least-impacted stream sites to develop quantitative biological criteria for determining classification or compliance status relative to the narratives in the VWQS's. Monitoring data were reduced to "metrics" or "indices" representing characteristics of biological structure and function. Findings were presented as categorical ratings (i.e. excellent, good, fair, poor) which determine the degree to which a site supports the aquatic life use designated in the VWQS. In water quality standards jargon, this becomes an assessment of "aquatic life use support" (ALUS). Rankings were determined based on comparison with minimally or least-impacted

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[2] "Classification status" refers to the water quality management values and uses as articulated in the Vermont Water Quality Standards and the extent to which those values and uses applicable to aquatic life are supported.

[3] Compliance determinations are based on upstream-downstream sampling of macroinvertebrates and periphytic algae as collected on artificial substrates. Resulting data are subjected to a series of comparative analyses with specific endpoints for determining "significant alteration". The procedures are highly prescriptive and site-specific. Because one of the main objectives of the protocols is to remove the influence of habitat heterogeneity from the analysis by using artificial substrates, the methods are not comparable to natural substrate sampling methods as a general means of evaluating ambient biological condition.

conditions (biological expectations) for wadeable streams in Vermont. Site-specific measures of biological condition were used in determining quality classification. Numeric criteria included community metrics that were assessed individually for macroinvertebrates (eg taxa richness, diversity) and collectively for fish (Index of Biotic Integrity). Sites exhibiting biological condition that departed only slightly from the least-impacted biological expectation were ranked as higher quality than sites showing greater departure from that condition (see **Appendix 10**).

Problem Statement: After more than ten years of implementing biological criteria in wadeable streams in Vermont on a more-or-less informal basis, several truths emerged as being self-evident: 1) language related to biological standards in the existing VWQS's did not encourage efficient decision-making; 2) not all wadeable streams are created equal; and 3) dichotomous decisions of aquatic life use support based on biological data, which ignore the environmental gradient from which the data were derived, are often unreasonable and not supported by good science. These observations led to the development of several initiatives aimed at recognizing differences among wadeable streams and revising language in the VWOS's to provide more precise and practical guidance for making aquatic life use support decisions in a manner consistent with good science and existing DEC practice to the extent possible. This document reports on the results of the following initiatives:

1. Revisions to the Vermont Water Quality Standards

2. Classification of wadeable streams in Vermont in a manner consistent with inherent biological differences among streams;

3. Identification and description of reference conditions within each identified biological classification;

4. Identification of measures of macroinvertebrate and fish community biological integrity that describe an heirarcy of increasing impact based on the degree of deviation from the reference condition.

Vermont Water Quality Standards Revisions Related to Aquatic Biota: It could be argued that efficiency and guidance precision are not necessarily appropriate goals for decision-making[4]. However, VTDEC water quality managers felt that some revisions to language in the VWQS's could result in more reasonable and scientifically defensible decisions regarding the determination of aquatic life use support in a manner consistent with State and Federal management goals and statutes.

Following several years of intense public process, revised water quality standards were adopted by the Vermont Water Resources Board, approved by the Vermont Legislature, and designated to become effective July 2, 2000. There were several significant changes to the language affecting biological standards: 1) the term "reference condition" was introduced to replace "background condition" as the basis for evaluating biological integrity; 2) within Class B waters, three water management types were established; 3) within Class A, two management types were established; 4) the concept of deviation from the reference condition as the means for evaluating aquatic life use support was introduced; 5) the terms "minimal", "minor", and

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[4] Sometimes the discussions, arguments, and counter-arguments associated with the decision-making process in the absence of clearly defined endpoints can be extremely rewarding and result in well thought out and defensible decisions. At other times, however, this process can end in frustration with no reasonable decision resulting. In any case, such a process is often very intense and resource consuming and is not particularly practical for routine decision-making.

“moderate” were introduced to describe an hierarchy of ranges of deviation from the reference condition appropriate to specific water classes or water management types.

The revised standards identify Class A and Class B waters. Two Class A management Types are created to distinguish between drinking water supplies (Class A - Water Supply) and those waters designated Class A for ecological reasons (Class A - Ecological). Three water management Types, representing three levels of protection, are included under Class B waters. Until waters are designated as specific water management Type through the basin planning process, the operative biological criterion for all Class B waters is a slightly modified (the term “reference condition” replaces “background condition”) statement from the 1997 Water Quality Standards (effective 4/21/97).

VTDEC General Approach to Biocriteria Development - General Theoretical Considerations: The essential theory behind biocriteria includes the presumption that there exists a biological condition of, in this case, wadeable streams that represents conditions least impacted by human activities. An informal set of biological criteria has been developed using fish and macroinvertebrate community biometrics, for evaluating compliance with the State's Class B water quality standards. As presently employed, the criteria apply to Class B wadeable streams with minimal respect to potential differences in reference community makeup. As a result, the criteria may not reflect important distinctions between stream ecotypes. The potential exists then, that the criteria as historically applied may not be flexible enough to be effectively used across all stream types without resultant errors in aquatic life use support findings (what may be an appropriate standard of comparison for a high elevation cold water trout stream may not apply to a low elevation warm water stream). Existing ecological classifications from other regions (eg Hughes et al 1987) do not adequately describe Vermont’s stream ecotypes. Consequently a more localized framework which specifically characterizes natural communities of Vermont’s waters is required. As we view it, the ideal classification system would categorize streams into groups which, in their natural or least disturbed (reference) condition, would exhibit similar biological characteristics *within* the groups, and distinctly different characteristics *between* groups. Groups would be determined on the basis of biological similarity using appropriate measures of similarity. The geophysical and chemical characteristics which best describe each biologically determined group would then be determined and serve as a label for each group. These group descriptors in combination would be non-overlapping and mutually exclusive and provide a clear and simple association between biological and geophysical/chemical characteristics. A stream site could be subsequently placed into a classification group after determining a relatively modest number of geophysical-chemical characteristics of that site.

It is important to point out at the onset that the attainment of the “ideal classification system” serves primarily as a goal toward which to strive. Because the range of environmental variables and relationships occur, across streams, as continua, rather than in discrete bundles, it is contrary to ecological theory to expect that any classification will be clean and exclusionary. There will always be outliers from typical stream types and zones of transition between classifications where “fuzzy theory” dominates and nothing is definitive. Our goal will be to identify the minimum number of reasonably discrete stream types and to reduce as much as possible the chaos represented by the transitional gradients between classes.
Many investigators have chosen to classify streams according to *a priori* regionalization based on physiographic regions (eg ecoregions). This approach is grounded on the assumption that aquatic community characteristics exhibit some degree of distinctiveness between those physiographic regions. Other investigators have chosen a *post-priori* approach by defining regions as groups of streams which exhibit a degree of biological homogeneity as determined from analysis of biological community data. These approaches are effectively and intelligently discussed by Gerritson(1995) and Norris(1995). Clearly, both approaches are valid and are not necessarily exclusive of one another.

VTDEC has chosen to pursue a *post-priori* regional approach for this project primarily due the the existence of a large body of high quality biological monitoring data representing a wide range of geographical distribution and stream types. We have employed a variety of methods, including Two Way Indicator Species Analysis, Canonical Correspondence Analysis, and best professional judgement to identify ecologically "discrete"[5] classes of wadeable streams for both macroinvertebrate and fish communities.

VTDEC General Approach to Biocriteria Development - Stream classification: "Not all wadeable streams are created equal" is a relatively straightforward observation. Identifying and describing the characteristics which create those inequalities is more complex. Certainly the concept of stream classification is not new. Neither are the basic ecological theories recognized as important to the classification of running waters. In 1934, W. E. Ricker proposed "An Ecological Classification of Certain Ontario Streams" (Ricker1934). Ricker's scheme is very attractive in its simplicity and common sense. He identifies a list of environmental variables that he believes determine the structure and function of ambient biological communities. He listed the following characteristics as being "more important" to determining biological classification: 1) geological characteristics of the watershed; 2) land use in the watershed; 3) current velocity (stream power); 4) substrate type; 5) water temperature; 6) volume of discharge (stream size). He recognized the inter-correlations between environmental variables. He noted that biological characteristics are determined by combinations of site-specific (e.g. substrate type) and landscape level (e.g. watershed geology and land use) characteristics. The basic dichotomies used by Ricker to differentiate stream classes include: large and small; warm and cold; swift and slow; hard and soft (alkalinity); stony and muddy. Each one of the basic descriptive dichotomies can be quantified with a vast array of categorical and continuous inter-correlated variables and sliced into any number of classifications. Most current classification systems are founded on these basic dichotomies.

VTDEC General Approach to Biocriteria Development - The Reference Condition: A clear and concise definition of "reference condition" is critical to the process of developing and implementing biological criteria (Hughes et al. 1986). In any discussion of the term "reference condition", it soon becomes clear that the reference concept carries with it considerable semantic baggage. Gerritsen (1995) summarized the theory of reference condition: "Reference conditions are not single cases, such as upstream or paired reference sites, but should reflect regional[6] conditions and regional variability under minimal human disturbance (i.e. pollution, habitat alteration, flow regulation, or stocking and harvesting activities)." Thus the reference condition "takes into account differences between systems and natural variability within the region, and establishes a range of expectations for unimpacted sites."

The revised VWQS's define the reference condition as follows:

> *Reference condition means the range of chemical, physical, and biological characteristics of*

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[5] The term "discrete" is used here in its biological sense, recognizing the fuzziness of the boundaries separating stream classes.

[6] We interpret the term "regional" as used here to be fairly broad in its meaning and refers to any scheme which creates classifications (or regions) of biological similarity. Thus region may refer to either a "physiographic region" based on regions of physiographic similarity, or a "classification grouping" based on groups of sites exhibiting a high degree of biological similarity. The degree of overlap between "regions" established using these methods is the subject of discussion between supporters of various classification schemes (Norris, 1995; Gerritson, 1995)

> *waters minimally affected by human influences. In the context of an evaluation of biological indices, or where necessary to perform other evaluations of water quality, the reference condition establishes attainable chemical, physical, and biological conditions for specific water body types against which the condition of waters of similar water body type is evaluated.*

While recognizing the need to define reference condition in the context of regional variability, Hughes, (1995) offers supplementary means of describing biological expectations that could be used to address specific problems. Such means include the use of:

- site-specific historical monitoring data [7]
- experimental laboratory data (e.g. chemical criteria derived from laboratory tests in effect describe reference conditions for specific chemicals)
- quantitative models (e.g., in-stream flow models currently used to evaluate hydrological modification impacts)
- upstream-downstream impact evaluations (e.g., current biological monitoring methodologies implemented by the VTDEC for determining compliance with the Indirect Discharge Regulations),
- and last, but perhaps most important, best professional judgement. Best professional judgement, or expert opinion, is critical to the implementation of any option for determining reference condition.

Reference sites and associated biological and physico-chemical information were selected from the existing VTDEC database for macroinvertebrate and fish assemblages as previously described. The selection of reference sites from the existing database was largely driven by best professional judgement based on the evaluation of known land use and human activity factors. The same individuals responsible for conducting this analysis also collected the data which composes the current VTDEC streams database. Initially a group consultation among VTDEC staff biologists was held to jointly develop a list of candidate reference sites.
Sites regarded as good candidates were those considered to have watersheds supporting a minimum of human activity and could be characterized as least-disturbed. Generally low elevation reference site candidates in the Champlain valley have greater portions of their watersheds characterized by agricultural activities. These activities include open pasture land with some row crops and contain streams with modified riparian zones. Conversely, higher elevation mountainous reference candidate sites have predominately forested watersheds with minimal, if any, agricultural activity and sparse, rural human settlement, but with a greater potential for impacts resulting from atmospheric deposition. Some candidate reference sites with larger drainage areas also had mostly forested watersheds with slightly more human activity in the watershed than in the small mountainous sites.

The candidate list was further scrutinized to ensure general site distribution throughout the State and representativeness of the range of environmental gradients common to Vermont. Best professional judgement was used to eliminate a number of these candidate sites in the interests of reducing redundancy.

The final reference site list selected for analysis included a total of 140 sites from 123 rivers and streams, representing approximately twenty-one percent of the available site pool (**Appendix 1**). Of the 140 sites, macroinvertebrate community data were available from 93 sites from 81 streams and rivers and fish

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[7] Vermont DEC has used historical biological data to establish limits of acceptable biological change related to land development. In developing a storm-water permit for proposed construction in a Class A watershed in Southern Vermont, eight years of historical macroinvertebrate monitoring data were used to describe the range of natural variability associated with a number of measures of biological condition for the receiving water. This range was used to set the site-specific reference condition for limits of acceptable change.

community data were available from 76 sites from 68 streams and rivers. Twenty-nine of the sites, representing 26 rivers and streams, have data from both fish and macroinvertebrate communities. There is at least one reference site located in each of the 17 designated drainage basins of Vermont.

One of the final 76 fish reference sites and eight of the final 93 macroinvertebrate reference sites have direct discharges located upstream. All discharges above the sites are small municipal waste treatment plants. The small size of discharge, high dilution with receiving water and distance between reference site and discharge combined to minimize potential biological impact at these sites. A suitable distance was maintained between the reference site location and any riparian degradation located upstream. Nearly all reference sites selected have intact riparian zones and are located on rivers which have unregulated flows.

VTDEC General Approach to Biocriteria Development - Deviation from reference conditions: In developing methods to discriminate degrees of impact from reference conditions, measures of community biological integrity are calculated from ambient monitoring data. These measures are referred to as “metrics”. Relevant metrics are selected based on their ability to discriminate between stream ecotypes and by their sensitivity to disturbance. Evaluations of impact can be made by evaluating a suite of metrics individually (multi-metric assessment) or by combining metrics into a composite score based on the average score of a suite of selected metrics (multi-metric index assessment). VTDEC uses both approaches in making impact evaluations.

1. Impacts to macroinvertebrate communities are assessed using multiple metrics but evaluating each metric independently rather than combining individual metric evaluations into an overall site score. The severity of impact is determined by comparing each metric to the range of reference values for that metric and evaluating the overall performance of the suite of metrics in relation to the reference condition and the biological integrity of the site.

2. Impacts to fish communities are assessed using indices based on multi-metric index evaluations. These “indices of biotic integrity” utilize multiple metrics to derive an overall site score that is used to characterize the severity of impact. Index scores are based on the performance of the suite of metrics making up the index.

The difference in approach for macroinvertebrate and fish communities is based in the relative complexity of the two communities and the amount of information that is contained in each metric. It is the opinion of VTDEC staff that significant deviation in a single metric of the relatively speciose macroinvertebrate communities has more biological significance than a similar observation in the relatively species depauperate fish community. Many Vermont streams contain less than five fish species, which limits the number of ways that the community can be measured and the amount of the information that is contained in each measurement or metric.

Biocriteria-Related Definitions from 7/2/2000 Vermont Water Quality Standards

Aquatic biota means all organisms that, as part of their natural life cycle, live in or on waters.

Aquatic habitat means the physical, chemical, and biological components of the water environment.

Biological integrity means the ability of an aquatic ecosystem to support and maintain, when consistent with reference conditions, a community of organisms that is not dominated by any particular species or functions (balanced), is fully functional (integrated), and is resilient to change or impact (adaptive), and which has the expected species composition, diversity, and functional organization.

Functional component of the aquatic ecosystem means a portion of the aquatic biological community identified by its role in the processing of energy within the aquatic ecosystem (e.g., primary producers, predators, detritivores, etc.).

Intolerant aquatic organisms means those organisms which are particularly sensitive to, and likely to be adversely affected by, the stress of pollution, flow modification or habitat alteration (e.g., mayflies and stoneflies).

Reference condition means the range of chemical, physical, and biological characteristics of waters minimally affected by human influences. In the context of an evaluation of biological indices, or where necessary to perform other evaluations of water quality, the reference condition establishes attainable chemical, physical, and biological conditions for specific water body types against which the condition of waters of similar water body type is evaluated.

Taxonomic component of the aquatic ecosystem means a portion of the biological community identified by a hierarchical classification system for identifying biological organisms that uses physical and biological characteristics (e.g., Insecta: Plecoptera: Perlidae: *Agnetina capitata*).

Tolerant aquatic organisms means organisms (e.g., midges and annelids) that, although they may be affected by the stress of pollution, flow modification or habitat alteration, are less sensitive and less likely to be adversely affected than are intolerant aquatic organisms.

MACROINVERTEBRATE BIOCRITERIA

This section outlines the general effort to determine distinct wadeable stream macroinvertebrate assemblage types by using the composition of the dominant species from reference-quality streams. Physico-chemical variables were identified which appeared to distinguish and shape these assemblage types. Using the above analysis, each reference stream was then placed in a stream assemblage type and the natural range in each community metric determined. Differences in the expected range of the community metrics were then compared between assemblage types. Following that analysis, the community metrics within each stream assemblage type were compared to a set of impacted streams. A subset of metrics was then selected which: 1) best described the structure and function of the reference condition; and 2) was able to discern biologically significant changes within the impacted sites.

Macroinvertebrate Methods

Sample collections: Methods used to collect aquatic macroinvertebrates in wadeable streams are documented in the Vermont Department of Environmental Conservation Field Methods Manual (VTDEC 1989). All macroinvertebrate samples are collected during the fall index period, from September to mid-October. A two-person field crew selects a representative riffle section in the stream reach to be sampled. (The majority of streams included in this analysis are hard-bottom riffle-pool type streams. However, a number of low gradient silt-and sand-dominated non-riffle streams were sampled and included in the stream typing phase of the analysis.) Physical characteristics recorded at each site include: stream width; depth; water velocity; water temperature; specific conductance; weather conditions; substrate composition; substrate embeddedness (riffle sites only); canopy cover; stream bank condition and immediate upstream land use. Observations noting relative stream discharge, precedent hydrological condition, and biological condition of the substrate/habitat are made. All data are entered onto a field sheet with appropriate site and sampling event identifiers, along with additional comments that may be pertinent to the site evaluation. A water sample is collected for pH and alkalinity determination and placed on ice for return to the laboratory.

Samples are collected using an 18 inch wide x 12 inch high D-frame net with a 500 u mesh size. The goal of sampling is to collect a sample that is representative of the overall biological integrity within the section of stream being sampled. The net is placed in the riffle at an appropriate location and an area immediately upstream of the net is thoroughly disturbed by hand, ensuring that all pieces of substrate are moved and rubbed clean of attached organisms. Moving up-stream, this is repeated at 4 to 5 locations within the riffle, representing a range of velocity and substrate type characteristic of that riffle. Each specific location is actively sampled for about 30 seconds, and active sampling is terminated at the end of two minutes. A stop-watch is used to record active sampling time. Time spent relocating to a new area within the riffle is not counted as part of the two minutes. The contents of the net are washed into a quart mason jar and preserved with 75% ethanol. The process is repeated, being careful to avoid areas previously disturbed. This "composite" sampling methodology effectively collects samples representative of the macroinvertebrate community of that riffle [8]. This sampling protocol is most comparable to the riffle-run sampling portion of Rapid Bioassessment Protocol III (RBPIII) as described in Plafkin et al. (1989).

Sample Processing: All methods used to process aquatic macroinvertebrate samples for this project are documented in the Vermont Department of Environmental Conservation Field Methods Manual (VTDEC

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[8] This sampling methodology is nominally identified as a kick net sample. This is technically a misnomer as no "kicking" is actually done. All substrate manipulation is done by hand. It is our opinion that this method of substrate manipulation, combined with the moving to different locations within the riffle, increases the representativeness of the sample and the precision of the sampling method. Sampling effort is extremely reproducible. It has been our experience that it is very unusual for the percent standard error of total organism abundance and taxa richness estimates using this methodology (combined with associated sample processing methods) to exceed 40% and 20% respectively. Data precision will be discussed separately.

1989). All sample processing is done in a laboratory setting. Processing includes picking organisms from the sample, sorting the organisms into taxonomic groups, identifying organisms to lowest possible taxonomic level, and entering data into the data management system.

An entire sample is thoroughly washed through a # 30-mesh brass sieve. The sample is then back-washed into a 12 x 18 inch white enamel tray that has been marked so as to delineate 24 numbered equal squares. The sample is spread evenly over the tray surface. A random number between 1 and 24 is selected and picking is started on that square in the tray. All organisms are removed from a square before proceeding to the next sequentially numbered square. Picking continues into subsequently numbered squares until a minimum of six squares (25 percent of the sample) have been picked. If less than 300 organisms have been picked at this point, picking continues until a total of 300 organisms have been removed or the entire sample has been picked, whichever comes first. Sub-sampling details are recorded on bench sheets. All picked samples are checked by a second biologist prior to discarding. Removed organisms are sorted to order and placed in appropriately labeled vials in alcohol for further identification. If the sample has not been totally picked, the remaining sample is qualitatively examined for Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa not found in the sub-sample. Organisms are removed, labeled, and stored separately from the sub-sampled organisms.[9] All organisms are subsequently identified to the lowest practicable taxonomic level by staff specializing in order-specific taxonomy. Identifications are recorded on laboratory bench sheets.

Data Management: Raw data from field and bench sheets are entered into an electronic data management system. The VTDEC electronic data management system is a custom-scripted Microsoft Access© data base specifically developed for the management of macroinvertebrate monitoring data. Raw taxonomic data from the bench sheets are converted to standardized abundance per unit sampling effort estimates to account for variations in sub-sampling methods. The data management system calculates and reports the mean percent composition and density per unit sampling effort of all taxa, the standard error (based on the minimum of two replicates) of all taxon abundance estimates, the functional group percent composition, and a wide range of community biometrics for each sampling event in a sample summary report. Taxa richness is manually adjusted for each sample to account for differing levels of taxonomic identification within a sample[10]. The biometrics are electronically transferred to a macroinvertebrate metrics data table and the adjusted taxa richness values are inserted. From this table a site summary report is generated, which includes all sampling events from a site over time. Other sampling event data and meta-data are entered and stored in linked tables. **Table 1** lists metrics and associated event data that are calculated and included in site summary reports.

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[9] Organisms removed from the sample as part of the EPT scan are not used in subsequent calculations of organism abundance or associated metrics calculated from abundance estimates. The primary purpose is for use in species distribution databases, and to aid in the BPJ of the biologist in interpreting the richness metrics.

[10] For example, the taxonomic bench sheet may list *Baetis tricaudatus* and *Baetis* immature. The management system script will count two taxa when calculating taxa richness, whereas it is more likely that the immature organisms are of the same taxon as the identified species; counting two species would overestimate the real taxonomic richness. We haven't figured out how to make this correction electronically yet as some judgement is required.

Table 1. Macroinvertebrate community metrics and physico-chemical measurements for each sampling event.

Community Metrics	*Physico-Chemical Parameters*
Relative Abundance	Temperature
Species % Composition	Specific Conductance
Order % Composition	pH and Alkalinity
Functional Group % Comp	Width and Depth
Mean EPT Taxa Richness	Water Velocity
Mean Taxa Richness	% Embeddedness
EPT/Richness Ratio	Periphyton (qual)
Biotic Index (0-10)	Bank Stability/Vegetation
Species Diversity	Substrate Size Distribution
# Dominant Genera	Canopy
% Dominant Genera	Drainage Area
EPT/EPT&Chironomid ratio	Ecoregion
% Hydropsychidae	Elevation
PPCS- Functional groups	Stream Order
PMA-Orders	

Reference Database Development: In order to select a database from which to draw information for this project, a number of data review activities were carried out on the data residing in the VTDEC electronic data base. This review was conducted in order to ensure that all data subsequently used for this project would be comparable, representative, and of high quality:

1. All sampling events in the database were screened in order to ensure consistent methodologies, a consistent level of sampling and analytical effort, and were representative of the "normal" range of ecological conditions[11]. Sites not considered wadeable (the main database contains sampling data from lakes, ponds and wetlands) were eliminated.

2. Data were removed from consideration which were generated from non-standardized methods as previously described (e.g. data from species-specific distributional surveys were excluded as were samples collected during non-index sampling periods).

Using the general guidance previously described for establishing reference conditions, DEC biologists identified a total of 93 sites from the VTDEC monitoring database to represent reference, or minimally affected, macroinvertebrate sites (**Appendix 1**). There were over 171 sampling events associated with

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[11] All sampling events are screened for representativeness. The most common source of high variability in our sampling programs has been related to hydrological extremes. Elevated flows during sampling can reduce the effectiveness of sampling methodologies, including reduced fishability or a reduction in kick net efficiency. Precedent hydrological extremes can result in significant short-term alterations to biological communities, particularly in moderate to high gradient turbulent streams.

these sites. All sampling events were conducted according to standard methods discussed previously. Data from each sampling event were reviewed for taxonomic consistency[12]. **Table 2** shows some of the adjustments and aggregations that were made to the database. Because species level identifications were not always possible across all sites, most of the aggregations involve lumping species at the generic level when it was felt that there was any degree of inconsistency over time in the accuracy of species taxonomy.

Additionally, immature animals identified to the family level were proportionally assigned to those genera present at a site. These aggregations and adjustments are critical when calculating metrics based on taxa richness and diversity:

Table 2. Macroinvertebrate Taxonomic Aggregations

Coleoptera	- *Optioservus, Oulimnius*, and *Stenelmis* were aggregated at the Genus level;
Trichoptera	- *Brachycentrus* and *Rhyacophila* aggregated at Genus level; - *Symphitopsyche bifida group* includes *S. morosa*; - *Symphitipsyche macleodi* includes *S. ventura*;
Plecoptera	- *Isoperla* and *Pteronarcys* aggregated at the Genus level; - Chloroperlidae, Leuctridae, Capniidae, and Peltoperlidae aggregated at the Family level;
Diptera	- *Cricotopus* and *Rheotanytarsus* aggregated at the Genus level; - *Simulium* aggregated into two groups; Group A- *S. fibrinflatum, S. jenningsi, S. aestivum/aureum*; Group B- *S. tuberosum, S. corbis, S. vittatum*;
Ephemeroptera	- *Baetis, Ephemerella, Seratella, Stenonema* agggregated at the Genus level;

The dominant macroinvertebrate taxa (a taxon as defined above comprising 3 percent or more of the community) were determined for each site/sampling event. From the 171 available sampling events, 100 (including all 93 sites) data points[13] were randomly selected to determine biologically-based community types within the reference streams database. A few temporal sampling events were included because of the limited number of data points with large drainage areas or of low gradient. A total of 97 dominant macroinvertebrate taxa were identified from the 100 sites/events. Physico-chemical measurements and information collected concurrently with the biological samples were determined for the above 100

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[12] In order to make legitimate comparisons of faunal assemblages across sites, it is important that identification of organisms be made at a consistent level between samples. As taxonomists come and go over the years, or as individual taxonomists develop experience over time and confidence waxes and wanes, the level of taxonomy within certain orders may vary from sample to sample. For example, with some samples the taxonomist may feel comfortable identifying Chloroperlid stoneflies to the generic or species level, while with other samples, for various reasons the taxonomist may feel that there are several genera present but not to the point of differentiating genera, and backing off to a family level identification.

[13] Number of events analyzed determined by statistical software data matrix size limitations.

reference site files (see **Table 1**).

Data Analysis: Two-way indicator analysis (TWINSPAN) was used to group sites into biological assemblage categories using a microcomputer version of this tool (PC Ord - McCune and Mefford 1997). TWINSPAN simultaneously classifies species groups and site groups and displays both on a two way table. Groups are defined by a level of similarity to each other. Associations between the macroinvertebrate assemblages, and physico-chemical variables was analyzed using canonical correspondence analysis (CCA) included in CANOCO version 3.12. This ordination method is used to analyze associations between species assemblage data and environmental data. The output figure places environmental and biological data along two synthetic axes which serve to separate the species according to their niche differences while indicating which environmental variables are most responsible for this differentiation. Canonical Correspondence analysis (CCA) was used to examine the biological gradients within the reference database and physico-chemical characteristics that most influenced those gradients. Correlation analyses were used to examine the strength of the relationships between physico-chemical characteristics and the environmental gradients identified through CCA. These analyses, in conjunction with the best professional judgement of DEC biologists, were used to identify biological stream types (assemblage types) and the physico-chemical parameters that most strongly characterized those assemblage types. Community metric values were summarized for each assemblage type using standard statistical descriptors, including mean, median, confidence intervals, and selected percentiles. The Kruskall-Wallis non-parametric ANOVA and the Dunn's's multiple comparison test (from Sigma Stat version 2.03) were used to examine the differences in the ranges of physico-chemical and biological attributes between assemblage types.

Non-Reference Database Development: A database of disturbed (non-reference) sites was assembled from the DEC monitoring database. These sites were selected based on the results of past monitoring and best professional judgement of DEC biologists (**Appendix 2**). Physico-chemical data and best professional judgement were used to assign all impact sites to the appropriate assemblage type. Community metrics for the impacted sites were summarized in the same manner as for the reference sites. Distributions of metric values from impacted sites were compared to distributions from reference sites in order to assist in the selection of metrics suitable for measuring impact and for determining the degrees of change from the reference condition consistent with language in the Vermont Water Quality Standards.

Threshold Biological Indices development: The reference and non-reference data bases developed above were used to guide the selection of a suite of metrics that were consistent with the following criteria:

1- Represent a range of both the structural and functional characteristics of the macroinvertebrate assemblage;
2- Demonstrate the least amount of natural variability within each reference stream type;
3- Respond in a relatively predictable manner to a variety of categories of environmental disturbance;

The distribution of the selected metrics within the reference and non-reference data bases were examined. The following factors were considered when evaluating appropriate threshold indices for each biological classification. Hierarchal deviations from the reference condition along a disturbance gradient were selected to be consistent with narrative biological criteria in the VWQS's.

1. The statistical significance of metric values between reference and impacted sites;

2. The median, range, 5th / 95th , 25th / 75th percentiles, of each metric of the minimally affected reference sites within each stream type;

3. The median, and 10th / 90th percentiles of metric values observed at sites considered to be impacted within each stream type;

4. The level of precision for each metric using the assessment methodology;

5. General metric water quality rating from other North East States, and literature;

6. The best professional judgement (BPJ) of the VTDEC biologists;

Results and Discussion

The final reference database used to characterize the macroinvertebrate community types consisted of 93 sites plus seven additional temporal events at seven sites, totaling 100site/events. Most have minimal human activity in the watersheds immediately above the sites. In order to gather data for larger watersheds and rivers, it was necessary to locate some reference sites in the lower reaches of watersheds with some human development well above the sampling site. Some of these sites may have what would be considered more than minimal human activity, but in the judgement of the authors this activity had only at most, a minor impact on the stream reaches sampled.

Statistical Analysis: An initial TWINSPAN analysis on the selected 100 reference site/events, and 97 weighted taxa (pseudospecies) show that 32 taxa were cosmopolitan across the first six major divisions. As a result the TWINSPAN divisions misaligned some sites by placing too much emphasis on taxa that were not good indicators of community types. Cosmopolitan species include the following: the mayflies *Stenonema sp, Seratella sp., and Baetis spps;* the stoneflies *Taeniopteryx spps* and *Isoperla spps,*; the caddis flies *Dolophiloides sp., Lepidostoma sp, and Symphitopsyche sparna;* and the dipterans *Hexatoma sp, Micropsectra sp. and Simulium tuberosum group*,as well as the following: *Lumbricus sp., Niadidae, Polypedilum avicep, Epeorus sp., Rithrogenia sp., Ehemerella sp., Paraleptophlebia sp., Antocha sp., Empididae, Bezzia sp., Eurylophella sp., Polycentropus sp., Ectopria sp., Cricotopus sp., Micrasema sp., Orthocladius sp., Thienemannemyia sp., Cladotanytarsus sp.*

A second TWINSPAN classification removing the 32 cosmopolitan taxa was run on the remaining 65 taxa across 100 sites. This TWINSPAN iteration shows four biologically distinctive macroinvertebrate assemblage stream classifications as shown in **Table 3.**

Table 3: A TWINSPAN ordination table showing four stream types as indicated by the 65 dominant macroinvertebrate taxa present across 100 stream sites. Indicator taxa for each stream type are bolded. The pseudospecies cut levels are 0, 0.1 , 0.4 , 3, 10, and 20.

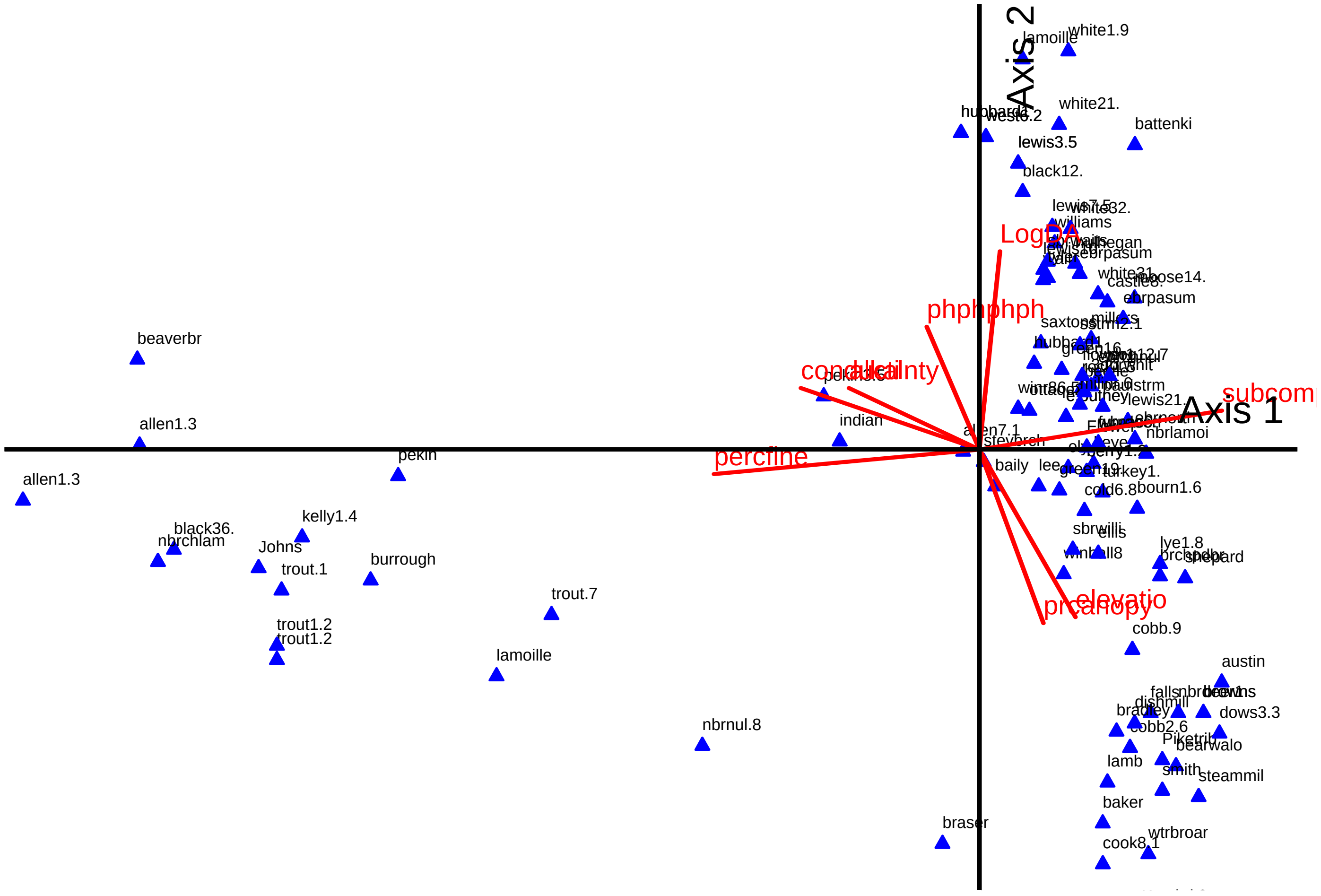
```
          045915999145 56 34778 2579  347234569577814 223356 12377888127435514  112122336666667362488889498919
          0558660495474966184875545589907267357112946789048732726901291318027212180034371234890506345366928331

Peltoper  25514422423411212-34211111-----3----2------11-------1-----1-------------------------------1-1-------------  00000
Ebrehmi   2----------1-113-34232-122--24----------------11-------------1----------------3-----1-----------------------  000010
E brevic  444----42---1--1----3----1-----------------1-----------1--2---1-------------------------------------------  000010
Parapsyc  42112--1212---------------1-------------------------------------------------------------------------  000010
S alhedr  -211-3-35--334-5--33------2----4-----3---4---2---3-----------------------------1------------------  000010
Smacleod  --2-1----4-314-----3-1--1-----------------------------------------------------------------------------  000010
Palaegap  ---1--------------------------------------------------------------------------------------------------  000010
Malireku  334233313433-111111-1-2-------11----1-111---------------1---------------------------------------  000010
Taenione  3-4----1----11-1--1-1-----------------------------1-------------1--------2--------------------------  000010
Oulimniu  223554544-441-325--442123----12--1-1--2--1-31-3--222--11-11122-2----3--123--1-----12---11-------------  000011
Apatania  -22443-244-334351212232-312---1221-2--1--212-22--331----1----32-1----12-1-----11-------------------  000011
Capniida  33211---2-11-2-3-2313-2-2-321111-1-11------1--1----1--22111---4125-1---------------1-----1------------  000011
Glossoso  ----4--4411----2-31-222-5242114221334--11-2-11421-32--22221--1113324424211---1--11---------------------  0001
Chlorope  46444634455531243344444443343332 43-33423333533234313-1-4224441----1-2-1-2-1-2---------1-11-----------  0001
T bavari  433-43443224-141-23343-2-4-343-343233-122-43433132--123-2-1-1-31-1--------21-11-1212223132132---4---  0010
Ptardell  ----2-2-1--114513416213-23-234----21-3211-211-1-11--112-111--1-1-1------121-21----11-1111------------  001100
Rhyacoph  444443324433533323413332344343321341-32334331332233233343322-41121-11-3-132233-111---3-22------------  001101
Leuctrid  6443424242542-2322414421332444432--33321-11--3----13122424232--1112--1-----1--1---------113--------2--  001101
Sslosson  -2------423-451-2433333214433444444444434435443444-444344345-43443444-33-344533---------241-----------  00111
Agnetina  ------21----432213-1233312433323322333122432232222 32-13312231132-31-----1--11-11-----11-------1-----  00111
Pelegans  ---3--------------------------------------------1--------------------4---44531-------------------  010000
Pconvict  ------------------------1-------------------------1-----------2--1---11-221-422------------------  010000
Chimarra  -----------------------------------------------------2445-55554-----4443211-1------------------  010000
Neoperla  -----------------------------------------------------------244421-----1323233------------------  010000
Psephenu  -------------------------221212--4-1-111-1-------1-1-4132-1-1341311-1224331-44342333---1-----------  010001
Tdiscolo  -------------------3----2---------11---12233322-2112--1233---2--111-24223131333213----------------  010001
Helicops  -------------------------21--1-----------------1-2--32244-1-1--123-3-44--23353244431-1------------  010001
S bronta  ---------------------------12----32311--31343-3334-22322332223 3-212-113133423234344 3---------------  010001
S morosa  ------------1------------3-----14342---112-32345334255333434-324--5543464544544445-4---------------  010001
Paragnet  ------------1--------1---1-22334134-2--1122122221113332211132-1-1--1-222333433323232221---------------  010001
Euorthoc  -------------2---------1-------2----1---2---1-1-1------112-4-3-------3-1---3----1-----------------  010010
Acroneur  -------1----3--1--2--1--2--21122-33-1--111111--1--1211--122-----21-243211221311211--2--------------  010010
Isogenoi  ------------3----1----------2-1-12-122--11243111112-11-1----1111--------11--21---------------------  010010
Brachyce  ---------------------224----------21-411434234464--34-3--1142--2------122355-4535----11------------  010011
Pseudocl  ----------1-----1-1--1-4222-42144443-4333234644444324333313333 43-24433333243232111-1211----1------3-  010100
Isonychi  ---------------1----------22-----1---111--11---1-433-32231432---4123444331-12334-56--------------4-  010100
Optioser  ---------1---44-5521--1-244-4324123655544--2343-22--114-1113453534443333434414434433411211-----12---  010101
Psilotre  --------1--11-21-1-1-1-1--2-1111-1-----11--2-----142--1-2-121-21--------33--1121------------------2-  010101
Stenelmi  ------------1------------1--------------------1------2--23443344322331125444424 4-3--1-1-21----2  01011
Cheumato  -----13-3---4-31-----------31-----------33334343-4444443434444443444534543343234344335 1---1--3-3---  01011
Rheotany  -------------1-------3--221-12--1----111-21--3111-1-31212-1-2-31-1--113-421-3--142312-12443--1-----  011
Atherix   -2-2-2-----22--244244-332211-1-21-311-111231112--21-2-1-121-21-22---1-1--212211--3121-2233444-3-----  100
Gomphida  ---11--11-22---111-21--1--111121----3--1----1---1-1-112222-1-------------31-------------2213--1---1-3-2  100
Tipula    --11-2-1---11--1--11-1-1--2215-1-------------------1----1-------122---------1----1---3324344---21--  101
Hydropsy  ----------------------------111---------------------1-----3--13-14---------1-3321441----41---2-2--2  110
Aspectro  -------------------------------------------------------------------------------------14-----12--2-  111000
Hydatoph  ---4-----------------------1------1---------1----------1-----1-1----------------1----222----4----44  111000
Centropt  ----------------------------------------------------------------------------------2---------4-3-  111000
Zavrelym  ------------------------1---------------------------------------------------------1111-----24--1-  111000
Dubiraph  -------------11------------3-------------1----1--------1-31---------111-1-111134112336434-13  111001
Paratany  --------------------------------------------------------1-------------------1--------12--44----  111001
Pscalaen  --------------------------------------------------------------------------------1- 144411-----334  111001
Simgrp a  -------------------1-----------------------1-------------------21--------------1---3116------3  111001
Lype      --------------1--------------------------------------------------------------------4-142---------  111001
Corduleg  ------------1---------------1------1------------------------------------------------34121---1--12  111001
Pisidium  --------------1--1-------1------1-------1--------------------3--2---------111---21323222--34434-4  111001
Hexageni  -------------------------------------------------------------------------------------1-2---4---  111001
Caenis    -----------------------------------------------------------------------1--1-1---21-1-6----1-  111001
Tanytars  ------------2----1----------123----------------------------1---112-------1---1---1----2422--2-2432-1-  11101
Physidae  ------------1-------------1-------1-1---------1---------------------------11------2-1-1-25-----  11101
Hyallela  --------------2----------------------------------------------1------------1-----------1--446-3--  111100
Gammarus  ----------------------------------------------------------------------------------------1----64--  111100
Helisoma  ----------------------------------------------------------------------------------------4-3----  111100
Sphaeriu  ---------------------------------------------------------------------------1---2--------3--5---  111101
Asellus   --------------------------------------------------------------4-------------------------22-4  11111
          0000000000000000000000000000000000000000000000000000000000000000000000000000000000001111111111111111
          0000000000000000000000000001111111111111111111111111111111111111111111111111111111110000000011111111
          0000000000000111111111111110000000000000000000000000000000000000000011111111111111111111110000111100000011
```

Small High Gradient Low Alkalinity Cold High Elevation **(SHG)**	Moderate High Gradient Moderate Alkalinity Cold Mid Elevation **(MHG)**	Large High Gradient High Alkalinity Warm Low Elevation **(WWMG)**	Low Gradient Slow Winders **(SW)**

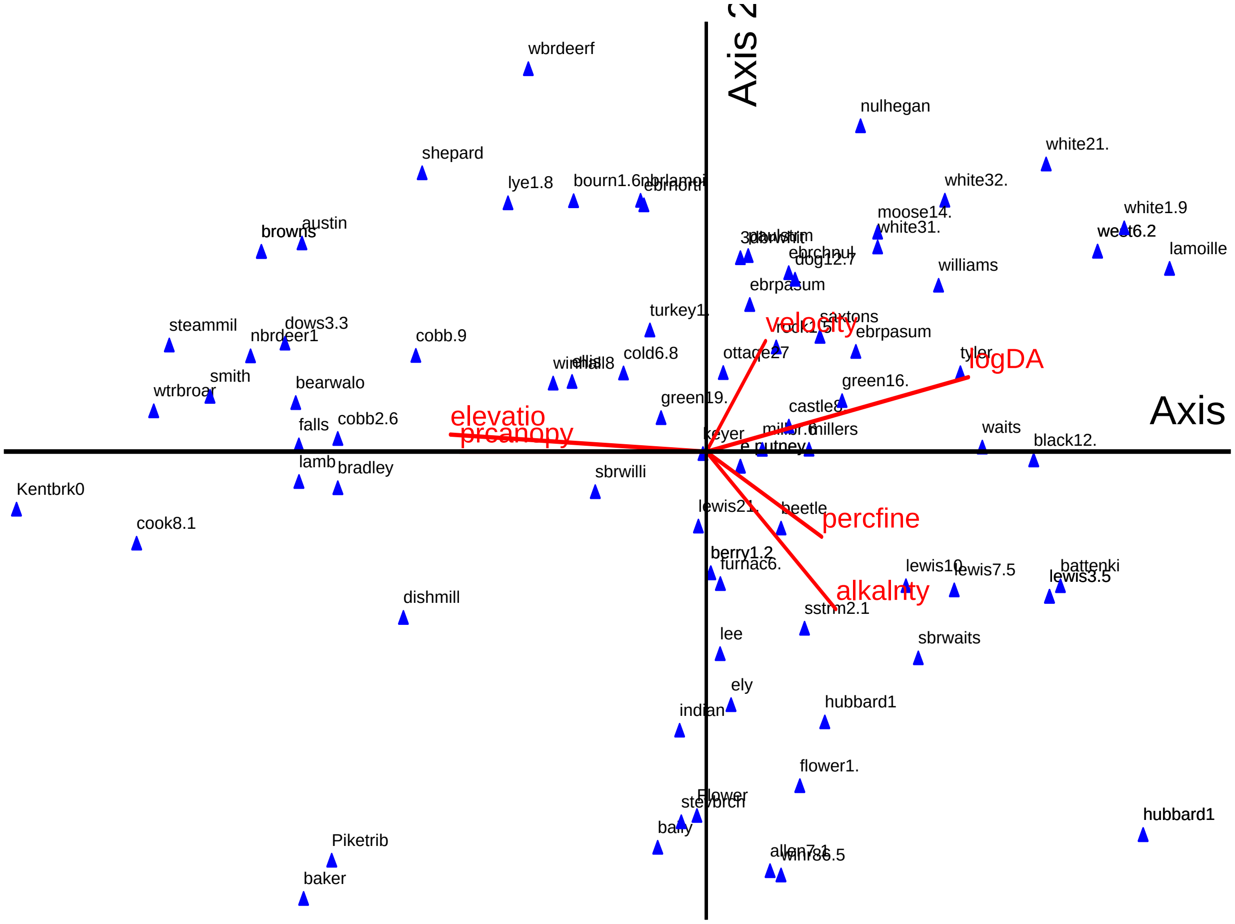
Canonical Correspondence Analysis on these 100 sites/events shows the relative influence of 10 environmental variables on the composition of the 65 remaining indicator taxa across sites (**Figure 1**). The percent fines, and dominant substrate particle size clearly separated out sixteen sites along the first primary axis. This distinction can be viewed as a separation of depositional from erosional sites and is most clearly associated with stream power factors such as stream gradient and substrate particle size. Site knowledge confirmed that this distinction is primarily one of low gradient sites with sand/silt dominated substrate versus higher gradient riffle sites dominated by gravel/cobble/boulder course substrate. This appears to be a reasonable biological distinction based on physical habitat implications.

Figure 1. Canonical correspondence analysis showing location of 100 sites/events as determined by the percent composition of 65 dominate macroinvetebrate taxa, and the relative influence of 10 environmental variables on 2 primary axes.



The sites with coarser substrates of gravel to boulder seem to be additionally influenced by the physicochemical attributes of drainage area, stream order, elevation, canopy cover, alkalinity, pH, and conductivity. In order to investigate these relationships more precisely, the 16 low-gradient sites that were most closely aligned with the first primary axis (per cent fines/substrate size) were removed from the data set and a second CCA conducted. The resulting CCA on the 84 remaining high gradient sites and their 44 associated macroinvertebrate taxa is presented in **Figure 2**. The output figure suggests that the taxonomic composition at these high gradient sites is strongly influenced by elevation, drainage area, stream order, canopy cover, pH, conductivity and alkalinity.

Figure 2. Canonical correspondence analysis of 84 high gradient stream site as arrayed by 44 dominate macroinvertebrate taxa on two axis, and the relative influence of the environmental variables of elevation, drainage area (log), stream order, canopy cover, pH, conductivity, and alkalinity.



In order to clarify what factors were most useful for making distinctions in stream types along the various environmental gradients identified by CCA, correlation analyses were conducted to describe relationships between important environmental factors as well as relationships between those factors and the CCA axes. An initial "correlation" was conducted using best professional judgement (BPJ). The distinction between "low gradient" and "high gradient" streams (in the 100 site dataset) as identified by CCA, is clearly a function of substrate composition and can be clearly defined by that characteristic.

The gradients identified by CCA of the 84 "high gradient" sites are more complex. Correlations between the physicochemical attributes are presented in **Table 4.** A high level of correlation occurs between the physical attributes of canopy cover, elevation, log drainage area, and stream order. Specific conductance, pH and alkalinity were also found to be highly correlated. From the above measures *canopy cover, elevation, log drainage area, and alkalinity* were selected as the best physico-chemical attributes to use in judging the placement of streams into an appropriate biological stream type. **Table 5** shows the final correlations between the selected physico-chemical attributes and the first two canonical axes from the high gradient sites based on weighted averaging of the species scores.

Table 4. A correlation matrix between the significant physico-chemical attributes (from CCA) which influence high gradient streams. Bold indicates r values greater than 0.6.

	Drainage Area (Log)	Stream Order	Elevation	% Canopy	pH	Alkalinity	Specific Conductance
Log D. A.	1						
Stream Order	**0.92**	1					
Elevation	**-0.68**	**-0.63**	1				
% Canopy	**-0.67**	**-0.66**	**0.60**	1			
pH	-0.30	0.23	-0.54	-0.45	1		
Alkalinity	0.13	0.05	-0.44	-0.36	**0.77**	1	
Sp. Cond.	0.16	0.07	-0.52	-0.311	**0.74**	**0.93**	1

Table 5. Inter-set correlations between four selected physico-chemical attributes and first two canonical axes from 84 high gradient reference sites.

Physico-chemical attribute	Axis 1	Axis 2
Log D A	**0.85**	0.27
Elevation	**-0.83**	0.13
% canopy	**-0.80**	0.00
Alkalinity	0.41	**-0.69**

Determination of stream types: Using the above analyses plus the best professional judgement of DEC biologists, the 100 reference sites were placed into four biologically based stream classes (**Appendix 3**). This was done by using the TWINSPAN divisions as a basis for distinguishing groups of sites exhibiting similar biological structure, with stream classification placement adjustments made based on the CCA and correlation analyses plus the best professional judgement (BPJ) of DEC biologists. The macroinvertebrate stream assemblage types will be referred to as Small High Gradient streams **- SHG,** Medium High Gradient streams **- MHG,** Warmwater Moderate Gradient streams **-WWMG**, and Slow Winding streams **- SW .**

TWINSPAN identified the SW stream sites as being the most unique in the first division of streams. CCA analysis also separated out the same stream sites on the first axis as being highly influenced by the physico-chemical attributes of percent fines, dominant substrate particle size and velocity. The data show streams in type SW are significantly higher in percent fines, and lower both in velocity and dominant particle size than all three of the high gradient stream types (**Table 6**). The percent fines in these streams averaged 85 percent of the substrate composition, with the average particle size between silt and sand. The velocity at these sites averaged 0.5 ft/sec. The other physico-chemical attributes ranged widely within this stream type, suggesting that additional stream types may be broken out from this grouping. TWINSPAN analysis also indicates that more biological community types may be encompassed by this broad stream classification as was found within the high gradient sites. At this time however, not enough sites have been sampled within this SW group to attempt any further classification or biocriteria development.

TWINSPAN analysis on the remaining 84 high gradient sites by 44 dominant taxa separated out the SHG streams in the first division, and the MHG streams from the WWMG streams in the second division. Drainage area, elevation and stream order were significantly different ($p<0.05$) between all three high gradient stream types. The SHG streams are also significantly different from the other two in percent canopy cover, pH, Alkalinity, and Conductivity. The statistical validity of this stream classification was assessed using multivariate ANOVA. Dunnet's test was used to test the hypothesis of no differences between the three proposed stream classifications. This statistical procedure revealed that a linear combination of the predictive variables (drainage area, elevation, % fines, velocity, % canopy, pH, alkalinity, and conductivity) produced a strong separation between the three stream types (Wilkes' $\Lambda = 0.18$, $F = 12.79$, $p<0.0001$). Linear discriminant functions were then developed using the eight predictive variables which can be used to mathematically allocate a sampling site to a stream class with a known probability of correct classification. A brief description of the stream types, their physico-chemical attributes and their indicator species as identified by TWINSPAN, CCA, and BPJ follows.

Small High Gradient Streams (SHG) - Small mountain headwater (1-3 order) streams typically at high elevation and highly canopied . They are high in gradient, and as a result their substrate is dominated by gravel/cobble/boulder with percent fines averaging 3 percent. The drainage areas average 10km^2 and the water chemistry is relatively soft, with alkalinity averaging 18 mg/l. Indicator and preferential taxa include the following:

Plecoptera- *Peltoperla sp., Malerikus sp., Chloroperlidae, Leuctidae*
Trichoptera- *Symphitopsyche macleodi, Parapsyche sp.*
Coleoptera- *Oulimnious sp.*
Diptera- *Eukiefferella brevicalar*

Medium High Gradient Streams (MHG) -Medium sized mountain streams in the 3rd-4th order range, typically found at moderate elevations (average 814 ft), with moderate (average 50 percent) canopy. The gradient is high with the substrate dominated by gravel/cobble/boulder, with percent fines averaging 6 percent. The drainage areas average 88km^2, and the water chemistry is usually moderate in alkalinity averaging 48 mg/l . Indicator and preferential taxa include the following:

Plecoptera- *Agnetina sp., Isogeniodes sp.*
Trichoptera- *Rhychophila sp., Symphitopsyche slossonae, Brachycentrus sp.,Glossosoma sp*
Coleoptera- *Promoresia tardella*
Diptera- Tvetenia bavarica

Warm Water Moderate Gradient Streams and Rivers (WWMG) - Large, valley streams 4-6th order in size or small streams within the Champlain Valley all at lower elevations (average 369 ft). Typically more open canopied (averaging 30 percent cover), and warmer based on the dominant species present. Gradients are moderate with substrates dominated by gravel/cobble/boulder, with percent fines averaging 7 percent. The drainage areas can range widely but are often quite large with an average size of 480km^2. Alkalinities are typically high, averaging 70 mg/l. Indicator and preferential taxa include the following:

Plecoptera- *Neoperla sp.*
Ephemeroptera- *Isonychia sp.*
Trichoptera- *Symphitopsyche morosa, Chimarra sp., Hydropsyche sp., Helicopsyche sp.,*
Coleoptera- *Stenelmis sp., Promerisia elegans*
Diptera- *Polypedilum convictum.*

Slow Winders (SW) - Low gradient streams with substrates dominated by sand/silt, averaging over 85 percent fines. Indicator and preferential taxa include the following:

Bivalvia-*Pisidium sp.*
Amphipoda- *Hyallela sp.*
Odonata- Cordulagaster *sp.*
Coleoptera- *Dubiraphia sp.*
Trichoptera- *Lype sp.*
Diptera- *Polypedilum scalaenum, Aspectrotanypus sp.*

The mean and range of physico-chemical attributes within each stream assemblage type are presented in **Table 6.** Differences in macroinvertebrate community metrics between three community types are presented in **Tables 7-12**.

Table 6. Physico-chemical attributes of the four macroinvertebrate community types. Data reported as the mean and minimum- maximum. Stream community types with the same letter are not significantly different ($p<0.05$) using the Kruskall-Wallis non-parametric ANOVA and the Dunn's multiple comparison test.

Parameter/ Community Type	**SHG** n=23	**MHG** n=43	**WWMG** n=18	**SW** n=16
Drainage area (km^2)	**10.5** 0.6-95 **A**	**87.5** 1-513 **B**	**480** 10-1781 **C**	**25** 4-60 **AB**
Elevation (ft)	**1535** 840-2500 **A**	**814** 290-1624 **B**	**369** 140-900 **C**	**560** 100-1339 **BC**
% Canopy Cover	**82.6** 30-100 **A**	**45** 10-90 **B**	**29** 10-80 **B**	**44** 10-80 **B**
% Fines (sand/silt)	**3.2** 0-12 **A**	**5.9** 0-15 **AB**	**7.2** 3-12 **B**	**85** 20-100 **C**
Dominant Substrate Size Category*	**5.1** 3-6 **A**	**5.0** 4-6 **A**	**5.0** 5-6 **A**	**1.5** 1-2 **B**
Velocity ft/sec	**1.4** .75-2.8 **A**	**1.4** 0.8-2.3 **A**	**1.5** 0.5-2.0 **A**	**0.5** 0.1-1.0 **B**
Order	**1.5** 1-3 **A**	**2.8** 1-4 **B**	**3.6** 1-5 **C**	**2.1** 1-3 **AB**
pH	**7.09** 6.29-8.07 **A**	**7.59** 6.46-8.38 **B**	**7.93** 6.95-8.41 **B**	**7.55** 6.45-8.41 **B**
Alkalinity (mg/l)	**18** 3-99 **A**	**48** 2.8-127 **B**	**69** 10-154 **B**	**91** 9-260 **B**
Conductivity (umhos)	**56** 19-206 **A**	**127** 22-293 **B**	**209** 53-450 **BC**	**262** 540-38 **C**

* The Dominant Substrate size was assigned a ranking from 1-6. 1=silt, 2=sand, 3=gravel, 4=coarse gravel, 5=cobble, 6=boulder.

Table 7. Macroinvertebrate community biometrics from three community types. The mean ± 95% confidence level, and the median and 25th-75th percentiles are reported.

Biometrics / Community Type	**SHG** n=40	**MHG** n=68	**WWMG** n=31
Density	**1231** ± 229 **1016** 699-1716	**1919.3** ± 226 **1797** 1259-2359	**2315** ± 487 **2244** 1224-3534
Richness	**40.1** ± 2 **40** 36-43.5	**47.6** ±1.6 **45.8** 43.3-51.8	**45.7** ± 3.4 **45.5** 38.9-51.4
EPT	**23.1** ± 0.9 **23.5** 21.5-25.3	**26.8** ± 0.8 **27** 24.5-28.5	**23.2** ± 1.3 **23** 20.5-25
EPT/Richness	**0.58 ±** 0.02 **0.6** 0.5-0.6	**0.57 ±** 0.01 **0.6** 0.5-0.6	**0.53** ± 0.05 **0.5** 0.4-0.7
No. Of Ephemeroptera Taxa	**6.4** ± 0.6 **6** 5-8	**9.07** ± 0.4 **9** 8-10	**9.2** ± 0.9 **9** 7.3-11
No. Of Plecoptera Taxa	**8.7** ± 0.6 **9** 8-9.5	**8.8** ± 0.5 **9** 7-10	**6.3** ± 1.2 **6** 5-7
No. Of Trichoptera	**11.8 ±** 0.8 **12** 10-14	**13.2** ± 0.7 **13** 11.5-16	**12.5** ± 1.4 **12** 9.3-15
% Dominant Genera	**21** ± 2.0 **20** 17-24	**20.5** ± 1.8 **20** 14.4-24	**21.2** ± 2.2 **19** 16.7-24.6
No. Of Dominant Genera	**7.9** ± 0.5 **7** 7-9	**7.6** ± 0.4 **7.5** 6-9	**8.3 ±** 0.6 **8** 7-9.8
Bioindex (0-10)	**2.3** ± 0.2 **2.3** 1.9-2.8	**3.1** ± 0.1 **3.2** 2.8-3.6	**3.7** ± 0.3 **4.0** 3.2-4.2
EPT / EPTC	**0.84** ± 0.03 **0.9** 0.8- 0.9	**0.86** ± 0.02 **0.9** 0.8-0.9	**0.9** ± 0.04 **0.9** 0.8-1.0
% Hydropsychidae	**6.2** ± 1.9 **4** 1.6 - 9.1	**17.2** ± 2.2 **15** 10.7-25.5	**18.7** ± 3.4 **18** 11.3-23.5
Species Diversity	**4.19** ± 0.1 **4.2** 4.0 - 4.4	**4.5** ± 0.1 **4.6** 4.3-4.8	**4.3** ± 0.1 **4.3** 4.1-4.6

Table 8. Results of the Dunn's multiple comparison tests between the three macroinvertebrate community types for biometrics. Stream types with the same number are not significantly different (p>0.05)

Biometrics / Community Type	**SHG** n=40	**MHG** n=68	**WWMG** n=31
Density	1	2	2
Richness	1	2	2
EPT	1	2	1
EPT/Richness	1	1-2	2
No. Of Ephemeroptera Taxa	1	2	2
No. Of Plecoptera Taxa	1	1	2
No. Of Trichoptera	1	2	1-2
% Dominant Genera	1	1	1
No. Of Dominant Genera	1	1	1
BIO INDEX (0-10)	1	2	3
EPT / EPTC	1	1	1
% Hydropsychidae	1	2	2
Species Diversity	1	1	1

Table 9. Percent composition of the taxonomic orders of macroinvertebrates from three community types. The mean ±95% confidence level, and the median and 25th-75th percentiles are reported.

Community Type	**SHG** n=40	**MHG** n=68	**WWMG** n=31
%Coleoptera	**8** ± 2.8 **4.3** 1.0 -12.3	**6** ± 1.4 **4.0** 1.2 - 7.5	**13** ± 2.9 **11.7** 6.8 - 18.1
%Diptera	**19** ± 3.2 **16.0** 12.4 -22.3	**18** ± 2.1 **16.9** 11.0 - 22.8	**13** ± 3.3 **12.9** 6.6 - 17.0
%Ephemeroptera	**23** ± 4.4 **19.4** 13.6 -30.4	**34** ± 3.0 **33.0** 25.7 - 43.2	**32** ± 5.5 **29.4** 22.6-44.2
%Trichoptera	**28** ± 4.4 **24.3** 18.7 -35.5	**33** ± 3.0 **33.3** 24.4 - 41.2	**33** ± 5.5 **29.4** 22.6 - 44.2
%Plecoptera	**21** ± 3.9 **18.7** 12.4 - 28.5	**8** ±1.5 **6.6** 4.6 - 9.6	**8** ± 5.4 **3.6** 2.0 - 7.6
%Oligochaeta	**<1** ± 0.3 **0.2** 0.0 - 0.3	**<1** ± 0.3 **0.1** 0.0 - 0.4	**1** ± 0.7 **0.1** 0.0 - 0.5
PMA-O	**76** ± 3 **76** 70 - 84	**81** ± 2 **83** 78 - 86	**80** ± 3 **81** 75 - 85
PPCS-O	**0.54** ±.004 **0.54** 0.44 - 0.64	**.54** ± 0.02 **.54** 0.52 - 0.64	**0.48** ± 0.03 **0.49** 0.42 - 0.53

Table 10. Results of the Dunn's multiple comparison tests between the three macroinvertebrate community types for percent composition of the Orders,PMA-O, and. PPCS-O. Stream types with the same number are not significantly different (p>0.05).

Community Type	**SHG**	**MHG**	**WWMG**
% Coleoptera	1	1	2
% Diptera	1	1	2
% Ephemeroptera	1	2	2
% Trichoptera	1	1	1
% Plecoptera	1	2	2
% Oligochaeta	1	1	1
PMA-O	1	2	1-2
PPCS-O	1-2	1	2

Table 11. Percent composition of the functional groups of macroinvertebrates from three macroinvertebrate community types. The mean ± 95% confidence level, and the median and 25th-75th percentiles are reported.

Community Type	**SHG** n=40	**MHG** n=68	**WWMG** n=31
Collector - Gatherer	**31** ± 4.8 **27.5** 18.0 - 40.7	**32** ± 3.0 **31.7** 22.0 - 41.7	**22** ± 3.7 **21.2** 13.0 - 27.6
Collector - Filterer	**18** ± 3.6 **17.1** 8.5 - 24.3	**30** ± 2.8 **29.8** 19.8 - 37.2	**36** ± 4.5 **32.9** 27.2 - 43.4
Predator	**19** ± 2.4 **18.3** 23.1	**13** ± 1.3 **11.9** 9.3 - 14.3	**7** ± 1.2 **5.5** 4.7 - 10.0
Shredder-Detrivore	**15** ± 3.0 **12.8** 9.3 - 17.6	**4** ± 0.9 **3.1** 1.7 - 5.1	**2** ± 1.4 **0.2** 0.1 - 0.6
Shredder-Herbivore	**1** ± 0.7 **0.5** 0.00 - 1.8	**1** ± 0.5 **0.7** 0.3 - 1.7	**5** ± 2.4 **2.8** 0.4 - 6.9
Scraper	**12** ± 2.9 **9.0** 4.4 - 18.5	**13** ± 1.8 **11.2** 7.9 - 16.8	**22** ± 3.4 **21.2** 14.7 - 28.5
PMA-F	**74** ± 2.4 **75** 69 - 80	**76** ± 1.7 **76** 71 - 80	**76** ± 3.5 **77** 68 - 84
PPCS-F	**0.59** ± 0.03 **0.60** 0.52 - 0.65	**0.64** ± 0.02 **0.64** 0.58 - 0.72	**0.56** ± 0.04 **0.58** 0.50 - 0.63

Table 12. Results of the Dunn's multiple comparison tests between the three macroinvertebrate community types for percent composition of the functional groups, PMA-F, and PPCS-F. Stream types with the same number are not significantly different (p>0.05).

Community Type	**SHG** n=40	**MHG** n=68	**WWMG** n=31
Collector Gatherer	1	1	2
Collector Filterer	1	2	2
Predator	1	2	3
Shredder-Detrivore	1	2	3
Shredder-Herbivore	1	1	2
Scraper	1	1	2
PMA-F	1	1	1
PPCS-F	1	2	1

Selection of Macroinvertebrate Biometrics and Criteria

Multiple metrics were selected and criteria established in order to protect both the structural and functional integrity of the aquatic macroinvertebrate assemblages for each community type. The need to evaluate a number of metrics is necessary to fully protect the biological integrity of the macroinvertebrate stream assemblage, and to increase the confidence level that the correct determination of impairment has been made. Different types of pollution insults will alter the assemblage structure or function in different ways. Pollution insults can generally be grouped into broad categories i.e. toxic, organic enrichment, and habitat alterations. These different categories of pollution insults will often effect only certain metrics of the macroinvertebrate assemblage integrity. As such a number of metrics have been selected which in combination meet the following criteria:

1- Represent a range of both the structural and functional characteristics of the macroinvertebrate assemblage;
2- Demonstrate the least amount of natural variability within each reference stream type;
3- Respond in a relatively predictable manner to a variety of categories of environmental disturbance;

The metrics selected and biocriteria thresholds for each water classification were chosen based on assessment of the following factors.

1. The statistical significance of metric values between reference and impacted sites;

2. The median, range, 5th / 95th , 25th / 75th percentiles, of each metric of the minimally affected reference sites within each stream type;

3. The median, and 10th / 90th percentiles of metric values observed at sites considered to be impacted within each stream type;

4. The level of precision for each metric using the assessment methodology;

5. General metric water quality rating from other North East States, and literature;

6. The BPJ of the VTDEC biologists;

The Mann-Whitney Rank Sum test (two sample, non-parametric T-test - see **Appendices 4-6**) was applied to determine significant differences in metrics between reference and non-reference data bases. If a metric could not show a significant difference ($p<0.05$) it was rejected from further consideration.

Tukey box plots and percentile distributions of metric values within reference and non-reference data bases were evaluated in order to select metrics that demonstrated the least variability within their distributions and the greatest separation between the mean reference and non-reference values. If a metric could not demonstrate impact in at least 10 percent of the non-reference sites it was rejected. Similar methods of using percentile descriptive statistics for determining degrees of deviation from the reference condition indicating impact have been applied by researchers in developing Indices of Biotic integrity (Plafkin 1989; Yoder 1995; Stribling et al. 1998). Using the degree of change in metrics observed between known impacted sites and an upstream reference site has also been a long established method of developing impairment criteria for a metric (Lenat 1987; Bode et al. 1994).

Metrics with an established, widespread and accepted use in the field of biomonitoring were selected over a newly-tested metric.

The best professional judgement of the DEC biologists as to the strength of the metric in Vermont streams was a critical factor in selecting a suite of metrics. **Appendices 4-6** list all the metrics evaluated and their descriptive statistics by stream type.

Following the above exercise, a total of 8 metrics were selected for each stream type. Because different pollutant types will often only effect certain aspects of macroinvertebrate community structure or function, not all metrics will demonstrate impairment at a site known to be impacted. It is for this reason that a number of metrics are used to ensure the protection of both the structural and functional integrity of the community.

Three taxonomic structure and compositional metrics were selected:

1. *Taxa (species)Richness;*

2. Ephemeroptera, Plecoptera Trichoptera Taxa richness (EPT);

3. *Percent Model Affinity at the Order level (PMA-O)*;

All of the taxonomic richness metrics were shown to be significantly different ($p<0.05$) between the reference streams and impacted streams for all three stream types. The reason for selecting *richness*, and the *EPT* index over the other taxonomic structure metrics is the greater difference between the means of the reference and impacted sites, and their proven acceptance in the field of biomonitoring. The taxonomic compositional metric PMA-O was selected over the other compositional metrics for similar reasons. The percent dominant taxa and percent Ephemeroptera were also shown to have strong potential; however, a significant number of outliers made these metrics less robust. They will continue to be calculated and used in the area of best professional judgement when developing weight of evidence findings.

Four indicator taxa and functional group metrics were selected:

1. *Bioindex (modified Hilsenhoff 0-10)*;

2. Percent Oligochaeta;

3. *EPT / EPT & Chironomidae (relative abundance)*;

4. *Pinkham Pearson Coefficient of Similarity based on functional groups (PPCS-F)*;

These four metrics consistently showed significant change between the reference and impact streams. The *Bioindex* and *EPT / EPT & Chironomidae* are also well established in the literature as good indicators of stream enrichment. The percent *Oligocheata* was selected because it identified impacted sites due to sedimentation. The *PPCS-F* showed significant differences ($P<0.05$) between the reference and impacted sites and incorporates shifts among all the functional groups.

A Spearman rank order correlation was run on the selected metrics to test for redundancy **(Appendices 8-10**). None of the metrics were significantly ($p<0.05$) correlated with an R^2 greater then 0.75, and no more then two metrics were ever correlated with an R^2 greater than 0.60 within any of the macroinvertebrate community types. This level of redundancy between, at the most, two metrics is an acceptable level, and shows that the series of seven metrics represent different attributes of community structure and function within a macroinvertebrate community type. The threshold biological indices for the selected metrics for each macroinvertebrate community type are presented in **Table 13**. For each macroinvertebrate community type, the metrics and criteria are graphically presented as box plots comparing the reference sites to the impacted sites in **Figures 3-5.**

Table 13. Macroinvertebrate assemblage threshold indices for three macroinvertebrate community types, and associated classes in Vermont. All criteria are either > or < and = the values listed. Extreme departures from the criteria thresholds indicates either a very poor biological condition or an assemblage of exceptionally high value.

	SHG			MHG			WWMG		
Metric	A1	B1	B2-3 A2	A1	B1	B2-3 A2	A1	B1	B2-3 A2
Richness	> 35	> 31	> 27	> 43	> 39	> 30	> 40	> 35	>30
EPT	> 21	>19	> 16	> 24	> 22	> 18	> 21	> 19	> 16
PMA-O	>65	> 55	> 45	> 65	> 55	> 45	> 65	> 55	> 45
BI	< 3.00	< 3.50	< 4.50	< 3.50	< 4.00	<5.00	< 4.25	< 4.75	< 5.40
% Oligo	< 2	< 5	< 12	< 2	< 5	< 12	< 2	< 5	< 12
EPT/ EPT+C	> 0.65	> 0.55	> 0.45	> 0.65	> 0.55	> 0.45	> 0.65	> 0.55	> 0.45
PPCS-FG	> 0.50	> 0.45	> 0.40	> 0.50	> 0.45	> 0.40	> 0.50	> 0.45	> 0.40
Density	>500	>400	>300	>500	>400	>300	>500	>400	>300

The threshold index values for the selected metrics were determined in the following manner;

The **Class A** thresholds were initially set to include at least 75 percent of the reference data base. The criteria were then slightly adjusted based on BPJ. Exclusion of the lower 25 percent of the values in the reference site distribution ensures against the influence of metric outliers within the reference sites. The presumption is that it is reasonable to expect, with some confidence, that the "best" 75 percent of the reference sites are " within the range of the natural condition". Streams which meet the Class A threshold criteria demonstrate that they are substantially meeting their biological potential.

The **Class B1** thresholds were initially set to include at least 95 percent of the reference data base. Exclusion of the lower 5 percent of the values in the reference site distribution ensures against the influence of metric outliers within the reference sites. The presumption is that it is reasonable to expect, with some confidence, that the "best" 95 percent of the reference sites show no more than a "minor change from the reference condition". Streams which meet the Class B1 threshold criteria demonstrate no more then a minor change from the reference condition.

The **Class B2-3 and A2** thresholds were generally set below the 95th percentile**,** or range of the reference value for each stream type. The Class B2-3 and A2 thresholds were then adjusted based on best professional judgment interpretation of the relationships between the range of reference values and the median, and 10th / 90th percentiles of the metric distribution from sites known to be impacted. The presumption is that it is reasonable to expect that a metric value representing more than a "moderate change from the reference condition" would fall at the extremes or outside the range of the reference data base distribution.

Figure 3. Tukey plots of the macroinvertebrate biometrics distributions for reference and impacted **SHG streams**. Boxes represent the 25th & 75th percentiles, with mean line, and 10th and 90th percentile bars. Outliers are shown as points.

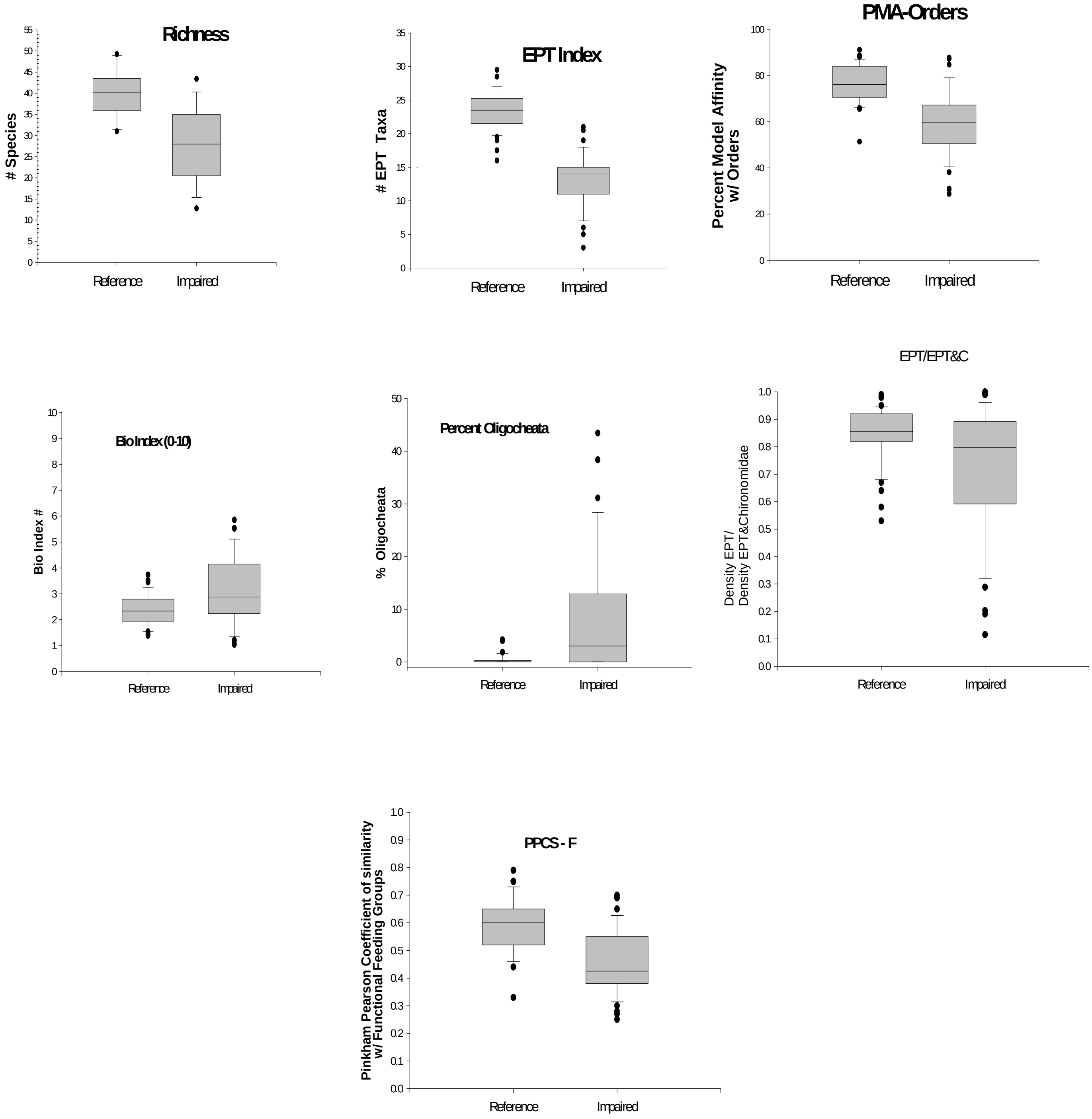


Figure 4. Tukey plots of the macroinvertebrate biometrics distributions for reference and impacted **MHG streams**. Boxes represent the 25th & 75th percentiles, with mean line, and 10th and 90th percentile bars. Outliers are shown as points.

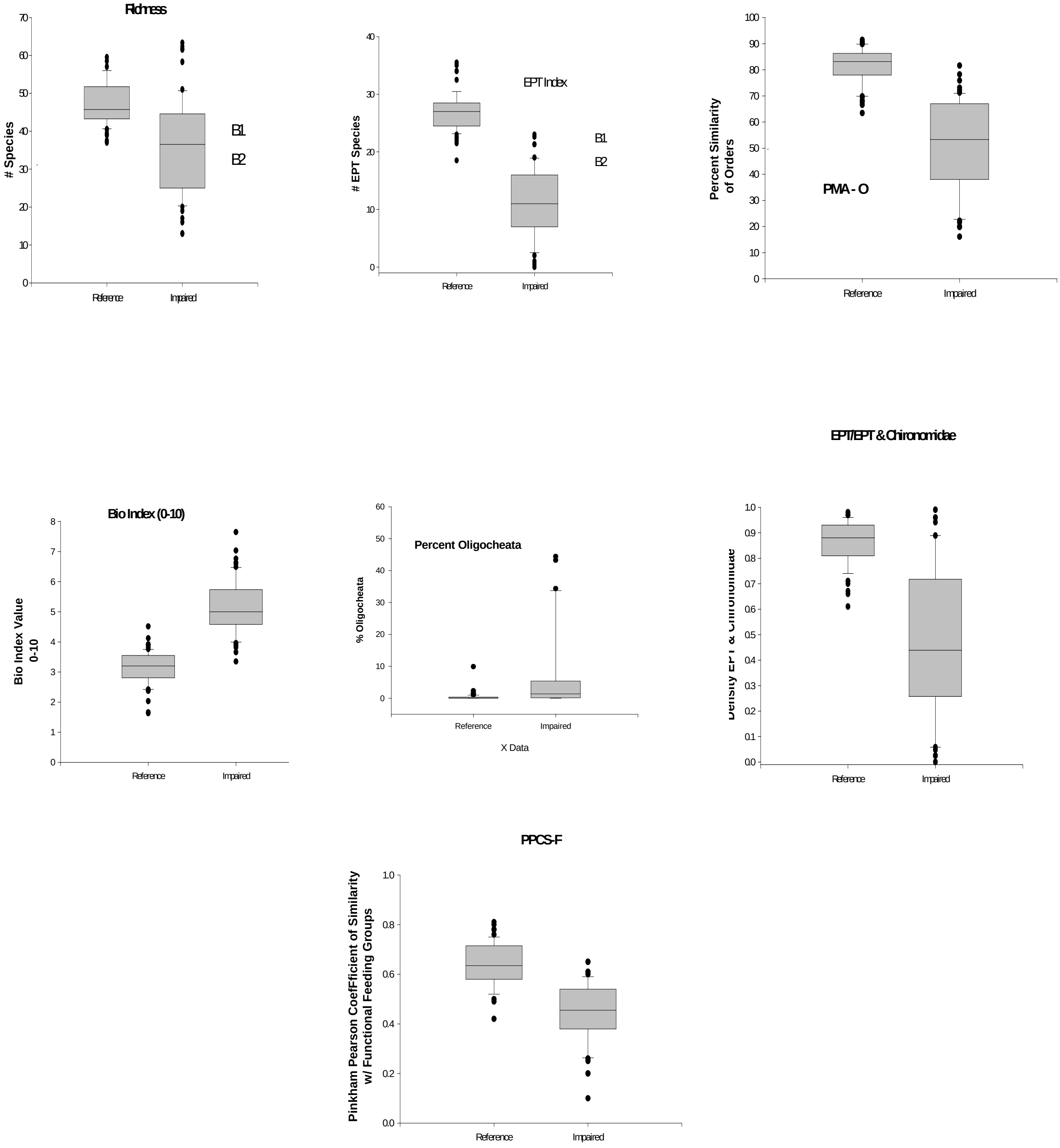
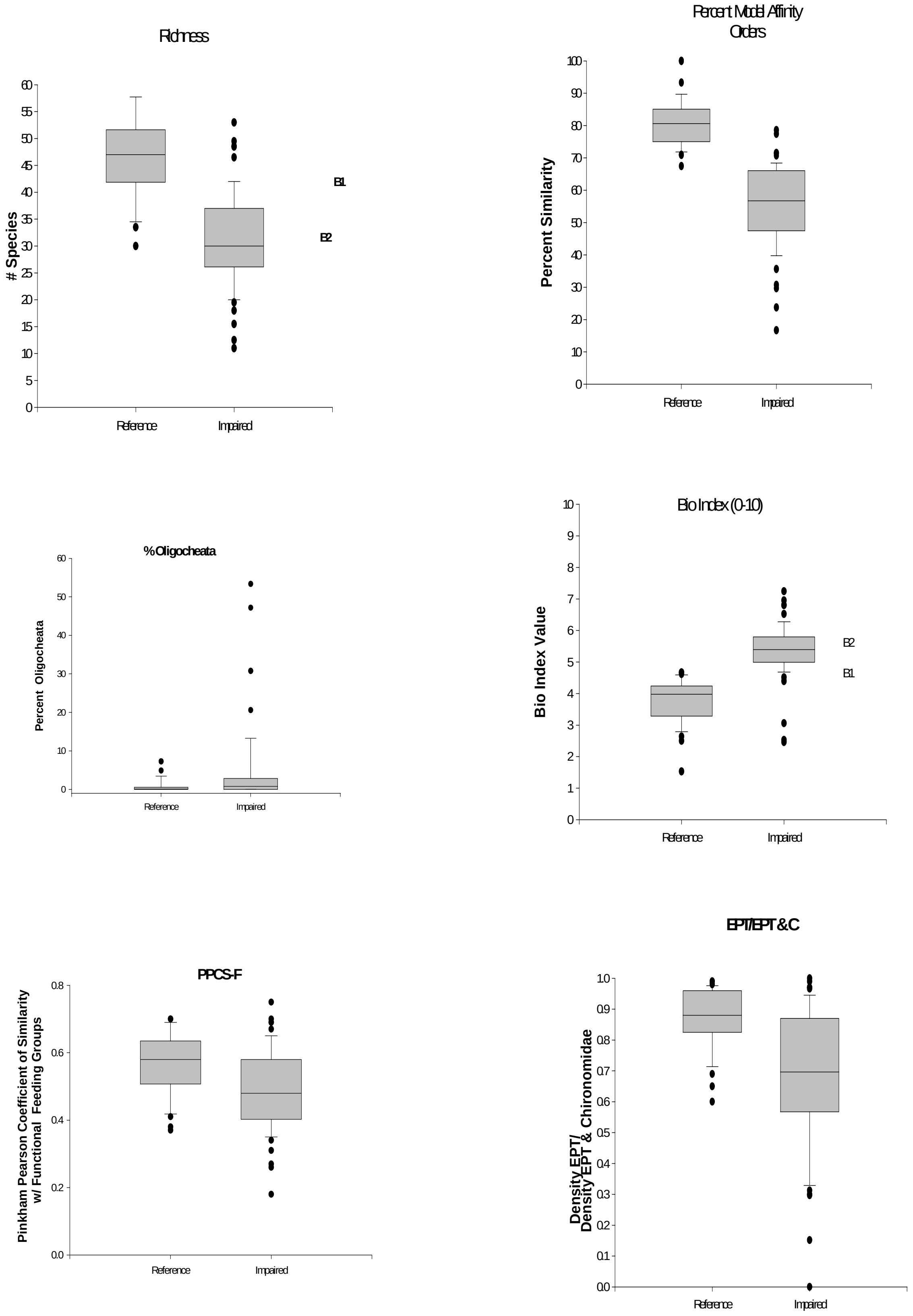


Figure 5. Tukey plots of the macroinvertebrate biometrics distributions for reference and impacted **WWMG streams**. Boxes represent the 25th & 75th percentiles, with mean line, and 10th and 90th percentile bars. Outliers are shown as points.



The following pages list the selected metrics, their ecological significance, interpretation, and calculation**.**

Density - Density is the relative abundance of animals in a sample (unit sampling effort). The relative abundance is a basic measure of a stream's secondary productivity. The density criteria was set very conservatively but is needed to ensure some basic level of macroinvertebrate productivity is maintained. The density will generally decrease due to both habitat and toxic impacts. It can also be relatively low in naturally unproductive streams which is why the minimum criteria is very conservative. Nutrient enrichment will often increase the overall density of a stream. It is an important metric to use in determining the causes and mechanisms of disturbances to the macroinvertebrate community.

> *Calculation:* Density is calculated by totaling the number of organism found in a sample. If less than the entire sample is processed, numbers tabulated in the sub-sample are adjusted to reflect "unit sampling effort" density. When replicate samples are collected from a site, this metric is calculated as the mean density by adding the density from each replicate and dividing by the number of replicates.

Taxonomic Structure and Compositional metrics

Richness- Species richness is the number of species in a sample unit. It is perhaps the most basic and accepted measure of assemblage diversity. Species richness will decrease when an assemblage is stressed from habitat degradation or poor water quality conditions (Plafkin et al. 1989). It can increase slightly in streams that are moderately enriched, and can also be naturally lower in smaller headwater streams (Bode et al.,1996). The richness expectation in the SHG streams was significantly lower then the larger stream types and the threshold biocriterion has been adjusted accordingly.

> *Calculation*: Richness is the total number of distinct taxa identified in a sample. Note: immature organisms identified to family or genus are not considered a distinct new taxa if a genus or species identification is determined within its group in a sample. When replicate samples are collected from a site, this metric is calculated as the mean richness by adding the taxa richness from each replicate and dividing by the number of replicates.

EPT Index- The EPT index is a subset of the above richness measure. It is the number of species in the sample in the generally more environmentally sensitive orders Ephemeroptera, Plecoptera, and Trichoptera. EPT richness will decrease when an assemblage is stressed from habitat degradation or poor water quality conditions (Lenat 1989). The number of EPT taxa will increase from slight enrichment, but are generally the first to decrease from moderate to extreme enrichment. The expected number of EPT species were found to be slightly lower in the SHG and WWMG stream types. The EPT biocriteria values for both these stream types have been adjusted slightly to account for the lower EPT expectation.

> *Calculation*: The number of distinct taxa identified in a sample from the insect orders Ephemeroptera, Plecoptera, Trichoptera. Note: immature organisms identified to family or genus are not considered a distinct new taxa if a genus or species identification is determined within its group in a sample. When replicate samples are collected from a site, this metric is calculated as the mean richness by adding the taxa richness from each replicate and dividing by the number of replicates.

Percent Model Affinity of Orders - (PMA-O) Is a measure of order level similarity to a model based on the reference streams (Novak and Bode 1992). The PMA-O decreases with increasing environmental stress on the macroinvertebrate assemblage. This is due to the general trend of decreasing abundance of the more pollution sensitive orders, and increasing abundance of the more pollution tolerant orders in highly polluted streams. The PMA-O reference condition was found to be relatively similar between the three stream types. The slightly lower affinity value from the SHG streams was not great enough to justify a threshold biocriterion adjustment.

> *Calculation*: PMA-O is calculated by determining the percent composition for each major group - Coleoptera, Diptera, Ephemeroptera, Plecoptera, Trichoptera, Oligochaeta, Other - at the assessment site and comparing them to the mean percent composition values from the reference condition (model). The model order percentages are then arrayed with the assessment site order percentages. The sum of the lower of the two values for each order is the PMA-O.

$$\text{PMA-O} = \sum \min (X_a \text{ or } X_r)$$

Where: X_a = the percent composition of order X from the assessment site;

X_r = the percent composition of order X from the appropriate reference condition;

Indicator taxa and functional group metrics

Hilsenhoff Biotic Index- BI (0-10) - The HBI is a measure of the macroinvertebrate assemblage tolerance toward organic (nutrient) enrichment (Hilsenhoff 1987). In many ways this index is both an indicator taxa metric and functional group metric, since those taxa which become more dominant in moderately enriched streams are those which are taking advantage of shifts in the available food base in the stream. There were significant differences in the reference condition between all three of the high gradient stream types. This may be due to both a natural shift in the food web from coarse allochthonous detritus and diatoms in SHG streams to one more dominated by fine particulate organic matter, and greater autotrophic production with a shift toward other algal groups in WWMG streams. These types of food web shifts have been described in detail in the literature and form the basis of the river continuum concept (Cummins 1974; Vannote et al.1980; Culp and Davies 1982). The threshold biocriteria values for each stream type were adjusted to reflect differences in the reference condition BI value.

> *Calculation :* The BI is calculated by: 1) multiplying the number of individuals of each taxon in a sample by that taxon's assigned tolerance value, as assigned by VTDEC after Hilsenhoff 1987; Bode 1996; 2) adding the total of all these taxon/tolerance value products; and 3) dividing the resulting sum by the total number of individuals of all taxa assigned a tolerance value. The resulting number is the Bio Index value.

$$\text{HBI} = \sum \frac{n_i\, a_i}{N}$$

Where: - "n" is the number of individuals of the "i"th taxon;
- "a" is the index value of that taxon;
- N is the total number of individuals in the sample;

% Oligochaeta - Percent Oligochaeta is a measure of the percent of the macroinvertebrate community made up of the Order Oligochaeta. The percent Oligochaetes in the community increases with increased amounts of sedimentation and nutrients or organic matter in the stream. Many Oligochaetes in streams are burrowers by habit and generally feed on organic particulates that settle on the bottom substrate in streams. The percent Oligochaetes in the reference streams is very low and not significantly different between stream types. The presence of relatively higher percent Oligochaetes was consistently found in impacted streams associated with high sedimentation indicators such as percent sand, embeddedness and siltation.

Calculation: The number (abundance) of Oligochaeta in a sample divided by the total number of animals in the sample.

EPT/EPT & Chironomidae -EPT/EPT plus Chironomidae is a measure of the ratio of the abundance of the intolerant EPT orders to the generally tolerant Diptera family Chironomidae. With increased ecological degradation often associated with non-point pollution causing stream warming, habitat impairment from silt/sediment, and enrichment, the more tolerant species of Chironomidae will dominate the stream community causing the ratio to decrease. This metric is less robust then some, in that it only demonstrated impairment in about 10 percent of the impacted sites.

Calculation: The number (abundance) of animals from the orders Ephemeroptera, Trichoptera and Plecoptera in a sample divided by the above plus the number of Chironomidae.

Pinkham-Pearson Coefficient of Similarity - Functional Groups - (PPCS-F) - The PPCS-F is a measure of functional feeding group similarity to a model based on the reference streams. It is similar in concept to the **PMA-O** in that a site is compared to a model of the composition of the functional feeding groups in the reference condition as opposed to order level taxonomic changes. Also the Pinkham-Pearson Coefficient of Similarity (Pinkham and Pearson 1976) was used as the similarity index. Significant departures in functional group similarity to the reference streams indicates that the energy pathways thru the aquatic ecosystem have been significantly altered compared to that of the reference stream model (Shackleford 1988).

Calculation: PPCS-F is calculated by first determining the percent composition of the six major functional groups (collector gatherer, collector filterer, predator, shredder-detritus, shredder-herbivore, scraper) as assigned by VTDEC after Merrit and Cummins (1996) and Bode (1996) at the assessment site. For each functional group determine the quotient of min/max between the assessment site and the reference model for the stream type. The sum of these quotients divided by six (# of functional groups) is the PPCS-F.

$$\text{PPCS-F} = 1/k \sum_{I=1}^{K} \text{minimum}(xia, xib)/\text{maximum}(xia, xib)$$

Where:
- k = the number of comparisons between stations (6)
- xi = the number of individuals in functional group I
- a, b = site a, site b

FISH BIOCRITERIA

Fish Community Methods

The general goal of this effort was to determine how the fish community reference condition changed with different stream types. Physico-chemical variables were identified which appeared to be associated with assemblage type. Sites were also classified in an attempt to delineate groups that exhibited similar metric values. Following that analysis, the derivation of both the Cold Water Index of Biotic Integrity (CWIBI) and the Mixed Water Index of Biotic Integrity (MWIBI) and their metric scoring results will be presented.

Sample Collections: Stream fish community sampling is generally conducted during the late-summer, early fall index period. Fish assemblages were sampled from small, cold and warm headwater streams supporting only one or two species, to larger streams, often tributaries of Lake Champlain, supporting 20 fish species. Fish were sampled in smaller streams with a backpack DC electrofishing unit. Larger, wider streams were sampled with a DC stream-side electrofisher using two anodes fished simultaneously by two crews. Each site was electrofished in a systematic fashion beginning at the downstream margin of the section and continuing upstream to the head in the section. One to three passes are made over the entire section. All stunned fish observed are captured and identified in the field where possible. All data used in the analysis originated from wadeable sites which were judged to have been representatively sampled. That is, an effort was made to include in the sampled section, all of the dominant habitat types characteristic of the surrounding stream reach. Generally the wider the channel width (the larger the river) - the longer the sampled section . No data are available for deeper sites which require boat-mounted electrofishing methods to sample. Reference and impacted sites ranged in section length from 40 to 200m. Approximately 90% of the reference sections fished were over 70m in length with only one section less than 50m. Where sites were sampled more than once, the data from the event which included the most species was selected to represent the site in this analysis.

The general method of fish assemblage sampling used by the VTDEC has been to sample stream sections which are representative in habitat of the overall stream reach in which they are located. For example, if a stream reach is of high gradient with a predominance of riffles, then the section selected for sampling will reflect that character by including a predominance of riffles. An effort has also been made to include all habitat types within a section so that the full assemblage present may be sampled. For most sites a subjective habitat analysis is conducted which includes visual estimates of quality and quantity of the following physical factors: in-stream cover; substrate composition; pool-riffle ratio; bank stability; type of dominant riparian vegetation; relative stream discharge; water clarity and percent canopy. Sampling efficiency is also noted in the field notes. For many sites, a more quantitative habitat evaluation is undertaken using a series of measurements of velocity, depth and substrate at regularly placed points on perpendicular transects. Physico-chemical parameters measured on-site include water temperature, specific conductance and alkalinity.

All fish are collected during sampling, identified in the field, examined for external anomalies, and released. Sampled stream reaches are isolated with block nets in the absence of natural barriers. All sampling and taxonomic Identifications are conducted by experienced biologists. On infrequent occasions, where identifications cannot be made in the field, voucher specimens are taken back to the laboratory for positive identification using Scott and Crossman (1973) and Smith (1985) and LaBar et al. (unpublished).

Comparability and representativeness are assured through the use of standardized sampling and evaluation methodologies, consistent site selection criteria, and the judicious use of best professional judgement Precision and accuracy of field collections of fish are difficult to evaluate through direct replication of a sampling effort. At sites where two or more sampling passes are made, relative accuracy is evaluated using standard population estimate calculations with estimates of standard error and 95% confidence limits. All field methods used to collect fish for this project are documented in VTDEC (1989 and 1994).

Data Management: Data are transcribed from field and bench sheets directly into an Access relational database program. This software calculates an array of population metrics, including those which comprise the current MWIBI and CWIBI. Also entered on the data sheet are site physical and chemical information, including a physical habitat analysis. A comprehensive sampling event report is generated for each event. The same individual conducts the sampling, enters the data onto the data sheet program and calculates the IBI scores.

Data Analysis: Two-way indicator analysis (TWINSPAN) was used to group sites into assemblage categories using a microcomputer version (PC Ord - McCune and Mefford 1997). TWINSPAN simultaneously classifies species groups and site groups and displays both on a two way table. Groups are defined by a level of similarity to each other. Canonical Correspondence analysis (CCA) was used to examine the biological gradients within the reference database and the physico-chemical characteristics that most influenced those gradients - CANOCO version 3.12. This ordination method is used to analyze associations between species assemblage data and environmental data. The output figure places environmental and biological data along two synthetic axes which serve to separate the species according to their niche differences while indicating which environmental variables are most responsible for this differentiation.

The Index of Biotic Integrity: The MWIBI and the CWIBI are composite indexes that measure the overall health of a fish assemblage. The MWIBI is applied to all streams of wadeable size that contain five or more native fish species. This index is comprised of nine community metrics which are individually scored and summed to yield a single score. Actual calculation of IBI scores is conducted by the supervising biologist, not a computer. Values range from 9 (poor) to 45 (excellent). The MWIBI was modified directly from a New England modification (Miller et al.1988) of Karr's original IBI (1981) which was developed for mid-western streams. To the greatest extent possible, the MWIBI retains the original theoretical framework of Karr's (1981) index. Since however, stream fish assemblages in the Northeast differ distinctly from those of the mid-west (Halliwell et al.1999), some metrics needed to be modified, while others were eliminated.

Since a minimum of five native species is required to apply the MWIBI, many waters with fewer species could not be evaluated using this approach. Most sampled stream sites with fewer than five fish species are from coldwater streams. Except for the very small examples, all warmwater streams support at least five species. Presently there is no IBI which can be effectively applied to warmwater streams supporting fewer than five native species. The CWIBI, derived during the initial stages of the current work, has been specifically formulated to evaluate assemblages with two to four species in small coldwater streams. Since point discharges into this stream type are rare, such an index needed to be particularly responsive to non-point discharges and general physical habitat degradation such as sedimentation and the removal of riparian vegetation.

Potential metrics for inclusion into the CWIBI were selected from existing cold and coolwater IBI's (primarily Lyon's et al.1996; Mundahl and Simon 1999; and Halliwell et al. 1999). Three candidate metrics were developed by the VTDEC. The raw values from each candidate metric were contrasted between reference and impacted sites. Statistical significance was used to determine whether a particular metric could sufficiently distinguish between reference and impact sites. Raw metric values which were not significantly different between reference and impacted sites ($p>0.05$, Mann-Whitney rank sum test, Sigma Stat version 2.03) were rejected from further consideration. Prior to the current analysis, several modifications had been made to the MWIBI during the course of applying the index to 276 sites over the last 12 years. These metrics have withstood the "test of time" and are believed to be the best metrics in reflecting assemblage health in Vermont streams. As a result, the process of choosing candidate metrics and selecting the candidates to form the current metrics which comprise the MWIBI will not be included here.

For both IBI's, metric redundancy was measured by correlating raw values for candidate metrics with each other using Spearman rank correlation. If values for two metrics from the reference data set were highly correlated (r>0.80) then the one with the lower ability to detect degradation (higher p:Mann-Whitney rank sum test) was excluded from the index (Mundahl and Simon 1998).

Assigning the scoring criteria for the MWIBI and CWIBI was conducted using a variety of approaches. Scoring criteria for metric 1 from the MWIBI (species richness) was accomplished by use of the maximum species richness lines (Karr et al. 1986). For metrics "borrowed" from other indexes, the same scoring criteria were used if they appeared to accurately reflect conditions from the current VTDEC database. If after examining the data, the original criteria was judged inappropriate, the ranges were modified to better suit Vermont data using best professional judgement. In assigning scoring criteria to new metrics (developed for the Vermont IBI's) the distribution of the data was examined using scatter-plots and data arraying (listing all values in order). For small coldwater streams (CWIBI) there was no relationship ($p<0.05$, Spearman rank order correlation) between raw metric values and upstream drainage area (stream size) or elevation. This meant that all metrics could be scored the using the same criteria without regard for these two parameters. For the MWIBI, some raw metric values varied by elevation and upstream drainage area. These metrics were differentially scored according to pre-determined breaks in site elevation and upstream drainage area.

The range of sensitivity of each metric to human disturbance over the full scope of impact was determined for both IBI's. The primary sensitivity range was identified by plotting each raw metric value against the IBI value for all sites. Each IBI was used as a measure of impact from which its respective metrics were plotted. Primary metric sensitivity is indicated by the area on the plot where the slope of the line is the steepest (Angermeier and Karr 1986). Indexes of Biotic Integrity are more sensitive to varying degrees of impact when their component metrics, in combination, span the expected range of perturbations. (Karr et al. 1986).

Reference sites varied in elevation from 102 to 2162 m and in site drainage from 10 to 298 km^2. Only native species which occurred at more than one site were used in the identification of assemblage types. Thirty-two of the total of 53 fish species were entered into the analysis. Rainbow and brown trout were excluded since they are non-indigenous species. Their inclusion in the analysis would be considered inconsistent with the reference ideal which describes conditions present in the absence of nonnative humans. The remaining 21 species occurred only once in the data set and where therefore dropped from the analysis.

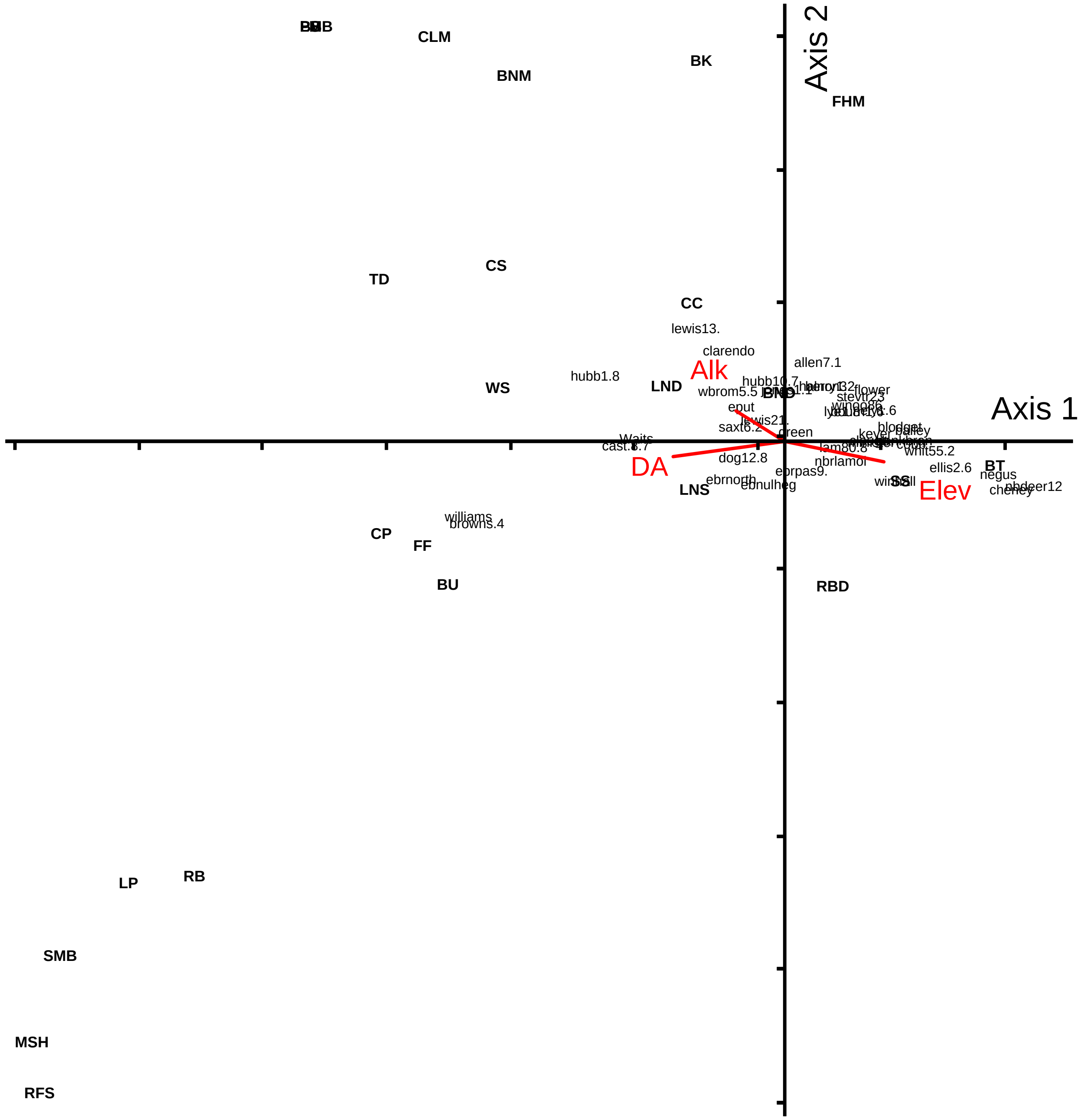
Results and Discussion

Statistical Analysis of Reference Sites

A CCA was applied to the data-set to identify important environmental variables that govern fish assemblage structure of streams and rivers. The resulting CCA bi-plot indicates that, of the variables included, site elevation and upstream drainage area appear to be the strongest factors on determining fish assemblage structure **(Figure 6)**. Axis 1 was defined largely by site drainage area and site elevation, while site drainage area and alkalinity characterized axis 2. Sites and species to the left of the plot are indicative of coldwater low alkalinity streams, and generally have smaller upstream drainages. Sites and species clustered to the left are indicative of low elevation warmwater assemblages, with higher alkalinities.

Data from sites lacking a complete set of environmental variables and sites with just brook trout were not included in the analysis. Additionally data form three low-gradient stream sites were not included in the final CCA plot. The effect of these sites on the triplot would have obscured differences in the remaining sites based on other variables. It is probable that when enough data is collected from low-gradient sites that these will form a new assemblage type Until more data is available for this stream type, however, these sites will not be considered.

Figure 6 . A Canonical correspondence analysis tri-plot for the first two axes showing fish species (bold) and locations. The bi-plot indicates that two major fish species-environment gradients exist. Environmental Gradient lines have been extended slightly in proportion to each other to facilitate labeling on the diagram. Eigenvalues for axis 1 is 0.562 and for axis 2 is 0.380. The first two axis explained 19.8 % of the total variance.



An evaluation of the first two divisions of the TWINSPAN output table **(Table 14)** showed site groupings which indicated clear trends in both biological and physico-chemical variables. These trends can be described as a progression in the table, from left to right, of decreasing site elevation and increasing site drainage area and alkalinity. Percent fines were higher in sites situated in the right half of the diagram while percent pool was highest in Group 4. Stream gradient was higher in the left side than the right. A left to right progression from low to high species richness is also clearly shown. The left to right progression then, can be best characterized as small, coldwater, high elevation, high gradient streams progressing into lower elevation, larger, moderate gradient streams, and finally to low elevation (Champlain Valley) warmwater streams of small to moderate size. In addition to using five, percent-composition-related pseudo-species, presence-absence data was also used to determine clusters. Using the P/A data yielded a very similar output table, describing essentially the same categories.

The generation of a stream classification based on similarities in assemblages is helpful in identifying gradients in site biological and physical properties across the breadth of the 76 site reference database The TWINSPAN classification generally supports the CCA results in depicting consistent changes in elevation and upstream drainage area as important factors in determining species composition of the assemblages. The strict assignment of breaks in metric scoring and IBI type to TWINSPAN-generated groups, however should not be made. Differences in species composition as interpreted by TWINSPAN, do not necessarily provide a direct translation of differences or trends in raw metric values. As an example, two reference sites may contain totally different species compositions, but have similar raw metric values, e.g. site 1: logperch and tessellated darter gives *two* benthic insectivore species while site two has longnose dace and slimy sculpin.- also *two* benthic insectivores.

Table 14 . A TWINSPAN output table showing the ordination of the 76 stream sites from fish collections. Groups derived from the first two levels of division (1-4) are divided by vertical lines. Pseudo-species cut levels are 0,5,10,20 and 40% composition.

```
                           1                 2                     3                   4
Sites ⇒              112333416675 3463 12223545452 712457445  11223677 122355575 14766613364466
Species ⇓          4926156778342639951842692805628707014582725355782466134001319105396793404318

Brook trout        55555555555552555553434324422431111-111---21--1111-111111--1-----1---------- 111
Slimy sculpin.     ------------115544454553553411444555542---1434-114--3-332-311---1------1---- 110
Longnose sucker    ---------------------1----1-----111111----1----1----1-1-1-111--------------- 10
Blacknose dace     ---------455-------13211-3455544423443-5555245522554555555454514131-11111-11 011
Longnose dace      --------------------2-1--113--44422121-----1111111-5234245243-4432-1-4-12--- 010
Creek Chub         -------------------2-2-1----11----------22413123221521111111--231--5411331-24 0011
White sucker       ---------------------------------------1-1212211311-11111-11--22241111111--- 00101
Rosyface shiner    --------------------------------------------------------------3----------1-- 00100
Mimic shiner       ----------------------------------------------------------------4---------2-- 00100
Mottled sculpin    ----------------------------------------------------1----------------------1 00100
Finescale dace     ---------------------------------------1--------------1--1----2-23----32-1-- 00100
Fallfish           -------------------------------------------2-1-1------1--1----2-23----32-1-- 00100
Burbot             --------------------------------------------1---------1--12---1--------2---- 00100
Rock bass          ---------------------------------------1--------1-------------2-----1-1111-- 00011
Common shiner      ----------------------------11----1-----1--2-1-11---111--121--1113-133213--- 00011
Chain pickerel     -------------------------------------------1----------------------1--------- 00011
Bluntnose minnow   -----------------------1--------------41--1---------------1--13-2114231111- 00011
Banded killifish   ---------------------------------------32--------------------------12-1---1- 00011
Tessellated darter ------------------------------------------------------------44331122212544-- 00010
Smallmouth bass    -------------------------------------------------------------1-31---1--11--- 00010
Logperch           ----------------------------------------1---------------------2--1--111-21-- 00010
Yellow perch       ------------------------------------------------------------------1-----22-- 00001
Silvery minnow     ------------------------------------------------------------------14-----4-- 00001
Sea lamprey        ------------------------------------------------------------------------14-1 00001
Pumpkinseed        ------------------------------------------------------------------34-1--111- 00001
Largemouth bass    ---------------------------------------------------------------------1--12-- 00001
Golden shiner      -----------------------------------------------------------------111------- 00001
Fathead minnow     ----------------------------------------1----------1-----1--------3-3--1---- 00001
Cutlips minnow     ----------------------------------------1----------------2-----------432---- 00001
Brook stickleback  -------------------------------------------------------------------1-------1 00001
Brown bullhead     ---------------------------------------1--------------------1------111--111- 00001
Redbelly dace      ---------------------1----3-------------3---------------------1----1------54 00000
                   0000000000000000000000000000000000000001111111111111111111111111111111111111
                   0000000000000000000111111111111111111110000000000000000000000000001111111111
                   0000000000001111111000000000001111111110000000000000000000001111110000000011
                   0000000001110011111000000001110001111110011111111111111111110011110000001101
                            011010000100000001011011000001010000000000111111111010001 00111101
```

Means	Group 1	Group 2	Group 3	Group 4
Elevation	1268	1044	606	218
Drain. Area	10.6	35.1	68.6	89.8
Alkalinity	22.4	40.1	63.6	107.0
%Fines	9.6	7.0	20.7	58.7
%Pool	47.7	35.7	45.8	64.3
Gradient	37.0	19.3	11.8	14.4

One approach towards more directly assessing how the IBI metrics change between the reference stream sites is to substitute raw metric values for species in TWINSPAN. The groupings created by the divisions would then be generated by real changes in metric values. **Table 15** shows a TWINSPAN on metrics and sites. The output table shows the primary division being made which separates higher richness sites from lower richness sites. The group to the left of the division contains mostly sites with five or more species, with the group to the right: mostly sites with less than five species. The MWIBI is currently applied to sites with five or more species, whereas the CWIBI is applied to sites with 2-4 species. The group on the right was further divided between sites with 2-4 species and sites with just one species (brook trout). This division corresponds directly to the existing criterion which separates sites evaluated by the CWIBI and sites where no IBI can be applied. This TWINSPAN then, essentially validates the current method of assigning one of the two IBI's, or neither, to site data.

Table 16 shows mean physico-chemical variables of the three categories of reference sites. The gradients in drainage area, pH-alkalinity, gradient and cold to warmwater illustrated by the species-based TWINSPAN output table are also depicted in the metric-based TWINSPAN output table. Mean site elevations between the categories show the same trend, but to a lesser extent. The group of MWIBI sites included a wide range of stream sizes and included both warm and coldwater assemblages. The resultant variation in raw metric values between these sites was addressed by examining 2 and 3-dimensional scatter plots of metric values by site elevation and drainage area. Where raw metric scores exhibited clear gradients in the scatter plots, lines were drawn which delineated groups of sites by elevation and/or drainage area. Metric expectations (criteria) were then developed for each elevation/site size group according to the plotted values for a particular metric within that group.

Table 15. A TWINSPAN ordination table showing a reference stream grouping based on raw MWIBI metric scores rather than fish species. The primary and one of the secondary divisions indicate the need for changes in metric scoring or use of different IBI's. The choice of applicable IBI is made by determining site species richness: note that where Richness=1 (one species) no IBI can be applied. The CW IBI is used with a site richness of 2- 4 native species (a "2" on the richness row in this table). For sites with greater than 4 species ("3" and "4" in the table) the MWIBI is used.

```
                           MWIBI                            CWIBI           No IBI
Metrics   Sites

          455623345555346222334554461223334444556  2233266 112      1111111776766767
          15858783067998203601434054979236267912027454521350511346891234678346089271
DELT      -----1---1----------------------------------------------------------------   11

CCWS      33554343355555344344244---33-2-1212-2-233-322--1--------------------------  101
GENFEED   555555555555554454444545543223323222233332323111-1----------2-------------  100

Ben.Ins.  ----2222211222222211222222222222212222221122222222221 1-1111111--1--------- 0111
Insect    53555555545555555555555555555555555555555555555555555555555555555--------- 0110
Richness  43324443344433334333344334323343333333332333333322222222222222222111111111 0110
Density   55545555555454444355444545335355555444455554554535555352453355454455333 12  010

Intolera  -----11111-11112121112222212222222122212122222222222222221222222211211111111  001

Top Carn  2---22-1-12243455554545545-32341313244155555522545545455455555555555555555  000

          00000000000000000000000000000000000000000000011111111111111111111111111111
          00000000000000011111111111111111111111111111100000000000000000000111111111
          00000000000011100000000000111111111111111111100011111111111111111000011111
          00001111111100100000000111000000000000011111100100000111111111111001100011
```

Table 16. Mean and range ( ) of physico-chemical variables for the three IBI- related stream groups for reference stream sites.

	Mixed Waters IBI (n=45)	Cold Water IBI (n=20)	No IBI, brook trout only sites (n=9)
Elevation (Feet)	655 (102-1880)	983 (416-2116)	1360 (930-2162)
Drainage (km^2)	68.8 (2-298)	13.4 (2-33)	8.9 (3-17)
Alkalinity(mg/l)	65.8 (3-227)	34.5 (2-96)	10.3 (1-27)
pH	7.6 (5.9-8.9)	7.3 (6.4-8.1)	6.5 (6.6-8.2)
% Fines	21.1 (0-100)	9.7 (0-30)	5.9 (0-20)
% Pool	43.6 (10-95)	42.8 (15-65)	43.6 (25-75)
Gradient[1]	12.1 (1-70)	31.8 (6-80)	36.1 (8-61)

[1] Measured as the drop in elevation from the sample location to a point 1,000 ft downstream.

The IBI's

The MWIBI has been applied in modified forms since 1987. It is used to evaluate assemblages from both warm and cold waters. Since the MWIBI has been used in various forms for 14 years - having "withstood the test of time" - a detailed examination of it's component metrics will not be presented here (see Langdon 1989 for an introduction of its use in Vermont). A summary of metric effectiveness, sensitivity to impact and redundancy is provided below, however. Since the CWIBI was developed from the present effort, a more complete discussion of that index will be presented.

Coldwater sites naturally containing only a single species (nearly always brook trout) are not, at this time, deemed suitable for application of any conventional IBI. The information content in a single-species assemblage is insufficient to construct a meaningful IBI which could be broadly applied across the state. Any attempts at evaluating the health of a brook trout-only assemblage is currently limited to comparing population estimates and age structure with historical on-stream data, or from a adjacent stream surrogate.

The CWIBI was developed to address the void created by the minimum species requirement if the MWIBI. The MWIBI was deemed ineffective at determining impact when applied to assemblage data with less than five species. Vermont streams evaluated by the CWIBI have higher elevation and gradient, smaller upstream drainage area and lower alkalinity than sites addressed by the MWIBI. The CWIBI addresses the need to assess stream fish assemblages containing two to four species. Such an index needs to be particularly responsive to non-point discharges and general physical habitat degradation such as sedimentation and the removal of riparian vegetation. The low-richness fish assemblages of these small coldwater streams have less biological information content and therefore may most appropriately be assessed with a reduced-metric IBI (after Lyons et al. 1996). While both IBI's for wadeable Vermont streams could be considered reduced metric versions when compared to the traditional 12 metric form, the six-metric CWIBI has only half the original metric number.

The MWIBI: The MWIBI is presented in **Table 17**. The ability of the MWIBI metrics to identify impact was validated using a Mann-Whitney rank sum test. Raw metric values from reference and impacted sites which naturally supported five or more species were included. Values of six of the nine current MWIBI metrics tested differed significantly ($p<0.001$) between reference and impacted sites (**Table 18**). Differences in raw values of the abundance metric were barely significant between reference and impacted sites ($p=0.051$, Mann-Whitney rank sum test). Densities were *higher* at impacted sites, which is just the opposite of the conventional metric assumption (Karr et al. 1986). This observation implies that the dominant impacts were characterized primarily by nutrient additions, resulting in increased productivity. This does seem to be the case with this groups of impacted stream sites, as most were moderately enriched from urban and agricultural influences. Additionally, far fewer sites suffered from toxic and low-flow impacts which would normally result in reduced abundance. Karr et al. (1986) found that this metric was not sensitive to milder impacts, responding only to the more severe perturbations. If enough impacted sites in the project data set had been severely impacted then, the abundance values would probably have been lower for this group. Since this metric is frequently used in IBI's (Mundahl and Simon 1999) and has been shown to respond to severe impacts (Karr et al. 1986), it will be retained in the MWIBI.

Species richness values from reference and impact sites were not significantly different. **Figure 7** shows that, in general, species richness increases with drainage area. As a result then, the effect of streams size would tend to mask differences from human disturbance, since all sites were included in the test. This metric has been noted to be sensitive to a wide range of impact. Karr et al.(1986) noted for warmwater populations, decreasing richness with increasing levels of disturbance, while the opposite noted from cold water streams (Lyon's et al. 1996; Mundahl and Simon 1999). It is suspected, however that for

Vermont streams, this metric may respond similarly to the density metric, in that it may be sensitive only to moderate to severe impact. This may be in part due to the naturally high proportion of tolerant species resident in New England rivers. Since fish assemblages of the Northeast are post-glacial, they tend to be relatively species-poor (Schmidt 1986). The species, which *were* able to re-colonize this area from sometimes distant refugia under harsh conditions, would necessarily have to exhibit a certain hardiness towards environmental perturbation. Less robust species may have not been suitably adapted to the rigors of migration and either never entered New England or if so, failed to persist. Consequently, the assemblages we find today may be more resistant to certain human-induced impacts, which would otherwise cause more observable reductions in species richness in waters supporting more diverse assemblages. Additionally the reported *increase* in species with increasing degradation reported from cold water streams (Lyons et al. 1996) may not take place as often in Vermont coldwater streams. Lyons et al. reported that the Wisconsin coldwater streams from that study were of low gradient, allowing coolwater and warmwater species to invade impacted sections. Much of the Vermont landscape is of high relief and many of its streams exhibit moderate to high gradient. In these streams, barriers to upstream migration are more likely to be found. Lyon’s et al (1996) similarly theorized that in high gradient areas, increasing impact would not result in increasing species richness and cites the study of Leonard and Orth (1986) on coolwater streams in West Virginia as an example. Since however, the species richness metric has registered a decline in a few cases of severe toxic impact in Vermont, it seems reasonable that it should be retained in the MWIBI.

The third metric that was not significantly different between reference and impacted sites was proportion of DELT (p=0.122, Mann-Whitney rank sum test). The impacted sites data-set contained few sites with potential for containing toxic contaminants, which are primarily responsible for producing DELT anomalies. Several investigators have observed increased levels of DELT anomalies have been associated with point source discharges of industrial or municipal origin (cited from Sanders et al. 1999). The fact that this metric was de-scored on only 3 of 38 impacted Vermont sites is seen more as an indicator of a lack of significant point discharges in Vermont than as a reason to remove it because of any inherent lack of sensitivity to disturbance.

Table 17. Mixed Waters Index of Biotic Integrity (MWIBI) for The Fish communities of Wadeable Vermont Streams

	For streams naturally supporting more than four native species		**Scoring Criteria**		
			5	**3**	**1**
	Species Richness and Composition				
1	Total number of native fish species		Follows maximum species richness lines (Appendix 12)		
2	Number and identity of native, intolerant species (*A non-native trout may be substituted when brook trout are absent*)	[Site Elevation >400 ft.]-	>1	1	0
		[Site Elevation <400 ft.]-	>0	-	0
3	Number and identity of native benthic insectivore species	[Site Elevation <400 ft. with site drainage <25 km^2]	1	-	0
		All other sites	>1	1	0
4	Proportion of individuals as white suckers and creek chubs		<11%	11-30%	>30%
	Trophic Composition				
5	Proportion of individuals as generalist feeders	[Site Elevation >500 ft.]-	<20%	20-45%	>45%
		[Site Elevation <500 ft.]-	<30%	30-60%	>60%
6	Proportion of individuals as water column and benthic insectivores *(score a "1" if blacknose dace is >60% of assemblage)*	[Site Elevation >500 ft.]-	>65%	30-65%	<30%
		[Site Elevation <500 ft.]-	>55%	20-55%	<20%
7	Proportion of individuals as top carnivores (*Nonnative trouts included)*	[cold water assemblage] -	>15%	5-15%	<5%
		[warm water assemblage with site drainage >25 km^2.] -	>10%	3-10%	<3%
		[warm water assemblage with site drainage <25 km^2.] -	0	-	-
	Fish Abundance and Condition				
8.	Proportion of individuals with Deformaties,. fin erosion, lesions or tumors		<1%	1-4%	>4%
9.	Abundance in Sample (one pass #100m^2) *(Nonnative species included)*	[Site Elevation <500 ft].	>20	10-20	<10*
		[Site Elevation >500 ft].			
		[Alk. >9 mg/l]	>10	7-10	<7*
		[Alk. <9 mg/l]	>6	3-6	<3*

*site scores "poor"

1 All sites within the Connecticut River drainage are to be scored as > 500 elevation

Index Scores

Index Scores	
Excellent	41-45
Very good	37
Good	33
Fair	25-27
Poor	<25

Conditions for Use

1. For wadeable streams only.
2. Site should naturally support at least five native species.
3. Only individuals more than 25mm TL are to be entered into the determination.
4. Only species with more than one individual captured are entered in metrics 2 and 3.
5. Stocked fish are not considered in determinations.

Table 18 Metric value medians and *means* for reference and impacted sites. Metric 1, species richness is clearly influenced by stream size as well as perturbation and must be scored using a MSRL which appears in **Appendix 12.**

MWIBI Metric	Reference Sites n=42	Impacted Sites n=30	Significantly[1] Different ?
1. Total Number of Native Species	7.0 *7.7*	7.5 *7.9*	N: p= 0.383
2. Number of Intolerant Species	2.0 *1.4*	0.0 *0.2*	Y: p<0.001
3. Number of Benthic Insectivore Species	2.0 *2.3*	1.0 *1.2*	Y: p<0.001
4. Percent as White Suckers and Creek Chub	5.0 *9.1*	31.0 *34.9*	Y: p<0.001
5. Percent as Generalist Feeders	10.0 *22.3*	61.4 *56.5*	Y: p<0.001
6. Percent of Insectivores	62.0 *64.1*	38.4 *41.2*	Y: p<0.001
7. Percent as Top Carnivores	6.0 *13.9*	0.0 *1.4*	Y: p<0.001
8. Percent with DELT Anomalies	0.0 *<0.1*	0.0 *1.0*	N: p=0.122
9. Abundance	23.0 *56.1*	97.0 *110.0*	Y: p=0.051
MWIBI	40.0 *38.3*	29.0 *28.0*	Y: p<0.001

1. Results of a Mann-Whitney-rank sum test.

The range of sensitivity to human disturbance was determined for all metrics by plotting each metric value against the MWIBI for all sites (**Figure 7)**. The MWIBI score was used as a measure of disturbance**.** Maximum sensitivity of a metric is indicated by the area on the plot where the slope of the line is the greatest (Angermeier and Karr 1986). Percent insectivores, number of intolerant species and number of benthic insectivores appear to be sensitive to moderate to the more severe impacts only. The creek chub-white sucker metric appears to respond to all levels of impact, while percent top carnivores and percent generalist feeders seem to be responsive only to mild to moderate impacts. There were no apparent trends in density and % DELT anomalies. The later two metrics have been observed to be sensitive only to moderate to severe impacts (Karr et al. 1986). Since the Vermont data set has relatively few of severely impacted sites, the failure of any clear tendencies in the plots used to determine sensitivity range should be expected. The remaining metrics appear to cover the range of apparent degradation with most being more sensitive to moderate to the more severe impacts (**Figure 8**) Karr et al. (1986) stated that when the sensitivities of component metrics taken in combination, span the expected range of perturbations, the result is a more accurate and effective index.

Figure 7. Raw metric values for each of nine metrics from the MWIBI from combined reference and impacted sites plotted against MWIBI values. These plots were used to identify primary sensitivity ranges of each MWIBI metric.

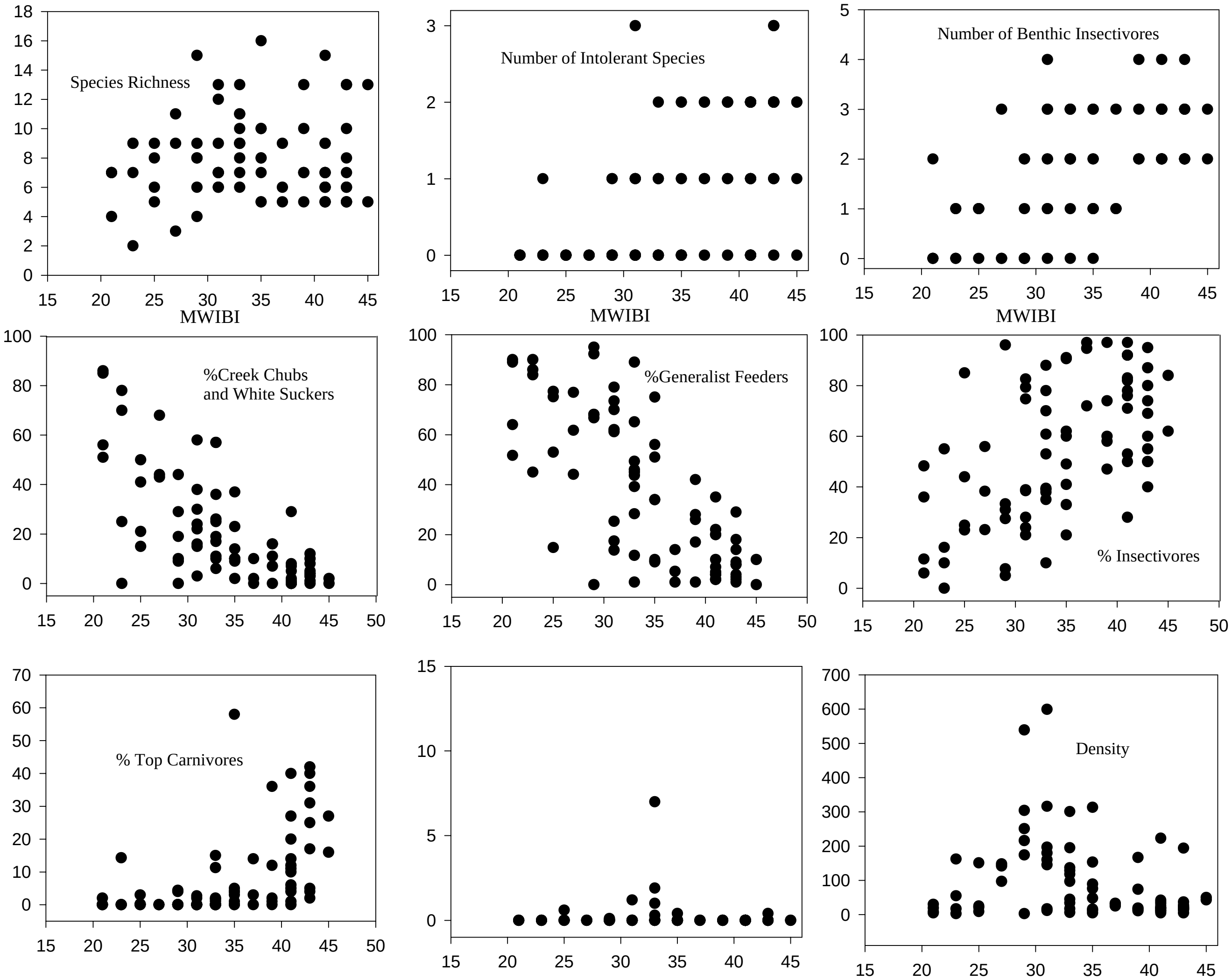


Figure 8. Relative metric sensitivity as determined by a graphic evaluation for the MWIBI.

Metric redundancy was examined by measuring the correlation (Spearman r) between the nine metrics from reference stream sites (**Table 19**) . Retention of redundant metrics is discouraged as they would contribute little to the sensitivity of the index (Karr et al. 1986). Mundahl and Simon (1999) used a criterion of r = 0.80 or more to identify sets of metrics which were redundant. In the current data set only one contrast between metrics gave a Spearman r of 0.80 or more : percent generalist feeders and percent insectivores. Since these metrics represent two of three trophic groups present in Vermont streams, it is not surprising that they exhibit a high degree of correlation. A plot of the two metrics showed a linear but asymmetrical relationship. While a one-to-one relationship was evident over a short area in the range, the rest of the points were broadly scattered. Because of this and in the interest of conforming to the IBI convention of providing metrics that account for all dominant trophic levels, it was decided that both metrics would be included.

The precision of any IBI is difficult to accurately describe since it would involve re-sampling the same section within a short time period. Re-sampling the same section may not result in as similar a sample because of the disturbance to the assemblage caused by the electroshocking. Waiting long enough for the population to stabilize, following an electrofishing pass, may allow for seasonal changes to take place, causing shifts in composition and density. As a result, any measured variation in scores would be in part due to sampling or seasonally-induced changes in assemblage composition. The closest approximation (although probably an over-estimate) of this variation is that from annual variation exhibited from a fixed site. MWIBI data is available from the same station between years for four reference locations (two warm and two coldwater sites) and is shown in **Table 20.** The MWIBI varied not more than 4 points at these four sites for the time periods listed. From this it may be concluded that index variation within the same year and season would probably be less than 4 points. The 95% confidence intervals for the three sites with enough data to analyze were ±1.1, ±1.6 and ± 1.8.

The MWIBI and CWIBI may be applied to wadeable moderate to high gradient warm and coldwater streams in Vermont. They should not be applied to low-gradient, sand-bottomed streams or very small warmwater streams supporting less than five native species.

Table 19. A Spearman rank order correlation matrix of correlation coefficients for MWIBI metrics for reference stream sites.

n=33	No. Intol. Spp.	No. of Benth. Ins. Spp.	C.chub-W.suck.	% Gen. Feeders	% Insect.	% Top Carn.	%DELT	Abun.
No. of Native Species	-0.49	0.23	0.48	0.70	-0.44	-0.24	0.28	0.37
No. Intolerant Species		0.24	-0.57	-0.72	0.48	-0.40	-0.18	-0.49
No. of Benthic Insectivore Spp			-0.20	-0.18	0.33	0.01	0.04	-0.40
% C. chub plus W.suckers				0.74	-0.66	-0.08	0.18	-0.12
% Generalist Feeders					-0.80	-0.27	0.26	0.35
% Insectivores						-0.21	-0.16	-0.17
% Top Carnivores							-0.23	-0.47
% DELT								0.33

Table 20. Temporal variation in the MWIBI and its nine component metrics at four sites.

Location	Year	MWIBI	Location	Year	MWIBI
Allen Brook Mean=39.4 (95% C.I.= ±1.1)	1987	39	Lewis Creek 3.7	1989	43
	1989	39		1990	45
	1990	39		1991	43
	1991	41		1992	45
	1992	39		1994	41
Browns River 0.4 Mean = 39.8 (95% C.I.= ±1.6)	1991	39	Winhall River	1993	41
	1993	37		1994	41
	1994	41		1995	41
	1995	41			
	1996	41			
Browns River 17.2 Mean = 42.6 (95% C.I.= ±1.8)	1991	45			
	1992	41			
	1993	43			
	1994	41			
	1995	43			
	1997	41			

The CWIBI: The stream sites targeted for this index are structurally simple. Minimally impacted sites are generally dominated by a top carnivore - brook trout and a benthic insectivore - slimy sculpin. These two species are classified as intolerant coldwater stenotherms (Halliwell et al. 1999). Species also present in lower numbers include the top carnivores, brown and rainbow trout and the insectivores, blacknose dace and longnose sucker. With increasing site disturbance, trout and sculpin are progressively replaced by blacknose dace, followed in importance by the generalists, creek chub and white sucker. All of the later three species are considered tolerant of impacted conditions (Halliwell et al. 1999).

The assemblages from the 18 reference stream sites were dominated by salmonids (mostly brook trout) and by slimy sculpin. Numbers of blacknose dace, creek chub and white suckers were generally minor components at these locations. Alternatively, the 11 impacted sites supported fewer brook trout and other salmonids. Blacknose dace became the dominant species at these sites with concurrent increases in creek chubs and white suckers as well.

Fourteen possible metrics were tested for use in the CWIBI. Eleven of the fourteen candidate metrics significantly differed between reference sites and impacted sites ($P<0.05$, Mann-Whitney rank sum test - **Table 21)** . Most of the comparisons were highly significant ($p< 0.001$). Metrics which were not significantly different between the two groups were judged to be of inadequate sensitivity to impacted conditions and were rejected.

Table 21. Metric value means for the CWIBI. All differences were significantly different (p<.001, Mann- Whitney rank sum test). The six-metric CWIBI scores were multiplied by 1.5 to correspond with the nine-metric MWIBI.

(Number of Sites)	Reference Sites 18	Impacted Sites 11
Number of Intolerant Species	1.8	0.8
% Coldwater Species	83	23
% Generalist Feeders	1	22
% Top Carnivores	49	21
Brook Trout Density (1 run/100m2)	12	0.4
Brook Trout Length Class Number	2.9	0.8
VT Coldwater IBI	41.1	14.4

Metric redundancy was examined by correlating the remaining 11 metrics from reference streams using the Spearman r. Since fish assemblages of these small coldwater streams are fairly simple and repetitive, significant correlations were expected between some of the candidate metrics. Strong (>0.80;) Spearman correlations were found between the per cent tolerant and per cent intolerant species metrics, and with each of these metrics to the per cent coldwater species. The per cent coldwater species metric was selected over the percent intolerant and percent tolerant because it included the longnose sucker as a positive scoring influence in the determination. This species is considered intermediate in tolerance and thereby would not figure in the index scoring in the two tolerance-related metrics. This species is a cold water stenotherm (Halliwell et al. 1999) and should be a good indicator species of impacts which significantly increase water temperature in coldwater streams. The metrics selected for use in the CWIBI showed no Spearman r's above 0.65 (**Table 22**).

Table 22. A Spearman rank order correlation matrix of correlation coefficients for CWIBI metrics for reference stream sites.

n=18	% Coldwater	% Generalist	% Top	Brook Trout	BKT age
No. of Intol. Species	0.53	0.00	-0.16	-0.21	0.12
% Coldwater Species		-0.65	0.38	0.13	0.16
% Generalist Feeders			-0.42	-0.05	-0.05
%Top Carnivores				0.29	0.20
Brook Trout Density					0.50

Although the per cent generalist feeder metric did not discriminate as sharply (p=0.032) as did most metrics between reference and impacted sites, it was selected for use because it provided the needed measure of tolerant species presence without being highly correlated with the per cent coldwater species metric. Three additional metrics were rejected for varying reasons which resulted in the retention of six metrics to compose the final CWIBI. The metrics which were not selected are presented in **Table 23** along with the reasons for their elimination. The proposed coldwater CWIBI is shown in **Table 24**. Three of the six CWIBI metrics were taken from a IBI for Wisconsin coldwater streams (Lyons et al.1996). The percent generalist feeder metric was taken from the Vermont MWIBI (with re-set criteria). The final two metrics involving brook trout were developed specifically for this index. The metrics included on the CWIBI are briefly discussed below. **Figure 9** shows plots of the CWIBI plotted against each of the six composite metrics. As with the MWIBI, the analysis of the resulting slopes provides an indication of where, in the range of minor to extreme degradation, each metric showed the greatest sensitivity.

Figure 9. Raw metric values from combined reference and impacted sites plotted against CWIBI values. These plots were used to identify primary sensitivity ranges of each CWIBI metric.

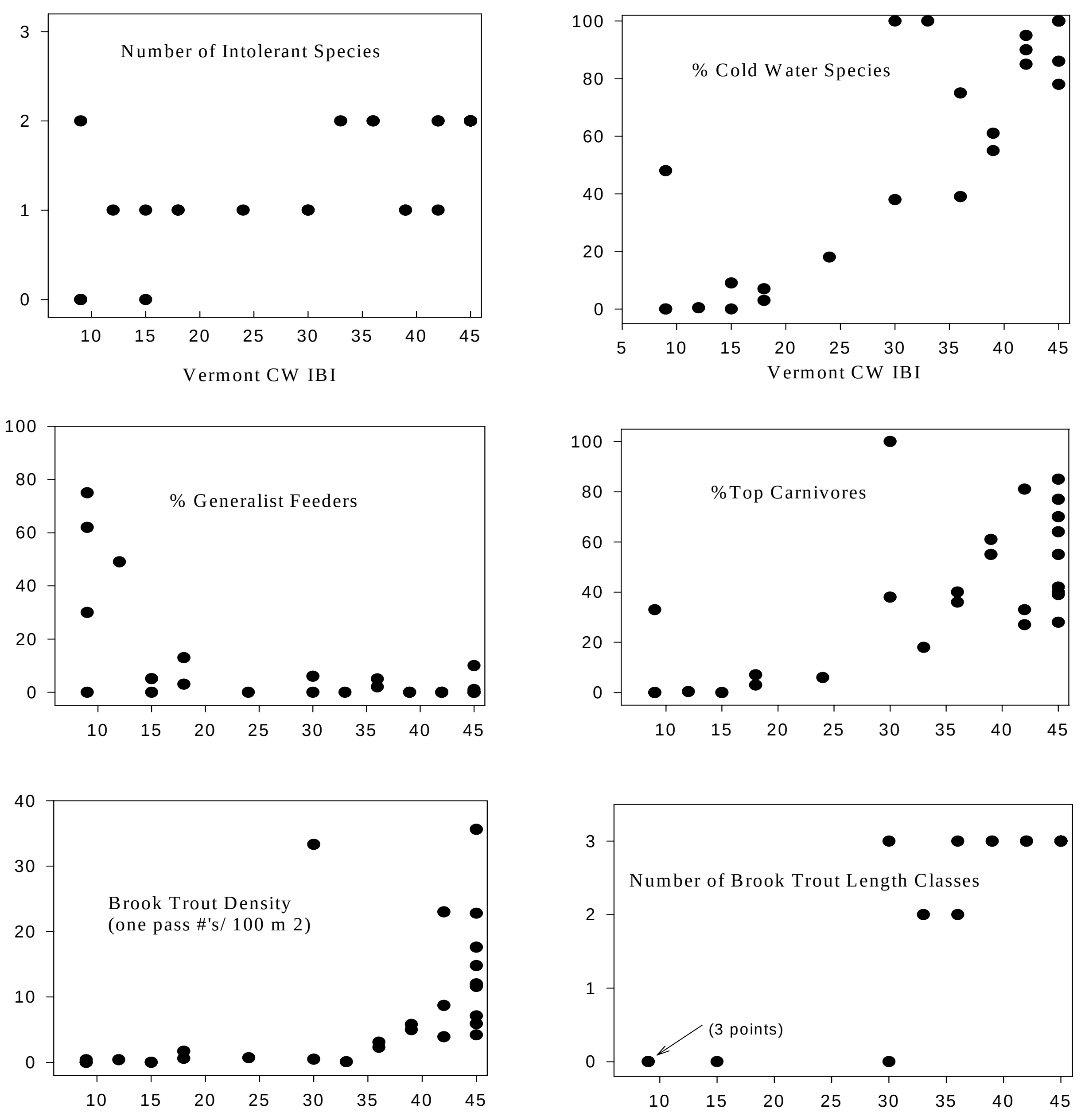


Table 23. Candidate metrics which showed significant ($p<0.05$, Mann-Whitney rank sum test) differences between reference and impacted sites but were rejected for other reasons

Metric Not Used	Reason
% Blacknose Dace	Did not sufficiently distinguish between reference and impacted sites
Total Density	Did not sufficiently distinguish between reference and impacted sites
% Benthic Insectivore % Slimy Sculpin	Both metrics were correlated with each other at r= 0.98 (Spearman). About 33 % of reference and slightly impacted sites do not naturally support benthic insectivores, which include slimy sculpin. A chance would exist, then, to de-score this metric at a site for a reason that is unrelated to habitat quality if one of these metrics were to be incorporated into the index..
% Intolerant Species % Tolerant Species	% Intolerant species and % tolerant species correlated with each other at r=0.89 and with % coldwater species at r = 0.99 and 0.90 respectively. Coldwater species was judged stronger because it included longnose sucker in the determination, whereas % intolerant species did not.
% Brook trout of total salmonids	Did not sufficiently distinguish between reference and impacted sites
% Brook Trout	Using *three* brook trout metrics appeared to overly weigh the six-metric index towards that species. Brook trout density should be a slightly better overall metric since it will be more sensitive to toxic impacts. Such impacts tend to drastically decrease fish density, sometimes without changing proportional species composition.

Table 24. An Index of Biotic Integrity for Small Vermont Coldwater Streams (CWIBI).

For coldwater streams naturally supporting from two to four native species	**7.5**	**4.5**	**3**
1. Number of intolerant species (one exotic trout species may be substituted for brook trout)	2	1	0
2. Proportion of individuals as coldwater stenotherms	> 75%	50-75%	< 50%
3. Proportion of individuals as generalist feeders	< 5%	5-9%	> 9%
4. Proportion of individuals as top carnivores	> 35%	25-35%	< 25%
5. Brook trout density (#s/100 m^2-1 pass)	>4.0	2.0-4.0	<2.0
6. Brook trout age class structure (young-of-the-year = < 100mm, adult=>100mm	yoy and adults present	yoy only	yoy absent

Metric Scores

Excellent	42-45
Very Good	36
Good	33
Fair	27
Poor	<27

Conditions for Use

1. Only fishes over 25 mm in length should be considered
2. Only naturally reproducing salmonids are to be considered
3. Only species represented by more than a single individual will be entered into metrics 1 and 6

Metric Descriptions

Metric 1: *Number of intolerant species*
Intolerant species are expected to be the first species to become reduced or eliminated following degradation (Karr et al. 1986). This metric, derived from the coldwater IBI of Lyon's et al.(1996), reflects the presence of slimy sculpin and brook trout. In the absence of brook trout, one of the two exotic trouts (brown or rainbow) could be substituted as an intolerant species. This metric appeared to be most sensitive to moderate to severe impacts (**Figure 10**)

Metric 2: *Proportion of individuals as coldwater stenotherms*
Coldwater species generally decline with increasing degradation (Mundahl and Simon 1998). Degradation of small Vermont streams is often characterized by the removal of riparian vegetation which may raise summer water temperatures beyond the point tolerated by the resident coldwater species. In small Vermont streams, coldwater stenotherms are represented by brook trout, slimy sculpin, brown and rainbow trout and longnose sucker. Lyons et al. (1996) included this metric in their coldwater IBI for small coldwater streams. Mundahl and Simon (1999) noted that this metric was one of the strongest discriminators of degradation in coldwater streams. Sensitivity of this metric was greatest to mild to moderate levels of impact.

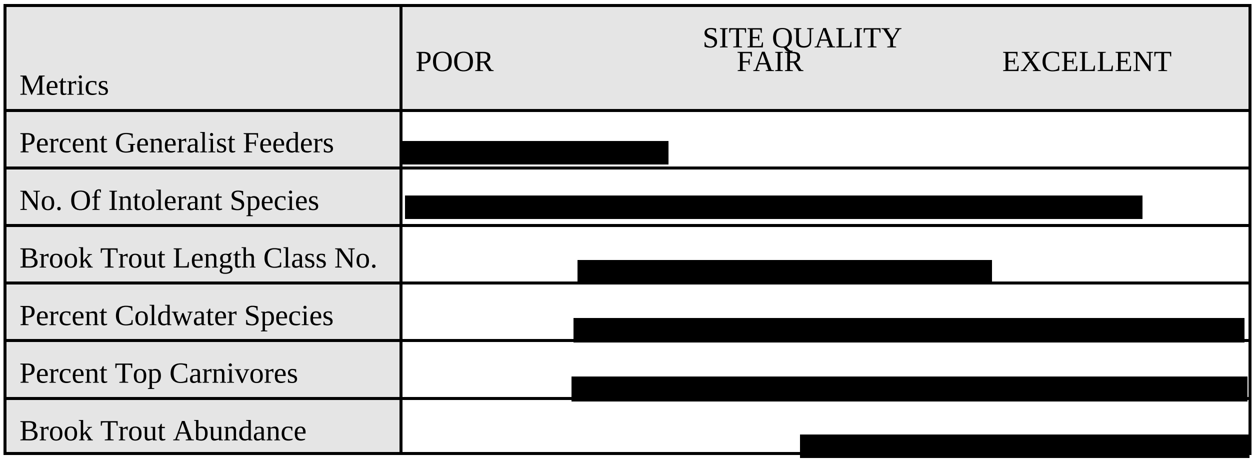
Metric 3: *Proportion of individuals as generalist feeders*
This metric was taken from the Vermont MWIBI with modified scoring criteria. The expectation is that

the proportion of generalist feeders increases as stream conditions degrade. This metric clearly discriminated (p<.001 Mann-Whitney rank sum test) between 36 impacted and 47 reference sites in a data set of larger cold and warm water streams from Vermont (VTDEC manuscript in preparation). Generalist feeders are more adaptable than are specialists to varying food supplies, which is often a characteristic in impacted streams (Fausch et al.1990). This metric was most sensitive to moderate to severe impacts.

Metric 4: *Proportion of individuals as top carnivores*
This is the third of Lyon's et al.(1996) metrics which is included in the CWIBI. Karr (1981) noted that numbers of top carnivores decrease with declining stream health. Top carnivores in small coldwater Vermont streams are represented by brook, brown and rainbow trout. This metric showed the greatest sensitivity to degradation which was judged to be mild to moderate.

Metrics 5 and 6: *Brook trout density and age class structure*
Two brook trout metrics were included in this six metric index to purposely weigh the index towards this important species. Occupying the top of food web, this intolerant, keystone species has been cited as a strong indicator of habitat and water quality (Steedman 1988; Lyons et al. 1996). The brook trout is the most common species in small coldwater Vermont streams, existing as the dominant native top carnivore. This highly revered species can be indicative of a near-pristine biological condition. Fausch et al. (1990) noted that in species-poor areas such as coldwater streams, that measures of abundance and reproductive success may be appropriate parameters for consideration in assessing the health of the fish community. Metrics 5 and 6 address these attributes for the most prevalent and recreationally important species found in these small coldwater streams. The expectation of metric five is that as stream degradation increases, the density of brook trout will decrease. With increasing degradation, metric six will show fewer age classes. Of particular importance is the reduction or loss of young-of-the-year fish, indicating a possible failure of that species to successfully reproduce. Brook trout abundance is more sensitive to mild to moderate levels of perturbation, while the age class metric is most sensitive to moderate degrees of impact.

Figure 10. Relative metric sensitivity as determined by a graphic evaluation for the VT CWIBI.



Applicability of the CWIBI : The dominance of salmonid and sculpin species at un-impacted Vermont stream sites is consistent with observations made elsewhere (Lyons et al. 1996; Maret 1998). Numbers of these species declined in Vermont streams with increasing stream impact. The reduction or loss of these species has also been observed with increasing degradation of coldwater streams (Steedman 1988; Lyons et al. 1996; Maret et al.1997; Mundahl and Simon 1999).

The CWIBI was designed to be a measure of water and habitat quality as well as an indicator of natural biodiversity. To that extent, non-native salmonids were included as positive influences on index scoring. The additional assemblage information gained from the inclusion of brown and rainbow trout facilitates the assessment of water and habitat quality. Considering these non-native species as a strictly negative influence on the CWIBI would be to overlook their real utility as indicators of environmental quality (Moyle and Marchetti 1999). They are classified as coldwater species, intolerant species and top carnivores (from Halliwell et al.1999). Since however, they are non-native, their presence is not considered, in the context of the CWIBI, as equal to that of brook trout. If at a particular site, numbers of brown and or rainbow trout are great enough to significantly suppress native brook trout (through assumed competition) the index will respond in metrics 5 and 6 by de-scoring the final index value. The presence of low numbers of the exotic trouts however, should not lead to a lower index score.

The CWIBI represents a reduced metric version of the original IBI paradigm. The application of an IBI with fewer than the customary 12 metrics has been cautioned against due to a possible loss of sensitivity to the range of possible impact (Miller et al. 1986). The use of fewer metrics, however reflects a decreased amount of information content in these simple, low richness assemblages (Simon and Lyons 1995). Reduced metric IBI's are commonly used to evaluate coldwater streams characterized by low species richness (i.e. Leonard and Orth 1986; Steedman 1988; Lyon's et al. 1996; Halliwell et al.1999). It is believed that while additional metrics may be potentially used in the CWIBI, the current version provides a workable tool in evaluating an here-to-fore un-quantified measure of biological integrity in small Vermont trout streams. All six component metrics have demonstrated sensitivity to impact. Taken in composite, the metrics appear to span the range of stream impact from severe (or as severe as we have measured) to mild. This characteristic has been cited as requisite for a accurate biological index (Karr et al. 1986).

The CWIBI is similar to the Wisconsin coldwater IBI of Lyon's et al. (1996). The Wisconsin metrics: number of intolerant species, per cent top carnivores and the percent coldwater species were used in the CWIBI with slightly re-scored criteria. The percent generalist feeders metric and the two brook trout metrics were substituted for per cent tolerant species and per cent salmonids as brook trout respectively. The two brook trout metrics are believed to incorporate additional information into the index in contrast to the use of the single metric, per cent salmonids as brook trout.

The current data set includes only two classes of streams - impacted and reference. Separating the impacted sites into two levels of impact, would provide additional information on how the CWIBI responds to a gradation of impacts. Too little temporal replication exists to evaluate annual variation in the index. Subsequent collections will add to the eventual analysis of this attribute. The Proposed CWIBI appears to effectively and consistently identify impacted conditions. This index will be validated with independent data which was not used in its development. An effort will be made in future work to assess as great a variety of impacts as possible.

Application of both IBI's: The MWIBI and CW IBI may be applied to wadeable moderate to high gradient warm and coldwater streams in Vermont. They should not be used to assess low-gradient, sand-bottomed streams or very small warmwater streams supporting less than five native species. These two stream types appear to be dominated by tolerant generalist feeders, regardless of the level of human impact present. Since the IBI model is sensitive to trophic level proportions and numbers of tolerant-intolerant species, even minimally impacted low gradient and small warmwater sites often generate low IBI scores. Subsequently, as the DEC database includes an increasing number of these sites, perhaps an additional evaluative tool (within or outside the current IBI model) may be developed to address these stream types.

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Appendix 1. A list of all reference streams used to calibrate fish and macroinvertebrate biological criteria.

Location	Community	Drainage km ^{2}	Elevation ft.	Town
Ball Mtn.Brk.-trib14	Fish	3.6	1631	Jamaica
Bean Brook	Fish	11	1620	Newark
Blodgett Brook	Fish	10.4	1100	Lemington
Browns River	Fish	225	351	Fairfax
Browns River	Fish	47	761	Underhill
Brush Brook	Fish	11	1161	Huntington
Cheney Brook	Fish	9.7	1880	Dover
Clarendon River	Fish	67.5	581	Clarendon
Clark Brook	Fish	6.5	1339	Granville
Clough Brook	Fish	24.4	1001	Bloomfield
Coggman Creek	Fish	26	130	West Haven
Dog River	Fish	84.9	750	Northfield
Ellis Brook	Fish	7.07	1831	Dover
Elmore Br.-Lamoille	Fish	39.4	783	Elmore
Green River	Fish	48.3	807	Guilford
Halnon Brook	Fish	8	416	Salisbury
Hancock Branch	Fish	21	1263	Hancock
Hancock Brook	Fish	16.8	1280	Worcester
Herrick Brook	Fish	13	1089	Middlesex
Jay Branch-Trib7	Fish	6.7	1397	Jay
Jones Brook	Fish	23	570	Middlesex
Lamoille River	Fish	55	1164	Greensboro
Lemon Fair River	Fish	24.6	298	Orwell
Lewis Creek	Fish	165.6	98	Ferrisburgh
Lewis Creek	Fish	159.8	108	Ferrisburgh
Lewis Creek	Fish	69.4	335	Hinesburg
Lily Brook	Fish	3.2	930	Troy
Little Otter Creek	Fish	2	450	Bristol
Mill Brook	Fish	91	669	Windsor
Mill River	Fish	162	600	Clarendon
Minister Brook	Fish	19.6	935	Worcester
Moose River	Fish	66.8	1159	Victory
Moose River	Fish	53.8	1319	Granby
Bourn Brook	Fish	11.1	2162	Winhall
N.Fork East Creek	Fish	35	190	Orwell
Negus Brook	Fish	4.3	1760	Dover
Neshobe River	Fish	54.5	359	Brandon
Otter Creek	Fish	298	568	Wallingford
S.Fork East Creek	Fish	2	400	Benson
Stickney Brook	Fish	8.3	872	Dummerston
Teney Brook	Fish	6	718	Rutland City
Thorpe Brook	Fish	7.5	102	Charlotte
Trout Brook	Fish	11	115	Milton
Trout Brook-trib	Fish	2.1	118	Milton
W.Br.Ompompanoosuc R.	Fish	98.5	830	Strafford
White River	Fish	14.3	1339	Granville
Youngman Brook	Fish	3.6	140	Swanton

Allen Brook	Both	9.5	160	Colchester
Allen Brook	Both	10.1	518	Williston
Bailey Brook	Both	5.9	1078	Hardwick
Berry Brook	Both	6	532	Richford
Bourn Brook	Both	18.26	900	Manchester
Castleton River	Both	128	401	Castleton
Cobb Brook	Both	12.4	1319	Jamaica
E.Br.North River	Both	103.3	880	Halifax
E.Br.Nulhegan River	Both	90	955	Bloomfield
East Putney Brook	Both	41.7	290	Putney
Ely Brook	Both	11.3	970	Vershire
Dowsville Brook	Both	8.1	1255	Duxbury
E.Brch.Passumpsic R.	Both	72.4	970	East Haven
N.Brch.Deerfield R.	Both	4.6	1939	Dover
Hubbardton River	Both	115	160	West Haven
Hubbardton River	Both	44	255	Hubbardton
Keyer Brook	Both	29.4	1110	Canaan
Lee River	Both	30	583	Jericho
Lewis Creek	Both	55.4	575	Starksboro
Lye Brook	Both	19.09	840	Manchester
N.Br.Lamoille River	Both	50	1050	Belvidere
Saxtons River	Both	62	495	Rockingham
Stevens Branch Trb23	Both	9	774	Williamstown
Tyler Branch	Both	155.5	407	Enosburgh
Waits River	Both	123.1	475	Bradford
Williams River	Both	222	308	Rockingham
Winhall River	Both	46.6	1470	Winhall
Winooski River	Both	33	1056	Cabot
Flower Brook	Both	21.4	1045	Danby
Austin Brook	Macroinvertebrates	9.5	1240	Granville
Baker Brook	Macroinvertebrates	0.6	1535	Chittenden
Batten Kill	Macroinvertebrates	432	540	Arlington
Bear Wallow Brook	Macroinvertebrates	3	1280	Granville
Beaver Brook	Macroinvertebrates	1.2	180	Weybridge
Beetle Brook	Macroinvertebrates	25	767	Troy
Black River	Macroinvertebrates	37.3	1156	Plymouth
Black River	Macroinvertebrates	266.8	834	Irasburg
Bradley Brook	Macroinvertebrates	3.4	1535	Warren
Browns River	Macroinvertebrates	6.1	1400	Underhill
Burroughs Brook	Macroinvertebrates	56.3	895	Danville
Cobb Brook	Macroinvertebrates	5.8	1571	Windham
Cold River	Macroinvertebrates	25.8	1330	Shrewsbury
Cook Brook	Macroinvertebrates	1.3	1709	Peru
Dish Mill Brook	Macroinvertebrates	5.1	1430	Burke
Dog River	Macroinvertebrates	86	745	Northfield
E.Brch.Passumpsic R.	Macroinvertebrates	184.8	740	Lyndon
Ellis Brook	Macroinvertebrates	20.7	1624	Dover
Falls Brook	Macroinvertebrates	6	1580	Killington
Furnace Brook	Macroinvertebrates	38	970	Chittenden
Green River	Macroinvertebrates	65.9	671	Guilford
Green River	Macroinvertebrates	44.1	1017	Halifax
Indian Brook	Macroinvertebrates	10.2	405	Essex
Johns River	Macroinvertebrates	19.9	689	Derby

Lamb Brook	Macroinvertebrates	4	2022	Readsboro
Lamoille River	Macroinvertebrates	1754.8	300	Georgia
Lamoille River	Macroinvertebrates	20	1339	Greensboro
Lee River	Macroinvertebrates	39.8	499	Jericho
Lewis Creek	Macroinvertebrates	178	140	Ferrisburgh
Kelly Brook	Macroinvertebrates	4.1	145	Swanton
Lewis Creek	Macroinvertebrates	115	300	Hinesburg
Kent Brook	Macroinvertebrates	0.6	2480	Killington
Mill Brook	Macroinvertebrates	41.8	308	Jericho
Millers Run	Macroinvertebrates	75.3	900	Sheffield
Moose River	Macroinvertebrates	207.1	1013	Concord
N.Br. Nulhegan River	Macroinvertebrates	54.7	1155	Ferdinand
Nulhegan River	Macroinvertebrates	391.1	900	Bloomfield
Ottauquechee River	Macroinvertebrates	66	1018	Bridgewater
Paul Stream	Macroinvertebrates	44	860	Brunswick
Pekin Brook	Macroinvertebrates	60.1	745	Calais
Trib. to Pike Hill Brook	Macroinvertebrates	1.3	1210	Corinth
Rock River	Macroinvertebrates	46.7	453	Newfane
Sacketts Brook	Macroinvertebrates	41.5	235	Putney
S.Br.Williams River	Macroinvertebrates	9.24	720	Chester
S.Branch Waits River	Macroinvertebrates	110.7	680	Bradford
Shepard Brook	Macroinvertebrates	17.5	1020	Fayston
Smith Brook	Macroinvertebrates	4.6	1920	Goshen
South Stream	Macroinvertebrates	45	842	Bennington
Steam Mill Brook	Macroinvertebrates	5.4	2066	Walden
Third Br.White River	Macroinvertebrates	74.09	820	Braintree
Trout Brook	Macroinvertebrates	12.2	100	Milton
Trout Brook	Macroinvertebrates	12	112	Milton
Trout Brook	Macroinvertebrates	6.5	122	Milton
Turkey Mtn.Brook	Macroinvertebrates	33.5	690	Townshend
W.Brch.Deerfield R.	Macroinvertebrates	94.9	1515	Readsboro
W.Trib.Roaring Brook	Macroinvertebrates	2	2120	Killington
Wells River	Macroinvertebrates	256	425	Wells
Wells River	Macroinvertebrates	142	780	Groton
West River	Macroinvertebrates	1048.6	312	Dummerston
White River	Macroinvertebrates	1781.3	460	Hartford
White River	Macroinvertebrates	1080	505	Royalton
White River	Macroinvertebrates	513	700	Stockbridge
White River	Macroinvertebrates	510	715	Stockbridge
Lewis Creek	Macroinvertebrates	145	220	Charlotte

Appendix 2. List of impacted streams, type of impact and community where stream was used to validate biological metrics.

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Stream Name	General impact type	Community
Adams Brook	enrichment, sedimentation	Macroinvertebrate
Allen Brook	urban nps	Fish
Bartlett Brook	urban nps	Both
Berry Brook	enrichment	Macroinvertebrate
Black River	organic enrichment	Macroinvertebrate
Branch Pond Brook	pH, acid rain	Macroinvertebrate
Browns River	pH, hydrology	Both
Browns River	sedimatation. habitatat degradation	Fish
Centennial Brook	urban nps	Macroinvertebrate
Chase Brook	habitat, sedimentation	Macroinvertebrate
Clay Brook	habitat, toxicity, iron seeps	Macroinvertebrate
Coburn Brook	toxicity, milkhouse	Both
Cold Brook	sedimentation, habitat	Fish
Colemen Brook	acidification	Macroinvertebrate
Deer Brook	enrichment	Both
Deerfield River	dewatering	Fish
Dog River	enrichment, toxicity	Macroinvertebrate
Dowesville Trib #1	habitat, logging	Macroinvertebrate
Dowsville Brk Trb#11	habitat, logging	Macroinvertebrate
E.Trib.Roaring Brook	enrichment	Macroinvertebrate
Ely Brook	acid mine toxicity	Both
Englesby Brook	urban, nps	Both
Godin Brook	enrichment	Macroinvertebrate
Godin Brook Trib#4	enrichment	Macroinvertebrate
Great Brook	hydrology	Macroinvertebrate
Hoosic River	Toxicity, enrichment	Macroinvertebrate
Indian Brook	urban nps	Both
Iron Stream	habitat, toxicity, iron	Macroinvertebrate
Jewett Brook	enrichment	Macroinvertebrate
Joe's Brook	dewatering	Fish
Laplatte River	enrichment	Macroinvertebrate
Little Otter Creek	enrichment, sedimentation	Macroinvertebrate
Lye Brook	pH, acid rain	Macroinvertebrate
Mad River	hydrology, flood	Macroinvertebrate
McCabes Brook	nps	Macroinvertebrate
Mill Bk (Fairfax)	enrichment	Macroinvertebrate
Mill Brook	enrichment	Macroinvertebrate
Mill River (Georgia)	habitat, enrichment	Both
Missisquoi River	enrichment, sedimetation	Macroinvertebrate
Moon Brook	urban, nps	Both
Morehouse Brook	habitat, urban nps	Macroinvertebrate
Muddy Brook	habitat	Both
Munroe Brook	urban nps, sedimentation	Both
N.Br.Berry Brook	enrichment, silage/milkhouse	Both
N.Branch Ball Mt.Brk	sedimentation	Macroinvertebrate
N.Brch.Deerfield R.	sedimentation, temp,	Macroinvertebrate

Stream Name	General impact type	Community
Newton Brook	enrichment	Both
Otter Creek	enrichment, toxicity	Macroinvertebrate
Ottauquechee River	dewatering	Fish
Passumpsic River	enrichment, flow	Macroinvertebrate
Pike Hill Brook	toxicity	Both
Potash Brook	urban nps, sedimentation	Macroinvertebrate
Poultney River	enrichment, nps	Macroinvertebrate
Rice Brook	habitat, sedimentation	Macroinvertebrate
Roaring Brook	sedimentation/ nonpoint	Macroinvertebrate
Sacketts Brook	toxicity	Both
Samsonville Brook	enrichment	Macroinvertebrate
Saxe Brook	enrichment	Macroinvertebrate
Soapstone Brk.Trib3	sedimentation	Macroinvertebrate
Sphear Brook	enrichment	Macroinvertebrate
Sterns Brook trib	enrichment	Both
Stevens Branch	toxicity, chlorine	Both
Stevens Branch Trb23	toxicity, chlorine	Both
Stevens Brook	toxicity, urban nps	Both
Stevensville Brook	pH, hydrology	Macroinvertebrate
Stone Bridge Brook	enrichment, temp	Macroinvertebrate
Stratton Pond Trib-1	sedimentation	Macroinvertebrate
Styles Brook	sedimentation	Macroinvertebrate
Sunderland Brook	urban nps, habitat	Both
Thatcher Brook	sedimentation	Fish
Trib to Joiner Bk.	Habitat, logging	Macroinvertebrate
Trib. to Muddy Brook	urban nps	Fish
Trout Brook	enrichment, temp	Macroinvertebrate
Unnamed Brook (Cambridge)	habitat, toxicity	Macroinvertebrate
W.Br.Ompompanoosuc R	toxicity	Both
Wanzer Brook	enrichment, habitat	Macroinvertebrate
West Hill Brook	hydrology	Macroinvertebrate
Whetstone Brook	urban runoff	Macroinvertebrate
White River	chlorine spill	Macroinvertebrate
Wild Branch	hydrology	Macroinvertebrate
Winooski River	toxicity-chlorine	Macroinvertebrate

Appendix 3. The 93 macroinvertebrate reference sites listed by stream type as defined by TWINSPAN, CCA and BPJ analysis. Temporal data was used at high gradient sites marked with an asterisk to establish range of community metrics within stream type.

SHG	MHG	WWMG	SW
Dowsville Brook 3.3	Rock River 1.5	*West River 6.2	*Allen Brook 1.3
*Bradley Brook 1.7	Mill Brook 0.6	*Allen Brook 7.1	Black River 36.4
*Cobb Brook 0.9	Turkey Mtn.Brook 1.0	*Lewis Creek 3.5	Burroughs Brook 2.9
Kent Brook 0.6	Shepard Brook 5.0	*Lewis Creek 7.5	Pekin Brook 0.2
Cobb Brook 2.6	*Winhall River 8.1	*Lewis Creek 10.6	Trout Brook 0.1
*Austin Brook 0.2	Sacketts Brook 3.5	Williams River 3.0	Trout 0.7
*Bear Wallow Brook 0.2	*East Putney Brook 0.3	*Hubbardton River 1.8	*Trout Brook 1.2
*Baker Brook 2.7	Saxtons River 6.2	*Hubbardton River 10.7	Lamoille River 83.5
Cook brk 8.1	Dog River 12.7	White River 1.9	Kelly1.4
Falls Brook 0.8	Stevens Branch Trb23 0.5	White River 21.8	Johns River 1.4
W.Trib.Roaring Brook 0.2	*Lewis Creek 21.4	Nulhegan River 0.2	Beaver Brook 1.6
*Lye Brook 1.8	Furnace Brook 6.3	Black River 12.1	
*Bourn Brook 1.6	S.Br.Williams River 1.3	Tyler Branch 2.2	
*Smith Brook 1.3	Ottauquechee River 27.5	*Lamoille River 15.7	
Pike Hill Trib 0.1	Cold River 6.8	Indian Brook	
W.Brch.Deerfield R. 1.8	White River 31.9		
*Lamb Brook 0.7	*White River 32.4		
N.Brch.Deerfield R. 12.6	3rd Branch White 12.7		
Steam Mill Brook 5.5	Third Br.White River 18.1		
Dish Mill Brook 2.1	*Castleton River 8.7		
Keyer Brook 1.0	Flower Brook 4.9		
*Browns River 20.8	Batten Kill 33.3		
	*Waits River 2.6		
	S.Branch Waits River 0.6		
	South Stream 2.1		
	*Ellis Brook 1.0		
	*E.Br.North River 11.7		
	*Green River 16.0		
	*Green River 19.9		
	*Wells River 0.6		
	Wells River 4.4		
	Wells River 10.5		
	*Moose River 14.3		
	*E.Brch.Passumpsic R. 1.7		
	E.Brch.Passumpsic R. 3.8		
	*Paul Stream 0.1		
	E.Br.Nulhegan River 0.7		
	*Berry Brook 1.2		
	Beetle Brook 1.1		
	Lee River 2.8		
	N.Br.Lamoille River 12.3		
	Bailey Brook 0.5		
	*Winooski River 86.5		
	Ely Brook		
	Millers Run		

Appendix 4. The biometrics of SHG streams comparing the reference sites to known impacted sites. The significant p values reported using the Mann-Whitney Rank Sum Test. Reference stream data reported is the *Rmedian*=median value from reference sites, *R5/95, min/max* = 5th or 95th percentile, and the minimum or maximum value from reference sites depending on expected direction of change in metric with impact. Impacted stream data reported is the *I median* = Median value from impacted sites, and the I 25/75, 10/90 = the 25th or 75th percentiles and the 10th or 90th percentiles depending on the direction of change in the metric with increasing impact. Those metrics marked with a + increase with impact; all others decrease. X= Metric dropped because P>0.05

		Reference Streams (n=40)		Impacted Streams (n=42)	
	p<0.05	R median	R5/95,min/max	I median	I 25/75,10/90
Density	.001	1016	458/325	222	138/47
Richness	.001	40.2	31/27.5	28	20.5/15.3
EPT	.001	23.5	18/15.5	14	11/7
EPT/Richness	.001	0.58	0.48/0.45	.46	0.40/0.30
#Ephemeroptera	.001	6	4/3	3	2/1
#Plecoptera	.001	9	5.5/4	5	3/2
#Trichoptera	.001	12	9/6	6	4/2
% Dominant +	.002	20	35/38	24	37/50
# Dominant	.04	7	6/6	7	6/4
BIO INDEX (0-10) +	.005	2.34	3.26/3.73	2.88	4.15/5.11
EPT / EPTC	.016	0.86	0.61/0.53	.80	0.59/0.32
%Hydropsycidae +	.212	x	x	x	x
Species Diversity	.001	4.2	3.78/3.12	3.68	3.15/2.70
%Coleoptera	.001	4.3	0.23/0	.60	0/0
%Diptera +	.08	X	x	x	x
%Ephemeroptera	.003	19	5.3/2.7	9.60	2.0/0.2
%Trichoptera	.002	24	8.9/7.4	14.3	9.4/6.6
%Plecoptera	.33	x	x	x	x
%Oligocheata +	.001	1.8	0.3/2.9	3.0	12.9/28
% Other +	.84	x	x	x	x
O-PMA	.001	76	66/51	60	50/40
O-PPCS	.001	0.54	0.38/0.35	.36	0.28/0.24
Collector - Gatherer +	.2	x	x	x	x
Collector - Filterer +	.7	x	x	x	x
Predator +	.8	x	x	x	x
Shredder-Detrivore	.4	x	x	x	x
Shredder-Herbivore +	.9	x	x	x	x
Scraper	.001	9.0	0.8/0.4	1.7	0.44/0
F-PMA	.001	75	56/62	65	55/48
F-PPCS	.001	0 .6	0.45/0.33	0.42	0.38/0.31

Appendix 5. The biometrics of MHG streams comparing the reference sites to known impacted sites. The significant p values reported using the Mann-Whitney Rank Sum Test. Reference stream data reported is the *Rmedian*=median value from reference sites, *R5/95, min/max* = 5th or 95th percentile, and the minimum or maximum value from reference sites depending on expected direction of change in metric with impact. Impacted stream data reported is the *Imedian* = Median value from impacted sites, and the I 25/75, 10/90 = the 25th or 75th percentiles and the 10th or 90th percentiles depending on the direction of change in the metric with increasing impact. Those metrics marked with a + increase with impact; all others decrease. X= Metric dropped because P>0.05

		Reference Streams (n=68)		Impacted Streams (n=58)	
	p<0.05	Rmedian	R 5/95,min/max	Imedian	I25/75,10/ 90 25/75,10/90
Density	.041	1797	727/620	1208	536/154
Richness	.001	45.8	39/37	36.5	25/20.3
EPT	.001	27	22/18.5	11	7/2.5
EPT/Richness	.001	0.56	0.47/0.43	0.28	0.22/0.12
#Ephemeroptera	.001	9	5/7	4	2/1
#Plecoptera	.001	9	3/5	2	0/0
#Trichoptera	.001	13	7/8	6	4/2
% Dominant taxa +	.001	20	35/40	27	34/49
# Dominant taxa	.184	x	x	x	x
BIO INDEX(0-10) +	.001	3.2	3.91/4.51	5.00	5.74/6.47
EPT / EPTC	.001	0.88	0.61/0.70	0.44	0.26/0.06
%Hydropsycidae +	.4	X	x	x	x
Species Diversity	.001	4.55	3.83/3.40	3.91	3.4/2.5
%Coleoptera	.164	x	x	x	x
%Diptera +	.001	16.9	44/31	42	64/74
%Ephemeroptera	.001	33	16/9	5	1.2/.2
%Trichoptera	.001	33	11/9	21	6/1
%Plecoptera	.001	7	3/1	.3	0/0
%Oligocheata +	.001	0.1	1/10	1	5/34
% Other +	.038	0.5	2/5	1	4/10
O-PMA	.001	83	68/63	53	38/23
O-PPCS	.001	0.58	0.52/0.39	0.31	0.23/0.15
Collector - Gatherer +	.096	x	x	x	x
Collector - Filterer +	.113	x	x	x	x
Predator +	.001	12	6/4	7	4/2
Shredder-Detrivore	.001	3	0.4/0	1	0.1/0
Shredder-Herbivore +	.001	0.7	5/10	7	16/27
Scraper	.152	x	x	x	x
F-PMA	.001	76	64/46	66	58/43
F-PPCS	.001	0.64	0.52/0.42	0.45	0.38/0.26

Appendix 6. The biometrics of WWMG streams comparing the reference sites to known impacted sites. The significant p values reported using the Mann-Whitney Rank Sum Test. Reference stream data reported is the *Rmedian* = median value from reference sites, *R5/95, min/max* = 5th or 95th percentile, and the minimum or maximum value from reference sites depending on expected direction of change in metric with impact. Impacted stream data reported is the *Imedian* = Median value from impacted sites, and the I 25/75, 10/90 = the 25th or 75th percentiles and the 10th or 90th percentiles depending on the direction of change in the metric with increasing impact. Those metrics marked with a + increase with impact; all others decrease. X= Metric dropped because P>0.05

		Reference Streams (n=31)		Impacted Streams (n=55)	
	p<0.05	Rmedian	R 5/95,min/max	Imedian	I 25/75,10/90
Density	.011	2244	440/354	1397	732/183
Richness	.001	45.5	30/27.5	30	26/20
EPT	.001	23	18.5/15.5	9.5	6/3
EPT/Richness	.001	0.50	0.37/0.34	0.30	0.19/0.13
#Ephemeroptera	.001	9	6/4	3	2/0
#Plecoptera	.001	6	3/2	1	0/0
#Trichoptera	.001	12	7/4	6	4/2
% Dominant +	.002	19	30/36	24	32/48
# Dominant	.001	8	6/6	7	6/5
BIO INDEX (0-10) +	.001	3.98	4.62/4.67	5.39	5.80/6.27
EPT / EPTC	.001	0.89	0.65/0.60	0.70	0.57/0.33
%Hydropsycidae +	.002	18	36/39	32	41/57
Species Diversity	.001	4.34	3.94/3.51	3.73	3.24/2.37
%Coleoptera	.02	12	3/1	6	2/1
%Diptera +	.001	13	32/33	24	40/50
%Ephemeroptera	.001	29	10/6	3	0.6/0
%Trichoptera	.02	32	54/59	41	52/66
%Plecoptera	.001	4	1/0.7	0.2	0/0
%Oligocheata +	.04	0.1	5/7	0.8	3/13
% Other +	.001	0.6	3/4	2.8	10/20
O-PMA	.001	80	67/65	57	47/40
O-PPCS	.001	0.49	0.38/0.37	0.36	0.29/0.21
Collector - Gatherer +	.46	x	x	x	x
Collector - Filterer +	.06	x	x	x	x
Predator +	.56	x	x	x	x
Shredder-Detrivore	.86	x	x	x	x
Shredder-Herbivore +	.34	x	x	x	x
Scraper	.001	21	7/5	11	2/1
F-PMA	.002	77	60/56	70	64/52
F-PPCS	.009	0.58	0.37/0.37	0.48	0.43/0.35

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Appendix 7. Spearman rank order correlation between the selected metrics in the SHG stream type. An x indicates the correlation was not significant at p<0.05.

	EPT	PMA-O	Bio Index	EPT/EPT&c	% Oligochaeta	PPCS-F
Richness	0.67	x	x	-0.34	x	0.35
EPT		x	x	x	x	0.45
PMA-O			x	x	x	0.61
Bio Index				-0.38	x	x
EPT/EPT&c					x	x
% Oligochaeta						x

Appendix 8. Spearman rank order correlation between the selected metrics in the MHG stream type. An x indicates the correlation was not significant at p<0.05.

	EPT	PMA-O	Bio Index	EPT/EPT&c	% Oligochaeta	PPCS-F
Richness	0.59	0.26	x	-0.43	0.24	0.35
EPT		0.36	x	x	x	0.28
PMA-O			x	x	x	0.37
Bio Index				-0.28	x	x
EPT/EPT&c					x	x
% Oligochaeta						x

Appendix 9. Spearman rank order correlation between the selected metrics in the WWMG stream type. An x indicates the correlation was not significant at p<0.05

	EPT	PMA-O	Bio Index	EPT/EPT&c	% Oligochaeta	PPCS-F
Richness	x	x	0.49	-0.66	x	x
EPT		x	x	x	x	x
PMA-O			x	x	x	x
Bio Index				-.070	x	x
EPT/EPT&c					x	x
% Oligochaeta						x

Appendix 10.

Macroinvertebrate biocriteria *previously* used for determining the biological integrity of the aquatic biota for wadeable streams and rivers in Vermont. Method used 2 min. kick net sample sub-sampled in laboratory by picking one quarter of sample, if sub-sample is less than 300 animals then additional sub-sample is picked until a minimum of 300 animals are in the sub-sample. The proportion of sample picked is then recorded. Identifications are done in the laboratory to the genus - species level. The overall biological integrity of a stream is determined by evaluating the rating and degree of each metric and the number of metrics which are found to be in an acceptable or unacceptable range.

Metric Rating	**Mean Richness**	**Mean EPT**	**Bio Index**	**Diversity**
Very Poor	*<15*	*<8*	*≥3.50*	*<1.50*
Poor	*15-19*	*8-12*	*3.01-3.49*	*1.51 - 2.24*
Fair	*20-29*	*13-17*	*2.75-3.00*	*2.25 - 2.99*

Unacceptable (fails Class B Standards)

Acceptable (meets Class B Standards)

Metric Rating	**Mean Richness**	**Mean EPT**	**Bio Index**	**Diversity**
Good	30-39	18-22	2.01-2.74	3.00-3.99
Very Good	40-49	23-25	1.51-2.00	4.00-4.49
Excellent	≥50	>25	≤1.50	>4.50

Metric Rating	**% Dominant Genera**	**#EPT/#EPT&Chiro**	**# EPT/# Chiro**	**EPT/R**
Poor	*$55*	*<.25*	*#.50*	*#.30*
Fair	*$40 <55*	*>.25<.45*	*>.5 <1.00*	*>.30 #.45*

Unacceptable (fails Class B Standards)

Acceptable (meets Class B Standards)

Metric Rating	% Dominant Genera	#EPT/#EPT&Chiro	# EPT/# Chiro	EPT/R
Good	$25 <40	>.45<.75	>1 <2	>.45 #.60
Excellent	<25	>.75	>2	>.60

Appendix 11. Macroinvertebrate QA/QC methods:

The following practices provide a means of evaluating the precision, accuracy, comparability, and representativeness of the macroinvertebrate data used in this project. These activities are documented in”Vermont DEC Ambient Biomonitoring Activities - Work/QA Project Plan” (DEC, 1994).

- Precision is determined by field replication. All samples are collected, at a minimum, in duplicate. The mean of replicate samples is the value used for incorporation into the working database. Samples with a relative standard error (RSE) of greater than 40% for abundance estimates and 20% for taxa richness may be eliminated from the database.

- Accuracy in the lab, all samples picked are checked for completeness by a second biologist. Standard taxonomic keys are used for all identifications and each ID is assigned a confidence level. A reference collection of all species identified is maintained and all samples are archived in their entirety. ID’s are corroborated in-house as well as through external experts when appropriate. A random sub-sample of completed samples are re-identified to check consistency.

- Comparability and representativeness are assured by maintaining consistent standardized sampling and processing methods, and always sampling only during the fall index period.

Appendix 12. Maximum Species Richness Lines (MSRL's) for the two elevation zones in Vermont. Metri scoring appears on right side of graphs.