

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

## **- Development Phase -**

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### **Executive Summary**

February 10, 2004

(Updates 11/14/01 and 2/10/03 versions)

#### **Part 1: Overview - executive summary**

Revisions to the Vermont Water Quality Standards (VWQSs, effective date July 2, 2000) have introduced language relative to the use of biological criteria. The concept of reference condition has been established as the benchmark for determining support of designated uses related to the aquatic biota. The language in the revised standards requires that the regulatory agency, the Vermont Department of Environmental Conservation (VTDEC), establish procedures for making determinations of biological use support in the context of the reference condition and departures from the reference condition that are consistent with narrative criteria in the VWQS's. This document describes the methods by which the VTDEC has developed biological criteria for wadeable streams in Vermont using information on aquatic macroinvertebrate and fish communities. Criteria reflect the following language from the revised VWQSs:

The Class A1 (ecological) criteria reflect the reference condition consistent with the language "minimal impacts from human activity".

The Class B - Water Management Type 1 criteria reflect minor changes from the reference condition and in the best professional judgment of VTDEC biologists is consistent with the language "minor changes in the relative proportions of taxonomic and functional components; relative proportions of tolerant and intolerant components are within the range of the reference condition".

The Class B, Class A 2 (water supply) and Class B - Water Management Types 2 and 3 reflect the language "moderate change (from the reference condition) in the relative proportions of tolerant, intolerant, taxonomic and functional components" as well as historical application language "no change from the reference condition that would have undue adverse effect on the composition of the aquatic biota."

The objectives of this effort have been to:

- A. establish a means of categorizing wadeable streams according to the macroinvertebrate and fish communities found within the reference (minimally impacted) condition;
- B. evaluate and describe the physico-chemical attributes which best describe and differentiate identified stream categories;
- C. describe the biological metrics (measures of biological community) within the reference

stream categories and evaluate how they differentiate between the reference biological condition of identified stream categories;

D. evaluate the response of selected biological metrics to environmental impact ;

E. select appropriate measures of biological condition and establish criteria describing departure from the reference condition that can be used to evaluate aquatic life use support status consistent with VWQS designated uses and management types;

In order to accomplish the objectives stated above, the following tasks were conducted:

A. The existing VTDEC biological data base was prepared for use;

B. Reference sites were selected;

C. Stream categorizations within the reference sites were statistically and qualitatively examined using biological and physico-chemical attributes;

D. Biological and physico-chemical attributes within identified reference categories were evaluated and described;

E. Biological attributes (metrics) were described and evaluated in both the reference and non-reference condition;

F. Criteria were then set for each stream category using biological attributes (metrics) which best determined relative departure from the reference condition;

This document presents the results of those tasks. Differences in ecological information content of macroinvertebrate and fish communities primarily related to differences in the diversity of species dictate differential treatment of the two assemblages. Therefore, macroinvertebrate and fish communities have been treated separately in Parts 2 and 3 respectively.

## **Part 2: Macroinvertebrates - executive summary**

### **Data Base - Sampling, Sample Processing and Analysis, and Data Management:**

*Sampling:* Macroinvertebrate samples are extracted from representative riffles within stream reaches being evaluated. A D-frame net with 500u mesh openings is used in a standardized kick-net sampling procedure. Two minutes (timed) of active sampling is carried out in such a way that a composite sample representing the full range of water velocity within the riffle is collected. Sampling effort is comparable to RBP III as described in Plafkin et al (1989). Physico-chemical attributes are recorded for each sampling event.

*Sample Processing and Data Management:* All macroinvertebrate processing is conducted in a laboratory setting. Preserved (ethanol) samples are spread out on a gridded tray and organisms are picked from the debris in a methodical manner. Minimum sub-sample requirements are at least 25

percent of the entire sample **and** a minimum of 300 organisms (or the entire sample if less than 300 organisms). All organisms are identified to the lowest practicable taxonomic level (usually genus or species). Data are entered into an electronic data base which standardizes sub-sampling effort, calculates sampling error (based on replicate sampling at a site), and calculates a prescribed list of community metrics. QA/QC practices are employed regularly throughout the process.

*Data Base:* The Vermont Department of Environmental Conservation (VTDEC) biological data base contains validated macroinvertebrate data from wadeable stream sites throughout Vermont. Data were collected for a variety of reasons related to VTDEC programs including discharge compliance monitoring and general status and trends monitoring.

#### **Reference Site Selection:**

From the site file described above, VTDEC biologists identified a total of 93 minimally impacted, or reference sites. All sites selected were, in the best professional judgment of VTDEC biologists, minimally impacted by human activity. While the majority of identified reference sites have minimal human activity in the upstream watershed, many sites have significant activity upstream, including direct discharges, roading, town and village development, and agriculture. In all cases, however, the potential impacts at the selected reference sites were judged to be minimal. Due to multi-year sampling at many sites, macroinvertebrate data from more than 170 sampling events were available at these 93 sites.

#### **Reference Stream Categorization Methods and Results:**

*Methods:* Macroinvertebrate and physico-chemical data from 100 sampling events, including data from all 93 sites, were used to categorize reference streams according to biological and physico-chemical attributes. Two-way Indicator Species Analysis (TWINSPAN) was used to group sites into biological assemblage categories; Canonical Correspondence Analysis (CCA) was used to explore the relationships between biological and physico-chemical attributes.

*Results:* Four general macroinvertebrate community categories of wadeable streams were identified by evaluating the macroinvertebrate data. These categories were established by combining best professional judgment with the results of the TWINSPAN and CCA analyses. Drainage area, elevation, % canopy, and alkalinity were the best physico-chemical discriminators between stream categories.

The four macroinvertebrate wadeable stream categories are described as follows:

- 1. Small High Gradient Streams (SHG)** - Small headwater streams typically found at higher elevation averaging 1535 ft, highly canopied first to third order streams. They are high in gradient, and as a result, their substrate is dominated by gravel, cobble, and boulder with generally only about three percent fines. The drainage areas average around 10km<sup>2</sup>, and the water chemistry is relatively soft, with alkalinity averaging around 18mg/l.
- 2. Medium-size High Gradient Streams (MHG)** -Medium sized streams in the third to fourth order range, typically found at moderate elevations averaging 814 ft, with an average 50% canopy. The gradient is high with the substrate dominated by gravel, cobble, and boulder, and only about six percent fines. The drainage areas average 88km<sup>2</sup>, and the water chemistry is usually moderate in alkalinity averaging 48mg/l.
- 3. Warm Water Moderate Gradient Streams and Rivers (WWMG)** - Larger streams fourth to sixth order in size or small streams within the Champlain Valley all at lower elevations averaging 369 ft. Typically more open canopied and warmer than SHG and MHG streams based on the dominant macroinvertebrate species. Gradients are moderate with substrates dominated by gravel, cobble, and boulder, and averaging seven percent fines. The drainage areas range widely but are often quite large with an average size of 480km<sup>2</sup>. Alkalinities are typically high, averaging 70mg/l.
- 4. Slow Winders (SW)** - Low gradient streams with substrates dominated by sand/silt.

The Type 4 stream category, the “slow winders”, clearly differed from the other three higher gradient stream categories, being characterized by low gradient and sandy substrate. Due to few data points within this category, no further evaluation of biocriteria was made at this time.

In order to evaluate the degree to which metric reference expectations (criteria) vary between stream categories, 30 macroinvertebrate metrics within each of the three remaining high-gradient stream categories were statistically characterized and compared across categories. The 30 metrics included measures/indicators of community structure, community function, and the relative importance of tolerant and intolerant organisms within the community. Data from all valid sampling events at all 93 reference sites were used (n = 40, 68, and 31 for SHG, MHG, and WWMG respectively) in order to factor both spatial and temporal variability into the statistical summaries for each stream category. While many of the metrics were able to discriminate one stream category from the other two (Dunn’s multiple comparison test; p<0.05), only three metrics were significantly different between all three high gradient stream categories: Hilsenhoff biotic index; percent predators; and percent shredder-detritivores.

## **6. Metric Evaluation and Selection:**

In order to evaluate the response of metrics to known impact, VTDEC biologists identified 73 sites (155 sampling events) from the main data base that were known to be impacted. Types and degree of impact varied. Habitat alterations related to sedimentation and hydrology, and nutrient enrichment were the primary types of impact noted. The physico-chemical descriptors of the reference stream categories were used to assign each impacted stream site to its most probable stream category (n = 42, 58, and 55 for SHG, MHG, and WWMG respectively). Because of overlap in the range of physico-chemical characteristics between reference stream sites, assigning a

“test” stream to a category requires some judgment. The distribution of each of the 30 metrics within each impacted stream category was statistically summarized in the same manner as the reference streams. The summary statistics for each of the 30 metrics within each stream category from the impacted streams were compared to those from the reference streams.

The statistical significance of metric response to impact was evaluated by using the Mann-Whitney rank sum test ( $p < 0.05$ ) to compare metric values between reference and impact conditions for each stream category. Many metrics showed significant statistical differences between reference and impacted sites (21, 24, and 25 metrics for SHG, MHG, and WWMG respectively). Percent community composition by functional group were the metrics least likely to detect impact while metrics related to taxa richness and tolerance/intolerance were the most consistent at detecting impact.

As a result of the above evaluations, eight metrics were selected for biocriteria development. Metrics were selected in combination meet the following criteria: address functional, structural and indicator components of biological integrity; demonstrate minimal natural variability within reference stream category; and respond to impact.

A total of 8 metrics were selected for each stream category.

- Density was selected as a general indicator of secondary productivity.
- Three taxonomic structure and compositional metrics were selected: *Taxa (species) Richness*, *Mayfly-Stonefly-Caddisfly Taxa Richness (EPT)*, and *Order-level Per Cent Model Affinity (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 categories.
- Four indicator taxa and functional group metrics were selected: *Bio Index*, *Percent Oligochaeta*, *Ratio of Mayflies-Stoneflies-Caddisflies to Chironomid Midges (EPT/EPT&C)*, and *Functional Reference Similarity (PPCS-F)*. These four metrics consistently showed significant change between the reference and impact streams.

Brief descriptions of these metrics follow:

**Density-** Is the relative abundance of animals in a sample.

**Richness-** Species richness is the number of species in a sample unit. Richness is calculated as the total number of distinct taxa identified in a sample. Note immature larva identified to family or genus are not considered a distinct new taxa if a genus or species identification is determined within its group.

**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 is calculated as the number of distinct taxa identified in a sample from the insect orders Ephemeroptera, Plecoptera, Trichoptera. Note same rules apply as above for richness in determining number of distinct taxa.

**Percent Model Affinity of Orders (PMA-O)** - PMA-O is a measure of order-level similarity to a model based on the reference streams (Novak and Bode, 1992). 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 to the mean percent composition values from the reference condition (model). Array the model order

percentages with the assessment site order percentages. The sum of the lower of the two values for each order is the PMA-O.

**Hilsenhoff Biotic Index- BI (0-10)** - The BI is a measure of the macroinvertebrate assemblage tolerance toward organic (nutrient) enrichment (Hilsenhoff, 1987). The BI is calculated by multiplying the number of individuals of a taxon by its assigned tolerance value (VTDEC BI values, Hilsenhoff 1987). The total of all these products divided by the total number of individuals of each taxon assigned a tolerance value is the BI value.

**% Oligochaeta** - The percent oligochaeta is a measure of the percent of the macroinvertebrate community made up of the Order Oligochaeta. Percent Oligochaeta is calculated by dividing the number (abundance) of Oligochaeta by the total number of animals in the sample.

**EPT/EPT & Chironomidae** -  $EPT/EPT + C$  is a measure of the ratio of the abundance of the intolerant EPT orders to the generally tolerant Diptera family Chironomidae.  $EPT/EPT + C$  is calculated by dividing the number (abundance) of animals from the orders Ephemeroptera, Trichoptera and Plecoptera, by the above plus the number of animals of the order Chironomidae in the sample.

**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** above; however, it measures functional feeding group changes instead of taxonomic changes to the macroinvertebrate assemblage. 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 (1991) at the assessment site. For each functional group determine the quotient of min/max between assessment site and the reference model for the stream category. The sum of these quotients divided by six (number of functional groups) is the PPCS-F.

## 7. Biological Criteria Evaluation and Selection:

The threshold index values for the selected metrics were determined in the following manner and are presented in **Table 1**;

The **Class A1** (ecological) thresholds were initially set to include at least 75 percent of the reference data base. The criteria were then slightly adjusted based on best professional judgment (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 B - WMT 1** 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 B - WMT 1 threshold criteria demonstrate no more than a minor change from the reference condition.

The **Class B - WMT's 2 and 3** and **Class A2** (water supply) criteria were generally set below the 95<sup>th</sup> percentile, or range of the reference value for each stream category. The Class B – WMT's 2 and 3 and Class A2 (water supply) criteria were then adjusted based best professional judgment interpretation of the relationships between the range of reference values and the median, and 10<sup>th</sup> / 90<sup>th</sup> 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.

**Table 1.** Macroinvertebrate assemblage threshold indices for three macroinvertebrate community categories and associated classes in Vermont. All criteria are either > or < and = the values listed. Extreme departures from the criteria thresholds indicate either a very poor biological condition or an assemblage of exceptionally high value.

Metric	SHG			MHG			WWMG		
	A	B1	B, B2-3 A2	A	B1	B, B2-3 A2	A	B1	B, 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

## **Part 3: Fish - executive summary**

### **General Overview:**

The general approach of this effort was to determine how the fish assemblage reference condition changes with stream or river category. As streams grow in size they exhibit an increased potential to support more fish species and thereby a different species composition. The species composition also is a function of geographic location within the state. The lower Champlain Valley streams support additional species not naturally occurring in rivers outside the valley. A minimally impacted site then will vary somewhat in its composition depending on certain physical variables which best describe the effects of geographic location and stream size.

### **Data Base - Sampling, Sample Processing and Analysis, and Data management:**

*Sampling:* Stream fish community sampling is conducted during the late summer to fall period. Fish are sampled in stream sections that are judged to be representative of the overall stream reach being assessed. Stream sections sampled are generally greater than 70 meters, and less than 200 meters, in length. Sampling is conducted by standard electroshocking methods. Assessment sections are shocked from downstream to upstream with either a back pack or streamside shocker, depending on stream size. One to three passes are made over the entire section. All stunned fish observed are captured, identified, examined for anomalies and released following completion of sampling. Physical-chemical attributes, including habitat observations or quantification, are made with each sampling event.

*Sample Processing and Data Management:* With the exception of difficult identifications, which are brought back to the laboratory for confirmation, all sample processing occurs in the field at the time of sampling. Data are transcribed from field sheets and entered into an electronic data base which standardizes sampling effort and calculates an array of population metrics. QA/QC practices are employed throughout the process.

*Index of Biotic Integrity:* The health or biological integrity of flowing water fish communities in Vermont streams is currently being evaluated by two indices of Biotic Integrity (IBI). The IBI concept was introduced in the 1980's for the mid-west US. Since then, many modifications have been produced for different regions. The IBIs used here have been calibrated to Vermont wadeable streams by assessing an array of proven metrics from other IBIs and selecting those that are most responsive to perturbation in Vermont streams.

Prior to the current work, a single IBI was used to assess stream fish assemblage integrity. This mixed-water IBI (MW IBI) is applied to all streams of wadeable size that contain five or more native fish species. This index contains nine metrics. Values range from 9 (poor) to 45 (excellent). Since a minimum of five native species is required to apply the MWIBI, many smaller, coldwater streams could not be evaluated using this approach. A new IBI designated as the Coldwater IBI, (CW IBI) was derived during the initial stages of the current project and has been specifically formulated to evaluate assemblages with two to four species in small coldwater streams. Sites naturally containing only a single species (nearly always brook trout) are not, at this time, deemed suitable for application of any conventional IBI.

*Data Base:* The VTDEC biological data base contains validated fish population data from wadeable stream sites throughout Vermont. Data were gathered for a variety of reasons related to VTDEC programs including discharge compliance monitoring, fish distribution surveys, and status and trend monitoring.

## Reference Site Selection:

VTDEC biologists identified a total of 76 minimally impacted, or reference sites from 71 streams. Reference sites varied in elevation from 102 to 2162 ft and in site drainage from 2 to 298 km<sup>2</sup>. All sites selected were, in the best professional judgment of VTDEC biologists, minimally impacted by human activity. While the majority of identified reference sites have minimal human activity in the upstream watershed, some sites have significant activity upstream, including direct discharges, roading, town and village development, and agriculture. In all cases, however, the potential impacts at the selected reference sites were judged to be minimal.

## Reference Stream Categorization Methods and Results:

Canonical correspondence (CCA) and Two-way Indicator Species (TWINSPAN) analyses were applied to the reference data set. Only native species which occurred at more than one of the 76 sites were used in the evaluation. Thirty-two of the total of 54 fish species encountered in these streams were entered into the analysis. Both tools indicated that site elevation and stream size were the most important variables in shaping fish assemblage characteristics. While these analyses identified where several potential divisions in the reference condition species composition were likely to occur along a stream size/elevation gradient, the delineations were not discrete enough to be able to describe a groups of species assemblages that were meaningful to this analysis.

Historical use of the Index of Biotic Integrity as well as the evaluation of a metric-based TWINSPAN analysis indicate that species richness is a critical categorization factor and requires the identification of three stream categories: 1) Brook trout only streams; 2) Cold-water streams containing from 2 to 5 species; 3) Streams containing 5 or more species. **Table 2** shows the distribution of physico-chemical factors associated with each of those three stream categories within the reference condition. The factors identified in this table are used to guide best professional judgment when categorizing a stream to its most probable IBI categories. Within the MW IBI additional “categorization” is accomplished by adjusting scoring based on elevation and stream size (drainage area).

**Table 2:** Mean and range ( ) of physico-chemical variables for the three IBI- related stream categories for reference stream sites.

	No IBI-Brook Trout only Sites (n=9)	Cold water IBI (n=19)	Mixed Waters IBI (n=48)
Elevation (Feet)	1360 (930-2162)	983 (416-2116)	655 (102-1880)
Drainage (km <sup>2</sup> )	8.9 (3-17)	13.4 (2-33)	68.8 (2-298)
Alkalinity (mg/l)	10.3 (1-27)	34.5 (2-96)	65.8 (3-227)
pH	6.5 (6.6-8.2)	7.3 (6.4-8.1)	7.6 (5.9-8.9)
% Fines	5.9 (0-20)	9.7 (0-30)	21.1 (0-100)
% Pool	43.6 (25-75)	42.8 (15-65)	43.6 (10-95)
Gradient <sup>1</sup>	36.1 (8-61)	31.8 (6-80)	12.1 (1-70)

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

**Metric Evaluation and Selection:**

*Mixed Water Index of Biotic Integrity:* The MW IBI has been applied in Vermont in various forms since 1987. It consists of nine metrics. Metrics were selected as indicators of community structure, function, and condition. **Table 3** shows the nine metrics and their response to impact by comparing mean metric values of the reference sites with mean values from impacted sites and calculating the Mann-Whitney U statistic ( $p > 0.05$ ).

**Table 3:** 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 Maximum Species Richness Line.

MWIBI Metric	Reference Sites n=43	Impacted Sites n=30	Significantly <sup>1</sup> 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	41.0 39.2	29.0 28.4	Y: $p < 0.001$

1. Results of a Mann-Whitney-U test.

The Mixed Water Index of Biotic Integrity for Vermont is presented in **Table 4** below.

**Table 4.** Mixed Waters Index of Biotic Integrity (MWIBI) for The Fish communities of Wadeable Vermont Streams

For streams naturally supporting more than four species		Scoring Criteria			
		5	3	1	
<b>Species Richness and Composition</b>					
1	Total number of native fish species		(Follows maximum species richness lines)		
2	Number and identity of native, intolerant species ( <i>A non-native trout may be substituted for brook trout when absent</i> )	[Site Elevation >400 ft.] - [Site Elevation <400 ft.] -	>1 >0	1 -	0 0
3	Number and identity of benthic insectivores	[Site Elevation <400 ft. with site drainage <25 km <sup>2</sup> ] All other sites	1 >1	- 1	0 0
4	Proportion of individuals as white suckers and creek chubs		<20%	20-40%	>40%
<b>Trophic Composition</b>					
5	Proportion of individuals as generalist feeders	[Site Elevation >500 ft.] - [Site Elevation <500 ft.] -	<20% <30%	20-45% 30-60%	>45% >60%
6	Proportion of individuals as water column and benthic insectivores ( <i>score a "1" if blacknose dace is &gt;60% of assemblage</i> )	[Site Elevation >500 ft.] - [Site Elevation <500 ft.] -	>65% >55%	30-65% 20-55%	<30% <20%
7	Proportion of individuals as top carnivores ( <i>Nonnative trouts included</i> )	[cold water assemblage] - [warm water assemblage with site drainage >25 km <sup>2</sup> .] - [warm water assemblage with site drainage <25 km <sup>2</sup> .] -	>15% >10% 0	5-15% 3-10% -	<5% <3% -
<b>Fish Abundance and Condition</b>					
8	Proportion of individuals with Deformities, fin erosion, lesions or tumors		<1%	1-4%	>4%
9	Abundance in Sample (one pass - #100m <sup>2</sup> ) ( <i>Nonnative species included</i> )	[Site Elevation <500 ft]. [Site Elevation >500 ft]. [Alk. >9 mg/l] [Alk. <9 mg/l]	>20 >10 >6	10-20 7-10 3-6	<10* <7* <3*
*site scores "poor"					

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

**Metric 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

*Cold Water Index of Biotic Integrity*: Fourteen possible metrics were evaluated for use with the CW IBI. Response to impact was evaluated by comparing mean values within the reference condition with mean values from a set of impacted sites. Metric redundancy was evaluated through Spearman rank correlation analysis.

The Coldwater Index of Biotic Integrity for Vermont is presented in **Table 5** below. **Table 6** shows the final metric selection with response to impact.

**Table 5:** An Index of Biotic Integrity for Small Vermont Coldwater Streams

<i>For coldwater streams naturally supporting from two to four native species</i>	<b>5</b>	<b>3</b>	<b>1</b>
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 <sup>2</sup> -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 26  
 Poor <26

**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

**Table 6:** Metric value means from reference and impacted sites for the VT CWIBI. All differences were significantly different ( $p < .001$ , Mann-Whitney U test). The six-metric CWIBI scores were multiplied by 1.5 to correspond with the nine-metric MWIBI.

(Number of Sites)	Reference Sites n = 18	Impacted Sites n = 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/100m <sup>2</sup> )	12	0.4
Brook Trout Length Class Number	2.9	0.8
VT Coldwater IBI	41.1	14.4

The following table (**Table 7**) shows the thresholds for MW IBI and CW IBI scores for associated WQ Classes and Water Management Types in Vermont. Extreme departures from the thresholds indicate either a very poor biological condition or an assemblage of exceptionally high value in relation to its associated WQ Classification.

**Table 7:** Fish community Index of Biotic Integrity (MWIBI and CWIBI) scoring thresholds for associated Water Quality Classes and Water Management Types - Vermont Water Quality Standards effective 7/2/2000

WQ Class or Water Management Type	A1 (ecological)	B - WMT1	B B - WMT 2-3 A2 (water supply)
	<b>Reference Condition</b>	<b>Minimal</b> Change from the Reference Condition	<b>Moderate</b> Change from the Reference Condition
Index of Biotic Integrity Score	.42	>36	>27