Alaska Stream Condition Index:
Biological Index Development for Cook Inlet
1997 – 2000 Summary

August 2001

Biological Monitoring Program

Alaska Stream Condition Index:
Biological Index Development for Cook Inlet 1997 – 2000 Summary

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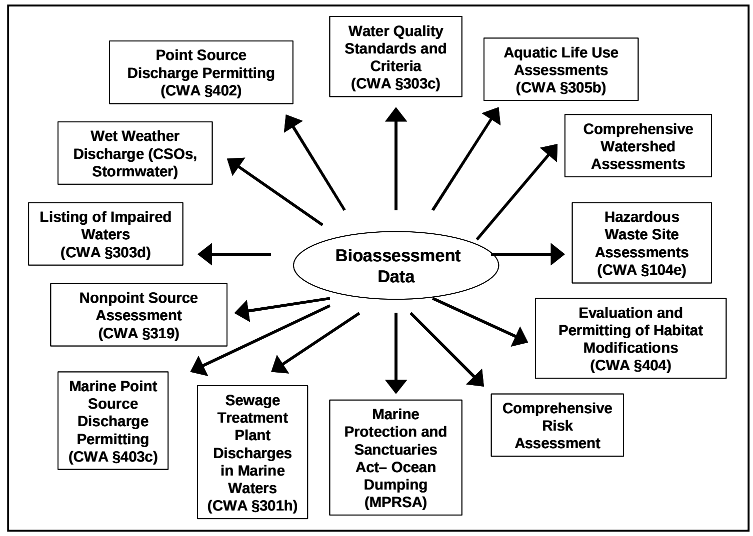
1. HIGHLIGHTS

Monitoring of biological communities integrates the effects of different pollutant stressors such as excess nutrients, toxic chemicals, increased temperature, and excessive sediment loading and thus provides an overall measure of the aggregate impact of the stressors. Biological communities respond to stresses of all degrees over time and, therefore, offer information on perturbation not always obtained with episodic water chemical measurements or discrete toxicity tests. The central purpose of assessing biological condition of aquatic communities is to determine how well a water body supports aquatic life.

Biological communities reflect overall ecological integrity (i.e., chemical, physical, and biological integrity). Therefore, bioassessment results directly assess the status of a waterbody relative to the primary goal of the Clean Water Act (CWA). Biological assessments are crucial to evaluating ecosystem health and provide crucial water quality planning information for managing more complex water quality problems (see graphic listing water quality programs).



Use of Bioassessment in State Water Quality Programs

In Alaska, bioassessment is in a developmental phase. This report describes the development of an Alaska Stream Condition Index (ASCI) that can be used by Alaska Department of Environmental Conservation (ADEC) for a variety of purposes. The highlights of this biological pilot program are:

- Three stream classes were determined for use as a framework for regional bioassessment throughout the Cook Inlet Ecoregion. The classes conform to a combination of gradient and substrate, thereby establishing benchmarks for aquatic faunal distribution and composition. The classes are *Low Gradient – fine substrate*, *Low Gradient – coarse substrate*, and *High Gradient*.
- The Alaska Stream Condition Index (ASCI) was developed for these three stream classes to include an aggregate of biological metrics, adjusted for the respective expectations of the aquatic faunal distribution and composition. Six metrics were identified for each of the *Low Gradient – fine substrate* and *High Gradient substrate* stream classes, and 8 metrics were obtained for the ASCI relevant for the *Low Gradient – coarse substrate* stream class.
- Narrative biological condition categories were established along the biological condition gradient to provide a basis for assessing the quality of the stream sites. These narrative categories relate to "excellent", "good", "fair", "poor", and "very

poor". The thresholds for these categories are linked to the population distribution of the reference sites to provide an estimate of regional expectations for the respective stream classes.

- One hundred and twenty three (123) stream sites were sampled in the Cook Inlet Ecoregion over a 4-year period. Of these 123 sites, 40% had good or excellent biological condition, 39% were fair, and 21% were poor or very poor. However, these statistics simply represent an assessment validation of streams selected to represent *a priori* assumptions of condition and quality.
- Six major watersheds or geographical areas were represented in the 4-year sampling program of the Cook Inlet Ecoregion: Anchorage metropolitan area, Matanuska River, Upper Susitna River, Lower Susitna River, Upper Kenai Peninsula, and Lower Kenai Peninsula. Of these, the majority of the impaired sites (fair and worse) were in Anchorage and the Upper Kenai. The best sites were in the Lower Susitna and Upper Kenai.
- This bioassessment framework, i.e., ASCI, has been tested on a variety of stream types typical of Alaska, and is ready to be validated and implemented in other parts of Alaska. The ASCI is based on EPA procedures and is a cost-effective biomonitoring tool designed to enable ADEC to better assess and monitor stream quality throughout Alaska.

The future of the ASCI is the broader development and refinement for statewide implementation. The link between the biological indicator and water regulation is integral to water resource protection. The following figure (Figure 1) illustrates how a quantitative scientific basis of reference data can be transformed to narrative descriptions of biological condition, and used in a regulatory context for water quality programs.

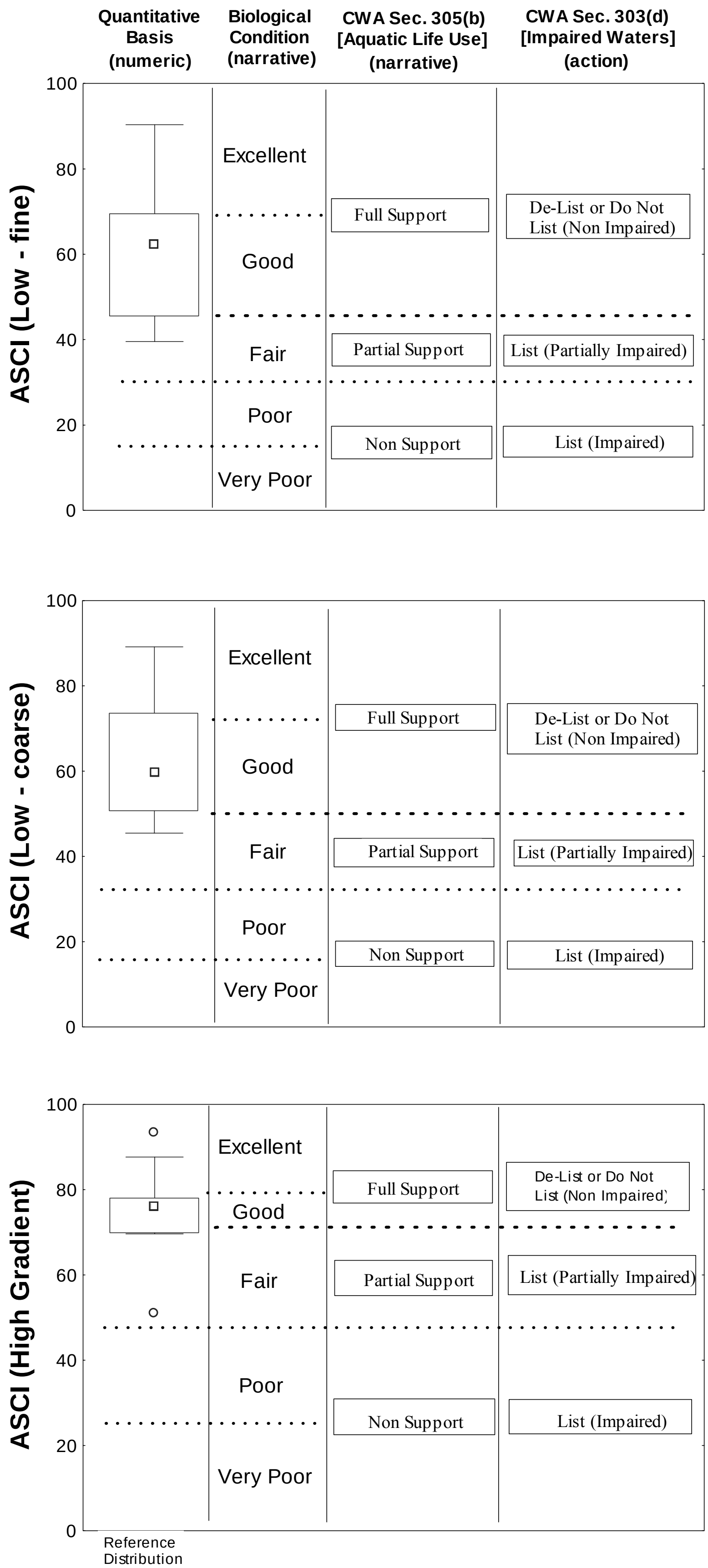


Figure 1. The Alaska Stream Condition Index for each of the 3 stream classes, based on quantitative reference distributions and transformed to narrative descriptions and regulatory actions.

2. THE APPLICATION OF STREAM BIOASSESSMENT IN ALASKA

Alaska Department of Environmental Conservation (ADEC) has undertaken an important initiative to enhance its aquatic monitoring and assessment program using biological information and to seek collaborative ventures with other agencies and environmental groups for water resource protection. In 1997, ADEC contracted the University of Alaska Anchorage's Environment and Natural Resources Institute (ENRI) to facilitate a major cooperative effort to develop bioassessment procedures and a framework for conducting cost-effective, scientifically valid stream assessments using an integration of biological, physical, and chemical data.

ENRI's biological monitoring and assessment program has been funded by the Alaska Department of Environmental Conservation (ADEC) under the USEPA Non-point Source Program (Clean Water Act Section 319). It is being supplemented with the assistance of volunteer professional biologists, citizen volunteers, and state and federal agency representatives. Cooperative partners have included the U.S. Department of Agriculture Natural Resource Conservation Service, Alaska Department of Natural Resources, Alaska Department of Fish and Game, ADEC, Native American Fish and Wildlife Society, Alaska Railroad, U.S. Geological Survey, Alaska Cooperative Extension Service and Matanuska-Susitna Borough, Anchorage, Wasilla, and Homer Soil and Water Conservation Districts, U.S. Environmental Protection Agency, Municipality of Anchorage, U.S. Geological Survey. ENRI continues to collaborate with many federal and state agencies as well as nonprofit organizations to meet water quality goals for biological integrity as set forth in the Clean Water Act.

The Clean Water Act of 1972 (PL-92-500) has as one of its primary goals the maintenance and restoration of biological integrity, which incorporates biological, physical, and chemical quality. This concept refers to the natural assemblage of indigenous organisms that would inhabit a particular area if it had not been affected by human activities. This integrity or naturally occurring structure and function of the aquatic community becomes the primary reference condition used to measure and assess waterbodies in a particular region.

> ***Biological integrity*** is commonly defined as "the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity and functional organization comparable to that of the natural habitat of the regions" (Karr and Dudley 1981, Gibson et al. 1996).

Through the 303(d) and Total Maximum Daily Load (TMDL) framework outlined in the Clean Water Act of 1972 (and revisions of 1977, 1987), those waters considered to be impaired and threatened must be identified and improved to meet their designated uses. The definition of impairment by natural resource management or regulatory agencies is typically based on attainment or non-attainment of numerical water quality standards associated with a waterbody's designated use. If those standards are not met (or attained), then the waterbody is considered to be impaired. Resident biota in a watershed function as continual natural monitors of environmental quality, responding to the effects of both episodic as well as cumulative pollution and habitat alteration. Conducting ambient biological surveys is one of the primary approaches to biomonitoring. These surveys, in turn, are used to measure the attainment of biological integrity. The assessment of ecosystem health cannot be done without measuring the attainment of biological integrity goals as directed by USEPA and characterized by the state of Alaska.

Careful measurement of the natural aquatic ecosystem and its constituent biological communities can determine the condition of biological integrity. Several key attributes are measured to indicate the quality of the aquatic resources. Biological surveys establish the attributes or measures used to summarize several community characteristics, such as taxa richness, number of individuals, sensitive or insensitive species, observed pathologies, and the presence or absence of essential habitat elements.

Biological measurements, called metrics, represent elements of the structure and function of the bottom-dwelling macroinvertebrate assemblage. Metrics change in some predictable way with increased human influence (Barbour et al. 1996). They include specific measures of diversity, composition, and functional feeding group representation and include ecological information on tolerance to pollution. Multimetric indices, such as the IBI, incorporate multiple biological community characteristics and measure the overall response of the community to environmental stressors (Karr et al. 1986, Barbour et al. 1995). Such a measure of the structure and function of the biota (using a regionally-calibrated multimetric index) is an appropriate indicator of ecological quality, reflecting biological responses to changes in physical habitat quality, the integrity of soil and water chemistry, geologic processes, and land use changes (to the degree that they affect the sampled habitat).

> This study was designed to address the following objectives:
>
> - Establish a framework for ADEC's statewide biological monitoring and assessment program.
> - Develop regional reference conditions as a basis for bioassessment for the Cook Inlet Ecoregion (i.e., Kenai Peninsula, Municipality of Anchorage and the Matanuska and Susitna river basins).
> - Provide ADEC with an Alaska Stream Condition Index for use in biological assessments of streams throughout Alaska.

Multimetric, macroinvertebrate indices of biotic integrity, variously called RBP (Rapid Bioassessment Protocol; Plafkin et al. 1989; Barbour et al. 1999), ICI (Invertebrate Condition Index; Ohio EPA 1989), B-IBI (Benthic IBI; Kerans and Karr 1994), and SCI (Stream Condition Index; Barbour et al. 1996; Major et al. 1997; Major and Houston 1998), have been developed for many regions of North America and are generally accepted for biological assessment of aquatic resource quality (e.g., Southerland and Stribling 1995; Karr 1991; Gibson et al. 1996). The framework of bioassessment consists of characterizing reference conditions upon which comparisons can be made and identifying appropriate biological attributes with which to measure the condition. Reference conditions are "best available" conditions where biological potential is at its highest for the particular region or area. These reference conditions are representative of sustainable ecosystem health.

The purpose of this study was to develop a multimetric biological index for Alaska streams. From this bioassessment framework that is calibrated for the Cook Inlet Ecoregion, further refinement and validation of other regions of Alaska could be done. Application of this biological index in Alaska would be for nonpoint source impact investigations, watershed assessments (305b), and listing/delisting of impaired waters (303d). The success in ADEC's implementation of bioassessment would be realized when a statewide program is in place to evaluate impacts to Alaska's water resources from multiple and cumulative stressors.

3. ESTABLISHING STREAM CLASSES AS A FRAMEWORK FOR BIOASSESSMENT

Biological systems naturally vary in composition and diversity of fauna depending on the characteristics and geomorphology of the surrounding environment (in this case, streams) in which they reside. Partitioning this natural variability into relatively homogenous classes can aid in detecting biological differences attributable to human impacts. However, before attributing biological differences to human impacts, the sampling error must be minimized (using standardized sampling techniques) and the natural effects must be recognized. We expect that natural characteristics of streams (gradient, substrate, habitat, etc.) can be used to categorize streams of similar biological potential. Such categorization (or classification) of streams provides a framework within which assessment of human impacts can proceed.

The stream classification process in this project used both biological and non-biological data from reference streams. Reference conditions were established using streams with undisturbed or minimally impaired watersheds. Criteria were established for reference and impaired site selection (Major et al. 1998). Similarities of the reference site biological communities were first quantified, then non-biological characteristics that "explained" similarities were sought. The explanatory variables defined the classes of streams with similar biological composition. This process was accomplished using ordination techniques and subsequent comparison of metric value ranges distributed among the classes.

Explanatory Variables

- gradient of stream
- substrate composition
- diversity of habitat for biota

We would expect different responses of aquatic organisms to stress in naturally variable systems. For instance, biota in low gradient streams may be more tolerant to dissolved oxygen fluctuations than those in high gradient streams. In addition, organisms in fine sediment are more tolerant to a wide range of stressors than those in coarse or hard substrate. Increased diversity of aquatic habitat induces an increased diversity of organisms.

Conclusions: Three stream classes (non-glacial) were identified for Alaskan streams in the Cook Inlet ecoregion:

1. **Low Gradient – fine substrate**
   Fine substrates, banks, and aquatic vegetation are predominant (> 40%) instream habitats.
2. **Low Gradient – coarse substrate**
   Boulders, cobbles, clean gravel, and large woody debris are predominant (> 40%) instream habitats.
3. **High Gradient (> 2% grade)**
   Steeper streams with riffles, hard substrates, and straight channels.

Justifications for the conclusions to support three stream classes are as follows:

- The ordination diagram illustrates a distinct cluster of *Low Gradient – fine substrate* samples with similar biological composition (Figure 1), using the explanatory variables mentioned above.

- The *Low Gradient – coarse substrate* and the *High Gradient* stream classes show considerable overlap in Figure 2, and no physicochemical variables could be found to explain the ordinal arrangement of streams of these two classes. Other physicochemical variables evaluated included ecoregion, river basin, apparent land use, drainage basin size, discharge, elevation, sample date, water chemistry, and estimated substrate composition. However, biological variables helped to differentiate classes (see below).

- The ordination uses genus level data for midges (Diptera: Chironomidae). This information was not available for all samples because midges were identified at the family level earlier in the sampling program. Experimental ordinations using family level midge data were not as distinct because the predominance of midges in the samples overpowered less numerous taxa.

- The taxa that were most influential in the ordination include Simuliidae, Baetidae, and *Zapada*, all of which were common in the samples.

- To the right of axis one were the samples that were collected from fine sediments, banks, and aquatic vegetation. These samples had lower relative abundance of EPT's (mayflies, stoneflies, and caddisflies) and higher relative abundance of non-insects and some of the midges. Many of the samples were collected from meandering, low gradient streams in the upper Kenai Peninsula.

- At the lower end of the axis two of the ordination were samples dominated by blackflies (Diptera: Simuliidae). At the left of the diagram were those samples dominated by EPT's.

- Earlier analysis suggested that two stream classes existed in sites with coarse substrates: *Low Gradient* and *High Gradient* (Major and Houston 1999, Major et al. 1998).

- Reference biological conditions are unique in each stream class, as can be illustrated with box and whisker plots of common metric values (Figure 3). Relative to the other two stream classes, the *Low Gradient – fine substrate* reference condition is characterized by fewer EPT and intolerant taxa, as well as fewer EPT and non-chironomid Diptera individuals. The *Low Gradient – coarse substrate* reference condition has greater numbers of EPT and intolerant taxa, more EPT individuals, and the lowest HBI values. The *High Gradient* reference condition has intermediate ranges of many metrics, but has greater numbers of non-chironomid Diptera individuals and fewer collectors.
- Although the reference metric distributions of the *Low Gradient – coarse substrates* and the *High Gradient* stream classes are similar in many respects, the responses of metrics to stress are very different among these two classes (see Chapter 4).

Analytical Methods

- *Non-metric Multidimensional Scaling (NMDS) Ordination* – Spatial array of sites based on similarity/difference of benthic composition and abundance.

- *Box-and-Whisker Plots* – Display of ranges of values for the biological data oriented by proposed stream classes.

See Appendix A for a full discussion of methods.

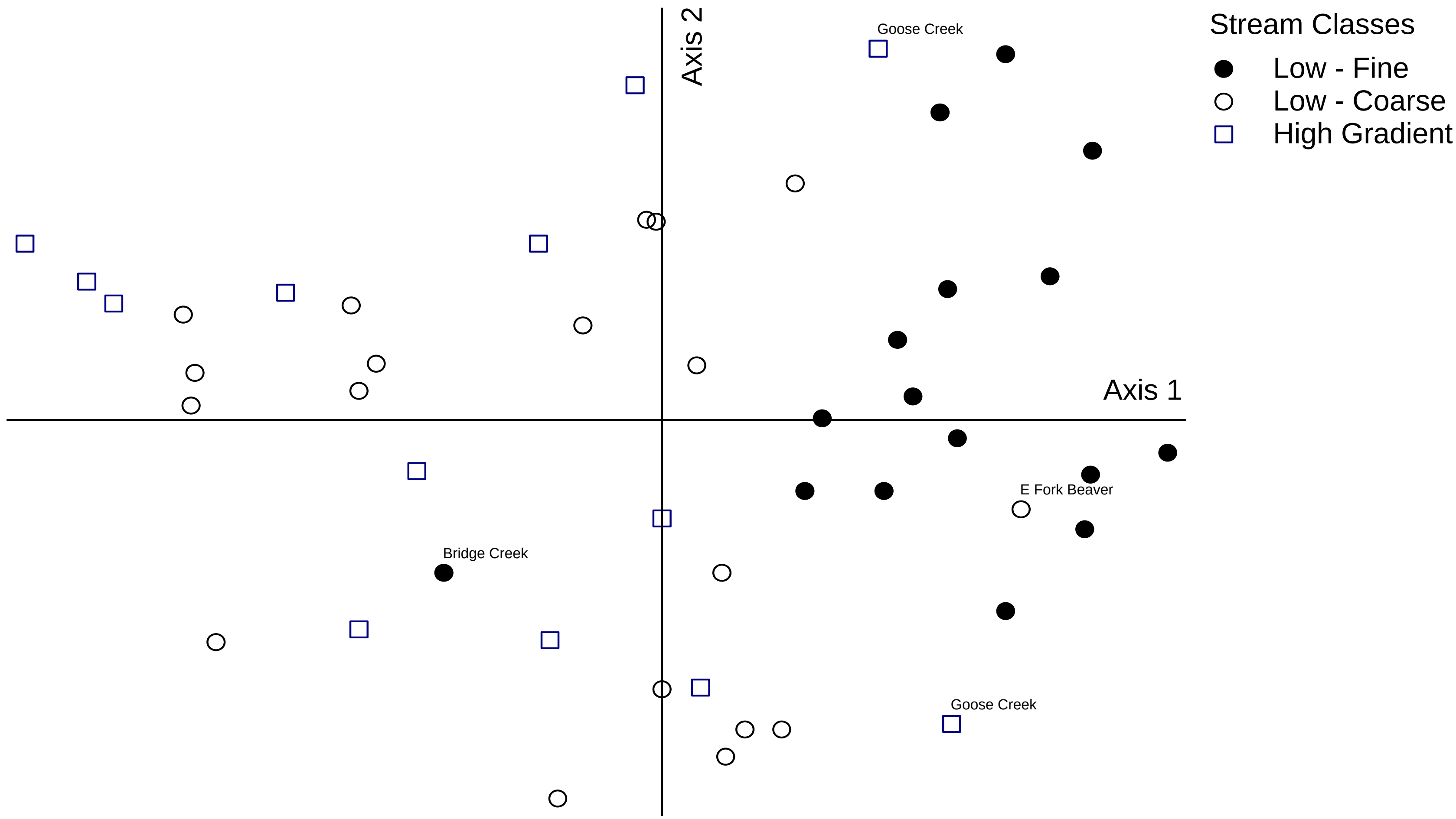


Figure 2. NMDS ordination of reference and subreference samples (Chironomid identifications at the genus level). Three stream classes have been identified, with the *Low Gradient – fine substrate* stream class distinctly grouped in this diagram. Only outlying samples are identified.

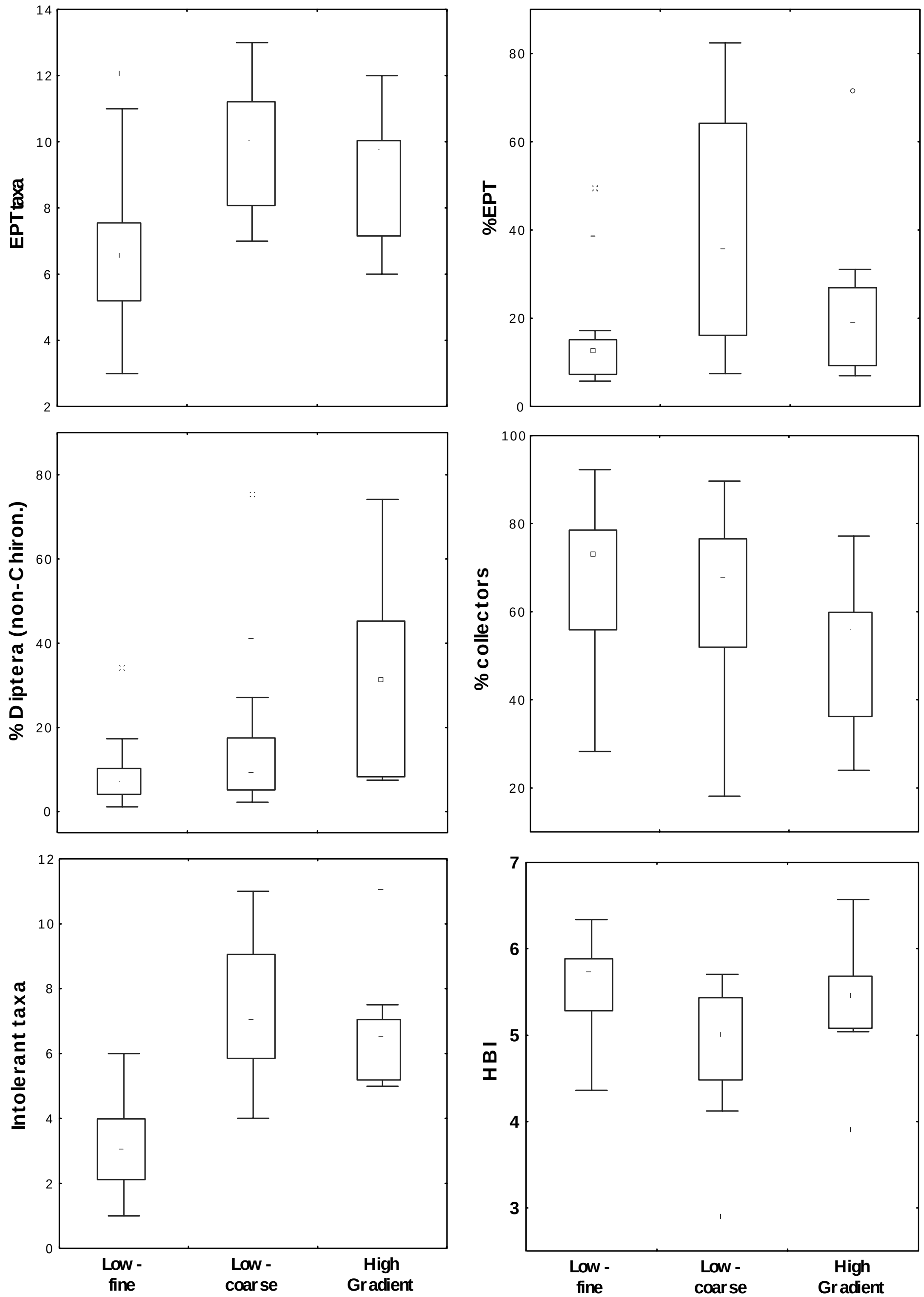


Figure 3. Distribution of metric values in populations of reference sites of three stream classes, illustrating some of the differences in composition that were not apparent in the NMDS ordination.

4. CHOOSING BIOLOGICAL METRICS

Biological metric evaluation involves comparison of the community metrics of reference sites to those of stressed sites. Differences in metric value distributions from reference and stressed sites illustrate the biological changes that occur when environmental insults are introduced. Metrics are evaluated for the consistency, degree, and biological significance of responses to increasing stress.

> **Biological Metrics**
>
> A biological metric is a measurement of community characteristics calculated from taxa enumeration or presence/absence data. Metrics usually change in some predictable way with increasing human influence.

In this project, 80 biological metrics from 4 metric categories were calculated and evaluated. A partial listing of the evaluated metrics is shown in Table 1. The metric categories were richness, composition, functional organization, and pollution tolerance. Richness metrics measure the diversity of taxa in specified taxa groups. In general, greater diversity is a sign of a healthy ecosystem. The presence of only a few taxa is indicative of a loss of pollution sensitive organisms. Composition metrics measure percent representation by a specified taxon or taxa group. Composition metrics can increase or decrease with increasing stress, depending on the characteristics of the taxa group being measured. As an example, the percentage of mayflies, stoneflies, and caddisflies (% EPT) usually decreases with increasing stress because these organisms are generally intolerant of disturbances and pollution. Functional organization metrics are measures of the predominant ways the community eats, moves, or reproduces. Either counts of taxa or percentages of individuals with the specified characteristic are calculated. Percent predators and swimmer taxa are examples of functional metrics. The responses of functional metrics to stresses are variable. Pollution tolerance metrics convey information on the ability of the community to withstand stress. The Hilsenhoff Biotic Index and % tolerant organisms are examples of pollution tolerance metrics. For these metrics, pollution tolerance values ranging from 0 (most sensitive) to 10 (most tolerant) have been assigned to each taxon.

> **Metric Categories:**
>
> *Richness* — counts of distinct taxa within selected taxonomic groups.
>
> *Composition* — proportions of individuals belonging to selected taxonomic groups.
>
> *Functional Organization* — counts or proportions of taxa based on mode of feeding, mechanism for mobility, or frequency of reproduction.
>
> *Pollution Tolerance* — counts, proportions, or weighted scores of taxa based on ability to survive exposure to pollutants.

The worth of each metric as an indicator of biotic condition was evaluated by comparing metric values from reference sites to those from stressed sites within each site class. Metrics with values that consistently and meaningfully distinguished between reference and stressed conditions were retained as possible index components. Reference conditions in cold streams with stable, climax vegetation in the watershed (e.g., alpine and oligotrophic streams) are generally nutrient and sediment poor. Thus, slight additions of nutrients and sediments to these systems may create conditions suitable to a greater diversity of organisms, even pollution sensitive ones. This potential response of the biota would result in increased metric values in the stressed sites (over the reference condition), when conventional response to pollutants is a decrease in value. Because metric values respond variably to stress, the values are interpreted as standardized scores that indicate worse or better conditions on a 0 to 100 scale.

Methods: Step 1, Metric Evaluation

- *Box-and-whisker plots* – visual assessment to determine discrimination of metric values between reference and stressed sites in each site class (see example, Figure 4 (example BW)).
- *Discrimination efficiency (DE)* – calculation of the percentage of stressed samples with metric values worse than the worst 25th percentile of reference values. A high DE is good.
- *Metric scoring* – conversion of metric values to scores based on responses to increasing stress. Scoring is on a linear scale from 100 (best) to 0 (worst).

Methods: Step 2, Candidate Metric Selection

Metrics are retained for further analysis if they:

- show value and score differences between reference and stressed sites;
- represent a unique aspect of the community (one of the metric categories); and
- are not redundant with other component metrics (see Chapter 5 for this analysis).

See a detailed discussion of methods in Appendix A.

Conclusion: Candidate Metrics

The following metrics were candidates for inclusion in the indexes:

Low Gradient – fine substrate	*Low Gradient – coarse substrate*	*High Gradient*
EPT taxa	EPT taxa	EP taxa
Trichoptera taxa	Ephemeroptera taxa	Plecoptera taxa
Shannon-Wiener index	Trichoptera taxa	Trichoptera taxa
% EPT	% EPT	Shannon-Wiener index
% EPT (no Baet. or Zap.)	% EP	% EPT
% Trichoptera	% Ephemeroptera	% Ephemeroptera
% Diptera	PEPHEM_NB	% Plecoptera
% Chironomidae	% Plecoptera	% *Zapada* + Baetidae
O/E (family 75%)	% *Zapada* + Baetidae	% Diptera
% collectors	Baetidae/Ephemeroptera	O/E (family 75%)
% filterers	% non-insects	% collectors
% dominant	O/E (family 75%)	% filterers
Beck's Index	% scrapers	% shredders + scrapers
HBI	% tolerant	% scrapers
% clingers	HBI	% shredders

Table 1. Partial listing of metrics evaluated for ability to discern reference from stressed stream conditions. Metrics that are not described are minor modifications of those listed below.

Metric	Metric Description
Richness	
Total taxa	Measure of the overall diversity of the macroinvertebrate assemblage
EPT taxa	Number of taxa in the insect orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)
EP taxa	Number of mayfly and stonefly taxa
Ephemeroptera taxa	Number of mayfly taxa
Plecoptera taxa	Number of stonefly taxa
Trichoptera taxa	Number of caddisfly taxa
Diptera taxa	Number of "true" fly taxa (includes midges at the family level)
Shannon-Wiener Index[1]	A measure of both diversity and evenness
Composition	
% EPT	Percent mayfly, stonefly, and caddisfly individuals in the sample
% EPT taxa	Percent mayfly and stonefly individuals in the sample
% EPT (no Baetidae or Zapada)	Percent EPT exclusive of 2 tolerant taxa
% Ephemeroptera	Percent mayfly nymphs
% Ephemeroptera (no Baetidae)	Percent Ephemeroptera exclusive of 1 tolerant taxon
% Plecoptera	Percent stonefly nymphs
% Trichoptera	Percent caddisfly larvae
% Zapada + Baetidae	Percent of 2 apparently tolerant taxa
Baetidae/Ephemeroptera	Baetid mayflies of all mayflies
% Diptera	Percent "true" fly larvae and pupae
% Chironomidae	Percent midge larvae and pupae (a subset of % Diptera)
% Oligochaeta	Percent aquatic worms
% non-insects	Percent non-insects
% dominant taxon	Percent of the most abundant taxon
% model affinity (PMA)	Degree of agreement between sample composition and idealized model of composition of the reference condition
Observed/Expected taxa	Number of observed taxa of those commonly occurring in the reference condition (including variations of taxonomic resolution and threshold for defining "expected" taxa)
Functional Organization	
Collectors (% and taxa)	Percent of individuals and number of taxa that feed on detrital deposits or loose surface films
Filterers (% and taxa)	Percent of individuals and number of taxa that feed on suspended detritus
Predators (% and taxa)	Percent of individuals and number of taxa that prey on living organisms
Scrapers (% and taxa)	Percent of individuals and number of taxa that feed on attached organic matter
Shredders (% and taxa)	Percent of individuals and number of taxa that "shred" organic litter
Clingers (% and taxa)	Percent of individuals and number of taxa adapted for inhabiting flowing water, as in riffles
Semivoltine taxa	Number of taxa that require more than one year to reproduce
% multivoltine	Percent of individuals that reproduce more than once a year
Pollution tolerance	
% tolerant	Percent of sample considered tolerant of perturbation (tolerance values 7 - 10)

Table 1 (continued). Partial listing of metrics evaluated for ability to discern reference from stressed stream conditions. Metrics that are not described are minor modifications of those listed below.

Metric	Metric Description
Intolerant taxa	Number of taxa considered to be sensitive to perturbation (tolerance values 0 - 3)
Beck's Biotic Index	Weighted sum of intolerant taxa (= 2*number of most sensitive taxa + number of less sensitive taxa)
HBI	The average tolerance value of all individuals in the sample

[1] Shannon-Wiener Index = Σ -((n/N)*Log(n/N))/Log(2); where n is the number of individuals in a taxon and N is the number of individuals in the sample, summed for all taxa in the sample.

Justifications for the conclusions are as follows:

- 29 metrics showed potential for discriminating between reference and stressed conditions in at least one of the stream classes. 15 metrics were selected as candidates for use in an index for each stream class. For these metrics, applicable DE's were calculated (Table 2). Higher DE's indicate better separation between reference and stressed metric values. Figure 4 illustrates how DE is determined with respect to the reference sites in each stream class.

- Only 2 metrics performed well in all three site classes: % EPT and Observed/Expected taxa (O/E, family 75%). The O/E metric describes the percentage of taxa that are common to the reference condition but that do not occur in the evaluated sample. In the O/E (family 75%) metric, the common taxa are defined as those occurring in at least 75% of the reference samples when taxa are compiled at family or higher taxonomic levels.

- In the *Low Gradient – fine substrate* stream class, the metrics most responsive to stress (DE > 70%) were Trichoptera taxa, % Trichoptera, and O/E (family 75%).

- In the *Low Gradient – coarse substrate* stream class, the best performing metrics were % Ephemeroptera (without Baetidae) and the ratio (%) of Baetidae to Ephemeroptera.

- In the *High Gradient* stream class, 12 metrics evaluated had DE's greater than 70%. However, several metrics were unconventional in their response to increasing stress, i.e., increasing when they generally decrease or vice versa. The unconventional responses may be due to oligotrophic reference conditions that do not support a diverse fauna. The taxa that can inhabit such oligotrophic conditions are adapted to the lack of nutrients in these systems. Therefore, the introduction of minimal nutrient and/or sediment stresses may provide sufficient habitat conditions for a greater diversity and more intolerant individuals. Metrics in the pollution tolerance category were not evaluated because "tolerance" may have a different meaning in this site class.

- While these analyses identified 15 candidate metrics for each stream class, further listing of redundancy determination and iterative combinations of metrics for the most robust index was required. Therefore, the core metrics for the Alaska Stream Condition Index (ASCI) were determined as described in Chapter 5.

Table 2. Discrimination efficiency (DE) of 29 candidate metrics scores in three stream classes. DE's were not calculated for metrics with distributions that did not show discrimination in the box and whisker plots. The 25th percentile of reference sites was used to determine DE. Each metrics trend with increasing stress is listed as positive or negative.

	Low Gradient - fine		Low Gradient - coarse		High Gradient	
Metric	DE	Trend	DE	Trend	DE	Trend
EPT taxa	60.9	-	48.5	-	.	
EP taxa	.		.		45.5	+
Ephemeroptera taxa	.		63.6	-	.	
Plecoptera taxa	.		.		100.0	+
Trichoptera taxa	78.3	-	69.7	+	90.9	-
Shannon-Wiener	69.6	-	.		72.7	+
% EPT	47.8	-	33.3	-	100.0	+
% EP	.		48.5	-	.	
% Ephem. (no Baetidae)	.		75.8	-	.	
% EPT (no Baet. or Zapada)	69.6	-	.		.	
% Ephemeroptera	.		50.0	-	100.0	+
% Plecoptera	.		51.5	-	81.8	+
% Trichoptera	73.9	-	.		.	
% Zapada + Baetidae	.		43.9	-	100.0	+
Baetid/Ephemeroptera	.		71.2	+	.	
% Diptera	69.6	+	.		90.9	-
% Chironomidae	60.8	+	.		.	
% non-insects	.		57.5	+	.	
% dominant	60.8	+	.		.	
O/E (fam. 75%)	87.0	-	54.5	-	72.7	-
% collectors	52.2	+	.		63.6	+
% filterers	47.8	-	.		54.5	-
% shredders + scrapers	.		.		100.0	+
% scrapers	.		66.7	-	90.9	+
% shredders	.		.		90.9	+
% clingers	56.5	-	.		.	
% tolerant	.		50.0	+	.	
Beck's Index	56.5	-	.		.	
HBI	47.8	+	59.1	+	.	
Reference n	14		18		8	
Stressed n	23		66		11	

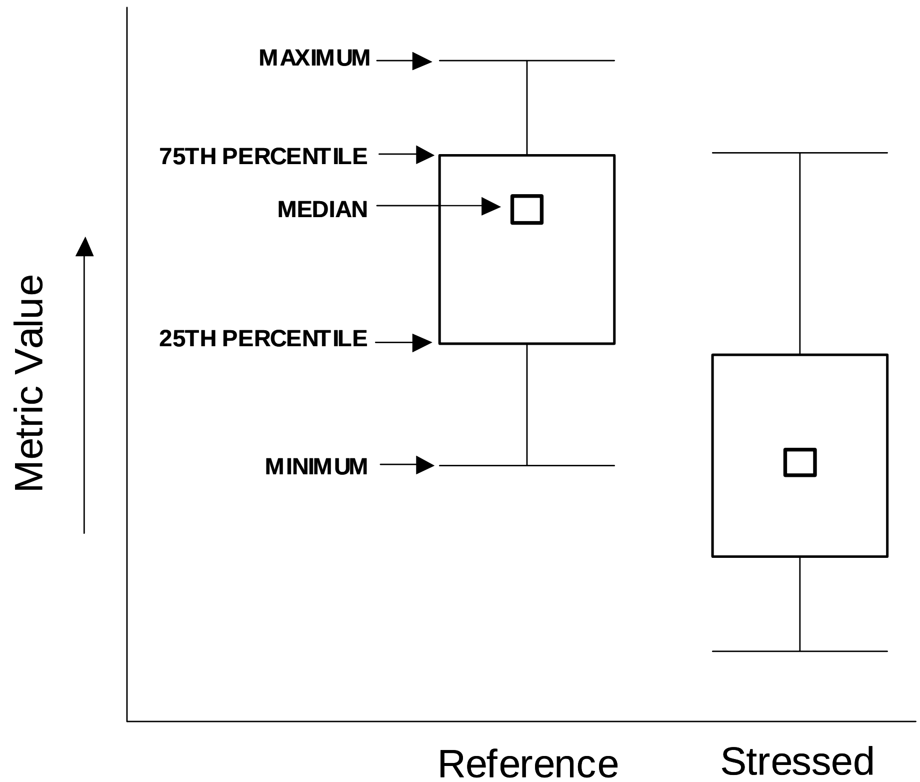


Figure 4. Example of a box and whisker diagram showing excellent metric discrimination between reference and stressed site conditions. Distribution statistics are annotated for the reference condition. The DE is the percentage of stressed sites that fall below the 25th percentile of reference sites.

5. AGGREGATING METRICS INTO A BIOLOGICAL INDEX

A biological index combines non-redundant biological metrics from different metric categories into a single numerical estimate of biological stream condition. Scores of the component metrics are averaged to give an index range of 0 to 100. The strategy for index development attempts to maximize the ability of the index to discern reference from stressed conditions while including meaningful and unique biological metrics. The use of a multimetric index facilitates the detection of impairment from multiple stressors because the component metrics have varying responses depending upon the perturbation (Karr et al. 1986, Barbour and Yoder 2000). Because metric responses differed among stream classes, index development was conducted separately for each stream class (i.e., *Low Gradient – fine substrate, Low Gradient – coarse substrate, High Gradient*).

Biological Index Development

- An index is specific to a stream class.
- Metrics with high discrimination efficiencies (DE's) are selected.
- Metrics from all metric categories are included (if possible).
- Scores of core metrics are averaged to form an index.
- Several index formulations are attempted.
- Only non-redundant metrics are included in each formulation.
- Metrics with high precision are preferred.
- The DE of each index formulation is calculated.
- The best index is identified as one with high DE, high precision, and meaningful metrics.

Methods: (see detailed discussion of methods in Appendix A)

Scoring – Metric values are standardized as scores from 0 (worst) to 100 (best) before being combined (averaged) in an index.

Correlation analysis – If metrics are correlated at a level of 0.8 or greater, they are redundant and are not used together in any index formulations.

Precision – Sampling variability can be quantified using data from field replicates. This allows specification of confidence ranges around observed metric and index values.

Index discrimination efficiency (DE) – The DE of an index is calculated as it is for individual metrics – as the percentage of stressed samples with scores worse than the 25^{th} percentile of reference scores.

Conclusions: Alaska Stream Condition Index (ASCI)

Core Metrics for each Stream Class:

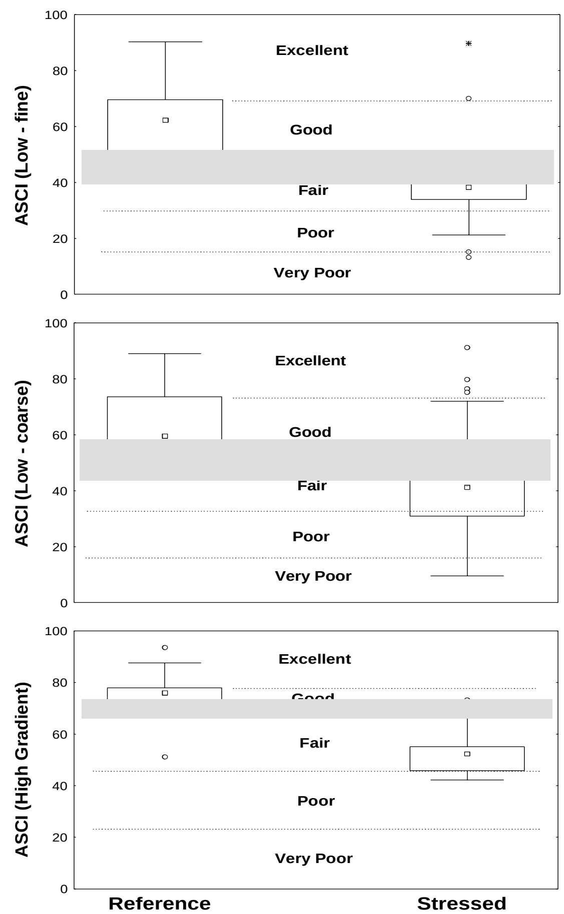
Low Gradient – fine substrate	*Low Gradient – coarse substrate*	*High Gradient*
Trichoptera taxa	Ephemeroptera taxa	Trichoptera taxa
% EPT (no Baetids or *Zapada*)	% Ephemeroptera (no Baetids)	EP taxa
% Diptera	% Plecoptera	% *Zapada* and Baetids
O/E (family 75%)	Baetidae/Ephemeroptera	% Diptera
% collectors	% non-insects	O/E (family 75%)
HBI	O/E (family 75%)	% collectors
	% scrapers	
	HBI	

Justification for the conclusions are as follows:

- In the *Low Gradient – fine substrates* stream class, the ASCI index contained 6 metrics from four metric categories: Trichoptera taxa, % EPT (no Baetidae or *Zapada*), O/E, % Diptera, % collectors, and the HBI. This index has a DE of 83% and a confidence range of 6.1 points. Distributions of core metric values are shown in Figure 5.

- The core metrics for the *Low Gradient – coarse substrate* stream class included 8 metrics from 4 metric categories: Ephemeroptera taxa, % Ephemeroptera (no Baetids), % Plecoptera, Baetidae of Ephemeroptera, % non-insects, O/E, % scrapers, and the HBI. This index has the lowest DE of the 3 stream classes (76%) and the highest confidence range of 7.5 points. Because of these factors, 8 metrics are included until further testing and validation are possible. Distributions of core metric values are shown in Figure 6.

ASCI in reference and stressed streams



Thresholds for the narrative ratings are based on percentiles of the reference distribution: 75th, 25th, and trisection of the scale below the 25th. The confidence range is shown in gray for an observation near the Good/Fair threshold.

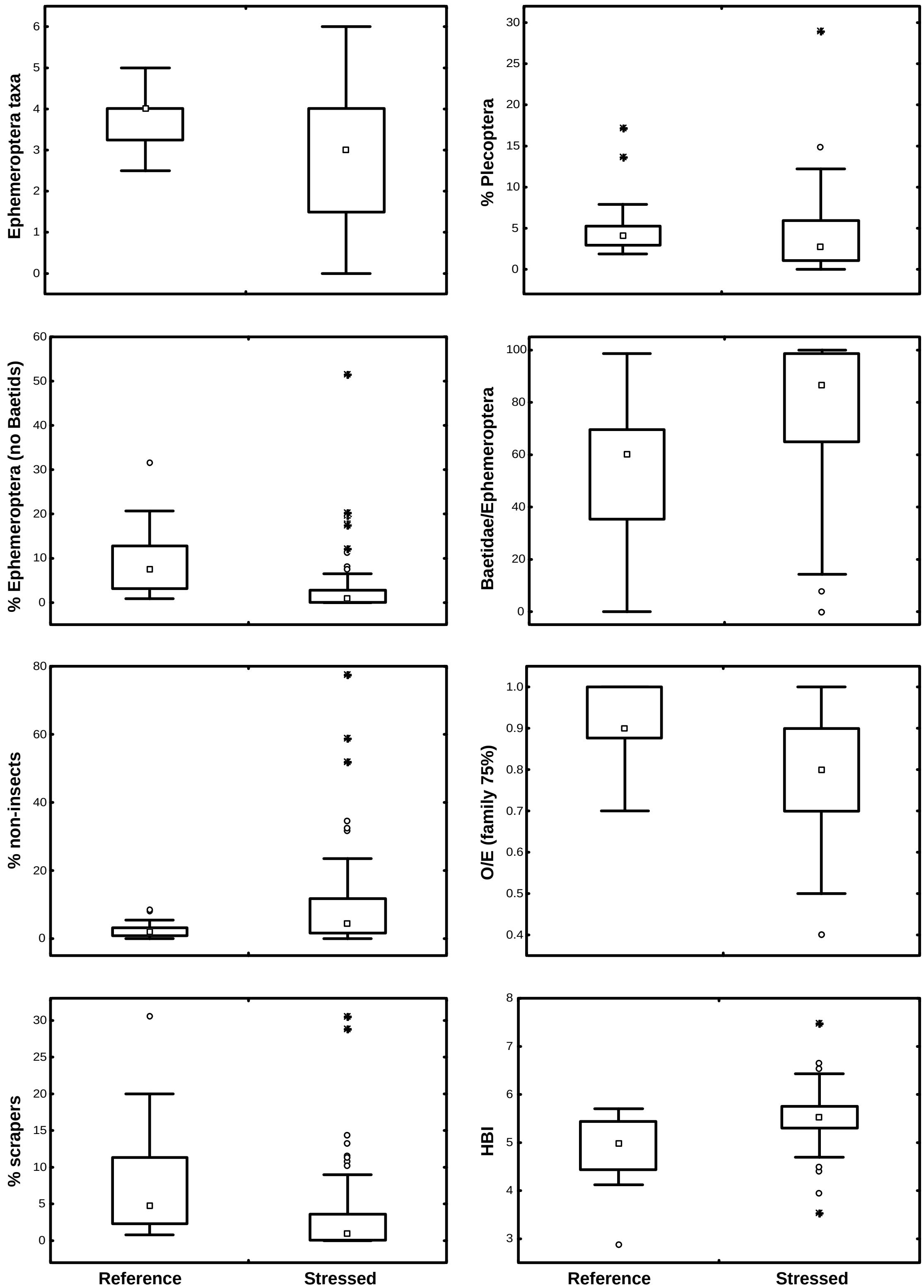


Figure 5. Distributions of core metric values in the *Low Gradient – fine substrate* stream class.

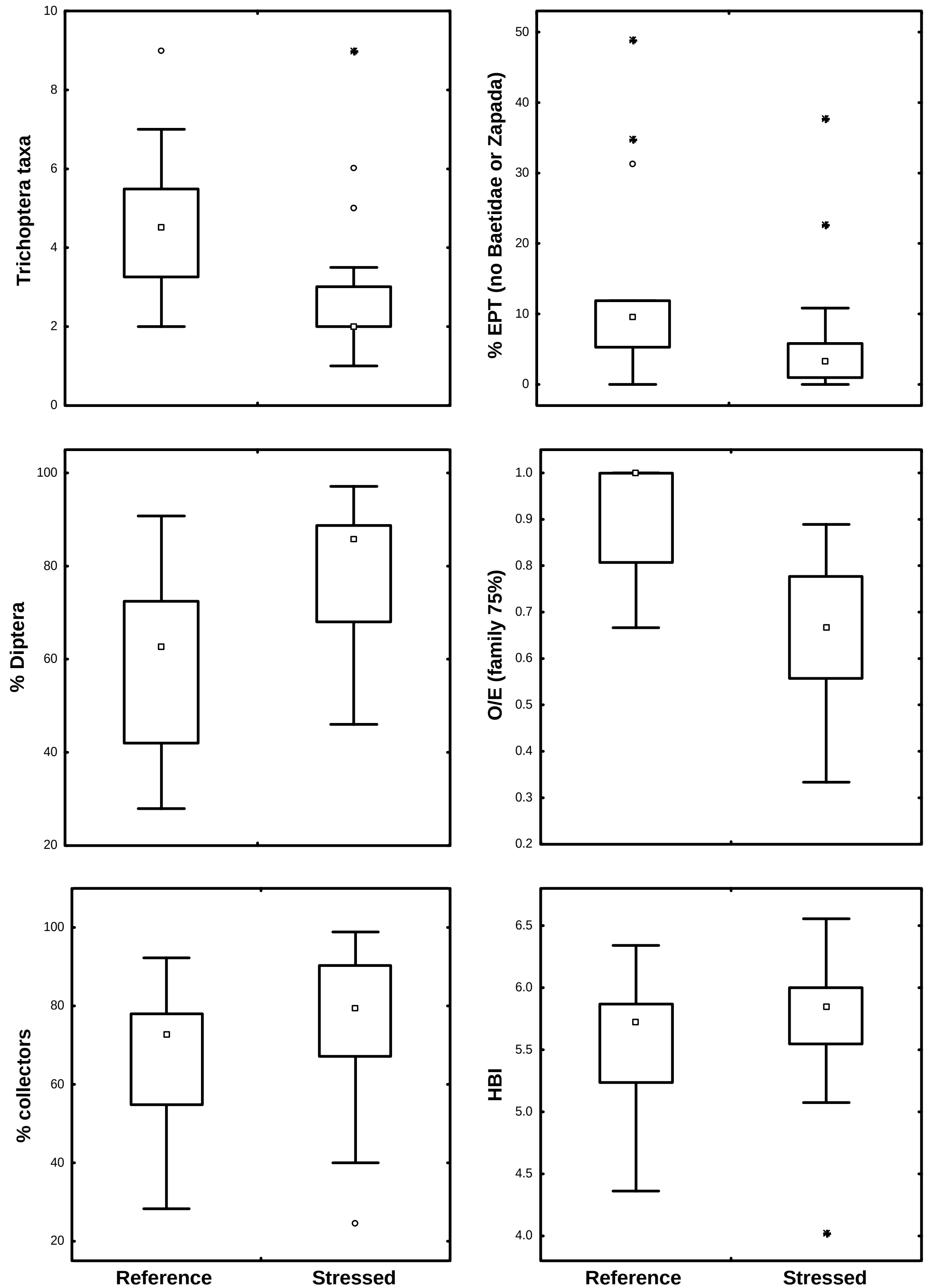


Figure 6. Distribution of core metric values in the *Low Gradient – coarse substrate* stream class.

- In the *High Gradient* stream class, 6 metrics from 3 metric categories compose the ASCI: Trichoptera taxa, EP taxa, % *Zapada* and Baetidae, % Diptera, O/E, and % collectors. No metrics from the pollution tolerance category were included. This index has a DE of 91% and a confidence range of 3.5 points. Distributions of core metric values are shown in Figure 7.

- Index values calculated from scored metrics are used to evaluate overall biological condition. To apply the index, a sample is first designated in one of the 3 stream classes. The core metrics of the stream class are scored using formulas provided in Table 3. Metric scores are averaged to derive an ASCI score.

- Examining core metrics and their contributions to the index can enhance interpretation of the ASCI. Specific community responses may then be interpreted in the context of the site and collection conditions.

- Index thresholds were established to define narrative biological condition categories, using the 25th percentile of reference scores as the threshold between "good" and "fair" conditions. The 75th percentile of reference scores was used as a threshold between "excellent" and "good" conditions and the range below the 25th percentile was evenly divided to obtain 3 levels of impairment ("fair", "poor", and "very poor"). Index scores for these thresholds are listed in Table 4.

- Index rating thresholds may be used as biocriteria for nonpoint source impact investigations, watershed assessments (305b), and listing/delisting of impaired waters (303d). The 25th percentile of reference ("good"/"fair" threshold) can be used as the critical threshold between acceptable and unacceptable conditions.

> *Biocriteria* are based on thresholds determined to differentiate impaired from non-impaired conditions. These thresholds may be subjective, but the appropriateness of the thresholds may be verified with index performance (DE) and precision estimates.

- An ASCI score that includes one of the narrative thresholds within its confidence range should be tentatively assessed at the observed rating and the rating should be confirmed with repeated sampling (more replicates at the site or annual resampling). Replicate sampling will increase precision. Assessors may also apply indexes specific to the other site classes, especially if the original site classification was questionable.

ASCI Application: Steps for Evaluating New Streams

1) Designate stream class for new stream.
2) Calculate core metrics for index.
3) Score and average metrics to obtain index score.
4) Rate stream condition and interpret rating.
5) Use component metrics to aid in cause and effect determination.

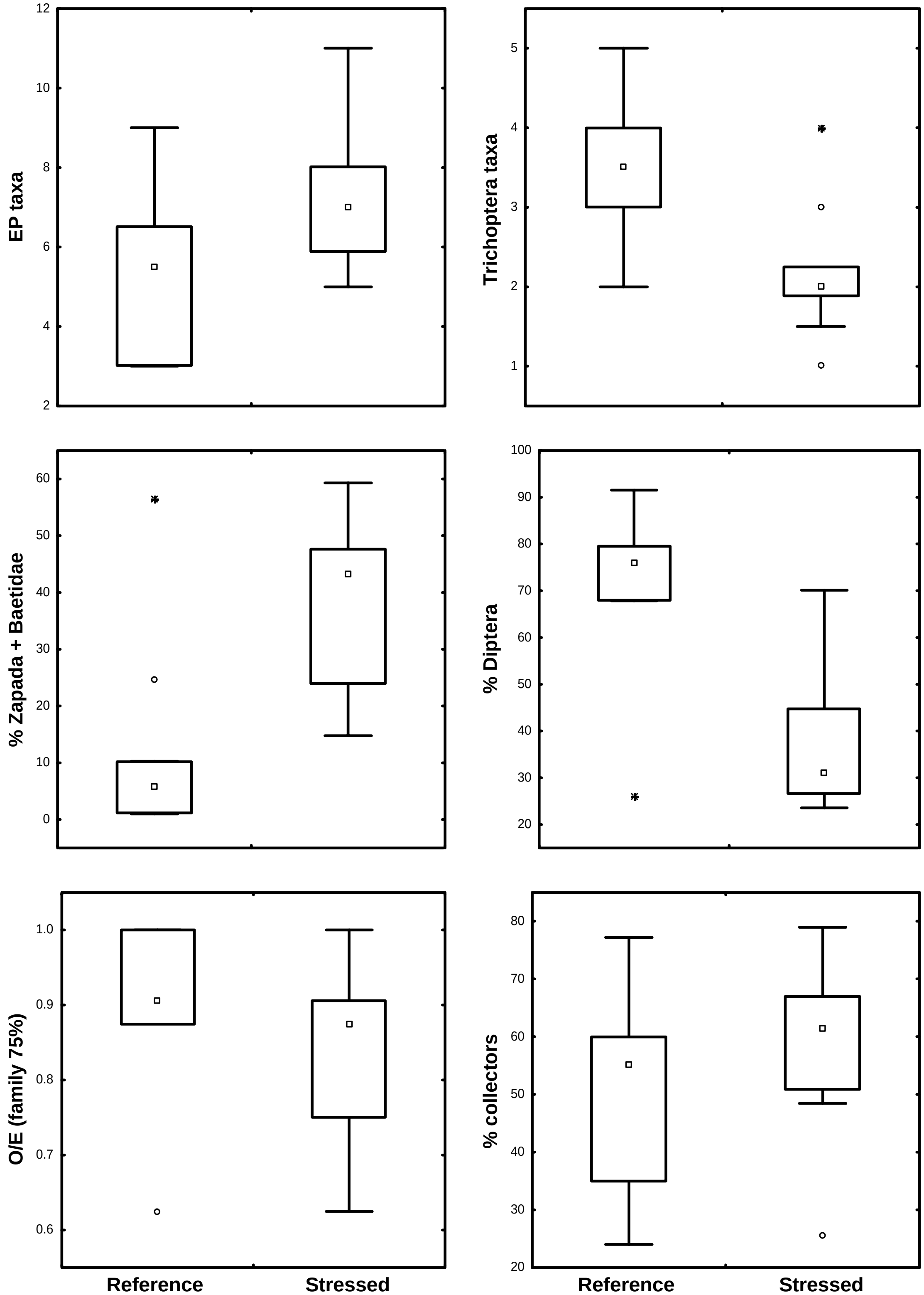


Figure 7. Distribution of core metric values in the *High Gradient* stream class.

Table 3. Scoring formulas for the selected index metrics in each site class, where X is the metric value. The scoring range is 0 to 100; any scores above this range should be set to 100, below the range should be set to 0.

Index Metric	Scoring formula
Low Gradient – fine substrates	
Trichoptera taxa	100 * X / 7
% EPT (no Baetidae or Zapada)	100 * X / 15)
% Diptera	100 * (100 – X) / 70
O/E (family 75%) [1]	100 * X
% collectors	100 * (100 - X) / 70
HBI	100 * (6.5 - HBI) / 2
Low Gradient – coarse substrates	
Ephemeroptera taxa	100 * X / 5.5
% Plecoptera	100 * X / 14
% Ephemeroptera (no Baetidae)	100 * X / 20
Baetidae / Ephemeroptera	100 * (100 - X) / 100
% non-insects	100 * (30 - X) / 30
O/E (family 75%) [2]	100 * X
% scrapers	100 * X / 15
HBI	100 * (6.5 - X) / 2
High Gradient	
EP taxa	100 * (12 - X) / 9
Trichoptera taxa	100 * X / 5
% Baetidae and Zapada	100 * (70 - X) / 70
% Diptera	100 * X / 90
O/E (family 75%) [3]	100 * X
% collectors	100 * (100 - X) / 75

1) The expected taxa in the *Low Gradient – fine substrate* stream class are: Oligochaeta, Hydracarina, Amphipoda, Chironomidae, Simuliidae, Baetidae, Brachycentridae, Leptoceridae, and Limnephilidae.
2) The expected taxa in the *Low Gradient – coarse substrate* stream class are: Hydracarina, Chironomidae, Simuliidae, Baetidae, Ephemerellidae, Heptageniidae, Chloroperlidae, Nemouridae, Perlodidae, and Limnephilidae.
3) The expected taxa in the *High Gradient* stream class are: Chironomidae, Simuliidae, Tipulidae, Baetidae, Ephemerellidae, Heptageniidae, Chloroperlidae, and Limnephilidae.

Table 4. Ratings and corresponding ASCI ranges in three stream classes. Thresholds are based on percentiles of the reference ASCI scores.

Rating	Lower Thresholds	Low - fine	Low - coarse	High Gradient
Excellent	75^{th}	69.6 or better	73.6 or better	78.0 or better
Good	25^{th}	45.1 – 69.5	50.4 – 73.5	69.7 – 77.9
Fair	2/3 of 25^{th}	30.0 – 45.0	33.6 – 50.3	46.5 – 69.6
Poor	1/3 of 25^{th}	15.0 – 29.9	16.8 – 33.5	23.2 – 46.4
Very Poor	0	14.9 or worse	16.7 or worse	23.1 or worse

6. ASSESSMENT OF THE BIOLOGICAL CONDITION OF ALASKA STREAMS

One-hundred-twenty-three (123) stations were sampled along 83 streams and rivers of the Kenai Peninsula, Municipality of Anchorage, and Matanuska/Susitna basin. Assessments using the ASCI show that 46% of the streams (combining multiple sites) received ratings of Good or Excellent. Others streams received ratings of Fair (38%), Poor (14%), and Very Poor (2%). The ASCI had an overall DE of 83% for sites (averaging multiple samples). The DE of 83% signifies that the ASCI (i.e., biological information) was able to correctly identify 83% of the samples as being reference or stressed (determined *a priori* by non-biological data).

- The percentages by ASCI rating stated above do not imply complete coverage of the streams and rivers of the region. The program sampling design specified that reference and stressed streams would be sampled. For regional assessment of all streams and rivers, a random sampling design is required.

- 83% of the reference sites received a rating of "Good" or "Excellent" and 83% of the stressed stations received a rating of "Fair" or worse (Figure 8).

- Frequencies of ratings by river basin show that Anchorage has the most Poor and Very Poor stations (Figure 9). Other basins have predominantly "Fair" or "Good" ratings. The most "Excellent" stations are found in the Upper Kenai basin. These data are not appropriate for basin-wide assessments. A different sampling design would be required to extrapolate to broader assessments.

- When ASCI scores are averaged for all stations along a stream or river, the resulting biological condition ratings are generalized to account for different stream classes along the watercourse. A listing of the 83 streams and rivers with generalized ratings is shown in Table 5). Refer to Appendix B (Data) for more detailed rating information.

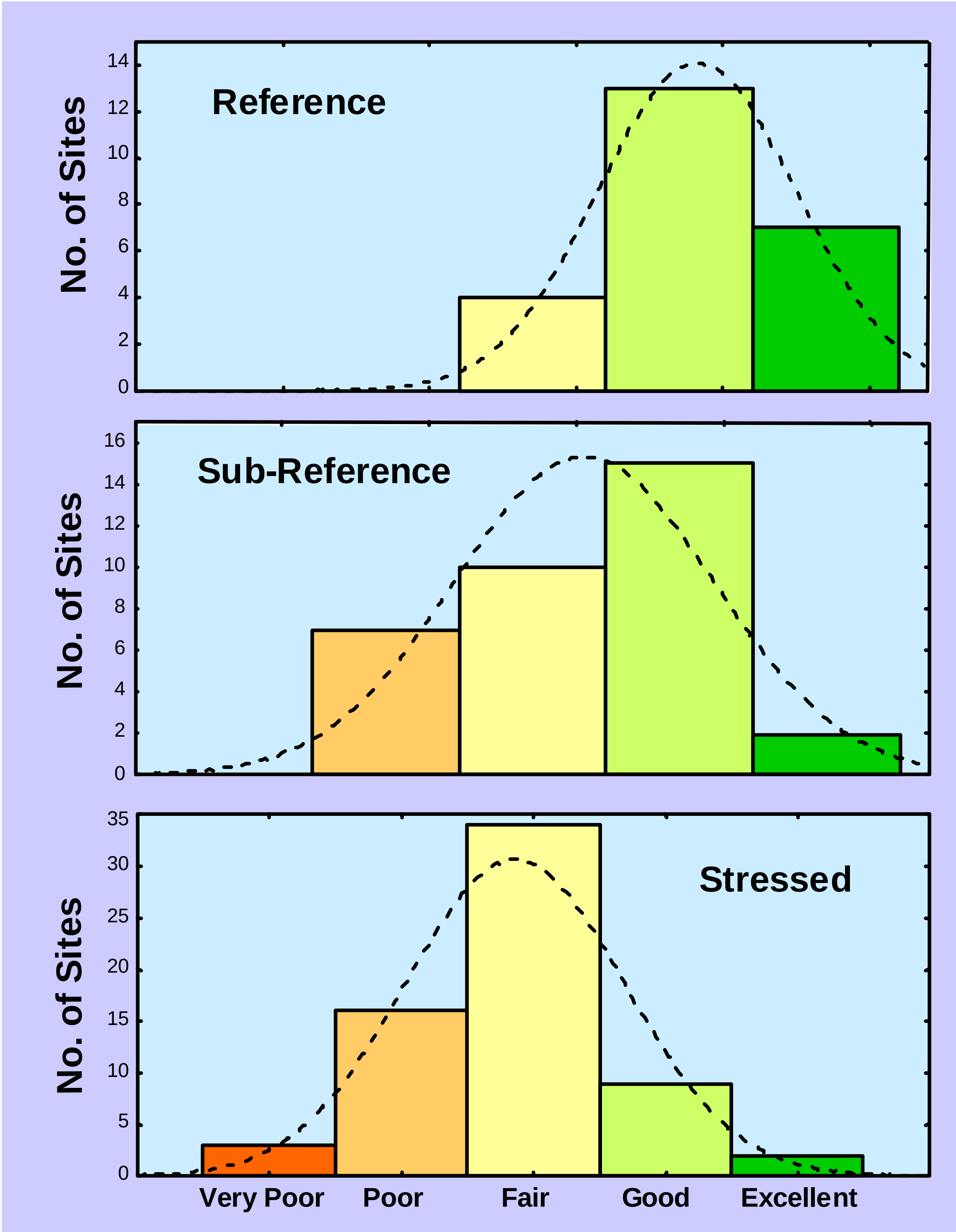


Figure 8. Frequencies of sites by ASCI rating in *a priori* site conditions.

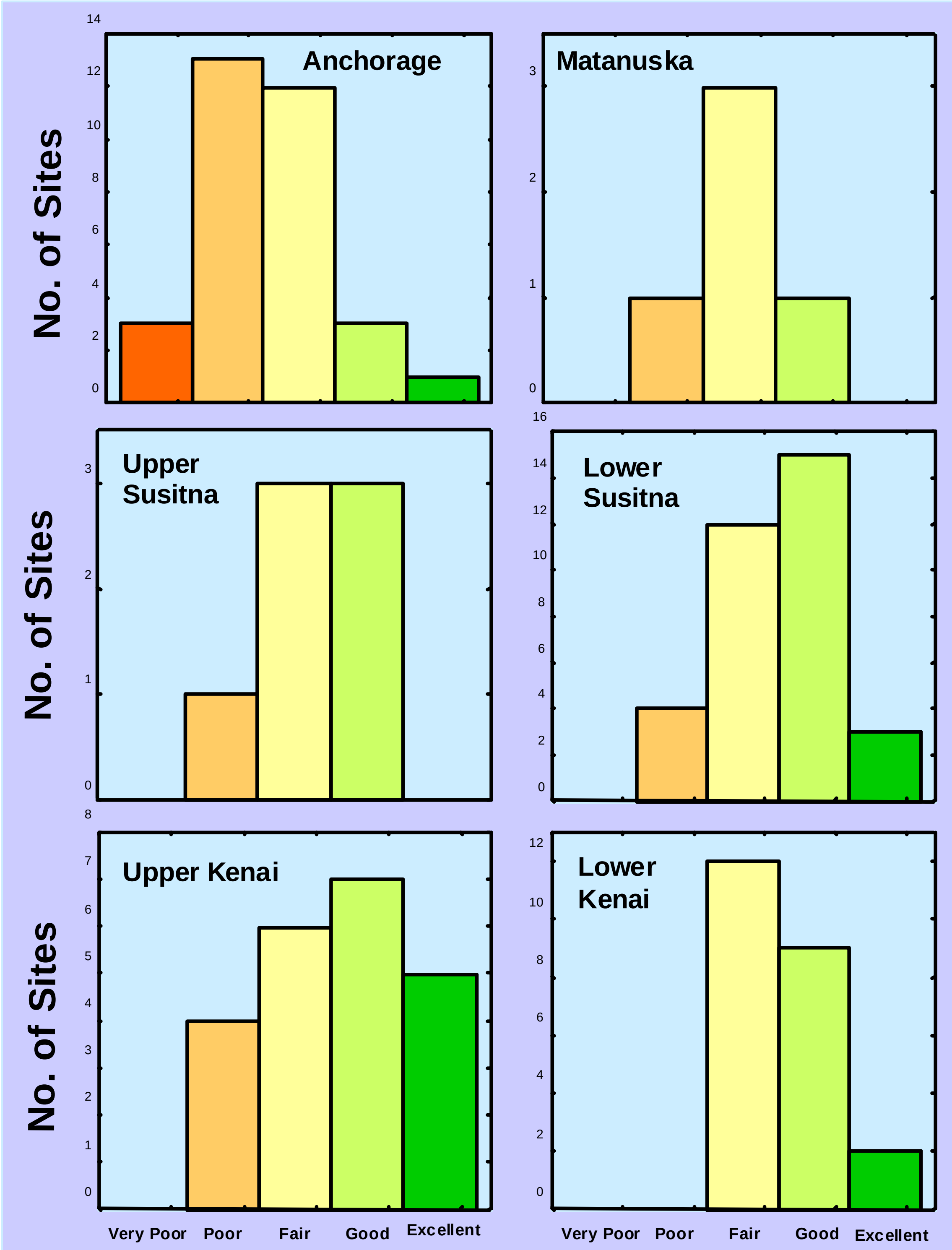


Figure 9. Frequencies of sites by ASCI rating in each of six river basins.

Table 5. Streams and rivers assessed with the ASCI. The ASCI scores are averages of all samples collected along the stream. The narrative ratings are therefore not site specific. See Appendix B for metric and index data on individual samples.

Waterbody Name	Site ID	Stream Class	*a priori* Cond.	ASCI	Rating
Anchor River	kpanc	Low - coarse	Stressed	40.6	Fair
Answer Creek	msans	Low - coarse	Reference	58.9	Good
Bear Creek	kpbea	Low - coarse	Reference	61.6	Good
Beaver Creek	kpbvr	Low - coarse	Reference	63.1	Good
Beaver Creek, Soldotna	kpbve	Low - fine	Stressed	48.4	Good
Bishop Creek	kpbis	Low - fine	Stressed	39.9	Fair
Bodenburg Creek	msbod	Low - coarse	Stressed	41.2	Fair
Bridge Creek	kpbri	Low - fine	Sub-Ref.	48.3	Good
California Creek	macal	High Gradient	Sub-Ref.	38.9	Poor
Campbell Creek	macam	Low - coarse	Stressed	44.5	Fair
Caswell Creek	mscas	Low - fine	Sub-Ref.	69.5	Good
Chakok River	kpcha	Low - coarse	Stressed	59.6	Good
Chase Creek	mscha	Low - coarse	Sub-Ref.	53.8	Good
Chester Creek	mache	Low - coarse	Stressed	29.9	Poor
Chickaloon River	kpchi	High Gradient	Reference	81.6	Excellent
Cottonwood Creek	mscot	Low-fine/coarse	Stressed	62.9	Good/Fair
Creekside Cabin	kpcre	Low - coarse	Stressed	41.0	Fair
Crooked Creek	kpcrk	Low - fine	Sub-Ref.	38.9	Fair
Deadhorse Creek	msdea	High Gradient	Sub-Ref.	64.1	Fair
Deception Creek	msdec	Low-coarse/High	Sub-Ref.	52.6	Fair
Deep Creek	kpdee	Low - coarse	Stressed	43.6	Fair
Diamond Creek	kpdia	Low - fine	Stressed	31.1	Fair
East Fork Beaver Creek	kpefb	Low - coarse	Sub-Ref.	20.7	Poor
East Fork Moose River	kpefm	Low - fine	Reference	53.6	Good
Fish Creek	msfis	Low - coarse	Stressed	50.1	Fair
Flynn Creek	msfly	High Gradient	Sub-Ref.	71.4	Good
Fritz Creek	kpfri	Low - coarse	Stressed	68.1	Good
Funny River	kpfun	Low - coarse	Stressed	35.6	Fair
Glacier Creek	kpgfc	Low - coarse	Unknown	55.7	Good
Gold Creek	msgol	High Gradient	Sub-Ref.	42.5	Poor
Goose Creek	msgoo	High Gradient	Reference	76.7	Good
Grey's Creek	msgre	Low - coarse	Sub-Ref.	32.2	Poor
Lake Creek	mslak	Low - fine	Stressed	37.6	Fair
Lane Creek	mslan	High Gradient	Sub-Ref.	59.6	Fair
Little Campbell Creek	malca	Low - fine	Stressed	15.2	Poor
Little Indian Creek	kplin	High Gradient	Reference	51.1	Fair
Little Meadow Creek	mslme	Low - coarse	Stressed	33.2	Poor
Little Rabbit Creek	malra	High Gradient	Stressed	52.3	Fair
Little Susitna River	mslsu	Low - coarse	Stressed	71.9	Good
Little Willow Creek	mslwi	Low - coarse	Sub-Ref.	53.3	Good
Lucille Creek	msluc	Low - fine	Stressed	41.2	Fair
McKenzie Creek	msmck	High Gradient	Sub-Ref.	70.9	Good
McNeil Creek	kpmcn	High Gradient	Reference	76.6	Good
McRoberts Creek	msmcr	High Gradient	Stressed	71.1	Good
Meadow Creek, Anchorage	mamea	High Gradient	Stressed	50.2	Fair
Meadow Creek, Mat-Su	msmea	Low - fine	Stressed	39.9	Fair
Middle Fork Chester Creek	mamch	Low - coarse	Stressed	9.5	Very Poor

Table 5 (continued). Streams and rivers assessed with the ASCI. The ASCI scores are averages of all samples collected along the stream. The narrative ratings are therefore not site specific. See Appendix B for metric and index data on individual samples.

Waterbody Name	Site ID	Stream Class	*a priori* Cond.	ASCI	Rating
Montana Creek	msmon	Low - coarse	Sub-Ref.	51.0	Good
Moose Creek, Kenai	kpmoo	Low - coarse	Reference	58.1	Good
Moose Creek, Mat-Su	msmoo	High Gradient	Stressed	42.3	Poor
Moose Creek, Petersville	msmop	Low - coarse	Sub-Ref.	51.6	Good
Moose River	kpmor	Low - fine	Reference	46.0	Good
Mystery Creek	kpmys	Low - coarse	Reference	83.8	Excellent
Nikolai Creek	kpnik	Low - coarse	Reference	57.6	Good
Ninilchik River	kpnin	Low - coarse	Stressed	37.1	Fair
North Fork Anchor River	kpnfa	Low - coarse	Stressed	46.9	Fair
North Fork Campbell Creek	manfc	Low - coarse	Ref/Sub-Ref	57.4	Good
North Fork Little Campbell	manlc	Low - fine	Stressed	13.3	Very Poor
Otter Creek	kpott	High Gradient	Reference	87.6	Excellent
Rabbit Creek	marab	High Gradient	Stressed	52.6	Fair
Seven Egg Creek	kpsve	High Gradient	Reference	72.4	Good
Sheep Creek	msshe	Low - coarse	Sub-Ref.	69.9	Good
Sherman Creek	msshr	High Gradient	Sub-Ref.	65.3	Fair
Ship Creek	mashi	Low-coarse/High	Stressed	34.7	Poor/Fair
Slikok Creek	kpsli	Low - fine	Stressed	35.9	Fair
Soldotna Creek	kpsol	Low - coarse	Stressed	24.9	Poor
South Fork Campbell	masfc	Low - coarse	Sub-Ref.	62.8	Good
South Fork Chester Creek	masch	Low - coarse	Stressed/Sub-Ref	24.5	Poor
South Fork Eagle River	masfe	High Gradient	Sub-Ref.	54.7	Fair
South Fork Little Campbell	maslc	Low - fine	Stressed	30.4	Fair
Stariski Creek	kpsta	Low - coarse	Stressed	43.5	Fair
Swanson River	kpswa	Low - fine	Ref/Sub-Ref	58.9	Good
Trapper Creek	mstra	Low - coarse	Reference	48.8	Fair
Troublesome Creek	mstro	High Gradient	Sub-Ref.	73.7	Good
Twitter Creek	kptwi	High Gradient	Sub-Ref.	62.2	Fair
Unnamed Crk. at Parks Hwy MP 121	ms121	High Gradient	Sub-Ref.	84.6	Excellent
Unnamed Crk. at Parks Hwy MP 140	ms140	High Gradient	Sub-Ref.	75.3	Good
Unnamed Trib. to Montana Creek	msumo	Low - coarse	Sub-Ref.	63.0	Good
Wasilla Creek	mswas	Low - coarse	Stressed	49.1	Fair
West Fork Moose River	kpwfm	Low - fine	Sub-Ref.	84.1	Excellent
Willow Creek	mswil	Low-fine/coarse	Stressed	50.6	Fair/Good
Wolverine Creek, site 01	mswol	High Gradient	Stressed	49.5	Fair
Wolverine Creek, site 02	mswol	Low - coarse	Sub-Ref.	32.0	Poor

7. THE FUTURE OF THE ALASKA STREAM CONDITION INDEX

Implementation Recommendations

- The ASCI should be calibrated for other parts of Alaska. The basic premise and framework for assessment and monitoring would remain the same. However, the benchmark for judging biological condition would be adjusted for different biological expectations.

- Implementing a probabilistic design within the Cook Inlet Ecoregion where the ASCI has been calibrated would provide a cost-effective means of addressing the attainment of Aquatic Life Use Support (ALUS) designations for 305(b) assessments.

- Standard Operating Procedures (SOPs) have been established for the ASCI and are documented as per Major and Barbour (2001). These SOPs should be used on a statewide basis to provide quality assurance on ecological data sampling and processing.

Technical Recommendations

- The index development process used all available data. The index performed reasonably well on the same data that was used in calibration, but a test of the index should be performed using an independent data set. The new data would ideally come from reference and stressed sites that had not been sampled between 1997 and 2000. The difficulty in accessing new areas has been recognized, but repeat visits to the same sites do not provide sufficiently independent data.

- The tolerance values, feeding groups, habits, and voltinism characteristics associated with each taxon was referenced to values and characteristics in use outside of Alaska. The applicability of these characteristics in Alaska should be carefully scrutinized, especially the tolerance values and voltinism characteristics. Because of ecoregional differences and taxa distributions, taxa characteristics in Alaska may be quite different from those recognized in the contiguous U.S.

- Multivariate analytical techniques were only briefly explored for the current analysis. The Rivpacs method (Wright et al. 2000) requires precise nonbiological data in order to calculate probabilities of membership within a site class (or cluster). Consistent taxonomic resolution is even more important with multivariate methods as compared to multimetric methods because evaluations are made based on taxa composition only instead of taxa characteristics.

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APPENDIX A

METHODS

Appendix A

Methods

Program Design

Sample sites were selected throughout the Kenai Peninsula, Municipality of Anchorage, and the Matanuska and Susitna river basins to represent either reference or stressed conditions. Reference conditions are found in streams with undisturbed or minimally impaired water quality, streambeds, riparian zones, and drainage basins. After applying non-biological site criteria developed during the pilot study (Major et al. 1998), the list of reference sites was reduced using professional judgement to identify exceptionally high quality sites. The criteria for stressed sites were applied without modification. This process resulted in three *a priori* stream conditions: reference, sub-reference, and stressed.

Samples from reference sites were used to develop the reference condition for index development and to identify stream classes. Stressed samples were used to determine how biological metrics responded to environmental insults (or how the stressed community differed from the reference condition). The degree and direction of metric response in the presence of stressors determined the usefulness of metrics for index development.

Sampling Methods

Field sampling, laboratory processing, and habitat assessment follow Standard Operating Procedures Edition 5 for the Alaska Stream Condition Index (Major and Barbour 2001) that are modifications of the U.S. EPA's Rapid Bioassessment Protocols (Barbour et al. 1999). In general, benthic macroinvertebrate sampling consists of capturing organisms in a D-frame net after suspension in the water column by disturbance of substrate. Twenty substrates are sampled within the 100 meter site and the subsamples are composited. Habitat/substrate types present in the reach are sampled proportionately, so those organisms from all significant and productive habitats are collected. In the laboratory, the sample is spread over a gridded pan and 4 grid areas are randomly removed. Organisms are sorted from debris and identified to the lowest level practical (genus preferred). If less than 300 organisms are picked in the first 4 grids, additional grids are sorted until at least 300 organisms are identified. Field chemistry is recorded *in situ* using Hydrolab® or similar equipment. Instream and riparian habitat conditions are assessed (scored) and other physical characteristics of the water channel, substrate, and drainage basin are measured or observed. Field crew training and equipment calibrations are routinely performed to minimize sampling error.

Analytical Methods

After data compilation and quality control, analysis proceeded in three main steps: 1) establishing stream classes as a basis for bioassessment, 2) choosing biological metrics, and 3) aggregating metrics into a biological index.

Establishing Stream Classes as a Basis for Bioassessment

The stream classification process uses both biological and non-biological data from undisturbed or minimally impacted streams (reference and sub-reference). Similarities of the biological samples are first quantified, then non-biological characteristics that "explain" similarities are sought. Alternative classification schemes were examined with multivariate ordination of the sampling sites based on their species composition, following methods outlined in Jongman et al. (1987) and Ludwig and Reynolds (1988). The determination of stream classes was confirmed by comparing distributions of common metric values among the proposed classes.

The first step in ordination is development of a similarity matrix. The relative abundance of each taxon within a sample is compared to the relative abundance of taxa from a second sample and a dissimilarity coefficient is calculated. This comparison is made for all pairs of samples and generates the sample-to-sample dissimilarity matrix. The Bray-Curtis dissimilarity coefficient (see text box) was used in this analysis.

> ***Bray Curtis Dissimilarity Coefficient:***
>
> BC = 1-2W/(A+B),
>
> where W is the sum of taxa abundances common to both samples and A and B are the sums of taxa abundances from individual samples.

Non-Metric Multi-Dimensional Scaling (NMDS) ordination interprets the dissimilarity coefficients as distance measures and plots samples in a multi-dimensional space, such that samples with similar biological compositions appear closer together on the diagram. The NMDS ordination (McCune and Mefford 1995) follows the procedure of Kruskal (1964). Categorical and continuous non-biological characteristics associated with each sample can be displayed on the same diagram to facilitate recognition of natural site classes. Stream classes (clusters of adjacent samples) were considered on the basis of similar ecoregions, river basins, elevation, Rosgen stream type, substrate composition, habitat availability, water chemistry and other parameters that were consistently recorded in the reference and sub-reference sites. Explanatory variables were chosen to describe stream classes based on visual assessment of the diagrams. Those variables that showed maximum separation and minimum overlap of sample clusters were chosen to describe the stream classes. This method has been shown to be robust for ordination of species composition (e.g., Kenkel and Orloci 1986, Ludwig and Reynolds 1988) and has been used successfully for classification of stream communities (e.g., Barbour et al. 1996; Reynoldson et al. 1997).

The comparison of relative taxa abundance within the samples was complicated when taxa were identified to variable taxonomic levels. Taxa identified at the family level could not be compared to taxa within the same family identified at the genus level because the uniqueness of the specimens in those cases is vague. Rare genera were either lumped at the higher taxonomic level or eliminated from the analysis, depending on the predominance of other lower level identifications. If most identifications were made at the genus level, family level data from the same family was eliminated. An effort was made to minimize data deletion, resulting in a considerable amount of taxa lumping and comparing abundance at the family level or higher.

Specimens of the midge family (Diptera: Chironomidae) were identified at the family level when the monitoring program was initiated. Ordination analysis was first conducted using all reference samples with midges identified at the family level. Because the midge family is dominant in many samples, the preliminary ordinations were driven by relative midge abundance. Ordination was repeated with fewer reference samples, those with midges identified at genus level. The final set of samples used in the site classification ordinations included reference and sub-reference samples that had midge identifications at the genus level.

After identification of stream classes using ordination techniques and consideration of factors documented in previous reports (Gerritsen et al. 2000), box and whisker plots of reference metric values were used to confirm the uniqueness of the classes. If differences in medians, intra-quartile ranges, and extreme ranges were not apparent, stream classes were re-examined.

Choosing Biological Metrics

Biological metric evaluation involves comparison of the metrics of reference sites to those of stressed sites. Differences in metric value distributions from reference and stressed sites illustrate the biological changes that occur when environmental insults are introduced. Metrics were evaluated for the consistency, degree, and biological significance of responses to increasing stress. The evaluation used two methods: comparison of box and whisker plots and calculation of discrimination efficiency (DE).

The box and whisker diagrams of the metric values were visually assessed to find those that showed a reasonable separation between the reference and stressed conditions. If the boxes (the intra-quartile ranges) were offset with little or no overlap, then the separation was considered sufficient and the metric was retained for further assessment. If the separation was vague, with overlap of the intra-quartile ranges, the metric was either dropped from the analysis. If separation was good but the direction of metric response was inexplicable, then the metric was also dropped. Average metric values were used when samples were replicated.

After initial screening of metric value distributions for adequate separation between reference and stressed samples, the discrimination efficiencies (DE's) of the most responsive and meaningful metrics were calculated. DE's are numerical indicators of the degree of separation between reference and stressed metric scores (see text box). Metrics with high DE's and meaningful responses (understandable trends with increasing stress) were considered candidates for inclusion in multimetric indexes.

> Discrimination Efficiency (DE)
>
> DE = the percentage of stressed samples with metric scores worse than the worst 25^{th} percentile of reference scores.

Aggregating Metrics into a Biological Index

Index development involves aggregation of metrics into a single numerical indicator of relative biological condition. An ideal index would include several highly discriminating, precisely sampled, non-redundant metrics (at least one from each category) and would be applicable throughout Alaska. Thus, selection of appropriate core metrics for the ASCI depended on several factors:

- High individual metric discrimination efficiencies,
- Representation by metrics from all metric categories (if possible),
- Uniqueness of metric values (elimination of redundant metrics),
- Precision of the index (variability),
- Similarity of core metrics across stream classes (if possible), and
- High index discrimination efficiency.

Within each stream class, several possible sets of metrics existed that would satisfy the factors listed above. Several alternative indexes were formulated using each of the possible combinations of metrics. The set of metrics that outperformed alternative sets was identified as the core metric set for the ASCI. The analytical methods used to evaluate each alternative index include calculation of metric DE's (accomplished while selecting candidate metrics), metric scoring and aggregation, metric correlation analysis, precision analysis, and calculation of index DE's.

Metrics were aggregated into indexes by averaging metric scores. Because each metric has a unique range of values from taxa counts, percentages, or calculated formulas, the values were standardized (scored) before aggregation. Scoring assigns the highest score (100) to the optimal metric value and the lowest score (0) to the worst metric value. Optimal values are defined as the 5^{th} or 95^{th} percentile of metric values (discounting 5% of values as possible outliers). The worst values are defined as the worst plausible or worst common values (discounting outliers). Scores for intermediate values are interpolated and outlying values are assigned optimal or worst scores.

Metric redundancy was checked using a Pearson product-moment correlation analysis for all candidate metrics within each stream class. Metrics were considered redundant if the correlation coefficient (r) was greater than 0.8 or less than –0.8. Redundant metrics were not used together in any index formulation.

Replicate field samples were collected for roughly 10% of the samples per year. The precision of the core metrics and the ASCI was estimated from these replicates. Precision is thus a quantification of the sampling error and does not account for inter-annual variability. The data from replicates within each stream class were entered into an Analysis of Variance (ANOVA). Using replicate pair identifiers as the treatment, the mean square error (MSE) and the root mean square error (RMSE) were calculated. The MSE and RMSE were interpreted as estimates of the variance and standard deviation (respectively) of the replicates.

The coefficient of variability (CV) was calculated as the RMSE divided by the mean of the replicate pairs for each core metric and the ASCI in each site class. A higher CV indicates greater variability with respect to the average value. The range around the observed mean within which the true mean can be expected with 90% confidence was calculated for single, duplicate, and triplicate observations.

90% confidence range =

$$\frac{1.64*RMSE,}{\sqrt{n}}$$ where n is the number of replicates.

High index discrimination was a primary consideration when selecting core index metrics. Index DE was calculated as it was for individual metrics; as the percentage of stressed samples having scores worse than the 25^{th} percentile of reference.

Stream condition ratings derived from the index

Rating stream condition based on reference index score distributions is a widely accepted method of identifying biological conditions that are similar to reference, below average of reference, or significantly different from reference. The 25^{th} percentile of reference is commonly identified as a critical threshold between acceptable and non-acceptable conditions. In this study, we identified the 25^{th} percentile of reference as the threshold between "Good" and "Fair" conditions. Additional thresholds were defined at the 75^{th} percentile of reference ("Excellent" and "Good"), and equal divisions of the range below the 25^{th} percentile into three parts ("Fair", "Poor", and "Very Poor").

APPENDIX B

SUPPORTING BIOLOGICAL DATA

Appendix B

Supporting Biological Data

Table B. Index and core metrics for assessed samples.

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	Trichoptera taxa	Trich. Taxa score	%EPT (no Baet. or [illegible])	%EPT (nBZ) score	% Diptera	% Diptera score	O/E (family 75%)	O/E score	% collectors	% collectors score	HBI	HBI score
kpbis01-600	L-F	Str.	35.5	2	28.6	0.6	4.3	71.2	41.1	0.7	66.7	67.4	46.6	6.0	25.7
kpbis01-697	L-F	Str.	42.4	5	71.4	2.7	18.3	81.2	26.9	0.8	77.8	89.4	15.1	5.6	44.8
kpbis01-699	L-F	Str.	41.8	2.5	35.7	1.9	12.9	67.9	45.9	0.9	88.9	70.4	42.2	6.0	25.3
kpbri01-699	L-F	sub-R.	48.3	5	71.4	5.2	34.6	78.8	30.3	0.8	77.8	75.0	35.7	5.7	39.9
kpbve01-600	L-F	Str.	51.1	2	28.6	0.3	2.0	46.0	77.1	0.9	88.9	73.0	38.5	5.1	71.3
kpbve01-699	L-F	Str.	45.8	3	42.9	5.1	34.0	85.8	20.3	0.8	77.8	24.7	100.0	6.6	0.0
kpcrk01-697	L-F	sub-R.	40.5	3	42.9	5.5	36.5	89.1	15.6	0.8	77.8	80.3	28.2	5.7	42.1
kpcrk01-699	L-F	sub-R.	28.3	1	14.3	1.2	7.8	92.5	10.7	0.7	66.7	54.2	65.4	6.4	5.0
kpcrk02-697	L-F	sub-R.	38.4	3.5	50.0	4.9	32.8	83.7	23.2	0.7	66.7	89.9	14.4	5.6	43.1
kpcrk02-699	L-F	sub-R.	50.1	2.5	35.7	7.3	49.0	64.9	50.2	0.8	77.8	71.9	40.2	5.5	47.5
kpcrk03-699	L-F	sub-R.	37.4	2.5	35.7	4.2	28.1	87.7	17.5	0.7	72.2	65.8	48.9	6.1	22.0
kpdia01-699	L-F	Str.	31.1	3	42.9	0.8	5.5	97.1	4.1	0.6	55.6	45.2	78.2	6.5	0.0
kpefm01-600	L-F	Ref.	50.0	4	57.1	5.3	35.4	62.5	53.6	0.7	66.7	64.8	50.3	5.8	36.7
kpefm01-697	L-F	Ref.	47.1	9	100.0	5.2	34.4	90.8	13.2	0.9	88.9	92.3	11.1	5.8	34.8
kpefm01-699	L-F	Ref.	63.9	3	42.9	5.3	35.4	32.2	96.8	1.0	100.0	28.2	100.0	6.3	8.0
kpmor01-799	L-F	Ref.	56.4	5	71.4	9.9	66.1	77.0	32.8	1.0	100.0	81.5	26.4	5.7	41.4
kpmor02-799	L-F	Ref.	42.0	3.5	50.0	4.2	28.0	81.0	27.2	0.8	83.3	74.7	36.2	6.0	27.4
kpmor03-799	L-F	Ref.	39.5	2	28.6	5.8	38.5	81.2	26.8	0.8	77.8	74.4	36.6	5.9	29.0
kpsli01-600	L-F	Str.	44.8	3	42.9	3.3	21.7	87.0	18.6	0.7	66.7	44.9	78.7	5.7	40.2
kpsli01-697	L-F	Str.	24.6	3	42.9	3.4	22.6	94.0	8.6	0.3	33.3	94.7	7.5	5.8	32.7
kpsli01-699	L-F	Str.	38.2	2	28.6	7.9	53.0	86.3	19.5	0.7	72.2	79.5	29.2	6.0	27.0
kpswa01-600	L-F	Ref.	79.9	7	100.0	34.8	100.0	49.7	71.8	1.0	100.0	73.8	37.4	5.1	70.5

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	Trichoptera taxa	Trich. Taxa score	%EPT (no Baet. or [illegible])	%EPT (nBZ) score	% Diptera	% Diptera score	O/E (family 75%)	O/E score	% collectors	% collectors score	HBI	HBI score
kpswa01-699	L-F	Ref.	78.1	4	57.1	48.9	100.0	35.6	92.1	1.0	100.0	84.8	21.7	4.5	97.8
kpswa03-699	L-F	Ref.	64.2	5	71.4	11.9	79.2	62.8	53.1	1.0	100.0	82.4	25.2	5.4	56.0
kpswa04-600	L-F	Ref.	43.1	2	28.6	0.0	0.0	52.7	67.6	0.8	77.8	57.4	60.9	6.0	24.1
kpswa04-799	L-F	Ref.	65.2	5	71.4	11.8	78.6	67.9	45.9	0.9	88.9	52.0	68.5	5.7	38.0
kpswa05-600	L-F	Ref.	74.0	6	85.7	9.0	60.0	48.1	74.1	1.0	100.0	40.8	84.5	5.7	39.8
kpswa06-600	L-F	Ref.	60.8	4	57.1	10.7	71.3	62.8	53.2	1.0	100.0	71.7	40.4	5.6	42.8
kpswa06-699	L-F	Ref.	90.4	7	100.0	31.2	100.0	27.9	100.0	1.0	100.0	70.4	42.2	4.4	100.0
kpswa10-600	L-F	sub-R.	46.3	3	42.9	4.8	32.1	71.5	40.7	0.9	89.0	74.2	36.8	5.8	36.4
kpswa10-699	L-F	sub-R.	50.4	2	28.6	1.9	12.5	31.5	97.9	0.7	66.7	62.9	53.0	5.6	44.0
kpwfm01-799	L-F	sub-R.	84.1	6	85.7	19.5	100.0	43.9	80.2	1.0	100.0	50.2	71.1	5.1	67.5
malca01-500	L-F	Str.	15.2	1	14.3	0.2	1.2	92.0	11.4	0.4	44.4	98.9	1.6	6.1	18.4
manlc04-500	L-F	Str.	13.3	1	14.3	0.0	0.0	95.0	7.1	0.3	33.3	96.5	5.0	6.1	20.0
maslc01-500	L-F	Str.	21.2	2	28.6	1.1	7.1	91.8	11.7	0.6	55.6	96.1	5.6	6.1	18.4
maslc02-500	L-F	Str.	35.4	2	28.6	7.1	47.1	88.2	16.8	0.6	55.6	87.5	17.9	5.6	46.2
maslc04-500	L-F	Str.	34.8	2	28.6	4.4	29.1	87.3	18.1	0.7	66.7	81.7	26.2	5.7	40.1
mscas01-598	L-F	sub-R.	65.8	5	71.4	11.8	78.9	62.0	54.3	0.8	77.8	56.6	62.0	5.5	50.5
mscas01-600	L-F	sub-R.	73.1	4	57.1	12.5	83.3	37.9	88.7	0.8	77.8	41.0	84.3	5.5	47.6
mscot01-598	L-F	Str.	70.2	6	85.7	22.7	100.0	68.2	45.5	0.8	77.8	71.3	41.0	5.1	71.0
mscot01-600	L-F	Str.	89.8	9	100.0	37.8	100.0	55.2	64.0	0.9	88.9	40.0	85.7	4.0	100.0
mslak01-600	L-F	Str.	37.6	1	14.3	3.2	21.4	66.1	48.5	0.7	66.7	69.3	43.9	5.9	30.6
msluc01-598	L-F	Str.	44.8	2	28.6	5.6	37.3	63.6	51.9	0.7	66.7	88.8	16.0	5.1	68.2
msluc01-600	L-F	Str.	40.1	3	42.9	2.9	19.5	86.9	18.8	0.8	77.8	65.7	49.0	5.8	32.6
msluc03-600	L-F	Str.	38.8	2	28.6	2.7	18.3	75.0	35.7	0.8	77.8	86.3	19.6	5.4	53.1
msmea01-598	L-F	Str.	34.9	5	71.4	1.9	12.9	90.6	13.4	0.8	77.8	93.2	9.7	6.0	24.0
msmea01-600	L-F	Str.	45.0	3.5	50.0	6.4	42.4	75.3	35.4	0.8	77.8	76.9	32.9	5.9	31.4
mswil01-700	L-F	Str.	35.7	1	14.3	10.8	72.1	75.7	34.7	0.4	44.4	96.8	4.5	5.6	43.9

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	Ephemeroptera taxa	Ephem. taxa score	% Plecoptera	% Plecoptera score	% Ephem. (no Baetidae)	% Ephem. (nB) score	Baetidae / Ephemeroptera	Baet. / Ephem. score	% non-insects	% non-insects score	O/E (family 75%)	O/E score	% scrapers	% scrapers score	HBI	HBI score
kpanc01-600	L-C	Str.	43.4	3	54.5	3.7	26.5	1.3	6.6	81.5	18.5	2.9	90.3	1	100	1.1	7.1	5.6	43.9
kpanc01-697	L-C	Str.	41.4	4	72.7	3.4	24.5	0.0	0.0	100.0	0.0	0.6	97.9	0.9	90	0.0	0.0	5.6	45.7
kpanc01-799	L-C	Str.	37.1	4.5	81.8	0.7	4.9	1.4	6.9	93.4	6.6	0.7	97.7	0.75	75	1.0	6.9	6.2	17.3
kpbea01-600	L-C	Ref.	78.0	4	72.7	3.1	22.2	20.7	100.0	63.9	36.1	2.1	93.1	1	100	20.0	100.0	4.4	100.0
kpbea01-697	L-C	Ref.	60.3	4	72.7	5.1	36.3	7.5	37.4	76.3	23.7	0.8	97.3	1	100	5.6	37.4	4.9	77.8
kpbea01-699	L-C	Ref.	46.4	4	72.7	2.3	16.1	0.9	4.5	98.6	1.4	3.2	89.5	0.9	90	0.9	6.0	4.7	91.1
kpbvr01-600	L-C	Ref.	60.9	3	54.5	4.3	30.6	9.6	48.2	50.0	50.0	8.6	71.4	1	100	9.3	61.9	5.1	70.7
kpbvr01-697	L-C	Ref.	50.1	4	72.7	1.9	13.3	3.5	17.3	60.6	39.4	0.8	97.3	1	100	2.4	16.0	5.6	44.4
kpbvr01-699	L-C	Ref.	78.4	4	72.7	2.7	19.3	31.5	100.0	59.1	40.9	1.8	94.0	1	100	30.6	100.0	4.3	100.0
kpcha01-600	L-C	Str.	47.2	4	72.7	2.5	18.1	1.7	8.4	64.7	35.3	1.1	96.3	1	100	1.1	7.5	5.7	39.3
kpcha01-699	L-C	Str.	72.0	4	72.7	9.4	67.4	17.6	88.1	67.1	32.9	0.0	100.0	0.9	90	3.8	25.2	3.9	100.0
kpcre01-699	L-C	Str.	41.0	2	36.4	1.5	10.7	1.5	7.5	96.7	3.3	3.0	90.0	0.7	70	1.5	10.0	4.4	100.0
kpdee01-600	L-C	Str.	36.7	3	54.5	1.4	10.0	1.4	7.0	88.9	11.1	4.9	83.7	0.8	80	0.7	4.6	5.7	42.3
kpdee01-697	L-C	Str.	60.0	5	90.9	3.3	23.4	4.9	24.6	45.5	54.5	1.6	94.5	0.9	90	6.1	41.0	5.3	60.8
kpdee02-600	L-C	Str.	42.4	3	54.5	6.9	49.6	1.4	6.9	86.7	13.3	6.3	79.2	0.9	90	1.0	6.9	5.7	38.3
kpdee02-697	L-C	Str.	38.1	2	36.4	2.7	19.4	0.4	1.9	96.4	3.6	1.2	96.1	0.9	90	0.4	2.6	5.4	54.5
kpdee02-799	L-C	Str.	40.8	5	90.9	2.2	15.4	1.1	5.4	86.7	13.3	0.5	98.2	1	100	0.0	0.0	6.4	3.4
kpefb01-699	L-C	sub-R.	20.7	1	18.2	0.0	0.0	0.0	0.0	100.0	0.0	49.0	0.0	0.4	40	7.7	51.0	5.4	56.6
kpfri01-697	L-C	Str.	56.1	3	54.5	2.5	18.1	7.6	38.0	64.7	35.3	0.3	98.9	1	100	7.0	46.4	5.3	57.9
kpfri01-699	L-C	Str.	80.2	4	72.7	9.8	70.2	19.6	98.2	62.4	37.6	2.7	91.1	1	100	10.7	71.4	4.5	100.0
kpfun01-600	L-C	Str.	38.2	2	36.4	2.2	15.4	0.7	3.6	50.0	50.0	6.8	77.3	0.9	90	0.7	4.8	5.9	27.9
kpfun02-600	L-C	Str.	33.1	2	36.4	2.4	17.0	0.2	0.8	91.7	8.3	3.8	87.2	0.8	80	0.2	1.1	5.8	34.2
kpgfc01-697	L-C	Unk.	55.7	2	36.4	30.2	100.0	0.4	2.2	50.0	50.0	1.7	94.3	0.6	60	0.4	2.9	4.1	100.0
kpmoo01-600	L-C	Ref.	57.4	3.5	63.6	3.7	26.2	8.2	40.8	86.5	13.5	0.7	97.7	0.85	85	4.8	32.3	4.4	100.0
kpmoo01-697	L-C	Ref.	51.8	4	72.7	2.0	14.2	7.6	37.8	74.0	26.0	0.4	98.7	0.7	70	3.2	21.2	5.0	74.0
kpmoo01-699	L-C	Ref.	65.0	3	54.5	13.6	97.3	7.7	38.4	85.8	14.2	2.9	90.5	0.93	93.3	4.8	31.7	4.1	100.0
kpmys01-697	L-C	Ref.	78.5	4	72.7	7.9	56.4	15.5	77.3	29.6	70.4	1.4	95.4	0.9	90	13.7	91.5	5.0	74.2
kpmys01-699	L-C	Ref.	89.1	5	90.9	13.7	97.8	16.2	80.9	30.4	69.6	5.4	82.0	1	100	14.5	96.8	4.6	94.7

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	Ephemeroptera taxa	Ephem. taxa score	% Plecoptera	% Plecoptera score	% Ephem. (no Baetidae)	% Ephem. (nB) score	Baetidae / Ephemeroptera	Baet. / Ephem. score	% non-insects	% non-insects score	O/E (family 75%)	O/E score	% scrapers	% scrapers score	HBI	HBI score
kpnfa01-697	L-C	Str.	32.2	1	18.2	6.0	42.6	0.0	0.0	100.0	0.0	3.4	88.7	0.6	60	0.0	0.0	5.5	48.3
kpnfa01-799	L-C	Str.	61.6	6	100.0	3.5	25.3	11.4	57.1	34.1	65.9	12.2	59.3	0.9	90	10.2	68.2	6.0	26.8
kpnik01-600	L-C	Ref.	54.4	4	72.7	4.6	32.9	4.6	23.0	60.0	40.0	3.0	90.1	1	100	3.9	26.3	5.5	50.1
kpnik01-697	L-C	Ref.	45.4	3	54.5	3.9	28.1	1.2	5.9	40.0	60.0	0.0	100.0	0.7	70	0.8	5.2	5.7	39.8
kpnik01-699	L-C	Ref.	72.8	4.5	81.8	17.2	100.0	10.6	53.0	59.9	40.1	2.0	93.4	0.9	90	3.7	24.5	2.9	100.0
kpnin01-697	L-C	Str.	32.1	2	36.4	1.1	8.2	0.8	3.8	90.5	9.5	2.3	92.4	0.6	60	0.8	5.1	5.7	41.6
kpnin01-699	L-C	Str.	42.2	3	54.5	2.9	20.5	3.7	18.4	86.6	13.4	9.0	69.9	1	100	3.3	21.9	5.7	38.6
kpsol01-600	L-C	Str.	21.0	1	18.2	0.0	0.0	0.0	0.0	100.0	0.0	58.9	0.0	0.5	50	28.8	100.0	6.7	0.0
kpsol01-697	L-C	Str.	26.3	1	18.2	0.8	5.5	0.0	0.0	100.0	0.0	2.3	92.4	0.6	60	0.0	0.0	5.8	34.8
kpsol01-699	L-C	Str.	12.9	1	18.2	0.0	0.0	0.0	0.0	100.0	0.0	22.5	25.0	0.5	50	0.0	0.0	6.3	10.0
kpsol02-600	L-C	Str.	21.6	1	18.2	0.0	0.0	0.0	0.0	100.0	0.0	32.4	0.0	0.5	50	11.5	76.9	5.9	27.8
kpsol02-697	L-C	Str.	30.8	2	36.4	0.3	2.4	0.0	0.0	100.0	0.0	0.0	100.0	0.7	70	0.0	0.0	5.8	37.4
kpsol02-699	L-C	Str.	36.6	2	36.4	0.0	0.0	1.3	6.3	25.0	75.0	8.8	70.8	0.7	70	0.0	0.0	5.8	34.0
kpsta01-600	L-C	Str.	44.3	4	72.7	4.1	29.5	0.6	3.2	95.2	4.8	2.2	92.6	0.9	90	0.6	4.2	5.4	57.3
kpsta01-697	L-C	Str.	40.0	2	36.4	2.1	14.8	1.3	6.6	86.4	13.6	0.7	97.5	0.85	85	0.9	5.9	5.3	59.9
kpsta01-799	L-C	Str.	46.2	4	72.7	2.6	18.5	1.0	5.2	66.7	33.3	5.7	81.0	1	100	1.0	6.9	5.5	51.8
macam04-599	L-C	Str.	48.4	1	18.2	10.0	71.8	1.0	4.8	0.0	100.0	4.3	85.6	0.6	60	0.0	0.0	5.6	46.5
macam06-500	L-C	Str.	42.3	0	0.0	10.9	78.0	0.0	0.0	0.0	100.0	22.7	24.4	0.7	70	0.0	0.0	5.2	66.0
macam08-500	L-C	Str.	43.0	3	54.5	8.8	63.2	1.3	6.6	62.5	37.5	13.3	55.8	0.8	80	1.3	8.8	5.8	37.3
mache02-599	L-C	Str.	40.3	2	36.4	5.8	41.4	0.4	1.8	96.0	4.0	4.7	84.3	0.8	80	0.4	2.4	5.1	72.0
mache04-699	L-C	Str.	30.2	1.5	27.3	29.1	100.0	0.0	0.0	100.0	0.0	23.5	21.7	0.65	65	0.0	0.0	6.0	27.4
mache08-500	L-C	Str.	19.2	1	18.2	2.4	16.8	0.0	0.0	100.0	0.0	34.5	0.0	0.7	70	0.0	0.0	5.5	48.2
mamch02-500	L-C	Str.	9.5	1	18.2	1.1	7.9	0.0	0.0	100.0	0.0	77.6	0.0	0.5	50	0.0	0.0	7.5	0.0
manfc07-500	L-C	Ref.	50.8	2.5	45.5	4.7	33.3	2.1	10.6	0.0	100.0	8.0	73.2	0.8	80	1.5	9.9	5.4	53.7
manfc10-699	L-C	sub-R.	52.3	2.5	45.5	4.5	31.8	2.4	11.9	8.3	91.7	1.4	95.2	0.9	90	1.2	8.0	5.6	44.5
manfc12-500	L-C	Ref.	74.4	4	72.7	5.3	38.1	15.2	75.8	49.3	50.7	1.6	94.5	0.9	90	13.5	90.2	4.8	83.6
masch01-500	L-C	Str.	25.1	1	18.2	2.2	15.9	0.0	0.0	100.0	0.0	20.9	30.4	0.8	80	0.4	3.0	5.4	53.4
masch03-500	L-C	Str.	22.5	1	18.2	6.5	46.1	0.0	0.0	100.0	0.0	52.1	0.0	0.8	80	0.0	0.0	5.8	36.1

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	Ephemeroptera taxa	Ephem. taxa score	% Plecoptera	% Plecoptera score	% Ephem. (no Baetidae)	% Ephem. (nB) score	Baetidae / Ephemeroptera	Baet. / Ephem. score	% non-insects	% non-insects score	O/E (family 75%)	O/E score	% scrapers	% scrapers score	HBI	HBI score
masch05-500	L-C	Str.	13.9	1	18.2	0.0	0.0	0.0	0.0	100.0	0.0	19.1	36.3	0.4	40	0.5	3.3	6.2	13.5
masch06-500	L-C	Str.	29.3	1	18.2	1.0	7.3	0.0	0.0	100.0	0.0	20.3	32.3	0.4	40	13.2	88.0	5.5	48.8
masch09-500	L-C	Str.	25.0	1	18.2	2.2	15.8	0.0	0.0	100.0	0.0	14.2	52.5	0.7	70	0.0	0.0	5.6	43.6
masch13-500	L-C	sub-R.	25.8	1	18.2	1.4	9.9	0.0	0.0	100.0	0.0	5.1	83.0	0.6	60	0.0	0.0	5.8	35.5
masfc11-500	L-C	sub-R.	51.6	5	90.9	4.1	29.1	3.4	16.9	86.8	13.2	3.4	88.7	0.9	90	2.4	15.8	5.1	67.9
masfc11-699	L-C	sub-R.	73.9	5.5	100.0	12.5	89.0	8.8	43.8	81.5	18.5	2.9	90.2	1	100	7.5	50.0	4.4	100.0
mashi03-500	L-C	Str.	20.5	4	72.7	0.4	2.7	0.4	1.9	85.7	14.3	31.7	0.0	0.7	70	0.4	2.5	6.5	0.0
msans01-598	L-C	Ref.	58.9	4	72.7	3.7	26.5	6.8	34.1	21.4	78.6	1.9	93.8	0.9	90	4.6	31.0	5.6	44.6
msbod01-598	L-C	Str.	41.1	2	36.4	2.4	16.8	2.7	13.5	91.3	8.7	2.0	93.3	0.7	70	3.0	20.2	5.1	70.0
msbod01-600	L-C	Str.	41.3	2	36.4	2.2	15.8	3.6	18.0	75.0	25.0	1.7	94.5	0.7	70	3.6	23.9	5.6	46.7
mscha01-598	L-C	sub-R.	53.8	4	72.7	4.7	33.4	3.0	14.9	30.0	70.0	3.8	87.2	1	100	2.1	14.2	5.7	37.8
mscot02-598	L-C	Str.	30.8	1.5	27.3	0.1	1.0	0.2	1.0	97.6	2.4	5.1	83.0	0.5	50	4.5	29.8	5.5	52.0
mscot02-600	L-C	Str.	23.5	1	18.2	0.0	0.0	0.0	0.0	100.0	0.0	1.9	93.7	0.4	40	0.0	0.0	5.8	36.3
mscot03-598	L-C	Str.	32.7	3	54.5	0.0	0.0	0.7	3.7	92.6	7.4	4.1	86.5	0.5	50	1.1	7.4	5.5	52.1
mscot03-600	L-C	Str.	47.3	3	54.5	12.2	87.2	0.8	3.9	92.3	7.7	4.7	84.3	0.7	70	0.8	5.2	5.2	65.8
msdec02-600	L-C	sub-R.	38.1	2	36.4	2.2	16.1	0.7	3.7	66.7	33.3	0.7	97.5	0.8	80	0.0	0.0	5.7	37.9
msfis01-598	L-C	Str.	48.1	4	72.7	4.8	34.6	1.8	8.8	75.4	24.6	1.1	96.2	0.8	80	1.3	9.0	5.3	59.0
msfis01-600	L-C	Str.	52.1	3	54.5	14.8	100.0	1.1	5.6	71.4	28.6	4.5	85.1	0.9	90	0.6	3.7	5.5	49.6
msgre01-598	L-C	sub-R.	38.7	2	36.4	0.5	3.3	0.9	4.6	33.3	66.7	0.5	98.5	0.7	70	0.0	0.0	5.9	30.6
msgre01-600	L-C	sub-R.	25.7	1	18.2	0.0	0.0	0.0	0.0	100.0	0.0	1.9	93.8	0.6	60	0.0	0.0	5.8	33.4
mslme01-598	L-C	Str.	29.1	2	36.4	0.2	1.3	0.3	1.6	90.0	10.0	6.2	79.2	0.55	55	2.5	16.6	5.8	32.8
mslme01-600	L-C	Str.	37.3	3	54.5	1.7	12.0	0.8	4.2	94.4	5.6	6.7	77.6	0.9	90	0.8	5.6	5.5	48.5
mslsu01-598	L-C	Str.	53.9	6	100.0	5.5	39.0	1.9	9.6	76.0	24.0	0.6	97.9	1	100	1.0	6.4	5.4	54.2
mslsu01-700	L-C	Str.	75.3	6	100.0	10.0	71.8	12.3	61.6	37.2	62.8	5.0	83.3	0.8	80	11.4	76.1	5.2	66.6
mslsu02-598	L-C	Str.	65.2	5.5	100.0	9.0	64.0	5.3	26.7	51.1	48.9	1.4	95.5	0.95	95	4.8	31.7	5.3	60.0
mslsu02-700	L-C	Str.	91.2	5	90.9	7.7	54.7	51.7	100.0	7.7	92.3	2.4	92.0	1	100	30.6	100.0	3.5	100.0
mslsu03-598	L-C	Str.	69.5	4	72.7	8.0	57.3	8.0	40.1	14.3	85.7	3.2	89.3	1	100	8.6	57.0	5.4	53.7
mslsu03-700	L-C	Str.	76.5	5	90.9	4.0	28.5	20.3	100.0	19.7	80.3	11.3	62.3	1	100	9.0	59.8	4.7	90.2

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	Ephemeroptera taxa	Ephem. taxa score	% Plecoptera	% Plecoptera score	% Ephem. (no Baetidae)	% Ephem. (nB) score	Baetidae / Ephemeroptera	Baet. / Ephem. score	% non-insects	% non-insects score	O/E (family 75%)	O/E score	% scrapers	% scrapers score	HBI	HBI score
mslwi01-598	L-C	sub-R.	50.2	3	54.5	10.6	75.9	1.2	6.1	79.2	20.8	9.7	67.6	0.85	85	0.3	1.7	4.7	89.8
mslwi01-700	L-C	sub-R.	56.3	5	90.9	3.7	26.6	6.3	31.6	56.4	43.6	3.0	90.1	0.9	90	2.6	17.3	5.3	60.6
msmon01-598	L-C	sub-R.	51.0	4	72.7	7.9	56.4	2.6	13.2	80.0	20.0	2.6	91.2	1	100	0.5	3.5	5.5	51.1
msmop01-598	L-C	sub-R.	51.6	4	72.7	2.5	17.6	4.5	22.5	15.4	84.6	4.9	83.6	0.8	80	2.0	13.7	5.7	37.7
msshe01-598	L-C	sub-R.	69.9	3	54.5	18.9	100.0	11.8	59.2	34.1	65.9	9.6	67.8	1	100	1.8	11.7	3.7	100.0
mstra01-598	L-C	Ref.	48.8	4	72.7	3.8	27.5	3.0	15.0	65.0	35.0	4.7	84.3	0.9	90	2.1	14.2	5.5	51.4
msumo01-598	L-C	sub-R.	63.0	4	72.7	9.0	64.4	6.3	31.4	59.0	41.0	1.6	94.8	0.9	90	5.5	36.6	5.0	73.0
mswas01-598	L-C	Str.	45.0	3	54.5	4.3	30.8	2.1	10.6	78.0	22.0	1.7	94.2	0.9	90	2.1	14.2	5.6	43.6
mswas01-600	L-C	Str.	39.1	2	36.4	0.9	6.2	3.1	15.3	75.0	25.0	3.1	89.8	0.7	70	3.1	20.4	5.5	49.6
mswas02-600	L-C	Str.	59.0	5	90.9	5.2	37.1	6.5	32.5	77.9	22.1	4.8	84.1	1	100	5.6	37.5	5.1	67.7
mswas04-600	L-C	Str.	53.2	4	72.7	9.9	70.8	3.3	16.5	73.1	26.9	13.2	56.0	1	100	0.5	3.1	4.9	79.9
mswas05-600	L-C	Str.	47.8	3.5	63.6	4.3	30.5	2.7	13.5	71.6	28.4	8.8	70.7	0.95	95	1.8	11.9	5.1	68.8
mswas10-598	L-C	Str.	52.3	3	54.5	3.5	25.2	5.3	26.5	65.1	34.9	1.4	95.3	0.9	90	4.9	33.0	5.3	58.6
mswas10-600	L-C	Str.	47.6	4	72.7	8.0	57.3	2.4	11.8	75.0	25.0	18.4	38.7	1	100	0.5	3.1	5.1	72.4
mswil04-598	L-C	Str.	43.6	4	72.7	5.2	36.9	0.0	0.0	100.0	0.0	3.0	89.9	0.9	90	0.0	0.0	5.3	58.9
mswil04-700	L-C	Str.	79.8	6	100.0	3.1	22.0	17.8	89.0	44.7	55.3	0.3	98.9	0.9	90	14.4	95.9	4.8	87.2
mswol02-600	L-C	sub-R.	32.0	2	36.4	2.9	20.4	0.3	1.6	96.4	3.6	4.4	85.2	0.7	70	0.0	0.0	5.7	39.2

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	EP taxa	EP taxa score	Trichoptera taxa	Trichop. taxa score	% Baetidae + Zapada	% Baet.+ Zap. score	% Diptera	% Diptera score	O/E (family 75%)	O/E score	% collectors	% collectors score
kpchi01-697	HG	Ref.	93.5	3	100.0	4	80	4.6	93.4	91.5	100.0	0.9	87.5	24.0	100.0
kpchi01-699	HG	Ref.	69.7	9	33.3	3	60	10.3	85.3	77.2	85.8	1.0	100	59.9	53.4
kplin01-699	HG	Ref.	51.1	7.5	50.0	2	40	56.5	19.3	26.0	28.9	1.0	100	48.8	68.3
kpmcn01-699	HG	Ref.	76.6	3	100.0	3	60	6.1	91.2	74.6	82.8	0.6	62.5	52.6	63.2

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	EP taxa	EP taxa score	Trichoptera taxa	Trichop. taxa score	% Baetidae + Zapada	% Baet.+ Zap. score	% Diptera	% Diptera score	O/E (family 75%)	O/E score	% collectors	% collectors score
kpott01-697	HG	Ref.	87.6	5	77.8	5	100	5.4	92.3	73.2	81.3	0.9	87.5	34.8	86.9
kpsve01-697	HG	Ref.	72.4	5	77.8	3	60	24.5	65.0	67.8	75.4	1.0	100	57.9	56.2
kptwi01-699	HG	sub-R.	62.2	6	66.7	2	40	25.3	63.8	38.6	42.9	1.0	100	54.9	60.1
macal02-600	HG	sub-R.	34.9	7	55.6	2	40	70.4	0.0	9.6	10.6	0.6	62.5	69.6	40.6
macal04-600	HG	sub-R.	42.9	7	55.6	2	40	60.5	13.5	11.5	12.8	0.8	75	54.4	60.8
malra02-500	HG	Str.	52.3	7.5	50.0	2	40	43.3	38.1	30.9	34.4	1.0	100	61.5	51.3
mamea02-500	HG	Str.	46.5	5	77.8	2	40	59.3	15.3	26.8	29.8	0.8	75	68.9	41.4
mamea04-500	HG	Str.	58.1	7	55.6	3	60	46.0	34.3	38.2	42.4	0.9	87.5	48.4	68.7
mamea06-500	HG	Str.	46.1	6	66.7	2	40	46.5	33.6	26.2	29.2	0.6	62.5	66.4	44.7
marab04-699	HG	Str.	52.6	8	44.4	3	60	51.3	26.8	27.6	30.7	1.0	100	59.6	53.8
masfe01-699	HG	sub-R.	54.7	10	22.2	4	80	34.9	50.2	19.4	21.6	0.9	87.5	50.0	66.7
mashi10-599	HG	Str.	48.9	11	11.1	2	40	29.8	57.4	51.2	56.9	1.0	100	78.9	28.1
ms12101-598	HG	sub-R.	84.6	4	88.9	5	100	0.7	99.0	93.6	100.0	0.9	87.5	75.9	32.2
ms14001-598	HG	sub-R.	75.3	7	55.6	5	100	3.4	95.2	90.2	100.0	0.9	87.5	89.8	13.5
msdea01-598	HG	sub-R.	64.1	6	66.7	3	60	11.1	84.2	68.5	76.1	0.8	75	83.0	22.7
msdec05-598	HG	sub-R.	63.6	7	55.6	2	40	13.1	81.3	65.4	72.6	1.0	100	75.8	32.2
msdec05-600	HG	sub-R.	70.6	9	33.3	3	60	7.8	88.8	71.2	79.1	1.0	100	53.4	62.2
msfly01-598	HG	sub-R.	71.4	7	55.6	5	100	13.6	80.6	67.4	74.9	0.9	87.5	77.5	30.0
msgol01-598	HG	sub-R.	42.5	9	33.3	2	40	57.9	17.2	27.8	30.9	1.0	100	75.0	33.3
msgoo01-598	HG	Ref.	78.0	6	66.7	4	80	1.2	98.3	80.1	89.0	0.9	87.5	65.0	46.6
msgoo01-600	HG	Ref.	75.4	6.5	61.1	4	80	1.0	98.6	79.6	88.4	0.9	93.8	77.2	30.4
mslan01-598	HG	sub-R.	59.6	8	44.4	5	100	46.7	33.2	36.7	40.8	1.0	100	70.9	38.9
msmck01-598	HG	sub-R.	70.9	6	66.7	4	80	14.5	79.3	66.8	74.2	1.0	100	81.3	25.0
msmcr01-598	HG	Str.	69.0	7	55.6	4	80	24.6	64.9	55.4	61.6	0.9	87.5	51.6	64.6
msmcr01-600	HG	Str.	73.2	7	55.6	2	40	14.7	78.9	70.1	77.9	0.9	87.5	25.5	99.3
msmoo01-598	HG	Str.	42.3	8	44.4	1	20	41.6	40.6	23.6	26.2	0.8	75	64.4	47.5
msshr01-598	HG	sub-R.	65.3	6	66.7	3	60	3.5	95.0	86.0	95.5	0.6	62.5	90.8	12.3
mstro01-598	HG	sub-R.	73.7	9	33.3	7	100	6.0	91.5	56.7	63.0	1.0	100	59.2	54.4

Site-Date (myy)	Stream Class	*a priori* Condition	ASCI	EP taxa	EP taxa score	Trichoptera taxa	Trichop. taxa score	% Baetidae + Zapada	% Baet.+ Zap. score	% Diptera	% Diptera score	O/E (family 75%)	O/E score	% collectors	% collectors score
mswol01-598	HG	Str.	44.8	5.5	72.2	1.5	30	58.3	16.7	28.1	31.2	0.9	87.5	76.8	30.9
mswol01-600	HG	Str.	54.3	10	22.2	2	40	21.7	69.0	42.6	47.4	0.9	87.5	55.4	59.4