Preliminary Index of Biological Integrity (IBI) for Periphyton in the Eastern Sierra Nevada, California – Draft Report

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February 28, 2007
(updated August 2008)

EXECUTIVE SUMMARY

Use of periphyton, or attached benthic algae, as an indicator of stream water quality has provided an important source of information for the detection and assessment of environmental degradation in streams and rivers. The growth and type of algae in streams responds rapidly to both chemical pollutants (toxics, nutrients) and physical habitat disturbance (loss of structural diversity, bank erosion, sedimentation, elevated temperature), providing a biological measure of changing environmental quality.

In the eastern Sierra Nevada mountains, replicate samples collected from cobble-size substrates in riffle habitats of over 100 streams over a period from 1996 to 2004 provided the basis for development of a preliminary index of biological integrity (IBI) - a means of quantifying the biological quality of streams. The index combined standardized scores from of 5 separate indicator metrics that were found to provide the clearest relationship to a composite habitat disturbance gradient. Acceptable standards for biological integrity were based on the least disturbed or reference streams of the region, which were defined as those with low exposure to watershed-scale disturbance (fewest upstream road crossings), and/or minimal local reach-scale bank erosion from livestorck grazing In order to compare streams of similar size, the study streams were also divided into two groups, with channel widths less than or greater than 400 cm. Impairment of test sites in both small and large streams was evaluated based on the distribution of reference sites, with any test sites falling 2 standard deviations below the reference mean IBI judged to be in impaired condition [this report includes a listing of streams for the region as supporting, partially supporting, or not supporting (impaired) of the biological integrity defined by the IBI]. Total chlorophyll content was used as a separate indicator of an enriched algal community and while reference streams were typically below 1 μg chlorophyll a cm^{-2} at least half of all test streams were above this level. Total richness diversity was observed to increase over the range of site disturbance, suggesting that the extent of degradation among streams sampled may still have been below the level of severity that limits diversity of algae. For this reason, diversity measures may be difficult to interpret as components of periphyton IBIs. Sedimentation was found to be one of the most important factors in reducing biological integrity, evident in lower IBI values in streams with median particle size below 40 mm.

INTRODUCTION

The use of periphyton as quantitative indicators of environmental quality in rivers and streams is a reliable monitoring tool of ecosystem integrity because attached algae form the autotrophic base of aquatic food webs. Benthic algae have short generation times and patchy microdistributions and so they respond rapidly to chemical and physical changes and can be used to detect impacts at localized spatial scales (Lowe and Pan 1996). Varied tolerances to different stressors, especially among diatoms, also may permit the interpretation of causes and degrees of environmental degradation (Hill et al. 2001, Fore 2003). Dense blooms of algae have long been associated with eutrophication through nutrient enrichment and organic sediment loading in lakes and rivers, so the abundance of algae has often been viewed as a sign of poor water quality and used as an indicator of impairment (Dodds et al. 1998, Busse et al. 2006). Use of composite diatom community metrics (such as species richness) for assessment of ecological conditions was first applied in classic pollution studies of rivers (Patrick et al. 1954) and has now been integrated into many programs of water quality testing, regulation and management (Stevenson and Rollins 2006).

Water quality standards based on aquatic life uses have been developed for many states and in national assessments based on macroinvertebrates and fish but fewer have included algae. Periphyton monitoring tools have been developed for the states of Kentucky, Montana, and Oklahoma (Stevenson and Bahls 1999), and more recently for the mid-Atlantic region (Fore 2003) and Idaho (Fore and Grafe 2002). Following collection from benthic substrate surfaces, the taxonomic composition of the assemblage is identified from a sample (diatoms and/or soft-algae), and these counts are then used to calculate metrics such as taxa richness or species indicative of stress tolerance. An Index of Biological Integrity (IBI) is a composite score, or multi-metric index, that combines the normalized values of separate measures of community composition, tolerance and function. Multi-metric scores are a means of summarizing different kinds of information about a community of organisms into a single value (Karr and Chu 1999). This creates a simplified system for assessing biological integrity by comparison to the range of IBI values that are found in references streams – those meeting defined criteria representing the natural state of streams and watersheds for a given region (Stoddard et al. 2006).

METHODS

Stream Periphyton Sampling, Processing and Counting

Periphyton sampling consisted of collections taken from three different cobble-size rocks, randomly selected from riffle zone locations. Entire rock surfaces (upper surfaces, sides and exposed lower surfaces) were scrubbed into a small volume of stream water using nylon or wire brushes until cleaned (usually 3-5 minutes). A 20 ml subsample of this was taken and placed into a plastic vial and preserved with buffered formaldehyde to a final concentration of 3-4%. Surface areas (cm^2) based on length, width, height, and perimeter were calculated for each rock sampled so that cell densities could be estimated. Subsamples were taken from each collection both for periphyton as above ($cells/cm^2$), and another fraction filtered (GFA, 1.6 micron retention) for chlorophyll *a* (ug/cm^2) analyses. Some of the triplicate diatom and soft algae samples were counted separately to obtain measures of spatial variability within sites, and the others were combined into site composite samples before counting. Prior to calculating metrics, the counts from sites with separate replicates were combined into a single composite cumulative sample. Whether derived from replicate samples combined before or after counting, most samples analyzed had in excess of 500 cells counted (of total diatoms and soft algae), representing a standard level of counting effort (Stevenson and Smol 2003). In preparation for counting, periphyton samples were homogenized and divided equally into soft algae and diatoms according to Stevenson and Bahls (1999). Proportions were noted when samples were not split evenly and adjusted in final density estimates. Diatoms were oxidized and acid-cleaned, and permanent Hyrax® slide mounts were prepared for each collection. When possible, a minimum of 300 diatom cells was counted under oil immersion at 1000x magnification from each slide preparation. In low-density samples, diatom richness was estimated by counting until no new species were observed for 100 specimens as outlined by Stevenson and Bahls (1999). Taxa used in metrics for number of halophic and eutrophic diatoms, and % motile diatoms are included in Table 1. Diatom identifications were made from Patrick and Reimer (1966, 1975), Krammer and Lange-Bertalot, (1986, 1988, 1991a, 1991b), and Sonneman et al. (1999). Diatom nomenclature followed that of Fourtanier and Kociolek (1999), Kingston (2000), and Andresen et al. (2000). Soft algal analyses were performed with a Palmer counting cell and taken to the lowest possible taxonomic level. A minimum of 300 soft algal cells were counted under oil immersion at 40x magnification from each sample. Estimates for soft algae richness followed that of diatoms in low-density samples. Soft algae identifications were made from Prescott (1962), Rivers (1978), Dillard (1993), and Lawrence and Seiler (2002). Protocols for cell density estimates followed that of Stevenson and Bahls (1999).

IBI Development

Reference screening

Based on maps, field data, and knowledge of land use status, each of 130 study sites sampled between 1996 and 2004 was placed into a reference or test group. References met the following criteria: either less than 0.2 road crossings/km of perennial upstream channel length within the watershed, and/or less than 25% bank erosion within the reach and having no known point-sources of pollution, and judged to be least exposed to land use disturbance among sites in the region. Test sites did not meet one or both the reach-scale and watershed measures of disturbance, and had known exposure to pollution or intensive local land use disturbances, or was part of a study design to test for impairment. Site disturbance was also independently evaluated from surveys of physical habitat so that reference and test groups could be compared for multiple measures of overall habitat quality.

Site disturbance.

In addition to separation of the streams into reference and test populations, individual measures of site disturbance were derived from habitat and water quality surveys, and were further combined into an integrated site disturbance index (ISDI, Table 2). Summation of these ISDI scores was used to evaluate correlations in the response of different periphyton metrics to a gradient of combined environmental stress exposure (Table 3). The influence of natural environmental gradients on periphyton community metrics were also examined with regard to elevation, channel slope, upstream channel length, stream width, and dissolved silica content (required for diatom growth). To describe site disturbance, each site had detailed physical habitat transects and water quality measurements taken at the same time as periphyton collections (Herbst and Silldorff 2007, in revision).

Table 1. Listing of diatom indicator taxa

Halophilic Species	Eutrophic Species	% Motile Diatoms
Amphipleura rutilans	*Bacillaria paxillifer*	*Navicula* spp.
Amphora coffeaeformis	*Craticula gregaria*	*Nitzschia* spp.
Anomoeoeis sphaerophora	*Craticula molestiformis*	*Surirella* spp.
Craticula halophila	*Didymosphenia geminata*	
Ctenophora pulchella	*Entomoneis alata*	
Cyclotella meneghiana	*Eolimna minima*	
Cymatopleura solea	*Fallacia pygmaea*	
Denticula elegans	*Luticola mutica*	
Entomoneis alata	Mayamaea atomus	
Entomoneis paludosa	*Melosira varians*	
Mastogloia elliptica	*Navicula salinarum*	
Mastogloia smithii	*Nitzschia capitellata*	
Navicula capitatoradiata	Nitzschia frustulum	
Navicula peregrina	*Nitzschia linearis*	
Navicula salinarum	*Nitzschia palea*	
Navicula tripunctata	*Nitzschia sigmoidea*	
Nitzschia compressa	*Nitzschia umbonata*	
Nitzschia epithemoides	*Rhoicosphenia abbreviata*	
Nitzschia pusilla	*Rhopalodia gibba*	
Nitzschia reversa	*Sellaphora pupula*	
Nitzschia sigma	*Sellaphora seminulum*	
Surirella striatula	*Staroneis phoenicenteron*	
	Stephanodiscus niagare	

Metric selection

Metric performance was evaluated primarily by correlation with known stressor gradients, expressed as the composite ISDI measure of site disturbance. Metrics were selected based on expected correlated responses with site disturbance (Table 3) and consistent performance in both small and large stream types. Selected metrics were re-scaled to three categories corresponding to the lower, middle, and upper ranges of each distribution (trisection method; Karr and Chu 1999). Scores were assigned with respect to the expected response to site disturbance, giving lower values to the ranges of the indicator metrics associated with disturbed conditions (Table 4). The equal-weighted metrics were then summed as multi-metric IBI scores for each site, and these increase with improving biological condition. Levels of biological condition were then defined using statistical properties of the reference distribution (Table 5). Chlorophyll *a* quantity ($\mu g/cm^2$) was also used as a separate indicator of enrichment among the study sites, and can be related to productivity and "fouling" of habitat by excess algae (reducing habitat availability to invertebrates and fish). These periphyton metrics and habitat variables are presented here as a preliminary data set that will be re-assessed as new sample data are added and watershed-scale measures of disturbance can be integrated.

Table 2. Integrated Site Disturbance Index (ISDI) = sum of scores for each site

	Scores and corresponding metric range			
Physicochemical Variable	**0**	**1**	**2**	**Disturbance Indicated**
% riparian cover (reduction)	>50%	25-50%	<25%	bank vegetation loss
temperature (°C)	<15	15-20	>20	warming, low oxygen
substrate uniformity as %FSG	<50%	50-75%	>75%	low substrate diversity
% eroded banks	<20%	20-40%	>40%	instable channel
% cobble embedded	<20%	20-40%	>40%	burial of habitat
% leaf + wood (reduction)	>10%	5-10%	<5%	organic resource loss
conductivity (µS)	<100	100-200	>200	pollutants, nutrients
% macrophytes	<5%	5-20%	>20%	rooting in silt, low flow

Range = 0 to 14

Group >>	A	B	C	D	E	F
ISDI range	0-1-2-3	4-5	6-7	8-9	10-11	12-13-14

RESULTS

The distribution of total algae diversity (richness) across sites within the reference and test groups showed an increase with lower channel slope and longer upstream length (Figure 1A and B), indicating that this was in part a natural function of stream size (sites further downstream in any watershed will have both lower slopes and longer upstream channels). Stream length increase and gradient decrease also corresponds to the gradient of site disturbance (ISDI). In order to separate these natural effects from disturbance, sites were grouped into similar environments according to stream size. Large streams (>400 cm average width) were separated from small streams (<400 cm) such that most of the former were low gradient downriver sites, while the latter group was mostly shorter headwater streams of moderate gradient (Figure 2). The grouping of all streams by ISDI range and accordance with reference and test site designations is shown in Figure 3.

All of the periphyton metrics tested for small streams produced significant correlations with the ISDI gradient, but only a more limited set were related to the composite measure of habitat quality among large streams (Table 3). Although richness measures (total and diatom) were correlated with disturbance, these metrics increased in response to declining habitat quality, contrary to expected response, so were excluded from consideration as metrics that could be clearly interpreted. Based on the metrics that could be applied in both small and large stream types, and that relation to the disturbance gradient also provided separation between reference and test groups, five variables were found that could be used in forming an IBI. Two variables were indicators of tolerance to habitat quality – (1) the percent motile diatoms, representing taxa that are capable of moving out of deposited sediments, and (2) a combination of groups of taxa that are tolerant of dissolved mineral content (halophilic), and of enrichment in nutrients (eutrophic). The other metrics used the densities of two taxa that are common and typically associated with mixed conditions of poor habitat quality (the diatom *Nitzschia palea* and the filamentous green algae *Stigeoclonium lubricum*), and one taxon (the diatom *Fragilaria arcus*) that is considered sensitive and usually found only in undisturbed environments. The presence or absence of these taxa, as well as their density, also provides information on the extent of biological change associated with environmental degradation (Figure 4).

Table 3. Correlations of periphyton response metrics with site disturbance (ISDI)

[Using Spearman's *r*]	**Small Streams (N=78)**		**Large Streams (N=52)**	
	[43 Reference, 35 Test]		[34 Reference, 18 Test]	
METRIC	***r*-value**	**p-value**	***r*-value**	**p-value**
Total taxa	.622	.000	.535	.000
Diatom taxa	.596	.000	.436	.001
Total density	.500	.000	.137	.332
% Motile diatoms	**.387**	**.000**	**.539**	**.000**
No. Halophilic diatoms	.388	.000	.297	.032
No. Eutrophic diatoms	.444	.000	.192	.173
No. Halophilic + Eutrophic	**.533**	**.000**	**.263**	**.060**
Density *Achnanthidium minutissima*	.396	.000	-.145	.306
Density *Fragilaria arcus*	**-.412**	**.000**	**-.410**	**.003**
Density *Nitzschia palea*	**.591**	**.000**	**.568**	**.000**
Density soft algae	.352	.002	-.032	.820
Density *Cladophora*	.307	.006	-.165	.243
Density *Stigeoclonium*	**.450**	**.000**	**.324**	**.019**
% Filamentous green	.381	.001	-.088	.533
Density *Clado.* + *Stigeo.*	.450	.000	.033	.817
Density cyanobacteria	.316	.005	-.068	.638
Chlorophyll a ($\mu g\ cm^{-2}$)	.517	.000	.491	.000
IBI	**-.658**	**.000**	**-.672**	**.000**

The scoring range for the selected periphyton metrics was produced using the trisection approach (Barbour et al. 1999), and these values summed to produce an IBI score for each site. Thresholds of reporting classes of biological integrity were based on the 25th percentile of the reference range to define the boundary between supporting and partially supporting (unimpaired classes), and 2 standard deviations below the reference mean as the boundary below which streams would be considered not supporting, or impaired (Table 5). Though the same metrics were used for both small and large stream groups, the threshold criteria vary according to the distribution of reference site data. All streams surveyed are ranked according to this system in the appendix.

Table 4. Periphyton metrics used for preliminary IBI for Lahontan Region

Expected response to disturbance and re-scaled score (sum range = 5-25)		Scoring category		
Metric	**Response**	**1**	**3**	**5**
% Motile diatoms	Increase	>30	15-30	<15
No. halophilic + eutrophic diatoms	Increase	8-11	5-7	0-4
Density *Fragilaria arcus*	Decrease	absent	$<10^3$	$>10^3$
Density *Nitzschia palea*	Increase	$>10^5$	10^3-10^5	$<10^3$
Density *Stigeoclonium*	Increase	$>10^4$	$<10^4$	absent

The IBI metrics also showed separation of reference and test sites over the gradient of ISDI scores (Figure 5). The standardized and combined scores of these metrics further distinguished the reference and test classes, and the resulting IBI distributions were significantly non-overlapping for both small and large stream groups (Figures 6 and 7, notched box plots show no notch overlap). Using chlorophyll *a* as an independent indicator, test sites showed significantly higher biomass than test sites (Figures 8 and 9). For both stream groups, most reference sites had < 1.0 µg cm^{-2}, and about half of test sites had > 1.0 µg cm^{-2}.

Table 5. Scoring thresholds for condition classes of biological integrity

Statistical criterion=	>25th percentile Ref	>2 SD Ref mean and <25th percentile Ref	<2 SD Ref mean
Reporting class=	Supporting	Partially Supporting	Not Supporting
Small streams	≥15	10-14	≤9
Large streams	≥17	13-16	≤12

IBI scoring had strong negative correlations with site disturbance (Table 3 and Figure 10), and was less variable compared to component metrics (coefficients of variation among reference streams were 24.6% for the small size class, and 18.9% for large streams; all metrics were higher). IBIs could also be clearly separated among site disturbance (ISDI) groupings comparing the least-disturbed A and B groups to the most-disturbed E and F groups (Figure 11). All AB group sites were supporting or at least partial supporting (5 of 60), whereas among EF group sites, 13 of 30 were not supporting, 10 were partial, and only 7 were supporting. IBIs for all streams combined comparing reference to test showed scores below IBI=15 could only be partially supporting (the 25th percentile, Figure 12). One of the most consistent habitat predictors of low IBIs was small sediment particle size (Figure 13).

DISCUSSION

Using a limited set of periphyton community metrics to explore responses to stream site disturbance, an IBI was developed here to quantify biological differences between predetermined reference and test sites. Metrics with consistent and expected responses to a compound index of local site-level disturbance (ISDI) were standardized and summed to produce the multimetric index. Low and high groupings of ISDI scores corresponded closely with where the best and worst biological integrity was observed. The analyses also detected and minimized the influence of natural environmental gradients on periphyton diversity (stream size, watershed location), requiring preparation of separate IBIs for small and large streams (< or > 400 cm width). Condition classes scaled to IBI scores of the reference distribution permitted preliminary assessment of impaired conditions for a list of test sites in the Lahontan Region (Figure 14, Appendix).

The increased richness of diatoms and total species that was observed over disturbance gradients (Table 3) runs counter to general expectations, but is not unprecedented in studies of benthic algae (Stevenson 1984, Fore 2003, Fore and Grafe 2002, Hill et al. 2001). The nutrient-poor waters of many undisturbed streams may restrict the productivity and type of periphyton that such habitats can sustain, so moderate levels of nutrient enrichment and organic pollution may provide a subsidy that enhances richness. The level at which this type of pollution may become a stress rather than a subsidy is unclear and requires experimental test. We observed richness to increase in response to higher amounts of fine and sand substrate comprising benthic substrates (smaller D-50, Figure 13), as well as to a compound index (ISDI) of physical and chemical habitat degradation that did not directly include nutrients. The physical environments of many of the poorest habitats were severely damaged by livestock grazing and channel erosion, yet harbored higher periphyton diversity. Although sedimentation typically reduces macroinvertebrate diversity as habitat space is reduced, microhabitat at the diatom scale may support higher density and species richness on small particles that create increased surface areas relative to substrate volume. Such surfaces may also concentrate adsorbed organic matter and charged nutrient ions. The diversity response as an indicator may differ according to the type of degradation - increasing with physical disturbance and enrichment (C, N, P), and decreasing on lethal exposure to toxic chemical pollutants. In any case, the different conceptual paradigms may be amendable to experimental manipulations. Does intermediate disturbance permit overlapping assemblages of both tolerant and sensitive species? If evenness is greatest at moderate disturbance, does richness correspond? Does diversity initially increase with productivity, then decrease with competitive exclusion? Are there far more tolerant taxa of diatoms than there are sensitive, such that stress, especially of mixed types, promotes conditions for more species to persist? Are there shading and silicate limitations operating in many forested and granitic reference habitat environments of the Sierra? An understanding of these questions will be necessary before periphyton richness can be used as a reliable indicator. In addition, it will be important to establish whether the accrual of algal species with downstream progression into lower gradient sites along watersheds is a general phenomenon, attributable to a greater upstream species pool for colonization, greater microhabitat diversity in larger streams, and higher light levels and nutrient availability (Biggs 1996).

Approximately half of test streams of both small and large size groups showed chlorophyll *a* values above 1 $\mu g\ cm^{-2}$. In studies of regularly sampled streams in New Zealand, about 40% of streams exposed to a moderate intensity of agricultural land use showed chlorophyll *a* in excess of 10 $mg\ m^{-2}$ (Biggs 1995), equivalent to the 1 $\mu g\ cm^{-2}$ observed in test sites here. This suggests that perhaps half of Lahontan test streams might be similarly exposed to moderate levels of agricultural land use such as grazing with the potential to create nutrient loading. Most of the highly agricultural streams in the New Zealand study had >100 $mg\ m^{-2}$, an amount found in only a few samples in the Lahontan data set, and these were sampled by alternate methods (coring tube) as cobble substrates were not available for sampling (Adobe Creek and Cottonwood Lower – supporting and partial supporting, respectively, according to IBI scores). Chlorophyll *a* levels that have been considered unacceptable for recreation and aesthetics are in the range of 100-150 mg m^{-2} (=10-15 µg cm^{-2}; Busse et al. 2006). Chlorophyll *a* extremes may indicate nuisance algae problems, but such outliers may not be as useful in assessing gradients of habitat degradation.

Of 53 total test sites, 24 were judged to be unimpaired, 14 partial supporting, and 15 of these impaired – with a distinct corresponding to expected impairment based on ISDI groupings E and F (where 13 of 30 were not supporting). Most of the poor-performing reference sites were in ISDI group C, and some were marginal at meeting selection criteria. This variation in the reference condition also reflects the natural range of spatial variability that can be expected and so provides a fairer standard in assessment of impaired condition. Temporal variability was also incorporated in the standard as some reference sites were repeat samples from different years (see appendix listings).

The approach taken in this preliminary periphyton IBI was based on relatively few metrics, with species responses examined using broad ranges of density, was scaled using a simple trisection method, and may have been somewhat biased by using variable counts among the samples. Low counts among low-productivity reference sites in particular may have led to lower richness estimates. Future refinement of the periphyton IBI will need to address how these methods can be improved. Use of the periphyton IBI might also be calibrated to different kinds of environmental impact, and should be used as a complement to assessment in the Lahontan Region using macroinvertebrate communities (Herbst and Silldorff 2007, in revision).

Alternative approaches for resolving issues with data and analysis, and further studies:

- Scale metrics continuous (10 =75th or 50th %tile references, 0= 10th %tile of tests)
- Select from broader range of metrics (add indicator groups), and use more in IBI
- Base metrics on analysis of relative abundance rather than densities
- Develop watershed-scale measures of disturbance to supplement the local site-level features used to describe environmental degradation
- Control for effect of sample count by using fixed-count re-sampling, and meet a minimum count target (500 total, with at least 300 diatoms) for all samples to avoid underestimating richness

- Expand data set with additional surveys, esp. low gradient, large reference sites, and sites with severe chemical pollution or nutrient enrichment
- Testing IBI with a validation data set of new reference and test sites to confirm placement of these samples within the distribution of the development data set used in this study
- Comparison to assessments of the same sites that have been based on benthic macroinvertebrate bioassessment (both IBI and predictive model ratings of impairment)
- Examine the response of richness among sites that are degraded primarily by physical disturbance in contrast to those exposed to toxic chemical pollutants
- Compare between-replicate variability from the samples that were counted separately prior to the practice of pooling all three periphyton samples taken at each site (these combined samples are listed as composites in the Appendix). This will help document the inherent variability in the method.
- Need for a methods comparison of the 3 whole-stone riffle samples to the 11 rock disc-samples taken in reach-wide benthos protocol (inter-method comparability)
- Develop a listing of sensitive taxa based on the environmental tolerance summaries compiled by Lowe (1974) and use these to develop other metrics

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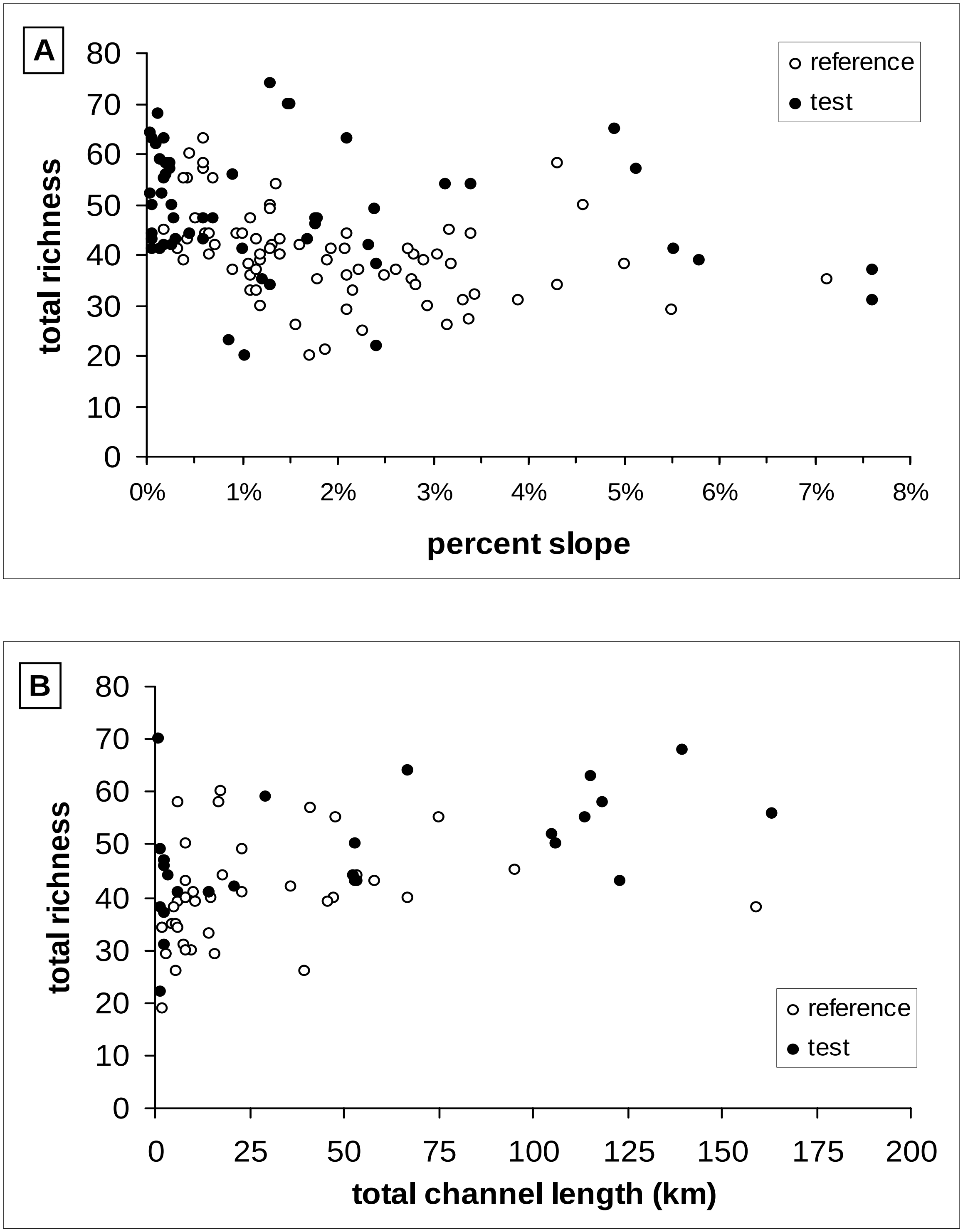


Figure 1. A: The dependence of total species richness on natural gradient of slope, and B: The increase in species richness with channel length (scales with watershed size).

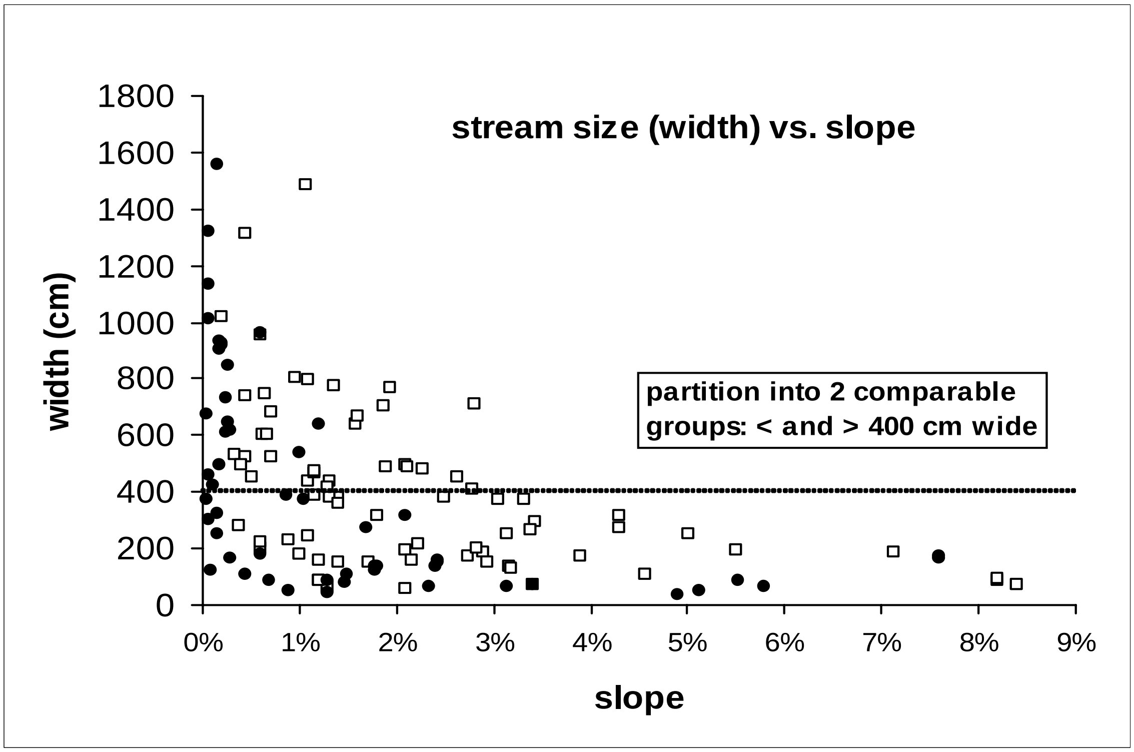


Figure 2. Distribution of stream sites by size (mean width) versus slope, and division into primarily upper and lower watershed sites.

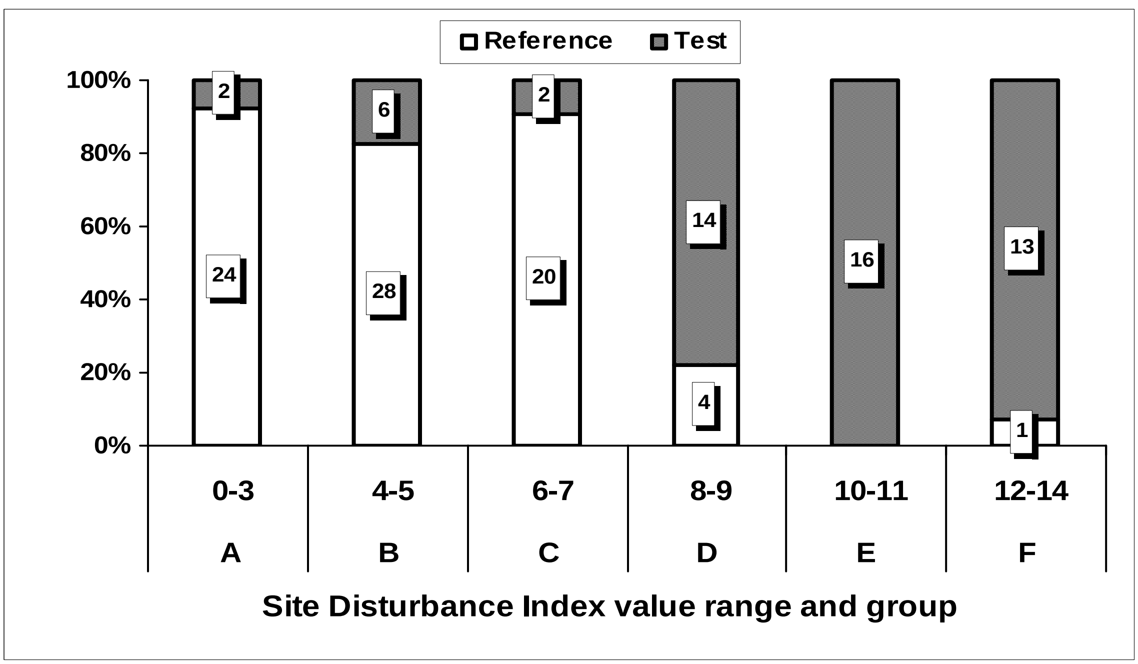


Figure 3. The reference and test site composition of site disturbance ranges and groupings for all streams. Label numbers on bars refer to number of sites in each group. Reference sites are mostly within the range of 0-7 and groups ABC, and test sites are mostly in the range of 8-14 and groups DEF.

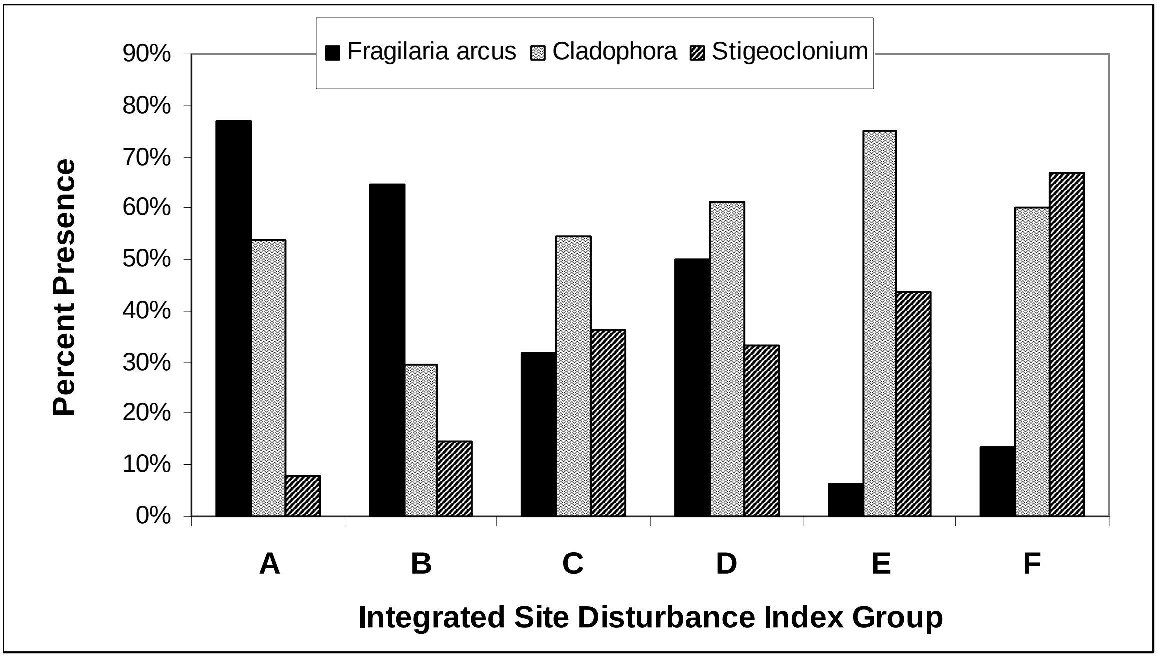


Figure 4. Presence of selected indicator algae along a site disturbance gradient. Bars show percent of sites within each group where each taxon was present. The diatom *F.arcus* frequency decreased with disturbance, while the green filamentous algae became more prevalent.

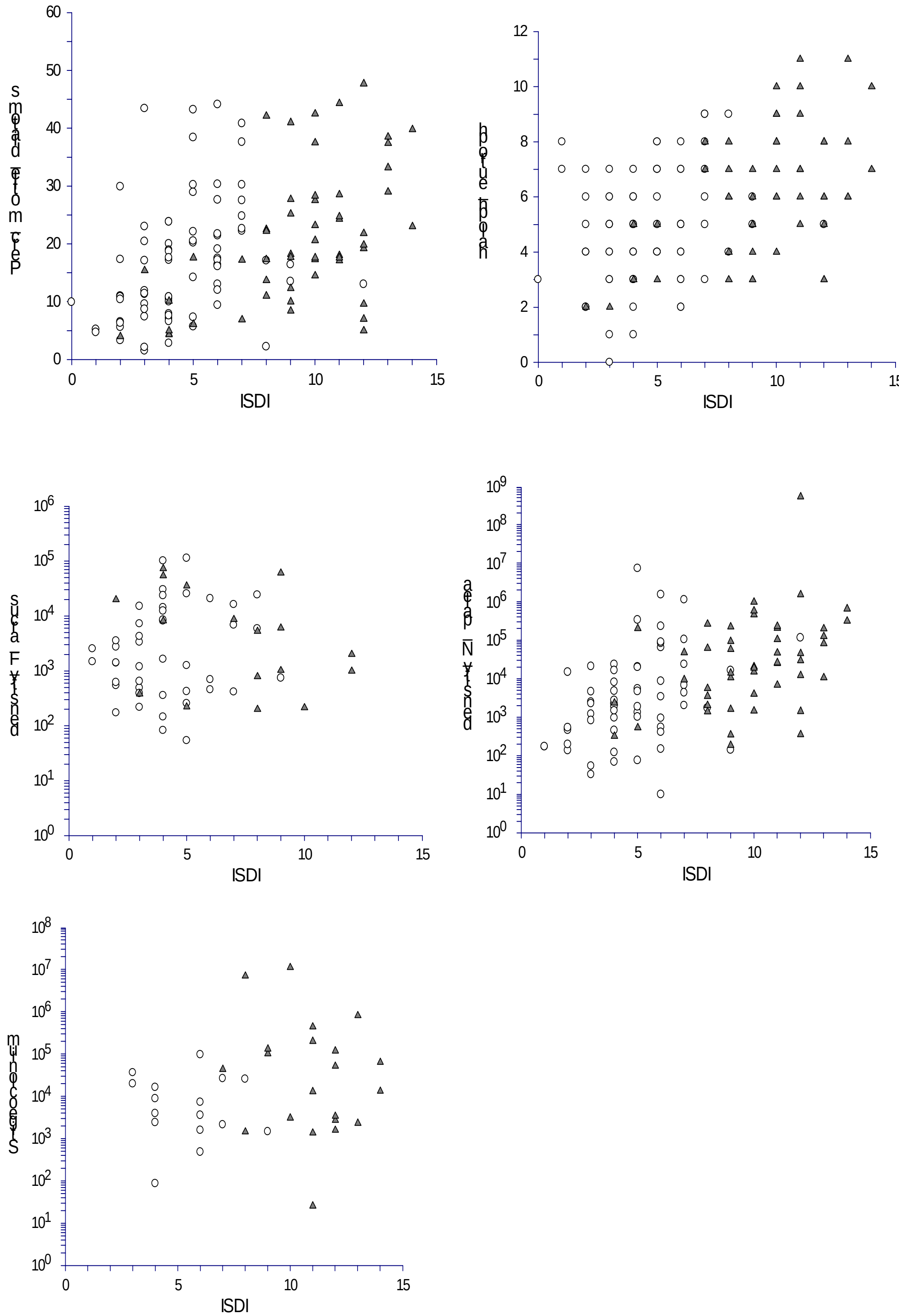


Figure 5. Periphyton IBI metrics relative to site disturbance gradient, inclusive of all streams (small and large size groups). Reference = open circles, Test = shaded triangles.

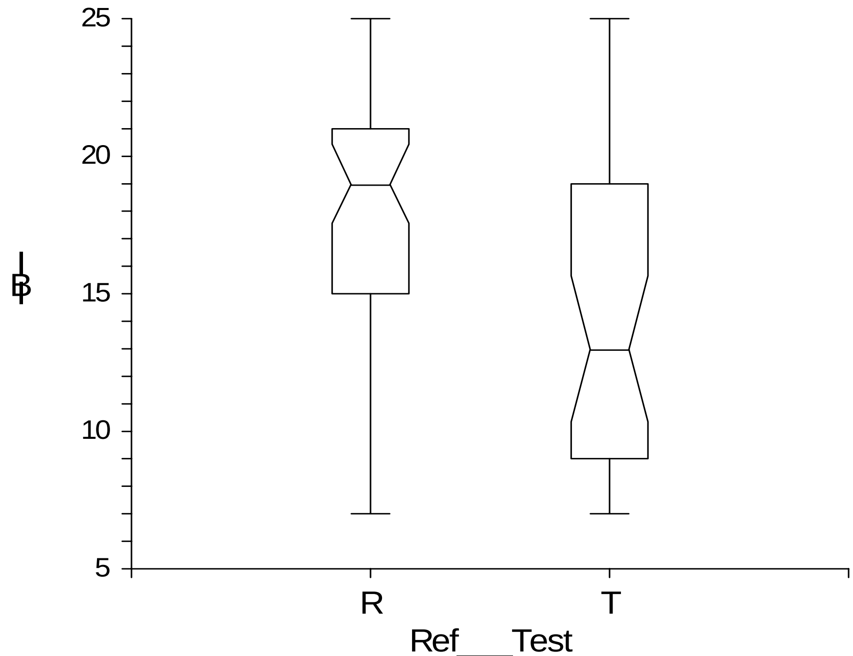


Figure 6. IBI box-whisker plots for small stream reference and test groups.

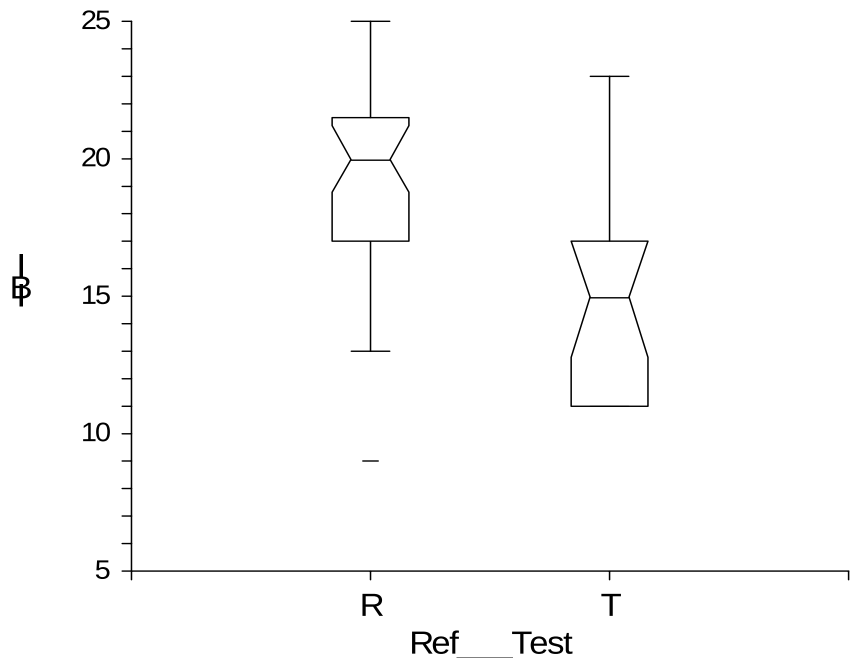


Figure 7. IBI box-whisker plots for large stream reference and test groups.

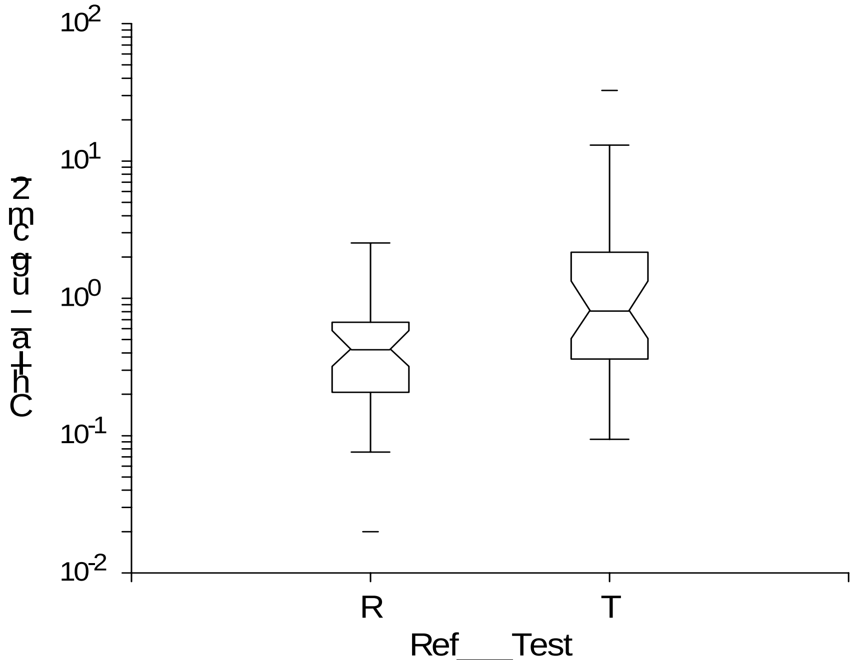


Figure 8. Small stream Chlorophyll *a* for Reference and Test groups. Note median line and distributions of these box-and-whisker plots separate many R and T sites below and above 1 µg cm^{-2}, respectively.

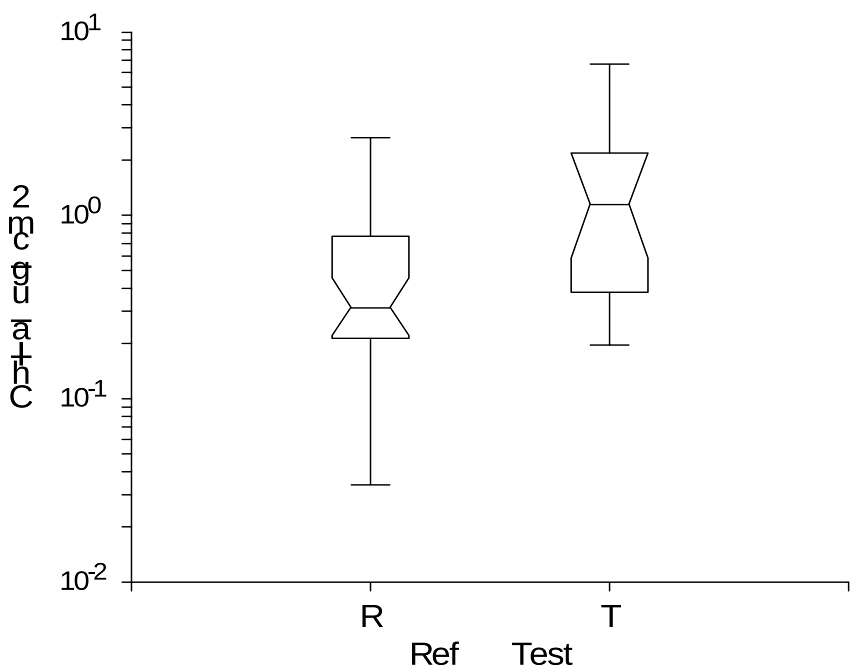


Figure 9. Large stream Chlorophyll *a* for Reference and Test groups. Note median line and distributions of these box-and-whisker plots separate many R and T sites below and above 1 µg cm^{-2}, respectively.

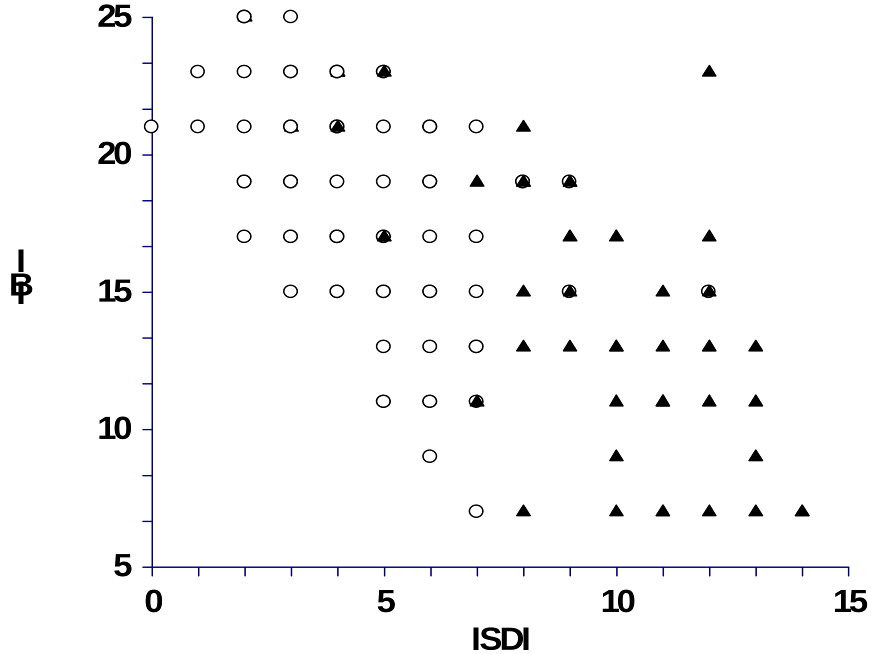


Figure 10. IBI distribution among all streams in relation to site disturbance index. Open circles are references, solid triangles are test sites.

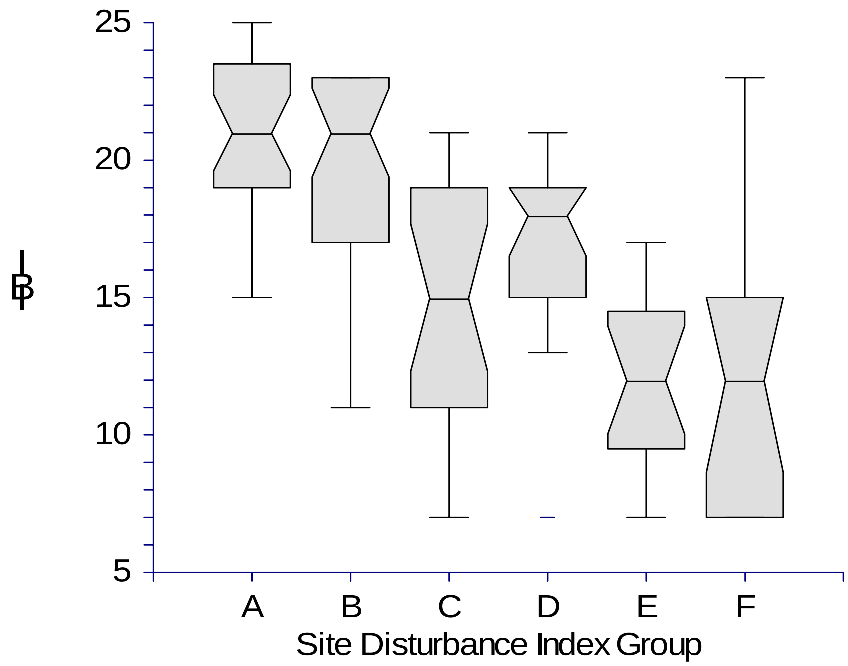


Figure 11. IBI distributions by site disturbance group. Clear difference between high integrity groups AB (0-5), and those above ISDI>10 (group E and F), many of which did not support biological integrity relative to the reference condition sites.

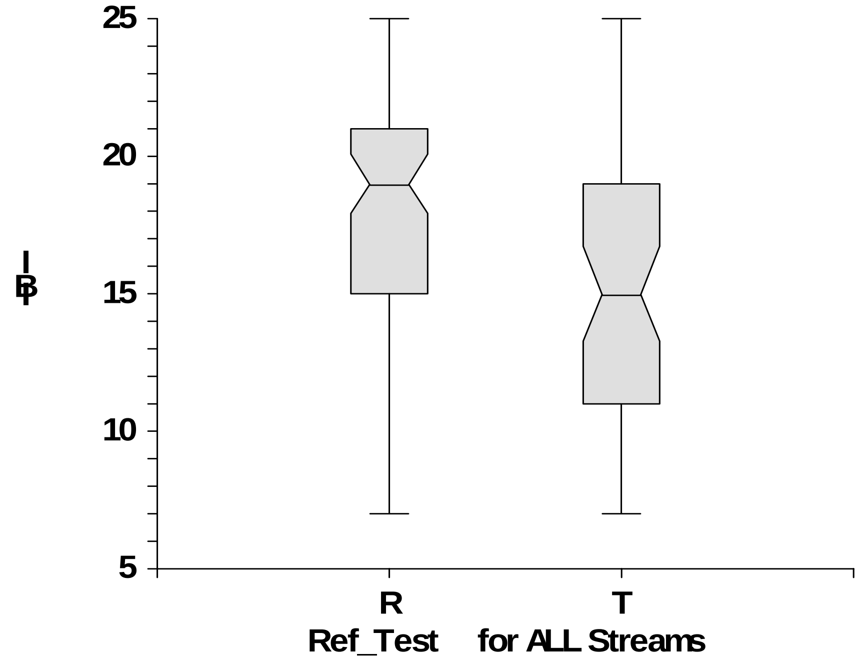


Figure 12. Reference and Test group IBIs for all streams combined.

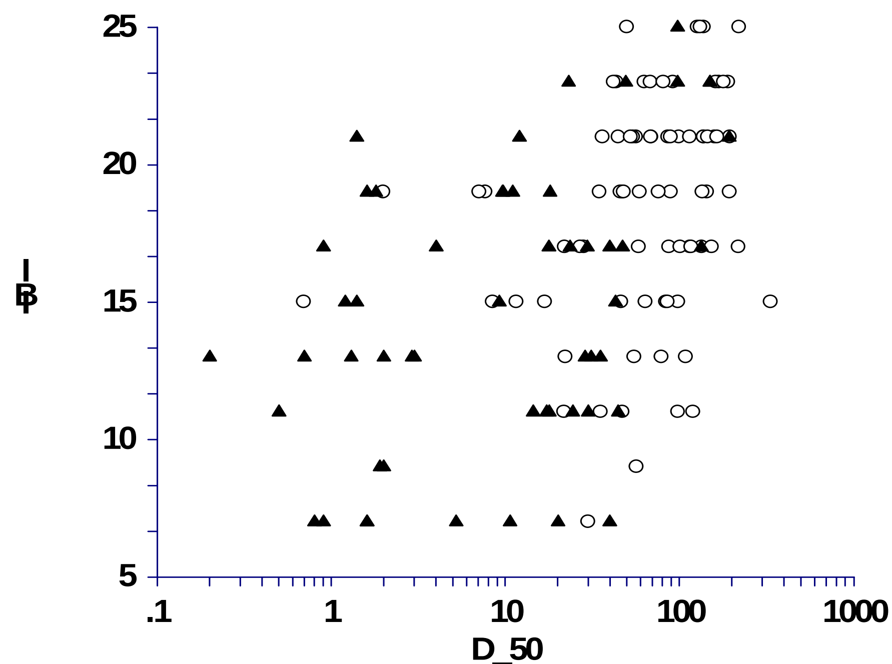
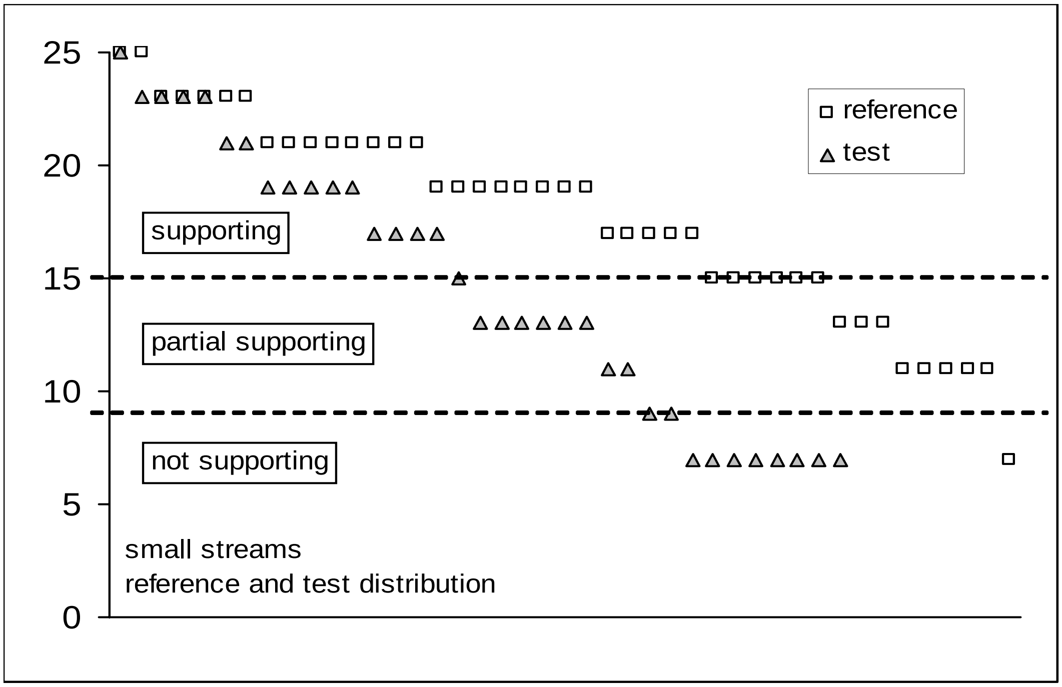


Figure 13. Relation of IBI scores to median particle size of sites (D-50 in mm on log scale). Note that below D-50 of 40-50 mm, many sites fall below IBI=15, below which all sites are only partially supporting of biological integrity.



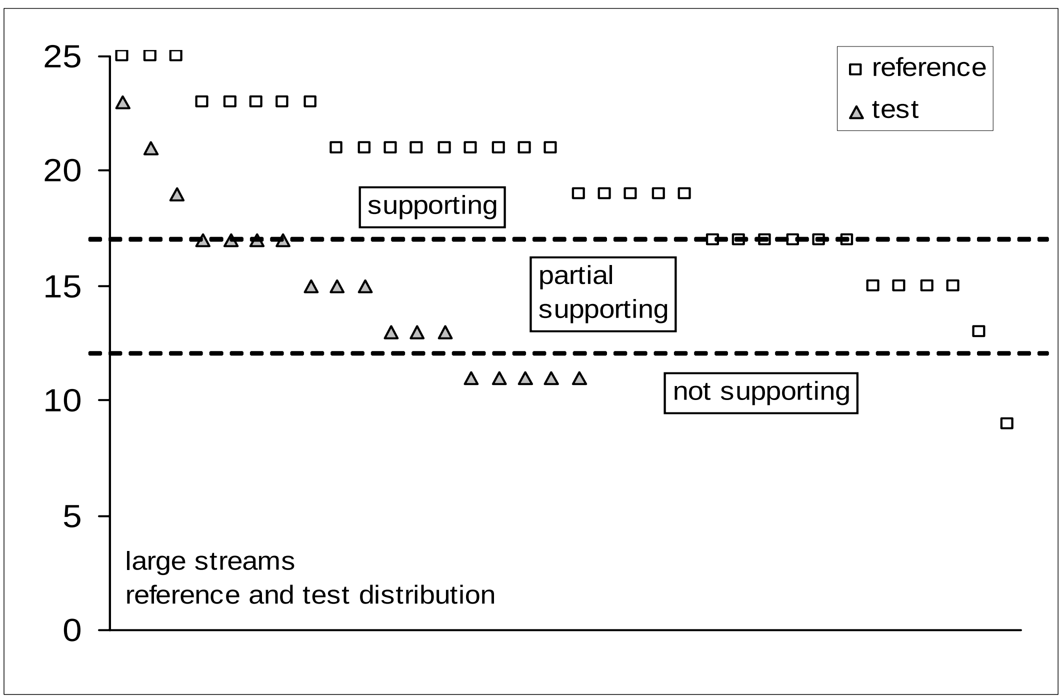


Figure 14. Ranked-order distributions of reference and test sites relative to biological condition thresholds for small streams (above), and large streams (below).

APPENDIX: Alphabetical Listing of Stream Sites by Size Class, Periphyton IBI scores and Condition Class

SMALL STREAM CLASS (<400 cm width)

Stream and Site Name	Date	R or T	ISDI	ISDI Group	IBI	Condition
Adobe Cr below Highway	2-Aug-96	T	11	E	15	supporting
Alder Cr. Meadow	11-Jul-01	R	5	B	19	supporting
Arastra Cr meadow	16-Jul-98	R	5	B	15	supporting
Aurora Cr Lower (Composite)	16-Jul-98	R	6	C	11	partial supporting
Bagley Valley Cr lower meadow (Composite)	8-Jul-99	T	13	F	7	not supporting
Bagley Valley Cr Restoration (Composite)	24-Jul-02	T	9	D	17	supporting
Bagley Valley Cr Restoration (Composite)	30-Jul-03	T	10	E	17	supporting
Bagley Valley lower control (Composite)	30-Jul-03	T	10	E	11	partial supporting
Bagley Valley lower control (Composite)	24-Jul-02	T	10	E	7	not supporting
Bagley Valley lower control (Composite)	8-Jul-99	T	8	D	7	not supporting
Bear Cr. Lower	30-Aug-00	R	4	B	23	supporting
Bear Cr. Lower	10-Jul-01	R	5	B	21	supporting
Bodie Cr Middle Exclosure	23-Jul-96	T	14	F	7	not supporting
Bodie Cr Stateline	16-Jul-98	T	11	E	7	not supporting
Burcham Cr. above Road	25-Aug-97	R	7	C	13	partial supporting
Charity Valley Cr next to trail (Composite)	19-Aug-04	R	6	C	15	supporting
Clearwater Cr. Middle	10-Jul-97	T	11	E	7	not supporting
Clearwater, Lower Warm Springs	26-Jul-96	T	13	F	9	not supporting
Cold Creek below Powerline Trail	23-Aug-01	R	2	A	23	supporting
Cottonwood Cr. Sweetwater Meadow	13-Aug-99	R	5	B	13	partial supporting
Cottonwood Lower, Bodie Hills	29-Jul-96	T	11	E	11	partial supporting
Deep Cr. above W. Walker	27-Aug-01	R	4	B	21	supporting
Deep Creek below Lobdell Lake	31-Jul-96	R	5	B	23	supporting
Dexter Canyon Cr. below ranch	3-Sep-96	T	10	E	9	not supporting
Dog Cr below road	9-Jul-97	R	6	C	21	supporting
Dunderberg Cr meadow above road	18-Aug-97	R	4	B	17	supporting
E. Martis Cr Forest margin (Composite)	28-Jul-04	R	6	C	11	partial supporting

Stream and Site Name	Date	R or T	ISDI	ISDI Group	IBI	Condition
Elder Cr lower (Composite)	10-Jul-03	R	3	A	19	supporting
Glass Cr lower	23-Jul-97	R	4	B	15	supporting
Golden Canyon Cr above trail xing (Cmp)	8-Jul-03	R	2	A	25	supporting
Heavenly Valley Cr. above Powerline Trail	30-Jul-01	T	3	A	21	supporting
Hidden Valley Cr. above Confluence	30-Jul-01	R	0	A	21	supporting
Hilton Cr below Park	12-Aug-98	T	7	C	19	supporting
Hot Springs Cr. above Grover	22-Aug-01	R	3	A	19	supporting
Juniper Cr above Road Crossing	10-Jul-01	R	7	C	13	partial supporting
Kinney Cr above Silver Trib (Composite)	17-Aug-04	R	2	A	25	supporting
Kirman Cr. upper	19-Aug-99	T	14	F	7	not supporting
Lacey Cr. Confined Section	12-Jul-01	R	4	B	23	supporting
Lower Hot Cr. north bend	17-Jul-98	T	12	F	7	not supporting
Main stem Martis Cr old reference (Composite)	26-Jul-04	R	7	C	11	partial supporting
Main stem Martis Cr old reference (Composite)	10-Jul-01	R	7	C	7	not supporting
Marble Canyon Cr. below Exclosure	7-Jul-97	T	9	D	19	supporting
Mill Cr Central (Composite)	1-Jul-03	R	5	B	15	supporting
N Canyon Cr below confluence (Composite)	14-Jul-98	R	12	F	15	supporting
Nye Cr at Road 141	15-Jul-98	T	12	F	13	partial supporting
O'Harrel Canyon Cr below exclosure	19-Aug-96	T	10	E	13	partial supporting
O'Harrell Canyon Cr. below exclosure	29-Jun-99	T	10	E	13	partial supporting
Pole Cr. Tributary Reference	31-Aug-00	R	4	B	21	supporting
Poore Cr. 1/3 Grazing use	31-Jul-97	T	11	E	13	partial supporting
Rough Cr below confluence (Composite)	12-Jul-98	R	5	B	11	partial supporting
Sagehen Cr. below field station	1-Sep-00	R	4	B	17	supporting
Sagehen Cr. below field station	12-Jul-01	R	5	B	11	partial supporting
Saxon Cr. above Oneidas	22-Aug-01	R	3	A	19	supporting
Schaeffer Trib to Martis Cr above Silver (Cmp)	27-Jul-04	R	2	A	17	supporting
Slinkard Cr below Exclosure	30-Jul-97	T	9	D	17	supporting
Slinkard Cr Restoration Area (Composite)	5-Aug-02	R	9	D	15	supporting

Stream and Site Name	Date	R or T	ISDI	ISDI Group	IBI	Condition
Slinkard Cr. Restoration Area	27-Jul-00	R	6	C	17	supporting
Slinkard Restoration Area (Composite)	29-Jul-03	R	8	D	19	supporting
Spratt Cr. above Road Crossing	6-Sep-00	R	4	B	21	supporting
Squaw Cr. S. Fork below headwall	9-Jul-01	T	4	B	23	supporting
Squaw Cr. S. Fork below headwall	29-Aug-00	T	4	B	23	supporting
Squaw Cr. lower meadow	9-Jul-01	T	12	F	23	supporting
Squaw Cr. lower meadow	28-Aug-00	T	9	D	13	partial supporting
Squaw Cr. middle meadow	9-Jul-01	T	12	F	17	supporting
Squaw Cr. middle meadow	29-Aug-00	T	9	D	19	supporting
Squaw Cr. Moraine	28-Aug-00	T	8	D	19	supporting
Squaw Cr. North Fork below Silverado	9-Jul-01	R	3	A	23	supporting
Squaw Cr. upper meadow	29-Aug-00	T	8	D	19	supporting
Summer Cr Meadow (Composite)	7-Jul-98	R	7	C	21	supporting
Swauger Cr.above E. Fork	17-Aug-99	R	2	A	19	supporting
Trib 1 SKC above SKC (Composite)	31-Jul-03	R	6	C	19	supporting
Trib 1 SKC above SKC (Composite)	23-Aug-00	R	4	B	19	supporting
Trib 1 SKC above SKC (Composite)	23-Jul-02	R	7	C	17	supporting
Trout Cr Bennett Flat	11-Jul-01	T	8	D	21	supporting
Trout Cr below Fountain Place	23-Aug-01	R	3	A	21	supporting
W. Martis Cr. below golf course (Composite)	28-Jul-04	T	10	E	13	partial supporting
Wilson Cr above 395 (Composite)	10-Jul-98	T	2	A	25	supporting
Wilson Cr below confluence (Composite)	10-Jul-98	T	4	B	23	supporting

LARGE STREAM CLASS (>400 cm width)

Stream and Site Name	Date	R or T	ISDI	ISDI Group	IBI	Condition
Blackwood Cr. above HWY 89 (Composite)	29-Jul-04	T	5	B	17	supporting
Buckeye Cr above WRID fence	11-Sep-00	T	12	F	11	not supporting
Buckeye Cr. below WRID fence	11-Sep-00	T	13	F	11	not supporting
Coldstream Cr. upper Gravel Pit	1-Sep-00	R	7	C	15	partial supporting
Convict Cr lower SNARL	16-Jul-99	R	4	B	17	supporting
Convict Cr lower SNARL (Composite)	21-Sep-04	R	2	A	19	supporting
Convict Cr lower SNARL (Composite)	1-Jul-04	R	3	A	17	supporting
Convict Cr. Lower SNARL	21-Jun-01	R	4	B	21	supporting
Convict Creek, lower SNARL	10-Jul-96	R	6	C	21	supporting
Deadman Cr above Big Springs Campgound	6-Jul-99	R	4	B	21	supporting
Desert Cr Lower (Composite)	15-Jul-98	R	3	A	17	supporting
E. Carson R. above Bagley Valley	7-Sep-00	R	6	C	19	supporting
E. Walker Cr fenced HRM	10-Sep-96	T	13	F	13	partial supporting
E. Walker R. below WRID fence	4-Aug-00	T	11	E	11	not supporting
General Cr. below Loop Road	30-Aug-00	R	4	B	23	supporting
Green Lower	1-Aug-96	R	4	B	23	supporting
Independence Cr below Road	13-Jul-01	R	2	A	25	supporting
Lee Vining Cr lower central channel (Cmp)	2-Jul-03	R	3	A	25	supporting
Lee Vining Cr. Moraine Campground	3-Aug-00	R	6	C	19	supporting
Little Truckee R. at upper Perazzo	31-Aug-00	R	6	C	13	partial supporting
Little Truckee R. below Coldstream	13-Jul-01	R	5	B	17	supporting
Little Walker Cr. above camp	21-Aug-96	R	5	B	17	supporting
McGEE Cr campground moraine	21-Jul-99	R	2	A	21	supporting
Murray Canyon Cr Below confl (Composite)	9-Jul-03	R	2	A	25	supporting
North Fork Prosser Cr. below USFS Boundary	11-Jul-01	R	9	D	19	supporting
Parker Cr. bench below lake	26-Jul-01	R	3	A	23	supporting
Perrazzo Cr. meadow	12-Jul-01	R	8	D	19	supporting
Pleasant Valley Cr below USGS boundary (Cmp)	18-Aug-04	R	3	A	21	supporting

Stream and Site Name	Date	R or T	ISDI	ISDI Group	IBI	Condition
Prosser Cr. below Confluence	31-Aug-00	R	6	C	15	partial supporting
Robinson Cr. above Twin Lakes	31-Aug-01	R	1	A	21	supporting
Robinson Cr. above WRID fence	11-Sep-00	T	10	E	17	supporting
Robinson Cr. below WRID fence	24-Jul-00	T	12	F	13	partial supporting
Robinson Cr. Honeymoon Flat	25-Jul-00	R	4	B	17	supporting
Rock Cr Tuff Campground	22-Jun-99	R	1	A	23	supporting
Rush Cr. above Hwy 395	26-Jul-01	R	3	A	21	supporting
Rush Cr. Bottomlands	2-Aug-00	T	5	B	23	supporting
Silver King Cr. above Valley	23-Aug-00	R	5	B	23	supporting
Taylor Cr below Fallen Lead	31-Jul-01	T	4	B	21	supporting
U. Owens R. above bridge	17-Aug-00	T	8	D	15	partial supporting
U. Owens R. above Mono Tunnel	20-Jul-00	T	9	D	17	supporting
U. Owens R. below Benton Crossing	18-Aug-00	T	10	E	17	supporting
U. Owens R. below Benton Crossing	24-Aug-99	T	11	E	11	not supporting
U. Owens R. below Mono Tunnel	19-Jul-00	T	12	F	15	partial supporting
U. Owens River Inaya lower (Composite)	31-Aug-04	T	9	D	15	partial supporting
U. Truckee R. at State Park (Composite)	30-Sep-99	R	6	C	9	not supporting
U. Truckee R. Barton above bridge lower	29-Sep-99	T	7	C	11	not supporting
U. Truckee R. Celio Lower	22-Sep-00	R	4	B	15	partial supporting
U. Truckee R. Sunset Stable Lower	20-Sep-00	T	8	D	13	partial supporting
U. Truckee R. Sunset Stable Upper	20-Sep-00	T	9	D	19	supporting
Virginia Cr. Upper Meadow	11-Jul-97	R	4	B	21	supporting
West Walker R. upper confluence	20-Aug-99	R	3	A	15	partial supporting
Wolf Cr. above Trailhead	7-Sep-00	R	6	C	21	supporting