4.1 Exploratory Classification Analysis

The classification investigation proceeded through ordination of taxa and metrics in reference sites so that samples could be organized by similar biological characteristics. Non-metric multidimensional scaling (NMS) ordination was used to find sites with similar taxa. Principle components analysis (PCA) was used to organize sites by similar metric values, using 45 selected metrics. In each of these ordinations, the biological gradients were mapped in two dimensions, with each axis describing orthogonal composite aspects of the community. Any strong associations of environmental factors with the axes prompted further investigation of the factors as possible classification variables.

Level 4 ecoregions were fairly distinct for reference SNEP sites using presence/absence ordinations. On the first axis of the NMS ordination, sinuosity, longitude, land slope, and substrate characteristics, and percent water and wetland cover in the watershed are the major correlated natural variables that might be useful for site classification. Drainage area was also correlated but might not be appropriate for classification. In more disturbed non-reference sites, watersheds were up to 189 km^2. If drainage area was used in site classification, the reference condition derived mostly from small sites (<25 km^2) might represent a natural condition that would not be applicable to large non-reference sites. Sinuosity was on the same axis as land slope and drainage area. These three variables are often related, as large catchments are generally in flatter valleys with low slopes and meandering streams.

Longitude is related to ecoregion and could be used as a continuous variable for classification whereas ecoregions could define categorical classes. However, there was no distinctive break-point or threshold along the longitudinal gradient and the categorical ecoregions would be better classification variables than longitude.

To explore the effects of environmental variables on metric distributions, a PCA was performed with 45 metrics that represented a variety of metric formulations and taxa characteristics. The PCA identified the same variables on the first axis as were identified in the NMS of taxa presence absence, though in a slightly different order of importance. These included sinuosity, land slope, percent water and wetland cover in the watershed, longitude, and drainage area. Substrate characteristics were also correlated, though not as strongly.

4.2 Classification Summary

Classification schemes related to Level 4 ecoregions and drainage area were considered but ruled out based on results from the NMS and PCA analyses. Level 4 ecoregion did not cluster distinctly in the PCA ordination of metrics. Moreover, defining site classes based on Level 4 ecoregions might be untenable because it would result in small sample sizes for index calibration. All the reference sites in the NBL were <15 km2, which is smaller than the bulk of stressed sites, suggesting that a classification scheme based on drainage area or ecoregion would result in insufficient comparable samples for index calibration.

Continuous variables that showed potential for classification included: annual air temperature (PRISM 1981-2010), sinuosity, longitude, land slope, substrate types, and drainage area. Because there are no clear break-points to distinguish classes based on the continuous variables, scores for individual metrics that showed strong correlations with these natural variables were adjusted during index development (see Section 5.1).

5 Index Development

During the calibration of the SNEP low gradient IBI, a parallel project (statewide MassDEP low gradient IBI development) was also underway. Several members of the SNEP workgroup were also members of the MassDEP workgroup. There was also overlap across the two datasets (the SNEP samples were included in the statewide MassDEP IBI dataset). Thus, the two projects were not completely independent and often were informing one another, as described in the ensuing sections.

Index development consisted of the following steps:

- Metric scoring
- Metric selection
- Index compilations and performance evaluation
- Selection of final IBI
- Index verification

5.1 Metric scoring

Evaluation and selection of metrics typically involve testing of many more metrics than end up in the final index. We calculated and evaluated over 150 metrics (Appendix B). Formulae were applied to the metrics to standardize them to a 100-point scoring scale (as in Hughes et al. 1998, and Barbour et al. 1999). The scoring scale was based on the percentile statistics (and minimum values) of metric values across all sites (as opposed to only reference sites). For metrics that decreased with increasing stress (referred to as 'decreasers'; an example is the number of intolerant taxa metric), we used the following equation in which the 95th percentile was the upper end of the scoring scale and the minimum possible value (zero) was the lower end:

$$Decreaser\ metric\ score = 100 * \frac{Metric\ value - minimum\ possible\ value}{95th\ percentile - minimum\ possible\ value}$$

For metrics that increased with increasing stress (referred to as 'increasers'; an example is the number of tolerant taxa metric), we used the following equation in which the 95th percentile was the upper end of the scoring scale and the 5th percentile was the lower end:

$$Increaser\ metric\ score = 100 * \frac{95th\ percentile - metric\ value}{95th\ percentile - 5th\ percentile}$$

A metric adjustment procedure was implemented for metrics that were strongly correlated with the classification variables (drainage area, mean annual air temperature (PRISM 1981-2010), longitude, percent wetland and open water in the watershed, mean land slope in the watershed). The procedure included the following steps:

1. Run a Spearman correlation analysis on all metrics and classification variables
    a. Include all reference samples
2. Identify metrics that were correlated at $|r| > 0.50$.