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Development of indices of biotic integrity for high-gradient wadeable rivers and headwater streams in New Jersey

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ABSTRACT

The New Jersey Bureau of Freshwater and Biological Monitoring (BFBM) has been conducting Fish Index of Biotic Integrity (FIBI) monitoring on rivers and streams (with drainage area > 12.95 km^2) in the Northern part of the state since 2000 using Karr's original IBI format with several regional modifications. In an effort to increase the overall performance of the IBI and to assess smaller headwater streams, a new design and approach to metric development was evaluated on approximately 230 high gradient streams. This design, developed by Thomas Whittier and Robert Hughes, has been implemented for numerous Western U.S. studies, as well as the Connecticut multi-metric indices (MMI). Analysis resulted in two distinct stream classes; a coldwater community (Headwaters IBI) with drainage area < 10.36 km^2 consisting of brook trout, sensitive salamanders, and native crayfish and cool/warmwater fish communities (Northern FIBI) with drainage area > 10.36 km^2. Over 140 metrics from ten ecological classes were tested for signal to noise, range, responsiveness, and redundancy. A total of eight metrics representing seven ecological classes were selected for the Northern FIBI and six metrics representing five ecological classes were selected for the Headwaters IBI. These two indices provide the Bureau with an effective and sensitive biological tool to monitor and assess all non-tidal, wadeable streams in the Piedmont, Highlands, and Ridge and Valley physiographic ecoregions.

1. Introduction

One of the primary goals of the Clean Water Act is to maintain and restore biological integrity of all water bodies within the United States. Water resource managers commonly use biological assessments to evaluate water quality and to assess whether water bodies are meeting the goals of the Clean Water Act (Simon, 1999). The advantages of using biological monitoring over chemical monitoring have been well documented (Karr, 1981; Barbour et al., 1999) and the use of fish as biological indicators has grown in popularity since the inception of Karr's Index of Biotic Integrity (IBI) in 1981. Karr's IBI was a multimetric index designed to assess water quality conditions in warm midwestern streams using fish assemblages (Karr, 1981; Karr et al., 1986). Karr's IBI has since been regionally modified to account for differences in assemblage structure and function (Miller et al., 1988) and has been applied by water resource managers worldwide (Roset et al., 2007; Ruaro and Gubiani, 2013). In the United States, it is common for water resource managers to have one or several biomonitoring programs focused on differing trophic levels of aquatic organisms (i.e., algae, macroinvertebrates, fish), as the United States Environmental Protection Agency (U.S. EPA) recommends the use of at least two assemblages to provide information on aquatic life use; the health of streams for aquatic organisms (U.S. EPA, 2003).

The New Jersey Department of Environmental Protection (DEP) has been conducting biological monitoring using aquatic macroinvertebrates since 1992. In an effort to further enhance the Department's biological monitoring program and to supplement existing benthic macroinvertebrate monitoring, the New Jersey Fish Index of Biotic Integrity (FIBI) was established in 2000. The FIBI was developed by the U.S. EPA Region 2 (Kurtenbach, 1994) and was based on Karr et al. (1986) original design with regional modifications. The index was then implemented by the DEP Bureau of Freshwater and Biological Monitoring (BFBM) on high gradient streams greater than 12.95 km^2 in contributing watershed drainage size. The FIBI was later recalibrated by BFBM in 2005 to increase the overall sensitivity and performance. This process involved replacing unresponsive metrics, altering feeding and tolerance guild designations for species, and modifying scoring criteria within Karr's rapid bioassessment framework. Although the recalibrated design greatly increased the sensitivity of the index, several metrics remained unresponsive to anthropogenic stressors. In addition, small first and second order headwater streams less than 12.95 km^2 remained largely unassessed for vertebrates and an index was

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warranted to support and describe aquatic life use in headwater streams of New Jersey.

Several states in New England including Vermont (Langdon, 2001), New Hampshire (Neils, 2007), and Connecticut (Kanno et al., 2010) have developed coldwater IBIs for these small headwater streams, typically dominated by brook trout *Salvelinus fontinalis* and slimy sculpin *Cottus cognatus*. Additionally, Lyons (2006) created a cool/warmwater headwater IBI to assess water quality in Wisconsin, but found too few fish to accurately assess streams less than 4 km^2 in watershed area or 4–10 km^2, if stream slope exceeded 1%. As a result of depauperate fish assemblages in very small streams, biologists began supplementing fish data with other vertebrate and invertebrate taxa, most notably streamside salamanders, to accurately assess the biological integrity of headwater streams. The Maryland Biological Stream Survey developed a streamside salamander IBI (Southerland et al., 2004), while others (Ohio Environmental Protection Agency, 2002; Whittier et al., 2007; Pont et al., 2009) have included multiple vertebrate taxa groups such as fish and amphibians into a composite IBI.

In 2004, the Academy of Natural Sciences of Drexel University was contracted by NJDEP and EPA to pilot a project aimed to develop bioassessment criteria for headwater streams using fish, salamanders, crayfish, and frogs as bio- indicators (Keller et al., 2012). In 2013, NJDEP completed the validation of the Headwaters IBI sampling protocol and began monitoring a fixed network of sites in 2014, thus enabling the Department to monitor all wadeable, non-tidal streams north of the fall line for aquatic life use.

In an effort to provide continuity between the Fish IBI and Headwaters IBI programs and to evaluate new analysis techniques, an innovative structured approach developed for western U.S. streams and rivers was reviewed (Whittier et al., 2007; Stoddard et al., 2008; MPCA, 2014). Several states have adopted this approach in the development or refinement of fish IBI's including Connecticut (Kanno et al., 2010) which completed coldwater and mixed-water multi-metric indices (MMI) and Minnesota (MPCA, 2014) which developed several state indices. We followed this same approach in the development of two New Jersey specific multi-metric indices that will serve as more sensitive/responsive replacements to those previously developed indices for non-tidal high gradient wadeable streams and rivers in the northern part of the state.

The development of two new size specific IBIs in the state of New Jersey presented several challenges. New Jersey is the most densely populated state in the United States (United States Census Bureau, 2011) and its freshwaters are relatively deficient in fish species compared to other regions of the United States. New Jersey's logging, agriculture, and industrial practices of the past and present day, coupled with rapid land development, have greatly altered aquatic systems throughout much of the state. As a result, natural conditions are scarce and the lack of reference streams further complicates the development of an IBI (Hughes et al., 1998). In addition, extensive stocking of nonnative sportfish, such as brown trout *Salmo trutta* and black basses *Micropterus* sp. has resulted in the naturalization of a number of species and has contributed to the declines of many native fishes (Rahel, 2000; Gozlan et al., 2010; McKenna et al., 2013).

The purpose of this study was to develop two indices of biotic integrity for the higher gradient streams of northern New Jersey that could be used to accurately assess water quality and aquatic life use across varying sizes of wadeable streams. A new northern Fish IBI for larger wadeable streams was developed which is more sensitive and responsive to anthropogenic stressors. Secondly, a new Headwaters IBI was developed to assess smaller order streams that are often low in fish richness and therefore cannot be accurately assessed solely with a fish based IBI. The development of these IBIs will allow all wadeable steams north of the fall line to be assessed for aquatic life use.

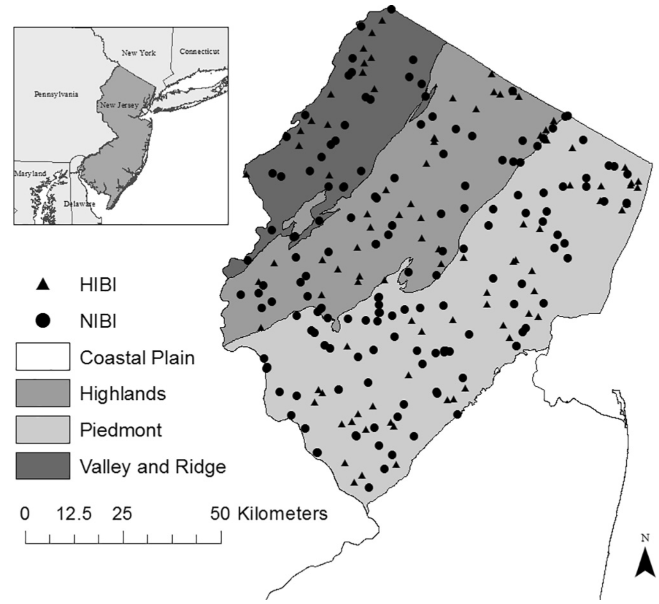


Fig. 1. Map of northern New Jersey depicting sampling locations for Headwaters IBI (triangle) and Northern Fish IBI (circle) development.

2. Materials and methods

2.1. Study area

Data were collected from wadeable streams north of the fall line which runs roughly south-east to north-west from Trenton, NJ to Raritan Bay. The fall line separates the Coastal Plain from the Piedmont ecoregions. Streams in the northern part of the state are characterized by high gradient, cobble/boulder streams in the Piedmont, Highlands, and Ridge and Valley ecoregions (Fig. 1).

2.2. Site selection

We used the least disturbed condition approach to select sites to evaluate both the Headwaters and Northern Fish IBI. The least disturbed condition approach uses the "best" available sites given the current conditions of aquatic resources in regards to landscape characteristics, EPA RBP habitat score for high gradient streams, chemistry, and biological communities (Stoddard et al., 2006). Sites were grouped in disturbance categories using criteria based on land-use characteristics and total habitat score (Table 1). The total habitat score is a visual based habitat assessment performed at each site using the format given in the Rapid Bioassessment Protocols (Barbour et al., 1999) for high gradient streams. Sites that did not fit the criteria of least impaired (LI) or most impaired (MI) were characterized as intermediate.

Table 1
Land use characteristics and total habitat score used to define least disturbed and most disturbed for each Index of Biotic Integrity.

Index	Condition	Least Impaired	Most Impaired
Fish IBI		N = 23	N = 25
	% Forest + %Wetland	≥60	< 40
	% Urban	≤20	> 60
	% Impervious cover	≤4	≥20
	Total Habitat Score	≥145	< 115
Headwater IBI		N = 35	N = 20
	% Forest + %Wetland	> 70%	< 30%
	% Urban	< 20%	> 70%
	% Impervious cover	< 5%	> 20%
	Total Habitat Score	Optimal or Suboptimal	Marginal or Poor

2.3. Electrofishing

Electrofishing was conducted at every site within a 150-meter stream reach to collect fish samples representative of the resident assemblage. Single pass pulsed DC backpack electrofishing was employed on all headwaters and fish IBI streams. This sampling methodology has been established for NJ and has been proven to provide the most effective and efficient method for collecting a representative sample of the resident fish assemblage. Gear ranged from 1–2 backpack units on small streams to 2–3 backpacks or a barge unit for medium to large rivers. A block net was employed at the furthest upstream point of the reach to impede fish movement outside of the sample reach. Electrofishing was conducted in an upstream manner in an effort to sample all available habitat types. All stunned fish were netted and placed in livewells. At headwater streams, salamanders, crayfish, and frogs were collected in addition to fish during electrofishing. All biota sampled by electrofishing were identified to species and enumerated. All fish were identified to species, examined for external anomalies or DELTs (Deformities, eroded fins, lesions or tumors), sport fish were measured for total length (TL; mm), and all specimens were released. Hatchery raised trout, easily identified by their numerous fin deformities, pale coloration, and missing scales/skin abrasions were not included in either dataset or analysis. Any fish, amphibian, or crayfish not readily identified in the field (except for New Jersey listed threatened or endangered species), were preserved in 10% formalin for later identification in the laboratory.

2.4. Amphibian and crayfish search

Additional sampling was conducted to target salamanders, frogs, and crayfish at each headwater stream site. The use of multiple sampling methods increases the likelihood to detect the presence of stream salamander species (Mattfeldt and Grant, 2007). Over the course of the study, several methods were applied to assess the most accurate, precise and cost-efficient sample; (1) kick netting, (2) turning a set number of objects, (3) a timed sample, where a crew of two individuals searched 10 meters of stream for 10 min in the stream and up to 1 m from the stream edge, and (4) an area constrained survey (ACS), was conducted by two individuals along a transect (15 m × 1 m area in the water and a 15 × 1 m area along the shore) on a randomly selected stream bank to sample a total area of 30 m^2. The primary sampling techniques used in this study were the timed sample as recommended by Keller et al. (2012), and the ACS, which is the most cost efficient and collects the most species (Strain et al., 2009). If the 15 m^2 ACS search area on shore contained insufficient moveable cover, then the search area was moved onto the adjacent floodplain. With each technique, available cover (rocks, logs, debris) greater than 96 cm^2 were turned by hand and all crayfish, salamanders, and frogs encountered were captured with the aid of dip nets. All objects turned in the survey were returned to their original position to reduce habitat disturbance. Taxa observed that escaped catchment are recorded and identified to the lowest taxonomic level based on observed characters. All biota sampled by were identified to species, enumerated, and released unless positive identification was not feasible in the field. Any amphibians not readily identified in the field (except for New Jersey listed threatened or endangered species), were preserved in 10% formalin for later identification in the laboratory.

2.5. Habitat and water chemistry

The gradient of the 150-m stream reach was measured using standard surveying equipment (sighting level, tripod, and stadia) and techniques described in Bovee and Milhous (1978). Habitat was assessed at every Headwater and Fish IBI site using the EPA Rapid Habitat Assessment Form for high gradient streams (Barbour et al., 1999). Hach Quanta or Hach MS-5 or YSI 556 water quality meters were used to measure in situ dissolved oxygen, water temperature, conductivity, and pH at each site.

2.6. Data

2.6.1. Site selection and IBI class determination

Cluster analysis was used to determine the number of distinct fish assemblages in northern NJ streams. All fish data from least and intermediate impaired streams were included in the analysis. Fish species that were collected in less than 5% of the samples (Bellucci et al., 2011) were considered rare and were subsequently removed from the dataset. A final data matrix consisting of 35 taxa and 158 sites was used in the analysis. Proportional abundance data was arcsine square-root transformed prior to running cluster analysis using PC ORD Version 6.08 (MjM Software Design, Gleneden Beach, OR). The analysis used the Sorensen distance measure with flexible beta linkage method (beta = −0.25; Kanno and Vokoun, 2008). Cluster groups were further evaluated using Indicator Species Analysis of presence/absence data in PC ORD. The Dufrene and Legendre's (1997) method was used to provide an indicator value for each species in each cluster group.

In order to assign streams as headwaters or non-headwaters, streams were separated into two size classes based on the cluster analyses of the resident fish assemblages. Streams less than 10.4 km^2 in drainage, typically Strahler orders 1–3 were classified as headwaters. Fish populations in these small streams are naturally depauperate requiring additional taxa to supplement the development of a biotic index. The Headwaters IBI was developed using a combination of fish, salamanders, frogs, and crayfish as bio-indicators.

The Northern Fish IBI was developed for those medium to large high gradient wadeable streams. These streams are greater than 10.4 km^2, typically Strahler orders 4 and 5, and contain adequate fish assemblages which enabled the development of a fish based biotic index.

2.6.2. Headwaters IBI (HIBI)

Fish, crayfish, and amphibian data were collected from 96 headwater streams north of the fall line (Fig. 1) between 2003 and 2013 by The Academy of Natural Sciences at Drexel University (Keller et al., 2012) and the New Jersey Department of Environmental Protection-Bureau of Freshwater and Biological Monitoring (NJDEP-BFBM). All headwater sites were sampled between May 1 and November 1. Disturbance criteria based on land use/land cover and habitat score were used to establish least impaired and most impaired sites (Table 1). A total of 35 least impaired and 20 most impaired sites were identified for statistical analysis as described in Section 2.7.

2.6.3. Northern Fish IBI (FIBI)

Fish assemblage data were collected from 137 sites (> 10.4 km^2) between 2006 and 2014. Sites were sampled from June through early October. This dataset consisted of a combination of targeted (fixed) sites for the assessment of long term trends and randomly selected (probabilistic) sites to provide an overall assessment of the state. Disturbance criteria based on land use/land cover and habitat score were used to establish least impaired and most impaired sites (Table 1). A total of 23 least impaired and 25 most impaired sites were identified for statistical analysis as described in Section 2.7.

2.7. Metric analysis and selection

2.7.1. Range

To insure metrics with low richness values or identical values were not included, each metric was evaluated for the number of species, percent zero values, and the maximum identical value using the entire datasets. In several instances, it was necessary to remove ubiquitous species which can often overshadow other members of the resident assemblage (MPCA, 2014) and their removal also reduces the number of identical metric values among sites. Metrics with less than three species collected were eliminated due to poor differentiation between sites (Kanno et al., 2010). Metrics with greater than 75% zero values or identical values were also eliminated (Whittier et al., 2007).

2.7.2. Signal-to-noise

To insure responsiveness (signal) and precision (noise) of metrics, we analyzed data with signal to noise ratio calculated as:

$$S/N = \frac{(F-1)}{C_1}$$

where the F-statistic is derived from ANOVA and c_1 is the number of times repeat sites were visited (Kaufmann et al., 1999). Signal (S) is a measure of variability within the dataset, which must have a sufficient number of sites across the stressor gradient. A low signal is an indication that a metric lacks responsiveness to the types of stressors typically encountered. Noise (N) was measured at repeat site visits, which ranged between one to five years. Fluctuations in data from repeat sampling is an indication a metric lacks adequate precision. Metrics with S:N ratio < 3 lacked responsiveness and repeatability and were therefore rejected from further testing (Whittier et al., 2007).

The headwater dataset contained 10 sites that were revisited between 2009 and 2014, while the fish IBI signal to noise analysis was performed on fifty-six sites that were revisited between the years 2005 and 2013. An attempt was made to normalize data prior to signal to noise analysis typically using log10, log10(x + 1), and arcsine square root transformations. Revisit sampling was not completed within the same sampling season with most sites spanning several years between revisits.

2.7.3. Relationship with stream size and gradient

All metrics from both indices were analyzed for significant relationship with drainage size using linear regression of least impaired sites. Headwater sites were also evaluated for significant relationship with stream gradient. Metrics with a significant relationship and r^2 values greater than 0.25 were adjusted using the mean value from least impaired sites along with the linear regression model (Roth et al., 2000). The following equation was used to calculate the adjusted metric value:

$$\text{adjusted value} = \text{mean reference} + \text{observed} - \text{predicted}$$

where predicted value = slope * log10 (drainage area in mi^2) + y-intercept (Tech and Inc., 2007).

2.7.4. Correlation to disturbance gradient

Each metric was evaluated for significant relationships with percent forest and percent impervious cover (IC) from NJDEP land-use/land-cover data, habitat score (Rapid Bioassessment Protocol (RBP) habitat rating), and human population density (2010 census population data) using Pearson Correlation analysis. The number of HIBI and FIBI sites used in the correlation matrix ranged from 54–96 to 127–137 sites respectively. Those metrics with significant correlations were included in further analyses.

2.7.5. Responsiveness

All metrics which passed other screening procedures outlined in Sections 2.7.1–2.7.4 were evaluated using one-way analysis of variance (ANOVA) to compare differences in means among least impaired sites and most impaired sites. Prior to the analysis, each dataset was checked for normal distribution and an attempt was made to normalize data when necessary using log10, log10(x + 1), and arcsine square root transformations. The F-statistic from the ANOVA was used as the primary criteria for selecting the strongest metric within each ecological class.

2.7.6. Redundancy

A Pearson correlation matrix was used to evaluate redundancy among metrics using data from the least impaired sites. A correlation coefficient of $r = |0.75|$ was used as a cut-off for metric elimination (Emery et al., 2003), and those metrics that approached the threshold were evaluated for "conceptual redundancy". Metrics were considered conceptually redundant and would not pass the screening criteria if both metrics represented similar aspects of biological integrity (MPCA, 2014). Metric pairs with correlation coefficients greater than 0.75 were re-evaluated to determine if an alternate metric from the same ecological class could be substituted. In general, the most responsive metric was kept and the weaker metric was eliminated. Although the F-statistic from ANOVA between least impaired and most impaired data was the primary indicator of responsiveness, correlation coefficients and signal to noise results were also considered in metric selection and replacement.

2.8. IBI scoring

A continuous scoring technique developed by Tech and Inc. (2007) was used for both headwaters and fish IBI metrics. Continuous scoring uses the distribution of data to determine upper and lower thresholds to score sites on a scale from 0 to 100 (Blocksom, 2003). Sites in which scores are negative values and those scoring above the upper threshold are normalized to 0 and 100 respectively. The total index score is derived from averaging all individual metric scores. All metrics exhibiting a significant relationship with drainage size were adjusted according to the aforementioned linear regression methods (Section 2.7.3) prior to scoring.

Each metric was scored on a continuous scale using the 95th percentile of reference and impaired data as the upper threshold and zero as the lower threshold for metrics that decrease with an increase in stress:

$$\text{Score} = 100 \times \text{Metric Value}/\text{95th Percentile}$$

Metrics that increase with an increase in stressor levels were scored using the 5th percentile of reference as the upper limit using the formula:

$$\text{Score} = 100 \times (\text{95th Percentile} - \text{Metric Value})/(\text{95th Percentile} - \text{5th Percentile}).$$

3. Results

3.1. Fish assemblage characteristics

A total of 66 aquatic vertebrate species (58 fish species, 8 amphibian species) and 6 crayfish species were collected and included in the analysis from 233 sites.

Cluster analysis was used to identify six distinct fish assemblages which enabled us to distinguish between coldwater and cool/warmwater fish assemblages (Fig. 2, Appendix C). Based on results from cluster analysis, indicator species analysis, and linear regression, we determined a drainage area cutoff of 10.4 km^2 based on the characterization of cold and cool water assemblages where brook trout were no longer a dominant species. Assemblages 4 and 6 consist of brook trout/slimy sculpin and blacknose dace *Rhinichthys atratulus*/creek chub *Semotilus atromaculatus* assemblages, which tended to be the dominant fish assemblages in unaltered headwater (coldwater) streams. Reference sites with fewer than 5 species and 100 individuals are deemed to not accurately reflect the variation in small streams (Roth et al., 1998), and therefore should not be used in the development of a fish based IBI. Fish species richness was commonly below 5 for reference streams draining less than 10.4 km^2, further supporting the break between indices and use of other aquatic indicator taxa to develop a reliable IBI.

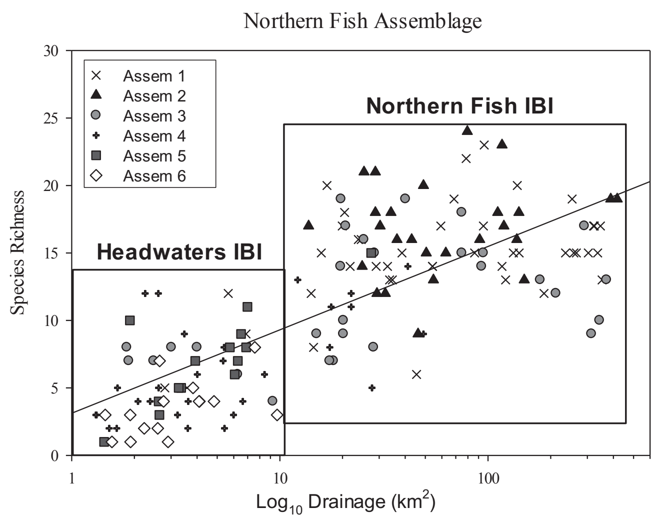


Fig. 2. Linear regression of fish species richness and drainage area (km^2) for New Jersey high gradient streams. New Jersey high gradient fish assemblages 1 through 6 are derived from cluster analysis (Appendix C).

3.2. IBI development

A total of 68 HIBI and 80 FIBI candidate metrics were tested for inclusion in the final indices. Overall mean HIBI and FIBI scores for most impaired and least impaired sites were significantly different (ANOVA; $p \leq 0.001$; Fig. 3). Total Fish and Headwaters IBI scores indicated good overall discrimination efficiency, 96% and 100% respectively (Fig. 3). Overall, both IBI's responded positively to general stressor indicators and land use gradients, such as percent urban land use (Fig. 4). The Headwater IBI and Fish IBI were comprised of 6 and 8 metrics respectively (Tables 2 and 3). The HIBI contained no metrics from the thermal stream flow ecological classes and Fish IBI contained no metrics in the non-native or indicator species ecological classes (Appendix A).

3.2.1. Headwater IBI

The HIBI was comprised of two proportional richness metrics (Table 2), two proportional abundance metrics, one taxonomic richness, and one density metric. No metrics included in the final HIBI were corrected for watershed area or reach gradient.

The number of intolerant vertebrate species had the highest F-value (38.8) and second highest discrimination efficiency (95%) of all metrics selected and within the group of taxonomic richness metrics tested (Table 2). Mean intolerant vertebrate species metric values were significantly different for all pairwise comparisons between disturbance categories (Tukey's test; $p < 0.05$; Fig. 5). This metric consisted of the most sensitive aquatic vertebrates in New Jersey's streams. The number of intolerant species typically decreases with increasing anthropogenic disturbance, thus intolerant species are vulnerable to degradation in stream water quality and habitat (Karr et al., 1986). This metric had a high discrimination efficiency (95%) and was significantly positively correlated with percent forest land cover in the watershed ($r = 0.69$; $p < 0.001$). This metric was also negatively correlated with percent urban land cover ($r = -0.65$; $p < 0.001$) and impervious cover in the watershed ($r = -0.60$; $p < 0.001$).

The proportion of vertebrate species as top carnivores metric represents the trophic ecological class of metrics. The presence of top predators in an aquatic system indicates that there is a diverse and healthy balance of trophic levels (Karr, 1981; Langdon, 2001). In small headwater streams where carnivorous fish are absent, salamanders are often the dominant vertebrate predator (Southerland et al., 2004). This metric had an F-value of 25.0 and was significantly negatively correlated with percent urban land cover ($r = -0.46$; $p < 0.001$) and impervious cover in the watershed ($r = -0.51$; $p < 0.001$).

The percent of tolerant fish metric represented the tolerance ecological metric class and had the highest signal to noise ratio (31.2) and second highest F-value (31.0; Table 2). The percent of tolerant fish increases with increasing anthropogenic disturbance. The percent tolerant fish metric was significantly correlated with percent urban land cover ($r = 0.64$; $p < 0.001$) and percent impervious cover in the watershed ($r = 0.77$; $p < 0.001$).

The proportion of total richness as native metric represents the nonnative ecological class of metrics. Native richness typically decreases with increasing anthropogenic disturbance. This metric had the third highest F-value (30.4) and second highest discrimination efficiency (89%; Table 2). The proportion of total richness as native metric was positively significantly correlated with percent forest ($r = 0.62$; $p < 0.001$) and negatively correlated with percent urban land use ($r = -0.64$; $p < 0.001$) and percent impervious cover in the watershed ($r = -0.62$; $p < 0.001$).

The percent native crayfish metric represents the composition ecological class of metrics. This metric had the highest discrimination efficiency (100%). Mean percent native crayfish metric scores were significantly different for all pairwise comparisons between disturbance categories (Tukey's test; $p < 0.05$). The percent native crayfish metric was significantly negatively correlated with% impervious cover ($r = -0.52$; $p < 0.001$) and urban land use ($r = -0.50$; $p < 0.001$)

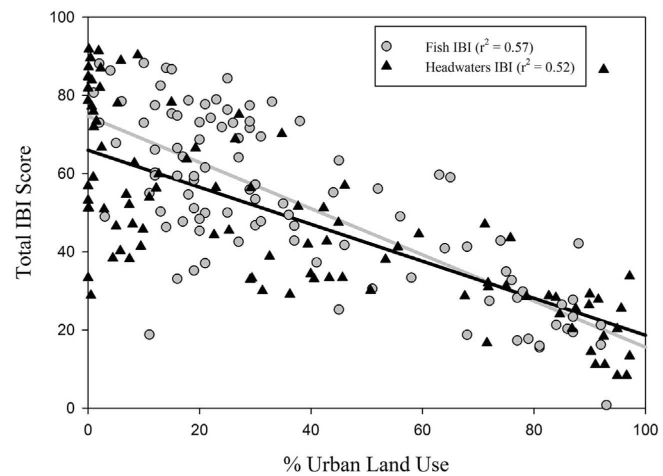


Fig. 4. Linear relationship between percent urban land use and total IBI score for Fish IBI (grey, circles) and Headwaters IBI (black, triangles).

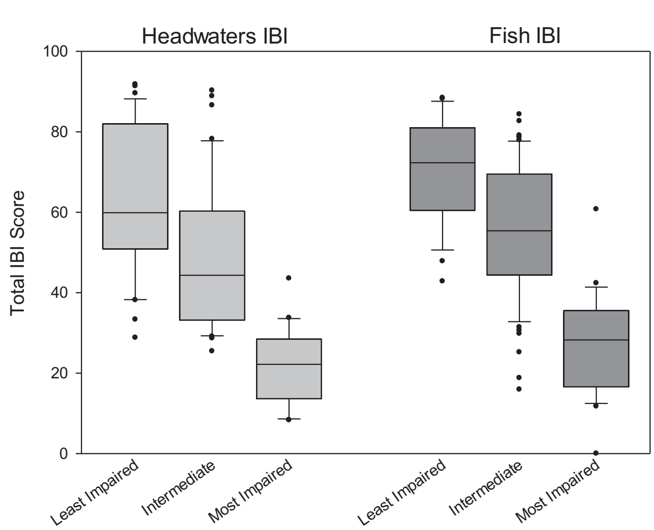


Fig. 3. Total Headwater IBI and Fish IBI score for each disturbance class.

Table 2
Metrics included in the Headwaters IBI for high gradient streams (drainage area < 10.4 km^2).

Metric	Ecological Class	Response to stress	S:N	5th percentile	95th percentile	F-value	% DE
Intolerant Vertebrate Richness	Taxonomic Richness	Decrease	14.3	0.0	3.0	38.8	95
Proportion of Vertebrate Richness as Top Carnivore[a]	Trophic	Decrease	17.8	0.0	38.0	25.0	79
% Tolerant Fish Individuals	Tolerance	Increase	31.2	0.0	96.1	31.0	89
Proportion of Total Richness as Native	Non-Native	Decrease	3.1	58.3	100.0	30.4	89
% Native Crayfish	Composition	Decrease	3.2	0.0	100.0	43.1	100
Brook Trout Density (individuals/100 m^2)	Composition/Indicator Species	Decrease	1.6	0.0	10.1	7.1	0

[a] Excludes American eel.

in the watershed and positively correlated with forested land use ($r = 0.49$; $p < 0.001$).

The density of brook trout metric represents the composition and indicator species ecological classes of metrics. This metric had the lowest F- value (7.1) out of all 6 metrics included in the HIBI (Table 2). This metric failed the screening criteria for S:N ratio of < 3; however, the number of repeat headwaters sites was limited (N = 10), and were mostly intermediate and impaired sites where few brook trout were collected. The discrimination efficiency for this metric was 0% for this metric because the 25th percentile for least disturbed sites was 0.00 for the density of brook trout metric (Table 2). There were many sites categorized as "least impaired" that did not have populations of brook trout, thus the 25th percentile was 0.00 for that metric. The brook trout density metric was significantly correlated with% impervious cover in the watershed ($r = -0.32$; $p = 0.015$). This metric was passed through the screening process, however, due to the high discriminatory ability between most impaired and least impaired sites, the sensitivity to land use alterations, and the ecological significance of brook trout in headwater systems.

3.2.2. Fish IBI

The Fish IBI consisted of four proportional richness metrics, three proportional abundance metrics, and one tolerance index. Of the final metrics included in the Fish IBI, three were corrected for watershed area and two have ubiquitous species excluded. Metrics were selected from seven different ecological categories and although a number of non-native and indicator species metrics were evaluated including nonnative species richness and proportional abundance of green sunfish *Lepomis cyanellus*, low F-statistics and poor results prevented using a metric in either of these two ecological categories (Appendix A1.2).

Several metrics in the richness and stream flow ecological categories passed all screening procedures, but the proportion of rheophilic species out performed all other metrics and had the highest F-value (99.5; Table 3). The rheophilic species metric was modified to remove tessellated darter *Etheostoma olmstedi*, a ubiquitous fish species in New Jersey, and then adjusted due to a significant relationship with drainage size ($r^2 = 0.33$; $p = 0.004$). This metric was highly correlated with habitat score ($r = 0.65$; $p < 0.001$) and exhibited 100% discrimination efficiency between least impaired and most impaired scores (Table 3; Fig. 6).

The reproduction category was represented by the proportional richness of lithophilic spawners which had the second highest F-value (68.6) among those metrics selected. Pairwise comparisons indicated least impaired sites differed significantly from intermediate and most disturbed sites and intermediate sites differed significantly from most disturbed sites (Tukey's test; $p < 0.001$; Fig. 6). This metric was positively correlated with percent forest ($r = 0.60$; $p < 0.001$) and negatively correlated with impervious cover ($r = -0.64$; $p < 0.001$). Lithophilic species require clean natural substrate for spawning and are good indicators of impairments due to siltation and channelization, as studies have shown significant impacts to this reproductive guild as a result of alterations to natural substrates (Berkman and Rabeni, 1987; Ohio EPA, 1987; Rabeni and Smale, 1995).

The proportional abundance of Cyprinidae species responded well to all testing criteria, had a high F-value (62.0), and was one of two composition metrics included in the final IBI. The metric had a significant relationship with drainage size ($r^2 = 0.31$; $p = 0.006$) and was therefore adjusted according to the aforementioned methods. Data from least impaired sites differed significantly from intermediate and most impaired sites and intermediate sites differed significantly from most impaired sites (Tukey's test; $p < 0.001$; Fig. 6). Cyprinid species are typically the most abundant taxa group of healthy stream assemblages in New Jersey and are very good indicators of disturbance in trophic structure and impacts to the benthic macroinvertebrate assemblage (Karr et al., 1986).

The proportional abundance of the top three taxa was also included as a composition metric due to its ability to identify poor diversity and dominance of a couple of taxa in stressed environments. Blacknose dace were removed from this metric, as this species is ubiquitous and often quite numerous even at healthy sites. Despite recommendations to only select one composition metric per IBI (Whittier et al., 2007), the dominance metric is not family based (darter, sucker, sunfish) like many found in traditional IBI's. Typically impaired streams tend to be dominated by a few species such as green sunfish, white sucker *Catostomus commersonii*, mummichog *Fundulus heteroclitus*, and/or banded killifish *Fundulus diaphanus*, while the abundance of remaining taxa is often quite low. Despite having the lowest F-value (33.7) of those metrics included in the IBI, this composition metric differed significantly in all pairwise comparisons among least, intermediate, and most

Table 3
Metrics included in the Fish IBI for high gradient streams (drainage area > 10.4 km^2).

Metric	Ecological Class	Response to stress	S:N	5th percentile	95th percentile	F-value	% DE
% Rheophilic Species[a]	Richness/Stream Flow	Decrease	12.5	−2.6	27.2	99.5	100
% Coldwater & Nontolerant Coolwater Species[b]	Thermal	Decrease	12.1	−2.3	85.5	43.6	80
% Generalist Feeders[a]	Trophic	Increase	6.5	28.4	78.0	56.2	88
Tolerance Index	Tolerance	Increase	16.4	4.5	9.3	56.4	92
% Lithophilic Spawners[a]	Reproduction	Decrease	13.2	5.6	69.0	68.6	96
% Cyprinidae[b]	Composition	Decrease	11.3	−10.9	75.3	62.0	88
% Dominant 3 Taxa[b]	Composition	Increase	7.5	28.9	92.4	33.7	88
% Benthic Insectivore Species[a]	Habitat	Decrease	16.0	5.0	37.8	50.3	96

[a] Proportion of Species.
[b] Proportion of Individuals.

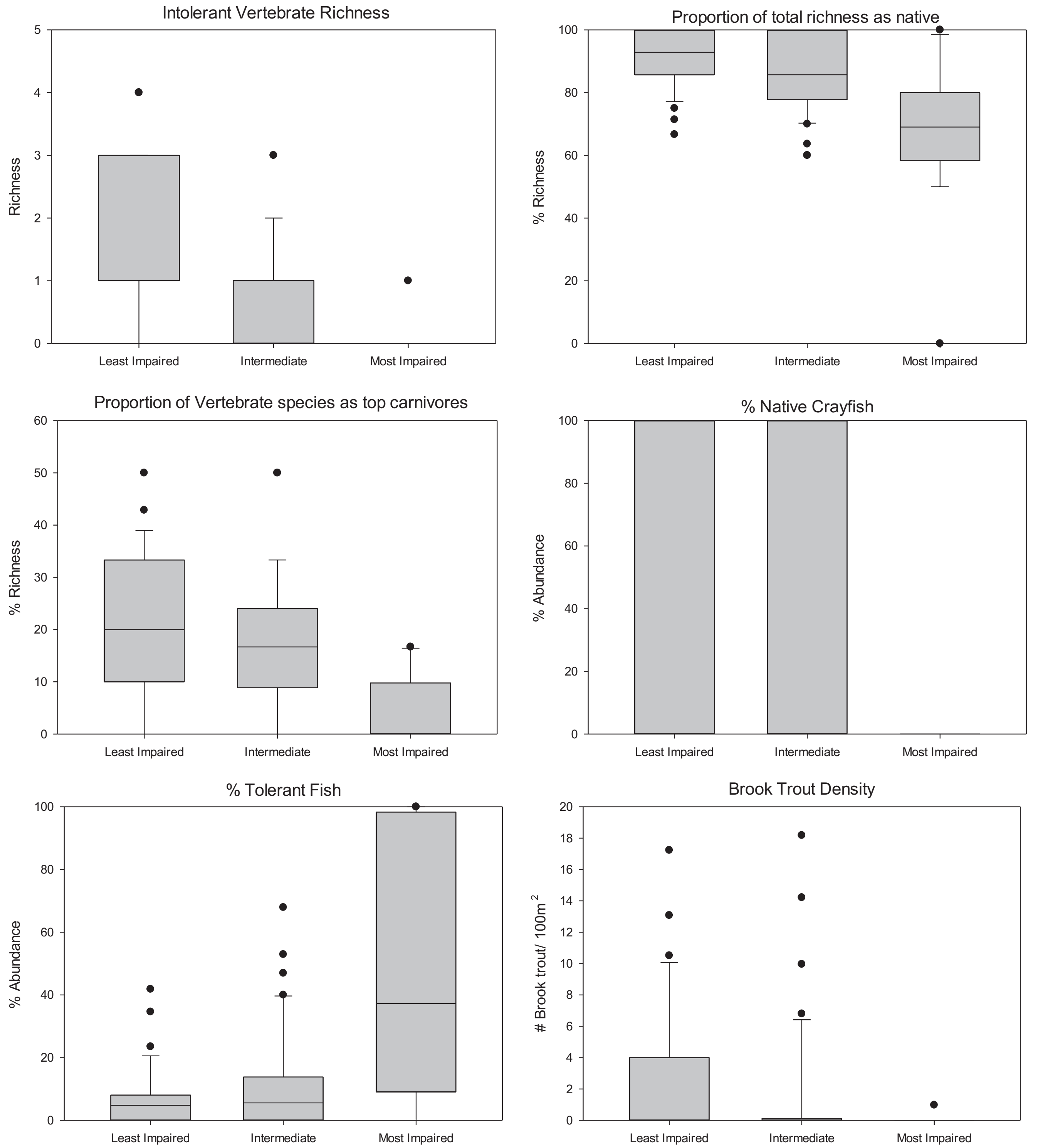


Fig. 5. Box-and-whisker plots showing the distribution of metric values for the final six selected HIBI metrics. Rectangles represent the 25th and 75th percentiles, bars are 5th and 95th percentiles, dots represent outliers, and horizontal midlines are medians.

impaired sites (Tukey's test; $p < 0.001$).

The thermal category was represented by two thermal groups combined into one proportional abundance metric. The percent cold and nontolerant coolwater species represents taxa with narrow thermal tolerances which are unable to survive largescale human disturbance. This will become an important metric to assessing thermal shifts in stream communities, and in particular those higher quality (sentinel) sites that are monitored routinely in an effort to assess potential impacts from climate change. Much emphasis has been placed on climatic models and the implications to coldwater fish species due to a changing climate (Ficke et al., 2007; Jones et al., 2013), but studies have shown coolwater assemblages may be just as sensitive to temperature shifts (Lyons et al., 2014).

The tolerance index is the sum of the products of each species proportional abundance and tolerance value in which sensitive, intermediate, and tolerant species are assigned tolerance values of 0, 5, and 10 respectively (Sindt et al., 2011). This metric outperformed tolerance richness and percent tolerance abundance metrics and had the highest

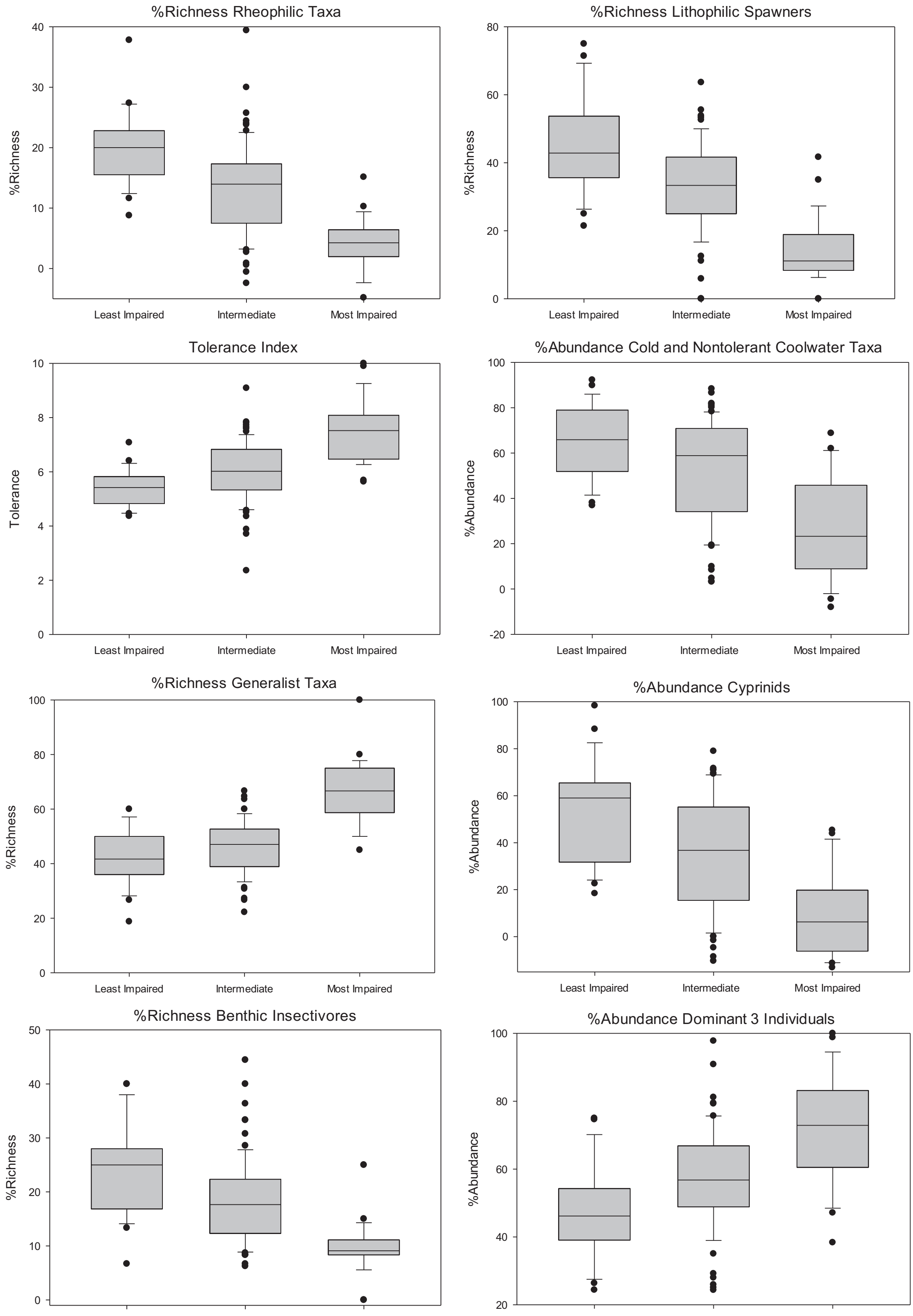


Fig. 6. Box-and-whisker plots showing the distribution of metric values for the final eight selected FIBI metrics. Rectangles represent the 25th and 75th percentiles, bars are 5th and 95th percentiles, dots represent outliers, and horizontal midlines are medians.

signal to noise ratio (16.38) of any metric tested (Table 3). The index was also negatively correlated with overall habitat score ($r = -0.58$; $p < 0.001$) and %forest ($r = -0.54$; $p < 0.001$) in the watershed and exhibited a significant difference in pairwise comparisons among least and most impaired and intermediate and most impaired sites (Tukey's test; $p < 0.001$).

Performance results for the proportional richness of generalist taxa were the strongest of the three negative response metrics, which increase with degradation. This metric was highly correlated with impervious cover ($r = 0.68$; $p < 0.001$) and human population density ($r = 0.69$; $p < 0.001$) and inversely correlated with habitat score ($r = -0.61$; $p < 0.001$; Table 3).

The proportional richness of benthic insectivores exhibited a relatively high signal to noise (15.95) and very good discrimination between least and most impaired sites (96%; Table 3). This metric is useful at detecting degradation in habitat and substrate, as sediment loading from bank erosion, channelization, or land use practices not only impacts the natural habitat for these benthic fish, but also the macroinvertebrate communities these specialized feeders rely on. Degradation of natural substrate from siltation is often reflected by a loss of benthic species richness (Karr et al., 1986) and abundance (Berkman and Rabeni, 1987).

Overall, final redundancy analysis resulted in a high Pearson's correlation between% abundance cold non-tolerant coolwater taxa and % abundance Cyprinidae taxa ($r = 0.70$; $p < 0.001$). Despite significant correlation between metrics, values were below the established threshold of $r = |0.75|$ (Emery et al., 2003).

4. Discussion

The traditional Rapid Bioassessment Protocol (RBP) methods for testing metrics and developing scoring criteria involve selecting or modifying a handful of candidate metrics from distinct categories (richness/composition, trophic composition, and abundance/condition) and developing maximum species richness (MSR) scoring plots, which are fit by eye and highly subjective based on professional judgment (Barbour et al., 1999). In contrast, the new "structured approach" developed by Whittier et al. (2007) involves testing large numbers of metrics from numerous ecological classes thus incorporating a variety of different metric types and variants, such as proportional richness, tolerance indices, percent dominance, catch per unit effort (CPUE), species diversity, and proportional abundance into the testing procedures. Traditional RBP analysis typically involved examination of box plots and testing for significant differences among reference and impaired sites, while new procedures also evaluate the variability within a dataset compared to the seasonal variability (S/N), essentially evaluating the responsiveness and the precision of metrics. The goal of the structured approach is to select only highly significant metrics based on F-values from ANOVA testing among least impaired and most impaired sites. This approach has proven successful in developing many newly created IBIs for areas where no previous IBI existed or in areas where biological assessments are being pioneered such as in Amazonian streams (Chen et al., 2017), Brazilian streams (Carvalho et al., 2017; Terra et al., 2013), South Dakota's northern glaciated plains (Krause et al., 2013), and high gradient headwater streams in New Jersey (this study).

Of the 80 Northern IBI metrics tested, 7 of the 10 original NJ RBP metrics were tested using the new structured approach. All 7 metrics failed testing procedures mainly due to poor response against stressor gradients, but also poor discrimination among least and most impaired sites and a few were not the strongest metric in the ecological class. Although the original NJ Fish IBI metrics could discriminate the ends of the stressor gradient, the index lacked sensitivity in the intermediate range.

The development of this NJ Northern Fish IBI resulted in the testing and selection of metrics similar to those in Connecticut's mixed-water MMI (Kanno et al., 2010) and Minnesota's Southern Rivers Fish IBI (MPCA, 2014). Similar to Connecticut, the rivers and streams that comprise the Fish IBI ($> 10.4\,km^2$) account for a combination of coolwater and warmwater fish assemblages. Species rich Midwest states, like Minnesota which has over 140 native fish species, have developed numerous different indexes based on watershed size, gradient, and thermal classifications (MPCA, 2014). In contrast, New Jersey's extensive landscape alteration, historical/recent fish stocking, and more importantly naturally, low species richness ($N = 54$), prevents discriminating individual indexes for each of the four major Northern IBI fish assemblages identified in the cluster analysis (Fig. 2; Appendix C).

The Headwater IBI developed for New Jersey's headwater streams shares similar metrics to those that were created for coldwater streams in Wisconsin (Lyons et al., 1996), Vermont (Langdon, 2001), and Connecticut (Kanno et al., 2010). All aforementioned IBIs included a measure of brook trout relative abundance because they are an intolerant, coldwater stenotherm that has been documented as an indicator of good water quality throughout its native range (Lyons et al., 1996; Langdon, 2001; Kanno et al., 2010). Coldwater habitat in New Jersey is very limited and has been greatly altered which has resulted in brook trout being present in only 8.5% of New Jersey's subwatersheds (New Jersey Division of Fish and Wildlife, 2005). Due to the limited number of stream miles that would be designated as "coldwater," we elected to use the term "headwaters" to represent streams less than $10.4\,km^2$ regardless of thermal classification. Similarly to Lyons et al. (1996) and Langdon (2001) the HIBI also includes top carnivore and intolerant species metrics, the only difference being that the HIBI includes amphibians and crayfish.

The inclusion of streamside salamanders into the HIBI greatly enhanced the ability to assess aquatic life use in small streams in New Jersey. Whittier et al., (2007) and Pont et al. (2009) also incorporated both amphibian and fish fauna into their multi-metric indices in western United States streams. Plethodontid stream salamanders are great indicators of environmental stress due to their biphasic life cycle, ubiquity, philopatry, lack of lungs and moist permeable skin (Welsh and Ollivier, 1998; Southerland et al., 2004). Stream salamanders have been shown to be responsive to multiple anthropogenic stressors and habitat disturbances such as urbanization, impervious surface cover, logging, siltation, and road crossings (Willson and Dorcas, 2003; Ward et al., 2008; Barrett and Price, 2014). Salamanders can be vulnerable to toxins in the environment both in the water and on land as they respire using gills as larvae or cutaneously through their skin as adults (Petranka, 1998). Salamanders are the dominant vertebrate predator in fishless headwater streams (Davic and Welsh, 2004). Therefore, as fish richness decreases, salamanders become a vital bio-indicator. Streamside salamander abundance is typically much higher in fishless streams or those devoid of an aquatic predator, as species like brook trout have been documented as a major predator of streamside salamanders (Resetarits, 1995, 1997; Barr and Babbitt, 2002). A stream salamander IBI developed for Maryland watersheds less than 300 acres was proven successful in evaluating fishless streams which periodically become dry (Southerland et al., 2004). The HIBI in this study was not developed to assess fishless streams; however, the use of both fish and salamanders allows for a better representation of the structure and function of an aquatic vertebrate assemblage.

Incorporating crayfish into the HIBI provides additional sensitive taxa to these naturally species- poor environments. Non-native crayfish have many negative impacts on freshwater ecosystems including displacement of native crayfish and amphibians, predation on fish eggs, amphibian eggs, invertebrates, and macrophytes (Holdich, 1999). Common crayfish *Cambarus bartonii bartonii* in Maryland were positively correlated with stream gradient, forested land, and cooler stream temperatures (Kilian and Ciccotto, 2014). Similarly, Keller et al. (2012) noted a significant positive correlation between native crayfish and forested land. Additionally, in this study the percent native crayfish metric was positively correlated with forested land and reach gradient and negatively correlated with impervious cover.

The HIBI is widely applicable to high gradient streams in New Jersey less than 10.4 km^2 in drainage size. Streams with drainage areas less than 1.29 km^2 or reach gradients greater than 7% have a greater chance of being fishless. All streams were sampled when holding water; however, fish were not caught at 3 of the 96 sites in our headwater network. The size of drainage areas from these fishless sites ranged from 0.36 km^2 to 0.94 km^2 with reach gradients ranging from 7.14% to 10.29% (Keller et al., 2012). Further research is warranted to evaluate whether or not the HIBI is a valid tool to assess fishless streams. Streams scoring on the higher end of the HIBI in general had higher densities of brook trout, presence of a sensitive salamander and common crayfish. Streams scoring lower on the HIBI were comprised of highly tolerant fishes, non-native fish and crayfish, and absence or low abundance of amphibians.

5. Conclusions

The development of a Headwater IBI and a northern Fish IBI provides a valid assessment tool to evaluate stream condition in all freshwater, non-tidal, wadeable streams in the Piedmont, Highlands, and Ridge and Valley physical provinces in New Jersey. A biological condition gradient (BCG) for northern New Jersey wadeable streams is being finalized, which will assist with the development of IBI ratings based on the appropriate Tier assignments. Additional parametric and nonparametric analysis results have been included in the Appendices D–F.

Acknowledgments

We would like to specifically thank David Keller and Richard Horwitz from The Academy of Natural Sciences of Drexel University for piloting the project and sampling methods for the Headwater IBI. We also like to thank the NJ Fish workgroup consisting of James Kurtenback, USEPA; Johnathan Kennen, USGS; Nicholas Procopio, NJDEP; Kevin Berry, NJDEP; Thomas Belton, retired NJDEP; and David Keller and Richard Horwitz from The Academy of Natural Sciences of Drexel University for their comments and review of this work.

Appendix A: Candidate metrics tested

See Tables A1.1 and A1.2.

Table A1.1
List of the 68 candidate metrics tested for inclusion in the Headwaters IBI.

- Taxonomic Richness
  - Number of top carnivore fish species
  - Number of intolerant fish species
  - Number of coldwater fish species
  - Number of fluvial specialist fish species
  - Number of intermediate fish species
  - Number of lithophilic fish spawners
  - Number of minnow species
  - Number of native lithophilic fish spawners
  - Number of native fish species
  - Number of benthic invertivore fish species
  - Number of coolwater fish species
  - Number of total fish species
  - Number of macro-habitat generalist fish species
  - Number of warmwater fish species
  - Number of general feeder fish species
  - Number of fluvial dependent fish species
  - Number of tolerant fish species
  - Number of vertebrate species
  - Number of native vertebrate species
  - Number of intolerant vertebrate species
  - Number of tolerant vertebrate species
  - Number of top carnivore vertebrate species
- Thermal
  - Percent of coldwater fish individuals
  - Percent of coolwater fish individuals
  - Percent of warmwater fish individuals
- Trophic
  - Percent of top carnivore fish individuals
  - Percent of benthic invertivore fish individuals
  - Percent of general feeder fish individuals
  - Percent of vertebrate top carnivore individuals
  - Proportion of vertebrate richness as top carnivore
  - Proportion of non-tolerant vertebrate species as top carnivore
- Tolerance
  - Percent of intolerant fish individuals
  - Percent of intermediate fish individuals
  - Percent of tolerant fish individuals
  - Percent of vertebrate intolerant individuals
  - Percent of vertebrate tolerant individuals
  - Tolerance Index
- Stream flow
  - Percent of fluvial specialist individuals, except blacknose dace
  - Percent lithophils
  - Percent native lithophils
  - Percent of fluvial specialist individuals
  - Percent of macro-habitat generalist fish individuals
  - Percent of fluvial dependent fish individuals
  - Percent rheophilic species
  - Percent rheophilic species (excluding blacknose dace)
- Non-native
  - Percent of non-native top carnivore fish individuals
  - Percent of non-native macrohabitat generalist fish individuals
  - Percent of non-native vertebrate individuals
  - Percent of non-native individuals (fish and crayfish)
  - Percent of non-native general feeder fish individuals
  - Percent of non-native warmwater fish individuals
  - Proportion of vertebrate species as non-native
  - Proportion of total richness as native non tolerant species
  - Proportion of total richness as native
- Indicator species and Composition
  - Percent of pioneer fish individuals
  - Percent of most abundant species
  - Percent of brook trout individuals
  - Percent of blacknose dace individuals
  - Percent Family Rhinichthys individuals
  - Percent of individuals of the most abundant species
  - Percent of white sucker individuals
  - Number of Native Crayfish Species
  - Percent Native Crayfish
  - CPUE Common Crayfish
  - Number Salamander and Sensitive Frog Species
  - Number Salamander and Sensitive Frog Species minus Two lined salamander
  - Brook trout density (#individuals/100 m^2)
  - Number of brook trout size classes

Table A1.2
List of the 80 candidate metrics tested for inclusion in the Fish IBI.

Taxonomic Richness
- Number of total species
- Number of resident species (total minus eel)
- Number of native species
- Number of non-native species
- Number of top carnivore species
- Number of general feeder species
- Number of benthic invertivore species
- Number of intolerant species
- Number of intermediate species
- Number of tolerant species
- Number of rheophilic species
- Number of rheophilic species, minus blacknose dace
- Proportion of rheophilic species, minus blacknose dace
- Proportion of rheophilic species, minus tessellated darter
- Number of rheophilic species, minus blacknose dace & t. darter
- Number of lithophilic species
- Number of native lithophilic species
- Number of native nontolerant species

Thermal
- Percent of coldwater individuals
- Percent of coolwater individuals
- Percent of native coolwater individuals
- Percent of non-tolerant coolwater individuals
- Proportion of non-tolerant coolwater species
- Percent of warmwater individuals
- Percent of native warmwater individuals
- Number of coldwater species
- Number of coolwater species
- Number of warmwater species
- Number of coldwater species

Percent of coldwater and nontolerant coolwater individuals

Tolerance
- Percent of intolerant individuals
- Percent of intermediately tolerant individuals
- Percent of tolerant individuals
- Tolerance index

Composition
- Percent of Family Cyprinidae individuals
- Percent of insectivorous Cyprinidae individuals
- Percent of Family Centrarchidae individuals
- Percent of dominant individuals
- Percent of top 2 dominant individuals
- Percent of top 3 dominant individuals
- Percent of top 2 dominant individuals, minus blacknose dace
- Percent of top 3 dominant individuals, minus blacknose dace

Stream flow
- Percent of rheophilic individuals
- Percent of rheophilic individuals, except blacknose dace & tessellated darter
- Percent of rheophilic individuals, except tessellated darter

Reproduction
- Percent lithophilic spawners
- Percent native lithophilic spawners
- Percent lithophilic spawners, minus white sucker
- Proportion lithophilic spawning species, minus white sucker

Indicator species
- Percent of blacknose dace individuals
- Percent of white sucker individuals
- Percent of white sucker and creek chub individuals
- Percent of pumpkinseed individuals
- Percent of tessellated darter individuals
- Percent of longnose dace individuals
- Percent of bluegill individuals
- Percent of largemouth bass individuals
- Percent of common shiner individuals
- Percent of fallfish individuals
- Percent of green sunfish individuals

Habitat
- Number of benthic insectivore species
- Proportion of benthic insectivore species
- Number of benthic insectivore species, minus tessellated darter
- Number of native non-tolerant benthic insectivore species
- Number of native non-tolerant benthic insectivore species, minus t. darter
- Percent of native non-tolerant benthic insectivore species
- Percent of native non-tolerant benthic insectivore species, minus t. darter
- Percent of benthic insectivore, proportional richness

Trophic
- Percent of top carnivore individuals
- Percent of general feeder individuals
- Proportion of general feeder species
- Percent of non-tolerant general feeder individuals
- Percent of benthic invertivore individuals
- Percent generalist feeders, proportional richness

Non-native
- Percent of non-native individuals
- Percent of non-native generalist individuals
- Percent of non-native general feeder individuals
- Percent of non-native top carnivore individuals
- Percent of non-native warmwater individuals
- Percent of invasive individuals

Appendix B: Metric designations

See Table B1.

Table B1
Fish, amphibian and crayfish used in metric calculations and their respective ecological designations. Ecological designations are as follows: A = non-native, N = native, C = coldwater, C-W = coolwater, W = warmwater, BI = benthic insectivore, FF = filter feeder, GF = generalist feeder, H = herbivore, I = insectivore, O = omnivore, TC = top carnivore, PF = parasitic/filterer, Litho = lithophilic, Rheo = rheophilic.

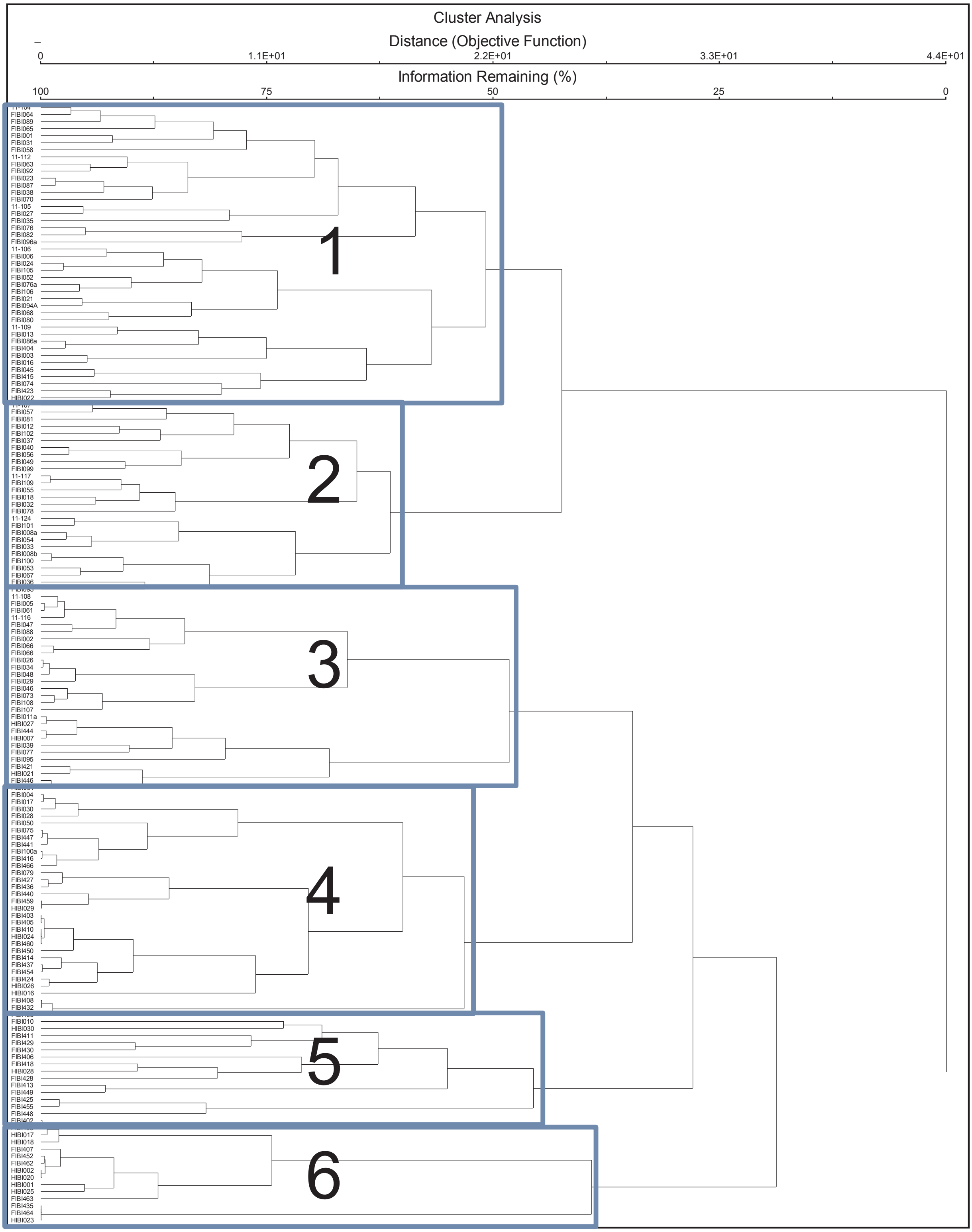
Species name		Origin	Temperature	Tolerance	Trophic	Reproduction	Stream Flow
Fish							
American brook lamprey	*Lethenteron appendix*	N	C-W	I	FF	Litho	Rheo
American eel	*Anguilla rostrata*	N	W	T	TC		
Banded killifish	*Fundulus diaphanus*	N	W	T	GF		
Black crappie	*Pomoxis nigromaculatus*	A	W	M	TC		
Blacknose dace	*Rhinichthys atratulus*	N	C-W	M	GF	Litho	Rheo
Bluegill	*Lepomis macrochirus*	A	W	M	GF		
Bluespotted Sunfish	*Enneacanthus gloriosus*	N	W	M	I		

(*continued on next page*)

Table B1 (*continued*)

Species name		Origin	Temperature	Tolerance	Trophic	Reproduction	Stream Flow
Bluntnose minnow	*Pimephales notatus*	A	W	T	GF		
Bridle shiner	*Notropis bifrenatus*	N	W	M	I		
Brook trout	*Salvelinus fontinalis*	N	C	I	TC	Litho	Rheo
Brown bullhead	*Ameiurus nebulosus*	N	W	M	GF		
Brown trout	*Salmo trutta*	A	C	I	TC	Litho	
Chain pickerel	*Esox niger*	N	W	M	TC		
Comely Shiner	*Notropis amoenus*	N	W	M	I	Litho	
Common carp	*Cyprinus carpio*	A	W	T	GF		
Common shiner	*Luxilis cornutus*	N	C-W	M	I	Litho	
Creek chub	*Semotilus atromaculatus*	N	C-W	M	GF	Litho	
Eastern creek chubsucker	*Erimyzon oblongus*	N	W	M	BI		
Cutlips minnow	*Exoglossum maxillingua*	N	W	I	BI	Litho	
Eastern mudminnow	*Umbra pygmaea*	N	W	M	GF		
Eastern silvery minnow	*Hybognathus regius*	N	W	M	H		
Fallfish	*Semotilus corporalis*	N	C-W	M	I	Litho	Rheo
Fathead minnow	*Pimephales promelas*	A	W	T	GF		
Golden shiner	*Notemigonus crysoleucas*	N	W	M	GF		
Goldfish	*Carassius auratus*	A	W	T	GF		
Green sunfish	*Lepomis cyanellus*	A	W	T	GF		
Largemouth bass	*Micropterus salmoides*	A	W	M	TC		
Longnose dace	*Rhinichthys cataractae*	N	C-W	M	BI	Litho	Rheo
Margined madtom	*Noturus insignis*	N	C-W	I	BI		
Mud sunfish	*Acantharchus pomotis*	N	W	M	I		
Mummichog	*Fundulus heteroclitus*	N	W	T	GF		
Northern hog sucker	*Hypentelium nigricans*	N	W	I	BI	Litho	
Northern pike	*Esox lucius*	A	W	M	TC		
Oriental weather fish	*Misgurnus anguillicaudatus*	A	W	M	BI		
Pirate perch	*Aphredoderus sayanus*	N	W	M	I		
Pumpkinseed	*Lepomis gibbosus*	N	W	T	GF		
Rainbow trout	*Oncorhynchus mykiss*	A	C	I	TC	Litho	Rheo
Redbreast sunfish	*Lepomis auritus*	N	W	M	GF		
Redfin pickerel	*Esox americanus*	N	W	M	TC		
Rock bass	*Ambloplites rupestris*	A	C-W	M	TC	Litho	
Satinfin shiner	*Cyprinella analostana*	N	W	M	I		
Sea lamprey	*Petromyzon marinus*	N	C-W	M	PF		
Shield darter	*Percina peltata*	N	C-W	I	BI	Litho	
Slimy sculpin	*Cottus cognatus*	N	C	I	BI		Rheo
Smallmouth bass	*Micropterus dolomieu*	A	C-W	M	TC	Litho	
Spotfin shiner	*Cyprinella spiloptera*	N	W	M	I		
Spottail shiner	*Notropis hudsonius*	N	W	M	I		
Striped bass	*Morone saxatilis*	N	W	M	TC		
Swallowtail shiner	*Notropis procne*	N	W	M	I	Litho	
Tessellated darter	*Etheostoma olmstedi*	N	C-W	M	BI		Rheo
Walleye	*Sander vitreus*	A	C-W	M	TC	Litho	
Western mosquitofish	*Gambusia affinis*	A	W	T	I		
White catfish	*Ameiurus catus*	N	W	M	TC		
White crappie	*Pomoxis annularis*	A	W	M	TC		
White perch	*Morone americana*	N	C-W	M	TC		
White sucker	*Catostomus commersonii*	N	C-W	T	GF	Litho	
Yellow bullhead	*Ameiurus natalis*	N	W	M	GF		
Yellow perch	*Perca flavescens*	N	C-W	M	TC		
Amphibians							
Bullfrog	*Lithobates catesbeianus*	N		T	TC		
Green Frog	*Lithobates clamitans*	N		T	I		
Longtail Salamander	*Eurycea longicauda longicauda*	N		I	I		
Mountain Dusky Salamander	*Desmognathus ochrophaeus*	N		I	I		
Northern Dusky Salamander	*Desmognathus fuscus*	N		I	I		
Northern Red Salamander	*Pseudotriton ruber ruber*	N		I	TC		
Northern Two-lined Salamander	*Eurycea bislineata*	N		T	I		
Northern Spring Salamander	*Gyrinophilus porphyriticus porphyriticus*	N		I	TC		
Pickerel Frog	*Lithobates palustris*	N		M	I		
Red-spotted Newt	*Notophthalmus viridescens viridescens*	N		M	I		
Crayfish							
Allegheny Crayfish	*Orconectes obscurus*	A		T			
Common Crayfish	*Cambarus bartonii bartonii*	N		I			
Red Swamp Crayfish	*Procambarus clarkii*	A		T			
Rusty Crayfish	*Orconectes rusticus*	A		T			
Spinycheek Crayfish	*Orconectes limosus*	N		M			
Virile Crayfish	*Orconectes virilis*	A		T			
White River Crayfish	*Procambarus acutus*	N		M			

Appendix C: Cluster analysis of New Jersey fish assemblages



Appendix D: Nonparametric analysis

Fish IBI nonparametric Spearman correlation and Kruskal-Wallis (KW) test results.

Metric	Ecological Class	Spearman Correlation w/ Population Density n = 127	Spearman Correlation w/ Forest n = 137	Spearman Correlation w/IC n = 137	Spearman Correlation w/ Habitat n = 137	Mann-Whitney LI vs MI n = 48	K-W H Statistic LI vs MI n = 48
% Rheophilic Species-Tessellated Darter (drainage size corrected)[a]	Taxonomic Richness	−0.38	0.55	−0.39	0.67	$p < 0.001$	33.5
% Cold/NonTolerant Coolwater Species (drainage size corrected)[b]	Thermal	−0.43	0.50	−0.44	0.56	$p < 0.001$	22.6
% Generalist Species[a]	Trophic	0.48	−0.47	0.49	−0.57	$p < 0.001$	28.4
Tolerance Index	Tolerance	0.44	−0.54	0.46	−0.56	$p < 0.001$	28.5
% Lithophilic Species-White Sucker[a]	Reproduction	−0.56	0.57	−0.56	0.63	$p < 0.001$	30.4
% Cyprinidae (drainage size corrected)[b]	Composition	−0.55	0.50	−0.55	0.54	$p < 0.001$	27.8
% Top 3 Dominant Species-Blacknose Dace[b]	Composition	0.54	−0.28	0.47	−0.41	$p < 0.001$	19.6
% Benthic Insectivore Species[a]	Habitat	−0.49	0.49	−0.51	0.57	$p < 0.001$	26.4

[a]Proportion of Species.[b]Proportion of Individuals.

Headwaters IBI nonparametric Spearman correlation and Kruskal-Wallis (KW) test results.

Metric	Ecological Class	Spearman Correlation w/ Urban n = 96	Spearman Correlation w/ Forest n = 96	Spearman Correlation w/ IC n = 62	Spearman Correlation w/ Habitat n = 96	Mann-Whitney LI vs MI n = 55	K-W H Statistic LI vs MI n = 55
Intolerant Vertebrate Richness	Taxonomic Richness	−0.61	0.66	−0.53	0.50	$p < 0.001$	59.0
Proportion of Vertebrate Richness as Top Carnivore	Trophic	−0.47	0.46	−0.46	0.49	$p < 0.001$	103.0
% Tolerant Fish Individuals	Tolerance	0.59	−0.50	0.77	−0.56	$p < 0.001$	571.5
Proportion of Total Richness as Native	Non-Native	−0.38	0.35	−0.43	0.34	$p = 0.001$	153.5
% Native Crayfish	Composition	−0.54	0.54	−0.53	0.50	$p < 0.001$	90.0
Brook Trout Density (individuals/ $100\,m^2$)	Composition/ Indicator Species	−0.33	0.34	−0.29	0.34	$p = 0.002$	202.5

Appendix E: Pearson correlation matrix

Fish IBI Pearson correlation matrix for metric redundancy.

	%BI Sp	%Dominant 3	%Cyprinidae	%Lithophilic Sp	Tolerance Index	%Generalist Sp	%Cold NonTol Cool
%Rheophilic Sp	0.48	−0.15	0.10	0.58	−0.23	−0.49	0.08
%Cold NonTol Cool	0.13	−0.47	0.70	0.56	−0.57	0.13	
%Generalist Sp	−0.43	0.20	0.06	−0.42	0.08		

Tolerance Index	−0.17	0.39	−0.54	−0.48
%Lithophilic Sp	0.33	−0.48	0.64	
%Cyprinidae	0.29	−0.48		
%Dominant 3	0.00			

Headwaters IBI Pearson correlation matrix for metric redundancy.

	Intolerant Vertebrate Richness	Proportion of Vertebrate Richness as Top Carnivore	% Tolerant Fish Individuals	Proportion of Total Richness as Native	% Native Crayfish
Proportion of Vertebrate Richness as Top Carnivore	0.414				
% Tolerant Fish Individuals	−0.008	−0.075			
Proportion of Total Richness as Native	−0.205	0.1	−0.167		
% Native Crayfish	0.463	0.044	−0.191	0.053	
Brook Trout Density (individuals/100 m^2)	0.462	0.153	−0.136	−0.358	0.349

Appendix F: Spearman correlation matrix

Fish IBI Spearman correlation matrix for metric redundancy.

	%BI Sp	%Dominant 3	%Cyprinidae	%Lithophilic Sp	Tolerance Index	%Generalist Sp	%Cold NonTol Cool
%Rheophilic Sp	0.57	−0.06	−0.01	0.60	−0.29	−0.50	0.10
%Cold NonTol Cool	0.07	−0.53	0.67	0.66	−0.56	0.12	
%Generalist Sp	−0.47	0.18	0.14	−0.34	0.15		
Tolerance Index	−0.19	0.40	−0.46	−0.55			
%Lithophilic Sp	0.35	−0.50	0.58				
%Cyprinidae	0.24	−0.45					
%Dominant 3	0.07						

Headwaters IBI Spearman correlation matrix for metric redundancy.

	Intolerant Vertebrate Richness	Proportion of Vertebrate Richness as Top Carnivore	% Tolerant Fish Individuals	Proportion of Total Richness as Native	% Native Crayfish
Proportion of Vertebrate Richness as Top Carnivore	0.42				
% Tolerant Fish Individuals	0.02	−0.179			
Proportion of Total Richness as Native	−0.078	0.034	−0.256		
% Native Crayfish	0.452	−0.021	−0.008	0.282	
Brook Trout Density (individuals/100 m^2)	0.694	0.378	−0.115	−0.014	0.456

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