Transitional Water Fish Assemblage Index of Biotic Integrity for New Hampshire Wadeable Streams

NEW HAMPSHIRE DEPARTMENT OF Environmental Services

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Transitional Water Fish Assemblage Index of Biotic Integrity for New Hampshire Wadeable Streams

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1. INTRODUCTION

The following document describes the development of a transitional water fish assemblage Index of Biotic Integrity (TWIBI) for New Hampshire wadeable streams. A transitional water fish assemblage is meant to describe an assemblage that neither resides in a strict coldwater, nor warmwater environment. Rather, transitional water fish assemblages reside in sections of rivers and streams "transitioning" away from a coldwater assemblage (few species, dominated coldwater specialists) and towards a warmwater assemblage (increased species richness, dominated by warmwater generalists). As the name suggests, transitional water fish assemblages share the biological attributes of two distinct fish assemblage types making them difficult to define with absolute certainty, and therefore, subsequently locate *a priori* purely based on their physical characteristics or geographic proximity.

The TWIBI is a numeric interpretation of the narrative water quality criteria as stated in New Hampshire Department of Environmental Services Administrative Rules Env – Wq 1700 covered under the statutory authority given in RSA 485-A:8, VI. Specifically, the narrative standard is detailed in section 1703.19 as:

> **Env-Ws 1703.19 <u>Biological and Aquatic Community Integrity</u>.**
>
> **(a)** The surface waters shall support and maintain a balanced, integrated, and adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of similar natural habitats of a region.
>
> **(b)** Differences from naturally occurring conditions shall be limited to non-detrimental differences in community structure and function.

The product of the TWIBI development process detailed in this document will ultimately be used to assess, in part, the health of applicable aquatic communities. Specifically, assessments under this authority will be made for aquatic life use (ALU) determinations as required for 305(b)/303(d) reporting to the United States Environmental Protection Agency (EPA). Additional applications include, but are not limited to the establishment of permit limits, determination of non-point source water quality impacts, water quality planning, and ecological risk assessment (Barbour et al. 1999).

As a two-part narrative criterion, the goal of index development was to first identify the natural structure and function of the fish assemblages residing in the pertinent natural habitats [1703.19(a)], and second, to determine when a detrimental departure from the natural condition has occurred [1703.19(b)]. The basic approach taken for TWIBI development was the identification of a suitable reference condition and establishment of a natural range of variation within this reference condition (=identification of natural structure and function). Once identified, a reference condition threshold was established below which the biological condition includes detrimental changes in overall aquatic community structure and function (=departure from natural condition). Transitional water fish assemblages not meeting or exceeding the reference condition threshold would be considered to demonstrate significant unnatural community structure and function alterations and consequently not attaining the narrative water quality standard in 1703.19 for ALU.

2. GENERAL PROCESS FOR TWIBI DEVELOPMENT

Indices of biological integrity for fish assemblages have been developed using a variety of approaches over the past twenty years (Karr 1981; Leonard and Orth 1986; Lyons et al. 1996; Mundahl and Simon 1999; Langdon 2001; Daniels et al. 2002; Hughes et al. 2004, and Whittier et al. 2007). While these approaches differ in their objectivity, data analysis approaches, and final index evaluation system, most follow the same basic developmental principles to arrive at a final condition index to characterize the overall structure and function of the fish assemblage.

For New Hampshire, the process of developing a numeric index that interprets the biological condition of transitional fish assemblages was similar to that described by Barbour et al. (1995) and included five basic steps:

1) ***Reference sites selection*:** An *a-priori* process used to select sites with minimal human impacts in order to establish the minimally impacted biological condition.

2) ***Transitional water fish assemblage identification*:** The determination of indicator species, assemblage diversity, applicable area, and non-biological factors that describe this assemblage type.

3) ***Identification of biological response indicators (metrics)*:** The selection of the best ecological measures of community structure and function. Generally known as metric selection.

4) ***Establishment of index scoring criteria and thresholds*:** A comparison of reference and non-reference biological conditions for the purpose of determining when substantial unnatural impacts to ecological structure and function have occurred.

5) ***Validation of index:*** Testing of metric responses, comparison of reference and non-reference conditions, and testing of the proposed threshold with an independent dataset.

The end result of the development process is a numeric index that includes multiple response indicators (i.e. multi-metric) that are considered cumulatively to quantify the biological condition of applicable streams. The index should be sensitive to human disturbance in that it demonstrates declining biological conditions in response to increasing anthropogenic impacts.

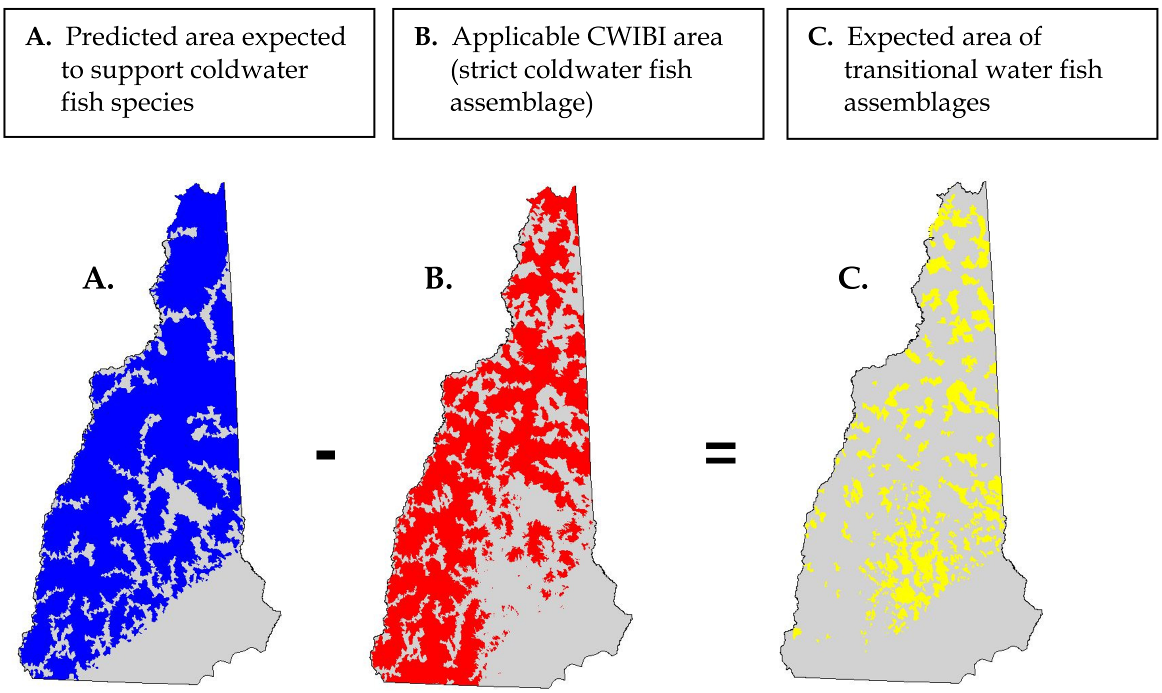
3. METHODS

3.1 Identification of Expected Transitional Water Fish Assemblage Areas

In order to avoid the difficulties in defining a distinct set of physical or geographic characteristics for rivers and streams that are expected to contain transitional water fish assemblages, areas supporting this fish assemblage type were identified through a process of elimination. First, the identification of the geographic boundaries of streams and rivers expected to support coldwater fish species year round were delineated using predictions from a logistic regression model based on latitude, longitude, and upstream drainage area (NHDES, 2007a). The areas not contained within these predictions are expected to contain warmwater fish assemblages and will be analyzed at a later date.

Next, the applicable areas of the New Hampshire strict coldwater fish assemblage index of biotic integrity (CWIBI) (NHDES, 2007b) were overlaid onto the expected coldwater fish species areas. The resulting, non-overlapping area was deemed to best define streams and rivers that are expected to contain transitional water fish assemblages (Map 1). Note, however, that by definition a transitional water fish assemblage is expected to support coldwater fish species throughout the year. Thus, transitional water fish assemblages were expected to resemble strict coldwater fish assemblages, primarily by the presence of coldwater fish species, but with differences in species richness and composition.

Map 1. Expected geographic distribution of 1st – 4th order streams expected to support coldwater fish species, applicable CWIBI area, and areas expected to support transitional water fish assemblages.



3.2 Comparison of Transitional and Strict Coldwater Assemblages

After the geographic boundaries were defined, all sites falling within the area were included in subsequent analyses. Once the final dataset was defined, the fish species composition and physical characteristics (latitude, longitude, elevation, and drainage area) of the transitional water fish assemblage reference sites were summarized and compared to reference sites included in the previously developed CWIBI in order to determine the level similarity or uniqueness. Species indicator analysis and non-metric multidimensional scaling (NMDS) were completed using PC-ORD (MjM software, Version 4) as a final step to confirm the need for separate condition indices (IBIs).

3.3 Dataset

The development of a condition index for the transitional water fish assemblage included a total of 164 sites located in 1st to 4th order rivers and streams. Data included in the development process originated from sampling performed by the NHDES and the New Hampshire Fish and Game Department (NHFGD). Of the original 164 sites, 29 were removed because fewer than 30 individuals were captured. The final dataset included 55 sites sampled by the NHDES from 1997 – 2007. At each site a representative sample reach of 150 meters was delineated and fish were collected in a single pass backpack electrofishing effort. All sites were part of the annual biological monitoring sampling program. For the NHFGD, data from two distinct programs was included. First, 43 sites were sampled as part the NHFGD's inland fisheries summer assessment program (SAP). These sites generally included a 100 – 150 meter sampling reach with fish collected during a single or multiple pass backpack electrofishing effort. Thirty-seven additional sites were included from NHFGD's Fishing-for-the-Future program (FFF). A similar sampling effort was employed for these sites with site selection focused on rivers and streams that had been previously stocked with coldwater gamefish species. Fieldwork for each of the programs above was completed primarily from 1995 – 2007.

The dataset was randomly broken into calibration and validation subsets. The calibration dataset included 31 reference sites, 27 minimally impacted sites, 31 moderately impacted sites, and 10 impacted (high) sites (Table 1). The validation dataset was designed to test the performance of the index and consisted of 11 reference, 9 minimally impacted, 10 moderately impacted, and 6 impacted sites. Reference sites were defined as "minimally disturbed" (Stoddard et al. 2006). Reference site identification and narrative impact ratings were based on the activities within the upstream drainage area and determined from a combination of a quantitative human disturbance rating system for the NHDES sites and aerial / topographic map inspection for NHFGD sites. Reference site determinations and impact ratings for NHFGD sites were finalized by the respective agency biologists familiar with the sample locations and their contributing drainage area.

Table 1. Number of sites in the calibration and validation datasets sampled by NHDES and NHFGD.

			Level of Impact			
Agency	**Project**	**Reference**	**Minimum**	**Moderate**	**High**	**Totals**
CALIBRATION						
NHDES	Annual Sampling	9	15	12	4	40
NH Fish and Game (NHFGD)	Annual Sampling (SAP)	14	6	9	4	33
	Fishing for the Future (FFF)	8	6	10	2	26
Totals		31	27	31	10	99
VALIDATION						
NHDES	Annual Sampling	4	3	6	2	15
NH Fish and Game (NHFGD)	Annual Sampling (SAP)	2	6	0	2	10
	Fishing for the Future (FFF)	5	0	4	2	11
Totals		11	9	10	6	36

For all sites as many fish as possible were collected during active sampling. After sampling was complete all fish were identified, enumerated, recorded, and immediately returned to the river or stream from which they were collected. Length and weight data were also collected for gamefish species for all NHFGD sites. For all sites, inclusion into the index development process required that each species had a minimum of two individuals. In addition, Atlantic salmon were excluded from the dataset since they only exist in New Hampshire rivers and streams through stocking efforts.

Finally, because salmonid fish species represent an integral component of any fish assemblage from which they are expected to occur, their origin and life stage are important to characterize when making condition assessments. While many of the sampling stations included in the dataset were known to contain both wild and stocked individuals, their origin was not always available from the data. Therefore, since wild salmonids in New Hampshire tend to be smaller than hatchery raised fish, a size limit was imposed to differentiate their origin where information was otherwise lacking. Based on input from NHFGD biologists, all salmonid individuals less than 180mm were considered wild (naturally produced) and subsequently retained for further analysis. In contrast, salmonid individuals greater than 180mm were assumed to be hatchery raised and excluded from further analysis. While, on occasion, wild salmonids certainly exceed 180mm in length in NH; such large, wild individuals are relatively uncommon.

With regards to life stage [young-of-year (YOY) or adult], where information was not available, a 90mm length threshold was established by the NHFGD whereby individuals less than 90mm were designated as YOY. Individuals exceeding 90mm in length were designated as adults.

Once final datasets were adjusted as described above, species richness, rank species abundance, and the number of individuals captured per site was compared between NHDES and NHFGD sites to ensure that the data sources were compatible. Kruskal-Wallis and Mann-Whitney U tests were used to determine if differences were detectable in environmental characteristics between the datasets.

3.4 Biological Response Indicators (Metrics)

Candidate metrics were selected from previously developed fish indices (Hughes et al. 2004; Karr 1981; Langdon 2001; Leonard and Orth 1986; Lyons et al. 1996; Mundahl and Simon 1999; Daniels et al. 2002; Whittier et al. 2007) and tested for their ability to respond to varying levels of human disturbance. Candidate metrics were classified into 8 major groups that included trophic class, tolerance to pollution, thermal preference, streamflow preference, species richness, reproductive strategy and success, assemblage composition, and origin (native or introduced) (Appendix A). For each metric, an expected response to impact was noted and used in the metric testing process. Expected responses were either positive (i.e. higher for reference than impacted sites) or negative (lower for reference than impacted sites). Species common names, scientific names and the respective ecological, pollution tolerances, thermal preferences, reproductive strategies, and origin for the most commonly encountered species are presented in Table 2.

Table 2. Names, abbreviations, origin, and autecological characteristics of fish species most commonly encountered at transitional water fish assemblage sampling locations. See Appendix B for explanation of abbreviations.

Common name	Scientific name	Abbreviation	Origin	Tolerance	Trophic class	Thermal preference	Reproductive Strategy[1]	Streamflow preference[2]	Streamflow preference[3]
Blacknose dace	*Rhinichthys atratulus*	BND	N	T	OI	ET	S_L	r	fs
Brook trout	*Salvelinus fontinalis*	EBT	N	I	TC	CW	S_L	r	fs
Brown trout	*Salmo trutta*	BT	I	I	TC	CW	S_L	r	fs
Burbot	*Lota lota*	BRB	N	M	TC	CW	S_L	x	mg
Creek chub	*Semotilus atromaculatis*	CC	N	T	GF	ET	S_L	x	fs
Common shiner	*Luxilus cornutus*	CS	N	M	GF	ET	S_L	x	fd
Fallfish	*Semotilus corporalis*	FF	N	M	GF	ET	S_L	x	fs
Lake chub	*Couesius plumbeus*	LC	N	M	GF	CW	S_L		mg
Longnose dace	*Rhinichthys cataractae*	LND	N	M	BI	ET	S_L	r	fs
Longnose sucker	*Catostomus catostomus*	LNS	N	M	BI	CW	S_L	x	fd
Rainbow trout	*Oncorhynchu s mykiss*	RT	I	I	TC	CW	S_L	r	fs
Slimy sculpin	*Cottus cognatus*	SS	N	I	BI	CW	H_D	r	fs
Spottail shiner	*Notropis hudsonius*	STS	I	M	OI	WW	S_L	l	mg
White sucker	*Catostomus commersoni*	CWS	N	T	GF	ET	S_L	x	fd

1 - Simon 1999; based on Balon 1975; 2 - from Stoddard et al. 2007; 3 - from Bain 1996

In order to determine the appropriateness of a candidate metric's inclusion into the final index a multi-step process was implemented that first included examining the distribution of metric values for reference and impacted sites. For each metric, reference and impacted site distributions were compared by first computing the 25th and 75th percentiles for reference sites and then determining the percentage of impacted site values that fell within the reference range. If greater than 60 percent of the impacted site values fell within the reference range the metric was eliminated (*Sensu* Whittier et al. 2007). Next, mean reference and impacted site metric values were compared and matched with the expected responses for individual metrics. Metrics that displayed observed responses counter to expected responses were also eliminated.

After the initial metric testing phase, all remaining metrics were evaluated with respect to natural environmental gradients to determine if any significant relationships were apparent. The objective of this step was to account for natural variation in metric values that was unrelated to the stressor gradient. To accomplish this, metric values from reference sites were regressed against environmental variables. For each candidate metric and environmental variable combination, regression significance was computed, data plots examined, and 75 percent prediction interval lines constructed to determine if a strong relationship existed.

The third phase of candidate metric testing included a detailed objective comparison between reference and test site distributions. First, significant differences between reference and impacted sites were determined from Mann-Whitney U tests for all metrics. The absolute value of the Z-scores for the respective candidate metrics were ranked from highest to lowest for each major metric category with the presumption that higher Z-scores indicated a more distinct stressor response (Whittier et al. 2007). Next, the mean, median, 75th, and 25th percentiles were computed for each metric and compared in five combinations (See appendix C). The total number and magnitude of correct responses for each metric was examined. These results were paired with the significance testing to arrive at an initial list of final metrics.

Once the final list of potential metrics was determined, redundancy testing was performed using the Spearman correlation coefficient. A target maximum correlation coefficient of 0.75 was established whereby metrics with coefficients greater than this value were considered excessively redundant requiring the selection of one or the other. In a limited number of cases some leniency was allowed in applying this rule in order to further consider candidate metrics for inclusion into the final index.

The final step in the metric testing phase included a review of the results from the steps outlined above. In some cases, similar metrics were interchanged in an attempt to balance the final index with regards to the number of positive and negative response metrics, major metric categories, and important fish assemblage characteristics. Final metric selection was designed to minimize metric redundancy, maximize the selection of metrics with the greatest separation between reference and impacted sites, and the inclusion of metric types that captured broad structural and functional ecological categories. Cumulative frequency distributions and box and whisker plots were constructed as a final visual aid in comparisons between reference and impacted sites for the selected metrics.

3.5 TWIBI scoring and threshold identification

Scores for individual metrics were established by reviewing the frequency distribution of reference and impacted sites. Specifically, three scoring categories (1, 3, and 5) were established to be consistent with previously developed fish indices by the Vermont Department of Environmental Conservation (VTDEC) (VTDEC 2004) with higher scores representing better condition. Then, for each metric, raw values for reference sites were examined using cumulative frequency distributions and the 25th (positive response metrics) or 75th (negative response metrics) percentiles in order to assign logical breakpoints for the metric scoring categories. Once categorical scoring thresholds were determined, scores were assigned to individual metrics for each site and a final index score computed by summing individual metric scores. A final TWIBI threshold for aquatic life use attainment was based on the 25th percentile index score for reference sites.

3.6 Final Index Score Performance Evaluation

As a final check on the ability of the index to discriminate along a human disturbance gradient, a Kruskal-Wallis test was completed for index scores across impact categories followed by Mann-Whintey U tests for pair-wise impact category comparisons. Finally, based on the recommended aquatic life use threshold, the number of sites meeting and failing to meet this threshold was determined for each site type. Contingency tables based on these outcomes were compared (Chi-square) for reference and test sites to determine if the distribution of sites exceeding and failing to meet the recommended criteria were significantly different from random expectations.

4. RESULTS

4.1 Transitional vs. Strict Coldwater Assemblages

The expected species composition and abundance of sites used in the development of the TWIBI was based on 31 reference sites from the calibration dataset and included 3,318 individuals from 14 species. Overall, blacknose dace was the most commonly collected species (87% of sites), followed by brook trout (77%), longnose dace (65%), longnose sucker (58%), and slimy sculpin (58%) (Table 3). The same suite of species also had the highest overall relative abundance [blacknose dace (25% of individuals), longnose dace (23%), slimy sculpin (17%), brook trout (8%), longnose sucker (8%)].

Table 3. Frequency of occurrence, total number of individuals, and rank abundance of fish species collected at transitional water fish assemblage reference sites. Rank of ranks is inverse ranking of sum of ranks for # sites present, percent of all individuals, average percent individuals/site.

Species	# Sites Present	of Sites Present	Rank	Total Number Individuals	% of All Individuals	Rank	Average % Individuals / Site	Rank	Sum of Rank	Rank of Ranks
BND	27	87.1	1	845	25.3	1	31.3	3	5	1
BRB	6	19.4	8	36	1.1	10	6.0	13	31	10
BT	3	9.7	10	28	0.8	11	9.3	11	32	12
CC	2	6.5	12	28	0.8	11	14.0	8	31	10
CS	2	6.5	12	27	0.8	13	13.5	9	34	13
CWS	8	25.8	6	50	1.5	9	6.3	12	27	9
EBT	24	77.4	2	282	8.4	4	11.8	10	16	4
FF	3	9.7	10	51	1.5	8	17.0	5	23	8
LC	5	16.1	9	234	7.0	6	46.8	1	16	4
LND	20	64.5	3	783	23.4	2	39.2	2	7	2
LNS	18	58.1	4	277	8.3	5	15.4	7	16	4
RT	7	22.6	7	114	3.4	7	16.3	6	20	7
SS	18	58.1	4	563	16.8	3	31.3	4	11	3
STS	2	6.5	12	11	0.3	14	5.5	14	40	14

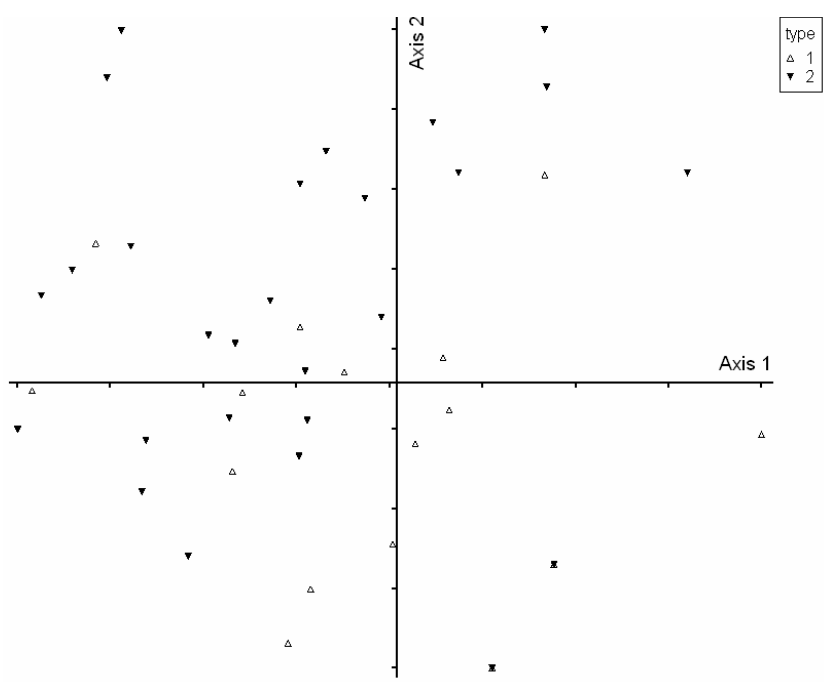
In comparison, the development of the CWIBI from 33 reference sites included 3,008 individuals from 10 species (NHDES, 2007a). The five species with the highest relative frequency, in decreasing order, from CWIBI reference sites was brook trout (94% of sites), slimy sculpin (76%), blacknose dace (37%), longnose dace (24%), and rainbow trout (12%) (Table 4). The overall relative abundance of these same species ranked highest among the CWIBI reference sites and were as follows: brook trout (33%), blacknose dace (31%), slimy sculpin (25%), longnose dace (4%), and rainbow trout (2%).

Table 4. Frequency of occurrence, total number of individuals, and rank abundance of fish species collected at coldwater fish assemblage sites. See Table 2 for explanation of "Rank of Ranks".

Species	# Sites Present	% of Sites Present	Rank	Total Number Individuals	% of All Individuals	Rank	Average % Individuals / Site	Rank	Sum of Ranks	Rank of Ranks
BND	12	36.6	3	934	31.1	2	15.4	3	8	3
BT	2	6.1	9	18	.6	8	.5	8	25	9
CWS	3	9.1	6	35	1.2	7	.8	7	30	7
CC	2	6.1	9	7	.2	10	.4	10	29	10
EBT	31	93.9	1	1006	33.4	1	49.9	1	3	1
LC	33	9.1	6	55	1.8	6	2.3	6	18	6
LND	8	24.2	4	125	4.2	4	3.4	4	12	4
LNS	3	9.1	6	17	.6	9	.5	9	24	8
RT	4	12.1	5	64	2.1	5	2.8	5	15	5
SS	25	75.8	2	747	24.8	3	24	2	7	2

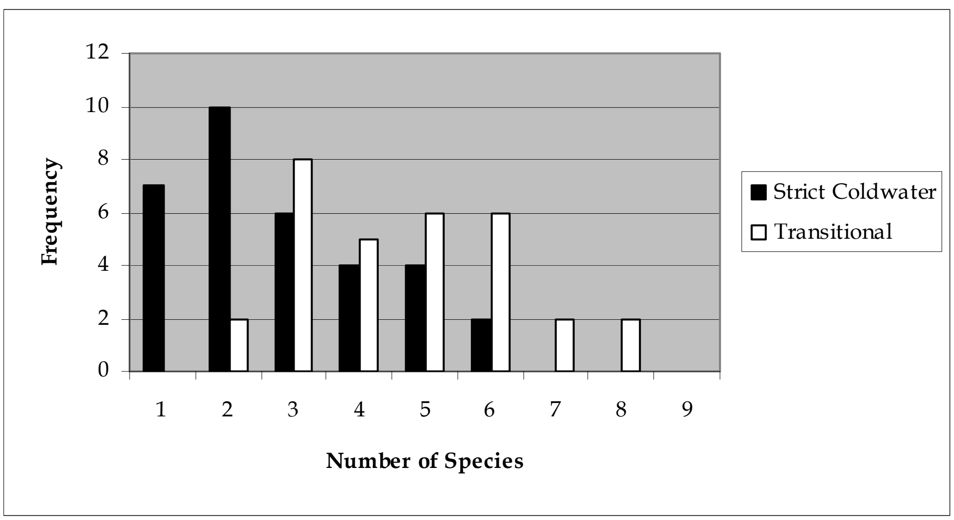
As suspected, reference sites for strict coldwater and transitional water fish assemblages shared similar overall species compositions. NMDS analysis of these assemblage types using species presence / absence data confirmed this finding (Figure 1). A general lack of site-grouping by category (fish assemblage type) is indicative of biological communities that share the same species compositions.

Figure 1. Nonmetric multidimeninal squaring ordination (NMDS) plot of cold- and transitional water fish assemblage reference site based on fish species presence absence. Final stress = 17.8; instability = 0.005. Open triangles (1) = coldwater reference sites; closed triangles (2) = transitional water reference sites.



While similarities were apparent among these assemblage types based solely on species presence or absence, significant differences in species richness (Mann-Whitney U; Z-score = -4.02, $p<0.0001$) were detected with transitional fish assemblages having more species (4.6) on average than strict coldwater assemblages (2.8) (Figure 2). When the relative frequencies and abundances of individual species were examined more closely, clear differences in transitional and strict coldwater assemblages were obvious. For example, while blacknose dace was regularly encountered at reference sites from both assemblage types, its relative frequency (percentage of species occurrences within each fish assemblage type) was much higher for transitional fish assemblage sites (87%) than coldwater fish assemblage sites (36%) (Figure 3). Additional species which were more frequently encountered at one assemblage type than another were longnose sucker, longnose dace, burbot, and white sucker. In addition, several species were exclusive to, or had higher relative abundances (percentage of species occurrences across assemblage types) in transitional than coldwater fish assemblage sites (Figure 3). Fallfish, pumpkinseed, common shiner, and burbot were all found only at transitional assemblage sites. Longnose sucker, longnose dace, white sucker, and blacknose dace all occurred in higher relative abundances at transitional than coldwater assemblage sites.

Figure 2. Number of fish species at cold- and transitional water reference sites.



Relative frequencies and abundances were combined to compute species indicator values (PC-ORD, MjM software). Higher indicator values are indicative of species with a strong membership to a particular assemblage type. For the transitional fish assemblage type, the species that served as the best indicators were blacknose dace, longnose sucker, longnose dace, and burbot. Each of these species had the highest indicator value differences among transitional and strict coldwater assemblage types and were also significantly different from indicator values produced from randomized data (Table 5).

Figure 3. Strict cold (black bars) and transitional (white bars) water fish assemblage fish species relative abundance (percentage of all sites) and frequency (percentage of assemblage-specific sites) at reference sites.

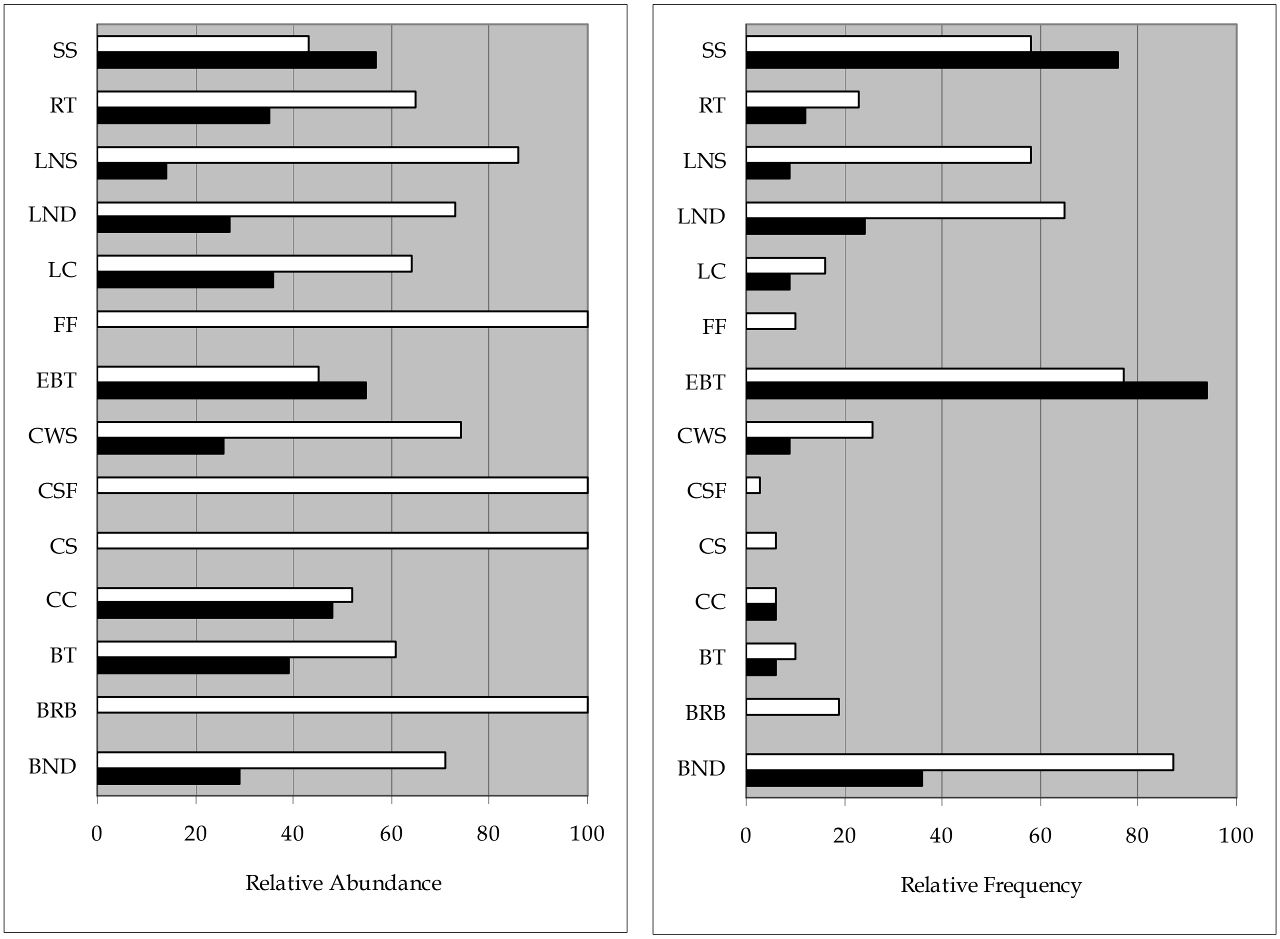


Table 5. Species indicator values (PC-ORD, MjM software) for fish species from strict cold- and transitional water assemblages. Randomized column reflects species specific indicator value after 1000 random reassignment of sites to an assemblage type using Monte Carlo simulations. p-value indicates level of significance between indicator values.

SPECIES	COLD	TRANSITIONAL	RANDOMIZED	p-value
BND	11	61	35	0.0010
BRB	0	19	8	0.0060
BT	2	6	8	0.6760
CC	3	3	6	1.0000
CS	0	6	4	0.2250
CSF	0	3	3	0.4700
CWS	2	19	13	0.1170
EBT	51	35	47	0.0700
FF	0	10	5	0.1080
LC	3	10	10	0.4570
LND	7	47	27	0.0040
LNS	1	50	22	0.0010
RT	4	15	13	0.2850
SS	43	25	39	0.1870

The environmental characteristics also differed when transitional and strict coldwater fish assemblage reference sites were compared. Upstream drainage area had the most significant difference between transitional and strict coldwater assemblage reference sites with mean drainage areas of 31 and 7 square miles, respectively (Mann-Whitney U test; Z-score = -5.64; p<0.0001) (Table 6). In addition, reference sites from transitional waters tended to be more northerly (Mann-Whitney; Z-score = -2.35; p=0.019) and westerly (Mann-Whitney U test; Z-score = -2.08; p=0.037) than from strict coldwaters. Elevation did not differ significantly between transitional and strict coldwater reference sites.

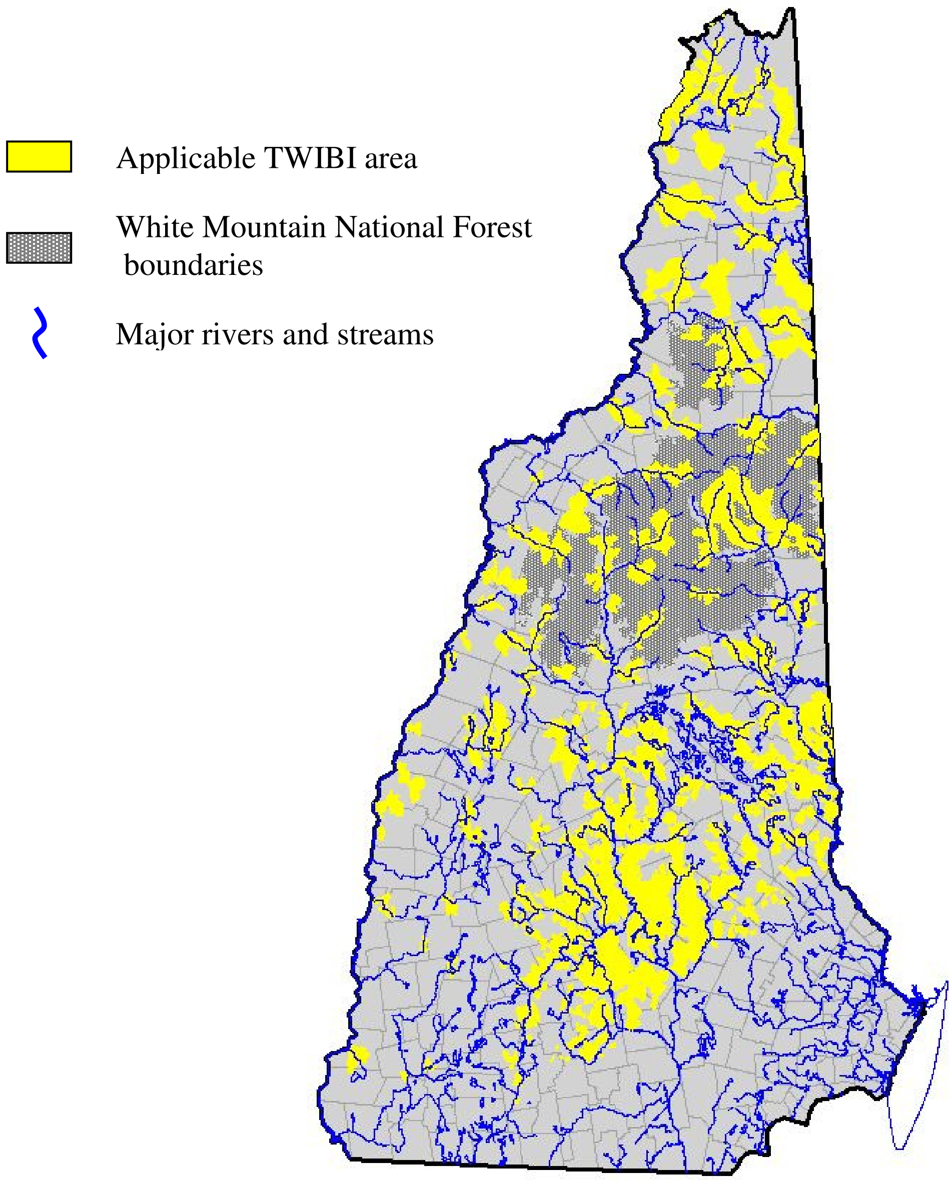
Table 6. Latitude (dd.dddd), longitude (dd.dddd), elevation (ft), and drainage area (sq. mi.) of reference sites for strict cold (CW) and transitional (TW) water fish assemblages. Asterisk indicates Mann-Whitney U test significantly different at p<0.05.

Fish Assemblage Type	N	Mean	Std. Error of Mean	Median	Minimum	Maximum
Latitude*						
CW	33	44.2071	0.10	44.3266	42.7313	45.1941
TW	31	44.5107	0.09	44.5317	43.3552	45.1084
Longitude*						
CW	33	71.4663	0.07	71.3699	71.0246	72.4281
TW	31	71.2747	0.03	71.2306	71.0351	71.7919
Area*						
CW	33	7.1	0.72	6.7	0.2	13.6
TW	31	31.3	2.85	34.7	3.7	63.5
Elevation						
CW	33	1157	73.14	1180	337	1999
TW	31	1063	65.40	1157	439	1658

4.2 Transitional Water Fish Assemblage Area

The area identified as expected to contain transitional water fish assemblages and subsequently applicable to the TWIBI was 1,622 square miles (Map 2). In total, the area represents 17.5% of the State of New Hampshire. The applicable TWIBI area is primarily located in central and northern sections of state with scattered areas along the western border of New Hampshire. The area identified in Map 2 is meant to serve as general guidance for determining when the TWIBI should be applied. However, for any given site, measures of latitude, longitude, and upstream drainage area will serve as the primary determinants in conjunction with the rules outlined in Section 3.1 when deciding if the TWIBI is the most appropriate fish index to assess the biological condition of the fish assemblage.

Map 2. Expected areas of transitional water fish assemblage occurrence and respective index of biological integrity (IBI) application.



4.3 Dataset Comparability

Prior to the index calibration phase, data source compatibility testing demonstrated a high level of similarity between data collected by the NHDES and the NHFGD. Mean species richness across all impact categories was not significantly different for sites sampled by the NHDES (4.4), the FFF

(5.2), and the SAP (5.1) (Kruskal-Wallis, p=0.67). Species composition was also similar with the three calibration data sources sharing the same top four species in terms of their rank abundance (Table 7). Overall, for each data source, blacknose dace was the most abundant species and comprised between 28 and 37 percent of all individuals collected. For the FFF dataset, slimy sculpin (14%) and longnose dace (13%) were the next most abundant species. For the SAP dataset, longnose dace (13%) and fallfish (11%) were the next most abundant species. The relative abundances of the top three species for each respective dataset accounted for between 55 to 67 percent of the individuals captured.

Table 7. Relative abundance and rank of species for sites sampled by the New Hampshire Department of Environmental Services (DES) and two programs (SAP, FFF) by the New Hampshire Fish and Game Department (NHFGD).

Species	DES			NHFGD SAP			NHFGD FFF		
	Individuals	Percent	Rank	Individuals	Percent	Rank	Individuals	Percent	Rank
BND	2127	36.9%	1	1903	31.8%	1	1123	28.1%	1
BRB	89	1.5%	10	57	1.0%	13	57	1.4%	13
BT	19	0.3%	14	40	0.7%	14	11	0.3%	14
CC	26	0.5%	12	109	1.8%	11	128	3.2%	9
CS	254	4.4%	5	492	8.2%	5	224	5.6%	7
CWS	203	3.5%	7	227	3.8%	8	286	7.2%	5
EBT	484	8.4%	4	249	4.2%	7	300	7.5%	4
FF	250	4.3%	6	665	11.1%	3	264	6.6%	6
LC	130	2.3%	8	178	3.0%	9	76	1.9%	11
LND	1153	20.0%	2	795	13.3%	2	517	12.9%	3
LNS	124	2.2%	9	596	10.0%	4	129	3.2%	8
RT	34	0.6%	11	82	1.4%	12	126	3.2%	10
SS	552	9.6%	3	354	5.9%	6	575	14.4%	2
STS	26	0.5%	12	132	2.2%	10	76	1.9%	11

The mean total number of individuals collected per sampling event was significantly different among the data sources with mean abundances of 105, 162, and 99 individuals at DES, FFF, and SAP sites, respectively (Kruskal-Wallis, p=0.036). However, since the index development process did not include absolute abundance metrics in the calibration phase (see section 4.4 below), the significant differences that were observed were not considered to be problematic. The similarity in site species richness and composition were considered adequate for combining the data sources in all subsequent aspects of index development.

4.4 Biological Response Indicators

The performance of 72 candidate metrics was tested using the calibration dataset to determine those best suited to describe the condition of a transitional water fish assemblage. Of these, 28 (38.9%) had both a sufficient non-overlapping range (< 60 percent of impacted sites contained within the 25^{th} and 75^{th} percentiles of the reference distribution) and the correct expected response when reference and impacted sites were compared (Table 8). Metrics that displayed substantial overlapping ranges between reference and impacted sites or a did not have the correct stressor response were excluded from further consideration. Of the eight major metric categories, the richness, reproductive, and non-native groups failed to produce at least one metric to be carried forward into to subsequent phases of metric testing.

Table 8. Number of candidate metrics in each major category and number retained for additional testing.

Metric Category	# Candidate metrics	# Retained for testing	%
Non-native	4	0	0.0
Composition / Indicator taxa	18	7	38.9
Reproduction	4	0	0.0
Trophic	8	3	37.5
Richness	2	0	0.0
Streamflow preference	17	4	23.5
Thermal preference	9	6	66.7
Tolerance	10	8	80.0
TOTAL	72	28	38.9

Possible relationships between metrics and natural environmental gradients were investigated for the remaining 28 metrics. The environmental variables included latitude, longitude, elevation, drainage area. Overall, a total of 12 significant ($p<0.05$) linear regressions were detected between individual metrics and environmental variables out of 112 combinations (28 metrics x 4 environmental variables). The remaining 28 candidate metrics were most frequently related to a site's elevation and latitude (4 each) as compared to other potential environmental gradients – area (3) and longitude (1). Of the 12 instances where significant metric-environmental variable relationships were detected, the highest observed R^2 value was 0.39 indicating that less than 50 percent of the variation was explained by the environmental variable. Further, in all cases where significant regressions were detected, the 75 percent prediction intervals for the minimum and maximum metric value demonstrated substantial overlap. Thus, it was concluded that none of the metrics required adjustment to take into account natural influences by environmental variables.

Twenty-four of the remaining 28 candidate metrics (86 percent) indicated either significantly higher (positive-response metrics) or lower (negative-response metrics) metric values for reference sites when reference and impacted sites were compared (Mann-Whitney U Test; $p<0.05$) (Table 9). Metrics that did not have significantly different responses (Mann-Whitney U Test $p>0.05$) between reference and impacted sites were excluded from further consideration into the index. Significant Mann-Whitney U tests were coupled with four separate measures of the magnitude of separation between metric values for reference and impacted sites (Appendix C). A decision was made to carry forward those metrics with the highest ranking based on the Mann-Whitney U test Z-scores and / or the greatest number of correct responses based on the degree separation between reference and impacted sites within each of the metric groups. For the thermal and tolerance metric groups an additional metric was retained for redundancy testing because these groups had the greatest number of metrics pass the first phase of testing (Table 8).

In all twelve metrics were selected for further consideration; eight were positive response metrics and four were negative response metrics (Table 9). Each metric, except for the per_T_sp and ct_CW_sp metrics, either ranked first or second in its respective metric group based on the Mann-

Whitney U test or had four or more correct test responses. The per_T_sp metric was retained because it was the best performing negative response metric in the tolerance metric group that regularly occurred at both reference and impacted sites in relative abundances greater than 20 eprcent (Table 8). Metric redundancy proved to be minimal with only three of the sixty-six possible metric combinations having inter-metric Spearman correlation coefficients in excess of 0.75 (Appendix D). However, of the three candidate metrics selected within the thermal category, the per_CW_sp and per_CW metrics were near the correlation coefficient threshold with the per_T_sp (-0.72) and EBT_SS (0.72), respectively. For this reason, the ct_CW_sp metric was considered to be the best representative from the thermal category as it had much lower correlation coefficients with the eleven other candidate metrics.

Table 9. Results of candidate metric testing between reference and impacted sites. Rank = Mann-Whitney U test Z-score rank within major metric category. # Correct responses = result of Mann-Whitney U test, mean, median, percentile testing (see appendix C). Bolded metrics carried forward through redundancy testing.

METRIC	Type	Expected response	Mean (reference)	Mean (impacted)	Mann-Whitney U Test		Rank	# Correct Responses
					Significance	Z-score		
BND	composition	-	25.5	29.0	0.277	N/A	N/A	1
CS	composition	-	0.7	5.7	<0.001	-4.39	2	2
CWS	composition	-	1.6	9.2	0.003	-2.99	5	2
CC_CS_FF	**composition**	**-**	**2.6**	**18.7**	**<0.001**	**-4.35**	**3**	**4**
CC_CS_FF_BND	composition	-	28.1	47.7	<0.001	-3.50	4	2
EBT	composition	+	11.7	2.5	0.004	-2.86	6	4
EBT_SS	**composition**	**+**	**29.2**	**6.4**	**<0.001**	**-4.50**	**1**	**5**
per_no_lotic	streamflow	-	4.1	5.7	0.113	N/A	N/A	2
per_r	**streamflow**	**+**	**80.6**	**48.6**	**<0.001**	**-3.53**	**2**	**4**
per_r_x	streamflow	+	95.9	94.3	0.113	N/A	N/A	2
per_fs_ex_bnd	**streamflow**	**+**	**57.0**	**34.8**	**<0.001**	**-3.82**	**1**	**3**
per_et	thermal	-	51.8	74.6	0.001	-3.25	6	2
ct_et_sp	thermal	-	2.1	4.2	<0.001	-3.88	3	5
per_et_sp	thermal	-	43.5	71.5	<0.001	-3.79	4	3
per_CW	**thermal**	**+**	**47.8**	**17.3**	**<0.001**	**-3.90**	**2**	**4**
ct_CW_sp	**thermal**	**+**	**2.6**	**1.1**	**<0.001**	**-3.57**	**5**	**5**
per_cw_sp	**thermal**	**+**	**54.9**	**18.8**	**<0.001**	**-4.44**	**1**	**5**
per_M	tolerance	-	38.3	51.4	0.262	N/A	N/A	2
per_T	tolerance	-	28.1	41.7	0.016	-2.42	7	2
per_tol_GF	**tolerance**	**-**	**2.6**	**12.7**	**<0.001**	**-3.68**	**2**	**5**
per_M_sp	tolerance	-	39.0	49.0	0.011	-2.53	6	4
per_T_sp	**tolerance**	**-**	**25.7**	**38.9**	**0.004**	**-2.91**	**5**	**3**
per_I	**tolerance**	**+**	**33.6**	**6.9**	**<0.001**	**-4.55**	**1**	**5**
ct_I_sp	tolerance	+	1.7	0.7	0.001	-3.38	4	4
per_I_sp	tolerance	+	35.3	12.1	<0.001	-3.57	3	4
per_GF	**trophic**	**-**	**8.5**	**31.2**	**<0.001**	**-4.18**	**1**	**5**
ct_GF_sp	trophic	-	0.7	2.2	<0.001	-3.91	2	5
per_BI	**trophic**	**+**	**48.3**	**26.2**	**<0.001**	**-3.65**	**3**	**4**

Metrics with the most separation between reference and impacted sites as well as a low level of redundancy were selected for inclusion in the index. Metric selection was also based on the inclusion of as many of the major metric categories as possible in order to reflect a transitional water fish assemblage with a balanced, integrated, and adaptive aquatic community structure and composition.

With these requirements in mind, a set of seven metrics was selected for inclusion into the index (Table 10). All seven metrics had significantly different values between reference and impacted sites (Mann-Whitney U test) and displayed three or more out of five correct performance responses. An eighth metric was added to reflect the age class structure of book trout. While not tested concurrently with the candidate metrics, a decision was made to include at least one metric that reflected the reproductive success of an important indicator species of the transitional water fish assemblage. As a final check on the degree of separation between reference and impacted sites, box plots were constructed for the metrics selected for inclusion into the TWIBI (Figure 4).

Table 10. Final metrics, abbreviations, and metric category selected for inclusion into the TWIBI. Mean, minimum, and maximum for reference (n=31) and impacted sites (n=10) for the calibration dataset.

			Reference			Impacted		
Metric	**Abbreviation**	**Category**	**mean**	**min**	**max**	**mean**	**min**	**max**
Percentage of Book trout and slimy sculpin	EBT_SS	Composition /Indicator taxa	29.2	0.0	100.0	6.4	0	50.0
Percentage of creek chub, common shiner, and fall fish	CC_CS_FF	Composition /Indicator taxa	2.6	0.0	33.0	18.7	0	53.5
Percentage of fluvial specialists excluding blacknose dace	per_fs_ex_bnd	Composition /Indicator taxa	57.0	2.6	100.0	34.8	9.7	89.1
Number of coldwater species	ct_CW_sp	Thermal preference	2.6	0.0	5.0	1.1	0.0	4.0
Percentage of tolerant species	per_T_sp	Tolerance	25.7	0.0	50.0	38.9	12.5	50.0
Percentage of benthic insectivores	per_BI	Trophic	48.4	0.0	83.5	26.2	0	58.9
Percentage of generalist feeders	per_GF	Trophic	8.5	0	41.5	31.2	7.7	66.2
Brook trout class age structure	EBT_age_class	Reproduction	-----	-----	-----	-----	-----	-----

4.5 Metric and TWIBI scoring

Raw metric values were converted to a numeric score based on the IBI schema established by the VT DEC (VTDEC 2004). Each metric from an individual site was eligible for one of three scoring categories (1, 3, 5) depending on the raw metric result. Low metric scores were used to reflect poorer assemblage condition. Metric score categories and corresponding raw metric thresholds were established by examining the cumulative frequency distributions of reference and impacted sites. For all metrics, a clear separation between reference and impacted sites was observed (Figure 5). Natural breakpoints in line slope for either reference or impacted cumulative frequency distributions were useful as an investigatory tool in identifying proposed scoring thresholds for most metrics.

Figure 4. Box and whisker plots of TWIBI metrics for reference and impacted sites from the calibration dataset. Upper extent of box is 75th percentile. Lower extent of box is 25th percentile. Line inside box is median. Upper whisker = [1.5 x (75th – 25th percentile]+ 75th percentile. Lower whisker = [1.5 x (75th – 25th percentile] – 25th percentile. Circles (O) indicate outlier points (1.5-3x interquartile range).

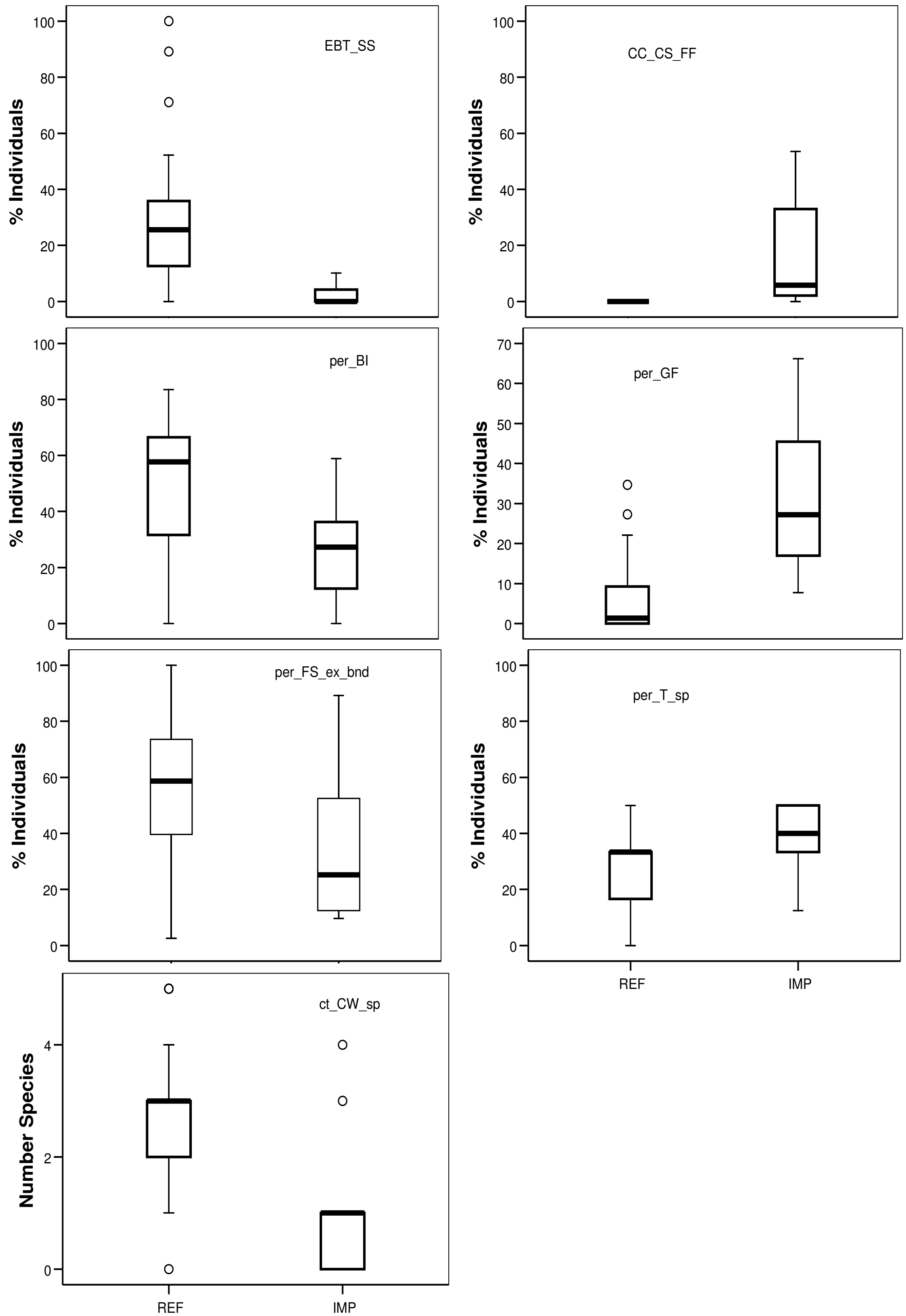
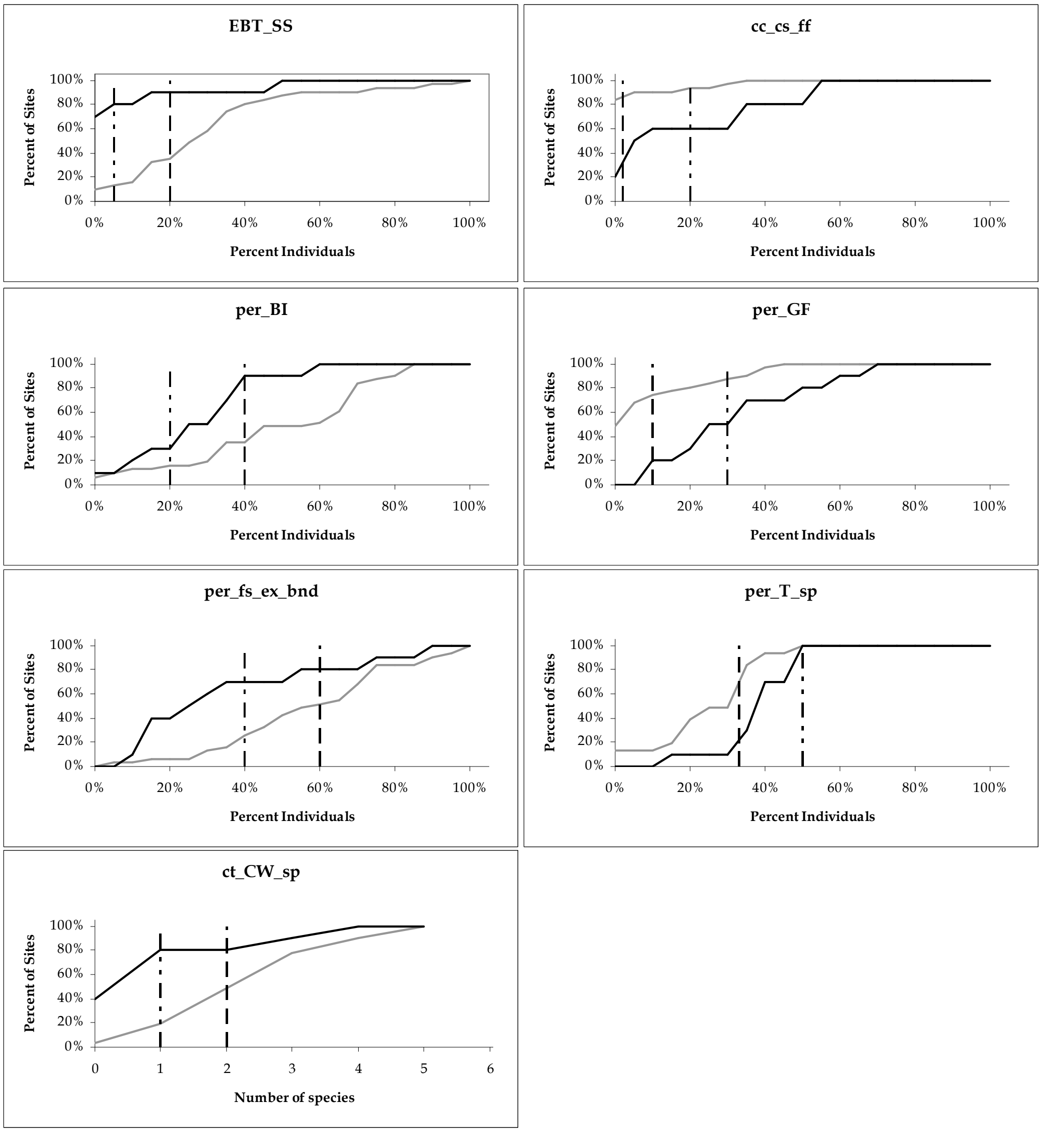


Figure 5. Cumulative frequency distributions of reference (grey lines) and impacted (black lines) sites from the calibration dataset and proposed scoring cutpoints. Long dashes = cut between 3 and 5 points. Short intermittent dashes = cut between 1 and 3 points.



For all metrics, a high percent of reference sites fell within the highest scoring category. For example, 65 percent of reference sites were within the highest scoring category for the percentage of benthic insectivore metric (per_BI), a positive response metric (Table 11). Conversely for the percentage of generalist feeders metric (per_GF metric), only 13 percent of reference sites were contained within the lowest scoring category. An attempt was made to include greater than 50 percent of reference sites and less than 20 percent of impacted sites in the highest scoring category. Logically, the proposed scoring thresholds generally also resulted in a much higher percentage of impacted sites in the lowest scoring category as compared to reference sites. The proposed scoring thresholds for each metric (Table 11) were designed to account for the raw analytical differences in the distribution of reference and impacted site data and reflect the associated structural and compositional responses of a transitional water fish assemblage to stressors.

Table 11. Proposed scoring cutpoints for TWIBI metrics including total number and percentage (in parentheses) of reference and impacted sites in each scoring category for the calibration dataset.

EBT_SS				CC_CS_FF			
Score	1	3	5	Score	1	3	5
Raw metric Threshold	<5%	5-20%	>20%	Raw metric Threshold	>20%	>2 -20%	</=2%
# Reference	4 (13)	7 (23)	20 (64)	# Reference	2 (6)	3 (10)	26 (84)
# Impaired	8 (10)	1 (10)	1 (10)	# Impaired	4 (40)	4 (40)	2 (20)
per_BI				**per_GF**			
Score	1	3	5	Score	1	3	5
Raw metric Threshold	<20%	20-40%	>40%	Raw metric Threshold	>30%	>10-30%	</=10%
# Reference	5 (16)	6 (19)	20 (65)	# Reference	4 (13)	4 (13)	23 (74)
# Impaired	3 (30)	6 (60)	1 (10)	# Impaired	5 (50)	3 (30)	2 (20)
per_fs_ex_bnd				**per_T_sp**			
Score	1	3	5	Score	1	3	5
Raw metric Threshold	<40%	40-60%	>60%	Raw metric Threshold	>/=50%	33-50%	<33%
# Reference	8 (26)	8 (26)	15 (48)	# Reference	2 (7)	14 (45)	15 (48)
# Impaired	7 (70)	1 (10)	2 (20)	# Impaired	3 (30)	6 (60)	1 (10)
ct_CW_sp				**EBT_age_class**			
Score	1	3	5	Score	1	3	5
Raw metric Threshold	0	1	>/=2	Raw metric Threshold	No YOY	YOY Only	YOY and Adult
# Reference	1 (3)	5 (16)	25 (81)	# Reference	15 (48)	1 (3)	15 (48)
# Impaired	4 (40)	4 (40)	2 (2)	# Impaired	7 (70)	1 (10)	2 (20)

For the brook trout age class metric (EBT_age_class), scoring categories mimicked those utilized in the CWIBI with one point assigned to sites where YOY are not captured, three points to sites where only YOY are captured, and five points to sites where both YOY and adults are captured. While brook trout were used exclusively in the development of the TWIBI, naturally occurring (not stocked) brown and rainbow trout may be substituted at sites where wild populations of brook trout are not observed. In addition, this flexibility was favored for future application of the TWIBI as successful reproduction of non-native salmonids still represents a positive indicator of assemblage condition. Further, the widespread introduction of these species occurred in the relative distant past (>100 years) and they have proliferated sporadically as naturalized species New Hampshire, especially in rivers and streams with larger drainages within applicable TWIBI areas.

Final TWIBI scores were computed by summing individual metric scores. The minimum score was seven and the maximum score was 40. TWIBI scores were significantly different across disturbance categories (Kruskal-Wallis Test; $\chi^2 = 33.04$, df = 3, $p<0.0001$) (Table 11). TWIBI scores were significantly different between all disturbance categories (Mann-Whitney U test, $p<0.01$) except for the moderate / impacted categorical comparison (Mann-Whitney U test, $p=0.45$) (Table 12).

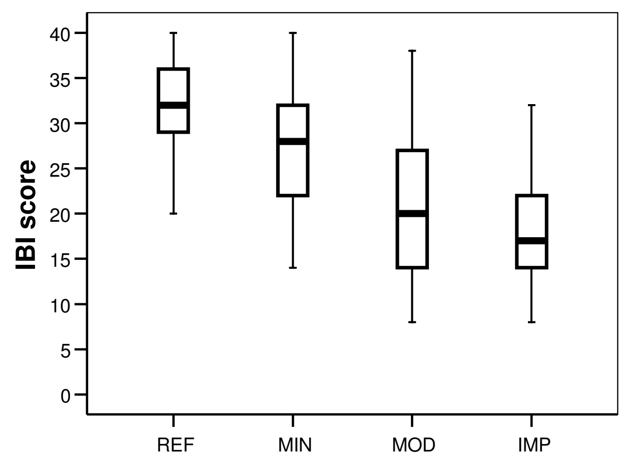
Table 12. TWIBI score disturbance category comparisons test results for calibration dataset.

Comparison	Test	Test Statistic	Significance
Overall	Kruskal-Wallis	$\chi2= 33.04$	p < 0.001
REF / MIN	Mann-Whitney U	Z = -2.69	p = 0.007
REF / MOD	Mann-Whitney U	Z = -4.75	p < 0.001
REF / IMP	Mann-Whitney U	Z = -4.06	p < 0.001
MIN / MOD	Mann-Whitney U	Z = -2.74	p = 0.006
MIN / IMP	Mann-Whitney U	Z = -2.94	p = 0.002
MOD / IMP	Mann-Whitney U	Z = -0.78	p = 0.445

The 25th percentile of reference sites was 5.5 index points higher than the 75th percentile for impacted sites (Figure 6). Mean reference and impacted site TWIBI scores were separated by 13.2 index points. Only one (10 percent) impacted site scored above the 25th percentile of reference sites and three (10 percent) of the reference sites scored below the 75th percentile of impacted sites.

Figure 6. TWIBI scoring summary for sites within each disturbance category (REF = Reference; MIN = minimum; MOD = moderate; IMP = Impacted) for the calibration dataset. Box and whisker plot - Upper extent of box is 75th percentile. Lower extent of box is 25th percentile. Upper whisker = [1.5 x (75th – 25th percentile]+ 75th percentile. Lower whisker = [1.5 x (75th – 25th percentile] – 25th percentile.

TYPE	N	Mean	Median	Percentiles			
				5	25	75	95
REF	31	31.6	32.0	21.2	28.0	36.0	40.0
MIN	27	27.0	28.0	14.8	22.0	32.0	40.0
MOD	31	21.0	20.0	9.2	14.0	30.0	35.6
IMP	10	18.4	17.0	8.0	14.0	22.5	32.0



4.6 IBI threshold determination

A pass-fail threshold for ALU attainment status (full support / non-support) was identified using TWIBI scores from the calibration dataset. As with previous biotic condition index thresholds established by the NHDES, the 25th percentile of the reference site index scores was utilized. With a proposed pass-fail threshold of 28, 26 of 31 (84 percent) reference sites exceeded the criterion, while 9 of 10 (90 percent) of impacted sites failed to achieve the criterion. Contingency tables indicated that the distribution of reference and test sites exceeding and failing to achieve the proposed criterion were significantly different (Table 13; χ^2; p<0.001).

Table 13. Observed and expected frequency of TWIBI threshold attainment (# above; equal to or above proposed criterion) and non-attainment (#below; below criterion) for reference and impacted sites from the calibration dataset. Chi-square critical value in parentheses (p=0.0001, df=1).

<table>
<tr><th>Site type</th><th></th><th># above</th><th># below</th><th>Total</th><th>Chi_square</th></tr>
<tr><td rowspan="2">Reference</td><td># observed</td><td>26</td><td>5</td><td rowspan="2">31</td><td rowspan="5">18.3
(10.828)</td></tr>
<tr><td># expected</td><td>20</td><td>11</td></tr>
<tr><td rowspan="2">Impacted</td><td># observed</td><td>1</td><td>9</td><td rowspan="2">10</td></tr>
<tr><td># expected</td><td>7</td><td>3</td></tr>
<tr><td></td><td>Total</td><td>27</td><td>14</td><td>41</td></tr>
</table>

For ease of communication, narrative categories were assigned based on the distribution of reference sites scores. Sites scoring in 36 or better received an "excellent" rating, sites scoring between 28 to 35 received a "good" rating, sites scoring between 22 to 28 received a "fair" rating, and sites scoring less than 22 received a "poor" rating. The narrative category ranges were based on the 25th and 75th percentiles (Figure 6) and are designed to discriminate, in simple terms only, the range of biotic conditions observed in transitional water fish assemblages. For the calibration dataset, these narrative ratings resulted in 15, 34, 18, and 32 sites being placed in the excellent, good, fair, and poor categories, respectively.

4.7 Validation Testing

A total of 36 sites were retained from the TWIBI calibration phase for the purpose of validating the performance of the index. An initial check of dataset comparability determined that the environmental characteristics were similar between datasets (Mann-Whitney U test; all comparisons, $p>0.05$). Similarly, raw values for the selected metrics did not differ between datasets (Mann-Whitney U test; all comparisons, $p>0.05$). Mean, median, and percentile comparisons confirmed Mann-Whitney U test results with only small differences observed in the environmental characteristics and raw metric values between the calibration and validation datasets (Tables 14 and 15).

Table 14. Environmental variable characteristics of sites included in the calibration and validation datasets.

DATASET	Env. Variable	N	Mean	Median	Percentiles			
					5	25	75	95
CALIBRATION	Latitude	99	43.9995	43.8562	42.9723	43.4399	44.5235	45.0924
	Longitude		71.5380	71.4933	71.0714	71.2306	71.7144	72.3301
	Elevation (ft)		929	924	412	604	1256	1579
	Area (sq. mi.)		24.7	22.5	2.2	7.9	37.8	58.9
VALIDATION	Latitude	36	44.0393	44.2668	42.9082	43.3267	44.6154	45.1760
	Longitude		71.6061	71.5179	71.1976	71.3873	71.8426	72.2855
	Elevation (ft)		986	1006	462	680	1296	1479
	Area (sq. mi.)		27.8	22.5	2.1	6.8	41.4	70.9

Table 15. Raw metric value distributions for sites included in the calibration and validation datasets.

DATASET	Metric	N	Mean	Median	Percentiles			
					5	25	75	95
CALIBRATION	ct_CW_sp	99	1.9	2.0	0.0	1.0	3.0	5.0
	per_BI		32.9	31.5	0.0	6.5	58.9	79.5
	per_GF		19.0	10.3	0.0	0.0	32.9	68.1
	per_fs_ex_bnd		47.1	46.0	5.1	25.6	70.0	90.8
	per_T_sp		32.0	33.3	0.0	20.0	40.0	60.0
	EBT_SS		20.6	12.3	0.0	0.0	30.8	73.1
	CC_CS_FF		11.0	0.0	0.0	0.0	15.1	50.2
VALIDATION	ct_CW_sp	36	2.0	2.0	0.0	1.0	3.0	5.0
	per_BI		33.7	38.6	0.0	11.0	53.1	74.4
	per_GF		21.3	15.5	0.0	0.0	37.1	73.4
	per_fs_ex_bnd		43.4	42.6	2.8	18.3	60.1	89.6
	per_T_sp		30.4	25.0	0.0	25.0	40.5	61.0
	EBT_SS		19.8	13.3	0.0	1.8	36.2	76.5
	CC_CS_FF		12.6	8.2	0.0	0.0	20.6	46.9

TWIBI scores for the validation dataset were significantly different across disturbance categories (Kruskal-Wallis Test; $\chi^2 = 11.87$, df = 3; p<0.008) (Table 16). TWIBI scores were significantly different between the reference / minimal disturbance and reference / impacted categories (Mann-Whitney U test, p<0.03) (Table 16). Overall, TWIBI scores for the validation dataset demonstrated fewer significant differences between disturbance categories than the calibration dataset (2 of 6 – validation; 5 of 6 – calibration).

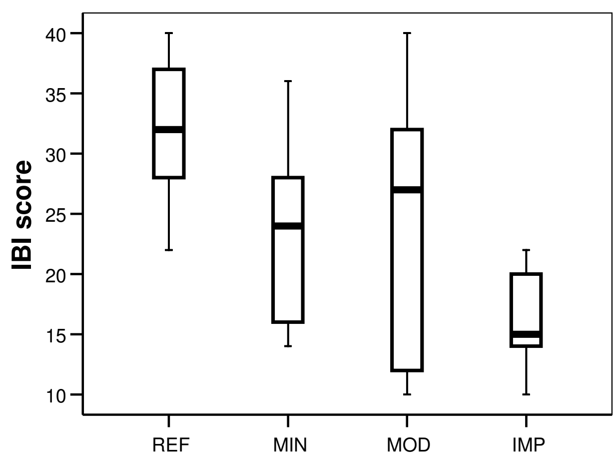
Table 16. TWIBI score disturbance category comparisons test results for validation dataset.

Comparison	Test	Test Statistic	Significance
Overall	Kruskal-Wallis	$\chi2= 11.87$	p = 0.008
REF / MIN	Mann-Whitney	Z = -2.25	p = 0.025
REF / MOD	Mann-Whitney	Z = -1.64	p = 0.114
REF / IMP	Mann-Whitney	Z = -3.28	p < 0.001
MIN / MOD	Mann-Whitney	Z = -0.08	p = 0.968
MIN / IMP	Mann-Whitney	Z = -1.91	p = 0.066
MOD / IMP	Mann-Whitney	Z = -1.37	p = 0.181

The 25th percentile of reference sites was 7.5 index points higher than for the 75th percentile of impacted sites (Figure 7). Mean reference and impacted site TWIBI scores were separated by 16.4 index points. None of the impacted sites scored above the 25th percentile of reference sites and none of the reference sites scored below the 75th percentile of impacted sites.

Figure 7. TWIBI scoring summary for sites within each disturbance category (REF = Reference; MIN = minimum; MOD = moderate; IMP = Impacted) for the validation dataset. . Box and whisker plot - Upper extent of box is 75th percentile. Lower extent of box is 25th percentile. Upper whisker = [1.5 x (75th – 25th percentile]+ 75th percentile. Lower whisker = [1.5 x (75th – 25th percentile] – 25th percentile.

TYPE	N	Mean	Median	Percentiles			
				5	25	75	95
REF	11	32.4	32.0	22.0	28.0	38.0	40.0
MIN	9	23.8	24.0	14.0	15.0	31.0	36.0
MOD	10	24.6	27.0	10.0	12.0	32.5	40.0
IMP	6	16.0	15.0	10.0	13.0	20.5	22.0



With the proposed pass-fail threshold of 28, 9 of 11 (82 percent) reference sites exceeded the criterion, while 3 of 6 (50 percent) of impacted sites failed to achieve the criterion. Contingency tables indicated that the distribution of reference and test sites exceeding and failing to achieve the proposed criterion were significantly different (Table 17; χ^2; p<0.005).

Table 17. Observed and expected frequency of TWIBI threshold attainment (# above; equal to or above proposed criterion) and non-attainment (# below; below criterion) for reference and impacted sites from the calibration dataset. Chi-square critical value in parentheses (p=0.0001, df=1)

Site type		# above	# below	Total	Chi_square
Reference	# observed	9	2	11	10.4 (7.879)
	# expected	6	5		
Impacted	# observed	0	6	6	
	# expected	3	3		
	Total	9	8	17	

5. SUMMARY AND RECOMMENDATIONS

The analysis of fish species relative abundance and frequency of occurrence from 135 wadeable streams in New Hampshire indicated that the definition of a distinct fish assemblage type, termed transitional water, is warranted. Transitional water fish assemblages in New Hampshire were closely allied to the previously identified strict coldwater fish assemblage (NHDES 2007a) in that they shared several of the most frequently encountered species (e.g. brook trout, slimy sculpin, blacknose dace). However, transitional water fish assemblages were found to have higher species richness (mean = 4.6) than strict coldwater fish assemblages (mean = 2.8) and frequently contained additional species not commonly encountered in strict coldwater environments (e.g. burbot, longnose dace, longnose sucker). Lyons et al. (2009) reported a similar finding with coolwater streams having approximately 1.2 times the species richness as coldwater streams in Michigan and Wisconsin. Yet these streams overlapped in their overall species composition with coldwater streams. However, unlike the coolwater streams identified by Lyons et al. (2009), which also shared the characteristics of warmwater streams, transitional water streams in New Hampshire more closely resembled coldwater streams with warmwater species only occasionally encountered in reference and minimally disturbed systems.

The observed difference between New Hampshire transitional water fish assemblages and the coolwater assemblages reported by Lyons et al. (2009) were a result in the approaches to define these communities. In this report, New Hampshire transitional water assemblages were defined as streams contained in areas expected to support coldwater fish species throughout the year based on a predictive model (NHDES 2007b), yet not part of the areas where the strict coldwater index of biotic integrity (CWIBI) was deemed applicable (NHDES 2007a). In contrast, Lyons et al. (2009) used species specific laboratory temperature preferences and field studies to define coolwater assemblages which included both coldwater and warmwater species.

Transitional water fish assemblages are capable of occurring statewide, yet their expected area of occurrence is focused in the central and northern sections of New Hampshire. The expected area of occurrence is dependent on a stream's longitude, latitude, drainage area, and to a lesser extent elevation. On average, transitional water rivers and streams had a drainage area 4.4 times the size of coldwater streams. Overall, the expected area of occurrence for transitional water fish assemblages represents approximately 18 percent of New Hampshire's land area with strict coldwater and warmwater fish assemblages expected to occur within 48 and 34 percent, respectively. In Michigan and Wisconsin, Lyons et al. (2009) reported that nearly 65 percent of the stream miles were expected to support coolwater fish assemblages. Thus, relative to Michigan and Wisconsin and in terms of the proportion of land area in New Hampshire, transitional water fish assemblages in New Hampshire are a relatively uncommon natural occurrence.

Streams and rivers where the TWIBI is the most applicable fish condition index will depend on a site's latitude, longitude, upstream drainage area, and elevation. However, because distinct boundaries in biological assemblages rarely exist, there may be instances when best professional judgement must be used before making a final decision of the most appropriate fish condition index to be applied in making an ALU determination. In particular, special attention will be paid to sites where the upstream drainage area is less than 15 square miles. As a general rule for these sites, when the natural species richness is equal to or less than 4 species, and one or more of these species includes naturally occurring salmonids or slimy sculpin, the CWIBI may be exchanged for the TWIBI. Conversely, some streams and rivers where transitional water fish assemblages are expected to occur, may be more appropriately assessed using a warmwater fish assemblage condition index. Examples would include flowing waters below natural impoundments, such as a wetland, or larger streams (>50 square miles) where the natural thermal regimes are too warm to support coldwater species. The exceptions outlined above will not apply to sites where apparent shifts in the fish assemblage are potentially linked to anthropogenic impacts.

An eight metric condition index proved useful in discriminating between reference and presumed impacted sites with overall index scores displaying an inverse relationship to the level of human disturbance. The selection of eight metrics was within the range of previously developed fish IBIs (Leonard and Orth 1986; Lyons et al. 1996; Langdon 2001; Daniels et al. 2002; Hughes et al. 2004; Whittier et al. 2007,), yet lower than the classic biotic index developed by Karr (1981). A predetermined number of metrics was not targeted prior to index development; rather the number included in the index was based on performance and redundancy testing for individual metrics. Overall, metrics associated with thermal preference, tolerance, and trophic class were most successful at differentiating between reference and impacted sites.

Unlike many previous IBIs (Leonard and Orth 1986; Lyons et al. 1996; Langdon 2001; Daniels et al. 2002), but similar to Whittier et al. (2007) overall species richness did not prove useful in discriminating between reference and impacted sites. The exclusion of overall richness as a metric in the TWIBI for New Hampshire was, in part, believed to be a reflection of the naturally low fish species diversity statewide. In addition, transitional waters, as defined above, represent streams and rivers with coldwater thermal regimes, and in turn, may serve as a natural restriction in the ability of warmwater species to thrive in these environments, thus further restricting a finite pool of fish species.

Similar to the CWIBI, a brook trout age class metric was included in the TWIBI to reflect the level of reproductive success by an important native, top carnivore, gamefish species. Based on the results, 48 percent of reference sites and only 20 percent of impacted sites had both adult and YOY brook trout, respectively. Unlike the other seven metrics included in the index, the brook trout age class metric is based only on presence or absence, rather than a percentage of species or individuals within a particular group. The presence of naturally occurring adults and young-of-year (YOY) was considered important in preserving the viability of this important indicator species, while the presence of just YOY was given an intermediate score, and lack of YOY was given the lowest score.

In recognition that non-native salmonids (brown and rainbow trout) naturally occur, on occasion, in transitional water fish assemblages, these species should be included in conjunction with brook trout when computing the TWIBI for all applicable metrics (per_fs_ex_bnd, ct_CW_sp) except the EBT_SS and brook trout age class metric. For the EBT_SS metric, brown and rainbow trout are to be excluded without exception. For the brook trout age class metric, brown and rainbow trout may be included in metric computation when brook trout are absent. The decision to allow the limited inclusion of non-native salmonids in the TWIBI reflects past fishery management actions which included the widespread stocking of these species, especially in suspected coldwater streams and rivers having larger drainage areas. In many cases, for waters where temperatures remained cold enough annually, these species established naturally reproducing populations and have proliferated. As a result, their presence represents a positive indicator of biological condition and should be reflected in the overall index score. However, the inclusion of recently stocked individuals is not permitted for any salmonid species. This includes brook trout and Atlantic salmon. These actions reflect recent fishery management decisions and not a natural ecological consequence of environmental conditions (Halliwell et al. 1999).

The index, as constructed, represents one that minimizes inter-metric redundancy and maximizes efficiency. None of the metrics included in the TWIBI had a correlation coefficient in excess of 0.75. The lack of metric redundancy indicates that each component of the index represents a unique expression of the ecological characteristics of the fish assemblage. Further, the individual metrics selected for inclusion into the index proved to be responsive to increases in environmental stressors based on the narrative impact rating categories. Of the eight metrics included in the index, each was able to clearly separate reference and impacted sites and was among the strongest indicators in doing so based on an objective testing process. While this process differs from that employed by Whittier et al. (2007), both attempt to achieve the same result; namely the selection of metrics, across broad ecological categories, that combine to represent the important qualities of an minimally impacted biological community and capable of detecting a departure from this condition.

Overall the TWIBI developed for New Hampshire streams bears some resemblance to the mixed waters index used by the Vermont Department of Environmental Conservation (VTDEC 2007) in terms of the total number of metrics (NH – eight, VT – nine), individual metrics, and index threshold (NH – 28, VT – 30). The similarity of these two indices is partially a reflection of fish assemblage similarity. With the exception of the Champlain drainage in Vermont, New Hampshire and Vermont share many of the same fish species. In both states, water temperature, ultimately, represents the primary natural environmental factor that structures fish assemblages. Elevation and watershed size, while important in structuring fish assemblages, are more appropriately considered proximal variables that influence water temperature. Thus, where the thermal regimes are similar, the resulting native fish assemblages in Vermont and New Hampshire streams are likely to be composed of many of the same species or of species filling similar ecological niches. In turn, while separate indices have been developed by their respective state agencies, they are likely transferable across state lines, and, more importantly, the results can be compared. Furthermore, it does not seem implausible that either of these indices could be applied throughout the New England states with minor adjustments where similar thermal regimes can be identified. Daniels et al. (2002) recommended similar a similar application of his Mid-Atlantic Slope IBI with modification in order to account for the natural ecosystem features and study objectives.

The recommended index threshold of 28 was based on the twenty-fifth percentile of all reference sites and corresponds to previously developed biological indices for fish and macroinvertebrates in New Hampshire. Hughes et al. 2004 provided examples of how manipulating threshold criteria can lead to varying amounts of stream miles considered to be impaired. Without a doubt the selection of any statistical threshold (i.e., x-percentile, # standard deviations) is a subjective decision that implies a level of confidence in the index's performance, natural variability, sampling efficiency, and an acceptable reduction in biological condition. For the TWIBI, and other biological indices developed by the NHDES, it is believed that a twenty-fifth percentile threshold is acceptable for the determination of aquatic life use. A lower or higher threshold would likely be under- or overprotective of the resource, respectively. Thus, the selection of this threshold is an attempt to balance an acceptable biological condition while concurrently taking into account largely uncontrollable sources of index variability such as sampling effectiveness, unmeasured components of ecosystem health (i.e. trophic dynamics), and regional environmental impacts.

Mean index scores from the calibration dataset were 32 for reference sites and 18 for impacted sites. Based on these results, and in conjunction with those observed from the validation dataset, it can be concluded that the index was capable of clearly distinguishing changes in fish assemblage structure and function as the level of disturbance increased. The selection of the 25th percentile of reference site index scores as a criterion translated into 9 of 10 impacted sites from the calibration dataset failing to achieve the threshold of 28. Overall, the threshold chosen for the TWIBI was determined to be appropriate in defining an acceptable versus unacceptable level of departure from the "natural" condition. However, as with any biological index, an "attainment" threshold is a human-imposed decision criterion along a gradient of ecological structure and function. As a result, a single numeric representation of overall assemblage condition should be considered in concert with the actual raw data when making final impairment or regulatory decisions.

The TWIBI establishes a proposed set of guidelines to define a unique fish assemblage, a suite of metrics to measure biological condition, and a criterion to determine the level of departure from minimally impacted sites. These guidelines, measures, and associated thresholds are, however, based on current environmental conditions. In evaluating the data, geographically widespread unnatural perturbations to these conditions include regional and global impacts such as acid deposition and climate change, respectively. The effects of these impacts are difficult, if not impossible, to account for, and therefore, should be considered as unknown elements that may have contributed to the geographic boundaries of the transitional water fish assemblage defined herein, as well as metric selection and threshold determination. Further, as these impacts are likely to continue, and perhaps worsen, modifications to the index will be necessary to account for changes in natural fish distributions, assemblage structure and function, and expectations in biological condition.

The TWIBI will serve as a partial numeric interpretation of the NHDES's current narrative water quality criteria relating to the biological integrity (Env - Wq 1703.19) of aquatic communities for 1st through 4th order wadeable streams meeting the definition of a transitional water fish assemblage. The index is designed to accurately and precisely describe the biological condition of this assemblage type through eight unique ecological measures (metrics). Other indices, such as the NHDES' benthic IBI, or physical and chemical water quality measures may be coupled with the TWIBI for the determination of aquatic life use and used in completing federally-required water quality reports, state-level regulatory actions, permit limits, and general water quality planning activities.

6. REFERENCES

Bain, M. B. 1996. Fish codes and classes: File documentation, sources, and notes. New York Cooperative Fish and Wildlife Research Unit, Cornell University, Ithaca, NY.

Balon, E. K. 1975. Reproductive guilds of fishes: a proposal and definition. Journal of the Fisheries Research Board of Canada 32:821-864.

Barbour, M. T, J. B. Stribling, and J. R. Karr. 1995. Multimetric approach for establishing biocriteria and measuring biological condition. Pages 63-77 *in* W. S. Davis and T. P. Simon, editor. Biological assessment and criteria: Tools for water resource planning and decision making. CRC Press, Boca Raton, Florida.

Barbour, M. T., J. Gerristen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, second edition. EPA 841-B-99-002. U. S. Environmental Protection Agency; Office of Water; Washington, D.C.

Daniels, R. A., K. Riva-Murrary, D.B. Halliwell, D.L. Vana-Miller, M.D. Bilger. 2002. An index of biological integrity for northern mid-Atlantic slope drainages. Transactions of the American Fisheries Society131:1044-1060.

Halliwell, D. B., R. W. Langdon, R. A. Daniels, J. P. Kurtenbach, and R. A. Jacobson. 1999. Classification of freshwater fish species of the Northeastern United States for use in the development of indices of biological integrity, with regional applications. Pages 301-333 *in* T. P. Simon, editor. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, Florida.

Hughes, R. M., S. Howlin, and P. R. Kaufmann. 2004. A biointegrity index (IBI) for coldwater streams of western Oregon and Washington. Transactions of the American Fisheries Society 133:1497-1515.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:28-30.

Mundahl, N. D. and T. P. Simon. 1999. Development and application of an index of biotic integrity for coldwater streams of the upper Midwestern United States. Pages 383-411 *in* T. P. Simon, editor. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, Florida.

NHDES. 2007a. Coldwater fish assemblage index of biotic integrity for New Hampshire wadeable streams. New Hampshire Department of Environmental Services, Concord, NH. Publication #R-WD-07-33.

NHDES. 2007b. Predicted coldwater fish indicator species presence in New Hampshire wadeable streams. New Hampshire Department of Environmental Services, Concord, NH. Publication #R-WD-07-38.

Langdon, R. W. 2001. A preliminary index of biological integrity for fish assemblages of small coldwater streams in Vermont. Northeastern Naturalist 8:219:232.

Leonard, P. M. and D. J. Orth. 1986. Application and testing of an index of biotic integrity in small, coolwater streams. Transactions of the American Fisheries Society 115:401-414.

Lyons, J., L. Wang, and T. D. Simonson. 1996. Development and validation of an index of biotic integrity for coldwater streams in Wisconsin. North American Journal of Fisheries Management 16:241-256.

Lyons, J., T. Zorn, J. Stewart, P. Seelbach, K. Wehrly, and L. Wang. 2009. Defining and characterizing coolwater streams and their fish assemblages in Michigan and Wisconsin, USA. North American Journal of Fisheries Management 29:1130-1151.

Simon, T. P. 1999. Assessment of Balon's reproductive guilds with application to Midwestern North American freshwater fishes. Pages 97-121 *in* T. P. Simon, editor. Assessing the sustainability and biological integrity of water resources using fish communities. CRC Press, Boca Raton, Florida.

Stoddard, J. L., D. P. Larsen, C. P. Hawkins, R. K. Johnson, and R. H. Norris. 2006. Setting expectations for the ecological condition of streams: The concept of reference condition. Ecological Applications 16:1267-1276.

VT DEC. 2004. Biocriteria for fish and macroinvertebrate assemblages in Vermont wadeable streams and rivers – Development phase. Agency report. Water Quality Division, Biomonitoring and Aquatic Studies Section, Waterbury, Vermont.

Whittier, T. R., R. M. Hughes, J. L. Stoddard, G. A. Lomnicky, D. V. Peck, and A. T. Herlihy. 2007. A structured approach for developing indices of biotic integrity: Three examples from streams and rivers in the western USA. Transactions of the American Fisheries Society 136:718-735.

Appendix A. Candidate metrics and their respective abbreviation, expected response organized by major category.

Metric	Abbreviation	Expected response	Metric group	Variable type
Percent introduced generalist feeder individuals	per_intro_GF	-	aliens	continuous
Percent introduced top carnivore individuals	per_intro_TC	-	aliens	continuous
Percent introduced warmwater individuals	per_intro_WW	-	aliens	continuous
Perccent introduced macrohabitat generalists	per_intro_mg	-	aliens	continuous
Number of Cyprinid species	num_cyp_sp	-	Composition	discrete
Percentage of Cyprinid species	per_cyp_sp	-	Composition	continuous
Percentage of the dominant species	per_dom_sp	-	Composition	continuous
Percentage of Blacknose dace	BND	-	Composition	continuous
Percentage of Burbot	BRB	+	Composition	continuous
Percentage of Brown trout	BT	+	Composition	continuous
Percentage of Creek chub	CC	-	Composition	continuous
Percentage of Common shiners	CS	-	Composition	continuous
Percentage of White suckers	CWS	-	Composition	continuous
Percentage of Brook trout	EBT	+	Composition	continuous
Percentage of Fallfish	FF	-	Composition	continuous
Percentage of Longnose dace	LND	+	Composition	continuous
Percentage of Longnose suckers	LNS	+	Composition	continuous
Percentage of Rainbow trout	RT	+	Composition	continuous
Percentage of Slimy sculpin	SS	+	Composition	continuous
Percentage of Brook trout and Slimy sculpin	EBT_SS	+	Composition	continuous
Percentage of Creek chub, Common shiner, Fallfish	CC_CS_FF	-	Composition	continuous
Percentage of Creek chub, Common shiner, Fallfish, Blacknose dace	CC_CS_FF_BND	-	Composition	continuous
Percentage of speleophil spawners (hole nesters) (Balon 1975 - B.2.7)	per_hol_dig		reproduction	continuous
Percentage of non-obligate substrate spawners (Balon 1975 - A.1.4)	per_nob_sub		reproduction	continuous
Percentage of polyphil spawners (nest builders on misc. materail (Balon 1975 - B.2.2)	per_nst_sub		reproduction	continuous
Percentage of phytophil spawners (obligatory plant spawners) (Balon 1975 - A.1.5)	per_ob_plt		reproduction	continuous
Percentage of simple lithophilic spawner individuals (non-guarding rock/gravel spawners) (Balon 1975 - A.1.2 & A.1.3)	per_simp_litho	+	reproduction	continuous
Percentage of specialized lithophilic spawner individuals (guarding rock/gravel spawners) (Balon 1975 - B.1.3)	per_spec_litho	+	reproduction	continuous
Number of fluvial specialist species (Based on Bain 1996)	num_fs_sp	+	richness	discrete
Number of fluvial dependant species (Based on Bain 1996)	num_fd_sp	+	richness	discrete
Number of fluvial specialist + dependant species (Based on Bain 1996)	num_fs_fd_sp	+	richness	discrete
Total number of species	num_sp_all	+	richness	discrete
Total number of native (non-introduced) species	num_sp_nat	+	richness	discrete
Number of macrohabitat generalist species (Based on Bain 1996)	num_mg_sp	-	richness	discrete
Percentage of fluvial dependant + specialist species (Based on Bain 1996)	per_fd_fs_sp	+	streamflow	continuous
Percentage of macrohabitat generalist species (Based on Bain 1996)	per_mg_sp	-	streamflow	continuous
Percentage of fluvial specialist individuals less blacknose dace	per_fs_ex_bnd	+	streamflow	continuous
Percentage of rheophilic individuals (Based on Whittier et al. 2007)	per_r	+	streamflow	continuous
Percentage of flowing water preferring individuals (Based on Whittier et al. 2007)	per_x	+	streamflow	continuous

Appendix A (con't).

Metric	Abbreviation	Expected response	Metric group	Variable type
Percentage of rheophilic + flowing water preferring individuals (Based on Whittier et al. 2007)	per_r_x	+	streamflow	Continuous
Percentage of non-lotic individuals	per_no_lotic	-	streamflow	continuous
Percentage of fluvial dependant individuals (Based on Bain 1996)	per_fd	+	streamflow	continuous
Percentage of fluvial specialist individuals (Based on Bain 1996)	per_fs	+	streamflow	continuous
Percentage of macrohabitat generalist individuals (Based on Bain 1996)	per_mg	-	streamflow	continuous
Percentage of fluvial dependant + specialist individuals (Based on Bain 1996)	per_fd_fs	+	streamflow	continuous
Percentage of coldwater species	per_cw_sp	+	thermal	continuous
Percentage of eurythermal species	per_et_sp	-	thermal	continuous
Percentage of warmwater species	per_ww_sp	-	thermal	continuous
Percentage of coldwater individuals	per_CW	+	thermal	continuous
Number of coldwater species	num_CW	+	thermal	discrete
Percentage of warmwater individuals	per_WW	-	thermal	continuous
Number of warmwater species	num_WW	-	thermal	discrete
Percentage of eurythermal individuals	per_ET	-	thermal	continuous
Number of eurythermal species	num_ET	-	thermal	discrete
Percentage of intolerant species	per_I_sp	+	tolerance	continuous
Percentage of moderately tolerant species	per_M_sp	-	tolerance	continuous
Percentage of tolerent species	per_T_sp	-	tolerance	continuous
Percentage of intolerant individuals	per_I	+	tolerance	continuous
Number of intolerant species	num_I	+	tolerance	discrete
Percentage of moderately tolerant individuals	per_M	-	tolerance	continuous
Number of moderately tolerant species	num_M	-	tolerance	discrete
Percentage of tolerant individuals	per_T	-	tolerance	continuous
Number of tolerant species	num_T	-	tolerance	discrete
Percentage of tolerant generalist feeder individuals	per_tol_GF	-	trophic	continuous
Percentage of benthic insectivore individuals	per_BI	+	trophic	continuous
Percentage of generalist feeder individuals	per_GF	-	trophic	continuous
Percentage of obligate insectivore individuals	per_OI	+	trophic	continuous
Pecentage of top carnivore individuals	per_TC	+	trophic	continuous
Number of benthic insectivore species	ct_BI_sp	+	trophic	discrete
Number of generalist feeder species	ct_GF_sp	-	trophic	discrete
Number of obligate insectivore species	ct_OI_sp	+	trophic	discrete
Number of top carnivore species	ct_TC_sp	+	trophic	discrete

Appendix B. Autecological fish characteristics.

Origin		Thermal Preference		Tolerance		Reproductive strategy[1]	
Abbreviation	*Type*	*Abbreviation*	*Type*	*Abbreviation*	*Type*	*Abbreviation*	*Type*
N	Native	CW	Coldwater	I	Intolerant	S_L	Simple Lithophil (coarse substrate spawners, non-guarders)
I	Introduced	ET	Eurythermal	M	Moderately Tolerant	H_D	Hole nester
		WW	Warmwater	T	Tolerant		

Trophic Class		Streamflow Preference[2]		Streamflow Preference[3]	
Abbreviation	*Type*	*Abbreviation*	*Type*	*Abbreviation*	*Type*
BI	Benthic insectivore	L	Prefers large rivers	fd	Fluvial dependant
GF	Generalist feeder	R	Rheophilic – prefers fast flowing waters	fs	Fluvial specialist
OI	Obligate insectivore	X	Prefers flowing waters	mg	Macrohabitat generalist
TC	Top carnivore				

1- Simon 1999; based on Balon 1975
2- Whittier et al. 2007
3- Bain 1996

Appendix C. Details of objective metric testing combinations for partial determination of metrics selected for inclusion into the TWIBI.

For positive (+) response metrics:

Combination	Compare	Mathematical Expression	Response Evaluation	Expected Response
1	Means	Mean (Reference sites) – Mean (Impacted sites)	Sign (+ / -) and magnitude	+
2	Medians	Median (Reference sites) – Median (Impacted sites)		
3	25th vs. 75th percentiles	25th percentile (Reference sites) – 75th percentile (Impacted Sites		
4	Mean vs. 75th percentile	Mean (Reference sites) – 75th percentile (Impacted sites)		
5	Median vs. 75th percentile	Median (Reference sites) – 75th percentile (Impacted sites)		

For negative (-) response metrics:

Combination	Compare	Mathematical Expression	Response Evaluation	Expected Response
1	Means	Mean (Impacted sites) – Mean (Reference sites)	Sign (+ / -) and magnitude	+
2	Medians	Median (Impacted sites) – Median (Reference sites)		
3	25th vs. 75th percentiles	25th percentile (Impacted sites) – 75th percentile (Reference Sites		
4	Mean vs. 75th percentile	Mean (Impacted sites) – 75th percentile (Reference sites)		
5	Median vs. 75th percentile	Median (Impacted sites) – 75th percentile (Reference sites)		

Appendix D. Spearman's correlation coefficients for 20 candidate metrics. Bolded text indicates metrics included in the TWIBI. Grey shaded cells indicate correlation coefficients >0.75.

METRIC↓→		**EBT_SS**	**CC_CS_FF**	per_r	**per_fs_ex_bnd**	per_cw_sp	**ct_CW_sp**	per_CW	per_I	per_tol_GF	**per_T_sp**	**per_GF**	**per_BI**
EBT_SS	**Correlation Coefficient**		**-0.01**	0.06	**0.16**	0.45	**0.35**	0.72	0.93	0.04	**-0.37**	**0.05**	**-0.14**
	Sig. (2-tailed)		**0.938**	0.752	**0.391**	0.011	**0.054**	0.000	0.000	0.851	**0.041**	**0.800**	**0.467**
CC_CS_FF	**Correlation Coefficient**	**-0.01**		-0.38	**-0.25**	-0.40	**-0.06**	-0.25	-0.08	0.62	**0.32**	**0.51**	**-0.48**
	Sig. (2-tailed)	**0.938**		0.035	**0.166**	0.027	**0.752**	0.167	0.677	0.000	**0.080**	**0.003**	**0.006**
per_r	Correlation Coefficient	0.06	-0.38		0.63	-0.08	-0.39	-0.25	0.10	-0.43	-0.08	-0.68	0.23
	Sig. (2-tailed)	0.752	0.035		0.000	0.660	0.031	0.183	0.593	0.015	0.688	0.000	0.208
per_fs_ex_bnd	**Correlation Coefficient**	**0.16**	**-0.25**	0.63		0.06	**0.07**	0.00	0.31	-0.28	**-0.40**	**-0.41**	**0.61**
	Sig. (2-tailed)	**0.391**	**0.166**	0.000		0.734	**0.722**	0.994	0.092	0.132	**0.028**	**0.024**	**0.000**
per_cw_sp	Correlation Coefficient	0.45	-0.40	-0.08	0.06		0.68	0.75	0.55	-0.38	-0.72	-0.13	0.01
	Sig. (2-tailed)	0.011	0.027	0.660	0.734		0.000	0.000	0.001	0.034	0.000	0.477	0.948
ct_CW_sp	**Correlation Coefficient**	**0.35**	**-0.06**	-0.39	**0.07**	0.68		0.57	0.50	0.04	**-0.53**	**0.25**	**-0.04**
	Sig. (2-tailed)	**0.054**	**0.752**	0.031	**0.722**	0.000		0.001	0.004	0.832	**0.002**	**0.180**	**0.835**
per_CW	Correlation Coefficient	0.72	-0.25	-0.25	0.00	0.75	0.57		0.78	-0.08	-0.53	0.10	-0.10
	Sig. (2-tailed)	0.000	0.167	0.183	0.994	0.000	0.001		0.000	0.669	0.002	0.610	0.599
per_I	Correlation Coefficient	0.93	-0.08	0.10	0.31	0.55	0.50	0.78		-0.02	-0.49	-0.01	-0.12
	Sig. (2-tailed)	0.000	0.677	0.593	0.092	0.001	0.004	0.000		0.915	0.005	0.944	0.504
per_tol_GF	Correlation Coefficient	0.04	0.62	-0.43	-0.28	-0.38	0.04	-0.08	-0.02		0.60	0.57	-0.21
	Sig. (2-tailed)	0.851	0.000	0.015	0.132	0.034	0.832	0.669	0.915		0.000	0.001	0.258
per_T_sp	**Correlation Coefficient**	**-0.37**	**0.32**	-0.08	**-0.40**	-0.72	**-0.53**	-0.53	-0.49	0.60		**0.06**	**-0.14**
	Sig. (2-tailed)	**0.041**	**0.080**	0.688	**0.028**	0.000	**0.002**	0.002	0.005	0.000		**0.753**	**0.452**
per_GF	**Correlation Coefficient**	**0.05**	**0.51**	-0.68	**-0.41**	-0.13	**0.25**	0.10	-0.01	0.57	**0.06**		**-0.29**
	Sig. (2-tailed)	**0.800**	**0.003**	0.000	**0.024**	0.477	**0.180**	0.610	0.944	0.001	**0.753**		**0.108**
	N	**31**	**31**	31	**31**	31	**31**	31	31	31	**31**		**31**
per_BI	**Correlation Coefficient**	**-0.14**	**-0.48**	0.23	**0.61**	0.01	**-0.04**	-0.10	-0.12	-0.21	**-0.14**	**-0.29**	
	Sig. (2-tailed)	**0.467**	**0.006**	0.208	**0.000**	0.948	**0.835**	0.599	0.504	0.258	**0.452**	**0.108**	

Appendix E. TWIBI sites, associated characteristics, and index scores.

Master ID	Project	Agency ID	Stream Name	Town	Disturbance Category	Site_type	repeat (n=no; r= repeat	Elevation (ft)	Drainage area (sq. mi.)	Longitude (dd.dddd)	Latitude (dd.dddd)	Probability supports coldwater fish species	TWIBI score
s101	DES	00C-50	CLARK BROOK	HAVERHILL	IMP	VALIDATION	n	483	17.1	72.0245	44.0895	0.995	14
s102	DES	98P-79	CHURCHILL BROOK	BROOKFIELD	REF	CALIBRATION	n	578	4.9	71.0714	43.5472	0.670	20
s103	DES	00M-18	TANNERY BROOK	BOSCAWEN	IMP	CALIBRATION	n	283	8.0	71.6290	43.3234	0.727	18
s104	DES	98P-79	CHURCHILL BROOK	BROOKFIELD	REF	VALIDATION	r	578	4.9	71.0714	43.5472	0.670	28
s105	DES	00M-38	WILLEY BROOK	WOLFBORO	MIN	CALIBRATION	n	611	6.6	71.1598	43.6234	0.770	14
s107	DES	00M-8	SANBORN BROOK	LOUDON	MOD	CALIBRATION	n	604	5.3	71.3873	43.3267	0.624	10
s109	DES	01M-01	OWL BROOK	HOLDERNESS	MIN	CALIBRATION	n	631	7.2	71.6292	43.7322	0.960	32
s114	DES	98S-65	BEECH RIVER	OSSIPEE	MIN	CALIBRATION	n	520	15.0	71.1565	43.7271	0.722	20
s115	DES	01M-07	MOOSILAUKE RIVER	WOODSTOCK	MIN	CALIBRATION	n	815	17.1	71.7119	44.0292	0.983	32
s116	DES	99C-23	PARTRIDGE BROOK	WESTMORELAND	MOD	CALIBRATION	n	459	16.5	72.4311	42.9497	0.677	12
s117	DES	01M-17	COCKERMOUTH RIVER	GROTON	MIN	VALIDATION	n	670	13.3	71.8499	43.7074	0.959	36
s118	DES	99M-18	BEAVER BROOK	ALTON	MOD	CALIBRATION	n	580	7.5	71.2006	43.5344	0.688	20
s119	DES	01S-13	SACO RIVER-EAST BRANCH	BARTLETT	REF	CALIBRATION	n	520	39.6	71.1591	44.0979	0.642	32
s120	DES	99M-28	DUDLEY BROOK	DEERING	MOD	CALIBRATION	n	831	8.7	71.8083	43.0997	0.577	24
s121	DES	03M-TREND03	SANBORN BROOK	LOUDON	MOD	CALIBRATION	r	604	5.3	71.3873	43.3267	0.624	14
s122	DES	99M-46	SMITH BROOK	GRAFTON	MOD	CALIBRATION	n	839	9.4	71.9525	43.5581	0.955	20
s123	DES	03M-TREND03	SANBORN BROOK	LOUDON	MOD	VALIDATION	r	604	5.3	71.3873	43.3267	0.624	20
s124	DES	99M-47	TIOGA RIVER	BELMONT	MOD	CALIBRATION	n	783	4.1	71.4282	43.4806	0.823	10
s125	DES	03M-TREND03	SANBORN BROOK	LOUDON	MOD	VALIDATION	r	604	5.3	71.3873	43.3267	0.624	12
s126	DES	99M-48	NIGHTHAWK HOLLOW	GILMANTON	MOD	VALIDATION	n	709	3.9	71.3532	43.4292	0.742	12
s127	DES	04c-01	BLOW ME DOWN BROOK	CORNISH	MOD	CALIBRATION	n	423	25.0	72.3765	43.5159	0.937	18
s128	DES	99M-5	STIRRUP IRON BRO	BOSCAWEN	MIN	CALIBRATION	n	417	5.9	71.6610	43.3759	0.825	22
s129	DES	04c-11	AMMONOOSUC RIVER	CARROLL	MOD	VALIDATION	n	1549	37.0	71.4712	44.2690	0.935	32
s130	DES	99M-51	AMEY BROOK	HENNIKER	IMP	CALIBRATION	n	427	12.0	71.8211	43.1919	0.627	14
s131	DES	05A-13	SWIFT DIAMOND RIVER	SECOND COLLEGE GRANT	REF	CALIBRATION	n	1360	26.3	71.0853	44.9426	0.997	28
s133	DES	05A-15	DEAD DIAMOND RIVER	SECOND COLLLEGE GRANT	REF	CALIBRATION	n	1512	40.4	71.1610	44.8664	0.989	24

Master ID	Project	Agency ID	Stream Name	Town	Disturbance Category	Site_type	repeat (n=no; r= repeat	Elevation (ft)	Drainage area (sq. mi.)	Longitude (dd.dddd)	Latitude (dd.dddd)	Probability supports coldwater fish species	TWIBI score
s134	DES	99M-8	BRADLEY BROOK	ANDOVER	MIN	CALIBRATION	n	853	2.2	71.8247	43.4067	0.928	28
s136	DES	99M-8	BRADLEY BROOK	ANDOVER	MIN	CALIBRATION	r	853	2.2	71.8247	43.4067	0.928	26
s137	DES	05C-11	OTTER BROOK	LANCASTER	IMP	CALIBRATION	n	924	24.9	71.5412	44.4812	0.994	20
s139	DES	06c-13	LITTLE SUGAR RIVER	CHARLESTOWN	MOD	VALIDATION	n	343	29.3	72.3853	43.3057	0.775	34
s141	DES	06c-17	MINK BROOK	HANOVER	IMP	VALIDATION	n	538	15.5	72.2679	43.6908	0.985	20
s146	DES	NH HEX 28.03	AMES BROOK	ASHLAND	MOD	CALIBRATION	n	515	5.6	71.6308	43.6920	0.957	24
s147	DES	07m-03	PUNCH BROOK	FRANKLIN	MIN	CALIBRATION	n	412	8.5	71.6690	43.4128	0.820	26
s149	DES	07m-07	COLLINS BROOK	FRANCESTOWN	MIN	VALIDATION	n	811	7.2	71.8308	43.0124	0.518	16
s151	DES	07m-09	HAYWARD BROOK	CONCORD	MOD	CALIBRATION	n	329	12.1	71.5575	43.2885	0.549	16
s152	DES	NH HEX 35.01	CHURCHILL BROOK	BROOKFIELD	MOD	CALIBRATION	n	533	7.0	71.0613	43.5499	0.621	30
s153	DES	07m-11	SALMON BROOK	SANBORNTON	IMP	CALIBRATION	n	589	18.2	71.6218	43.5159	0.731	16
s155	DES	07m-13	S BR BAKER RIVER	WENTWORTH	MIN	CALIBRATION	n	793	31.3	71.9320	43.8185	0.909	26
s157	DES	07s-05	COLD RIVER	CENTER SANDWICH	MOD	CALIBRATION	n	668	29.8	71.3645	43.8562	0.707	24
s163	DES	98C-1	INDIAN STREAM	PITTSBURG	REF	CALIBRATION	n	1307	63.5	71.4097	45.0924	0.986	36
s165	DES	98C-1	INDIAN STREAM	PITTSBURG	REF	VALIDATION	r	1307	63.5	71.4097	45.0924	0.986	28
s167	DES	98C-10	SIMMS STREAM	COLUMBIA	MIN	CALIBRATION	n	1266	28.0	71.4933	44.8494	0.999	38
s169	DES	98C-10	SIMMS STREAM	COLUMBIA	MIN	CALIBRATION	r	1266	28.0	71.4933	44.8494	0.999	40
s173	DES	98C-14	STRATFORD BOG BROOK	STRATFORD	REF	CALIBRATION	r	1011	16.9	71.5379	44.6783	0.999	40
s175	DES	98C-15	NASH STREAM	STRATFORD	MIN	CALIBRATION	n	1377	38.4	71.4539	44.6758	0.990	40
s179	DES	98C-18	UPPER AMMONOOSUC RIVER	BERLIN	REF	CALIBRATION	n	1157	48.7	71.2879	44.5235	0.912	34
s181	DES	98C-18	UPPER AMMONOOSUC RIVER	BERLIN	REF	CALIBRATION	r	1157	48.7	71.2879	44.5235	0.912	34
s183	DES	98C-19	AMMONOOSUC RIVER	CARROLL	MOD	VALIDATION	n	1466	43.5	71.4927	44.2647	0.892	32
s185	DES	98C-2	INDIAN STREAM	PITTSBURG	REF	VALIDATION	n	1229	67.4	71.4354	45.0744	0.980	26
s187	DES	98C-2	INDIAN STREAM	PITTSBURG	REF	VALIDATION	r	1229	67.4	71.4354	45.0744	0.980	32
s191	DES	98C-36	ISREAL RIVER	JEFFERSON	MIN	VALIDATION	n	1057	70.5	71.4989	44.4125	0.593	28
s193	DES	98C-36	ISREAL RIVER	JEFFERSON	MIN	CALIBRATION	r	1057	70.5	71.4989	44.4125	0.593	22
s195	DES	98C-4	PERRY STREAM	PITTSBURG	REF	CALIBRATION	n	1579	24.4	71.3200	45.1043	1.000	36
s197	DES	98C-6	CONNECTICUT RIVER	PITTSBURG	MIN	CALIBRATION	n	1644	59.9	71.2071	45.1190	0.984	36

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s199	DES	98C-6	CONNECTICUT RIVER	PITTSBURG	MIN	CALIBRATION	r	1644	59.9	71.2071	45.1190	0.984	34
s303	F_G_SAP	19990706--MMW-Pine Island Brook-efish	PINE ISLAND BROOK	LOUDON	MOD	CALIBRATION	n	774	0.3	71.4886	43.2942	0.753	22
s305	F_G_SAP	19990707-1000-MMW-Gues Meadow -Efish	GUES MEADOW	LOUDON	MOD	CALIBRATION	n	714	4.4	71.4675	43.3454	0.717	14
s307	F_G_SAP	19990707--MMW-Shaker Brook-efish	SHAKER BROOK	LOUDON	MOD	CALIBRATION	n	700	1.6	71.4877	43.3293	0.763	14
s309	F_G_SAP	19990714--MMW-Academy Brook-efish	ACADEMY BROOK	LOUDON	IMP	CALIBRATION	n	743	12.3	71.4385	43.3654	0.552	8
s311	F_G_SAP	20010622-1200-WILLEY-Willey Brook-efish	WILLEY BROOK	WOLFEBORO	MIN	CALIBRATION	n	716	6.6	71.1600	43.6225	0.770	24
s313	F_G_SAP	20050725-1030-USACE-Salmon Brook-efish	SALMON BROOK	SANBORNTON	IMP	CALIBRATION	n	753	20.6	71.6321	43.4971	0.670	16
s315	F_G_SAP	20050725-1220-USACE-Weeks Brook-efish	WEEKS BROOK	SANBORNTON	MIN	CALIBRATION	r	237	3.9	71.6628	43.5001	0.915	30
s317	F_G_SAP	20050726-1030-USACE-Knox Brook-Efish	KNOX BROOK	SANBORNTON	REF	CALIBRATION	n	1169	4.2	71.6833	43.5223	0.926	30
s319	F_G_SAP	20050726-1400-USACE-Blake Brook-Efish	BLAKE BROOK	NEW HAMPTON	REF	CALIBRATION	n	686	3.7	71.7144	43.5791	0.951	36
s321	F_G_SAP	20050727-1030-USACE-Needle Shop Brook-Efish	NEEDLE SHOP BROOK	HILL	MOD	CALIBRATION	n	995	8.0	71.6958	43.5292	0.905	22
s329	F_G_SAP	20060802-1115-BWR-Mill Brook Lower-Efish	MILL BROOK	SALISBURY	REF	CALIBRATION	n	677	4.3	71.7919	43.3552	0.880	30
s331	F_G_SAP	20060809-1030-BWR-Frazier Brook-Efish	FRAZIER BROOK	DANBURY	MOD	CALIBRATION	n	419	18.4	71.8956	43.4705	0.831	18
s333	F_G_SAP	20060818-1000-BWR-Mountain Brook-Efish	MOUNTAIN BROOK	ANDOVER	REF	VALIDATION	n	562	6.7	71.7957	43.4587	0.910	22
s335	F_G_SAP	20061008-1300-CHURCH-Churchill Brook-Efish	CHURCHILL BROOK	BROOKFIELD	MOD	CALIBRATION	n	981	7.9	71.0512	43.5447	0.586	20
s337	F_G_SAP	20010807--BCW-Cold River Reach 2-efish	COLD RIVER	SANDWICH	MIN	CALIBRATION	r	981	29.7	71.3681	43.8567	0.713	28
s339	F_G_SAP	20010808--USFS-Wild River-Efish	WILD RIVER	BEANS PURCHASE	REF	CALIBRATION	n	968	19.8	71.0857	44.2916	0.961	34
s343	F_G_SAP	20010814--USFS-Wild River-Efish	WILD RIVER	BEANS PURCHASE	REF	CALIBRATION	n	460	40.2	71.0552	44.3185	0.793	24
s345	F_G_SAP	20020810--SDWS-Swift Diamond Lower-efish	SWIFT DIAMOND RIVER	DIXS GRANT	REF	CALIBRATION	n	439	37.8	71.1821	44.8696	0.992	28
s347	F_G_SAP	20020810--SDWS-Swift Diamond Middle-efish	SWIFT DIAMOND RIVER	DIXS GRANT	REF	CALIBRATION	n	555	33.3	71.2095	44.8770	0.995	28
s353	F_G_SAP	20030820--SDWS-Swift Diamond Lower-efish	SWIFT DIAMOND RIVER	DIXS GRANT	REF	CALIBRATION	n	549	37.8	71.1821	44.8696	0.992	38
s355	F_G_SAP	20050720-1030-Cold-Cold River-Efish	COLD RIVER	ACWORTH	MOD	CALIBRATION	n	709	17.1	72.2384	43.2129	0.809	12
s377	F_G_SAP	20050817--USFS-Upper Ammo River-Efish	UPPER AMMO RIVER	BERLIN	REF	CALIBRATION	n	767	23.3	71.3255	44.4829	0.990	30
s379	F_G_SAP	20050823--JOHNS-Johns River (Meadow Site)-Efish	JOHNS RIVER	WHITEFIELD	IMP	VALIDATION	n	767	31.1	71.6096	44.3718	0.985	22
s381	F_G_SAP	20050823--JOHNS-Johns River (Railroad site)-Efish	JOHNS RIVER	WHITEFIELD	IMP	CALIBRATION	n	355	29.6	71.5959	44.3688	0.986	22
s383	F_G_SAP	20050823--JOHNS-Johns River (u/s of dam in town)-Efish	JOHNS RIVER	DALTON	MOD	CALIBRATION	n	576	54.6	71.6231	44.3828	0.890	18

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s385	F_G_SAP	20050908--JOHNS-Johns River (Brown Street)-Efish	JOHNS RIVER	WHITEFIELD	IMP	CALIBRATION	n	986	50.1	71.6224	44.3772	0.923	24
s387	F_G_SAP	20050908--JOHNS-Johns River (Sand Pit Site)-Efish	JOHNS RIVER	DALTON	MIN	CALIBRATION	n	779	58.1	71.6277	44.3936	0.862	20
s389	F_G_SAP	20050929-1100-BCW-Swift River-efish	SWIFT RIVER	TAMWORTH	REF	VALIDATION	n	620	26.5	71.2905	43.8862	0.753	40
s391	F_G_SAP	20060810-1240-JV-S. Branch Baker River-Efish	BAKER RIVER	WENTWORTH	MIN	CALIBRATION	n	589	38.6	71.9156	43.8241	0.832	18
s395	F_G_SAP	20060810-1400-JV-S. Branch Baker River-Efish	BAKER RIVER	DORCHESTER	MIN	CALIBRATION	n	651	18.5	71.9245	43.8001	0.967	16
s425	F_G_SAP	20060922-1020-BCW-Swift River-Efish	SWIFT RIVER	TAMWORTH	REF	CALIBRATION	n	1236	25.2	71.2972	43.8927	0.785	40
s427	F_G_SAP	20070817-1100-REG1-Swift Diamond River Lower-Efish	SWIFT DIAMOND RIVER LOWER	DIXS GRANT	REF	CALIBRATION	n	1000	37.8	71.1815	44.8695	0.992	30
s429	F_G_SAP	20070817-845-REG1-Swift Diamond River Upper-Efish	SWIFT DIAMOND RIVER UPPER	DIXVILLE	REF	CALIBRATION	n	945	21.2	71.2306	44.9037	0.999	30
s431	F_G_SAP	20070818-1130-REG1-Little Dead Diamond River Lower-Efish	LITTLE DEAD DIAMOND RIVER LOWER	ATKINSON AND GILMANTON ACADEMY GRANT	REF	CALIBRATION	n	1013	15.0	71.1233	44.9747	0.999	36
s433	F_G_SAP	20070819-1400-REG1-Dead Diamond River (Brungot's)-Efish	DEAD DIAMOND RIVER (BRUNGOT'S)	ATKINSON AND GILMANTON ACADEMY GRANT	REF	CALIBRATION	n	1256	47.8	71.1333	44.9799	0.987	34
s435	F_G_SAP	20070913-930-REG1-Bog Brook-Efish	BOG BROOK	WHITEFIELD	IMP	VALIDATION	n	964	18.7	71.6220	44.3770	0.996	16
s443	F_G_SAP	20070925-900-REG1-John's River (Meadow Site)-Efish	JOHN'S RIVER (MEADOW SITE)	DALTON	MOD	CALIBRATION	n	889	54.5	71.6223	44.3824	0.891	20
s505	F_G_FFF	31582-FFF-Rand Brook-Hillsboro 030-EFISH	RAND BROOK	GREENFIELD	MIN	VALIDATION	n	884	2.1	71.8444	42.9505	0.566	14
s507	F_G_FFF	31987-FFF-Boglie Brook-Hillsboro 084-EFISH	BOGLIE BROOK	PETERBOROUGH	MIN	VALIDATION	n	879	1.7	71.9183	42.9111	0.582	26
s509	F_G_FFF	31987-FFF-Hardy Brook-Hillsboro 072-EFISH	HARDY BROOK	PETERBOROUGH	MIN	CALIBRATION	n	1084	4.2	71.9229	42.9077	0.523	18
s511	F_G_FFF	31987-FFF-Sand Hill Brook-Hillsboro 067-EFISH	SAND HILL BROOK	PETERBOROUGH	MIN	VALIDATION	n	984	2.2	71.9342	42.8917	0.558	22
s513	F_G_FFF	31987-FFF-Sand Hill Brook-Hillsboro 068-EFISH	SAND HILL BROOK	PETERBOROUGH	MIN	CALIBRATION	n	966	2.2	71.9335	42.8915	0.557	22
s515	F_G_FFF	31988-FFF-Hardy Brook-Hillsboro 073-EFISH	HARDY BROOK	PETERBOROUGH	MOD	CALIBRATION	n	880	4.3	71.9250	42.9101	0.527	14
s517	F_G_FFF	31989-FFF-Alexander Brook-Hillsboro 032-EFISH	ALEXANDER BROOK	GREENFIELD	MIN	VALIDATION	n	859	3.1	71.8371	42.9587	0.548	14
s519	F_G_FFF	31996-FFF-Sand Brook-Hillsboro 115-EFISH	SAND BROOK	HILLSBORO	IMP	VALIDATION	n	1031	8.3	71.9011	43.1563	0.718	14
s525	F_G_FFF	9/19/1985-FFF-Hayes Brook-Strafford 009-EFISH	HAYES BROOK	NEW DURHAM	MOD	CALIBRATION	n	1229	2.9	71.1121	43.4399	0.613	34
s533	F_G_FFF	30567-FFF-Partridge Brook-Cheshire 026-EFISH	PARTRIDGE BROOK	WESTMORELAND	IMP	CALIBRATION	n	1293	23.2	72.4636	42.9723	0.580	14

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s535	F_G_FFF	30573-FFF-Ashuelot River-Cheshire 012-EFISH	ASHUELOT RIVER	MARLOW	MOD	VALIDATION	n	938	19.7	72.1997	43.0923	0.612	10
s537	F_G_FFF	30916-FFF-Bloods Brook-Sullivan 017-EFISH	BLOODS BROOK	PLAINFIELD	MOD	CALIBRATION	r	938	16.2	72.2715	43.5687	0.970	30
s545	F_G_FFF	30923-FFF-Chase Brook-Sullivan 001-EFISH	CHASE BROOK	UNITY	MOD	CALIBRATION	n	1139	19.9	72.3301	43.2729	0.855	8
s547	F_G_FFF	32308-FFF-Blood Brook-Sullivan 009-EFISH	BLOOD BROOK	PLAINFIELD	MOD	CALIBRATION	n	1017	16.2	72.2715	43.5687	0.970	30
s549	F_G_FFF	32308-FFF-Little Sugar River-Sullivan 008-EFISH	LITTLE SUGAR RIVER	UNITY	MOD	CALIBRATION	n	1017	24.8	72.3457	43.2836	0.805	38
s551	F_G_FFF	32308-FFF-South Branch Sugar River-Sullivan 009-EFISH	SOUTH BRANCH SUGAR RIVER	GOSHEN	MOD	CALIBRATION	n	1026	29.7	72.1544	43.3045	0.618	34
s563	F_G_FFF	36019-FFF-Indian Stream-Coos 072-EFISH	INDIAN STREAM	PITTSBURG	REF	VALIDATION	n	1275	41.4	71.3493	45.1768	0.999	40
s565	F_G_FFF	36019-FFF-Indian Stream-Coos 073-EFISH	INDIAN STREAM	PITTSBURG	REF	VALIDATION	r	1271	41.4	71.3493	45.1759	0.999	32
s567	F_G_FFF	36020-FFF-Perry Stream-Coos 064-EFISH	PERRY STREAM	PITTSBURG	MIN	CALIBRATION	n	1344	30.5	71.3061	45.0724	0.999	28
s569	F_G_FFF	36034-FFF-Bog Brook-Coos 035-EFISH	BOG BROOK	STRATFORD	REF	VALIDATION	n	1353	16.7	71.5358	44.6808	0.999	38
s573	F_G_FFF	36341-FFF-Johns River-Coos 015-EFISH	JOHNS RIVER	WHITEFIELD	MIN	CALIBRATION	n	1032	54.4	71.6225	44.3794	0.890	28
s575	F_G_FFF	36342-FFF-Clear Stream-Coos 069-EFISH	CLEAR STREAM	MILLSFIELD	REF	CALIBRATION	n	1352	21.8	71.2335	44.8085	0.998	34
s577	F_G_FFF	36350-FFF-Johns River-Coos 016-EFISH	JOHNS RIVER	WHITEFIELD	IMP	VALIDATION	n	1377	59.1	71.6317	44.4075	0.861	10
s579	F_G_FFF	36353-FFF-Hamm Branch-Grafton 008-EFISH	HAMM BRANCH	FRANCONIA	MIN	CALIBRATION	n	1379	30.7	71.7511	44.2146	0.979	28
s581	F_G_FFF	36353-FFF-Israel River-Coos 005-EFISH	ISRAEL RIVER	JEFFERSON	REF	CALIBRATION	n	1374	22.5	71.4183	44.3689	0.988	38
s587	F_G_FFF	36355-FFF-Stearns-Coos 093-EFISH	STEARNS	MILAN	REF	CALIBRATION	n	1389	34.7	71.1274	44.5317	0.960	30
s593	F_G_FFF	36382-FFF-Perry Stream-Coos 062-EFISH	PERRY STREAM	PITTSBURG	MIN	VALIDATION	n	1412	28.5	71.3134	45.0843	0.999	24
s595	F_G_FFF	36762-FFF-Moose River-Coos 083-EFISH	MOOSE RIVER	GORHAM	REF	VALIDATION	n	1303	22.6	71.2199	44.3905	0.980	36
s597	F_G_FFF	36767-FFF-Wild River-Coos 074-EFISH	WILD RIVER	BEANS PURCHASE	REF	CALIBRATION	n	1272	43.5	71.0351	44.3254	0.734	22
s601	F_G_FFF	36769-FFF-Ammonoosuc R.-Coos 025-EFISH	AMMONOOSUC R.	CARROLL	IMP	CALIBRATION	n	1566	34.7	71.4566	44.2628	0.943	32
s603	F_G_FFF	36769-FFF-Ammonoosuc R.-Coos 026-EFISH	AMMONOOSUC R.	CARROLL	MOD	CALIBRATION	n	1566	43.6	71.4945	44.2645	0.892	30
s605	F_G_FFF	37473-FFF-Israel River-Coos 008-EFISH	ISRAEL RIVER	JEFFERSON	MOD	VALIDATION	n	1062	73.4	71.5184	44.4190	0.550	22
s607	F_G_FFF	37474-FFF-Ammonoosuc River-Coos 054-EFISH	AMMONOOSUC RIVER	CARROLL	MOD	VALIDATION	n	1042	62.0	71.5228	44.2696	0.624	32
s609	F_G_FFF	37474-FFF-Hamm Branch-Grafton 009-EFISH	HAMM BRANCH	FRANCONIA	MOD	CALIBRATION	n	1607	29.5	71.7568	44.2029	0.981	18
s611	F_G_FFF	37476-FFF-Upper Ammonoosuc-Coos 084-EFISH	UPPER AMMONOOSUC	BERLIN	REF	CALIBRATION	n	1027	46.6	71.2892	44.5149	0.923	36
s613	F_G_FFF	37481-FFF-Moose River-Coos 082-EFISH	MOOSE RIVER	GORHAM	REF	VALIDATION	n	1027	22.5	71.2217	44.3881	0.980	34

Master ID	Project	Agency ID	Stream Name	Town	Disturbance Category	Site_type	repeat (n=no; r= repeat	Elevation (ft)	Drainage area (sq. mi.)	Longitude (dd.dddd)	Latitude (dd.dddd)	Probability supports coldwater fish species	TWIBI score
s615	F_G_FFF	37481-FFF-Peabody River-Coos 078-EFISH	PEABODY RIVER	GORHAM	REF	CALIBRATION	n	1658	40.8	71.1806	44.3631	0.871	36
s621	F_G_FFF	37489-FFF-Phillips Brook-Coos 022-EFISH	PHILLIPS BROOK	STARK	REF	CALIBRATION	n	1454	37.6	71.3274	44.6480	0.984	30
s623	F_G_FFF	37490-FFF-Simms Stream-Coos 052-EFISH	SIMMS STREAM	COLEBROOK	MIN	CALIBRATION	n	1453	33.1	71.5124	44.8728	0.998	32
s625	F_G_FFF	37831-FFF-Indian Stream-Coos 068-EFISH	INDIAN STREAM	PITTSBURG	REF	CALIBRATION	n	1523	58.9	71.3870	45.1084	0.991	22
s627	F_G_FFF	37832-FFF-Mohawk River-Coos 050-EFISH	MOHAWK RIVER	COLEBROOK	MOD	VALIDATION	n	1310	30.0	71.3879	44.8701	0.998	40
s629	F_G_FFF	37832-FFF-Simms Stream-Coos 051-EFISH	SIMMS STREAM	COLEBROOK	MIN	VALIDATION	n	1394	33.2	71.5173	44.8744	0.998	34
s631	F_G_FFF	37855-FFF-Israel River-Coos 010-EFISH	ISRAEL RIVER	JEFFERSON	MOD	CALIBRATION	n	1399	56.1	71.4830	44.3871	0.824	32