NGP Reference Site Project

Final Completion Report

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Project Description

The South Dakota Department of Environment and Natural Resources (SD DENR) is responsible for assessing water bodies for compliance with the Clean Water Act. This includes evaluation of physical, chemical and biological integrity. Contemporary approaches used to assess and monitor biotic integrity require comparative data from minimally disturbed reference sites. These reference sites should be validated against sites of known condition (targeted sites) and across a range of stream conditions (random sites). Regional indices of biotic integrity would further enhance the utility of biological data through optimal selection of monitoring invertebrate and fish metrics against stream stressors operating within a particular biophysical setting. The State has further identified the following needs relative to future uses of biological monitoring and assessment data to include (1) identification of biological response thresholds related to nutrients (nitrogen and phosphorus) for use in developing attainable nutrient criteria for wadeable streams, (2) incorporation of bioassessment methods in watershed management plans (Section 319 program) in order to evaluate individual BMP and overall program effectiveness and (3) incorporation of regional biological data in Use Attainability Assessments (UAA) to evaluate support for aquatic life uses.

This project focused on wadeable, perennial streams within the Northern Glaciated Plains Ecoregion (NGP). The NGP occupies approximately one-third of the state's surficial drainage area and is located entirely in the eastern glaciated portion of South Dakota (Figure 1). Climate within this ecoregion is subhumid and natural vegetation is primarily mixed and tall grass prairie species. Intermittent and linear wetlands provide drainage to large numbers of prairie pothole basins. Much of the land area has been tilled for agricultural production, although larger areas of cattle grazing do occur in the northern portions of the ecoregion. Mean annual precipitation ranges from 53 to 69 cm (Bryce et al. 1998).

South Dakota DENR identified a population of 2,546 wadeable perennial streams within the Northern Glaciated Plains ecoregion (Larson 2009). Watershed condition of these streams was evaluated using the Analytical Tools Interface for Landscape Assessment (ATtILA) ARCVIEW extension and GIS shapefiles of landscape features. Candidate sites were also validated on the ground using rapid assessment methodologies. Candidate reference sites were selected from this target population and those reference sites were sampled on a rotation for three years. However, these candidate reference sites had not been field validated against streams of known condition. Furthermore, the department was seeking integrated use of biological data in order to discriminate biological impairment within the stream population. While periphyton, macroinvertebrate and fish samples were collected and quantified from candidate reference sites, optimal biological metrics and indices of biotic integrity had not been calculated. Thus, DENR sought the development of a biological monitoring tool kit which would (1) facilitate development of regional indices of biotic integrity and (2) support analysis and interpretation of biological data relative to changes in water quality and habitat within the NGP.

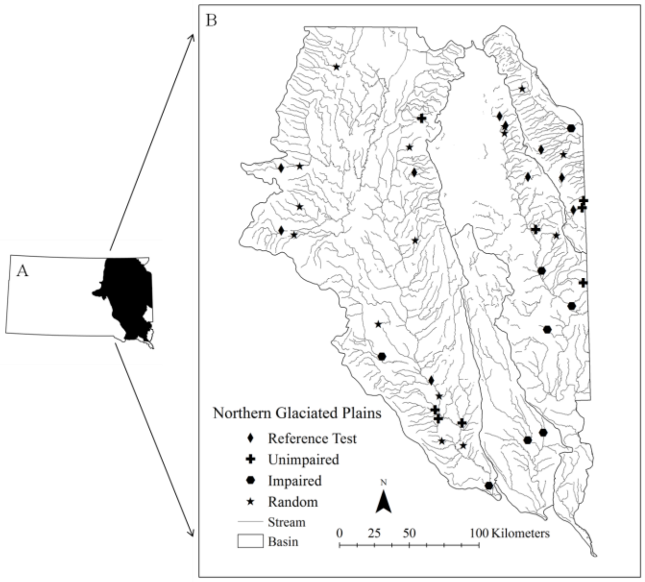


Figure 1. The Northern Glaciated Plains ecoregion (A) and wadable stream sites sampled to develop indices of biotic integrity and validate reference sites in eastern South Dakota (B).

Project Results by Task

Validation Site Selection

We received water quality, habitat and biological data for candidate reference sites directly from SD DENR. Targeted impaired sites (n=8), targeted unimpaired sites (n=8) and random sites (n=15) were selected from the NGP target population (n=2,546). All sites within the target population were ranked based upon the number and frequency of water quality violations. Those sites falling within the upper 5^{th} percentile in terms of number of parameters and frequency of violations were then ranked based upon ATtILA watershed condition score. Those having the worst standards violation record and the lowest watershed condition scores were selected to represent our targeted impaired group (n=8). Similarly, those sites in the lower 5^{th} percentile (best water quality) of water quality parameters and frequency of violations and the highest watershed condition scores were selected to represent the targeted unimpaired group (n=8). In addition to these site groupings of known condition, we also selected 15 sites at random from the NGP wadeable stream population (n=2,546) irrespective of watershed condition score or history of standards violations. All sites were sampled twice, once in 2010 and 2011. A few of our sites were sampled twice in 2011 due to high water the previous year. All sites were sampled using the same methodology as that used for candidate reference streams and within the same seasonal sampling period.

Collection of Water Quality Data and Data Summaries

Water quality variables linked to criteria written to support beneficial stream uses in South Dakota were measured from each random and targeted site during July 2010 and July 2011 (Table 1). Water quality grab samples and multiparameter sonde measurements were collected at the X-point within each sampled stream reach. During the collection of water-quality samples, instantaneous stream flow measurements were also taken. A minimum of 10 percent of the water quality samples collected were duplicated and supplemented with blanks for quality assurance and quality control (QA/QC). All water quality samples were collected using the methods outlined in Standard Operating Procedures for Field Samples Volume 1 Tributary and In-Lake Sampling Techniques (SD DENR Water Resources Assistance Program, 2005).

Table 1. Water quality parameters collected from random and targeted wadeable stream sites of the Northern Glaciated Plains ecoregion in eastern South Dakota.

Parameter	Container	Preserved	Filtered	Lab
Tot Alkalinity	A Bottle (1 Liter)	None	N	SDSU
Tot Solids	A Bottle (1 Liter)	None	N	SDSU
Tot Suspended Solids	A Bottle (1 Liter)	None	N	SDSU
Tot Dissolved Solids	A Bottle (1 Liter)	None	N	SDSU
Tot Ammonia	B Bottle (1 Liter)	Sulfuric	N	DOH
Tot Nitrate	B Bottle (1 Liter)	Sulfuric	N	DOH
Tot Kjeldahl Nitrogen	B Bottle (1 Liter)	Sulfuric	N	DOH
Tot Phosphorus	B Bottle (1 Liter)	Sulfuric	N	DOH
Diss Na	C Bottle (1 Liter)	Nitric	Y	DOH
Diss Si	C Bottle (1 Liter)	Nitric	Y	DOH
Diss Ca	C Bottle (1 Liter)	Nitric	Y	DOH
Diss Mg	C Bottle (1 Liter)	Nitric	Y	DOH
Diss Sulfate	D Bottle (1 Liter)	None	Y	DOH
Diss Cl	D Bottle (1 Liter)	None	Y	DOH
Diss Fl	D Bottle (1 Liter)	None	Y	DOH
Sol Reactive Phosphorus	D Bottle (1 Liter)	None	Y	DOH
E. coli	E Bottle (DOH Bottle)	None	N	DOH
Dissolved Oxygen	Multiparameter Sonde	---	---	SDSU
Conductance	Multiparameter Sonde	---	---	SDSU
pH	Multiparameter Sonde	---	---	SDSU
Water Temperature	Multiparameter Sonde	---	---	SDSU

Candidate reference sites generally displayed good water quality relative to water quality standards and other stream classes across a number of parameters measured during this study (Table 2). As expected, targeted impaired sites displayed significantly greater specific conductance (~1.4x), ammonia-N (~3x), nitrate-N (~6x), total phosphorus (~2x), total suspended solids (~3.6x), and water temperatures (~1.2x) than either candidate reference or targeted unimpaired sites. Targeted impaired sites also had significantly lower dissolved oxygen (~33%) than that observed from targeted unimpaired sites. Targeted unimpaired sites generally displayed good water quality but did have elevated average ammonia-N and total suspended solids as compared to candidate reference sites (Table 2). Standards violations were observed for low dissolved oxygen and elevated water temperatures. However, other parameters did not violate standards for marginal warmwater fisheries.

Table 2. Comparison of water quality attributes among NGP stream classes in eastern South Dakota, 2010-2011. Results of KW ANOVA for comparison among site classes and means comparison test reported; Ref – candidate reference, TUnImp – targeted unimpaired, TImp – targeted impaired, Rnd – random.

Parameter		**Ref**	**TUnImp**	**TImp**	**Rnd**	**p-value**
Alkalinity	n	31	16	16	30	p = 0.539
(mg/L)	$\bar{x}$	272	263	283	278	
	x_{50}	284	268	278	276	
	x_{min}	171	152	125	184	
	x_{max}	351	336	390	408	
Conductance	n	26	16	16	30	p = 0.019
(µS/cm)	$\bar{x}$	1041	1380	1480	1498	TarB > Ref
	x_{50}	861	1202	1523	1350	
	x_{min}	479	690	761	513	
	x_{max}	2294	2592	2192	3120	
Diss. O_2	n	26	16	16	30	p = 0.019
(mg/L)	$\bar{x}$	7.3	8.4	5.6	4.7	TarG > TarB
	x_{50}	7.3	7.8	6.2	5.0	
	x_{min}	4.0	3.5	0.6	0.2	
	x_{max}	13.8	14.0	8.9	11.0	
Diss SO_4	n	19	16	16	30	p = 0.471
(mg/L)	$\bar{x}$	342	592	467	626	
	x_{50}	227	450	533	582	
	x_{min}	89	53	81	13	
	x_{max}	863	1320	880	1700	
NH_3-N	n	31	16	16	30	p = 0.019
(mg/L)	$\bar{x}$	0.026	0.032	0.067	0.126	TarB > Ref
	x_{50}	0.030	0.030	0.030	0.030	
	x_{min}	0.010	0.030	0.030	0.030	
	x_{max}	0.120	0.060	0.270	0.770	
NO_3-N	n	31	16	16	30	p = 0.003
(mg/L)	$\bar{x}$	0.158	1.063	1.175	0.560	TarG,TarB > Ref
	x_{50}	0.100	0.400	0.700	0.100	
	x_{min}	0.100	0.100	0.100	0.100	
	x_{max}	0.600	6.500	5.500	3.700	
SAR	n	19	16	16	30	p = 0.269
	$\bar{x}$	0.92	0.72	1.09	1.46	
	x_{50}	0.26	0.66	0.76	1.15	
	x_{min}	0.10	0.15	0.33	0.23	
	x_{max}	2.88	1.61	3.88	5.93	
TDS	n	31	16	16	30	p = 0.133
(mg/L)	x	855	1269	1090	1238	
	x_{50}	612	1008	1223	1170	
	x_{min}	626	399	490	265	
	x_{max}	2025	3347	1612	2814	
Total P	n	31	16	16	30	p = 0.001
(mg/L)	$\bar{x}$	0.390	0.280	0.565	0.713	TarB > TarG, Ref
	x_{50}	0.172	0.258	0.509	0.485	
	x_{min}	0.014	0.053	0.297	0.052	
	x_{max}	2.510	0.664	1.090	3.200	

Table 2 (continued)

Parameter		Ref	TUnImp	TImp	Rnd	p-value
TSS	n	31	16	16	30	$p < 0.001$
(mg/L)	$\bar{x}$	17.4	52.3	62.1	31.9	TarB,TarG > Ref
	x_{50}	11.0	48.7	43.5	19.0	
	x_{min}	1.5	3.8	7.2	2.4	
	x_{max}	100	167.6	255	108.5	
Water Temp	n	25	16	16	30	$p < 0.001$
(ºC)	$\bar{x}$	21.1	22.9	25.3	22.0	TarB > TarG,Ref
	x_{50}	21.0	22.5	25.3	21.0	
	x_{min}	13.4	14.0	19.8	16.7	
	x_{max}	29.5	29.7	34.8	30.4	
pH	n	22	16	16	30	$p < 0.091$
	$\bar{x}$	--	--	--	--	
	x_{50}	7.82	8.12	7.75	7.89	
	x_{min}	7.24	6.54	6.83	7.14	
	x_{max}	8.50	9.40	8.25	9.21	

Collection of Stream Habitat Data

Detailed physical habitat measurements were made from each site following collection of water chemistries and biological samples (SD DENR Water Resources Assistance Program, 2005). Habitat data were collected from the entire sample reach and eleven equally spaced transects placed at equidistant locations along the reach. On either end of a transect the riparian land use, dominant vegetation type, animal vegetation use, dominant bank substrate, and bank slumping (presence/absence) were recorded. Bed substrate measurements were collected from eight locations across each transect. Several measurements of the channel cross-section were collected to estimate stream width, depth, channel bottom and top width, water depth, channel slope, bank length, bank angle, bank height, bankfull width, bankfull depth, and width:depth ratio. Length of the banks that were vegetated, erosional or depositional, as well as horizontal length of over-hanging vegetation and undercut banks extending over the stream channel bed were measured with a meter tape from each bank. Measures of canopy cover were collected from six stations at each transect using a densiometer. Finally, the number of large woody debris (LWD) were tallied for the entire reach. All habitat data were entered onto digital datasheets in the field using a Panasonic Toughbook computer. Formulas built into the datasheet facilitated calculations. Each reach was photographed and a drawing of the reach was recorded.

Stream habitat conditions displayed few differences among stream classes (Table 3). We did observe greater preliminary mean stream width (~ 2x) and stream flow (~4x) from targeted impaired versus candidate reference sites. However, none of the other habitat variables varied significantly among stream classes.

Table 3. Selected stream habitat variables directly linked to aquatic biotic integrity from NGP streams of eastern South Dakota, 2010-2011. Results of KW ANOVA for comparison among site classes and means comparison test reported; Ref – candidate reference, TUnImp – targeted unimpaired, TImp – targeted impaired, Rnd – random. Wilcoxon Rank Sum Test used for bed and bank features where reference data were missing.

Parameter		**Ref**	**TUnImp**	**TImp**	**Rnd**	**p-value**
Canopy Cover	n	10	8	8	15	p = 0.477
(%)	$\bar{x}$	12.9	16.6	5.1	13.0	
	x_{50}	6.0	5.2	0.2	0.7	
	x_{min}	0	0	0	0	
	x_{max}	66	90	23.8	74.9	
Discharge	n	10	8	8	15	p = 0.019
(cms)	$\bar{x}$	0.099	0.358	0.404	0.055	TarB > Ref
	x_{50}	0.072	0.223	0.414	0.038	
	x_{min}	0.001	0.021	0.031	0	
	x_{max}	0.254	0.784	0.832	0.170	
PMSW	n	10	8	8	15	p = 0.002
(m)	$\bar{x}$	3.5	5.4	6.8	5.4	TarB > Ref
	x_{50}	2.8	4.6	6.2	4.5	
	x_{min}	2.0	3.5	3.9	2.0	
	x_{max}	7.8	8.9	11.7	13.1	
Woody Debris	n	10	8	8	15	p = 0.409
(#)	$\bar{x}$	1.9	1.3	3.3	0.8	
	x_{50}	0	0.5	0.3	0	
	x_{min}	0	0	0	0	
	x_{max}	14	6	21	6.5	
Fine Substrate	n	10	8	8	15	p = 0.608
(%)	$\bar{x}$	53.9	57.6	67.0	77.6	
	x_{50}	49.4	62.3	63.2	81.4	
	x_{min}	26.1	26.8	38.6	32.7	
	x_{max}	87.5	77.3	99.5	100	
BFW	n	-	8	8	15	p = 0.279
(m)	$\bar{x}$	-	11.4	9.0	6.0	
	x_{50}	-	6.0	7.5	5.9	
	x_{min}	-	4.1	4.7	1.8	
	x_{max}	-	44.4	17.8	15.1	
FPW	n	-	8	8	15	p = 0.130
(m)	$\bar{x}$	-	10.7	19.1	15.7	
	x_{50}	-	10.0	13.4	12.7	
	x_{min}	-	6.0	7.4	2.8	
	x_{max}	-	21.3	46.8	66.6	
WW	n	-	8	8	15	p = 0.195
(m)	x	-	5.7	8.3	5.1	
	x_{50}	-	4.9	6.7	4.7	
	x_{min}	-	3.9	3.6	1.8	
	x_{max}	-	10.1	22.1	14.1	
Entrenchment	n	-	8	8	15	p = 0.999
(Ratio)	$\bar{x}$	-	1.6	1.7	2.8	
	x_{50}	-	1.6	1.6	2.2	
	x_{min}	-	1.4	1.3	1.6	
	x_{max}	-	2.0	2.9	7.6	

Table 3 (continued)

Parameter		Ref	TUnImp	TImp	Rnd	p-value
Width:Depth	n	-	8	8	15	p = 0.721
(Ratio)	$\bar{x}$	-	12.8	13.9	10.8	
	x_{50}	-	10.7	13.5	8.2	
	x_{min}	-	5.9	6.8	5.0	
	x_{max}	-	28.7	30.6	17.7	
Bank Angle	n	-	8	8	15	p = 0.798
(°)	$\bar{x}$	-	32.8	31.3	22.7	
	x_{50}	-	29.9	34.7	25.6	
	x_{min}	-	26.0	14.3	4.6	
	x_{max}	-	45.0	41.9	39.9	

Collection of Periphyton, Macroinvertebrate and Fish Data

Sampling of periphyton, macroinvertebrates and fish was performed using the same methodology followed by SD DENR during their sampling of candidate reference sites. Reach-wide composite macroinvertebrate samples were collected from eleven transects at each site during 2010 and 2011 (SD DENR Water Resources Assistance Program, 2005). Transect spacing was derived from preliminary mean stream width (PMSW) measurements. If the PMSW was less than or equal to 10 m, transects were spaced three PMSWs apart. If the PMSW was greater than 10 m, transects were spaced two PMSWs apart. Depending on the width, depth and current velocity of the stream, one of four possible methods were used to collect macroinvertebrate samples. A sub-sample was collected from each transect with a D-framed, 500-µm mesh net by disturbing an area that was one net width wide and one net width long upstream of the net opening for 30 seconds. The net was positioned with the opening facing upstream, allowing displaced organisms to drift into the net. At each transect, the sample was collected at the left, center, or right location (25%, 50%, or 75% of the transect width, respectively). The sample was collected on the right side at transect #1, on the left at transect #2, at the center at transect #3, and so on, zigzagging upstream through the sampled stream reach. Some of the more sluggish flowing sites were sampled with the "natural substrate, pool/glide" method. This method is similar to that for riffle/run sites, with main difference being net orientation. At pool/glide sites, the net was swept through the water column, due to the sluggish stream flow, so that the organisms trapped in the net would not escape. At each transect, the net was continuously swept back and forth above the disturbed area for 30 seconds. After obtaining a sample at a transect, the contents of the net were rinsed into a bucket. After collecting the final sub-sample at the last transect, the net was thoroughly examined to ensure the removal of all organisms. The contents of the bucket were sieved to remove fine sediment, placed into the pre-labeled container(s), and preserved with 95% ethanol. Macroinvertebrate samples were subsampled to reach a minimum count of 500 individuals per sample. Sorted individuals were generally identified to genus or that level appropriate for monitoring analysis (U.S. EPA 2004). Voucher specimens of each unique taxon were retained for deposit into the South Dakota Aquatic Invertebrate Collection (SDSU).

Following water quality and macroinvertebrate collections, we collected a reach-wide, composite periphyton sample from the same transect locations at which the macroinvertebrate samples were collected (SD DENR Water Resources Assistance Program, 2005). Depending on the habitat type at each transect, one of two methods were used to collect the periphyton sample. In "erosional" (i.e. coarse substrate) type habitats, a piece of substrate (e.g. rock, wood) < 15 cm in diameter was removed from the stream bed and placed in a plastic funnel that drained into a 500 ml plastic, dark bottle. We defined a 12 cm^2 area on the upper surface of the substrate using an area delimiter and dislodged attached periphyton from the substrate into the funnel by scraping/brushing for 30 seconds. We washed the dislodged periphyton from the rock, delimiter, and funnel into the 500 ml container using stream water. In "depositional" (i.e. fine substrate) habitats, we defined a 12 cm^2 area of soft sediments using the area delimiter and vacuumed the top 1 cm of sediments with a 60-mL syringe. The syringe was then emptied into a 500 ml composite sample container. Three different types of laboratory samples were prepared from each 500 ml composite periphyton sample: 1) an ID/enumeration sample (to determine taxonomic composition and relative abundance), 2) a biomass sample (dry weight), and 3) a chlorophyll sample. To prepare the ID/enumeration sample from the composite sample, a 50 ml aliquot of the composite sample was placed into a pre-labeled 60 ml container and preserved with 0.5 ml of Lugol's solution. This sample was mailed to Rithron Associates for analysis. To prepare the chlorophyll sample from the composite sample, we filtered a 25 ml aliquot of the composite sample through a glass-fiber filter. The filter and filtrate were wrapped in aluminum foil and placed in a cooler with ice until the samples could be frozen. The biomass sample was prepared in the same manner as the chlorophyll sample. Chlorophyll samples were mailed to SD DENR for analysis.

Fish were collected after other biological samples but before the physical habitat assessment so as to minimize disturbance to the fish community prior to sampling. We collected fish with the seining or electrofishing method, depending on the stream channel conditions. If the stream channel contained significant obstructions, such as aquatic vegetation or large rocks, we used the electrofishing method. Otherwise, the seining method was used. With either method, a single pass was conducted in an upstream direction. We made every effort to collect fish observed from all habitat types available within the sampled reach. In very small streams (<2 m wide) it was possible to sample most of the available habitat, but in larger streams, we meandered in an upstream direction between habitat types. Two to three personnel conducted the survey, depending on the method used. When using the electrofishing method, one person carried the backpack unit and operated the anode, and another person netted fish. When using the seining method, two people held each end of the net, and a third person lifted the net over any obstructions encountered along the stream reach. Fish survey results were recorded on a data sheet, including the specimen length, weight and species name. All live fish after processing were immediately returned to the stream, unless they are needed as voucher specimens. Voucher specimens of each fish species were retained for quality control and assurance purposes and deposition in the Natural Heritage Fish Reference Collection for South Dakota in the Department of Natural Resource Management at SDSU.

Raw Counts of Periphyton, Macroinvertebrates and Fish

Periphyton samples were identified and enumerated by Rithron Associates as per the same protocols used for SD DENR candidate reference sites. Macroinvertebrate samples were sorted, identified and enumerated in the Environmental Biology Laboratory at SDSU. Counts and relative counts of each taxon were based upon a 500 individual subsample drawn at random from the whole sample (U.S. EPA 2004). Fish identifications and counts were generally made in the field as samples were drawn from field gear. However, some species and small specimens required transport back to the laboratory for identification and enumeration. Macroinvertebrate and fish vouchers were retained and deposited into collections on the SDSU campus.

Metrics of Community Condition

Counts of individual periphyton, macroinvertebrate and fish taxa were used to estimate community measures (= metrics) which in turn were used to generate indices of biotic integrity (e.g., Barbour et al. 1999; Whittier et al. 2007). Metrics of community structure/abundance, diversity, guild structure, pollution tolerance and condition were calculated for each taxonomic group (see digital periphyton, invertebrate and fish data files submitted to SD DENR).

Many periphyton (72), macroinvertebrate (90) and fish (254) metrics were calculated from sample counts. While calculation procedures and values for all metrics were included in data sheets submitted to SD DENR, most were not able to pass our screening process (below) and will not be summarized here.

Selection of Optimal Metrics

Metrics of each taxonomic group were screened following procedures outlined in Whittier et al. (2007). This process sequentially eliminates metrics based upon value ranges, signal:noise ratio, discriminatory power, redundancy and overlap in metric values between targeted unimpaired and impaired sites.

The range test eliminated 19.5%, 11.1% and 5.5%, respectively of periphyton, invertebrate and fish metrics (Figure 2). Any metrics with >75% identical values or richness metrics with fewer than 3 species were eliminated in this step. Basically, metrics which did not vary significantly among sites were eliminated. The signal:noise ratio test eliminated metrics with low variation between sites relative to variation within a site. Those metrics displaying high variation among repeated samples at the same site were eliminated. The signal:noise test eliminated 70.7% of periphyton, 60.1% of invertebrate and 45.3% of fish metrics from IBI consideration. The discrimination test evaluates the ability of a metric to discriminate between sites known to be in good versus poor condition. This test eliminated 9.8% of periphyton metrics, 23.3% of invertebrate metrics and 35.4% of fish metrics. At this point, all but one periphyton metric had been eliminated. The redundancy test eliminates metrics which are highly correlated with one another. These are metrics which explain much of the same variation in community characteristics among sites. The redundancy test eliminated 4.4% of invertebrate and 0.4% of fish metrics. The overlap test evaluates the distribution of metric values between sites known to be in good condition and those in poor condition. Overlapping distributions suggest insufficient separation to discriminate good from poor sites. The overlap test eliminated 1.1% of invertebrate metrics and 13.4% of fish metrics.

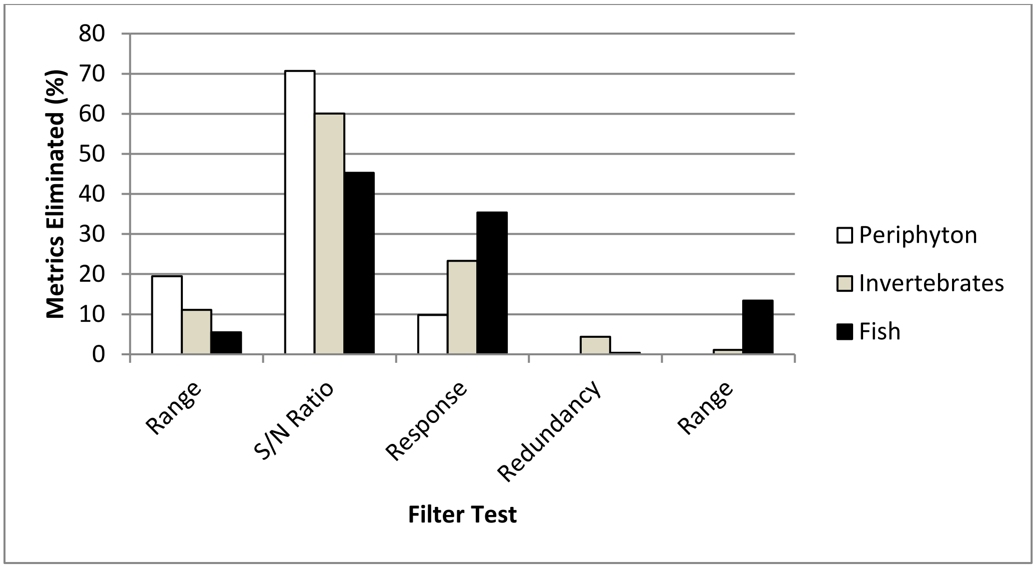


Figure 2. Comparative results of metric optimization for periphyton, macroinvertebrates and fish of the NGP ecoregion; percent of metrics eliminated at each step of the metric screening and optimization process.

Final invertebrate metrics passing all screening steps included the modified Hilsenhoff Biotic Index (-), percent abundance of climbers (+), percent abundance of insects (+) and Trichoptera generic richness (+). Final fish metrics included Centrarchidae species richness plus *Micropterus salmoides* (-), proportion of individuals which are nontolerant native invertivores (+), tolerant species richness (-), proportion of species which are lithophilic spawners (+), proportion of species which are alien fish (-) and the proportion of individuals which are native coolwater species (+). Invertebrate metrics represented tolerance, habit guild, composition and richness components of biotic integrity. Fish metrics represented composition, trophic guild, tolerance, reproductive guild, alien and habitat guild components.

Indices of Biotic Integrity for invertebrate and fish assemblages were derived from metrics which passed the screening process. Each of these optimal metrics was scored based upon the total distribution of values across all sites and sampling events. For positive metrics, values falling below the 10^{th} percentile were assigned a score of 0 while those falling above the 90^{th} percentile were assigned a score of 10. Metric values falling between the 10^{th} and 90^{th} percentiles were linearly interpolated to provide a continuous range of scores. Negative metrics were scored in a similar manner except that upper and lower thresholds were reversed. Scores for all metrics for a particular sampling event were summed and divided by the maximum possible score (= all metrics scoring 10) to generate a percentage or IBI score. Scores across all sites were adjusted so the highest score had a value of 100%. Site classes were assigned based upon quartile thresholds. Those sites with IBI scores equal to or above 75% were assigned a condition class of “Fully Supporting”. Those with scores falling between 50% and 75% were assigned the class “Slightly Impaired” and those with IBI scores falling below 50% were assigned the class “Not Supporting”.

Macroinvertebrate IBI scores ranged from a minimum of 24.3% to 100% ($\bar{x}$ = 52.5%) while fish IBI scores ranged from a minimum of 26% to 100% ($\bar{x}$ = 64.6%). The fish IBI consistently scored sites higher than the macroinvertebrate IBI (Figure 3). The average difference between fish and invertebrate IBI scores was 6% and the difference was significantly smaller from unimpaired sites as compared to candidate reference sites (KW ANOVA p = 0.030) but not different for the two other site classes.

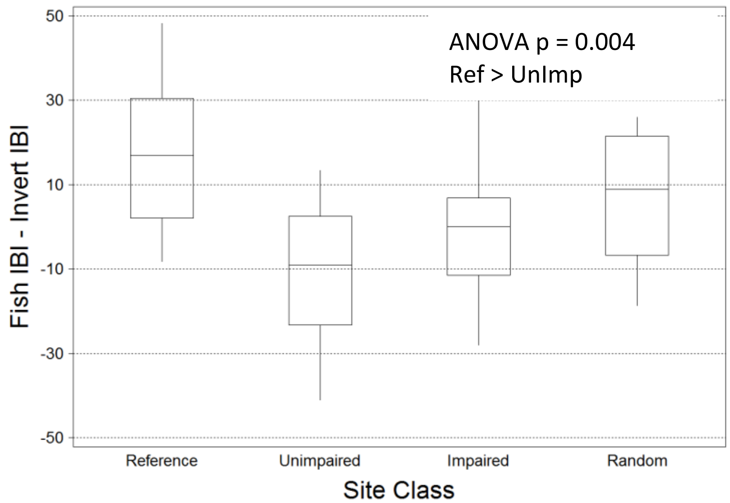


Figure 3. Difference between fish and invertebrate IBI scores by site class within the NGP of eastern South Dakota.

Both IBI's were able to successfully differentiate targeted unimpaired from targeted impaired sites (Figure 4). However, the difference between reference, targeted unimpaired and targeted impaired sites was much greater using the macroinvertebrate IBI than that observed using the fish IBI. Candidate reference site values were generally found to be lower than those observed from targeted unimpaired sites. This was especially noticeable from the invertebrate data.

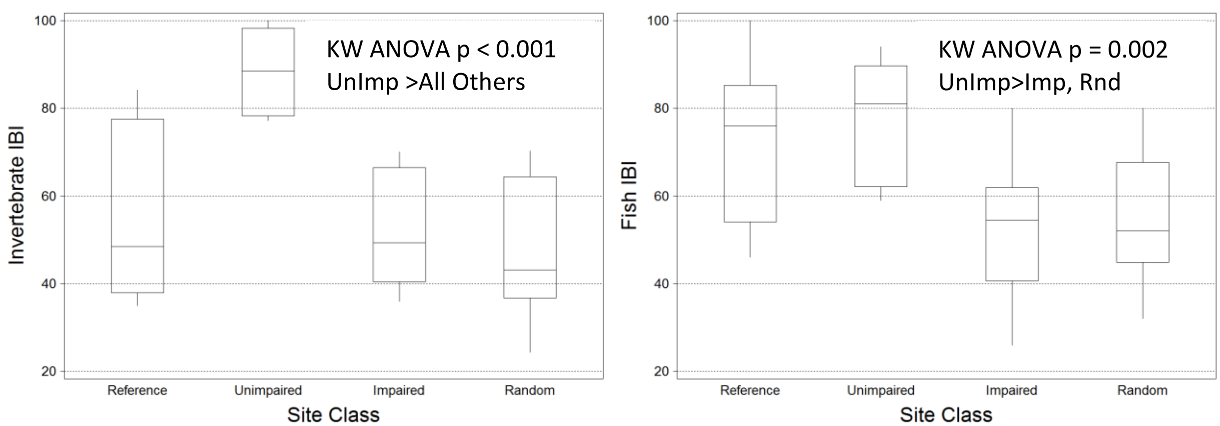


Figure 4. Comparison of invertebrate and fish IBI scores among stream classes of the NGP, eastern South Dakota.

IBI Relationships to Water Quality

We examined the rank correlation between fish and invertebrate IBI scores and selected water quality variables measured from each of our sites (Table 3). Reference site data was not utilized due to missing water quality and habitat data. Substrate size diversity, stream habitat diversity, sodium adsorption ratio, dissolved oxygen and total phosphorus concentrations were observed to be highly correlated with both invertebrate and fish IBI scores. Higher IBI scores were observed from sites with a mixture of substrate particle sizes, especially in sand and gravel size fractions. Higher IBI scores were also observed from stream reaches with a mixture and larger numbers of pools, runs and riffles versus uniform habitat throughout the reach. Lower IBI scores were observed from stream reaches with higher sodium adsorption ratios and total phosphorus concentrations.

Table 3. Spearman rank correlations between invertebrate and fish IBI scores and selected water quality and habitat measurements from targeted unimpaired, targeted impaired and random wadeable streams of the NGP, eastern South Dakota, 2010-2011. Candidate reference site data was not included in this analysis due to the high number of missing observations.

Parameter	**Invertebrate IBI**		**Fish IBI**	
	rho	p	rho	p
Substrate Size Diversity (H’)	0.487	0.007	0.479	0.008
SAR	-0.508	0.005	-0.387	0.035
Dissolved O_2	0.685	<0.001	0.486	0.007
Total P	-0.667	<0.001	-0.594	<0.001
N:P Ratio	0.610	<0.001	0.436	0.017
Channel Habitat Diversity (H’)	0.667	<0.001	0.662	<0.001

Invertebrate and fish IBI scores were both found to display a negative logarithmic relationship with total phosphorus concentrations (Figure 5). Linear regressions computed for both invertebrate and fish IBI’s relative to log transformed phosphorus were found to be significant. Log transformed total phosphorus explained between 27% and 36% of biotic integrity. Slopes and intercepts of both relationships were similar.

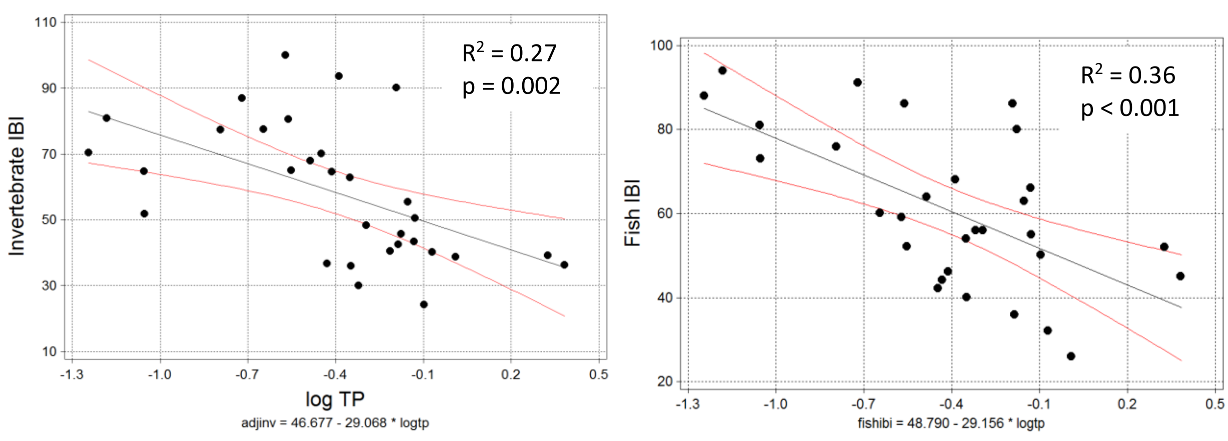


Figure 5. Relationship between invertebrate and fish IBI scores and total phosphorus concentrations observed in TarI, TarUI and Random wadeable streams of the NGP, 2010-2011.

Validation of Candidate Reference Sites

In general, we found poor agreement in biotic integrity between candidate reference and targeted unimpaired sites (Figure 4). A systematic analysis approach was used to identify and validate reference sites. We employed two-way cluster analysis using combined invertebrate and fish site mean counts to identify sites with similar community structure. That analysis revealed three main groupings of sites (Figure 6). Cluster group A was comprised of all but one targeted unimpaired, two targeted impaired and one random site. Cluster group B was comprised of a mixture of targeted impaired, random and candidate reference sites. This cluster appeared to include those sites in the worst condition. Cluster group C included most of the candidate reference sites, two random sites and one targeted unimpaired site.

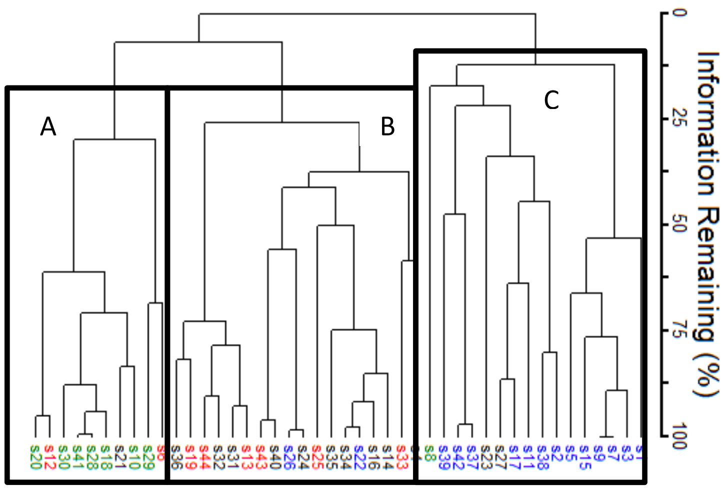
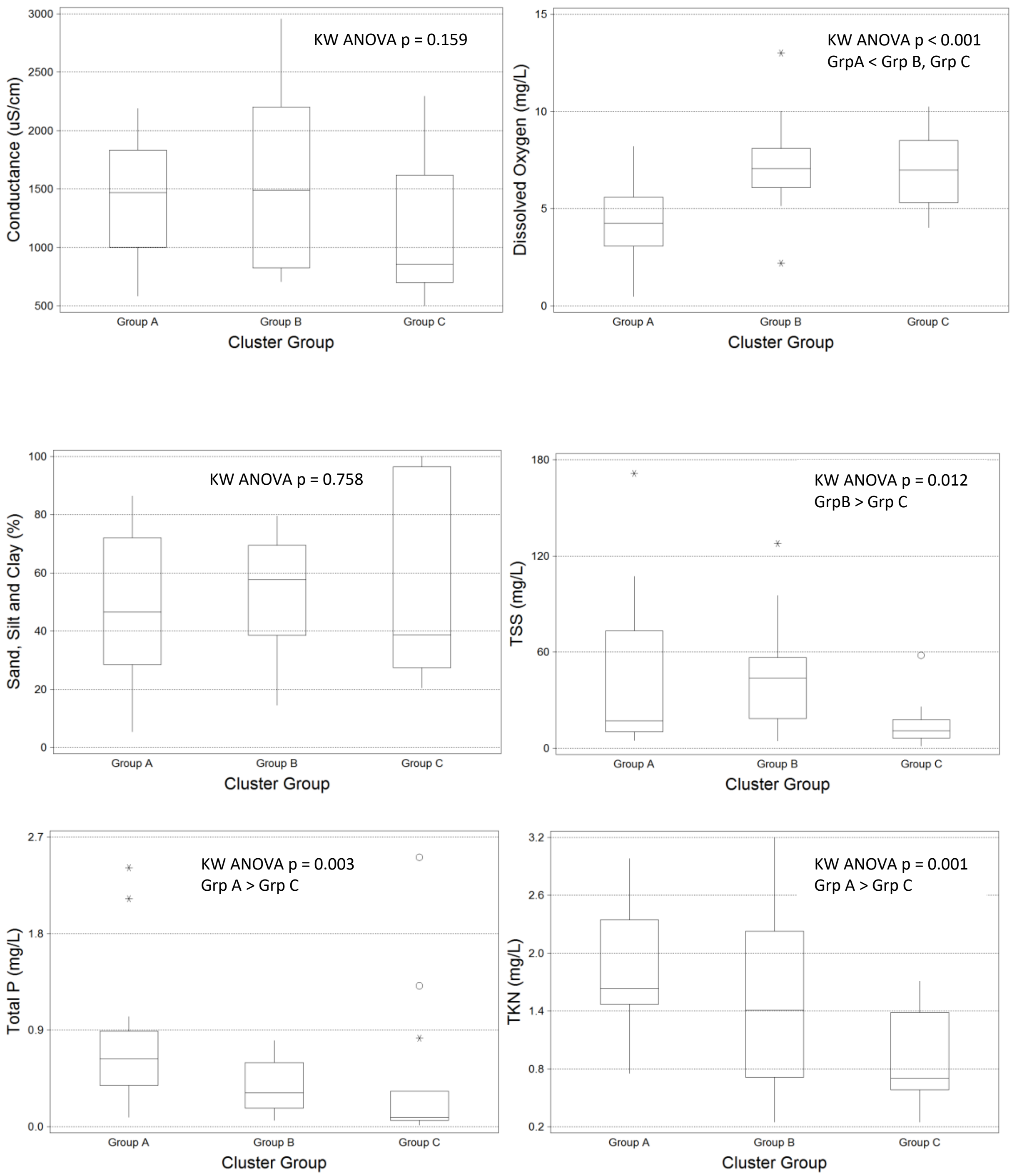


Figure 6. Cluster analysis dendogram showing groupings of sites based upon similarities in species composition and abundance for both invertebrates and fish. Blue – candidate reference sites, Green – targeted unimpaired sites, Red – targeted impaired sites, Black – random sites.

We added the cluster class variable to our IBI and habitat data sets and performed KW ANOVA to examine differences among the three cluster groupings. Our candidate reference site cluster grouping displayed better water quality and habitat conditions than the targeted impaired grouping (Figure 7). In many cases, the targeted unimpaired grouping displayed conditions equivalent to or somewhat lower than the candidate reference group.


KW ANOVA p = 0.159
Conductance (uS/cm)
3000
2500
2000
1500
1000
500
Group A
Group B
Group C
Cluster Group
KW ANOVA p < 0.001
GrpA < Grp B, Grp C
Dissolved Oxygen (mg/L)
15
10
5
0
Group A
Group B
Group C
Cluster Group
KW ANOVA p = 0.758
Sand, Silt and Clay (%)
100
80
60
40
20
0
Group A
Group B
Group C
Cluster Group
KW ANOVA p = 0.012
GrpB > Grp C
TSS (mg/L)
180
120
60
0
Group A
Group B
Group C
Cluster Group
KW ANOVA p = 0.003
Grp A > Grp C
Total P (mg/L)
2.7
1.8
0.9
0.0
Group A
Group B
Group C
Cluster Group
KW ANOVA p = 0.001
Grp A > Grp C
TKN (mg/L)
3.2
2.6
2.0
1.4
0.8
0.2
Group A
Group B
Group C
Cluster Group

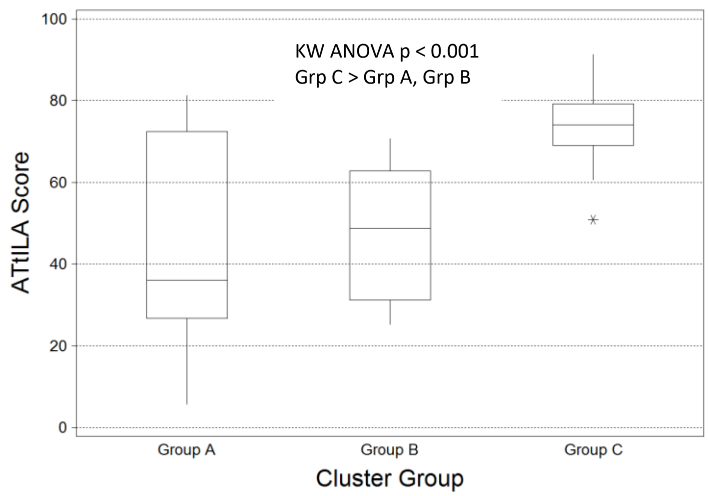


Figure 7. Physical and chemical habitat differences among biological site cluster groupings of wadable, perennial stream sites within the NGP of eastern South Dakota, 2010-2011.

We examined the distribution of invertebrate and fish IBI scores for the candidate reference cluster (Group C) and employed the third quartile value as a threshold for validating reference sites. Our validated reference sites (Table 4) all had mean invertebrate IBI scores greater than or equal to 77.6% and fish IBI scores greater than or equal to 85.3%. Three of these sites were candidate reference sites and four were targeted unimpaired sites.

Table 4. Validated wadable stream reference sites of the NGP in eastern South Dakota.

ComID	Site Class	Latitude	Longitude	Mean Invertebrate IBI	Mean Fish IBI
4081850	Cand Ref	45.46340	-97.04391	80.7	100
4084690	Cand Ref	45.22356	-96.79473	77.6	92
4085112	Cand Ref	45.12514	-96.83950	83.3	85.3
4112142	Tar Unimp	44.78802	-96.46658	80.7	94
4112768	Tar Unimp	44.78798	-96.46683	87	91
12668554	Tar Unimp	43.40183	-97.81482	100	86
12715986	Tar Unimp	45.34297	-97.92678	90.1	86

As a group, validated reference sites scored significantly above targeted impaired and random sites using both invertebrate and fish IBI's (Figure 8).

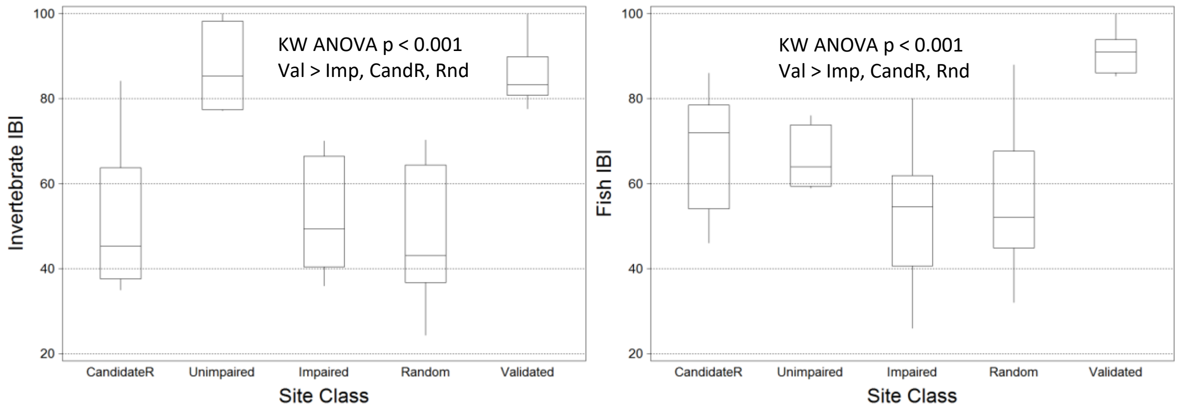


Figure 8. Invertebrate and fish IBI scores by site class within the NGP of eastern South Dakota, 2010-2011. CandR – candidate reference, TarG – targeted unimpaired, TarB – targeted impaired, Rnd – random, PropR – validated reference sites.

Calculation of Observed/Expected Ratios

Observed/Expected ratios and RIVPACS models were not developed from our data set due to insufficient number of sites. As new NGP sites are assessed using these IBI tools, we are hopeful that a sufficient number of sites and repeated visits will develop, allowing development of these tools.

Stream Assessment Toolbox

Part of our effort on this project included development of tools to facilitate collection of stream assessment data, calculation of regional IBI scores and stream condition classes and display of regional patterns in biotic integrity.

Contemporary stream assessment methodology requires data collection on a large number of stream attributes, including water chemistries, flow, stream and riparian habitat and biota. Field teams spend tremendous time in the field assessing each site, returning with large datasets which must be entered into a digital database. We adopted and modified a digital datasheet designed for field use on Toughbook computers in the field. This datasheet was developed in EXCEL allowing use on a variety of computer platforms. Field assessment variables were divided into logical groupings based upon the order in which they are collected in the field and efficient allocation of person-hours. Formulas were built into the spreadsheet facilitating calculation where necessary. The final sheet draws measurements from individual sheets to create a single record of field observations for a particular site and sampling event. A separate spreadsheet has been programmed to extract these records from user-defined site assessment files to create a combined set of stream assessment data. The digital data template and data extraction file combined save multiple hours of data entry and summary required before analysis can begin. The Toughbook computer and associated files allow environmental scientists to perform assessments in the field and return with working data files ready for analysis. Our field data template and data extraction file are included in the digital deliverables from this effort.

Invertebrate and fish master taxa lists have been created, providing a taxonomic breakdown of genera and species observed from the NGP and their ecological attributes. This master taxa list provides the template against which metrics are calculated for both IBI's. Both invertebrate and fish spreadsheets developed for this project provide a guide for future assignment of taxa to appropriate guilds and tolerance values. We also developed a separate IBI calculator spreadsheet which allows environmental scientists to enter the stream ID, date of collection and values for optimal IBI metrics for invertebrates and fish. Once metric values have been entered, formulas built into the spreadsheet will calculate the NGP IBI scores and assign stream condition classes. The IBI calculator spreadsheet is already populated with data from our NGP project, allowing environmental scientists to simply add new records and draw on the entire dataset for statistical summary. Our invertebrate and fish master taxa lists, IBI development file and IBI calculator are included in the digital deliverables from this effort.

Managers must convey their monitoring information to administrators and the general public. This communication often requires that data are summarized and simplified to convey those points most important to the target audience. We employed ArcGIS and kriging tools to develop regional maps of invertebrate and fish IBI patterns and ATtILA watershed condition scores within the NGP (Figure 9). These maps are coarse and their accuracy naturally improves with larger numbers of sampling sites covering this broad regional area. However, they do provide a means of examining broad spatial patterns and may facilitate allocation of monitoring and management resources to those areas in most need of help. To improve the accuracy of our invertebrate IBI map, we supplemented data collected from this effort with that collected from our previous intermittent headwater stream project. The fish map was created only with data from this effort. The ATtILA map was created with the entire target population (n=2,546). ArcGIS files, directions we followed to generate kriging maps and the maps we generated have been submitted with the digital deliverables from this project. We expect the accuracy of these maps to improve with time as DENR adds additional monitoring sites within the NGP.

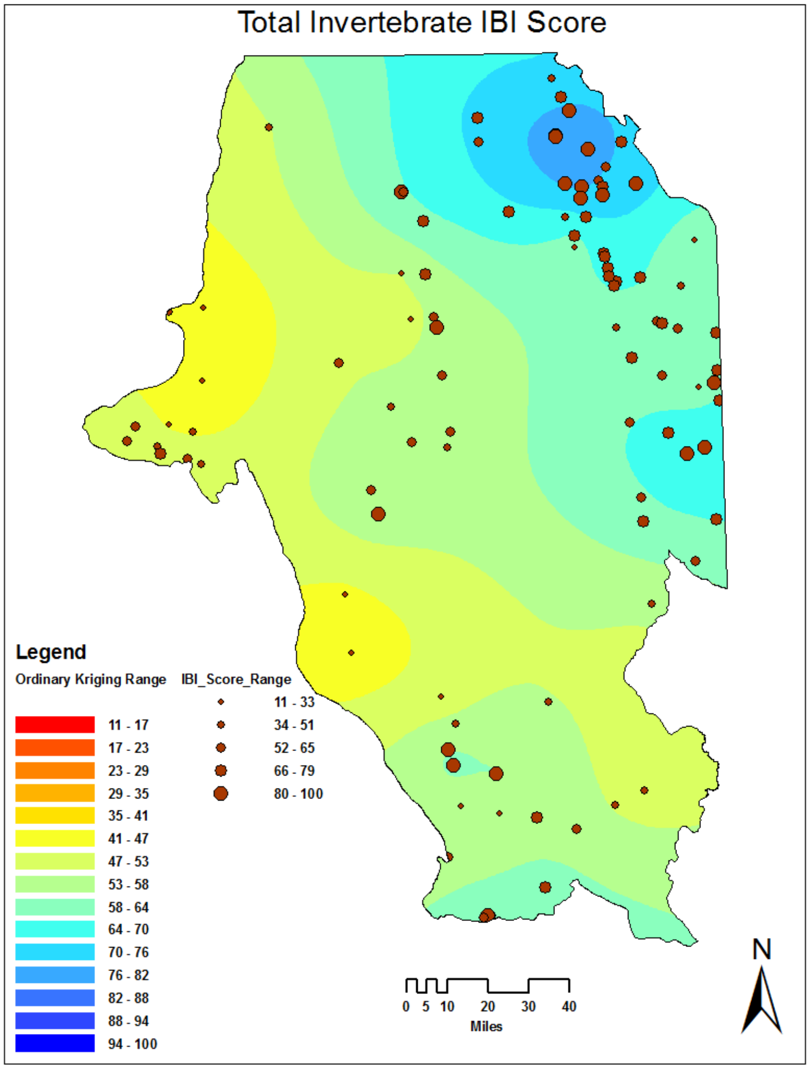


Figure 9. Kriging map generated from combined intermittent headwater stream and larger wadable stream invertebrate data for the NGP, eastern South Dakota. Bands of color on this map represent modeled IBI expectations within the NGP. Individual points are sampling sites which vary in size according to the mean IBI score for that site. Similar Kriging maps were generated using fish IBI scores and ATtILA watershed condition scores.

Experimental Stream Results

Ecosystem Effects of Native Coolwater Guild Fishes

Fish can have 'large and pervasive' effects on stream ecosystems (Vanni 2008). We proposed using experimental streams to compare the ecosystem effects of 3 species belonging to the native coolwater species guild in the Northern Glaciated Plains with 1 species belonging to the tolerant guild and with a fishless control. Central Stoneroller *Campostoma anomalum*, White Sucker *Catostomus commersoni* and Iowa Darter *Etheostoma exile*, belong to the native coolwater guild, and each of these three species represents a unique functional feeding guild, with potentially different effects on ecosystem structure and function. All three are benthic fishes; Central

Stoneroller is an herbivore, White Sucker is an omnivore, and Iowa Darter is an insectivore. Brassy Minnow *Hybognathus hankinsoni* is a tolerant guild fish and represented a potential future scenario in which nontolerant coolwater fishes are extirpated from a degraded stream. We predicted that as a result of climate change and other persistent anthropogenic stressors, in streams that were once populated with Central Stoneroller, White Sucker, and Iowa Darter, species loss may significantly alter biotic integrity and diminish ecosystem goods and services provided by streams.

Experimental streams simplify complex communities and allow researchers to focus on a particular interaction with replicated treatment combinations (Matthews et al. 2006). These systems are functionally and physically very similar to pool-riffle complexes in nearby natural streams (Bertrand et al. 2009; Gido and Matthews 2001; Gelwick and Matthews 1992). Experimental stream studies have successfully evaluated many mechanisms operating in stream ecosystems, examples of which are given along with their design in Matthews et al. (2006). An experimental approach allowed us to isolate the effects of individual species with replication.

We assessed effects of fishes belonging to the native coolwater guild during summer 2013. We collected preliminary data in fall of 2012 and found that even during cold fall water temperatures, Central Stoneroller, significantly moreso than White Sucker or Iowa Darter, altered trajectories of ecosystem structure and function, which influences material transport and storage in streams. We maintained water velocity at a constant rate of 0.15 m/s. Central Stoneroller, White Sucker, and Iowa Darter for the experiment were collected from Six-Mile Creek and other nearby streams in Brookings County, South Dakota. Fish were measured (total length; mm) and stocked in the experimental streams at ‘natural’ local densities of 5-10 g m^{-2} (e.g., Thompson 2008) on day 0 (26 June 2013). Each treatment was replicated 5 times, except White Sucker, for which only 4 replicates were possible, in a randomized design. Every other week, we measured ecosystem function with wholestream metabolism (NEP; Murdock et al. 2010), and once-per-month we measured nutrient retention (TN and TP). Every other week, we measured ecosystem structure with algal filament length and algal biomass (benthic chlorophyll *a*). After 7 weeks, we ended the experiment and removed all fish. Response variables were compared among treatments and control using repeated measures ANOVA (SPSS version 21).

Native coolwater guild fishes affected ecosystem structure. Algal filaments in stream pools were shortest in Central Stoneroller treatments, followed by Brassy Minnow, no fish, and Iowa Darter treatments respectively ($F_{4,19} = 7.87$, $P < 0.01$; Figure 1). Algal filament lengths in the White Sucker treatment were intermediate and not significantly different from any of the other fish or no fish treatments in post hoc comparisons. Riffle algal filament lengths were similar among treatments ($F_{4,19}$ = 0.73, P = 0.58).

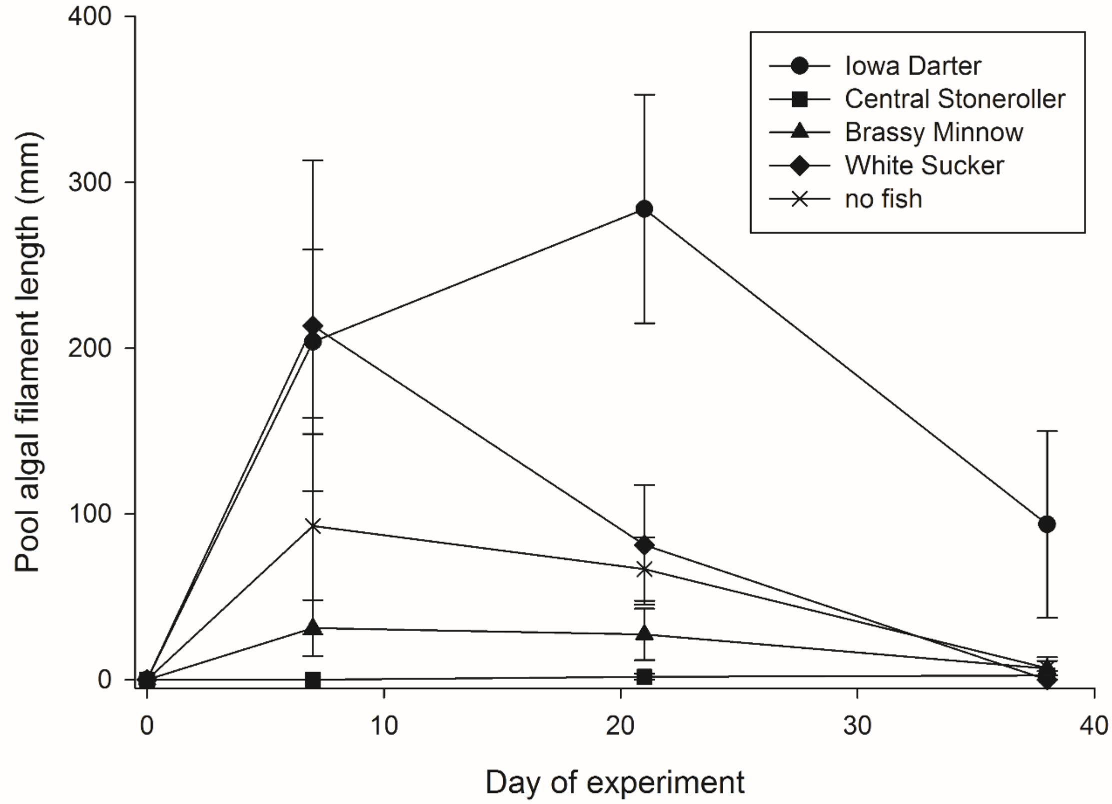


Figure 1.—Mean algal filament length measured in pools of experimental streams at the Dakota Ecosystem Studies Laboratory, Brookings, SD, during June-August 2013.

Algal biomass was similar among treatments (pools: $F_{4,18}$ = 1.92, P = 0.15; riffles: $F_{4,18}$ = 2.20, P = 0.11).

There was no evidence to suggest that individual species within the native coolwater guild had distinct effects on ecosystem function. Net ecosystem productivity was similar among native coolwater guild fish treatments, tolerant guild fish treatments, and a no fish control ($F_{4,2}$ = 2.75, P = 0.28). Nutrient retention was also similar among treatments and control (TN: $F_{4,19}$ = 0.91, P = 0.48; TP: $F_{4, 19}$ = 0.26, P = 0.90).

Individual fishes that comprise the native coolwater guild are not ecologically redundant. If climate change and other persistent anthropogenic stressors result in species loss within this guild, as detected by monitoring of biotic integrity, we can expect diminished ecosystem goods and services provided by streams. Whereas both Brassy Minnow and Central Stoneroller are benthic grazers, the tolerant Brassy Minnow does not graze algal filaments as effectively as the nontolerant Central Stoneroller. When Central Stoneroller maintain short algal turfs, photosynthetic biomass is converted into fish biomass and retained in the local stream reach, rather than self-shading, senescing, and elevating biological oxygen demand as it drifts downstream. Future analyses will compare gross primary production corrected for daily solar irradiance, respiration, and invertebrate community structure among treatments. Ongoing experiments are aimed at addressing the mechanisms that could drive extirpations in prairie stream ecosystems (critical swimming velocities, jumping ability).

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