

Selection and Validation of Regional Reference Sites and Development of Indices of Biotic Integrity for the Northwestern Great Plains Ecoregion

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 to include evaluation of physical, chemical and biological integrity. Established reference site biological data are needed to establish beneficial use support. Contemporary approaches used to assess and monitor biotic integrity require the establishment of a biological condition gradient which models biological responses along a gradient of increasing human-induced stress (EPA 2011). The State has previously engaged in efforts to identify habitat and biological reference sites and develop indices of biotic integrity for eastern South Dakota streams (Troelstrup 2010; Bertrand and Troelstrup 2013). The State has further identified the following needs relative to future uses of biological monitoring and assessment data: (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 study extended biotic integrity development into streams of the state's largest ecoregion, the Northwestern Great Plains (NWGP).

The NWGP ecoregion occupies nearly one-half of the state's surficial drainage area and is located entirely west of the Missouri River in South Dakota (Figure 1, Bryce et al. 1998). The ecoregion includes 10 LIV ecoregions which vary in potential natural vegetation, land form, soils and land use. Climate within this ecoregion is semiarid and natural vegetation is primarily mixed and short grass prairie species. Soils within this ecoregion are derived from shale, siltstone and sandstone. Topography is generally flat to rolling, although areas of buttes, badlands and river breaks provide greater relief. Much of the ecoregion is managed for cattle grazing, but spring wheat and alfalfa are also common crops. Larger areas of native grasslands are present. Agriculture is limited by erratic precipitation with mean annual accumulations ranging from 33 to 50 cm.

South Dakota DENR has identified a population of wadeable perennial streams in need of long-term monitoring and management within the NWGP. Included within this population are random sites selected for the U.S. EPA National Stream Assessment (NSA). We supplemented data collected from these NSA sites with data collected from 65 additional stream sites (Figure 1). Water quality, habitat and biological data collected from our sites were examined statistically to identify sites falling within the upper 10th percentile of site condition based upon field measurements. These candidate reference sites were validated against local habitat quality, GIS

(ATtILA) watershed assessment and multivariate analysis. We developed Indices of Biotic Integrity using invertebrate and fish data providing an integrated view of biological use attainment. A habitat quality index was developed to facilitate validation of indices of biotic integrity. Multivariate relationships between invertebrate community structure, habitat and water quality variables were completed to further validate IBI class assignments. Indices of biotic integrity, habitat quality indices and reference sites identified further the state's efforts to evaluate biological use criteria in support of assigned beneficial uses. Biotic integrity data will also be important in justification of proposed standards changes for those streams exhibiting what is believed to be natural elevation in selected water quality attributes.

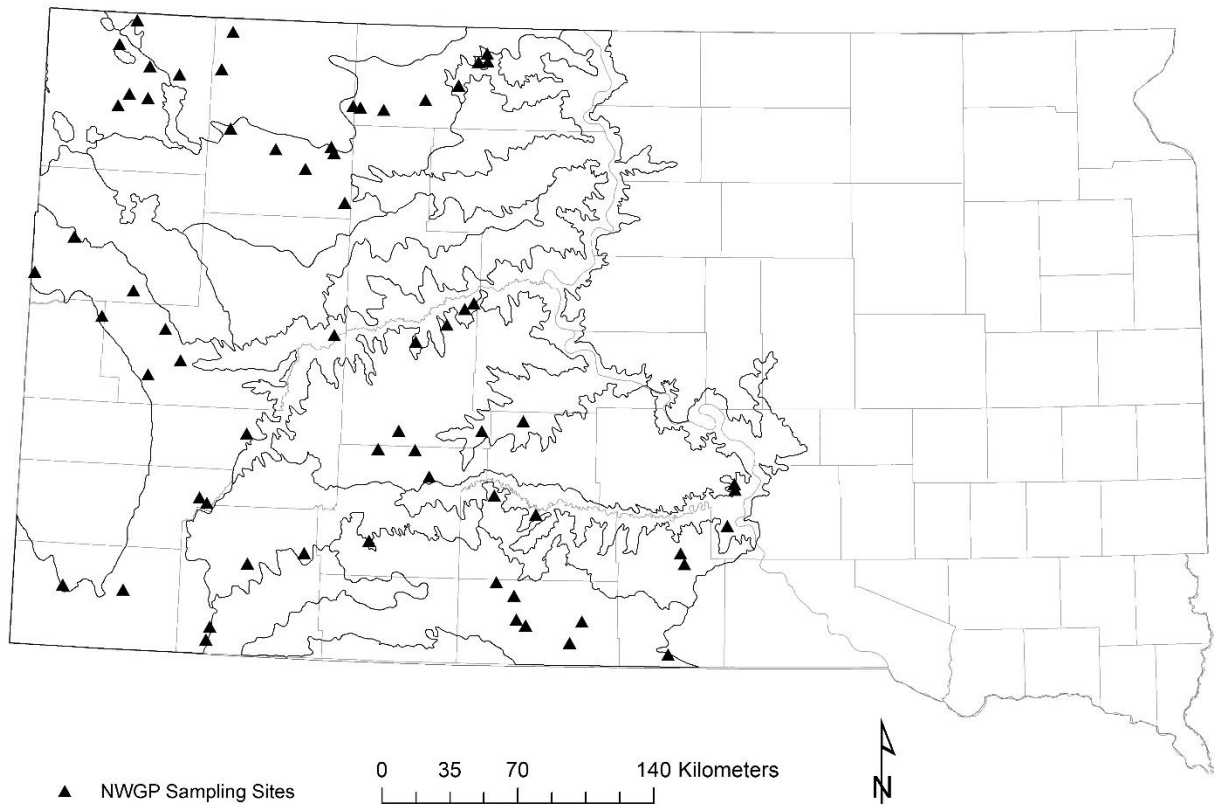


Figure 1. The Northwestern Great Plains ecoregion and wadable stream sites sampled to develop indices of biotic integrity and validate reference sites in western South Dakota. Lighter lines on map denote LIV ecoregion boundaries.

Project Results by Objective and Task

Objective 1: Employ a stratified (by LIV ecoregion), random selection of wadeable, perennial stream reference sites from within the NWGP.

Establishing the Target Population

U.S. EPA and SD DENR have engaged in the National Stream Assessment. This assessment draws on a large population of wadeable, perennial streams from which probability-based selections were made. We added 65 (including replacement sites) additional stream sites to those already identified for assessment by SD DENR (Figure 1). Because flow intermittency is more prevalent in western South Dakota, we utilized stream classification provided in NHDPlus to randomly select candidate perennial, wadable sites within each LIV ecoregion and then visited each site individually to confirm evidence of perennial flow during the first growing season. This effort expanded DENR's reference site development effort into the Northwestern Great Plains.

Drawing a Stratified, Random Sample

This study was focused on development of IBI's and candidate reference sites for perennial flowing, wadable streams of the NWGP. These sites were drawn randomly in proportion to the total stream miles within each LIV ecoregion. Sites located immediately below an impoundment or natural basin were excluded. If we were unable to get permission to sample a site, another was chosen at random from the same LIV ecoregion. This provided us with a probability-based random sampling of wadeable stream sites, allowing characterization of stream condition within each LIV ecoregion and across the NWGP as a whole. Any stream which appeared to demonstrate intermittency was replaced with another random site within the same LIV ecoregion. In addition, any selected site for which we could not get permission to sample was replaced with another random site within the same LIV ecoregion. The final list of sampled perennial stream sites (Figure 1) has been provided as a digital deliverable ([EXCEL – NWGP All Site Lat Longs](#)). This file also provides additional site attribute information which appears with the geodatabase provided as an additional digital deliverable. Watershed areas for our sampling sites ranged from 9.1 to 19,828 ha (Median = 318.5 ha).

Obtaining Landowner Permissions for Sampling

Landowner permissions were obtained for access to sampling sites based upon ownership records and communication with local natural resource agencies. A listing of landowners contacted is provided in the digital deliverable ([EXCEL – NWGP All Site Lat Longs](#)). Landowner permission was lost on four sites between the 2014 and 2015 sampling seasons. Those sites were replaced with four random sites from within the same LIV ecoregion, providing a total of 65 sites sampled during 2014 and 2015. This task was completed in 2013 and again at the end of the first sampling season in 2014.

Conducting Site Visits and Validating Candidate Sites

Following initial GIS-based selection of sampling sites in 2013, sites were visited and assessed visually for evidence of intermittency. Any site not demonstrating well defined channel features or showing evidence of intermittency was replaced with another random site within the same

LIV ecoregion. Every effort was made to interact with local landowners to facilitate intermittency determination.

Selection of Replacement Sites

Any site showing signs of intermittency or for which landowner permissions could not be attained were replaced with another random site within the same LIV ecoregion (see above).

Objective 2: Collection and Presentation of Water Quality Data from Selected Stream Sites

Water quality, physical habitat and biological samples were collected from a stratified, randomly generated sample of 65 total sites (including replacement sites) during the growing seasons of 2014 and 2015 (Figure 1). Each of these sites was sampled only once during each growing season and only under conditions of below bankfull hydrology. Samples were collected following Standard Operating Procedures (SOP) for Field Samplers, Volume II, Biological and Habitat Sampling (SD DENR, 2005).

Water Quality Data Collection

Variables linked to water quality criteria in support of beneficial stream uses in South Dakota were measured from each sampling site during the period June to August 2014 and 2015 (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 selected for quality assurance and quality control (QA/QC). These QA/QC samples included a duplicate and blank. 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 at random and targeted wadeable stream sites.

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 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
Dissolved Oxygen	Multiparameter Sonde	---	---	SDSU
Conductance	Multiparameter Sonde	---	---	SDSU
pH	Multiparameter Sonde	---	---	SDSU
Water Temperature	Multiparameter Sonde	---	---	SDSU
Turbidity	Nephelometer	---	---	SDSU

Water Quality Summary

Water quality samples were collected from 65 sites and 121 sampling events during the growing seasons of 2014 and 2015. All water quality data and quality assurance/quality control data are presented separately in a digital deliverable ([EXCEL – NWGP Water Chemistries](#)).

Our results indicated a total of 131 standards exceedances during 2014 and 2015 (Table 2). The majority (93) of those exceedances were for high levels of SAR. However, we also observed exceedances in relation to specific conductance, dissolved oxygen, sulfate, total dissolved solids, total suspended solids and water temperature. The greatest number of exceedances per visit were observed for LIV ecoregions 43a, 43c and 43h. LIV ecoregion 43i had the lowest number of exceedances.

Most of the water quality parameters we measured displayed significant differences among major river basins and LIV ecoregions (Table 3). The only parameters which did not vary among basins and LIV ecoregions were ammonia-N, water temperature and total suspended solids. The macroinvertebrate IBI (MIBI) was most highly correlated with SAR ($\rho = -0.44$) while the fish IBI (FIBI) was most highly correlated with dissolved sulfate ($\rho = -0.35$) and total suspended solids ($\rho = 0.33$).

Table 2. Observed standards exceedances by LIV ecoregion from water quality data collected in the NWGP, 2014-2015.

LIV Eco	Cond	DO	SAR	SO4	TDS	Temp	TSS	Total Exceedances	Exceedances Per Visit
43a	2	2	10					14	1.4
43c	8	3	29		2		2	44	1.5
43e			10				2	12	1.2
43f		1	8				1	10	1.0
43g			12	2	2	2	5	23	1.0
43h			6				2	8	1.3
43i		1					1	2	0.1
43j			18					18	1.0
Totals	10	7	93	2	4	2	13	131	1.5

Table 3. Summary statistics for water quality attributes within the NWGP ecoregion of South Dakota, 2014-2015. Results of KW ANOVA comparing site means among major river basins and LIV ecoregions within the NWGP. Spearman rank correlations between site means for IBI scores and each water quality variable.

Parameter			Basin	LIV Eco	MIBI rho	FIBI rho
Alkalinity (mg/L)	n	65				
	\bar{x}	320	p<0.01	p<0.01	-0.26	-0.12
	X ₅₀	283				
	X _{min}	130				
	X _{max}	823				
Conductance (μ S/cm)	n	65				
	\bar{x}	1954	p<0.01	p<0.01	-0.39	-0.28
	X ₅₀	1644				
	X _{min}	324				
	X _{max}	7285				
Diss. O ₂ (mg/L)	n	65				
	\bar{x}	8.1	p=0.40	p=0.12	0.08	0.30
	X ₅₀	8.3				
	X _{min}	2.6				
	X _{max}	11.3				
Diss SO ₄ (mg/L)	n	65				
	\bar{x}	777	p<0.01	p<0.01	-0.31	-0.35
	X ₅₀	467				
	X _{min}	5				
	X _{max}	4110				
NH ₃ -N (mg/L)	n	65				
	\bar{x}	0.033	p=0.20	p=0.06	-0.30	-0.05
	X ₅₀	0.025				
	X _{min}	0.025				
	X _{max}	0.213				
NO ₃ -N (mg/L)	n	65				
	\bar{x}	0.76	p<0.01	p<0.01	-0.02	0.01
	X ₅₀	0.20				
	X _{min}	0.10				
	X _{max}	6.00				
SAR	n	65				
	\bar{x}	34.5	p<0.01	p<0.01	-0.44	-0.14
	X ₅₀	27.2				
	X _{min}	0.8				
	X _{max}	135.9				
TDS (mg/L)	n	65				
	x	1243	p<0.01	p<0.01	-0.29	-0.26
	X ₅₀	1008				
	X _{min}	234				
	X _{max}	4381				
Total P (mg/L)	n	65				
	\bar{x}	0.21	p<0.01	p<0.01	-0.16	-0.05
	X ₅₀	0.15				
	X _{min}	0.02				
	X _{max}	1.02				

Table 3 (continued)

Parameter			Basin	LIV Eco	MIBI rho	FIBI rho
TSS (mg/L)	n	65				
	\bar{x}	85.0	p=0.06	p=0.03	-0.20	0.33
	x ₅₀	38.5				
	x _{min}	4.1				
	x _{max}	898.9				
Water Temp (°C)	n	65				
	\bar{x}	22.2	p=0.49	p=0.89	-0.22	0.03
	x ₅₀	22.4				
	x _{min}	12.6				
	x _{max}	27.5				
pH	n	65				
	\bar{x}	--	p<0.01	p<0.01	-0.22	0.11
	x ₅₀	8.09				
	x _{min}	7.42				
	x _{max}	8.81				

Macroinvertebrate and Fish Collections

Reach-wide composite macroinvertebrate samples were collected at each site from eleven transects (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 two possible methods were used to collect macroinvertebrate samples. A sub-sample was collected from each transect with a D-frame, 500- μ m 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 the 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 transect sample, 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 (500 μ m) to remove fine sediment, placed into pre-labeled container(s), and preserved with 95% ethanol (EXCEL – NWGP Invertebrate Data; Kuehl 2017).

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. 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. Fish less than approximately 25 mm in total length were not counted as part of the catch. We minimized handling stress by using a portable live well during collection, quickly sorting fish into wet containers, and replacing their water supply. All fish that were alive after processing were immediately returned to the stream, unless they were needed as voucher specimens. Voucher specimens of each fish species were retained for quality control and assurance purposes and deposited into the State Fish Collection and Database (SDSU). For fish that were identified with certainty to species level, several individuals of each species were preserved in 10% formalin solution. All fish that could not be identified to the species level in the field were preserved in a separate container in 10% formalin solution. These were returned to the laboratory for closer inspection and identification. Fish counts and IBI data sheets are presented separately in a digital deliverable ([EXCEL – NWGP Fish Data; Kaiser 2017](#)).

Physical Habitat Data Collection

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 at eight locations across each transect and assessed for substrate size using a gravelometer. Measurements along the channel cross-section at each transect 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 also made at each transect. Measures of canopy cover were collected from six stations at each transect using a spherical densiometer. Finally, the number of large woody debris (LWD) were tallied for the entire reach. Length and diameter of all pieces of LWD (> 5 cm diameter) were measured to calculate the volume of LWD within the reach. All field data were entered onto digital field sheets in the field and back-up files created

each night after sampling. Individual site digital data sheets are included separately along with the merged physical habitat data sheet ([EXCEL – NWGP Physical Habitat Data](#)).

We selected a subset of habitat parameters which best summarized habitat quality at each site (Table 6). These variables included entrenchment ratio, bank height, bank angle, percent length of bank eroded, length of overhanging vegetation, length of undercut banks, total riparian canopy cover, number of large wood debris in channel, percent of channel area covered by macrophytes and substrate particle size diversity. Substrate particle size diversity was calculated by applying the percentages of different substrate size classes at each sampling site to the Shannon-Weiner diversity index (Washington 1984). This index provides a measure of substrate diversity (variety of different size classes) and proportional representation (evenness) of classes at each site.

A principle components analysis was applied to selected habitat variables (above). Four principle components explained >70% of the variation in habitat conditions among our 65 sampling sites (Table 4). The variable with the highest loading on each of these four principle components was selected for habitat scoring. These variables included bank angle and percent macrophytic cover (PC1), total canopy cover (PC2), number of pieces of large woody debris (PC3) and substrate particle size diversity (PC4) (Table 5).

Table 4. Results of principle components analysis on stream habitat variables collected from wadable, perennial streams of the NWGP ecoregion in western South Dakota, 2014-2015.

Component	Eigenvalue	Percent Variance (%)	Cumulative Variance (%)	Highest Loading Variables
PC1	2.67748	26.8	26.8	Bank Angle, Percent Macrophytes
PC2	1.79870	18.0	44.8	Total Canopy Cover
PC3	1.51667	15.2	59.9	Large Woody Debris
PC4	1.06236	10.6	70.6	Substrate Size Diversity

Linearly interpolated scores were assigned to each of the above habitat variables as per Whittier et al. (2007) and the sum of these scores were rescaled to fall between 0 and 100. HQI scores ranged from 10.5 to 75.1 (Median = 37.6) across the NWGP. HQI scores were found to vary significantly among major river basins (KW ANOVA p=0.031), but not LIV ecoregions (KW ANOVA p=0.113).

Table 5. Summary statistics of optimal habitat metrics and HQI scores for wadable streams of the NWGP in western South Dakota.

Metric	Median	Range
Bank Angle (°)	30.7	8.5 – 62.8
Percent Macrophyte Cover*	9.9	0.0 – 102.2
Total Riparian Canopy Cover (%)	16.3	0.0 – 98.2
Pieces of Large Woody Debris	0.5	0 – 10
Substrate Size Diversity (H')	1.67	0.00 – 2.14
Habitat Quality Index (HQI)	37.6	10.5 – 75.1

*values represent the sum of area covered by emergent + submergent macrophytes

Table 6. Selected stream habitat variables directly linked to aquatic biotic integrity from NWGP streams of western South Dakota, 2014-2015. Results of KW ANOVA comparing site means among major river basins and LIV ecoregions within the NWGP. Spearman rank correlations between IBI scores for macroinvertebrates (MIBI) and fish (FIBI) and site means for each habitat variable.

Parameter			Basin	LIV Eco	MIBI rho	FIBI rho
Canopy Cover (%)	n	65	p=0.01	p=0.46	0.21	-0.07
	\bar{x}	22.0				
	x ₅₀	16.3				
	x _{min}	0				
	x _{max}	98.2				
Discharge (cms)	n	65	p=0.05	p<0.01	0.19	0.46
	\bar{x}	0.302				
	x ₅₀	0.105				
	x _{min}	-0.003				
	x _{max}	2.55				
Channel Width (m)	n	65	p=0.40	p=0.07	-0.06	0.30
	\bar{x}	4.7				
	x ₅₀	3.9				
	x _{min}	1.3				
	x _{max}	15.1				
Woody Debris (#)	n	65	p<0.01	p<0.01	0.17	0.01
	\bar{x}	1.4				
	x ₅₀	0.5				
	x _{min}	0				
	x _{max}	10				
Fine Substrate (%)	n	65	p<0.01	p=0.14	-0.24	-0.10
	\bar{x}	54.3				
	x ₅₀	55.0				
	x _{min}	0				
	x _{max}	98.2				
BFW (m)	n	65	p=0.24	p=0.52	-0.12	0.28
	\bar{x}	6.0				
	x ₅₀	5.1				
	x _{min}	1.7				
	x _{max}	16.3				
FPW (m)	n	65	p=0.21	p=0.50	-0.06	0.19
	\bar{x}	11.7				
	x ₅₀	9.1				
	x _{min}	3.4				
	x _{max}	60.7				
Bank Height (m)	n	65	p<0.01	p<0.01	<0.01	-0.25
	x	1.8				
	x ₅₀	1.5				
	x _{min}	0.4				
	x _{max}	6.5				
Entrenchment (Ratio)	n	65	p=0.20	p=0.36	0.24	-0.14
	\bar{x}	2.3				
	x ₅₀	2.1				
	x _{min}	0.9				
	x _{max}	7.2				

Table 6 (continued)

Parameter			Basin	LIV Eco	MIBI rho	FIBI rho
Width:Depth (Ratio)	n	65				
	\bar{x}	9.6	p=0.88	p=0.99	-0.27	0.35
	x ₅₀	8.1				
	x _{min}	2.2				
	x _{max}	36.7				
Bank Angle (°)	n	65				
	\bar{x}	32.6	p=0.30	p=0.30	-0.09	-0.14
	x ₅₀	30.7				
	x _{min}	8.5				
	x _{max}	62.8				

Objective 3: Develop Indices of Biotic Integrity Using Macroinvertebrate and Fish Assessment Data

Generating Counts of Macroinvertebrates and Fish from Sampled Sites

Macroinvertebrate samples were subsampled to reach a minimum count of 300 individuals per sample. Sorted individuals were generally identified to genus or that level appropriate for monitoring analysis (U.S. EPA 2004). Voucher specimens (n=222) were retained of each taxon for deposit into the South Dakota Aquatic Invertebrate Collection (SDSU). Digital data displaying raw and corrected counts (for subsampling) are included separately ([EXCEL – NWGP Invertebrate Data; Kuehl 2017](#)).

Total corrected abundance among our 65 sampled sites ranged from a minimum of 16 individuals to 22,992 individuals per sample (Mean = 1406, Median = 794). Number of invertebrate families ranged from 3 to 23 (Mean = 12, Median = 12) and the total taxonomic richness ranged from 3 to 43 (Mean = 22, Median = 21).

Calculating Metrics of Assemblage Condition

Counts of individual macroinvertebrate and fish taxa were used to estimate community measures (i.e., metrics) which in-turn were used to generate assemblage indices of biotic integrity (e.g., Barbour et al. 1999; [Kaiser 2017](#); [Kuehl 2017](#); Whittier et al. 2007). Metrics of community structure/abundance, diversity, guild structure, pollution tolerance and condition were calculated for each taxonomic group. A full listing of macroinvertebrate and fish metrics is provided and defined separately in digital data files ([EXCEL – NWGP Invertebrate Data; NWGP Fish Data; Kuehl 2017; Kaiser 2017](#)). We calculated and screened 103 metrics of invertebrate assemblage structure and 218 metrics of fish assemblage structure for further analysis (Kaiser 2017; Kuehl 2017).

Selection of Optimal Metrics and IBI Development

Macroinvertebrate and fish metrics were passed through a series of screening and selection steps as outlined in Whittier et al. (2007). This process sequentially eliminates metrics based upon the range of values, signal:noise ratio, correlation with natural gradients, discriminatory power and redundancy. Metrics passing this screening process were used to calculate assemblage-specific indices of biotic integrity ([EXCEL – NWGP Invertebrate Data](#); [NWGP Fish Data](#); [Kaiser 2017](#); [Kuehl 2017](#)). Final IBI scores were rescaled to fall between 0 (extremely poor) and 100 (excellent) relative to other sites within the NWGP.

We employed two different discrimination steps during metric optimization to identify macroinvertebrate assemblage metrics best suited to discriminate site conditions. The discrimination step of the optimization process evaluates the ability of a metric to discriminate between groups of sites which are known to be in good versus poor condition. In our first analysis, we utilized field habitat data to identify two groups of sites (poor versus excellent) and then tested the ability of each macroinvertebrate metric to discriminate between these groups ([EXCEL – NWGP Invertebrate Data](#); [Kuehl 2017](#)). We then independently identified two groups of sites (poor versus excellent) on the basis of watershed condition scores (upper versus lower quartile) generated from our ATtILA analysis (see below) and retested the ability of our metrics to differentiate between these two groups. These two discrimination analyses resulted in four optimal MIBI metrics based upon field habitat data, but only two based upon watershed condition scores. This suggested that macroinvertebrate assemblage structure may be more highly correlated with localized site characteristics and less sensitive to differences in overall watershed condition. Thus, we adopted the four metrics which passed the discrimination of poor and excellent sites on the basis of field habitat data for MIBI generation.

The four macroinvertebrate metrics which passed our screening process above included number of families, richness of collector-filterers, percent Odonata, Ephemeroptera and Trichoptera richness and non-Insecta richness (Table 7). Scores for these four metrics were summed and the sum re-scaled to generate values between 0 and 100 (larger values indicate better assemblage condition). Resulting macroinvertebrate IBI scores (MIBI) ranged from 2.6 to 93 and averaged 36.9 among all 65 study sites. Scores did not vary significantly among LIV ecoregions (KWANOVA, $p=0.057$) but did vary among major river basins (KW ANOVA, $p=0.033$) with streams of the Belle Fourche and Moreau basins having the highest scores and those of the Missouri and Grand with the lowest scores (Figure 2).

Table 7. Summary statistics of optimal invertebrate metrics and MIBI scores for wadable streams of the NWGP in western South Dakota.

Metric	Median	Range
Number of Families	12.0	4.6 – 23.0
Collector-Filterer Richness	3.5	0 – 13.5
Percent OET Richness	2.0	0 – 9.5
Non-Insect Richness	2.5	0 – 7.0
MIBI	35.8	2.6 – 93.1

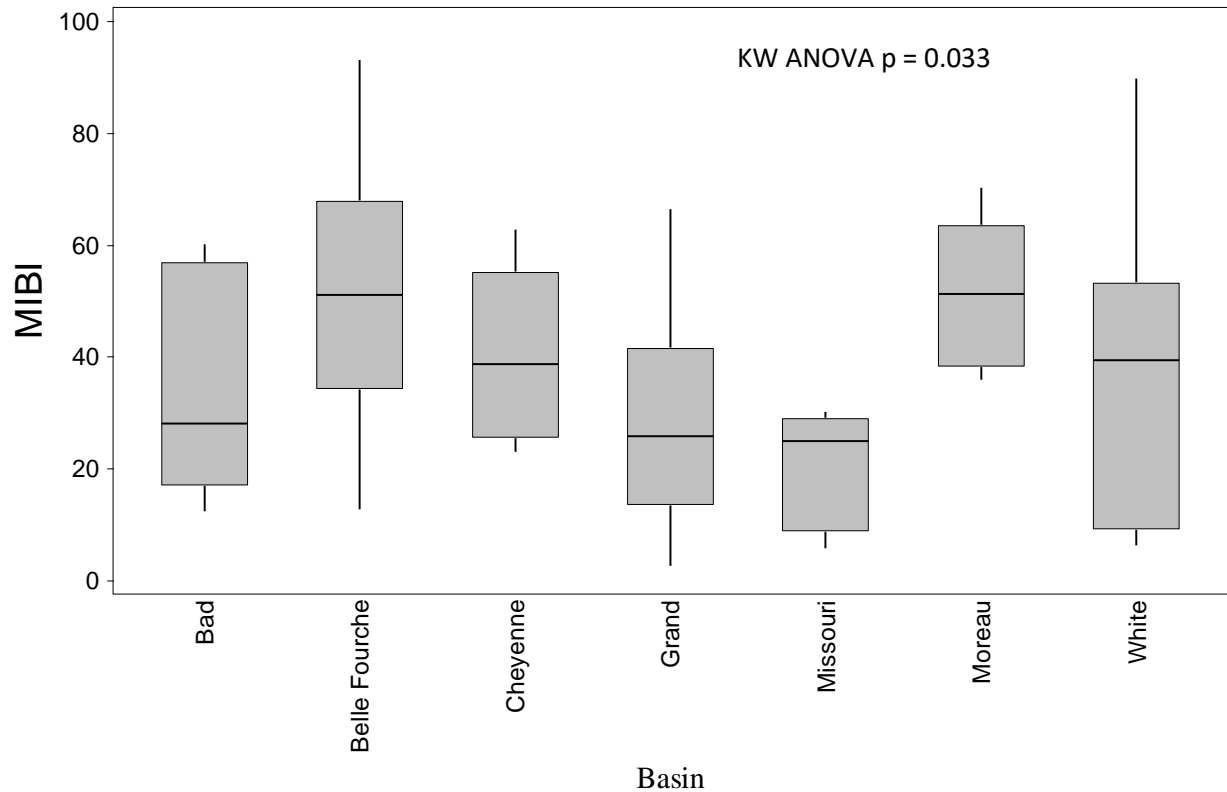


Figure 2. Comparison of MIBI scores among major river basins of the NWGP in western South Dakota.

The fish metric sequential screening process resulted in six fish metrics, including cyprinid insectivore species richness, proportion of individuals that are native large river fish, proportion of all fish species that are tolerant, abundance of alien fish, proportion of all species that are native lithophils and proportion of individuals that are *Rhinichthys obtusus* (Table 8). FIBI scores ranged from 16.7 to 76.7 and averaged 40.8 across all 65 study sites. FIBI scores were observed to vary significantly among LIV ecoregions (KW ANOVA $p < 0.01$; Figure 3). However, FIBI did not vary significantly among major river basins (KW ANOVA $p = 0.098$).

Table 8. Summary statistics of optimal fish metrics and FIBI scores for wadable streams of the NWGP in western South Dakota.

Metric	Median	Range
Cyprinid insectivore species richness	0.00	0 – 2
Proportion of individuals that are native large river fish	0.00	0.00 – 0.97
Proportion of all fish species that are tolerant	0.08	0.00 – 0.42
Abundance of alien fish	0.00	0 – 9
Proportion of all species that are native lithophils	0.21	0.00 – 1.00
Proportion of individuals that are <i>Rhinichthys obtusus</i>	0.00	0.00 – 1.00
FIBI	40.8	16.7 – 76.7

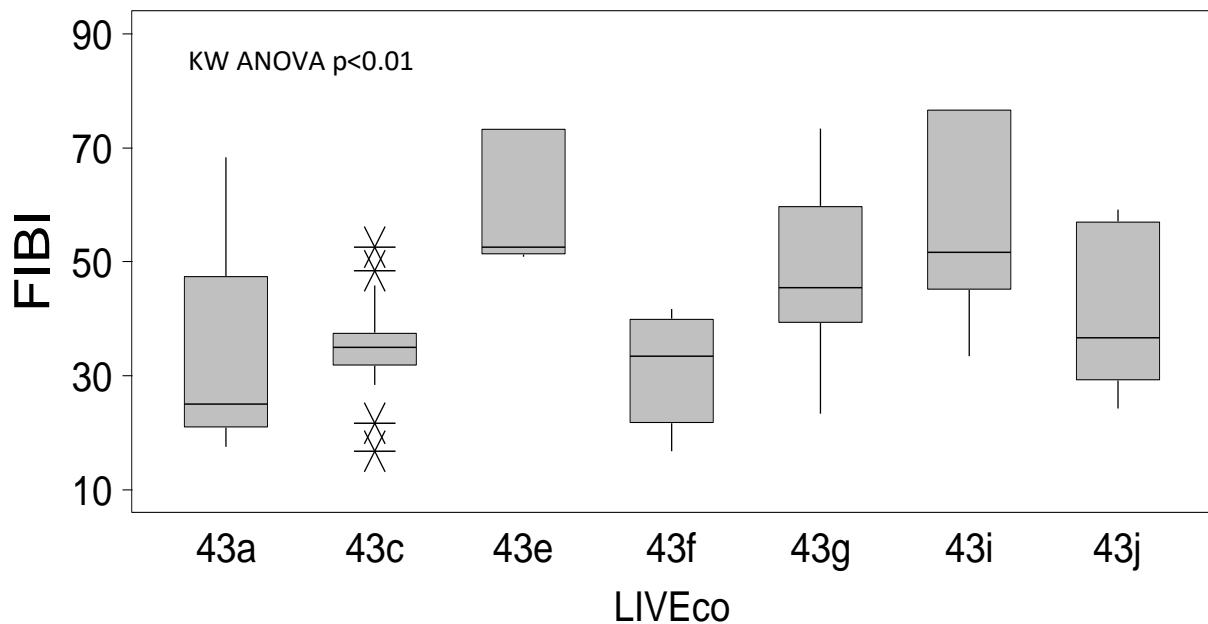


Figure 3. Differences in FIBI among LIV ecoregions of the NWGP in western South Dakota, 2014-2015.

Objective 4: Identify and Validate Candidate Reference Sites for the NWGP.

Identifying Candidate Reference Sites

Candidate reference sites were selected from the upper 25th percentile of sampled sites based upon HQI and assemblage IBI scores (Table 9). Only one sampled site was found to fall within the upper quartiles of HQI-MIBI-FIBI. Four sites were found to fall within both the HQI-MIBI and HQI-FIBI. Three sites were found to fall within the upper quartiles of MIBI-FIBI.

Table 9. Listing of stream sites within the upper quartiles of HQI(H), MIBI(M) and FIBI(F). H-M, falling within the upper quartile of HQI and MIBI but not FIBI; H-F, falling within the upper quartile of HQI and FIBI but not MIBI; M-F, falling within the upper quartile of MIBI and FIBI but not HQI; H-M-F, falling within the upper quartile of HQI, MIBI and FIBI.

H-M	H-F	M-F	H-M-F
125232731	125227052	137351925	126557559
126564276	126840937	154879617	
128629347	154730429	154879668	
151672715	154879371		

MIBI scores were found to be more strongly correlated with HQI scores across all 65 of our study sites (Figure 4). A significant linear relationship was observed between MIBI and HQI but

no significant relationship was found between FIBI and HQI. Thus, we place stronger emphasis on H-M and H-M-F sites (Table 9) as potential candidate reference sites.

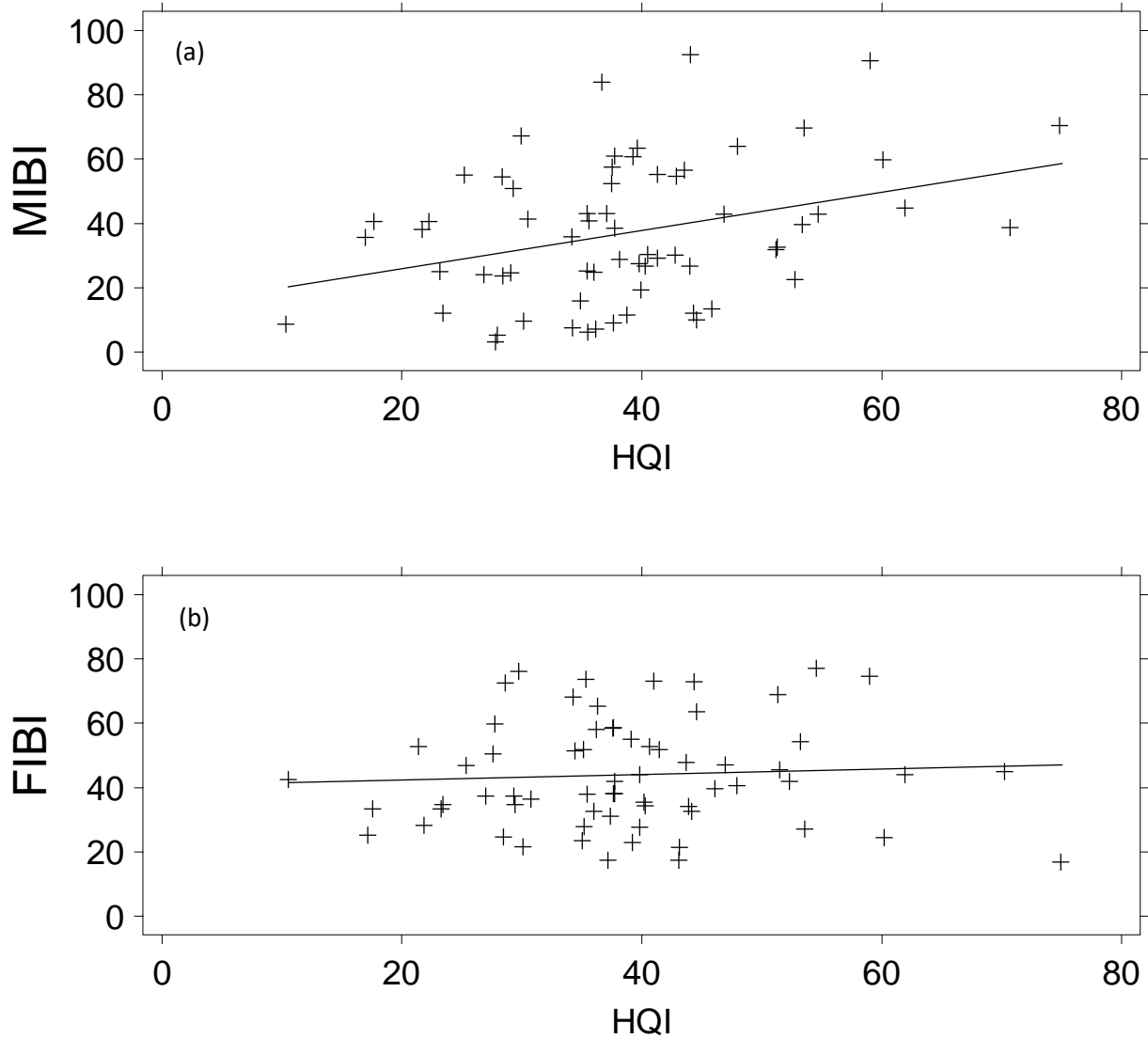


Figure 4. Relationships between MIBI (a) and FIBI (b) with HQI among wadable, perennial streams of the NWGP in South Dakota, 2014-2015.

Validating Candidate Reference Sites

The U.S. EPA Analytical Tools Interface for Landscape Assessment tool (ATtILA) has been used to identify candidate intermittent and perennial stream reference sites within the Northern Glaciated Plains ecoregion during previous studies (Troelstrup 2010; Bertrand and Troelstrup 2013). ATtILA watershed condition metrics were generated for all study site watersheds (n=65) and all HUC12's within the NWGP study area (n=1027). We chose HUC12 areas to establish the distribution of watershed conditions within the NWGP against which study area watersheds could be compared as the size distribution of HUC12 areas was similar to the size distribution of study watersheds. Metrics of watershed condition for all HUC12's and study area watersheds were generated using ATtILA ([EXCEL – NWGP ATtILA Data](#)) and passed through a sequential screening process to identify those metrics explaining the greatest variation in watershed characteristics. Final metrics used to generate scores included percent human use within the watershed, percent pasture/hay ground within the watershed, stream/road crossing density, herbaceous riparian cover within 30m of the channel, percent forest within 30m of the channel, percent barren ground on slopes greater than 9° within 30m of the channel, percent barren land within the watershed, percent shrubland within the watershed, percent impervious surface within the watershed, percent developed land within 30m of the channel, percent shrubland within 30m of the channel and percent agricultural land within the watershed on slopes greater than 9°. This collection of 16 metrics included measures of natural land cover and managed human use at both watershed and riparian scales. Details regarding metric generation, screening and watershed score generation can be found in Suehring (2017). Watershed condition scores generated from this process were rescaled to fall between 0 (poorest score) and 100 (best score). We expected candidate reference sites to have watershed condition scores equal to or above a value of 75.

Watershed condition scores (WCS) generated from ATtILA were observed to range from 0 to 100 throughout the NWGP ecoregion (Figure 5a; Mean = 68.2). Target population WCS distribution was skewed slightly to higher scores. WCS from our 65 study sites displayed a similar distribution but with a somewhat lower mean score of 57.8 (Figure 5b). WCS of our study sites did not quite vary significantly among LIV ecoregions ($p = 0.057$) nor did it vary significantly among major river basins ($p = 0.311$).

In general, there was little correspondence among sites scoring above 75 in ATtILA watershed condition, macroinvertebrate IBI and fish IBI (Table 10). Only one site was found to score above 75 in watershed condition and fish IBI. This same site had a poor macroinvertebrate IBI score. FIBI scores did display a weak positive correlation with WCS ($\rho = 0.196$) but this correlation was not significant ($p = 0.118$). Thus, we rely on the significant relationship between MIBI scores and site-specific HQI as validation that MIBI provides a means of assessing degraded biotic integrity from our western South Dakota sites.

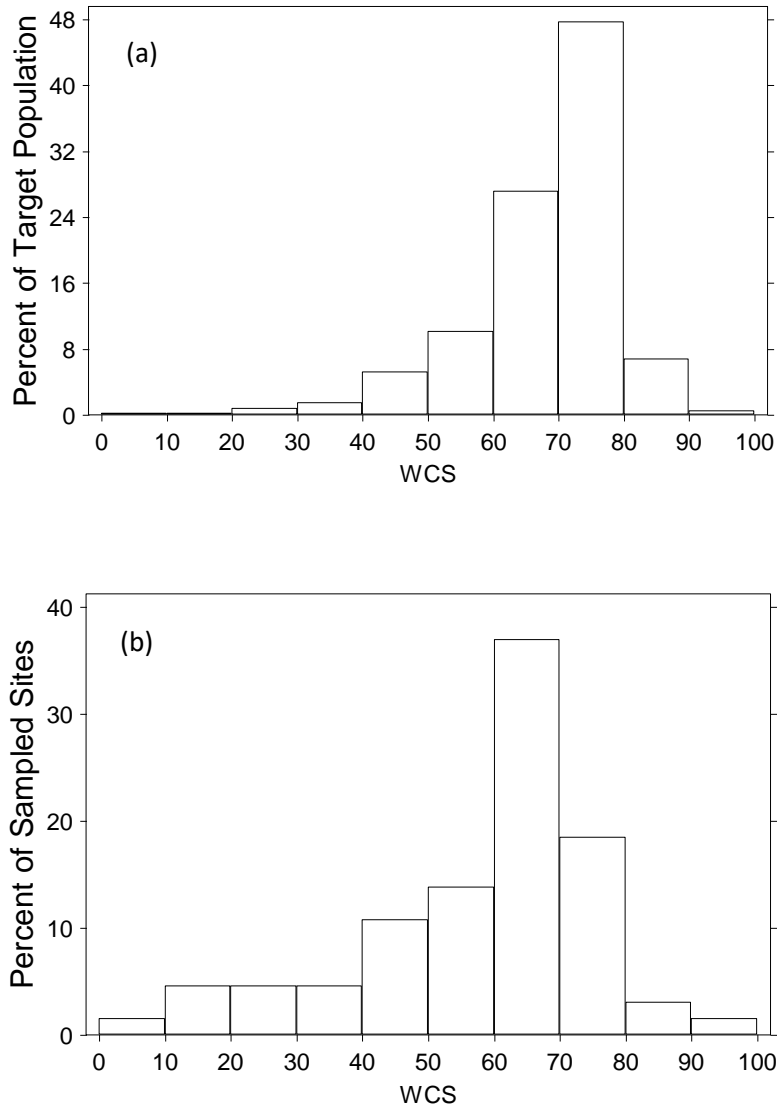


Figure 5. Distribution of watershed condition scores (WCS) generated from ATtILA analysis of landscape attributes for 1027 HUC12's (a) and our 65 sampled perennial stream watersheds (b) in western South Dakota.

Table 10. Sampling sites with watershed condition scores (WCS), macroinvertebrate IBI scores (MIBI) and fish IBI (FIBI) scores above 75 within the NWGP ecoregion of South Dakota.

WCS Streams	MIBI Streams	FIBI Streams
131704460	126557559	154730429
137351925	149713951	154730505*
143215959	154879617	
154730505*		
154853698		
154879371		

Our results suggest that fish and macroinvertebrate assemblages might be responding to drivers at different scales. MIBI metrics were better able to discriminate among sites based upon local habitat drivers while FIBI metrics appeared to be better predictors of larger-scale landscape differences among LIV ecoregions (Kaiser 2017; Kuehl 2017). While biotic integrity appeared to be well correlated with landscape metrics in small headwater catchments in eastern South Dakota (Troelstrup 2010), the relationship between IBI scores and watershed condition was observed to become poorer as watershed size increased (Bertrand and Troelstrup 2013; this study). Invertebrate assemblages in particular appear to be more responsive to localized management and habitat differences. Of course our design was constrained in terms of watershed (stream) size, so these conclusions are made within that context. In addition, watersheds in western South Dakota (this study) are generally less intensively managed than those which are heavily cropped throughout the eastern half of the state (Troelstrup 2010; Bertrand and Troelstrup 2013), compressing the stressor gradient over which biological response can occur. In general, our study watersheds in western South Dakota were in much better condition than many of those we examined in the eastern half of the state (Troelstrup 2010, Bertrand and Troelstrup 2013; this study). Much of western South Dakota is managed as hay ground and for livestock grazing while those of eastern South Dakota are exposed to a larger land-use stressor gradient. Pasture sizes in western South Dakota also tend to be much larger than those in the east, thus dispersing the effect of grazing animals on stream channels. Finally, western South Dakota is drier and more variable in terms of hydrology than eastern South Dakota (Bryce et al. 1998). Streams of western South Dakota display high frequency and duration of intermittency and wide intra-annual and interannual flow. This variable biophysical template supports assemblages of organisms which are well adapted to stressful conditions, further challenging development of IBI's capable of differentiating between natural variation and human stressors.

The overall goal of this effort was development of indices of biotic integrity which might be used in support of aquatic life uses in the NWGP ecoregion. Both macroinvertebrate and fish IBI's were produced following standard, published procedures consistent with those already developed in the eastern half of South Dakota. The MIBI, in particular, appears well suited to differentiate among sites with impaired local habitat and water quality. Datasets and products generated from this study along with similar efforts in eastern South Dakota (Troelstrup 2010; Bertrand and Troelstrup 2013) provide tools from which SD DENR can develop and implement their statewide biological monitoring program. While metrics comprising our IBI's do differ in some respects from those developed in adjacent states, several metrics we optimized for use in the NGP and NWGP IBI's are also utilized by Wyoming (Hargett 2011), North Dakota (Larsen 2013, Minnesota (MPCA 2014), Iowa (Wilton 2004), Nebraska (Bazata 2013) and EMAP-West (Stoddard et al. 2005). More importantly, both IBI's discriminate between biotic assemblages of impaired sites from those which are minimally impaired. IBI's developed in the eastern half of the state have already been implemented and improved through additional data collection (SD DENR 2016). This effort extends those capabilities to the NWGP, leaving only the Black Hills without a comparable IBI toolkit.

Extended analysis of WCS to help examine future landcover change scenarios under different policy directions was also achieved although outside the scope of our original project objectives

(Suehring 2017). This modeling analysis using futuristic projections of land-use change combined with ATtILA reanalysis of watershed condition may help water resource managers anticipate areas of the state most likely to witness future watershed degradation. Such modeling might allow planning for anticipated management needs in those watersheds deemed highly likely to become degraded. Water resource management agencies may then be better able to plan optimal implementation strategies and resource needs well in advance, making optimal use of limited monitoring and management dollars.

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