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Aquatic Invertebrate Monitoring at Knife River Indian Villages National Historic Site

Natural Resource Technical Report NPS/NGPN/NRTR—2013/778

ON THIS PAGE
Knife River before flowing into the Missouri River, Knife River Indian Villages National Historic Site
Photograph by: Lusha Tronstad, Wyoming Natural Diversity Database

ON THE COVER
Mark Andersen of the Wyoming Natural Diversity Database sampling aquatic invertebrates along the Knife River, Knife River Indian Villages National Historic Site
Photograph by: Lusha Tronstad, Wyoming Natural Diversity Database

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Abstract

The Knife River in central North Dakota is not supporting its designated use of recreation in the state because of high concentrations of fecal coliform along much of the river's length. About 60% of the length of the Knife River has fecal coliform concentrations higher than the recreation standard (200 colony forming units/100 mL). I measured basic water quality, fecal coliform concentrations, *Escherichia coli* concentrations, and aquatic invertebrate assemblages at three sites to estimate the ecosystem quality along the 5.5 km of the Knife River that flows through Knife River Indian Villages National Historic Site. Basic water quality was similar among sites. The Knife River was basic (pH ~8.4) and daytime dissolved oxygen concentrations were nearly 10 mg/L. Fecal coliform concentrations were at least one order of magnitude above the recreation standard, but *E. coli* concentrations were below the standard. The Macroinvertebrate Biotic Integrity Multimetric Index of North Dakota scored all sites within Knife River Indian Villages National Historic Site as least disturbed sites. The metric indicated that the Knife River within the park is in good condition compared to other rivers in western North Dakota. Individual metrics rated site #3 as lower quality compared to the other sites. Site #3 had much higher densities of aquatic invertebrates due mostly to Chironomidae (71%). Although the densities of many insect orders were similar among sites, several bioassessment metrics were highly influenced by Chironomidae. Site #3 had the highest benthic organic matter content, which may have been at least partially responsible for the higher density of Chironomidae. Management actions within the park will probably have minimal effects on fecal coliform concentrations. Working with landowners and towns within the watershed to change practices will be essential to reducing fecal coliform concentrations. Further studies are needed to identify the sources of fecal coliform (e.g., human, livestock, or wildlife) in the watershed.

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Introduction

Aquatic invertebrates have been used to monitor water bodies since the 12th century in Europe (Cairns and Pratt 1993) and the 1870s in the United States. Europeans developed the Saprobien system that used indicator organisms to identify the level of organic pollution (i.e., sewage) affecting a water body. In this approach, water bodies colonized exclusively by worms and blood midges (Chironomus) were considered severely polluted whereas waters colonized by mayflies and caddisflies were considered high quality. Ruth Patrick strongly influenced biomonitoring in the United States by developing methods using abundance and richness of multiple taxonomic groups (Patrick 1949).

Currently, two types of bioassessment methods are widely used in the United States. Multivariate or predictive models use statistical models to predict expected (reference) conditions and compare these values to observed conditions (e.g., Ode et al. 2008). To make the models, aquatic invertebrate data are matched to environmental factors not thought to be affected by anthropogenic activities (e.g., channel slope, elevation) at reference sites. To estimate the level of impairment at a site, observed data are compared to model predictions. On the other hand, multimetric indexes combine several bioassessment metrics into a single measure to estimate the level of impairment (Kerans and Karr 1994; Ode et al. 2008). Multimetric indexes are created by collecting biological samples at a range of reference sites. Based on the data from reference sites, the best metrics are chosen by eliminating correlated metrics, using a variety of measures (e.g., richness, habit, abundance), and selecting metrics that best differentiate the data. The selected metrics are scaled (e.g., 0-100), average values of the metrics are calculated, and thresholds are developed to estimate the level of impairment. To measure the ecosystem quality along a reach in question, biological data are collected, metrics are calculated, and a final average score is compared to thresholds. Multimetric indexes have been widely used to monitor biological assemblages, and were first developed for fish (Karr 1981) and later applied to aquatic invertebrates (Kerans and Karr 1994). Currently, most bioassessment programs in the United States use multimetric indexes, including North Dakota.

Recently, biologists are returning to interpreting ecosystem quality using single metrics. Interpreting single metrics is more straight forward and may provide insight into the mechanisms driving observed trends (Allan 2004). Tolerance values, habit, functional feeding groups, body size, and life history characteristics are examples of metrics that biologists interpret using a trait-based approach. Vandewalle et al. (2010) noted that higher abundance of invertebrates with multivoltine life cycles (taxa with >2 generations per year) correlated with a higher percentage of cropland in the floodplains of Europe rivers. Invertebrates with shorter lifespans are typically more resilient to stream disturbances. Similarly, Dolédec et al. (2006) found that New Zealand grassland streams with a higher percentage of land in agriculture had invertebrates with a shorter lifespan, more individuals that reproduced asexually, and more taxa with streamlined body shapes. Dolédec et al. (2006) reported that invertebrate traits explained more variation in land use compared to species composition. Using multiple approaches to interpret bioassessment metrics may provide the best perspective on ecosystem conditions.

Aquatic invertebrate are sensitive to a range of conditions, such as pollution from nutrients, trace elements, chemicals, bacteria, habitat degradation, land use, invasive species, and water regulation, making these animals ideal for estimating ecosystem quality of rivers. Aquatic invertebrates can be used for monitoring around the world and in nearly all aquatic habitats because these animals are diverse and ubiquitous. Invertebrates are relatively sedentary so their response reflects the conditions at the site. Some aquatic invertebrates are long lived and respond to ecosystem quality throughout their life. Chironomidae can develop from egg to adult in a matter of weeks, whereas a mussel can live >100 years. Invertebrates are food for many animals (e.g., fish, waterfowl, songbirds); therefore, changes in the food web can be observed through the invertebrate assemblage.

The Knife River in western North Dakota has high concentrations of fecal coliform and is not supporting its designated use of recreation (North Dakota Department of Health; http://www.ndhealth.gov/WQ/sw/). The Knife River runs through Knife River Indian Villages National Historic Site shortly before flowing into the Missouri River below Garrison Dam. The National Park Service was interested in how ecosystem quality changed as the river flowed through the park. The objectives of the study were to 1) measure how bacterial concentrations changed along the river, and 2) estimate ecosystem quality using aquatic invertebrates as the river flows through the park. I collected basic water quality, fecal coliform concentrations, *E. coli* concentrations, and aquatic invertebrate samples at three sites along the river within the park to answer these questions.

Study Area

The Knife River is ~193 km long tributary stream of the Missouri River Basin in west central North Dakota. The river originates in the badlands north of Dickinson, North Dakota and flows unimpeded into the Missouri River near Stanton, North Dakota. Spring Creek is the largest tributary of the Knife River (mean annual discharge 1946-2010 = 1.05 m^3/s; USGS National Water Information System, www.waterdata.usgs.gov) and flows into the Knife River near Beulah, North Dakota. The flow of the Knife River after the confluence with Spring Creek is 4.7 m^3/s (mean annual discharge 1930-2010; USGS National Water Information System, www.waterdata.usgs.gov).

Under the Clean Water Act of 1972, each river in the United States is assigned a class based on the designated uses of the water (e.g., drinking water, fisheries). The Knife River is a Class II water with a designated use of recreation; therefore, water quality must be maintained for safe human contact (e.g., swimming). The water quality of the river must be below standards set for each designated use. Currently, several reaches of the Knife River (118.5 km) are not supporting its recreation designation due to high concentrations of fecal coliform (http://www.ndhealth.gov/WQ/sw/). In addition, the recreation designation is not supported or is threatened by high concentrations of *E. coli* or fecal coliform in tributaries of the Knife River (Spring Creek, Coyote Creek, and Antelope Creek).

About 5.5 km of the Knife River flows through Knife River Indian Villages National Historic Site just before joining the Missouri River. Knife River Indian Villages National Historic Site was established in 1974 and includes 712 hectares. The park features archeological village sites surrounded by native short grass prairie and hardwood forest. I sampled three sites along the river on 29 August 2011: at the western boundary where the river flowed into the park (site #1), Awatixa fishing access (site #2), and at the eastern boundary where the river flows out of the park (site #3; Table 1; Figure 1).

Table 1. Location of each site along the Knife River (Datum NAD83).

Site	Zone	Easting	Northing
Site #1	14	0318996	5247736
Site #2	14	0319853	5246078
Site #3	14	0320477	5243986

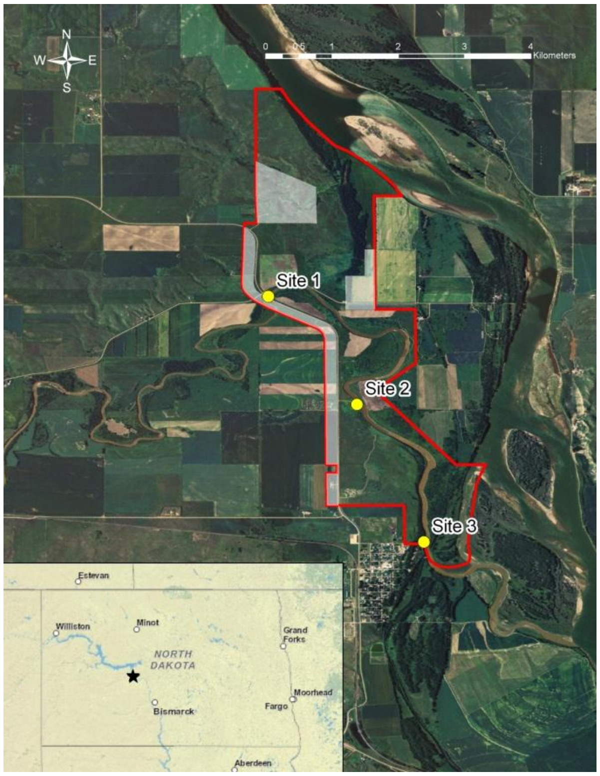

Figure 1. I sampled three sites (yellow dots) along the Knife River as it flows through Knife River Indian Villages National Historic Site (red boundary). Private land inside the historic site boundaries are shown in white. The Knife River flows into the Missouri River southeast of the historic site near the town of Stanton, North Dakota. The inset map shows the location of Knife River Indian Villages National Historic Site (star) in North Dakota.

The Knife River had a well-developed riparian area and the substrate was composed of fine sediments. The riparian area surrounding the Knife River was dominated by cottonwood (*Populus deltoides*) and green ash (*Fraxinus pennsylvanica*). The riparian area was often separated from the river by steep, mud banks, thus I sampled the river in places where the slope of the bank was more gradual. River flow was 2.6 m^3/s on the sampling day (USGS National Water Information System, www.waterdata.usgs.gov). Benthic substrates were composed of silt, sand, and clay. I collected large amounts of organic matter with each sample.

Methods

I measured basic water quality, water clarity, and bacterial concentrations to estimate conditions at each site. I measured basic water quality using a Yellow Springs Instrument (YSI) Professional Plus calibrated daily. Water clarity was estimated by lowering a Secchi disk into the water until the disk disappeared from sight. Finally, I collected two water samples from each site to measure the concentration of fecal coliform and *E. coli* using the Colilert method (SM9223B, Eaton and Franson 2005). Water samples were immediately placed on ice and shipped to the Wyoming Department of Agriculture Analytical Services. All samples were received by the laboratory within 30 hours of collection.

To measure the abundance and diversity of invertebrates in the Knife River, I collected aquatic invertebrates using a Hess sampler. I collected samples at three sites along the river within Knife River Indian Villages National Historic Site (Figure 1). Five samples were collected at each site along the shoreline of the river. Angradi et al. (2006) showed that samples collected along the shoreline of the Missouri River below Garrison Dam in North Dakota were the best samples to use for bioassessment of non-wadeable rivers. Sampled areas had been inundated since mid-March and were colonized by invertebrates. I placed the Hess sampler (500 µm mesh, 860 cm^2 sampling area, Wildlife Supply Company) into the substrate and agitated the sediment. Samples were preserved with ~80% ethanol and transported to the laboratory where invertebrates were sorted from debris. Each sample was checked by an additional qualified person to insure that all invertebrates were removed. Invertebrates were counted and identified under a dissecting microscope using appropriate keys (Needham et al. 2000; Smith 2001; Merritt et al. 2008; Thorp and Covich 2010).

To estimate ecosystem quality at each site, I calculated several bioassessment metrics using invertebrate data. Based on the data collected, previous studies (e.g., Resh and Jackson 1993; Kerans and Karr 1994), and models developed for North Dakota (Environmental Protection Agency 2009), I selected 22 metrics to compare sites (Table 2). I choose a variety of metrics including measures of richness, abundance, community diversity, pollution tolerance, habit, and functional feeding group. Pollution tolerance values of invertebrate taxa were taken from Bowles et al. (2008) and Barbour et al. (1999). Functional feeding group and habit were from Merritt et al. (2008). To distinguish among sites, I used ANOVA to compare abundance and bioassessment metrics for each sample (DataDesk6.1). Differences among sites were distinguished using Bonferroni multiple comparison tests, where differences were significant when $p < 0.0167$ (0.05/3; where I had three sites). Reported variance is standard error.

North Dakota developed the Macroinvertebrate Biotic Integrity Multimetric Index created under the Environmental Monitoring and Assessment Program (EMAP) West by the Environmental Protection Agency (Environmental Protection Agency 2009). These metrics have been developed for the rangeland plains of North Dakota (Northwestern Great Plains and Northwestern Glaciated Plains Ecoregions) in which Knife River Indian Villages National Historic Site is located. To develop the multimetric index, they collected aquatic invertebrate samples from a range of streams in western North Dakota and determined reference sites using landscape, physical habitat, and water chemistry data. After analysis, the six best metrics that represented a variety of measures (e.g., richness, habit) were chosen based on aquatic invertebrate data from these reference sites. These metrics were scaled from 0 to 100 based on the 5th and 95th percentiles of the data. The 25th percentile was the threshold for most disturbed sites and the 5th percentile was the threshold for least disturbed sites based on reference sites. These metrics were applied to non-reference reaches to place a site in one of three categories (least disturbed, moderately disturbed, and most disturbed). To do this, the average value of the six metrics (0-100 scale) were calculated and compared to thresholds.

I used the North Dakota Macroinvertebrate Biotic Integrity Multimetric Index for the rangeland plains to understand how sites along the Knife River compared to the greater region. I calculated the six metrics used by the rangeland plains index for each site along the Knife River. Using the index, I scored each metric (0-100) using the same scale as the Environmental Protection Agency (2009). Finally, I calculated an average value of the six metrics at each site and compared these values to the thresholds reported.

Table 2. The equations used to calculate bioassessment metrics. A variety of metrics were calculated that included measures of richness, abundance, community diversity, pollution tolerance, habits, and functional feeding group. EPT stands for the insect orders *Ephemeroptera, Plecoptera,* and *Trichoptera.* Hilsenhoff's Biotic Index (HBI) is used to estimate average pollution tolerance of an individual in the invertebrate assemblage. All richness metrics were calculated at the lowest taxonomic level used in the present study (typically genus).

Metric	**Equation**	**Predicted response to impact**
% Chironomidae	$= \left(\frac{abundance_{Chironomidae}}{total\ abundance}\right) \times 100$	Increase
% clingers	$\left(\frac{abundance_{clingers}}{total\ abundance}\right) \times 100$	Decrease
% clingers taxa	$\left(\frac{richness_{clingers}}{taxa\ richness}\right) \times 100$	Decrease
% EPT taxa	$= \left(\frac{richness_{EPT}}{taxa\ richness}\right) \times 100$	Decrease
% filterers	$\left(\frac{abundance_{filterers}}{total\ abundance}\right) \times 100$	Decrease
% gatherers	$\left(\frac{abundance_{gatherers}}{total\ abundance}\right) \times 100$	Decrease
% intolerant (0-5)	$\left(\frac{abundance_{tolerance0-5}}{total\ abundance}\right) \times 100$ Abundance of taxa with tolerance values 0 to 5.0	Decrease
% intolerant taxa (0-5)	$\left(\frac{richness_{tolerance0-5}}{total\ abundance}\right) \times 100$ Number of taxa with tolerance values 0 to 5.0	Decrease
% non-insects	$= \left(\frac{abundance_{non-insects}}{total\ abundance}\right) \times 100$	Increase
% predator taxa	$= \left(\frac{richness_{predators}}{taxa\ richness}\right) \times 100$	Decrease

Table 2. The equations used to calculate bioassessment metrics. A variety of metrics were calculated that included measures of richness, abundance, community diversity, pollution tolerance, habits, and functional feeding group. EPT stands for the insect orders *Ephemeroptera, Plecoptera*, and *Trichoptera.* Hilsenhoff's Biotic Index (HBI) is used to estimate average pollution tolerance of an individual in the invertebrate assemblage. All richness metrics were calculated at the lowest taxonomic level used in the present study (typically genus).

Metric	**Equation**	**Predicted response to impact**
% predators	$= \left(\frac{abundance_{predators}}{total\ abundance}\right) \times 100$	Decrease
% tolerant (6.0-7.0)	$\left(\frac{abundance_{tolerance6-7}}{total\ abundance}\right) \times 100$ Abundance of taxa with tolerance values 6.0 to 7.0	Increase
% tolerant (8.0-9.0)	$= \left(\frac{abundance_{tolerance8-9}}{total\ abundance}\right) \times 100$ Abundance of taxa with tolerance values 8.0 to 9.0	Increase
% tolerant (>7)	$= \left(\frac{abundance_{tolerant>7}}{total\ abundance}\right) \times 100$ Abundance of taxa with tolerance values >7	Increase
% tolerant taxa (>7)	$= \left(\frac{richness_{tolerant>7}}{taxa\ richness}\right) \times 100$ Number of taxa with tolerance values >7	Increase
EPT richness	Richness of mayflies, stoneflies, and caddisflies	Decrease
EPT/midge abundance	$= \frac{abundance_{EPT}}{abundance_{Chironomidae}}$	Decrease
HBI	$= \sum_{i=1}^{n} \frac{abundance_i \times tolerance_i}{total\ abundance}$	Increase
Taxa diversity	$= -\sum_{i=1}^{s} p_i \times \ln(p_i)$ Where p_i is the proportion of the i^{th} taxa	Decrease
Taxa evenness	$= \frac{taxa\ diversity}{\ln(taxa\ richness)}$	Decrease
Taxa richness	Number of taxa in a sample	Decrease
Total abundance	Total number of individuals (ind/m^2)	Decrease

Results

Basic water quality of the Knife River was similar among sites. All sites were supersaturated with oxygen, but site #2 had the highest dissolved oxygen concentration (Table 3). The pH level was basic (>7) and reducing conditions dominated (oxidation-reduction potential <200 mV) at all sites. Site #3 had the clearest water and site #2 had the most turbid water. Fecal coliform concentrations exceeded 2419.6 colony forming units (CFU)/100 mL at all sites. *E. coli* concentrations were highest at site #1 and decreased as the river flowed through the historic site. Water quality standards for the parameters I measured were met in the Knife River, except fecal coliform far exceeded the limit at all sites (Environmental Protection Agency 2003).

Table 3. Water quality at three sites along the Knife River at Knife River Indian Villages National Historic Site. Basic water quality was measured with a Yellow Springs Instrument Professional Plus sonde (oxidation-reduction potential = ORP). Average fecal coliform and *E. coli* concentrations were measured using the Colilert method (n = 2; CFU = colony forming units). All parameters were within North Dakota water quality (WQ) standards for recreation except fecal coliform.

		Knife River			**WQ**
Parameters	**Units**	**Site #1**	**Site #2**	**Site #3**	**standards**
Water temperature	°C	21.2	23.4	22.6	≤29.44
Dissolved oxygen	% saturation	104	137	118	
Dissolved oxygen	mg/L	8.6	10.9	9.6	≥5
Specific conductivity	µS/cm	2005	1938	1990	
pH		8.31	8.46	8.33	6 to 9
ORP	mV	70.5	71.1	159.8	
Secchi disk depth	cm	30	24	34	
Fecal coliform	CFU/100 mL	>2419.6	>2419.6	>2419.6	≤200*
E. coli	CFU/100 mL	49.75	21.6	16.05	≤126*

*Valid during the recreation season 1 May through 30 September

Insects (97%) were far more abundant than non-insect taxa (3%; Table 4). Diptera (1870 ind/m^2) were the most abundant order of insects followed by Hemiptera (810 ind/m^2), Ephemeroptera (320 ind/m^2), and Coleoptera (110 ind/m^2). Total invertebrate density was lowest at site #1 (2350 ind/m^2 ± 455) and highest at site 3 (4200 ind/m^2 ± 1185), but differences were not significant (P = 0.32, F = 1.3, df = 2).

Figure 2. Photos of a.) *Hydropsyche* (Hydropsychidae, Trichoptera), b.) *Nectopsyche* (Leptoceridae, Trichoptera), c.) *Dromogomphus spoliatus* (Gomphidae, Odonata), and d.) *Dromogomphus spinosus* (Gomphidae, Odonata) from the Knife River.

I collected 30 taxa of invertebrates from the Knife River at Knife River Indian Villages National Historic Site. At least four taxa of Diptera lived in the Knife River of which Chironomidae were the most abundant (91%). I identified three genera of Hemiptera in the family Corixidae (*Hesperocorixa*, *Palmacorixa*, and *Trichocorixa*), but most individuals were early instars. Three genera and families of Ephemeroptera lived in the river. *Caenis* (Caenidae) were most abundant (237 ind/m^2) followed by *Hexagenia* (Ephemeridae; 73 ind/m^2) and *Paracloeodes* (Baetidae; 7 ind/m^2). I collected two genera of riffle beetles (Elmidae), and *Dubiraphia* (112 ind/m^2) was far more abundant than *Stenelmis* (2 ind/m^2). Other orders of insects were present at low abundance including two genera of Trichoptera (*Hydropsyche*, Hydrospychidae and *Nectopsyche*, Leptoceridae; Figure 2 a and b), one genus of Megaloptera (*Sialis*,Sialidae), and three species of Odonata (*Dromogomphus spoliatus*, *Dromogomphus spinosus*, and *Chromagrion conditum*; Figure 2 c and d). I collected four Crustacea taxa of which Ostracoda (34 ind/m^2) were most abundant followed by Cyclopoida Copepoda (33 ind/m^2), Cladocera (7 ind/m^2), and *Hyalella* (Amphipoda; 1 ind/m^2). Four mollusk families live at low abundance in the Knife River (Physidae, Ancylidae, Planorbidae, and Sphaeriidae). Finally, I collected low abundance of Hydrocarina in the river (7 ind/m^2).

Table 4. Density (ind/m^2) and standard error of invertebrates at each site. An asterisk means that significant differences were detected among sites ($P < 0.05$) and a † indicates that the variable was natural log transformed to normalize variance for statistical analysis.

Taxa	**Site #1**	**Site #2**	**Site #3**
Hemiptera*†	1133±421	1058±337	247±105
Diptera*	865±97	1600±288	3151±887
Coleoptera*†	198±68	21±10	121±52
Ephemeroptera	135±28	400±143	416±82
Trichoptera	5±5	7±3	12±12
Megaloptera	0±0	2±2	2±2
Odonata	0±0	5±5	12±7
Crustacea	2±2	12±6	209±116
Gastropoda	0±0	9±4	26±26
Bivalvia	0±0	5±5	0±0
Hydrocarina	9±4	9±7	2±2
Insects	2335±455	3093±667	3960±1079
Non-Insects	12±4	35±10	237±138
Total	2347±456	3128±661	4198±1183

I calculated 22 bioassessment metrics for each sample at each site (Table 5). Of these, eight metrics detected differences according to site and six metrics detected differences among sites using multiple comparison tests. A higher proportion of individuals at site #3 were gatherers ($F = 24.7$, $df = 2$; Bonferroni, $P < 0.001$) and Chironomidae ($F = 43.3$, $df = 2$, Bonferroni, $P < 0.00001$) compared to the other sites. Similarly, a higher proportion of invertebrates with tolerance values of 6.0-7.0 lived at site #3 ($F = 38$, $df = 2$, Bonferroni, $P < 0.00001$). On the other hand, more predators lived at site #2 ($F = 16.9$, $df = 2$, Bonferroni, $P < 0.001$). A larger proportion of clinging invertebrates lived at site #1 compared to site #2 ($F = 5.3$, $df = 2$, Bonferroni, $P = 0.014$). Taxa evenness was higher at site #2 compared to site #3 ($F = 6.6$, $df = 2$, Bonferroni, $P = 0.016$). Taxa diversity (Shannon's Diversity Index) was highest at site #2 and lowest at site #3. Finally, the percent of invertebrates with tolerance values >7 was lowest at site #1.

Several bioassessment metrics had marginal P-values (≤ 0.11) based on the ANOVA comparing sites. EPT richness ($F = 2.7$, $df = 2$) was lowest at site #1. The average tolerance value for invertebrates in the assemblage (HBI) was highest at site #3 ($F = 3.6$, $df = 2$) and the number of taxa with tolerance values of 0-5.0 (sensitive taxa) was lowest at site 3 ($F = 2.6$, $df = 2$). The proportion of taxa with tolerance values >7 ($F = 2.8$, $df = 2$) was lowest at site #1 and taxa richness was higher at sites #2 and #3 ($F = 3.8$, $df = 2$).

Compared to other streams in western North Dakota, all the Knife River sites were scored as least disturbed using the Macroinvertebrate Biotic Integrity Multimetric Index of North Dakota. The Knife River sites scored 42 (site #1), 46 (site #2), and 48 (site #3). Score conditions increased as the river flowed through the park. According to the index, scores ≥38.2 are considered least disturbed, scores between 22.5 and 38.2 are considered moderately disturbed, and scores <22.5 are considered most disturbed (Environmental Protection Agency 2009).

Table 5. Average values and standard errors for bioassessment metrics for each site. I used ANOVA to detect differences among sites. If values were significantly different (P < 0.05), I used Bonferroni multiple comparison tests to distinguish among sites. Metrics with non-normal variance were natural log transformed for statistical analysis.

Metric	**Site #1**	**Site #2**	**Site #3**	***P*-value**	**Bonferroni**
% Chironomidae	31±3.2	46±3.5	71±2.4	<0.0001	3 vs. 1, 3 vs. 2
% clinger taxa	14±1.5	8.3±2.5	12.7±2.5	0.19	
% EPT taxa	26±4.2	26±1.3	27±3.4	0.98	
% gatherers	44±5.3	55±2.6	83±3.5	<0.0001	3 vs. 1, 3 vs. 2
% predator taxa	26±5.1	32±3.0	33±2.5	0.65	
% predators	11±2.5	24±1.8	7±2.1	0.0003	2 vs. 1, 2 vs. 3
% intolerant (0-5.0)	8.3±2.2	8.4±2.7	5.3±1.9	0.19	
% tolerant (6.0-7.0)	43±5.4	56±1.8	85±2.6	<0.0001	1 vs. 3, 2 vs. 3
% tolerant (8.0-9.0)	73±20	74±24	44±15	0.56	
% tolerant (>7)	13±2.3	22±2.0	18±1.5	0.03	
% tolerant taxa (>7)	30±2.8	41±4.15	44±5.5	0.097	
% intolerant taxa (0-5.0)	26±3.9	26±1.4	18±3.0	0.11	
EPT richness	2.2±0.4	3.0±0	3.0±0.3	0.11	
EPT/Chironomidae	0.20±0.04	0.30±0.11	0.18±0.04	0.44	
HBI	5.93±0.11	6.13±0.04	6.19±0.04	0.058	
Ln(% clinger)	9.3±3.0	1.2±0.82	2.5±0.75	0.014	1 vs. 2
Ln(% filterers)	0.30±0.30	0.86±0.52	3.3±1.7	0.26	
Ln(% non-insects)	0.51±0.18	1.6±0.63	4.1±2.3	0.15	
Taxa diversity	1.47±.14	1.75±0.05	1.25±.012	0.026	
Taxa richness	8.4±0.5	11.6±0.6	11.6±1.4	0.052	
Taxa evenness	0.69±0.05	0.72±0.02	0.52±0.05	0.012	3 vs. 2
Total abundance	2347±456	3128±661	4198±1183	0.32	

Discussion

Very little work has been published on the Knife River in North Dakota. A search of ISI Web of Knowledge http://thomsonreuters.com/products_services/science/science_products/a-z/isi_web_of_knowledge/ (keywords Knife River and North Dakota) revealed that only archeological work is available in the peer-reviewed literature. Rust (2006) sampled the aquatic invertebrates of the Knife River at Knife River Indian Villages National Historic Site as part of her thesis. Similar to the current study (97%), insects dominated the assemblage in the Knife River (98%; Rust 2006). However, Hemiptera (Corixidae) were the most abundant order of insects in her study (Rust 2006), whereas I found that Diptera were the most abundant order. Additionally, I collected a higher percentage of Chironomidae (49%) compared to Rust (2006; 12%). These differences may be due to sampling methods. Rust (2006) used a dip net and probably did not collect as many burrowing invertebrates as I did with a Hess sampler. Rust (2006) estimated that predators were the dominant functional feeding group in the river, whereas I calculated that gatherers were the most abundant group. Both the current study (10.5) and Rust (2006; 9) calculated a similar taxa richness. However, I calculated a lower HBI (6.1) compared to Rust (2006; 7.6). In other words, I calculated that an average invertebrate in the assemblage had a lower tolerance value to pollution, where 0 is an invertebrate that is highly sensitive to pollution and 10 is an extremely pollution tolerant taxa. I may have calculated a lower HBI values because I collected a higher density of benthic taxa, such as the burrowing mayfly *Hexagenia* and caddisflies, with lower tolerance values.

In general, the invertebrates in the Knife River were fairly abundant and diverse compared to other rivers in the western North Dakota. I collected more taxa in the Knife River compared to the Missouri River below Garrison Dam (Angradi et al. 2006). Mostly Chironomidae, Corixidae, and Oliogchaeta (worms) lived in the main channel of the Missouri River. A few other invertebrates lived in the backwaters of the Missouri River, including *Caenis* (mayfly), Coenagrionidae (damselfly), and Planorbidae (snail). The density of invertebrates along the shoreline of the Missouri River (2993 ind/m^2) was similar to the Knife River (3224 ind/m^2). Average invertebrate density and taxa richness in the Knife River (10.5 taxa) were higher than the Little Missouri River (963 ind/m^2; 5.3 taxa) near Theodore Roosevelt National Park (Tronstad 2013); however, these rivers differed in several ways. The Knife River was a deeper river with abundant organic matter in the substrates whereas the Little Missouri River typically contained less organic matter and was generally wider and shallower. Riparian and landscape vegetation differed between these areas. The Little Missouri River was dominated by cottonwood and willow (*Salix* spp.) in the riparian area, and the landscape was primarily grassland. Conversely, the riparian area and landscape at Knife River Indian Villages National Historic Site was dominated by cottonwoods and green ash. The Little Missouri River was sampled in the Badlands which is a dry area with erodible bedrock. Because of this, the discharge of the Little Missouri River is known to increase quickly. Additionally, the Knife River is located in a different ecoregion than the Little Missouri River and receives more annual precipitation. Despite these differences, some taxa were found in both rivers (*Paracloeodes*, Baetidae; *Caenis*, Caenidae; *Nectopsyche*, Leptoceridae; *Dubiraphia*, Elmidae; and *Dromogomphus*, Gomphidae)

Two multimetric indexes were developed for North Dakota, because bioassessment models developed for larger areas often do not perform well (Ode et al. 2008). A multimetric index was developed for the rangeland plains of North Dakota, which included the Knife River Basin. The six metrics used in the rangeland plains index were EPT richness, % clingers (abundance), % gatherers (abundance), % predator taxa, % taxa with tolerance values of 0 to 5.0, and % of individuals with tolerance values of 8.0-9.0 (Environmental Protection Agency 2009). According to this index, all sites along the Knife River in Knife River Indian Villages National Historic Site were considered least disturbed. In fact, multimetric scores increased as the river flowed through the park.

Using individual metrics, site #3 appeared to have lower quality compared to the other sites. Interpreting individual metrics for biomonitoring is often simpler and more informative than using an index composed of many metrics (Allan 2004). Site #3 had the highest % Chironomidae, % of individuals with tolerance values of 6.0-7.0, % tolerant taxa (tolerance values >7), and HBI value. Additionally, site #3 had the lowest % intolerant individuals (tolerance values 0-5.0), taxa diversity, % predators, and taxa evenness. The high density of invertebrates at site #3 was driven by Chironomidae (3046 ind/m^2). The density of other invertebrates did not decrease in general, but Chironomidae dominated the assemblage explaining why several metrics were highest (e.g., % Chironomidae) or lowest (% intolerant individuals, taxa diversity, % predators, and taxa evenness) at site #3. Chironomidae have a tolerance value of 6.0 (Bowles et al. 2008) which caused site #3 to have the highest percent of individuals with tolerance values between 6.0 and 7.0, and highly influenced the HBI values (6.2). Mayflies and caddisflies that tend to be sensitive to ecosystem quality reached their highest abundance at site #3, but their densities were swamped by the Chironomidae. One metric at site #3 that was not influenced by the high density of Chironomidae was the % of taxa with tolerance values >7.0. I collected several tolerant taxa (>7) at site #3 including *Culicoides* (Ceratopogonidae), *Sialis* (Sialidae), *Caenis* (Caenidae), *Hyalella*, Cladocera, Ostracoda, Copepoda (Crustacea), Ancylidae, Physidae, Planorbidae, and Spheariidae (Mollusk). Therefore, the ecosystem quality of site #3 may not be lower compared to the other sites, but the lower metrics were generally an artifact of high Chironomidae abundance.

The multimetric index developed for North Dakota scored site #3 highest, but several individual metrics rated this site lower. Five of the six metrics used in the North Dakota Macroinvertebrate Biotic Integrity Multimetric Index were not influenced by the high density of Chironomidae at site #3. Many Chironomidae are gatherers and the index used % gatherers to score sites. The % gatherers were predicted to decline in response to impacts, thus the higher the % gatherers the higher the score. In this case, the dominance of Chironomidae improved the multimetric score.

Higher organic matter content of substrates can increase the density of invertebrates. Organic matter can be both food and substrate for aquatic invertebrates (Allan 2001). How organic matter is used by invertebrates depends on the size of the organic matter and the invertebrates. Benthic organic matter varied from small to large in the Knife River, and was probably used as both food and organic matter. Site #3 had the highest organic matter content and the highest density of invertebrates. Egglishaw (1964) also found that more benthic organic matter correlated with higher densities of invertebrates. Similarly, Mackay and Kalff (1969) estimated that up to 5 times more invertebrates lived on leaves and detritus compared to sand, gravel, and cobbles and pebbles.

Unfortunately, I cannot distinguish how aquatic invertebrate assemblages in the Knife River varied with fecal coliform concentration, because exact concentrations were not measured at sites. Fecal coliform concentrations at all sites were at least one order of magnitude higher than the recreation standard. Previous studies showed that aquatic invertebrates can respond to fecal coliform concentrations. Invertebrate densities and fecal coliform concentrations were positively correlated in a study investigating the effects of feral hogs on streams in Louisiana (Kaller and Kelso 2006). They found fewer mayflies, and more Tanypodinae (subfamily of Chironomidae), snails, and riffle beetles in areas with higher fecal coliform concentrations. In the Knife River, the fewest mayflies and the most riffle beetles were collected at site #1 where the highest concentrations of *E. coli* were measured. Interestingly, *E. coli* concentrations decreased as the river flowed through the park, but I do not know if fecal coliform followed the same trend. Finally, Olive (1976) investigated the aquatic invertebrates of the Cuyahoga River in Ohio along a gradient of fecal coliform concentrations (130 – 11,000 FCU/100 mL). He sampled invertebrates and water chemistry monthly for a year. Besides fecal coliform, no other impairments were detected (e.g., trace elements, nutrients). Olive (1976) discovered that >3 times more taxa were located in reaches with lower concentrations of fecal coliform and that >50% of the taxa in these reaches were composed of intolerant taxa. About 25% of the taxa in the Knife River were intolerant and the fewest taxa were collected at site #1.

Some invertebrate bioassessment metrics were specifically designed to detect certain types of pollution. For example, Hilsenhoff's Biotic Index (HBI) was developed to detect organic pollution (Hilsenhoff 1987; 1988). According to the HBI scale, sites along the Knife River were fair to fairly poor quality. Organic pollution can come from many sources (e.g., wastewater discharge, livestock grazing), but organic pollution usually decreases dissolved oxygen concentrations and increases bacterial concentrations (Simon and Buikema 1997). Organic pollution may alter the invertebrate assemblage through different pathways. For example, some invertebrates, such as stoneflies, are sensitive to low dissolved oxygen concentrations. On the other hand, increased bacterial concentration may alter the food web, because aquatic invertebrates can probably eat these bacteria (Simon & Buikema 1997). After enriching a stream with cow manure, del Rosario et al. (2002) found that all invertebrates were consuming the manure, but the gathering mayfly, *Paraleptophlebia,* was enriched the most in California streams. Additionally, Chironomidae densities increased 5 times after cow manure additions.

The source of fecal coliform in the Knife River may be from livestock, wildlife, or human sources (e.g., wastewater treatment plant discharge, failing septic tanks). Urban areas can have a disproportionately large impact on rivers despite occupying a small area (Allan 2004). Several towns are located along the Knife River and Spring Creek, but these towns are small (<3500 people). High concentrations of these bacteria may enter the river if wastewater treatment plants or storm water discharge systems fail. Once these bacteria are in the river, they may multiply if conditions are suitable further increasing concentrations. The dominant land use in the area is agriculture (both crop and livestock production). Fecal coliform are gram negative bacteria that come from the gastrointestinal tract of animals; therefore, livestock and wildlife can be a source. Livestock or wildlife defecating in a stream can increase fecal coliform concentrations. Similarly, concentrated animal feeding operations (e.g., feedlots) near streams can dramatically increase bacterial concentrations. In a study of the Four Mill Run watershed in Virginia, Simmons et al. (2002) found that 17% of *E. coli* was from humans. The remainder was from waterfowl (37%), canine (9%), deer (10%), raccoons (15%), and other animals (12%).

Regardless of the source of fecal coliform, management changes by Knife River Indian Villages National Historic Site will probably have little effect on fecal coliform concentrations at the park. To be effective, management changes will likely have to occur across the watershed as a whole. If most of the bacteria are from livestock, management practices such as watering livestock away from the river and managing for well-developed riparian areas, can greatly decrease bacterial concentrations. Well-developed riparian areas can buffer the effects of surrounding land use (e.g., Sponseller et al. 2001; Feld 2013). If bacteria are primarily coming from urban areas, maintenance and repair of wastewater treatment plants, storm drains, or septic tanks may be required. To estimate the source of bacterial contamination, the ratio of certain bacterial groups can be used. For example when the ratio of fecal coliform to fecal streptococci is <0.1 the bacteria are likely from wildlife, if the ratio is between 0.1 and 0.7, the source of bacteria are likely from livestock (Muenz et al. 2006). But when the ratio is >4, bacteria are likely from humans.

Literature Cited

Allan, J. D. 2001. Stream Ecology: Structure and Function of Running Waters. Kluwer Academic Publishers, Boston.

Allan, J. D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual Review of Ecology Evolution and Systematics 35:257-284.

Angradi, T. R., E. W. Schweiger, and D. W. Bolgrien. 2006. Inter-habitat variation in the benthos of the Upper Missouri River (North Dakota, USA): implications for great river bioassessment. River Research and Applications 22:755-773.

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

Bowles, D. E., M. H. Williams, H. R. Dodd, L. W. Morrison, J. A. Hinsey, C. E. Ciak, G. A. Rowell, M. D. DeBacker, and J. L. Haack. 2008. Protocol for monitoring aquatic invertebrates of small streams in the Heartland Inventory and Monitoring Network. National Park Service, Fort Collins.

Cairns, J., and J. R. Pratt. 1993. A history of biological monitoring using benthic macroinvertebrates. Pages 10-27 in D. M. Rosenberg and V. H. Resh, editors. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York, NY.

del Rosario, R. B., E. A. Betts, and V. H. Resh. 2002. Cow manure in headwater streams: tracing aquatic insect responses to organic enrichment. Journal of the North American Benthological Society 21:278-289.

Dolédec, S., N. Phillips, M. Scarsbrook, R. H. Riley, and C. R. Townsend. 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society 25:44-60.

Eaton, A. D., and M. A. H. Franson. 2005. Standard Methods for the Examination of Water and Wastewater, 21st Centennial edition. American Public Health Association, Washington DC.

Egglishaw, H. J. 1964. The distributional relationship between the bottom fauna and plant detritus in streams. Journal of Animal Ecology 33:463-476.

Environmental Protection Agency. 2003. Bacterial water quality standards for recreational waters (freshwater and marine waters) status report, Office of Water, Washington DC.

Environmental Protection Agency. 2009. An ecological assessment of USEPA Region 8 streams and rivers. Environmental Protection Agency, Denver.

Feld, C. K. 2013. Response of three assemblages to riparian and catchment-scale land use: implications for designing catchment monitoring programmes. Freshwater Biology 58.

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomologist 20:31-39.

Hilsenhoff, W. L. 1988. Rapid field assessment of organic pollution with a family-level biotic index. Journal of the North American Benthological Society 7:65-68.

Kaller, M. D., and W. E. Kelso. 2006. Swine activity alters invertebrate and microbial communities in a coastal plain watershed. American Midland Naturalist 156:163-177.

Karr, J. R. 1981. Assessment of biotic integrity using fish communities. Fisheries 6:21-27.

Kerans, B. L., and J. R. Karr. 1994. A benthic index of biotic integrity (B-IBI) for rivers of the Tennessee Valley. Ecological Applications 4:768-785.

Mackay, R. J., and J. Kalff. 1969. Seasonal variation in standing crop and species diversity of insect communities in a small Quebec stream. Ecology 50:101-&.

Merritt, R. W., K. W. Cummins, and M. B. Berg, editors. 2008. An Introduction to the Aquatic Insects of North America, 4th edition. Kendall Hunt Publishing, Dubuque, IA.

Muenz, T. K., S. W. Golladay, G. Vellidis, and L. L. Smith. 2006. Stream buffer effectiveness in an agriculturally influenced area, southwestern Georgia: responses of water quality, macroinvertebrates, and amphibians. Journal of Environmental Quality 35:1924-1938.

Needham, J. G., M. J. Westfall, and M. L. May. 2000. Dragonflies of North America, Revised edition. Scientific Publishers, Gainesville, Florida.

Ode, P. R., C. P. Hawkins, and R. D. Mazor. 2008. Comparability of biological assessments derived from predictive models and multimetric indices of increasing geographic scope. Journal of the North American Benthological Society 27:967-985.

Olive, J. H. 1976. Chemical, physical, and biological assessment of water quality in the Cuyahoga River, Ohio USA 1973-1974. Ohio Journal of Science 76:5-15.

Patrick, R. 1949. A proposed biological measure of stream conditions, based on a survey of the Conestoga Basin, Lancaster County, Pennsylvania. Proceedings of the Academy of Natural Sciences of Philadelphia 101:277-341.

Resh, V. H., and J. K. Jackson. 1993. Rapid assessment approaches to biomonitoring using benthic macroinvertebrates. Pages 195-223 in D. M. Rosenberg and V. H. Resh, editors. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman & Hall, New York.

Rust, J. 2006. Establishing baseline data for aquatic resources in National Parks of the Northern Great Plains Network. Thesis. South Dakota State University.

Simmons, G. M., D. F. Waye, S. Herbein, S. Myers, and E. Walker. 2002. Estimating nonpoint fecal coliform sources in northern Virginia's Four Mile Run watershed. Pages 143-168 in T.

Younos, editor. Advances in Water Monitoring Research. Water Resources Publication, Highlands Ranch, Colorado.

Simon, K. S., and A. L. Buikema. 1997. Effects of organic pollution on an Appalachian cave: changes in macroinvertebrate populations and food supplies. American Midland Naturalist 138:387-401.

Smith, D. G. 2001. Pennak's Freshwater Invertebrates of the United States, 4th edition. John Wiley and Sons, Inc., New York.

Sponseller, R. A., E. F. Benfield, and H. M. Valett. 2001. Relationships between land use, spatial scale and stream macroinvertebrate communities. Freshwater Biology 46:1409-1424.

Thorp, J. H., and A. P. Covich, editors. 2010. Ecology and Classification of North American Freshwater Invertebrates, 3rd edition. Elsevier, New York.

Tronstad, L. 2013. Aquatic resources along the Little Missouri River near Theodore Roosevelt National Park, North Dakota. Natural Resource Technical Report NPS/NGPN/NRTR—2013/XXX. National Park Service, Fort Collins, Colorado.

Vandewalle, M., F. de Bello, M. P. Berg, T. Bolger, S. Doledec, F. Dubs, C. K. Feld, R. Harrington, P. A. Harrison, S. Lavorel, P. M. da Silva, M. Moretti, J. Niemela, P. Santos, T. Sattler, J. P. Sousa, M. T. Sykes, A. J. Vanbergen, and B. A. Woodcock. 2010. Functional traits as indicators of biodiversity response to land use changes across ecosystems and organisms. Biodiversity and Conservation 19:2921-2947.

Appendix A

List of invertebrate taxa collected in the Knife River at Knife River Indian Villages National Historic Site.

- Insects
  - Coleoptera
    - Elmidae
      - *Dubiraphia*
      - *Stenelmis*
  - Diptera
    - Chironomidae
      - Non-Tanypodinae
      - Tanypodinae
    - Ceratopogonidae
      - *Culicoides*
    - Simuliidae
  - Ephemeroptera
    - Caenidae
      - *Caenis*
    - Ephemeridae
      - *Hexagenia*
    - Baetidae
      - *Paracloeodes*
  - Hemiptera
    - Corixidae
      - *Hesperocorixa*
      - *Palmacorixa*
      - *Trichocorixa*
  - Megaloptera
    - Sialidae
      - *Sialis*
  - Odonata
    - Gomphidae
      - *Dromogomphus spoliatus*
      - *Dromogomphus spinosus*
    - Coenagrionidae
      - *Chromagrion conditum*
  - Trichoptera
    - Hydropsychidae
      - *Hydropsyche*
    - Leptoceridae
      - *Nectopsyche*
- Arachnida
  - Hydracarina
- Mollusca

- Gastropoda
  - Ancylidae
  - Physidae
  - Planorbidae
- Bivalvia
  - Sphaeriidae

Crustacea

- Amphipoda
  - *Hyalella*
- Cladocera
- Copepoda
  - Cyclopoida
- Ostracoda

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