National Park Service
U.S. Department of the Interior

Natural Resource Stewardship and Science

Aquatic Invertebrate Monitoring at Agate Fossil Beds National Monument

2012 Annual Report

Natural Resource Technical Report NPS/NGPN/NRTR—2014/874

ON THIS PAGE
Cody Bish and Lusha Tronstad retrieving Hester-Dendy samplers in the Niobrara River at Agate Fossil Beds National Monument.
Photograph by: Kristina Fox, National Park Service

ON THE COVER
Cody Bish recording basic water quality in the Niobrara River at Agate Fossil Beds National Monument.
Photograph by: Lusha Tronstad, Wyoming Natural Diversity Database

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May 2014

U.S. Department of the Interior
National Park Service
Natural Resource Stewardship and Science
Fort Collins, Colorado

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This report received informal peer review by subject-matter experts who were not directly involved in the collection, analysis, or reporting of the data. Data in this report were collected and analyzed using methods based on established, peer-reviewed protocols and were analyzed and interpreted within the guidelines of the protocols.

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Please cite this publication as:

Tronstad, L. 2014. Aquatic invertebrate monitoring at Agate Fossil Beds National Monument: 2012 annual report. Natural Resource Technical Report NPS/NGPN/NRTR—2014/874. National Park Service, Fort Collins, Colorado.

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Executive Summary

Aquatic invertebrates are excellent animals to use for monitoring ecosystem quality; however, what is the best method to sample aquatic invertebrates for such monitoring efforts? All samplers have advantages and disadvantages, and finding the sampler that minimizes bias and fulfills the objectives is crucial. The ecosystem quality of the Niobrara River at Agate Fossil Beds National Monument has been measured for 16 years using aquatic invertebrates colonizing Hester-Dendy samplers. Based on these measurements, three bioassessment metrics changed over time. HBI increased over the last 16 years, indicating that invertebrates living in the Niobrara River are more tolerant of pollution. EPT richness and the proportion of EPT taxa have declined over time, showing a decrease in the number of sensitive insects in the river. Hester-Dendy substrates are artificial multiplate samplers useful in rivers that are difficult to sample, but previous studies demonstrated that they bias results toward certain insect orders. Additionally, large debris dams form upstream of these samplers in the Niobrara River potentially altering samples. Therefore, I compared aquatic invertebrates collected using Hester-Dendy samplers and a Hess sampler in the Niobrara River. Hester-Dendy and Hess samplers collected a similar number of insects; however, Hess samples collected far more non-insect invertebrates. Bioassessment metrics calculated from Hess samples had higher taxa diversity, higher taxa richness, higher Hilsenhoff's Biotic Index (HBI) values, and a lower proportion of mayfly, stonefly, and caddisfly (EPT) taxa compared to Hester-Dendy samples. Taxa evenness and EPT richness were similar between the two samplers. I recommend collecting aquatic invertebrates using a Hess sampler in the Niobrara River at Agate Fossil Beds National Monument, because the Hess sampler will reduce the number of visits to each site reducing overall costs. Furthermore, Hess samples collect the natural density and diversity of invertebrates, and results are compared to other ecosystems. However, Agate Fossil Beds National Monument has 16 years of invertebrate data collected with Hester-Dendy samplers. Based on the monitoring objectives, managers will have to decide what method to continue monitoring with.

Acknowledgments

I thank Cody Bish, Kyle Hack, and Oliver Wilmot for field and laboratory assistance. I am grateful to Marcia Wilson, Kristina Fox, Patty Bean, and James Hill of the National Park Service who helped with logistics and field work, and gave me the opportunity to work at Agate Fossil Beds National Monument.

Introduction

Aquatic invertebrates are excellent indicators of ecosystem quality and have been used to monitor conditions since the 1870s (Cairns and Pratt 1993). Managers and scientists use aquatic invertebrates to monitor ecosystem quality, because these animals have several characteristics that make them ideal for the task. For example, aquatic invertebrates are relatively long lived (weeks to >100 years, Rosenberg and Resh 1993b). Unlike water samples that are collected periodically, aquatic invertebrates live in the stream year-round and represent conditions at that site. Water samples may miss discrete discharges of pollution, but aquatic invertebrates will respond to such events. These animals are relatively sedentary and are used to assess water quality at a location. Aquatic invertebrates are abundant, diverse, and easy to collect. Countless studies have measured that lower ecosystem quality can increase mortality, and decrease reproduction, survival, and fitness of aquatic invertebrates (Johnson et al. 1993). Some aquatic invertebrates are more sensitive to changes in ecosystem quality (i.e., stoneflies), while other are more tolerant (i.e., true flies). Changes in the diversity or community structure of aquatic invertebrates can be a sensitive measure of ecosystem quality, and these metrics are well-developed (Rosenberg and Resh 1993a).

The choice of what aquatic invertebrate sampler to use to monitor ecosystem quality can be a difficult decision that depends on many variables. All samplers have both advantages and disadvantages, but finding a sampler that minimizes bias and fulfills the objective is critical. Bioassessment studies use a variety of sampling methods, including kicknets, fixed-area samplers (e.g., Hess sampler), artificial substrates (e.g., Hester-Dendy samplers), and dipnets (Carter and Resh 2001). Deciding what sampler to use often depends on characteristics of the stream. For example, artificial substrates may be a good choice in ecosystems that are difficult to sample using other methods (De Pauw et al. 1986), such as large, deep rivers. The objective of the study determines what type of information should be collected. Dipnets and kicknets may only provide presence/absence data for aquatic invertebrates, but fixed area samplers can provide quantitative information on the density and biomass of these animals. Artificial substrates can be a useful technique to collect aquatic invertebrates; however, the samples collected do not represent natural assemblages or densities, and these samplers can be biased toward certain insect orders (Letovsky et al. 2012).

The National Park Service has been monitoring aquatic invertebrates at Agate Fossil Beds National Monument since 1989 using Hester-Dendy samplers. However, the National Park Service would like to consider other methods, because of difficulties collecting samples using artificial substrates and difficulties comparing results to other streams. For example, Hester-Dendy samplers calculate density as a function of surface area of all plates (e.g., 0.1 m^2 on 9 plates), whereas fixed area samplers report density as a function of surface area of benthic habitat (Hess samplers collect from 0.086 m^2 of stream bottom). Thus, invertebrate density calculated from artificial substrate samplers and fixed area samplers are not comparable.

Both fish and aquatic invertebrates suggest that ecosystem quality in the Niobrara River at Agate Fossil Beds National Monument has declined. One explanation for the decline is the invasion of

yellow flag iris (*Iris pseudacorus*; Bowles 2010, Bowles et al. 2013, Spurgeon et al. 2014). Yellow flag iris probably slows water velocity and increases organic matter in the stream leading to large daily and seasonal swings in dissolved oxygen concentrations. Another explanation for the decline in ecosystem quality is the introduction of invasive northern pike (*Esox lucius*) in the Niobrara River (Spurgeon et al. 2014). Pike are piscivores and likely reduced the fish assemblage from 11 species to 3 species between 1989 and 2011. Stasiak et al. (in prep) speculated that pike currently feed on crayfish, because other fish are scarce in the river. Introducing pike may have caused a trophic cascade that changed the abundance and assemblage of invertebrates in the Niobrara River. My objective was to compare invertebrates collected using Hester-Dendy samplers and a Hess sampler from 3 sites along the Niobrara River at Agate Fossil Beds National Monument. My specific questions were: 1.) How does the assemblage of invertebrates collected with Hester-Dendy samplers and a Hess sampler compare? 2.) How do the bioassessment metrics compare between these samplers? and 3.) How have the bioassessment metrics changed over time?

Study Area

The headwaters of the Niobrara River are located around Lusk, Wyoming, and flow eastward into Nebraska and eventually to the Missouri River near Niobrara, Nebraska. The Niobrara River Basin covers 32,600 km^2 of which the majority is grassland in northern Nebraska (Galat et al. 2005). Over 95% of the land within the basin is used for agriculture. The Niobrara River flows through Agate Fossil Beds National Monument in western Nebraska about 23 km from the Wyoming border. At this point the Niobrara River is a low order stream flowing through grassland. Agate Fossil Beds National Monument includes 2700 acres in a valley bottom, and 11 miles of river flows through the 4 mile wide park (Figure 1). The riparian vegetation in the Park is dominated by cattails (*Typha* sp.) and the invasive yellow flag iris. The substrate in the river consists of fine particles (e.g., sand, silt, and clay). Currently, pike, white suckers (*Catostomus commersonii*), and green sunfish (*Lepomis cyanellus*) inhabit the river within the park (Spurgeon et al. 2014); however, 9 fish species were collected at Agate Fossil Beds National Monument prior to 1990 (Spurgeon et al. 2014).

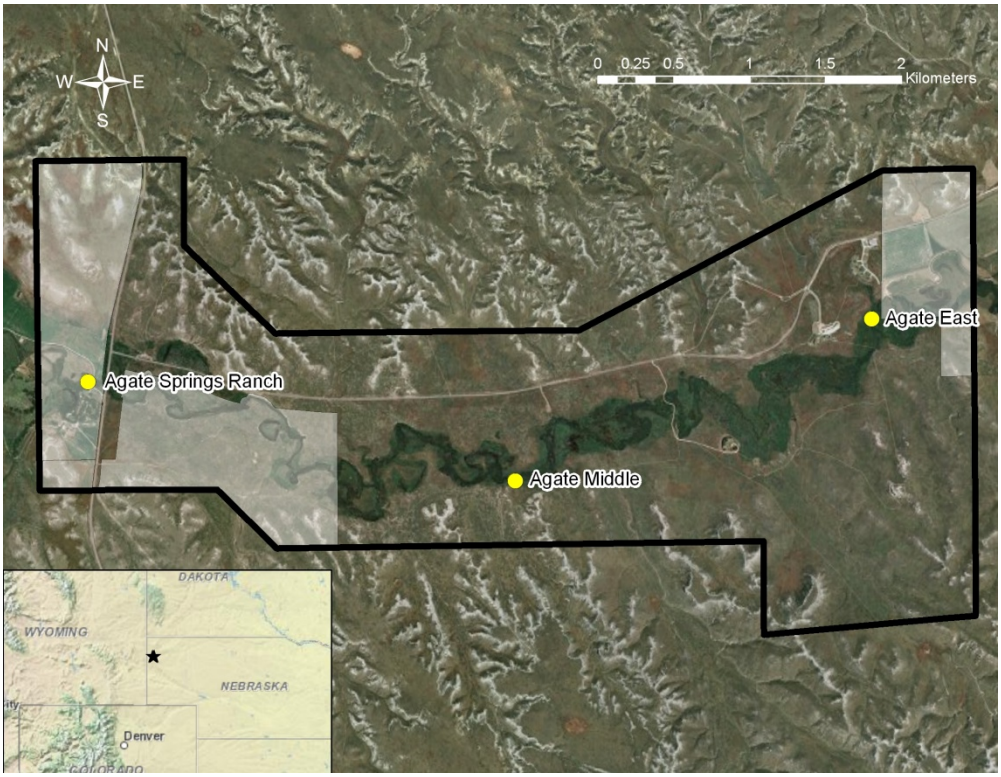

Figure 1. I sampled 3 sites along the Niobrara River at Agate Fossil Beds National Monuments. The black line is the Monument boundary and the transparent white areas are private land within the Monument boundary. The inset map shows the location of Agate Fossil Beds National Monument (star).

I sampled 3 sites along the Niobrara River (Figure 1, Table 1). The most upstream site (Agate Springs Ranch) is located near the west park boundary. Agate Springs Ranch has an overstory of plains cottonwood (*Populus deltoides*), and cattails are more abundant than iris (Figure 2a). The middle site, Agate Middle, is shallower and lacks an overstory (Figure 2b). Both iris and cattails are abundant here. Finally, Agate East, the site located before the Niobrara River flows out of the Park,

is the deepest site (Figure 2c). The riparian vegetation is dominated by iris with a few willow (*Salix* spp.).

Figure 2. Photos of a.) Agate Springs Ranch, b.) Agate Middle, and c.) Agate East.

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

Site	Ranch	Middle	East
Easting	599323	602143	604495
Northing	4697497	4693844	4697913

Methods

General Measurements

To assess the general habitat characteristics of the Niobrara River, I measured several features including general water quality, water clarity, sediment composition, and depth. I measured dissolved oxygen (percent saturation and mg/L), pH, water temperature, specific conductivity, and oxidation-reduction potential using a Yellow Springs Instruments (YSI) Professional Plus. The sonde was calibrated on-site before use. I measured water clarity by estimating the depth at which a Secchi disk disappeared from sight. The composition of sediment was estimated by sampling across the width of the stream channel and selecting the percent class for clay, silt, sand, gravel, cobble, boulder, bedrock, and hardpan/shale on a scale of 0 -7 (0 = none, 1 = trace, 2 = 1-5%, 3 = 5-25%, 4 = 25-50%, 5 = 50-75%, 6 = 75-95% and 7 = 95-100%; Peterson et al. 1999). Clay was defined as fine particles forming a ribbon after removing water, whereas silt did not form a ribbon. Sand was particles 0.06-2 mm in diameter, gravel was 2-64 mm in diameter, cobble was 64-256 mm in diameter, boulder was 256-4000 mm in diameter, bedrock was >4000 mm in diameter, and hardpan/shale was firm, consolidated fine substrate. I recorded the location of each site using a global positioning system (GPS; Garmin eTrex Vista HCx). Finally, I estimated water velocity (m/s; V) by measuring the depth of the water with a meter stick (3.2 mm width) parallel and perpendicular to flow across the width of the stream at 7 positions. By subtracting the 2 measurements, I calculated vertical displacement (D). The greater the vertical displacement of the water, the higher the water velocity. Velocity was estimated using the relationship:

$$V = \ln D * 0.304 + 0.405$$

Schlosser (1982) developed the above equation for a headwater stream in Illinois for vertical displacement between 0 and 20 mm (~0.25 to 1.5 m/s). To estimate discharge, I multiplied stream width, depth, and water velocity within 8 compartments of equal lengths.

Hester-Dendy Samples

I deployed 7 Hester-Dendy samplers (76 mm by 76 mm, 9 plates, Wildlife Supply Company) at each site on 5 July 2012 (Figure 3a). A rope was strung across the stream between 2 permanent posts and 7 loops were tied to separate the Hester-Dendy samplers. From each loop, another rope was tied with the Hester-Dendy samplers hanging about a foot below to allow for a drop in water level. I retrieved the samplers on 7 August 2012 by approaching the site from downstream and placing a dip net (150 µm mesh) under each sampler. Hester-Dendy samplers were immediately placed in a container with ~80% ethanol, and any organisms in the dip net were removed and placed in the same container. After returning to the laboratory, I dismantled the Hester-Dendy samplers to remove invertebrates that colonized the plates, rinsed samples using a 212 µm sieve, and preserved samples in 80% ethanol.

Hess Samples

To sample invertebrates that live in the emergent vegetation that is abundant along the margin of the Niobrara River, I collected 5 Hess samples (500 µm mesh, 860 cm^2 sampling area, Wildlife Supply Company) from each site on 5 July 2012 (Figure 3b). I placed the Hess sampler over cattails and/or

yellow flag iris to collect invertebrates that lived on the vegetation and in the surrounding sediment. The vegetation and sediment were vigorously agitated using our hands and a brush, and invertebrates were captured in the net of the Hess sampler. Samples were preserved in 80% ethanol.

Figure 3. Photos of a.) a Hester-Dendy sampler colonized by aquatic invertebrates and b.) processing an aquatic invertebrate sample collected with a Hess sampler.

Invertebrate analysis

Invertebrates were sorted from the debris and identified to genus (Insecta, Turbellaria, Isopoda, and Amphipoda), family (Decapoda, Pelecypoda (Bivalvia), Gastropoda), class (Annelida, Acarina) or phylum (Nematoda) with one exception (order: Collembola) according to Peterson et al. (1999). If invertebrates were numerous (>200 individuals) in any sample, I subsampled. First, I rinsed the sample through a 2 mm and a 212 µm (Hester-Dendy) or 500 µm (Hess) mesh sieves to separate the larger and less abundant invertebrates from the smaller and more abundant invertebrates. All invertebrates were removed and identified in the larger (>2 mm) portion of the sample. If invertebrates were numerous, I subsampled the contents of the sieve with the smaller mesh size using the record player method (Waters 1969). Invertebrates were identified under a dissecting microscope using Merritt et al. (2008) for insects, and Thorp and Covich (2010) and Smith (2001) for non-insect invertebrates.

Several bioassessment metrics have been calculated since 1989 to estimate ecosystem quality based on the invertebrates collected: HBI, EPT richness, proportion of EPT taxa (number of EPT taxa divided by the total number of taxa collected), taxa diversity (Shannon's index), taxa richness, and taxa evenness (Bowles 2010). To distinguish among sites, I used ANOVA to compare abundance and bioassessment metrics for each sampler. Differences among sites were distinguished using Bonferroni adjusted values. To evaluate differences between the two sampling devices, I used a two sample t-test to compare abundance and bioassessment metrics among sampler types. To analyze long-term bioassessment metrics for trends, I used functional data analysis (FDA). I plotted bioassessment metrics against time and calculated slopes and standard errors (SE) for each site. Average slopes and SE were averaged for each metric and confidence intervals were calculated for each average slope. Trends were significant when the confidence interval did not include zero. I used R (R Development Core Team 2013) including the packages *plyr* (Wickham 2011), *Matrix*

(Bates and Maechler 2013), and *vegan* (Oksanen et al. 2013) to calculate densities, bioassessment metrics, and make comparisons.

Results

In general, conditions were similar among sites. I measured higher water temperatures in July compared to August (Table 2). Conversely, dissolved oxygen concentrations were higher in August compared to July. Dissolved oxygen concentrations reached a minimum of 6.5 mg/L at 22:00 hours on 7 August 2012 and temperature varied between 24.6 and 17˚C during the night (Figure 4). pH was slightly basic and increased between July and August at each site. Specific conductivity was similar among sites and dates. Oxidation-reduction potential was higher in July (oxidizing conditions) and decreased in August. Total stream width and depth varied by date and site. Overall, Agate Springs Ranch was widest (2.7 m), and Agate Middle (2.3 m in July and 2.2 m in August) and Agate East (1.8 m in July and 2.5 m in August) were narrower. Agate Middle was shallowest in July and August (Table 3). Modeled water velocity was higher in August at all sites. Similarly, modeled discharged was higher in August (Table 4). Overall, the substrate in the Niobrara River was dominated by fine sediments (clay, sand, and silt). Agate Springs Ranch was dominated by silt and sand. The substrate at Agate Middle was primarily gravel, silt, and sand. Finally, Agate East was dominated by clay, sand, and silt.

Table 2. Water quality measured when Hess samples were collected and Hester-Dendy samplers were deployed (5 July), and when Hester-Dendy samplers were collected (7 August).

Site	Units	Ranch	Middle	East	Ranch	Middle	East
Date		5-Jul-12	5-Jul-12	5-Jul-12	7-Aug-12	7-Aug-12	7-Aug-12
Start Time		11:54	14:30	16:44	12:06	13:53	15:30
Water temperature	˚C	21.1	25.3	27.0	20.3	22.4	24.5
Dissolved oxygen	% saturation	86	90	84	108	136	120
Dissolved oxygen	mg/L	7.8	7.5	6.8	9.8	11.9	10.1
pH		8.14	7.95	8.17	8.28	8.27	8.41
Specific Conductivity	µS/cm	331.5	314.9	318.3	305.7	300.4	289.2
ORP	mV	202.7	216.7	222.1	196.5	177.1	177.8
Secchi Disk depth	cm	51	Bottom (38)	38	Bottom (53)	Bottom (17)	52

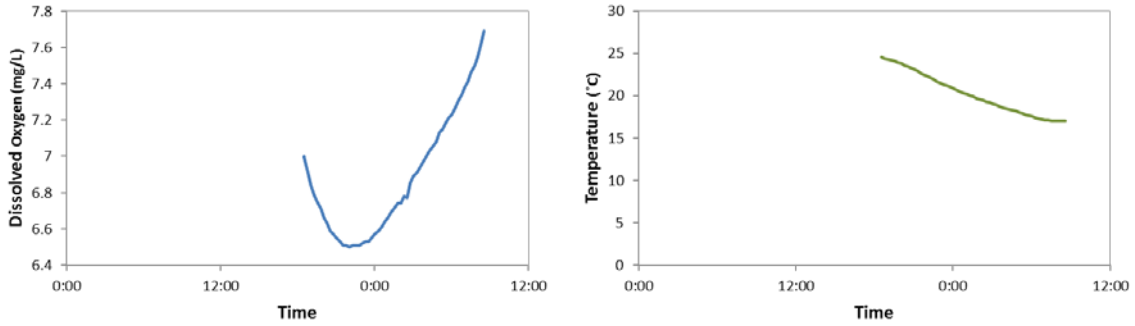

Figure 4. Dissolved oxygen concentrations and water temperatures at Agate East on 7-8 August 2012.

Table 3. Stream depth behind each Hester-Dendy sampler. Sampler 1 was on the south side of the Niobrara River and sampler 7 was on the north side of the river. Parallel depth is the actual water depth. Vertical displacement is an index of water velocity, where larger numbers indicate higher water velocity. Modeled water velocity was calculated using the relationship developed by Schlosser (1982).

	Hester-Dendy Samplers							
July 5, 2012	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**
Ranch								
Parallel depth (cm)	27.0	44.0	56.5	59.8	59.5	60.1	49.3	50.9
Vertical displacement (cm)	0.30	0.20	0.30	0.20	0.30	0.90	0.90	0.4
Modeled water velocity (m/s)	0.04	0.00	0.04	0.00	0.04	0.37	0.37	0.1
Middle								
Parallel depth (cm	31.0	36.0	38.2	38.6	39.0	39.5	31.0	36.2
Vertical displacement (cm)	0.50	0.50	0.40	0.40	0.80	0.50	0.20	0.5
Modeled water velocity (m/s)	0.19	0.19	0.13	0.13	0.34	0.19	0.00	0.2
East								
Parallel depth (cm	32.0	37.0	45.5	53.5	58.5	57.3	52.0	48.0
Vertical displacement (cm)	0.30	0.30	0.40	0.60	0.50	0.40	0.10	0.4
Modeled water velocity (m/s)	0.04	0.04	0.13	0.25	0.19	0.13	0.00	0.1
August 7, 2012								
Ranch								
Parallel depth (cm)	30.5	39.5	42.5	46.0	47.0	50.0	47.0	43.2
Vertical displacement (cm)	0.50	0.50	0.50	1.00	2.00	0.50	1.00	0.9
Modeled water velocity (m/s)	0.19	0.19	0.19	0.41	0.62	0.19	0.41	0.31
Middle								
Parallel depth (cm)	26.0	36.0	37.0	38.0	39.5	36.0	29.0	34.5
Vertical displacement (cm)	0.50	0.50	1.00	1.00	0.50	1.00	0.85	0.8
Modeled water velocity (m/s)	0.19	0.19	0.41	0.41	0.19	0.41	0.36	0.31
East								
Parallel depth (cm	45.0	54.0	63.0	73.0	77.0	84.0	81.0	68.1
Vertical displacement (cm)	0.50	0.75	0.50	0.50	0.50	0.50	1.00	0.6
Modeled water velocity (m/s)	0.19	0.32	0.19	0.19	0.19	0.19	0.41	0.24

Table 4. Estimated discharge of the Niobrara River calculated from measured stream depth, measured stream width, and modeled water velocity.

Modeled Discharge (m^3/s)		
	5-Jul-12	**7-Aug-12**
Ranch	0.15	0.20
Middle	0.08	0.15
East	0.09	0.33

I collected at least 21 taxa of invertebrates using Hester-Dendy samplers. Overall, Ephemeroptera, Crustacea, and Diptera were the most numerous invertebrates in decreasing order of abundance. Hester-Dendy samplers from Agate East (1980 ind/m^2) contained the most invertebrates and Agate Spring Ranch (790 ind/m^2) had the fewest, but densities were not different among sites (F = 2.7, df = 2, p = 0.07); however, I collected more taxa at Agate East compared to the other sites (Figure 5b; F = 18.2, df = 2, p = 0.018, Bonferroni: p <0.05). Taxa diversity (Figure 5a; F = 0.25, df = 2, p = 0.79) and taxa evenness (Figure 5a; F = 1.6, df = 2, p = 0.23) were highest at Agate Middle, but values were not different among sites (Table 5). I collected more EPT taxa at Agate East compared to the other sites (Figure 5b; F = 6.7, df = 2, p = 0.023; Bonferroni: p < 0.003). Similarly, Agate East contained the highest proportion of EPT taxa (Figure 5a; F = 1.5, df = 2, p = 0.25). The average tolerance value for an invertebrate in the assemblage was highest at Agate Middle (Figure 5b; F = 0.42, df = 2, p = 0.53).

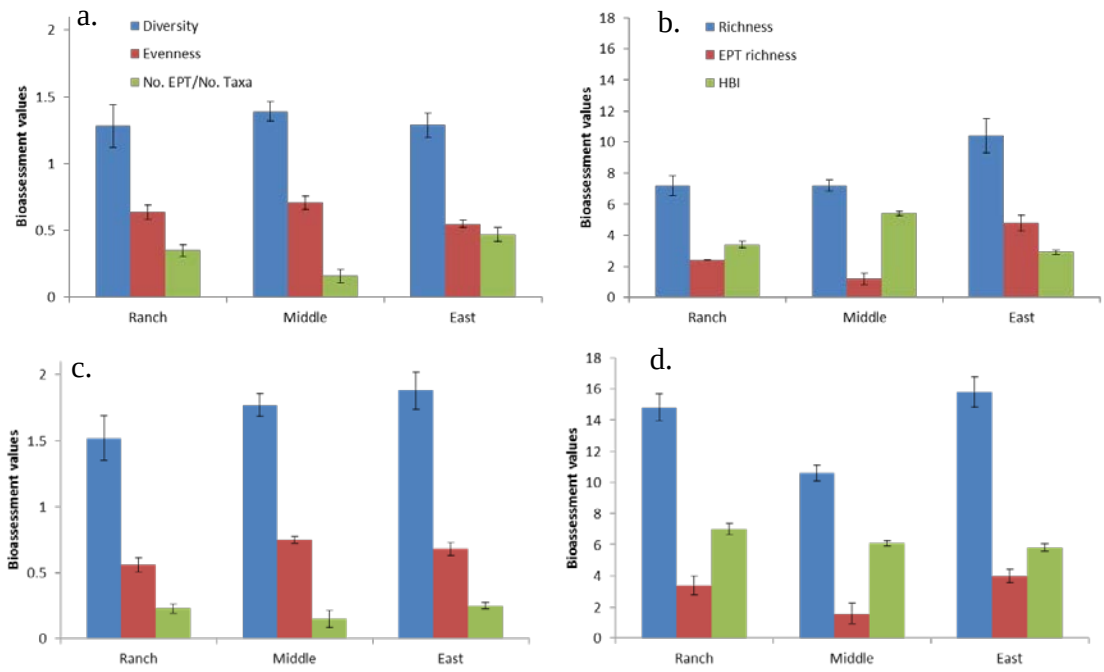

Figure 5. Invertebrate bioassessment metrics for 3 sites along the Niobrara River collected with a.) and b.) Hester-Dendy samplers, and c.) and d.) a Hess sampler. Higher values for taxa diversity, taxa evenness, number of EPT taxa/number of taxa, taxa richness, and EPT richness indicated better ecosystem quality, while lower values of HBI indicated higher ecosystem quality. Error bars are standard errors.

Table 5. Mean invertebrate bioassessment metrics and standard errors at each site along the Niobrara River collected with Hester-Dendy samplers or a Hess sampler.

Metric	Ranch	Middle	East
Hester-Dendy Samplers			
Taxa diversity	1.28±0.16	1.39±0.072	1.29±0.092
Taxa evenness	0.64±0.0530	0.71±0.050	0.55±0.028
No. EPT/No. taxa	0.35±0.045	0.16±0.051	0.47±0.052
Taxa richness	7.2±0.66	7.2±0.37	10.4±1.1
EPT richness	2.4±0.24	1.2±0.37	4.8±0.49
HBI	3.4±0.22	5.4±0.15	2.9±0.13
Hess Samples			
Taxa diversity	1.52±0.17	1.77±0.087	1.88±0.14
Taxa evenness	0.56±0.053	0.75±0.027	0.68±0.049
No. EPT/No. taxa	0.23±0.035	0.15±0.062	0.25±0.026
Taxa richness	14.8±0.86	10.6±0.51	15.8±0.97
EPT richness	3.4±0.60	1.6±0.68	4.0±0.45
HBI	7.00±0.36	6.09±0.16	5.82±0.23

I collected 33 taxa of invertebrates using a Hess sampler in the Niobrara River. Overall, Crustacea, Ephemeroptera, Diptera, and Annelids were the most numerous invertebrates in decreasing order of abundance. More invertebrates lived at Agate Ranch (7120 ind/m^2) compared to Agate Middle (1950 ind/m^2; $F = 3.1$, $df = 2$, $p = 0.04$, Bonferroni: $p = 0.038$). Taxa diversity (Figure 5c; $F = 1.85$, $df = 2$, $p = 0.20$) and taxa evenness were similar among sites (Figure 5c; Table 5; $F = 2.7$, $df = 2$, $p = 0.12$). Taxa richness was lowest at Agate Middle (Figure 5d; $F = 11.8$, $df = 2$, $p = 0.0015$, Bonferroni: $p < 0.01$). I collected the most EPT taxa at Agate East but differences were not significant (Figure 5d; $F = 0.33$, $df = 2$, $p = 0.57$). Similarly, the proportion of EPT taxa (Figure 5c; $F = 0.16$, $df = 2$, $p = 0.70$) were comparable among sites. The mean tolerance value of invertebrates was higher at Agate Springs Ranch compared to Agate East (Figure 5d; $F = 9.6$, $df = 2$, $p = 0.008$, Bonferroni: $p = 0.032$).

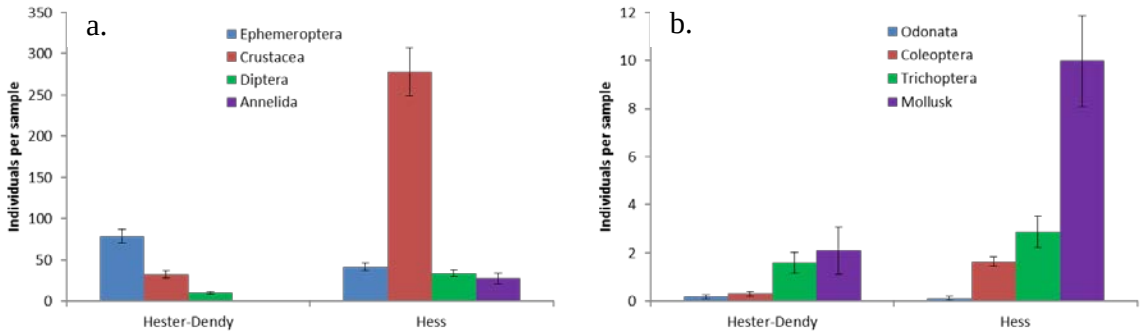

Figure 6. The abundance of a.) Ephemeroptera, Crustacea, Diptera, Annelida, b.) Odonata, Coleoptera, Trichoptera, and Mollusk calculated from Hester-Dendy samplers and Hess samples collected along the Niobrara River at Agate Fossil Beds National Monument. Error bars are standard errors.

I identified 47 invertebrate taxa from 4 phylum (Annelida, Mollusca, Nematoda, and Arthropoda) using both samplers in the Niobrara River (Appendix A, B). Hester-Dendy samplers collected 3 taxa not found in Hess samples (*Argia* and early instar Aeshnidae, Odonata; Cladocera, Crustacea). On the other hand, Hess samples collected 14 taxa not collected with Hester-Dendy samplers (Belostoma

and *Palmacorixa*, Hemiptera; *Calopteryx*, Odonata; *Colymbetes*, *Enochrus*, *Laccophilus*, and Lampyridae, Coleoptera; *Simulium*, *Culicoides*, and Tipulidae, Diptera; *Nectopsyche*, Trichoptera; Physidae and Sphaeriidae, Mollusca; Acari; Oligochaeta). More non-insects were collected in Hess samples compared to Hester-Dendy samplers (Figure 6; $F = 14.7$, $df = 1$, $p = 0.0007$); however, insects were equally abundant between samplers ($F = 0.07$ $df = 1$, $p = 0.80$). Hess samples contained more Crustaceans (Figure 6; $F = 11.7$, $df = 1$, $p = 0.002$), Diptera ($F = 8.5$, $df = 1$, $p = 0.007$), Mollusks ($F = 4.3$, $df = 1$, $p = 0.048$), and Annelids ($F = 11.6$, $df = 1$, $p = 0.002$). Taxa diversity ($t = -3.9$, d$f = 26$, $p = 0.006$), taxa richness ($t = -5.8$, d$f = 27$, $p < 0.001$), HBI ($t = -6.0$, d$f = 22$, $p < 0.001$), and the proportion of EPT taxa ($t = 2.4$, d$f = 23$, $p = 0.03$) differed between Hester-Dendy and Hess samples. Conversely, taxa evenness ($t = -0.6$, d$f = 28$, $p = 0.55$) and EPT richness ($t = -0.33$, d$f = 28$, $p = 0.75$) did not differ between Hester-Dendy and Hess samples. Despite differences between samplers for some metrics (Figure 7), long-term trends were similar (Figures 8 and 9; Table 6).

Bioassessment metrics were calculated from invertebrates collected with Hester-Dendy samplers for at least 16 years (1997-2011) in the Niobrara River at Agate Fossil Beds National Monument (Figure 8). Using the long-term data, I analyzed the metrics to estimate if any trends were evident over this period. I calculated that HBI values have increased over time, indicating that the invertebrate assemblage is composed of more tolerant taxa now compared to when monitoring began (Figure 8; Table 4). EPT richness and the proportion of EPT taxa decreased over this time period. A decrease in EPT richness indicated that fewer EPT taxa are being collected currently compared to the past when monitoring began. Similarly, a decrease in the proportion of EPT taxa signifies that a smaller proportion of the taxa collected are composed of mayflies, stoneflies, and caddisflies. In addition, I analyzed trends in the data by replacing 2010 through 2012 data with metrics calculated from Hess samples (Figure 9). The same trends were significant for both data sets (Table 6).

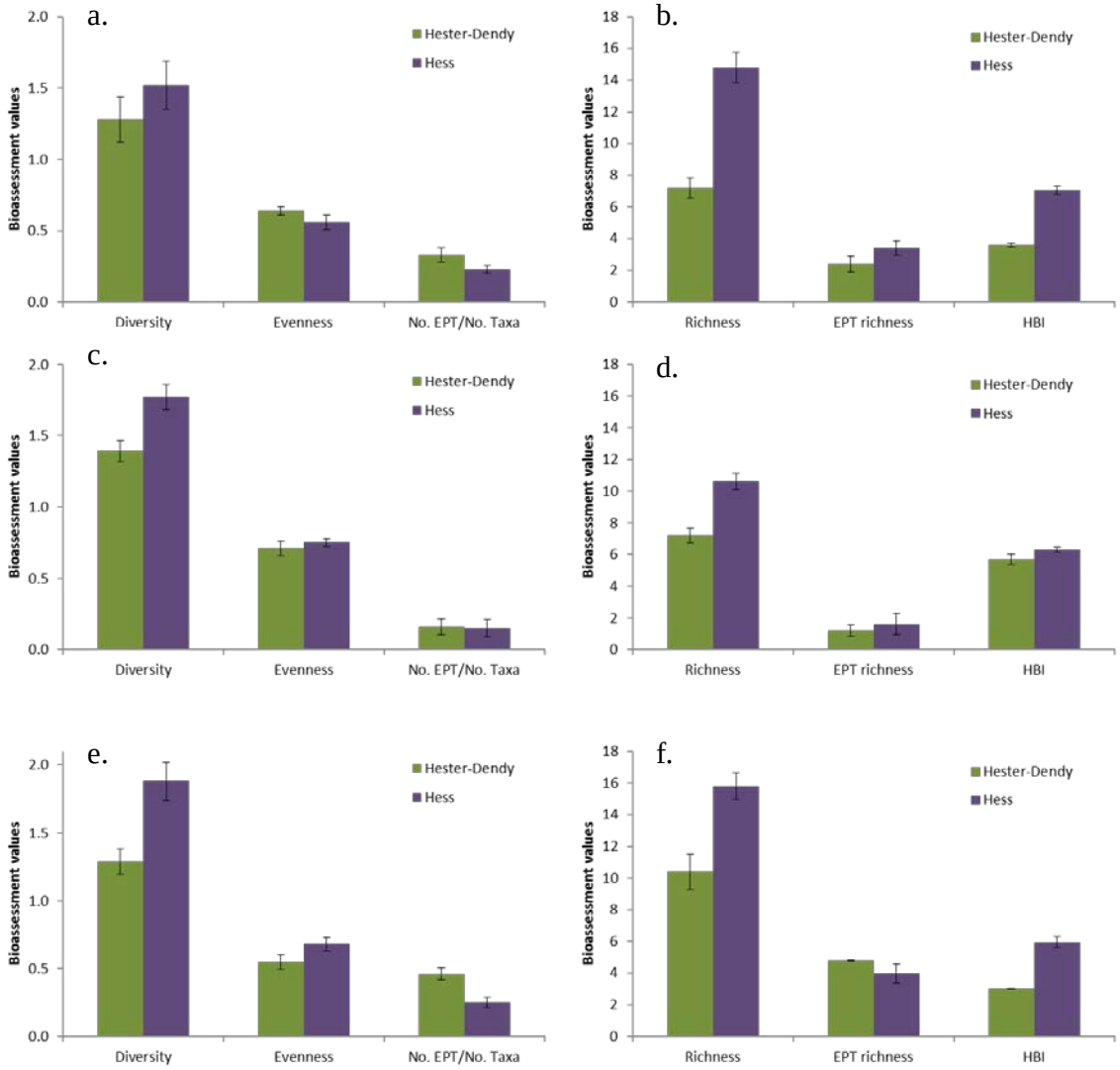

Figure 7. Invertebrate bioassessment metrics at a.) and b.) Agate Springs Ranch, c.) and d.) Agate Middle, and e.) and f.) Agate East collected along the Niobrara River with Hester-Dendy samplers and a Hess sampler. Higher values for taxa diversity, taxa evenness, number of EPT taxa/number of taxa, taxa richness, and EPT richness indicated better ecosystem quality, while lower values of HBI indicated higher ecosystem quality. Error bars are standard errors.

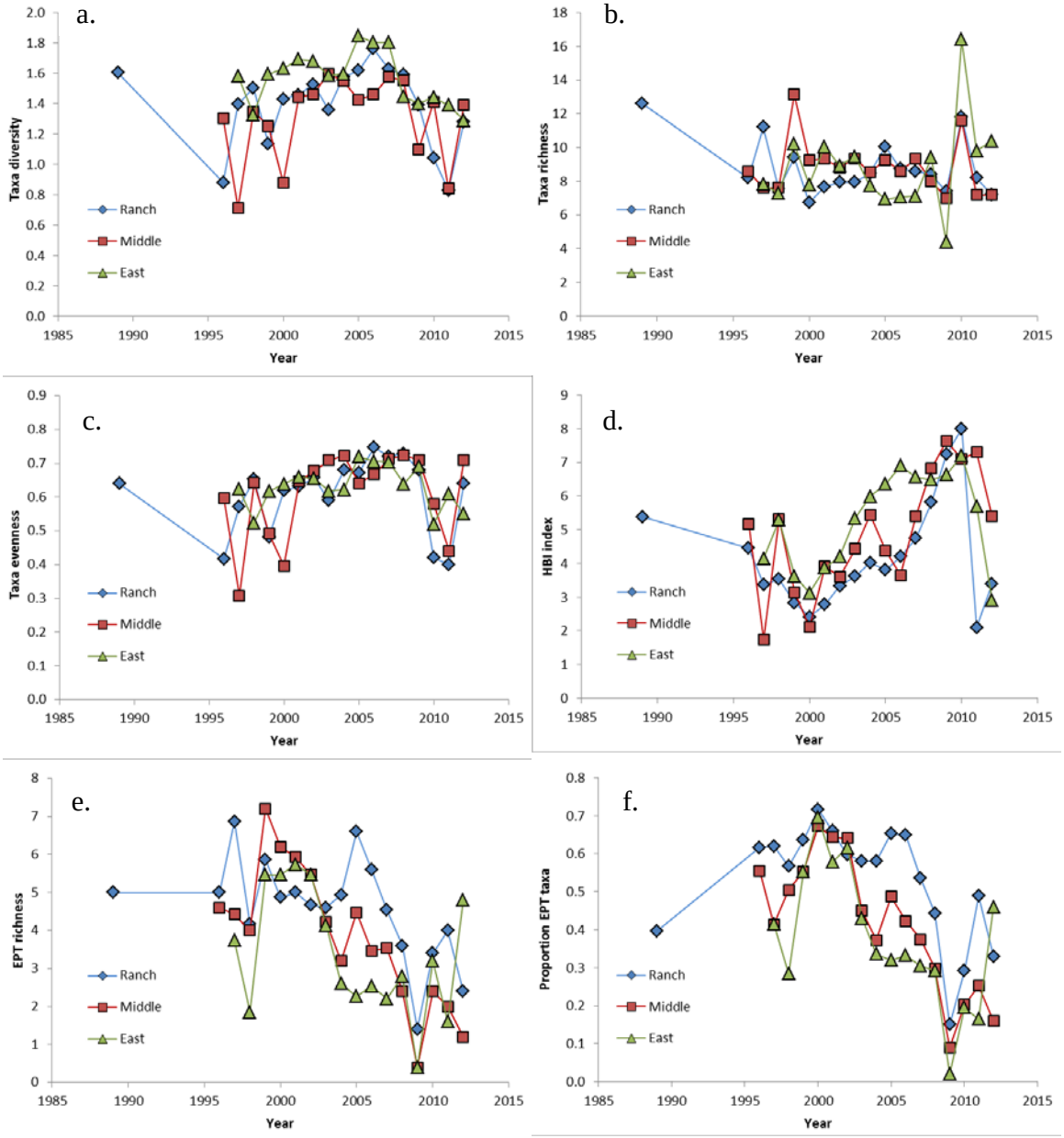

Figure 8. Invertebrate bioassessment metrics over time from the Niobrara River at Agate Fossil Beds National Monument. a.) Taxa diversity, b.) taxa richness, c.) taxa evenness, d.) HBI index, e.) EPT richness, and f.) the proportion of EPT taxa calculated from Hester-Dendy samplers. Past data (1989-2009) from Bowles (2010).

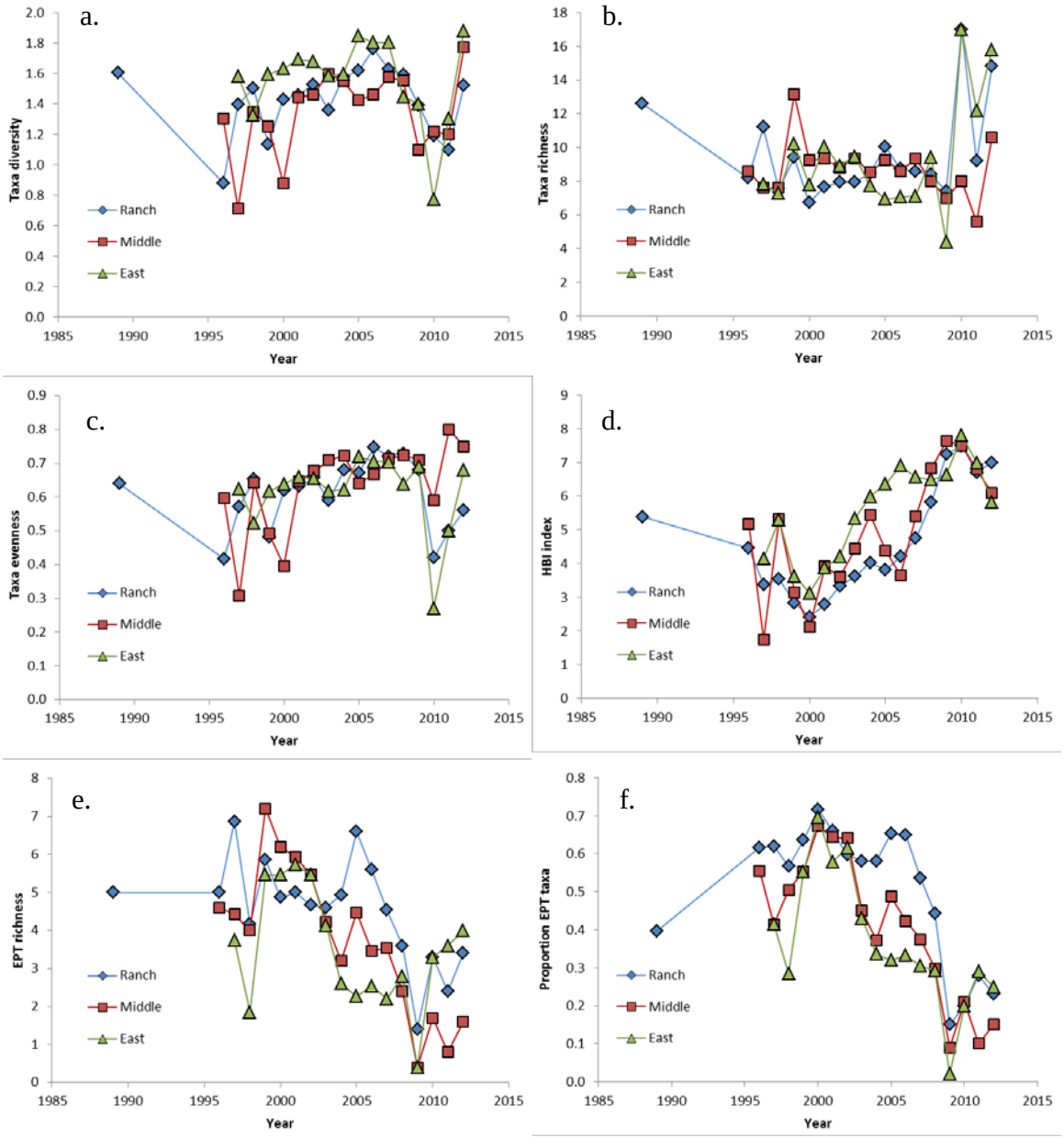

Figure 9. Invertebrate bioassessment metrics over time from the Niobrara River at Agate Fossil Beds National Monument. a.) Taxa diversity, b.) taxa richness, c.) taxa evenness, d.) HBI index, e.) EPT richness, and f.) the proportion of EPT taxa calculated from Hester-Dendy samplers (1989-2009) and Hess samples (2010-2012). Past data (1989-2009) from Bowles (2010).

Table 6. Functional data analysis of bioassessment metrics through time. Hester-Dendy samples were collected at all sited between 1997 and 2012. Hess samples were also collected at all sites between 2010 and 2012 and analyzed with Hester-Dendy data from 1997 to 2009. The slope and standard error (SE) of the slope are reported for each metric and site. The mean slope and SE were calculated for each bioassessment metric and compared to a confidence interval (CI). The relationship was significant (bold items) when the CI does not include zero.

	Hester-Dendy			Hess		
HBI	**Slope**	**Slope SE**	**CI**	**Slope**	**Slope SE**	**CI**
Ranch	0.05592	0.06391		0.14417	0.05436	
Middle	0.23798	0.06416		0.25284	0.06263	
East	0.13610	0.07131		0.26179	0.04679	
Mean	**0.14333**	**0.06646**	**0.0104 to 0.277**	**0.21960**	**0.05459**	**0.1104 to 0.3288**
Diversity						
Ranch	-0.00575	0.01080		0.00281	0.00921	
Middle	0.01012	0.01359		0.02096	0.01259	
East	-0.01116	0.00922		-0.01082	0.01511	
Mean	-0.00226	0.01120	-0.0247 to 0.0201	0.00432	0.01230	-0.0203 to 0.0289
Richness						
Ranch	-0.09266	0.06351		0.08545	0.11096	
Middle	0.00970	0.07901		-0.07797	0.07937	
East	-0.00019	0.14060		0.28090	0.16560	
Mean	-0.02772	0.09437	-0.2165 to 0.1610	0.09613	0.11864	-0.1412 to 0.3334
Evenness						
Ranch	0.00108	0.00449		0.00294	0.00414	
Middle	0.00970	0.00596		0.01667	0.00490	
East	-0.00019	0.00344		-0.00607	0.00615	
Mean	0.00353	0.00463	-0.0057 to 0.0128	0.00451	0.00506	-0.0056 to 0.0146
EPT						
Ranch	-0.12353	0.04600		-0.12218	0.04618	
Middle	-0.27918	0.05795		-0.30218	0.05904	
East	-0.14674	0.08291		-0.13778	0.07920	
Mean	**-0.18315**	**0.06229**	**-0.3077 to -0.0586**	**-0.18738**	**0.06147**	**-0.3103 to -0.0644**
Proportion EPT						
Ranch	-0.01037	0.00562		-0.01562	0.00624	
Middle	-0.02724	0.00542		-0.02966	0.00559	
East	-0.02146	0.00817		-0.02434	0.00725	
Mean	**-0.01969**	**0.00641**	**-0.0325 to -0.0069**	**-0.02321**	**0.00636**	**-0.0359 to -0.0105**

Discussion

Prairie streams can be difficult to sample for aquatic invertebrates. Prairie streams often have fine substrates, yet most quantitative aquatic invertebrate samplers are designed for streams with gravel or cobble substrate. One option for collecting aquatic invertebrates is using artificial substrate, such as Hester-Dendy samplers. Hester-Dendy samplers provide solid substrate in habitats that may lack such areas. Alternatively, these samplers may mimic snags or macrophytes that may occur along the margins of rivers. In the Niobrara River, Hester-Dendy samplers imitate the abundant cattails and iris in the riparian area. Invertebrate density is typically calculated based on the surface area of the plates; however, surface area in natural habitats (e.g., surface area of macrophyte leaves) is seldom calculated and would be extremely labor intensive. Therefore, density or biomass of aquatic invertebrates collected with Hester-Dendy samplers can only be compared to other ecosystems where Hester-Dendy samplers were also used.

Hester-Dendy samplers placed in the main channel of rivers probably have different invertebrates colonize them compared to when these samplers were placed in the riparian area. The riparian area differs from the main channel of the Niobrara River in several ways. For example, the riparian area is large (0.4 km wide in places), water velocity is much slower, and larger amounts of detritus probably accumulate along the edge of the stream. Macrophytes in the riparian area of the Niobrara River provide abundant substrate for aquatic invertebrates, but no aquatic plants live in the main channel. I placed Hester-Dendy samplers in the main channel of the river where water velocities were much higher and particulate organic matter does not accumulate. As a result, large debris dams can form while the Hester-Dendy samplers are being colonized. Debris dams were cleared weekly from the Hester-Dendy samplers which may disrupt colonizing invertebrates. Additionally, I have observed debris dams that were >0.3 m deep and >2 m in length when I retrieved the samplers. Because of these large debris dams, I collected taxa that normally would not be collected with a Hester-Dendy sampler, such as crayfish. Also, debris dams may cause higher variability in the samples because either more invertebrates (including debris) or fewer invertebrates (removing debris may displace individuals) may be collected depending on how the samples are processed. I have also observed Hester-Dendy samplers pushed out of the water entirely by debris dams. Therefore, Hester-Dendy samplers may induce greater variability in samples depending on conditions, personnel, and care in the field. Hester-Dendy sampler can be useful in large, deep rivers where other methods of sampling are difficult.

Hess samples collect natural densities of aquatic invertebrates that can be compared to other quantitative methods used in aquatic ecosystems (e.g., individuals per m^2 of stream bottom). Hess samplers are traditionally used similarly to Surber samplers, but they have the advantage of surrounding the sampling area. I chose to use a Hess sampler to collect aquatic invertebrates in the Niobrara River, because I could sample the macrophytes and sediments to estimate natural densities. I slipped the Hess sampler over the macrophytes at the edge of the main channel, and scoured the vegetation and sediment. Therefore, I sampled invertebrates that lived in both habitats (vegetation and sediment) and with multiple habits (e.g., crawlers, clingers, etc.). Hess samplers have shortfalls too; for example, Hess samplers cannot be used in deep water.

Aquatic invertebrates collected with Hester-Dendy samplers and a Hess sampler differed. Hess samples collected more taxa, higher diversity, higher HBI values, and a lower proportion of EPT taxa (Figure 7). I collected higher abundances of Ephemeroptera on Hester-Dendy samplers, but differences were not significant. Other studies have also found that Hester-Dendy samplers tend to select for EPT taxa and can elevate EPT metrics (Canton and Chadwick 1983, Letovsky et al. 2012). EPT richness from the Niobrara was similar between Hester-Dendy and Hess samples, but the proportion of EPT taxa was higher from Hester-Dendy samplers. The higher densities of benthic non-insect invertebrates (e.g., crustaceans, annelids, and mollusks) in Hess samples lower the proportion of the assemblage that was composed of EPT taxa. As a result, HBI values were higher in Hess samples, because the non-insect invertebrates generally had higher tolerance values. Additionally, I collected more taxa in Hess samples compared to Hester-Dendy samplers. Not all taxa colonize artificial substrates, because their habit does not allow them to live on the sampler (e.g., burrower), or conditions are not suitable on the sampler (e.g., water velocity to high, not enough food). Hester-Dendy samplers collected lower taxa diversity of invertebrates compared to kicknet samples in streams (McCabe et al. 2012, Letovsky et al. 2012). Additionally, kicknet samples were better at detecting change in Vermont streams, because of larger inter-site differences detected in kicknet samples (McCabe et al. 2012). Additionally, differences between Hester-Dendy and Hess samples may be from sampling dates. I collected Hess samples in July, because water levels in the river were low from severe drought. Hester-Dendy samples were deployed in July and collected a month later. However, previous work on the Niobrara River at Agate Fossil Beds National Monument found that aquatic invertebrate assemblages were similar in July and August (Bowles 2010).

Invertebrates collected with Hester-Dendy samplers and a Hess sampler were generally similar between 2011 and 2012, but the invertebrates collected in 2010 were more diverse and abundant (Tronstad 2012a, b). More taxa were collected using a Hess sampler in 2011 and 2012. Only 3 Hess samples were collected in 2010, because I was experimenting with sampler types which likely contributed to the lower (34 taxa) number of taxa collected compared to Hester-Dendy samples. Ephemeroptera was the most abundant invertebrate group collected in Hester-Dendy samples in 2011 and 2012, and Diptera were the most abundant group in 2010. Crustaceans were the most abundant invertebrate collected in Hess samples 2011 and 2012, and Diptera were the most abundant group in 2010. However, some of the bioassessment metrics calculated varied between years. For example, most HBI values were lower in 2012 compared to the previous 2 years, and EPT richness was generally higher in 2012 compared to 2011. These changes may reflect differences in conditions among years. For example, 2012 was an exceptional drought year with warm daily temperatures, little precipitation, and low water levels. I collected Hess samples earlier in 2012 compared to 2010 and 2011. Warmer water temperatures in 2012 likely decreased development time (e.g., Tronstad et al. 2010) and the stress of low water levels may prompt aquatic insects to complete development sooner (Tronstad et al. 2005). However, the composition of invertebrates was similar between years. Interestingly, the density of aquatic invertebrates were much higher in 2010 (Agate East Hester-Dendy 19,870 ind/m^2; Agate East Hess 31,510 ind/m^2) compared to 2011 and 2012. Higher densities of invertebrates colonized Hester-Dendy samplers in 2011, but I collected more invertebrates in Hess

samples in 2012. The reasons why I observed these differences is difficult to isolate because of the many factors measured and unmeasured that change among years.

Few long-term datasets of aquatic invertebrates exist, and these rare gems can be extremely useful for investigating changes in ecosystems (Jackson and Fureder 2006; Mazor et al. 2009). Long-term datasets can explain phenomenon that occur slowly, infrequently, subtly, or these datasets can help untangle complex issues in ecosystems (Jackson and Fureder 2006). The long-term dataset from the Niobrara River at Agate Fossil Beds National Monument may be used to understand how the ecosystem has changed and for what reasons. The Niobrara River dataset may also be used to investigate how ecosystem quality has changed through time. Mazor et al. (2009) used a 20 year dataset from 4 undisturbed streams in northern California to investigate trends in long-term bioassessment metrics. They found that some bioassessment metrics (Coleoptera richness, % intolerant taxa, % non-snail scrapers, % shredders, and proportion EPT) could have high coefficients of variation (CV = 16-246%). In the Niobrara River, at least 16 years of data exist and the CV is much lower for the metrics calculated (9-49%). Such variability in data is normal and may be caused by climatic variation, such as drought (Mazor et al. 2009).

Three of the 6 bioassessment metrics showed significant trends over the monitoring period. HBI increased over time, meaning that invertebrates in the assemblage tend to be more tolerant of pollution now compared to the past. Both EPT richness and the proportion of EPT taxa have declined over time. In general, EPT taxa are sensitive to pollution and a decline in sensitive taxa can signal a decrease in ecosystem quality. Both EPT metrics decreased in 2009, which may be due to a diesel spill that occurred upstream of Agate Springs Ranch on 23 June 2009. However, these metrics seem to be rebounding to values near 2008. Taxa richness decreased and HBI values increased in 2009 likely as a result of the diesel spill, and these metrics are rebounding. Interestingly, taxa diversity and taxa evenness showed little change in 2009, but taxa evenness was lower the following year. Based on the diesel spill, HBI, EPT richness, and the proportion of EPT taxa appeared to be the most sensitive metrics to pollution.

I recommend using a Hess sampler to collect aquatic invertebrates in the Niobrara River. Collecting invertebrates with a Hess sampler compared to Hester-Dendy samplers will reduce the number of visits to the sites along the Niobrara River from potentially 5 (deploying, 3 visits to clear debris dams, and retrieving) to only 1 (collecting). By collecting invertebrates on natural substrate there may be less variability in the samples, because of the difficulties using Hester-Dendy samplers in the Niobrara River. Sorting Hess samples will take a similar amount of time or slightly longer than Hester-Dendy samples; however, the time saved in the field will more than cover the cost of possibly increased sorting time. Hess samples should be collected in June, July or August, when water levels are high enough to extend into the riparian area, which will expedite sampling. Water levels need to be watched closely as annual variation in discharge and daily changes in irrigation withdrawals effect the river. Samples should be collected in July or August when possible, because the assemblages are most similar during these months (Bowles 2010).

Hess samples have several advantages over Hester-Dendy samplers in the Niobrara River; however, Hester-Dendy samples have been collected at Agate Fossil Beds National Monument for 16 years.

The long-term dataset at Agate Fossil Beds National Monument is invaluable, and may help untangle the mechanisms that have changed to Niobrara River over that time. Our results from the Niobrara River and the literature shows that Hester-Dendy samplers can bias the invertebrates collected compared to the natural assemblage (Canton and Chadwick 1983, Letovsky et al. 2012). Therefore, bioassessment metrics calculated from these two samplers will likely differ. What sampling device to continue with is a difficult decision and must be weighed carefully. The goals of the monitoring programs will help anwser this question. For example, if the goal is to compare the Niobrara River to other rivers or to assess the natural assemblage, then Hess samples are probably best to continue with. Conversely, Hester-Dendy samplers may be best to continue monitoring with if managers want to compare the conditions in the Niobrara with the past.

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Appendix A. Mean density (ind/m^2) of invertebrates collected from 3 sites along the Niobrara River at Agate Fossil Beds National Monument in 2012 using a Hess sampler.

Higher taxonomy	Family	Genus	Ranch	Middle	East
Acari			21	2	14
Amphipoda	Gammaridae	Gammarus	247	184	1019
Amphipoda	Hyalellidae	Hyalella	3674	749	1788
Bivalvia	Sphaeriidae		5	5	14
Coleoptera	Dytiscidae	Colymbetes	0	0	16
Coleoptera	Dytiscidae	Laccophilus	0	0	5
Coleoptera	Elmidae	Dubiraphia	0	0	9
Coleoptera	Gyrinidae	Gyrinus	2	0	5
Coleoptera	Hydrophilidae	Enochrus	0	0	2
Coleoptera	Hydrophilidae	Tropisternus	2	0	0
Coleoptera	Lampyridae		0	0	14
Crustacea	Copepoda	Cyclopoida	919	219	421
Decapoda	Cambaridae	Orconectes neglectus neglectus	84	142	247
Diptera	Ceratopogonidae	Culicoides	593	0	35
Diptera	Chironomidae	Blood midges	30	7	0
Diptera	Chironomidae	Midge pupae	0	0	9
Diptera	Chironomidae	Other midges	86	163	193
Diptera	Simuliidae	Simulium	5	0	40
Diptera	Tipulidae		2	2	7
Ephemeroptera	Baetidae	Baetis	30	0	358
Ephemeroptera	Baetidae	Early Instar Baetidae	5	5	7
Ephemeroptera	Caenidae	Caenis	0	0	5
Ephemeroptera	Ephemeridae	Hexagenia	523	5	93
Ephemeroptera	Heptageniidae	Heptagenia	109	0	293
Ephemeroptera	Leptophlebiidae	Paraleptophebia	9	0	0
Gastropoda	Ancylidae		79	123	119
Gastropoda	Physidae		2	0	0
Hemiptera	Belostomatidae	Belostoma	0	2	0
Hemiptera	Corixidae	Early Instar Corixidae	81	19	0
Hemiptera	Corixidae	Palmacorixa	35	7	0
Hirudinea			5	28	16
Odonata	Calopterygidae	Calopteryx	0	0	5
Oligochaeta			560	272	84
Trichoptera	Hydropsychidae	Cheumatopsyche	7	14	72
Trichoptera	Leptoceridae	Nectopsyche	0	0	5
Trichoptera	Rhyacophilidae	Rhyacophila	0	2	0
		Total density	7116	1949	4893

Appendix B. Mean density (ind/m^2) of invertebrates collected from 3 sites along the Niobrara River at Agate Fossil Beds National Monument in 2012 using Hester-Dendy samplers.

Higher Taxonomy	Family	Genus	Ranch	Middle	East
Coleoptera	Elmidae	Dubiraphia	0	0	2
Coleoptera	Gyrinidae	Gyrinus	0	4	0
Coleoptera	Hydrophilidae	Tropisternus	2	2	0
Crustacea	Cambaridae	Orconectes neglectus neglectus	0	4	6
Crustacea	Cladocera		6	0	0
Crustacea	Cyclopoida		32	186	56
Crustacea	Gammaridae	Gammarus	8	406	18
Crustacea	Hyalellidae	Hyalella	30	182	36
Diptera	Ceratopogonidae	Culicoides	20	0	2
Diptera	Chironomidae	Non-blood midges	42	118	110
Diptera	Chironomidae	Pupae	2	2	0
Diptera	Simuliidae	Pupae	0	2	0
Ephemeroptera	Baetidae	Early instar	6	4	36
Ephemeroptera	Caenidae	Caenis	0	0	2
Ephemeroptera	Ephemeridae	Hexagenia	2	0	90
Ephemeroptera	Heptageniidae	Heptagenia	290	0	424
Ephemeroptera	Leptophlebiidae	Early instar	338	4	1164
Hirudinea			0	0	10
Mollusca	Ancylidae		4	56	2
Odonata	Aeshnidae	Early instar	4	0	0
Odonata	Coenagrionidae	Argia	0	0	2
Trichoptera	Hydropsychidae	Cheumatopsyche	2	26	4
Trichoptera	Rhyacophilidae	Rhyacophilia	0	2	14
		Total density	788	998	1978

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

NPS 165/124721, May 2014

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