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Aquatic Invertebrate Monitoring at Agate Fossil Beds National Monument

2011 Annual Report

Natural Resource Technical Report NPS/NGPN/NRTR—2012/653

ON THE COVER
Niobrara River, Agate Fossil Beds National Monument
Photograph by: Lusha Tronstad, Wyoming Natural Diversity Database

Aquatic Invertebrate Monitoring at Agate Fossil Beds National Monument

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Executive Summary

Aquatic invertebrates are excellent animals to use for monitoring ecosystem quality; however, how to sample aquatic invertebrates for such monitoring efforts is a central question. 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 15 years using aquatic invertebrates colonizing Hester-Dendy samplers. These artificial substrate samplers are useful in rivers that are difficult to sample, but previous studies demonstrated that they bias results toward certain insect orders. Additionally, large debris dams formed upstream of these samplers in the Niobrara River potentially altering samples. Therefore, we compared aquatic invertebrates collected using Hester-Dendy samplers and a Hess sampler in the Niobrara River. Hester-Dendy and Hess samplers collected a similar invertebrate assemblage; however, Hess samples collected fewer mayflies, and fewer true flies, but more dragonflies and damselflies compared to Hester-Dendy samplers. Bioassessment metrics calculated using the two samplers were not statistically different. Three bioassessment metrics changed over time. Hilsenhoff's Biotic Index (HBI) increased over the last 15 years, indicating that invertebrates living in the Niobrara River are more tolerant of pollution. Mayfly, stonefly, and caddisfly (EPT) taxa richness and the proportion of EPT taxa have declined over time, showing a decline in the number of sensitive invertebrates. 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.

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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 qualities that make them ideal for the task. First, aquatic invertebrates are relatively long lived (weeks to >100 years, Rosenberg and Resh 1993b). Unlike water samples that are collected periodically, aquatic invertebrates collect water quality information all day long every day of their lives. Water samples may miss discrete discharges of pollution, but aquatic invertebrates will respond to such events. Second, these animals are relatively sedentary and are used to assess water quality at a location. Third, aquatic invertebrates are abundant, diverse, and easy to collect. Fourth, countless studies have shown that lower ecosystem quality can increase mortality, 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).

Aquatic invertebrates can be used to measure changes in ecosystem quality through time, because these animals are sensitive to water quality, invasive species, habitat degradation, and pollution (e.g., Rosenberg and Resh, 1993a). For example, the mayfly, *Hexagenia*, disappeared from Lake Erie in 1953 because eutrophication in the lake probably caused anoxic conditions in the sediments (Masteller and Obert 2000). Due to changes in environmental regulations, *Hexagenia* returned to Lake Erie in the 1990s. The assemblage structure of aquatic invertebrates changed when invasive lake trout (*Salvelinus namaycush*) dominated the Yellowstone Lake food web (Tronstad et al. 2010) and when invasive rainbow trout (*Oncorhynchus mykiss*) changed the feeding behavior of native Dolly Varden (*Salvelinus malma*) in Japan (Baxter et al. 2004). Increased transport of fine sediments in streams limited the recruitment of juvenile mussels in Swedish waters (Osterling et al. 2010). Finally, sensitive invertebrates were not observed at locations where copper, selenium, and cadmium concentrations were above the chronic limit for aquatic life (Tronstad and Reddy 2010).

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 kick nets, fixed-area samplers (e.g., Hess sampler), artificial substrates (e.g., Hester-Dendy samplers), grabs, and dip nets (Carter and Resh 2001). Deciding on 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. Dip nets and kick nets may only provide presence/absence data for aquatic invertebrates, but fixed area samplers and grabs 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 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 sampling methods, because of difficulties collecting samples using artificial substrates and difficulties comparing results to other rivers. In the Niobrara River, large debris dams form upstream of the samplers, biasing the invertebrates collected and introducing greater variability (Figure 1). Also, 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; Stasiak et al. in prep). 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 presence of invasive northern pike (*Esox lucius*) in the Niobrara River (Stasiak et al. in prep). 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. 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 three 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?

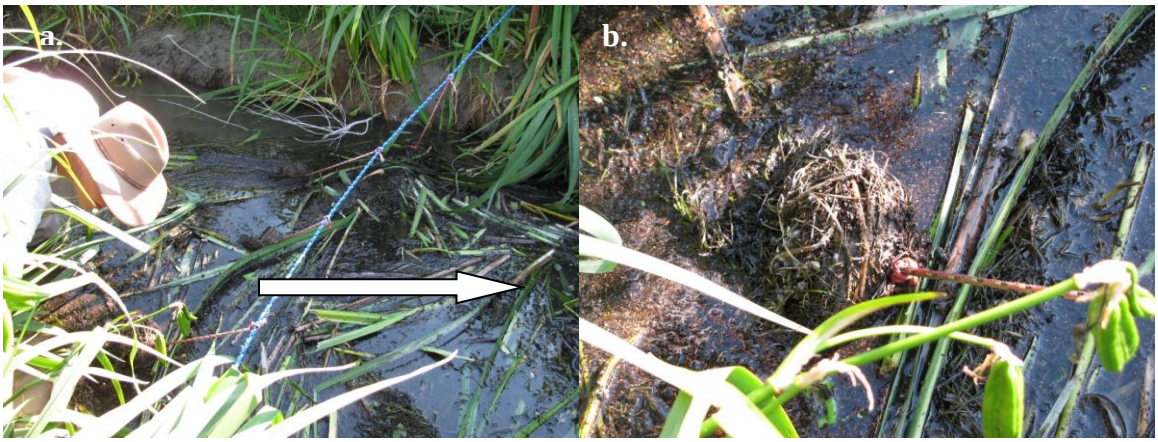

Figure 1. a) A debris dam (shown by arrow) caused by the Hester-Dendy samplers that extended >2 m upstream at Agate East. b) The debris dam likely increases variability in samples, such as the sampler shown that was pushed out of the water by the debris dam. Photos by Marcia Wilson.

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 about 1,100 ha in a valley bottom, and 18 km of river that flows through the 6 km wide park (Figure 2). The wetland vegetation in the Monument 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). Pike, white suckers (*Catostomus commersonii*), and green sunfish (*Lepomis cyanellus*) currently inhabit the river within the Monument (Stasiak et al. in prep).

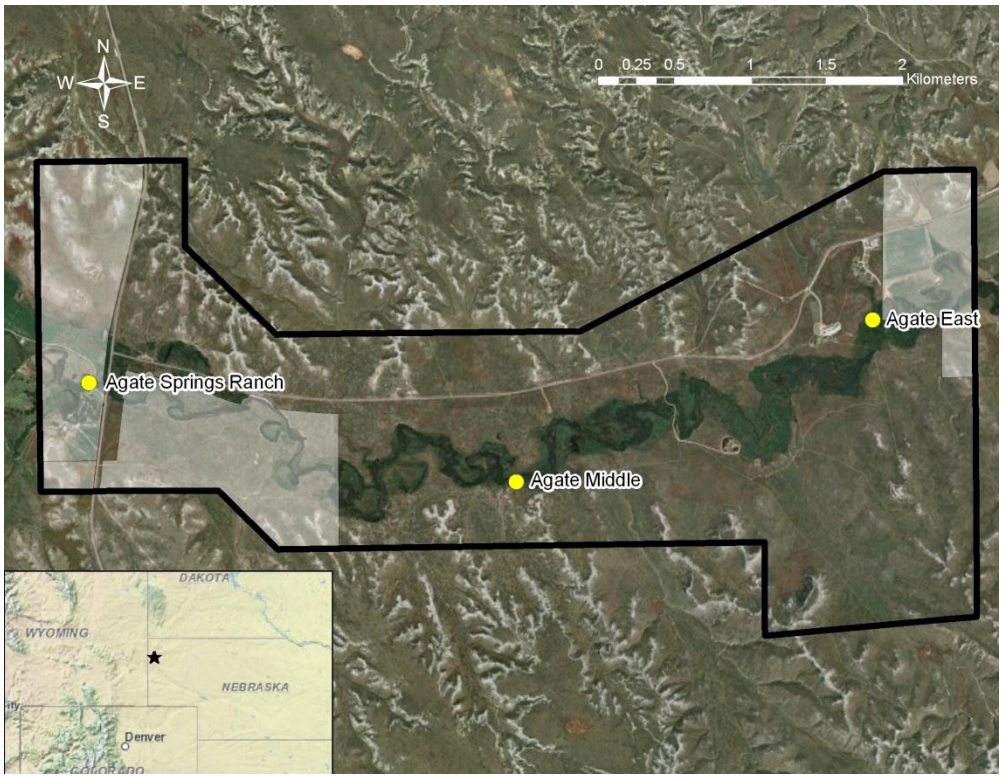

Figure 2. We sampled three sites along the Niobrara River at Agate Fossil Beds National Monument. The black line is the Monument boundary and the transparent white areas are private land within the Monument boundary. The inset map shows the location of Agate Fossil Beds National Monument (star).

I sampled three sites along the Niobrara River (Figure 2, Table 1). The most upstream site (Agate Springs Ranch) is located near the west Monument boundary. Agate Springs Ranch has an overstory of plains cottonwood (*Populus deltoides*), and cattails are more abundant than iris (Figure 3a). The middle site, Agate Middle, is shallower and lacks an overstory (Figure 3b). 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 3c). The wetland vegetation is dominated by iris with a few willow (*Salix* spp.).

Figure 3. Photos of a.) Agate Springs Ranch, b.) Agate Middle, and c.) Agate East. Location information located in Table 1.

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

Site description	Agate Springs Ranch	Agate Middle	Agate East
Easting	599323	602143	604495
Northing	4697497	4696844	4697913
Elevation (m)	1354	1350	1343

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 sediment 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 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. By subtracting the two measurements, I calculated vertical displacement (D); the greater the vertical displacement of the water, the higher the water velocity. V 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). Finally, I recorded the location and elevation of each site using a global positioning system (GPS; Garmin eTrex Vista HCx).

Hester-Dendy Samples

The National Park Service deployed five Hester-Dendy samplers (76 mm by 76 mm, 9 plates, Wildlife Supply Company) at each site on 25 July 2011 (Figure 4a). A rope was strung across the stream between two permanent posts and five 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 24 and 25 August 2011 by approaching the site from downstream and placing a dip net (212 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 Niobrara River, I collected five Hess samples (500 µm mesh, 860 cm^2 sampling area, Wildlife Supply Company) from each site on 24 and 25 August 2011 (Figure 4b). 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 4. Photos of a) Hester-Dendy sampler colonized by aquatic invertebrates and b) 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 two exceptions (order: Collembola, family: Diptera) according to Peterson et al. (1999). If invertebrates were extremely numerous (>500 individuals) in any sample, I subsampled as follows. First, I rinsed the sample through 2 mm and 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. Next, I subsampled the contents of the sieve with the smaller mesh size. 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: Hilsenhoff Biotic Index (HBI), Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness, proportion of EPT taxa (number of EPT taxa divided by the total number of taxa collected), taxa diversity (Shannon index), taxa richness, and taxa evenness (Bowles 2010). To distinguish among sites, I used ANOVA to compare abundance and bioassessment metrics for each sampler (DataDesk6.1). Differences among sites were distinguished using Bonferroni multiple comparison tests, where differences were significant when $p < 0.017$ (0.05/3; where I had 3 sites). To evaluate differences between the two sampling devices, I used ANOVA to compare abundance and bioassessment metrics among sampler types where differences were significant when $p < 0.05$. 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.

Results

In general, conditions were similar among sites. Agate Springs Ranch and Agate Middle had higher dissolved oxygen concentrations compared to Agate East (Table 2). Dissolved oxygen concentrations were collected in the afternoon with the exception of Agate East which was collected in the morning when dissolved oxygen concentrations are typically lowest. pH was slightly basic at all sites. Specific conductivity was similar among sites. Reducing conditions (<200 mV; oxidation-reduction potential) appeared to dominate in the Niobrara River. Water tended to be clearer at Agate Springs Ranch and Agate Middle compared to Agate East. Stream width was widest at Agate Middle (4.4 m) and narrowest at Agate Springs Ranch (2.7 m) and Agate East (2.5 m). Agate East was the deepest site and Agate Middle was the shallowest (Table 3). Estimated water velocity was highest at Agate Middle and lowest at Agate East. Overall, the substrate in the Niobrara River at each site was dominated by fine sediments (clay, sand, and silt). At Agate Springs Ranch, I estimated that 5-25% was silt and clay, and 50-75% was sand. At Agate Middle, 1-5% was sand, 5-25% was silt, and 1-25% was gravel. Agate East was composed of 5-25% silt, 75-95% was sand, and 5-100% was clay, depending on the position in the channel.

I collected 27 taxa using Hester-Dendy samplers. Overall, Ephemeroptera,Crustacea, Diptera, and Mollusks were the most numerous invertebrates in decreasing order of abundance. Hester-Dendy samplers from Agate East (4,786 ind/m^2) contained the most invertebrates and Agate Middle (1,526 ind/m^2) had the fewest, but densities were not different among sites ($F = 4.7$, $df = 2$, $p = 0.03$; Bonferroni, $p > 0.017$). Taxa diversity was highest at Agate East, and similar between Agate Middle and Agate Springs Ranch, but not significantly different among sites (Figure 4a; $F = 3.9$, $df = 2$, $p = 0.05$; Bonferroni, $p > 0.017$). Agate East had the highest taxa evenness (Figure 4a; $F = 2.3$, $df = 2$, $p = 0.14$) and the highest taxa richness (Figure 4b; $F = 2.2$, $df = 2$, $p = 0.15$), but values were similar among sites. I collected more EPT taxa at Agate Springs Ranch compared to Agate East (Figure 4b; $F = 8.9$, $df = 2$, $p = 0.004$; Bonferroni: $p = 0.006$). Similarly, Agate Springs Ranch contained the highest proportion of EPT taxa compared to the other sites (Figure 4a; $F = 26.7$, $df = 2$, $p < 0.0001$; Bonferroni: $p < 0.001$). The average tolerance value for invertebrates was higher at Agate East and Agate Middle, and much lower at Agate Springs Ranch (Figure 4b; $F = 72$, $df = 2$, $p < 0.0001$; Bonferroni: $p < 0.001$).

Table 2. Water quality and air temperature measured when invertebrate samples were collected.

Site	Units	Agate Spring Ranch	Agate Middle	Agate East
Date		24-Aug-11	24-Aug-11	25-Aug-11
Time		13:00	15:45	8:12
Dissolved oxygen	%	116	116	98
Dissolved oxygen	mg/L	8.8	8.9	7.7
pH		8.06	7.94	7.94
Specific conductivity	µS/cm	414.1	418.6	417
Conductivity	µS/cm	382.1	384	373
Water temperature	°C	21.0	20.7	19.6
ORP	mV	90.7	48.3	47.8
Secchi disk depth	cm	40	39	25.5
Air temperature	°C	34	34	26

Table 3. Stream depth behind each Hester-Dendy sampler. Sampler 1 was on the south side of the Niobrara River and sampler 5 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 velocity. An estimate of water velocity was calculated using the relationship developed by Schlosser (1982).

Sampler #	1	2	3	4	5	Mean
Agate Springs Ranch						
Parallel depth (cm)	52.0	64.5	64.0	53.0	43.0	55.3
Verticle displacement (cm)	0.0	0.5	1.0	1.5	3.5	1.3
Modeled water velocity (m/s)	0.00	0.19	0.41	0.53	0.79	0.38
Agate Middle						
Parallel depth (cm)	36.0	52.5	51.5	48.5	40.0	45.7
Verticle displacement (cm)	3.0	1.5	0.5	1.5	0.5	1.4
Modeled water velocity (m/s)	0.74	0.53	0.19	0.53	0.19	0.44
Agate East						
Parallel depth (cm)	79.5	71.5	70.5	57.5	49.5	65.7
Verticle displacement (cm)	1.7	0.5	1.0	1.0	0.0	0.8
Modeled water velocity (m/s)	0.57	0.19	0.41	0.41	0.00	0.31

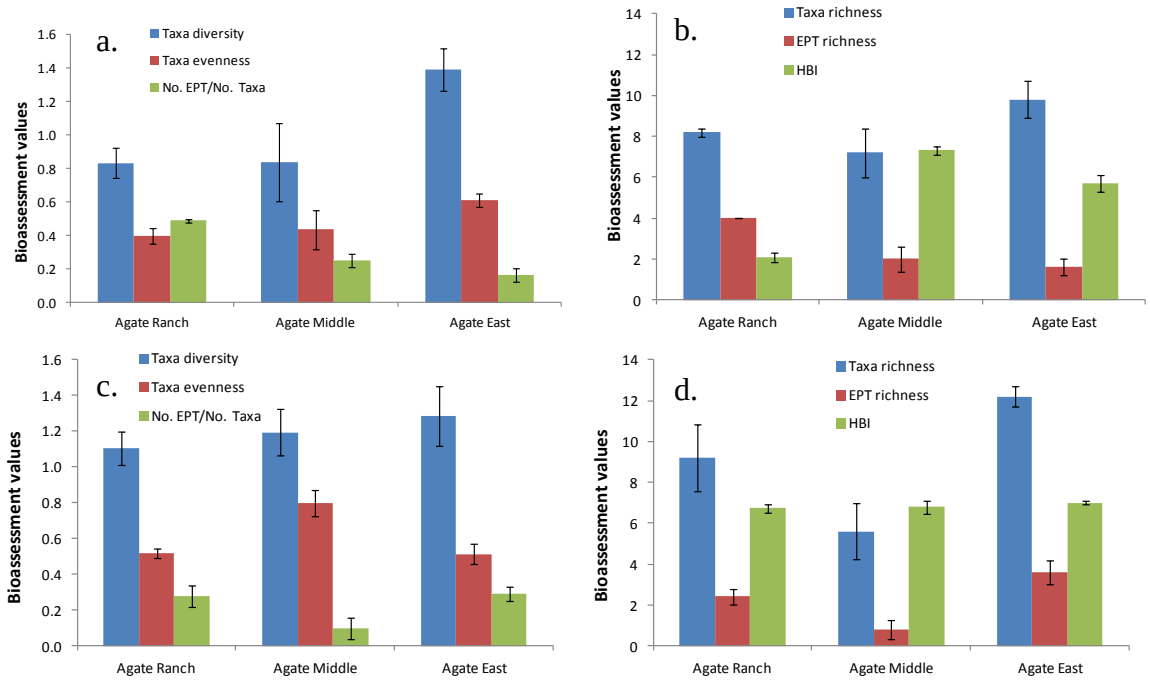

Figure 5. Invertebrate bioassessment metrics for three sites along the Niobrara River collected with Hester-Dendy samplers (a, b), and a Hess sampler (c, d). Higher values for taxa diversity, taxa evenness, number of EPT taxa/number of taxa, taxa richness, and EPT richness indicated better ecosystem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

I collected 29 taxa of invertebrates using a Hess sampler in the Niobrara River. Overall, Crustacea, Ephmeroptera, Diptera, and Mollusks were the most numerous invertebrates in decreasing order of abundance. Agate East had the highest density of invertebrates (3220 ind/m^2) and Agate Middle had the lowest density (345 ind/sampler), but densities were not different among sites (F = 3.2, df = 2, p = 0.07). Taxa diversity was similar among sites (Figure 5c; F = 0.07, df = 2, p = 0.93). Taxa evenness was highest at Agate Middle, but values were not statistically significant among sites (Figure 5c; F = 0.85, df = 2, p = 0.45). Similarly, taxa richness did not differ among sites (F = 2.3, df = 2, p = 0.14), but I collected the most taxa at Agate East and the fewest taxa Agate Middle (Figure 5d). I collected the most EPT taxa at Agate East but differences were not significant (Figure 5d; F = 3.3, df = 2, p = 0.07). The proportion of EPT taxa (Figure 5c; F = 1, df = 2, p = 0.4) and mean tolerance values (Figure 5d; F = 1, df = 2, p = 0.39) were similar among sites.

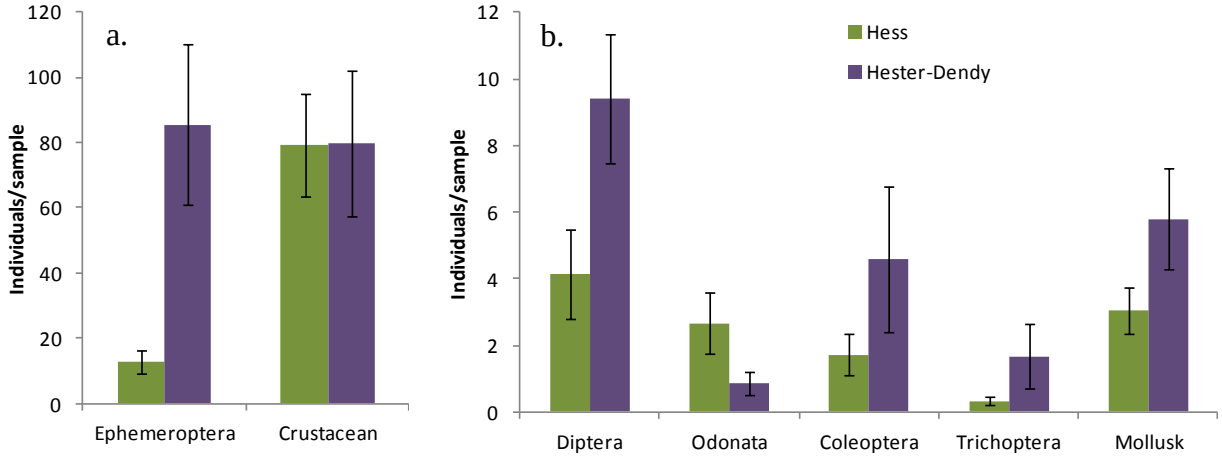

Figure 6. The density of a.) Ephemeroptera, Crustacea, b.) Diptera, Odonata, Coleoptera, Trichoptera, and Mollusk calculated from Hester-Dendy samplers and Hess samples collected along the Niobrara River at Agate Fossil Beds National Monument. Error bars are standard errors.

In general, Hester-Dendy and Hess samplers collected similar samples (Figure 6). I identified 34 invertebrate taxa from three phylum (Annelida, Mollusca, and Arthropoda) using both samplers in the Niobrara River (Appendix A). Hester-Dendy samplers collected four taxa not found in Hess samples (*Belostoma*, Hemiptera; *Bidessonotus* and *Sanfilippodytes*, Coleoptera; and blood midges, Diptera). On the other hand, Hess samples collected seven taxa not found in Hester-Dendy samplers (Cladocera, Crustacea; Physidae, Planorbidae, and Sphaeriidae, Mollusks; Oligochaeta; *Coptotmus*, Coleoptera; Tipulidae, Diptera). More insects were collected in a Hester-Dendy sample (102 ind/sample) compared to a Hess sample (22 ind/sample; $F = 15.5$, $df = 1$, $p = 0.0006$); however, non-insects were equally abundant between samplers (85 ind/sample; $F = 0.01$, $df = 1$, $p = 0.9$). Hess samples contained fewer Ephemeroptera ($F = 12.9$, $df = 1$, $p = 0.0013$) and Diptera ($F = 5.3$, $df = 1$, $p = 0.03$), but more Odonata (Figure 5; $F = 4.9$, $df = 1$, $p = 0.036$). Taxa diversity, taxa richness, taxa evenness, EPT richness, HBI, and the proportion of EPT taxa did not differ between Hess and Hester-Dendy samples ($p >> 0.05$). Because we did not find a difference between bioassessment metrics calculated using samples collected with Hester-Dendy samplers and a Hess sampler (Figure 7), long-term trends were similar (Figures 8 and 9).

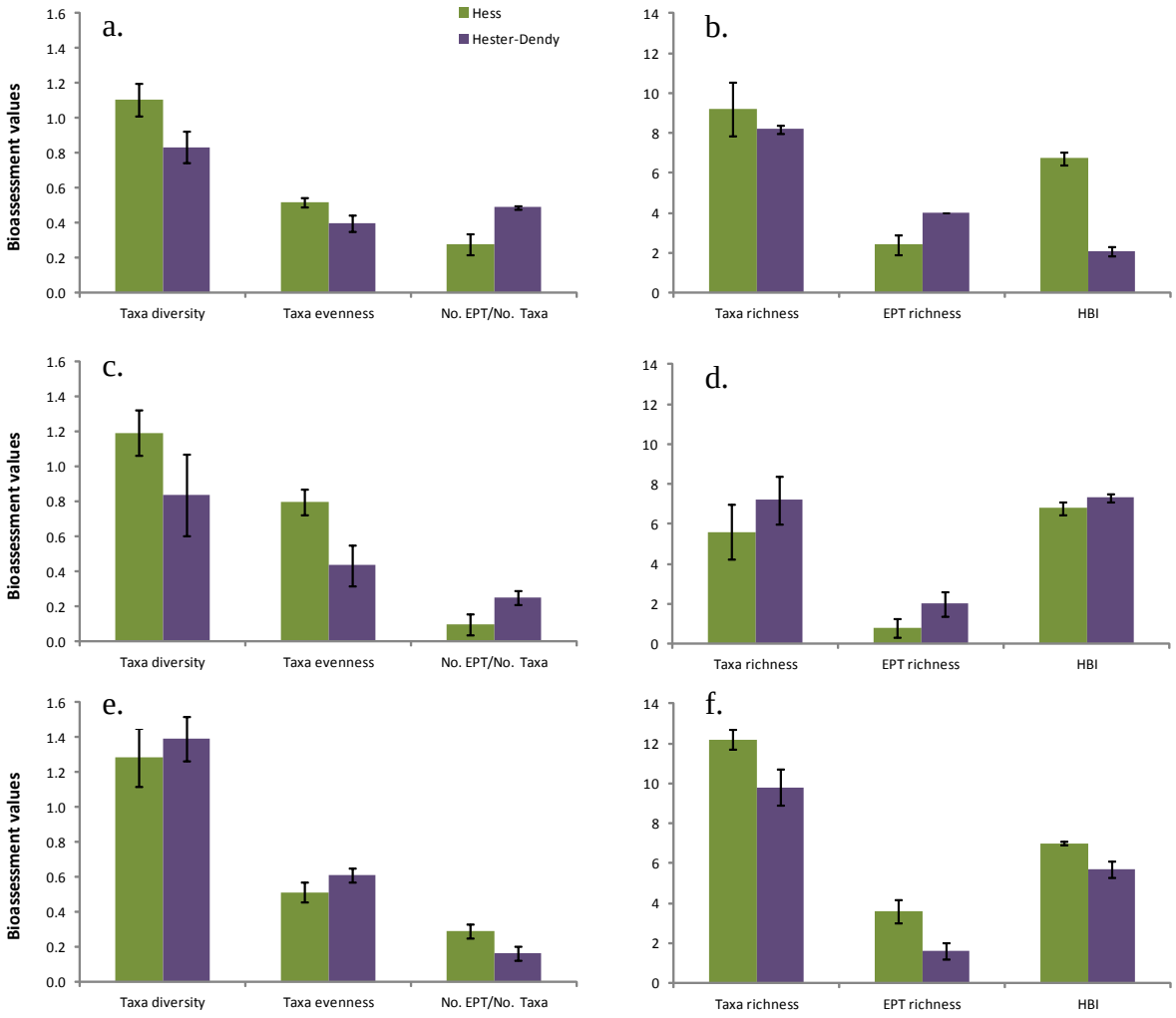

Figure 7. Invertebrate bioassessment metrics for three sites along the Niobrara River, Agate Springs Ranch (a, b), Agate Middle (c, d), and Agate East (e, f), collected with a Hess sampler and Hester-Dendy samplers. Higher values for taxa diversity, taxa evenness, number of EPT taxa/number of taxa, taxa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

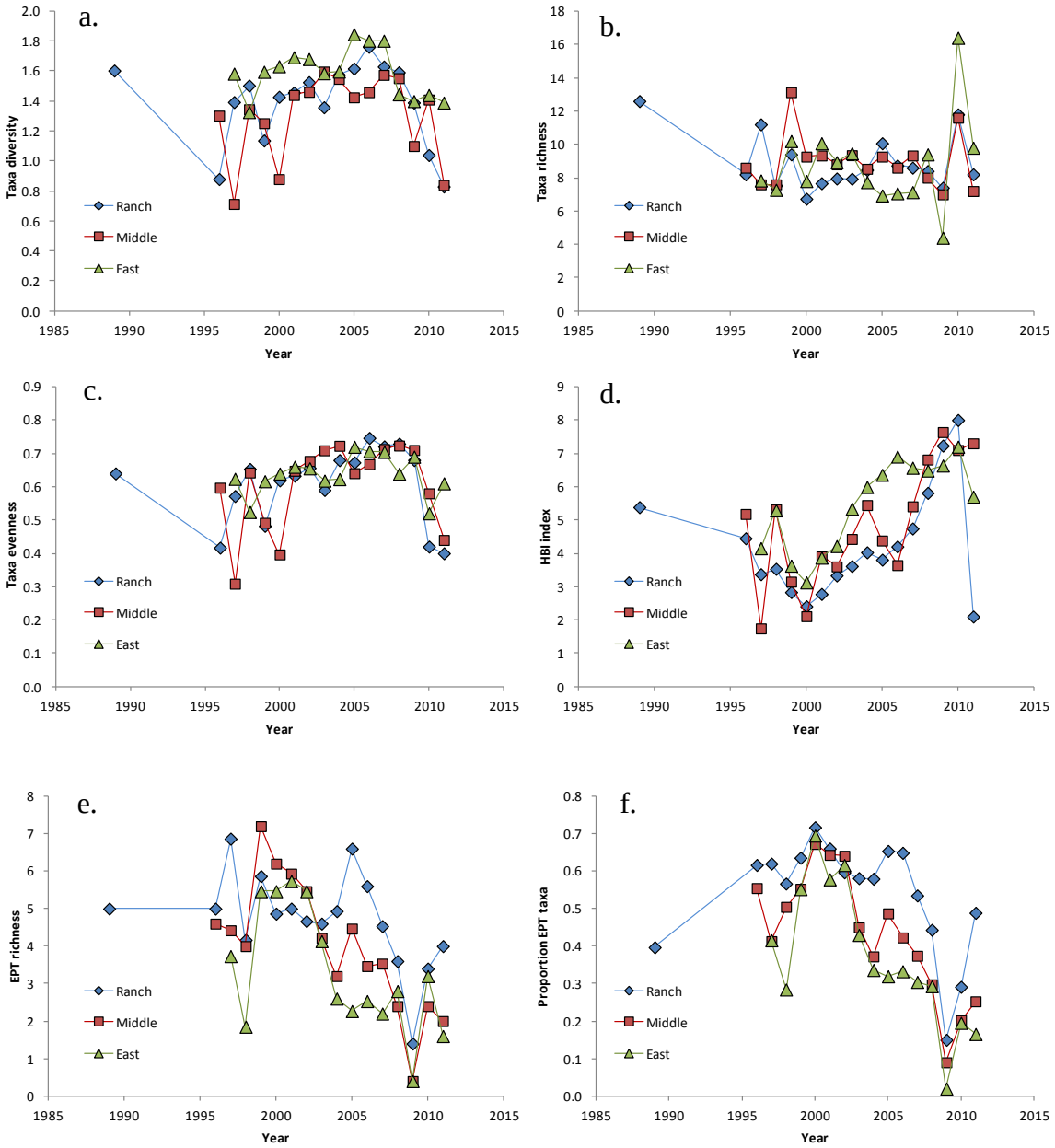

Figure 8. Invertebrate bioassessment metrics over time from the Niobrara River at Agate Fossil Beds National Monument. a.) Taxa diversity, b.) taxa richness, c.) taxa evenness, d.) HBI index, e.) EPT richness, and f.) the proportion of EPT taxa calculated from Hester-Dendy samplers. Past data (1989-2009)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

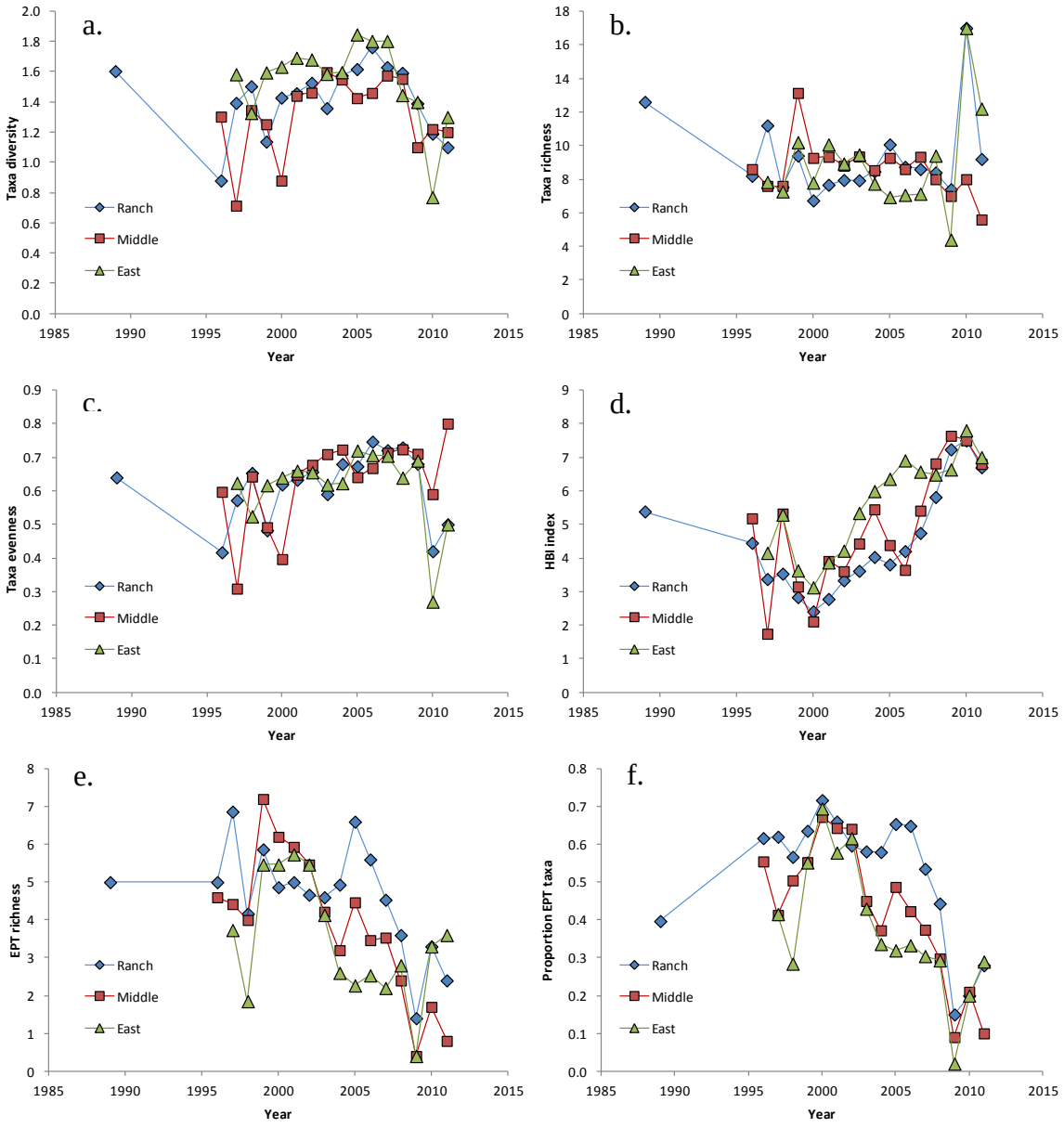

Figure 9. Invertebrate bioassessment metrics over time from the Niobrara River at Agate Fossil Beds National Monument. a.) Taxa diversity, b.) taxa richness, c.) taxa evenness, d.) HBI index, e.) EPT richness, and f.) the proportion of EPT taxa calculated from Hester-Dendy samplers (1989-2009) and a Hess sampler (2010-2011). Past data (1989-2009) from Bowles (2010).

Table 4. Functional data analysis of bioassessment metrics through time. 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 2010-2011			Hess 2010-2011		
HBI	**Slope**	**Slope SE**	**CI**	**Slope**	**Slope SE**	**CI**
Ranch	0.07797	0.06917		0.14257	0.05906	
Middle	0.27216	0.06925		0.26878	0.06982	
East	0.23282	0.05056		0.27818	0.04523	
Mean	**0.19432**	**0.06299**	**0.068 to 0.32**	**0.22984**	**0.05804**	**0.11 to 0.35**
Diversity						
Ranch	-0.00464	0.01196		0.00161	0.01018	
Middle	0.01016	0.01541		0.01447	0.01362	
East	-0.00571	0.00988		-0.02232	0.01553	
Mean	-0.00006	0.01242	-0.025 to 0.025	-0.00208	0.01311	-0.028 to 0.024
Richness						
Ranch	-0.07934	0.06983		0.00727	0.11000	
Middle	-0.03590	0.08765		-0.14002	0.08030	
East	0.11011	0.16040		0.18296	0.17200	
Mean	-0.00171	0.10596	-0.21 to 0.21	0.01674	0.12077	-0.22 to 0.26
Evenness						
Ranch	0.00072	0.00498		0.00228	0.00453	
Middle	0.00899	0.00675		0.01712	0.00555	
East	0.00214	0.00363		-0.00596	0.00683	
Mean	0.00395	0.00512	-0.0063 to 0.014	0.00448	0.00563	-0.0068 to 0.016
EPT						
Ranch	-0.10468	0.04923		-0.13090	0.05123	
Middle	-0.26922	0.06538		-0.30907	0.06679	
East	-0.21846	0.08214		-0.16632	0.08626	
Mean	**-0.19746**	**0.06558**	**-0.33 to -0.066**	**-0.20210**	**0.06809**	**-0.34 to -0.066**
Proportion EPT						
Ranch	-0.00904	0.00614		-0.01353	0.00671	
Middle	-0.02606	0.00612		-0.02918	0.00633	
East	-0.02861	0.00802		-0.02561	0.00821	
Mean	**-0.02124**	**0.00676**	**-0.035 to -0.0077**	**-0.02277**	**0.00708**	**-0.037 to -0.0086**

Bioassessment metrics were calculated from invertebrates collected with Hester-Dendy samplers for at least 15 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 ascertain if any trends were evident over this period. I calculated that HBI values have increased over this time, indicating that the invertebrate assemblage is composed of more tolerant taxa now compared to when monitoring began (Table 4). EPT richness and the proportion of EPT taxa decreased over this time period. A decrease in EPT richness indicates 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 and 2011 data with metrics calculated from invertebrates collected with a Hess sampler (Figure 9). The same trends were significant for both data sets (Table 4).

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 gravel or cobble bottom streams. 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 occur naturally in rivers. In the Niobrara River, Hester-Dendy samplers imitate the abundant cattails and iris in the wetland 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 the river probably have different invertebrates colonize them compared to if these samplers were placed in the wetland area. The wetland area differs from the main channel of the Niobrara River in several ways. For example, the wetland area is large (0.4 km wide in places), water velocity is much slower in the wetland area, and larger amounts of detritus probably accumulate in the wetland area. Macrophytes in the wetland area of the Niobrara River provide abundant substrate for aquatic invertebrates, but no aquatic plants live in the main channel. We placed Hester-Dendy samplers in the main channel of the river where water velocities were higher and large particulate organic matter is transported, causing large debris dams to form when the Hester-Dendy samplers are deployed. Debris dams were cleared weekly from the Hester-Dendy samplers; however, I have observed debris dams that were >0.3 m deep and >2 m in length when I retrieved the samplers (Figure 1). 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 (including debris) or fewer invertebrates (removing debris may displace individuals) may be collected depending on how the samplers are gathered. I have also observed Hester-Dendy samplers pushed out of the water by debris dams (Figure 1b). In fact, four of the five samplers deployed at Agate East were pushed out of the water to varying degrees when we collected the samples, which may explain the higher standard errors associated with Hester-Dendy samples at Agate East.

Hess samples collect natural densities of aquatic invertebrates that can be compared to other quantitative methods used in aquatic ecosystems (e.g., 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 that had 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 were generally similar. Bioassessment metrics calculated with these samplers were statistically no different. However, Hess samples tended to calculate higher taxa diversity, taxa evenness, taxa richness,

and HBI compared to Hester-Dendy samples (Figure 7). Conversely, Hester-Dendy samplers often had higher EPT richness and the proportion of EPT. We collected significantly more Ephemeroptera on Hester-Dendy samplers compared to the Hess sampler, which probably led to higher EPT richness in Hester-Dendy samples. 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). Additionally, we collected fewer taxa in Hester-Dendy samples compared to Hess samples, which may be because not all taxa colonize these artificial substrates. Letovsky et al. (2012) also noted lower taxa diversity of invertebrates that colonized Hester-Dendy samplers compared to kick net samples in Vermont.

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 four 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 15 years of data exist and the CV is much lower for the metrics calculated (16-42%). Such variability in data is normal and may be caused by climatic variation, such as drought (Mazor et al. 2009).

Three of the six 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. Agate Springs Ranch had a low HBI value in 2011, which comes from a high density of early instar *Paraleptophlebia* that colonized Hester-Dendy samplers. *Paraleptophlebia* are mayflies with very low tolerance values (1.2) where 0 indicates that the invertebrate is extremely sensitive to pollution and 10 denotes that the taxa is extremely tolerant of pollution. Hess samples did not collect the same high density of *Paraleptophlebia*, thus the HBI metric at Agate Springs Ranch calculated from Hess samples were similar to the past few years (Figure 9d) and did not influence the analysis to such a large degree (high leverage point; Table 4). 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.

I recommend using a Hess sampler to collect aquatic invertebrates in the Niobrara River. Collecting invertebrates with a Hess sampler compared to a Hester-Dendy sampler will reduce the number of visits to the sites along the Niobrara River from potentially five (deploying, 3 visits to clear debris dams, and retrieving) to only one (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. In general, a similar number of individuals were captured in each sample and bioassessment metrics calculated from Hester-Dendy and Hess samplers were similar. Hess samples should be collected in July or August, when water levels are high enough to extend into the wetland area, which will aid sampling. Water levels need to be watched closely as annual variation in discharge and irrigation withdrawals change annually, but in general samples should be collected in July or August when assemblages are similar (Bowles 2010).

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Appendix A. List of invertebrate taxa collected from the Niobrara River at Agate Fossil Beds National Monument in 2011. Taxa highlighted in yellow are taxa only collected by a particular sampler.

All Taxa collected	Taxa in Hess samples	Taxa in Hester-Dendy samples
Acari	Acari	Acari
Ancylidae	Ancylidae	Ancylidae
Baetis	*Baetis*	*Baetis*
Belostoma	*Caenis*	*Belostoma*
Bidessonotus	Ceratopogonidae	*Bidessonotus*
Blood midges	*Cheumatopsyche*	Blood midges
Caenis	Cladocera	*Caenis*
Ceratopogonidae	*Coenagrion/Enallagma*	Ceratopogonidae
Cheumatopsyche	*Coptotomus*	*Cheumatopsyche*
Cladocera	Cyclopoida	*Coenagrion/Enallagma*
Coenagrion/Enallagma	*Dubiraphia*	*Colymbetes*
Colymbetes	*Gammarus*	Cyclopoida
Coptotomus	*Gyrinus*	*Dubiraphia*
Cyclopoida	*Heptagenia*	*Gammarus*
Dubiraphia	*Hetaerina*	*Gyrinus*
Gammarus	*Hexagenia*	*Heptagenia*
Gyrinus	Hirudinea	*Hetaerina*
Heptagenia	*Hyalella*	*Hexagenia*
Hetaerina	*Laccophilus*	Hirudinea
Hexagenia	Oligochaeta	*Hyalella*
Hirudinea	*Orconectes neglectus negectus*	*Laccophilus*
Hyalella	Other midges	*Orconectes neglectus negectus*
Laccophilus	*Paraleptophlebia*	Other midges
Oligochaeta	Physidae	*Paraleptophlebia*
Orconectes neglectus negectus	Planorbidae	*Polycentropus*
Other midges	*Polycentropus*	*Sanfilippodytes*
Paraleptophlebia	*Simulium*	*Simulium*
Physidae	Sphaeriidae	
Planorbidae	Tipulidae	
Polycentropus		
Sanfilippodytes		
Simulium		
Sphaeriidae		
Tipulidae		

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