



Aquatic Invertebrate Monitoring at Agate Fossil Beds National Monument

2021 Data Report

Natural Resource Data Series NPS/AGFO/NRDS—2023/1389



**ON THIS PAGE**

Bryan Tronstad deploying Hester-Dendy samplers at the Agate East site along the Niobrara River at Agate Fossil Beds National Monument.

Photograph courtesy by Tresize Tronstad, Wyoming Natural Diversity Database, University of Wyoming

ON THE COVER

The Agate East site along the Niobrara River at Agate Fossil Beds National Monument with Hester-Dendy samplers deployed.

Photograph courtesy by Lusha Tronstad, Wyoming Natural Diversity Database, University of Wyoming

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Lusha M. Tronstad, Bryan P. Tronstad

Wyoming Natural Diversity Database
1000 East University Avenue
University of Wyoming
Laramie, Wyoming 82071

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Executive Summary

Monitoring ecosystems is critical to detect changes so that managers can address alterations. Freshwater streams are connected to the surrounding landscape and their health is a measure of the overall watershed integrity. Streams are the culmination of upland processes and inputs. Degradation, restoration and many other changes within the watershed can be detected in streams using biota living in these ecosystems. Aquatic invertebrates are excellent indicators of ecosystem quality because they are relatively long-lived, sessile, diverse, abundant, easy to collect and their tolerance to disturbance differ. Aquatic invertebrates were monitored at three sites along the Niobrara River at Agate Fossil Beds National Monument in 2021 completing 25 years of data collection. We collected invertebrates using two methods. Hester-Dendy samplers are artificial multi-plate samplers suspended in the water column to allow invertebrates to colonize and a Hess sampler is a metal tube that surrounds a known area to collect invertebrates on natural substrate and vegetation.

We identified 55 invertebrate taxa from four phyla (Annelida, Arthropoda, Mollusca, Nematoda) using both samplers in the Niobrara River (Appendix A and B). Hester-Dendy samplers collected one taxon not found in Hess samples and Hess samples collected 32 taxa not collected with Hester-Dendy samplers. Hess samples captured most taxa (98%) compared to Hester-Dendy samples (42%). Insecta, Crustacea, Annelida were the most abundant groups of invertebrates collected in the Niobrara River. The proportion of Insecta, Annelida, Mollusca, Coleoptera, Odonata and Hemiptera differed between Hester-Dendy and Hess samples ($p < 0.05$). The number of taxa (richness), diversity, the proportion of taxa from the orders Ephemeroptera, Plecoptera and Trichoptera (EPT; known to be sensitive to ecosystem changes) and Hilsenhoff's Biotic Index (HBI; $p \leq 0.003$) differed between sampler types, but taxa evenness and EPT richness ($p > 0.10$) did not. HBI at the sites ranged from 3.7 to 5.9 (very good to fair from Hilsenhoff 1987) using the Hester-Dendy and 5.2 (good from Hilsenhoff 1987) using the Hess sampler.

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Introduction

Aquatic invertebrates are excellent indicators of ecosystem quality and they have been used to monitor aquatic ecosystems since the 1870s (Cairns and Pratt 1993). Managers and scientists use aquatic invertebrates to monitor streams, because they have characteristics that make them ideal for the task. Aquatic invertebrates are relatively long lived (weeks to >100 years) and remain in the water for most of their life (Rosenberg and Resh 1993b). Unlike water samples collected periodically, aquatic invertebrates live in the water 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 also relatively sedentary making them ideal to assess water quality at a location. In addition, aquatic invertebrates are abundant, diverse and easy to collect. Lower ecosystem quality can increase mortality, and decrease reproduction, survival and fitness of aquatic invertebrates (Johnson et al. 1993). The characteristic that makes aquatic invertebrates so successful at monitoring ecosystem quality is that some aquatic invertebrates are more sensitive to changes (i.e., mayflies) while others are more tolerant (i.e., true flies; Barbour et al. 1999). Monitoring aquatic invertebrates can detect changes such as habitat degradation, changes in land use, altered food web, pollution or invasive species. Changes in the richness or assemblage structure of aquatic invertebrates can be a sensitive measure of ecosystem quality and these metrics are well-developed (Rosenberg and Resh 1993a).

Choosing which sampler to collect aquatic invertebrates and monitor ecosystem quality can be a difficult decision that depends on many variables. All samplers have advantages and disadvantages but finding a sampler that minimizes bias and fulfills the objectives 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 ecosystem. For example, artificial substrates may be a good choice in aquatic habitats that are difficult to sample using other methods, such as large, deep rivers (De Pauw et al. 1986). The type of information needed plays a major role. For example, qualitative data may be sufficient if the study is estimating conditions, but more rigorous quantitative sampling is needed to assess change over time. Qualitative samplers report proportional data on the invertebrate assemblage, but fixed area samplers can provide quantitative information on the density 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 monitored three sites along the Niobrara River at Agate Fossil Beds National Monument since 1997. Our objective was to calculate bioassessment metrics from invertebrates collected using Hester-Dendy multiple plate samplers and a Hess sampler in 2021 to estimate changes over time. Our specific questions were 1.) How did chemical and physical characteristics of the streams vary among sites, 2.) To what degree does the invertebrate assemblage and bioassessment metrics differ between samplers? and 3.) Do bioassessment metrics differ among sites? We collected five Hester-Dendy and Hess samples at each of the three sites to answer these

questions. Data can be used to address management decisions in the park and understand how the river changed over time.

Study Area

The headwaters of the Niobrara River are located near Lusk, Wyoming. The river flows eastward into Nebraska and eventually into the Missouri River near Niobrara, Nebraska. The Niobrara River Basin covers 32,600 km² of which the most 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 east of the Wyoming border. At this point the Niobrara River is a low order stream flowing through grassland. Agate Fossil Beds National Monument encompasses 2700 acres in a valley bottom and 10.5 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 (*Iris pseudacorus*). The substrate in the river predominantly consists of fine particles (e.g., sand, silt, and clay). Currently, northern pike (*Esox lucius*), white suckers (*Catostomus commersonii*) and green sunfish (*Lepomis cyanellus*) inhabit the river within the park; however, 9 other fish species were collected at Agate Fossil Beds National Monument prior to 1990 (Spurgeon et al. 2014).

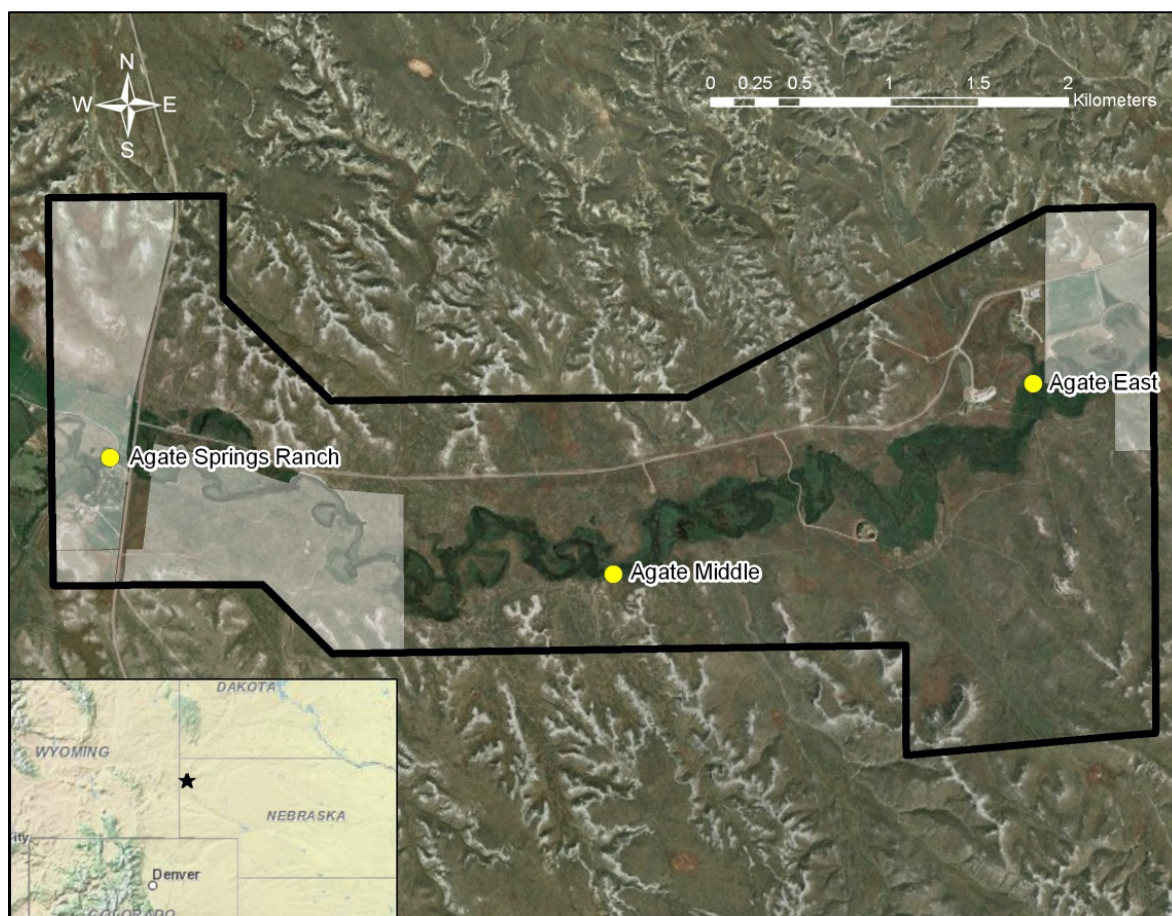


Figure 1. We sampled three sites along the Niobrara River at Agate Fossil Beds National Monuments. The black line is the Monument boundary and the transparent white areas are private land within the Monument boundary. The inset map shows the location of Agate Fossil Beds National Monument in Nebraska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

We sampled three sites along the Niobrara River at Agate Fossil Beds National Monument (Figures 1 and 2, Table 1). Aquatic invertebrates were first collected at Agate Springs Ranch in 1989 and again in 1996 when the Agate Middle site was added. A third site, Agate East, was added in 1997 and all sites have been monitored annually since that year. The most upstream site (Agate Springs Ranch) is located near the western park boundary. Agate Springs Ranch has an overstory of plains cottonwood (*Populus deltoides*) and cattails are more abundant than iris (Figure 2c; Appendix C). The central site, Agate Middle, lacks an overstory and has gravel substrate (Figure 2a; Appendix D). Both iris and cattails are abundant here. Finally, Agate East is located before the Niobrara River flows out of the park (Figure 2b; Appendix E). The riparian vegetation is dominated by iris with a few willows (*Salix* spp.) at Agate East. The river was treated in 2018 to reduce the iris growing there and riparian vegetation recolonized post-treatment.

Table 1. Location (Datum NAD83) of each site along the Niobrara River (± 5 m accuracy).

Coordinate	Ranch	Middle	East
Latitude	42.423	42.417	42.427
Longitude	-103.793	-103.759	-103.730
Elevation (m)	1354	1349	1343

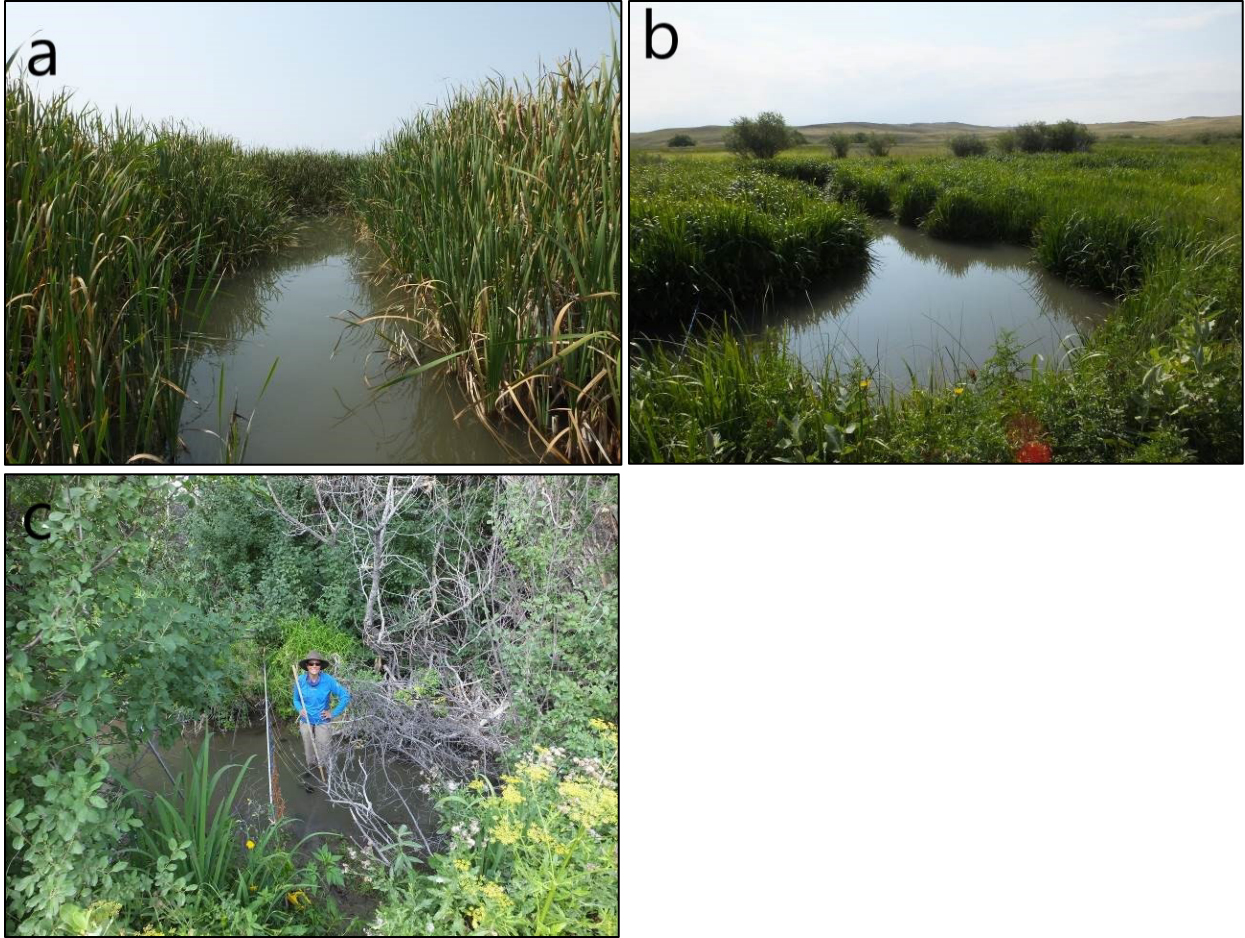


Figure 2. Photos of (a) Agate Middle, (b) Agate East and (c) Agate Springs Ranch.

Methods

General Measurements

We measured several variables to assess the general habitat characteristics of the Niobrara River including water quality, water clarity, sediment composition, depth and discharge. We measured dissolved oxygen concentration, pH, water temperature, specific conductivity and oxidation-reduction potential using a Professional Plus made by Yellow Springs Instruments. The sensors were calibrated on-site before use. We measured water clarity by estimating the depth at which a Secchi disk disappeared and using a calibrated turbidity meter (Hach 2100). The dominant substrate was assessed in the main channel and the bank of all sites using the soil texture test (Thien 1979). Clay was defined as fine particles forming a ribbon after removing water, whereas silt did not form a ribbon. Sand was characterized by particles 0.06–2 mm in diameter (sandpaper texture), 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. We recorded the location of each site using a global positioning system (GPS; Garmin Oregon 600). Finally, we estimated stream discharge (Q ; ft³/s) by measuring water depth (d ; ft) and water velocity (v ; ft/s) using a Marsh-McBirney Flo-Mate 2000 at 0.3 m intervals across the stream's width (w ; ft) and summing each interval to calculate discharge using the following equation:

$$Q = \sum d_i \times v_i \times w_i$$

Hester-Dendy Samples

We deployed 7 Hester-Dendy samplers (76 mm by 76 mm, 9 plates, Wildlife Supply Company) at each site. A rope was strung across the stream between two 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 at least 15 cm above the substrate. Debris dams were cleared from the samplers weekly. We retrieved the samplers after at least 30 days of colonization by approaching the site from downstream, and placing a dipnet (150 µm mesh) under each sampler and cutting the rope. Hester-Dendy samplers were immediately placed in a container with water. Any organisms in the dip net were removed and placed in the same container. We dismantled and scrubbed the Hester-Dendy samplers to remove invertebrates that colonized the plates within 24 hours of collection, rinsed samples using a 212 µm sieve and preserved samples in ~80% ethanol. Five samples were chosen for analysis based on their condition in the field (e.g., debris dams or touching bottom)

Hess Samples

We collected five Hess samples (500 µm mesh, 860 cm² sampling area, Wildlife Supply Company) from each site to sample invertebrates that lived in the emergent vegetation and sediment that is abundant along the margin of the Niobrara River. We placed the Hess sampler over cattails, iris or bull rush (*Scirpus* sp.) 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 elutriated in the field, preserved in 80% ethanol and returned to the laboratory for analysis.

Invertebrate analysis

Invertebrates were sorted from debris in white trays and identified under a dissecting microscope. We rinsed each sample through 2 mm and either a 212 (Hester-Dendy samples) or 500 μm mesh sieves (Hess samples) 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 in the smaller sieve, we subsampled the contents 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) for non-insect invertebrates. Invertebrate tolerance values were assigned to taxa from Barbour et al. (1999). Density was calculated as the area sampled by the Hess sampler (0.086 m^2) or the surface area of the Hester-Dendy plates (0.10 m^2).

Six bioassessment metrics have been calculated since 1989 to estimate ecosystem quality based on the invertebrates collected: taxa richness, taxa diversity (Shannon's index), taxa evenness (taxa diversity divided by natural log of taxa richness), Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, proportion of EPT taxa (number of EPT taxa divided by the total number of taxa collected) and Hilsenhoff's Biotic Index (HBI) for each replicate sample (Bowles et al. 2013). We used generalized linear models to compare invertebrate assemblage structure (e.g., density, proportion of a taxon) and bioassessment metrics among sites and between sampler types. If sites were significantly different ($\alpha < 0.05$), we used estimated marginal means (emmeans; Lenth 2021) to verify which sites differed from one another. The Gamma distribution best fit the data by visually inspecting histograms and using the *fitdistrplus* package (Delignette-Muller and Dutang 2015), and we used the Gamma distribution to analyze data. We calculated invertebrate abundances, densities, proportions and bioassessment metrics using R (R Development Core Team 2013) and the packages *plyr* (Wickham 2011), *Matrix* (Bates and Maechler 2013), and *vegan* (Oksanen et al. 2013).

Results

Generally, conditions were similar among sites and visits. Dissolved oxygen peaked at 9.41 mg/L during the day and minimum values reached 7.87 mg/L at night during two days in July (Figure 3a, b). Water temperature varied between 19.2 and 28.6°C in July (Figure 3c, d). Specific conductivity was higher in July than August and pH was highest at the Agate Springs Ranch (Table 2). Reducing conditions dominated all sites (oxidation-reduction potential < 200 mV). The stream channel at Agate East was deepest and Agate Middle was narrowest. Discharge was highest at the Agate East and higher in July. Water clarity was lower in August (shallower Secchi disk depths and turbidity measurements). Channel substrate was dominated by fines and gravel, and bank substrate was dominated by sand and silt.

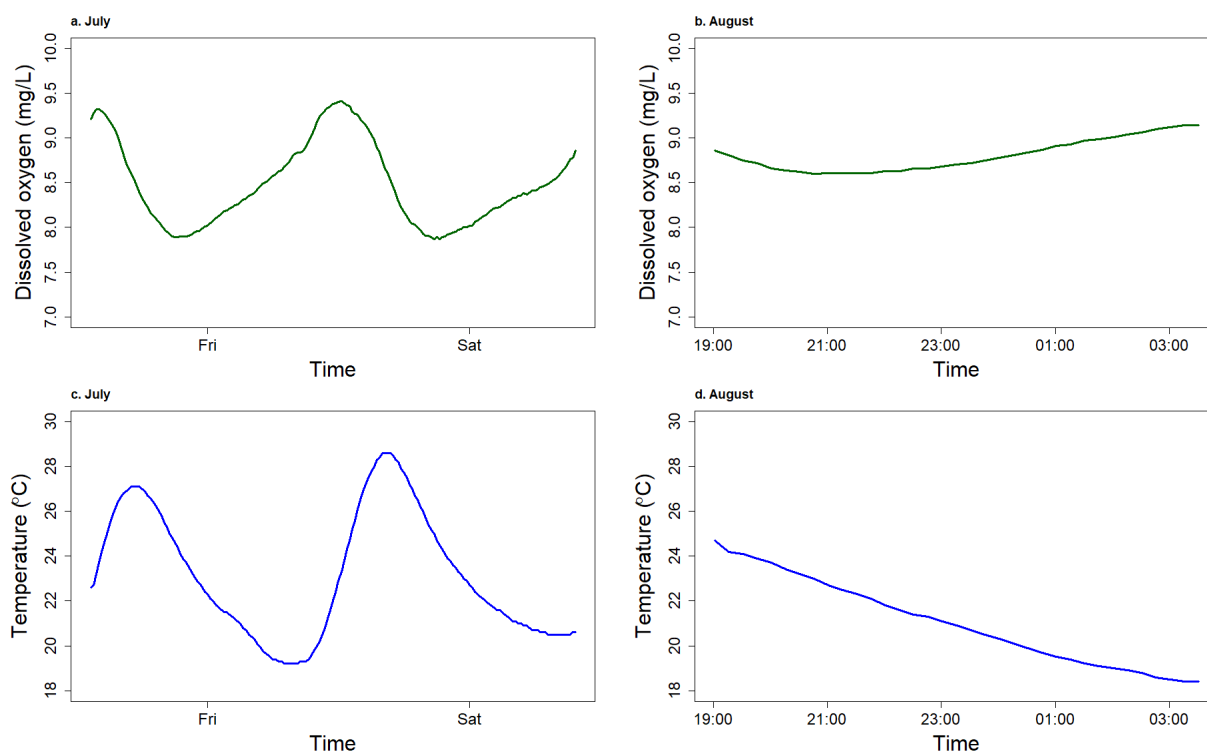


Figure 3. Dissolved oxygen (a, b) and water temperature (c, d) on 29–31 July 2021 when Hester-Dendy samplers were deployed (a, c) and 27–28 August 2021 when samplers were retrieved (b, d). The shorter August time was due to equipment malfunction.

Table 2. Water quality and site characteristics measured when Hester-Dendy samplers were deployed (July) and retrieved (August). Channel width is the width of the channel lacking emergent vegetation and dominant substrate is in the stream channel. Dissolved oxygen (DO), specific conductivity (SPC), water temperature and pH were measured with a Professional Plus made by Yellow Springs Instruments.

Parameter	Units	Ranch	Middle	East	Ranch	Middle	East
Date	–	29-Jul-21	29-Jul-21	29-Jul-21	28-Aug-21	28-Aug-21	29-Aug-21
Start time	–	16:00	14:00	17:00	8:30	13:00	9:00
Water temperature	°C	22.8	21.7	27	17.2	19.6	16.1
Dissolved oxygen	% saturation	103.4	96.5	96.6	87	91.1	84.6
Dissolved oxygen	mg/L	8.84	8.51	7.65	7.17	8.26	8.3
Specific conductivity	µS/cm	375.5	374.4	374.1	393.6	385.5	385.5
pH	–	8.1	7.97	8.26	8.15	8.17	8.27
ORP	mV	0	0	0	–	–	–
Secchi disk depth	cm	26	47.2	26	37	46	37
Turbidity	NTU	26.1	28.6	46.6	23	16.8	26.6
Max depth	ft	1.1	1.55	2.15	1.2	1.7	3.45
Channel width	ft	10.3	5.3	7.4	10.3	5.4	7.3
Discharge	ft ³ /s	2.7	84.0	2.6	4.9	5.8	5.3
Dominant substrate	–	Sand	Silt	Sand	Sand	Gravel	Sand
Air temperatures	°C	29.4	23.9	32.2	–	21.67	16.67

We identified 55 invertebrate taxa from four phyla (Annelida, Arthropoda, Molluska, Nematoda) using both samplers in the Niobrara River (Appendix A and B). Hester-Dendy samplers collected one caddisfly not found in Hess samples and Hess samples collected 32 taxa not collected with Hester-Dendy samplers (Appendix A and B). The proportion of Insecta ($t = 4.9$, $p < 0.0001$; Figure 4a), Annelida ($t = 6.4$, $p < 0.0001$; Figure 4b), Molluska ($t = 2.3$, $p = 0.03$; Figure 4c), Coleoptera ($t = 2.6$, $p = 0.01$; Figure 5a), Odonata ($t = 3.8$, $p < 0.001$; Figure 5c) and Hemiptera ($t = 2.8$, $p = 0.009$; Fig. 5f) differed between samplers. Proportions of Crustaceans ($t = 0.39$, $p = 0.70$; Figure 4d), Diptera ($t = 0.60$, $p = 0.56$; Figure 5b), Ephemeroptera ($t = 0.26$, $p = 0.80$; Figure 5d) and Trichoptera ($t = 1.4$, $p = 0.17$; Figure 5e) did not differ between samplers. Taxa richness ($t = 4.9$, $p < 0.0001$; Figure 6a), taxa diversity ($t = 6.1$, $p < 0.0001$; Figure 6b), proportion EPT taxa ($t = 3.2$, $p = 0.003$; Figure 7b) and HBI ($t = 3.2$, $p = 0.003$; Figure 7c) varied between sampler types. Evenness ($t = 0.86$, $p = 0.40$; Figure 6c) and EPT richness ($t = 1.7$, $p = 0.11$; Figure 7a) did not differ between Hester-Dendy and Hess samples.

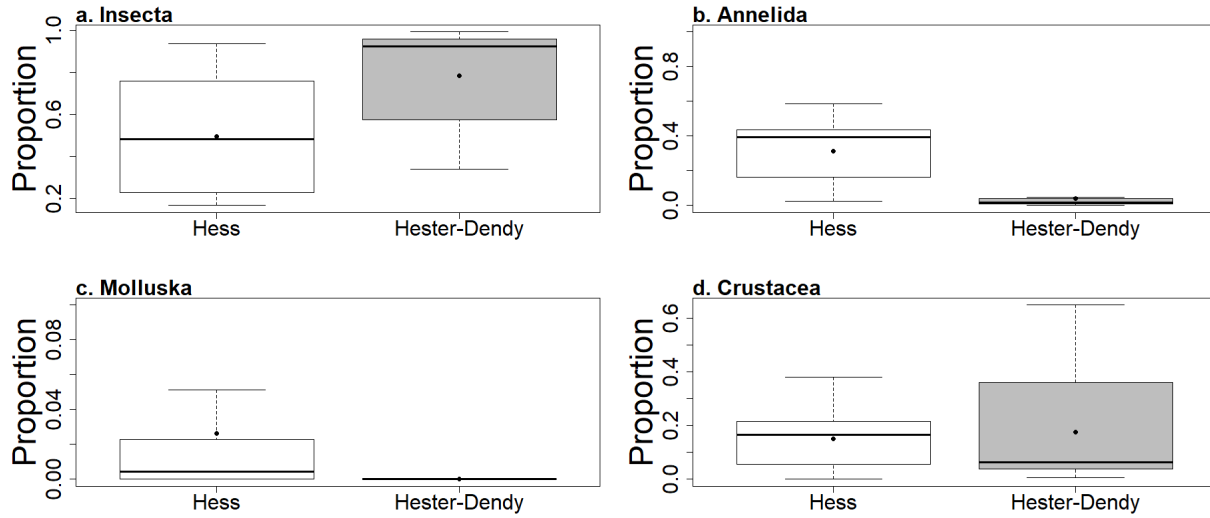


Figure 4. The proportion of (a) Insecta, (b) Annelida (c) Molluska, and (d) Crustacea collected by the two sampler types. Black circles represent mean values and bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles and whiskers indicate the lower and upper limits of the data excluding outliers.

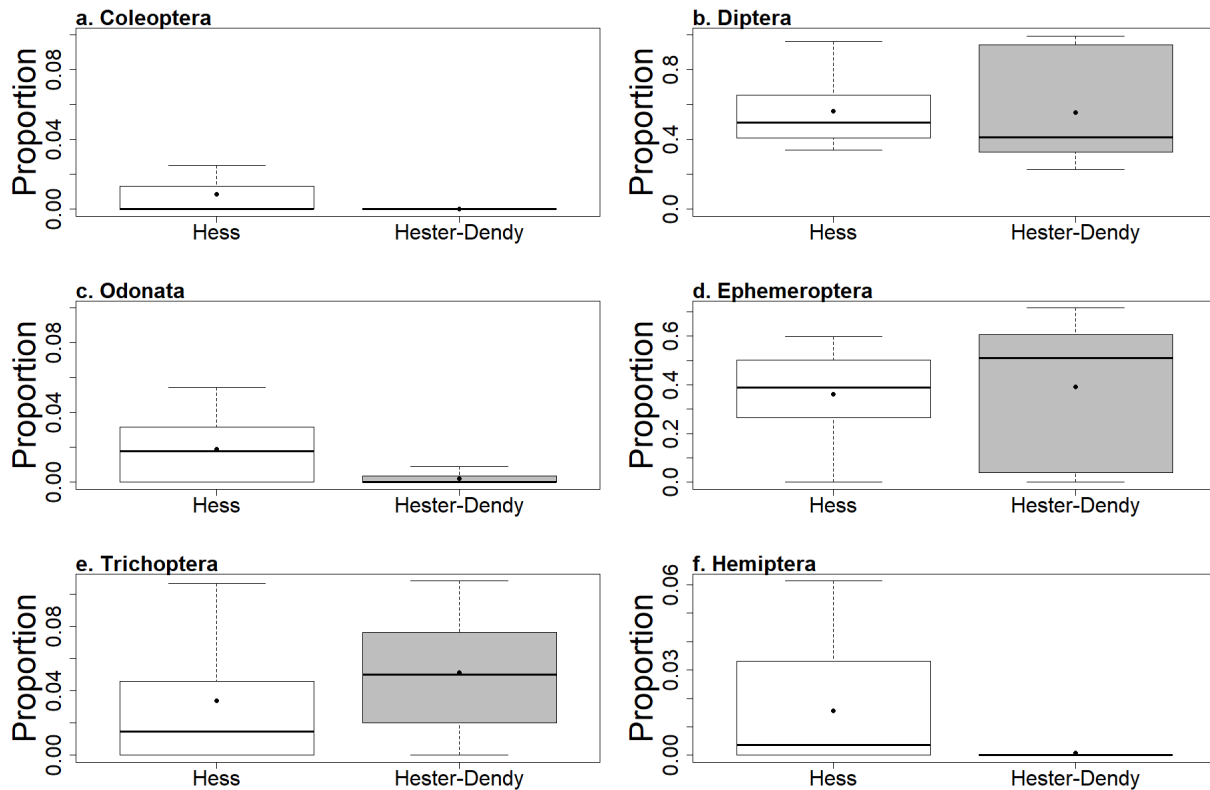


Figure 5. The proportion of (a) Coleoptera, (b) Diptera (c) Odonata, (d) Ephemeroptera, (e) Trichoptera and (f) Hemiptera collected by the two sampler types. Black circles represent mean values and bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles and whiskers indicate the lower and upper limits of the data excluding outliers.

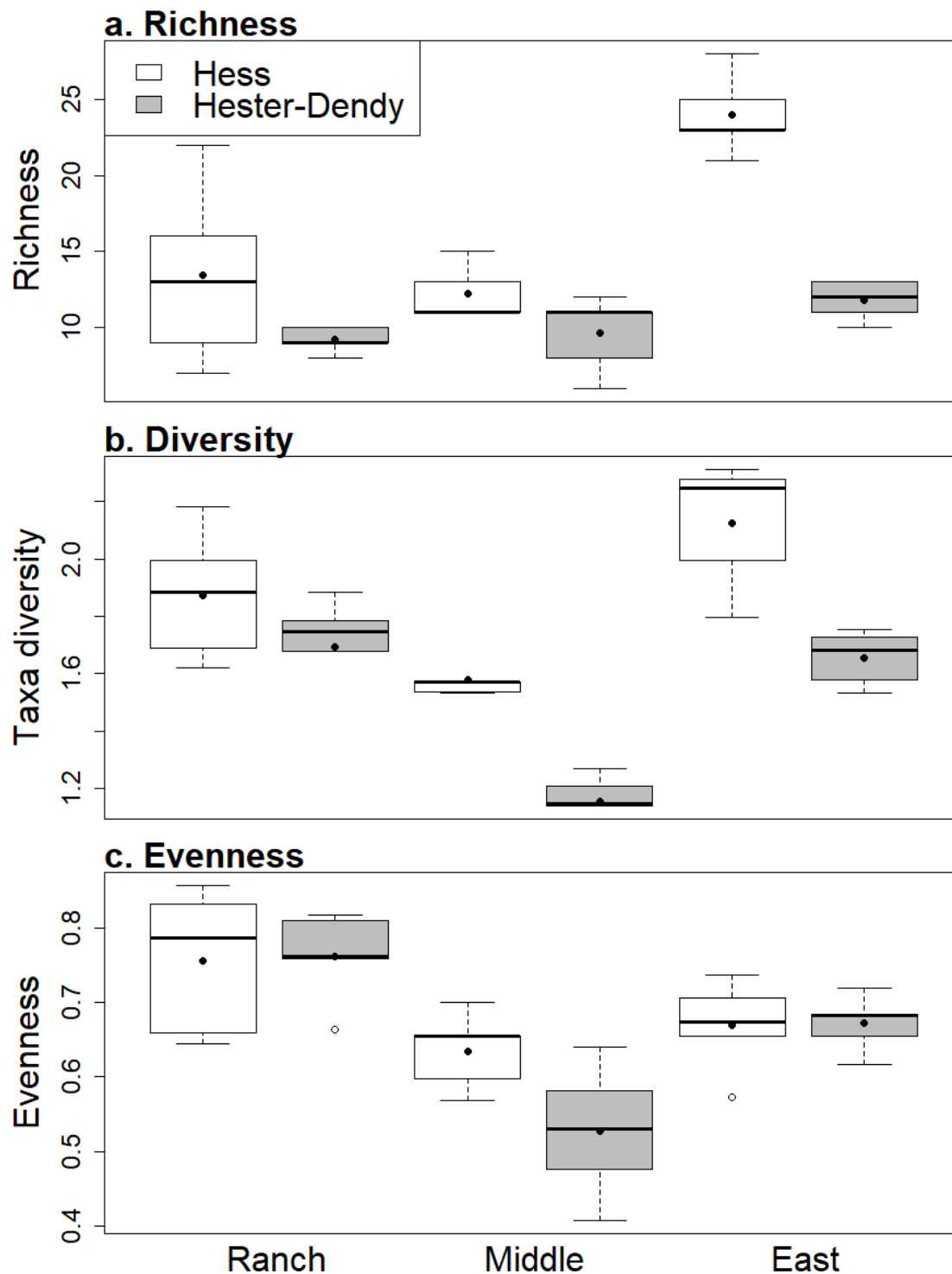


Figure 6. (a) Taxa richness, (b) taxa diversity and (c) taxa evenness calculated from Hess and Hester-Dendy samples collected from the Niobrara River in 2021. Higher values of all metrics indicate better ecosystem quality. Black circles represent mean values and bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles, whiskers indicate the lower and upper limits of the data excluding outliers and open circles are outliers.

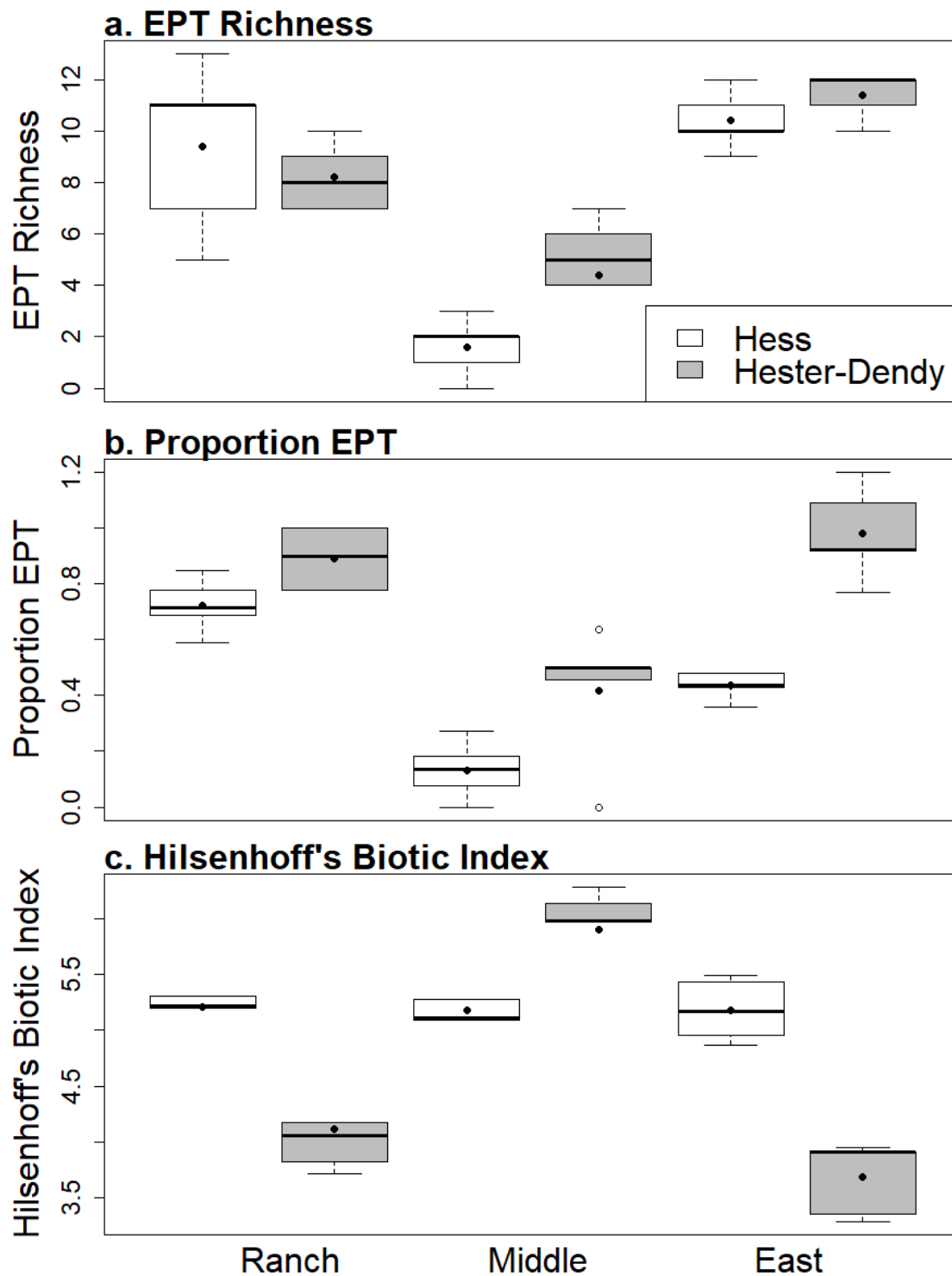


Figure 7. (a) Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, (b) proportion of EPT taxa and (c) Hilsenhoff's biotic index calculated from Hess and Hester-Dendy samples collected from the Niobrara River in 2021. Higher values of all metrics except HBI indicate better ecosystem quality. Black circles represent mean values and bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles, whiskers indicate the lower and upper limits of the data excluding outliers and open circles are outliers.

Using Hester-Dendy samplers, we collected 42% of the invertebrate taxa detected in the Niobrara River in 2021. Insecta (70% of individuals), Crustacea (26%) and Annelida (4%) were the most numerous invertebrate groups in Hester-Dendy samples. Of the insects, Diptera (60%), Ephemeroptera (35%) and Trichoptera (5%) were the most abundant orders (Appendix A). No beetles were captured in Hester-Dendy samplers. Hester-Dendy samples from Agate Middle (596 ind/sample; 5960 ind/m²) contained more invertebrates than Agate Springs Ranch (231 ind/sample; 2310 ind/m²) and Agate East (261 ind/sample; 2608 ind/m²; $t = 0.8-5.3$, $p = 0.0002-0.46$; emmeans, $p < 0.0006$). Taxa richness did not differ among sites ($t = 0.6-1.6$, $p = 0.12-0.51$; emmeans, $p = 0.54-0.99$; Table 3). Taxa diversity ($t = 1.8-2.6$, $p = 0.01-0.08$; emmeans, $p < 0.0001$), taxa evenness ($t = 0.04-2.0$, $p = 0.05-0.97$; emmeans, $p < 0.02$), EPT taxa richness ($t = 0.7-2.4$, $p = 0.02-0.47$; emmeans, $p = 0.003-0.11$) and the proportion of EPT taxa ($t = 4.6$, $p = 0.02$; emmeans, $p \leq 0.003$) were lowest at Agate Middle. Agate Springs Ranch had the highest proportion of EPT taxa ($t = 0.7-2.4$, $p = 0.02-0.47$; emmeans, $p = 0.003-0.11$). The average tolerance value for an invertebrate in the assemblage was highest at Agate Middle (HBI; $t = 1.6-5.6$, $p < 0.001-0.12$; emmeans, $p < 0.0001$).

We collected 98% of the invertebrate taxa detected using a Hess sampler in the Niobrara River. Overall, non-insect invertebrates (52%) dominated the invertebrate assemblage. Insects (48% of individuals), Crustacea (17%), Annelida (31%) and Gastropods (2%) were the most numerous invertebrate groups. Of the insects, Diptera (49%) were the most abundant followed by Ephemeroptera (43%), Trichoptera (4%), Odonata (2%), Hemiptera (1%) and Coleoptera (1%; Appendix B). The density of invertebrates collected with Hess samples at Agate Middle (138 ind/sample; 1602 ind/m²), Agate East (285 ind/sample; 3368 ind/m²) and Agate Springs Ranch (159 ind/sample; 1850 ind/m²) did not differ among sites ($t = 1.8-2.3$, $p = 0.04-0.09$; emmeans, $p = 0.10-0.90$). Taxa richness was highest at Agate East ($t = 0.6-1.6$, $p = 0.12-0.51$; emmeans, $p < 0.007$; Table 3). Taxa diversity was higher at Agate East than Agate Middle ($t = 1.8-2.6$, $p = 0.01-0.08$; emmeans, $p = 0.0004$). Taxa evenness did not differ among sites ($t = 0.04-2.0$, $p = 0.05-0.97$; emmeans, $p = 0.15-0.97$). Agate Middle had the fewest EPT taxa ($t = 0.7-2.4$, $p = 0.02-0.47$; emmeans, $p < 0.0001$). Agate Springs Ranch had the highest proportion of EPT taxa ($t = 4.6$, $p = 0.05$; emmeans, $p = 0.003$) and Agate Middle had the lowest proportion (emmeans, $p < 0.03$). The mean tolerance value did not vary among sites (HBI; $t = 1.6-5.6$, $p < 0.001-0.12$; emmeans, $p = 1$).

Table 3. Mean invertebrate bioassessment metrics and standard errors at each site along the Niobrara River collected with Hester-Dendy and Hess samplers in 2021.

Sample Type	Metric	Ranch	Middle	East
Hester-Dendy samples	Taxa richness	9.2±0.37	9.6±1.12	11.8±0.58
	Taxa diversity	1.69±0.09	1.16±0.04	1.65±0.04
	Taxa evenness	0.76±0.03	0.53±0.04	0.67±0.02
	EPT richness	8.2±0.58	4.4±1.21	11.4±0.40
	Proportion EPT taxa	0.89±0.05	0.42±0.11	0.98±0.07
	HBI	4.1±0.19	5.9±0.19	3.7±0.15
Hess samples	Taxa richness	13.4±2.66	12.2±0.80	24.0±1.18
	Taxa diversity	1.87±0.10	1.58±0.03	2.12±0.10
	Taxa evenness	0.76±0.04	0.64±0.02	0.67±0.03
	EPT richness	9.4±1.47	1.6±0.51	10.4±0.51
	Proportion EPT taxa	0.72±0.04	0.13±0.05	0.44±0.02
	HBI	5.2±0.10	5.2±0.13	5.2±0.12

Further Analysis

This 2021 data report is intended to provide a basic review of the data collected during the annual monitoring of invertebrates at AGFO. All invertebrate data found in this report are available from NGPN, as well as the archives found in the IRMA Data Store [DataStore - Generic Dataset - \(Code: 2283715\) \(nps.gov\)](#). For information on differences in sampling methods, invertebrate community trends over time and potential impacts to the macroinvertebrate community at AGFO, the 2010-2019 Aquatic Invertebrate, Water Quality, and Riparian Vegetation Status and Trends for Agate Fossil Beds National Monument report provides in-depth information on those topics.

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Appendix A

Table A-1. Mean number of individuals on a Hester-Dendy sampler collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2021.

Group	Order	Family	Genus	East	Middle	Ranch
Annelida	Hirudinea	–	–	1	0	0
Annelida	Oligochaeta	–	–	1	25	18
Arthropoda	Collembola	–	–	0	2	0
Crustacea	Amphipoda	Gammaridae	<i>Gammarus</i>	2	66	0
Crustacea	Cladocera	–	–	4	3	0
Crustacea	Copepoda	–	–	6	195	17
Insecta	Diptera	Cecidomyiidae	–	1	0	0
Insecta	Diptera	Ceratopogonidae	<i>Culicoides</i>	1	0	1
Insecta	Diptera	Ceratopogonidae	<i>Probezzia</i>	1	0	0
Insecta	Diptera	Chironomidae	<i>Non-Tanypodinae</i>	45	288	63
Insecta	Diptera	Chironomidae	<i>Tanypodinae</i>	32	2	4
Insecta	Diptera	Chironomidae	–	0	2	2
Insecta	Diptera	Empididae	<i>Hemerodromia</i>	1	0	0
Insecta	Diptera	Simuliidae	<i>Simulium</i>	0	2	14
Insecta	Ephemeroptera	Baetidae	<i>Baetis</i>	4	4	6
Insecta	Ephemeroptera	Caenidae	<i>Caenis</i>	5	1	1
Insecta	Ephemeroptera	Ephemeridae	<i>Hexagenia</i>	10	0	0
Insecta	Ephemeroptera	Heptageniidae	<i>Heptagenia</i>	12	0	35
Insecta	Ephemeroptera	Leptophlebiidae	<i>Paraleptophlebia</i>	122	7	66
Insecta	Hemiptera	Belostomatidae	<i>Belostoma</i>	0	0	0
Insecta	Hemiptera	Corixidae	–	0	1	0
Insecta	Odonata	Calopterygidae	<i>Calopteryx</i>	1	0	0
Insecta	Odonata	Calopterygidae	<i>Hetaerina</i>	2	0	0
Insecta	Odonata	Coenagrionidae	<i>Argia</i>	0	0	0
Insecta	Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i>	0	3	11
Insecta	Trichoptera	Leptoceridae	<i>Nectopsyche</i>	1	0	0
Insecta	Trichoptera	Polycentropodidae	<i>Polycentropus</i>	16	1	0
Insecta	Trichoptera	–	–	1	1	5
Nematoda	–	–	–	0	1	0

Appendix B

Table B-1. Mean density (ind/m²) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2021 using a Hess sampler.

Group	Order	Family	Genus	East	Middle	Ranch
Annelida	Hirudinea	–	–	12	19	0
Annelida	Oligochaeta	–	–	1272	753	86
Arachnida	Trombidiformes	Acari	–	12	0	0
Arthropoda	Collembola	–	–	38	23	0
Crustacea	Amphipoda	Gammaridae	<i>Gammarus</i>	523	237	7
Crustacea	Cladocera	–	–	174	35	0
Crustacea	Copepoda	–	–	67	123	0
Crustacea	Decapoda	Cambaridae	<i>Orconectes neglectus</i>	0	27	74
Crustacea	Ostracoda	–	–	12	0	0
Insecta	Coleoptera	Dytiscidae	<i>Liodessus</i>	10	0	0
Insecta	Coleoptera	Helophoridae	<i>Helophorus</i>	12	0	0
Insecta	Coleoptera	Hydraenidae	<i>Hydraenidae</i>	12	0	0
Insecta	Coleoptera	Lampyridae	<i>Lampyridae</i>	12	0	0
Insecta	Coleoptera	Melyridae	–	12	0	0
Insecta	Coleoptera	Staphylinidae	–	24	0	12
Insecta	Diptera	–	–	0	23	0
Insecta	Diptera	Ceratopogonidae	<i>Culicoides</i>	44	12	187
Insecta	Diptera	Ceratopogonidae	<i>Probezzia</i>	12	16	221
Insecta	Diptera	Chironomidae	–	35	2	70
Insecta	Diptera	Chironomidae	<i>Non-Tanypodinae</i>	246	221	449
Insecta	Diptera	Chironomidae	<i>Tanypodinae</i>	67	18	29
Insecta	Diptera	Culicidae	<i>Aedes</i>	12	0	0
Insecta	Diptera	Empididae	<i>Hemerodromia</i>	0	0	12
Insecta	Diptera	Ephydriidae	<i>Notiphila</i>	0	12	0
Insecta	Diptera	Limoniidae	<i>Eloeophila</i>	0	0	12
Insecta	Diptera	Limoniidae	<i>Epiphragma</i>	0	12	0
Insecta	Diptera	Limoniidae	<i>Limnophila</i>	12	43	47
Insecta	Diptera	Limoniidae	–	0	0	23
Insecta	Diptera	Limoniidae	<i>Paradelphomyia</i>	20	0	0
Insecta	Diptera	Limoniidae	<i>Pilaria</i>	0	0	151
Insecta	Diptera	Limoniidae	<i>Rhypholophus</i>	20	0	12
Insecta	Diptera	Muscidae	–	47	47	0
Insecta	Diptera	Simuliidae	–	12	0	0
Insecta	Diptera	Simuliidae	<i>Simulium</i>	0	0	41

Table B-1 (continued). Mean density (ind/m²) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2021 using a Hess sampler.

Group	Order	Family	Genus	East	Middle	Ranch
Insecta	Diptera	Tabanidae	<i>Chrysops</i>	0	12	0
Insecta	Diptera	Tipulidae	<i>Erioptera</i>	75	12	0
Insecta	Diptera	Tipulidae	<i>Tipula</i>	50	0	30
Insecta	Ephemeroptera	Baetidae	<i>Baetis</i>	226	49	535
Insecta	Ephemeroptera	Caenidae	<i>Caenis</i>	142	12	12
Insecta	Ephemeroptera	Ephemeridae	<i>Hexagenia</i>	98	0	39
Insecta	Ephemeroptera	Heptageniidae	<i>Heptagenia</i>	116	0	161
Insecta	Ephemeroptera	Leptophlebiidae	<i>Paraleptophlebia</i>	26	0	49
Insecta	Hemiptera	–	–	0	23	12
Insecta	Hemiptera	Belostomatidae	<i>Belostoma</i>	2	0	0
Insecta	Hemiptera	Corixidae	–	12	12	0
Insecta	Hemiptera	Corixidae	<i>Palmarcorixa</i>	35	0	0
Insecta	Hemiptera	Notonectidae	<i>Notonecta</i>	12	0	0
Insecta	Odonata	Calopterygidae	<i>Calopteryx</i>	12	12	0
Insecta	Odonata	Calopterygidae	<i>Hetaerina</i>	21	0	53
Insecta	Odonata	Coenagrionidae	<i>Argia</i>	2	0	0
Insecta	Odonata	Coenagrionidae	<i>Coenagrion/Enallagma</i>	29	18	0
Insecta	Trichoptera	–	–	0	0	12
Insecta	Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i>	0	0	82
Insecta	Trichoptera	Hydroptilidae	<i>Hydroptila</i>	0	0	24
Insecta	Trichoptera	Limnephilidae	<i>Homophylax</i>	0	12	0
Insecta	Trichoptera	Limnephilidae	<i>Limnephilidae</i>	12	0	0
Insecta	Trichoptera	Phryganeidae	<i>Ptilostomis</i>	64	0	0
Insecta	Trichoptera	Polycentropodidae	<i>Polycentropus</i>	12	0	0
Molluska	Bivalvia	Sphaeriidae	–	0	5	58
Molluska	Gastropoda	Lymnaeidae	–	140	12	0
Molluska	Gastropoda	Physidae	–	12	35	58
Molluska	Gastropoda	Planorbidae	–	12	0	0
Nematoda	–	–	–	15	51	18

Appendix C



Figure C-1. (a) Photos upstream, (b) left bank, (c) downstream and (d) right bank at Agate Springs Ranch.

Appendix D

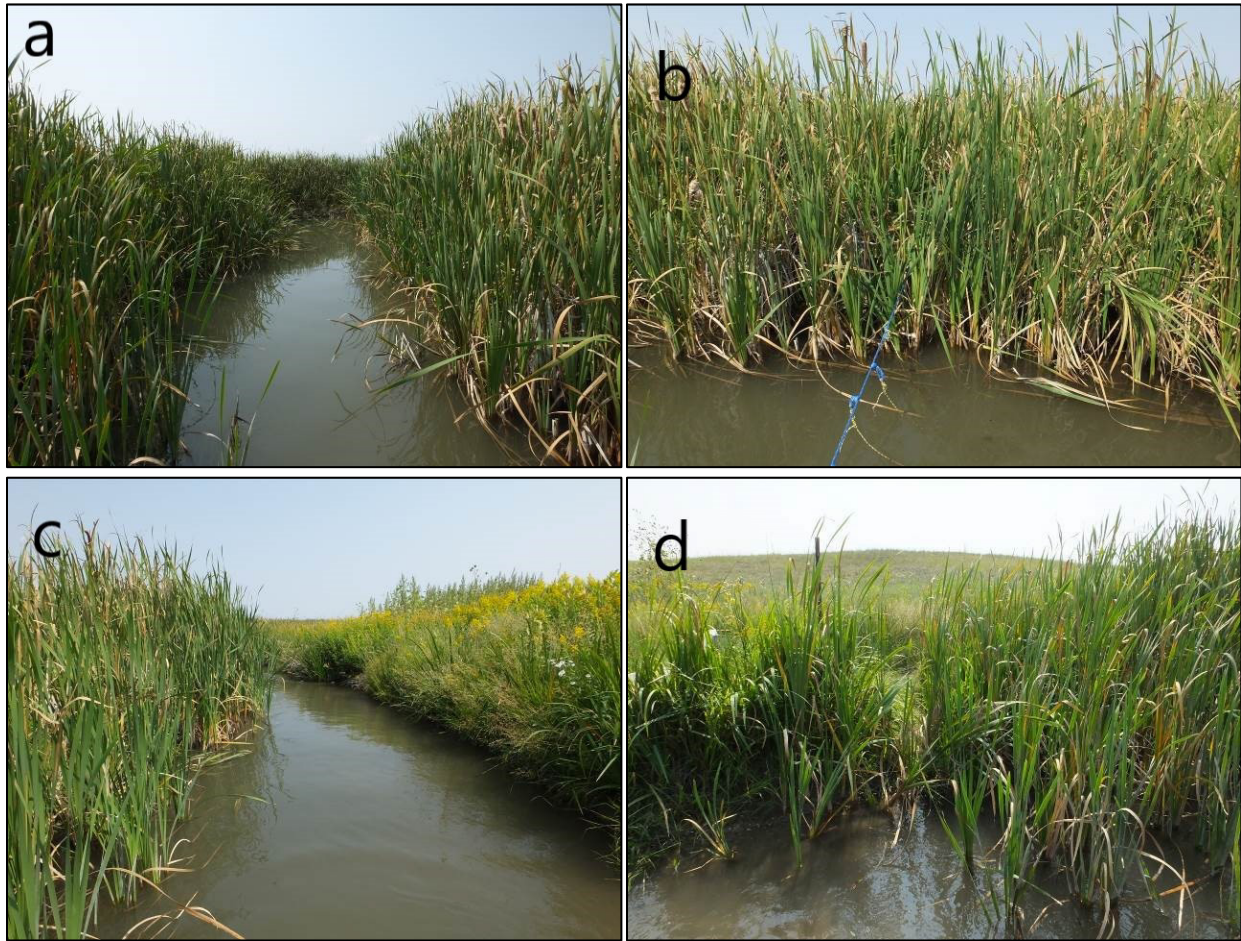


Figure D-1. (a) Photos upstream, (b) left bank, (c) downstream and (d) right bank at Agate Middle.

Appendix E

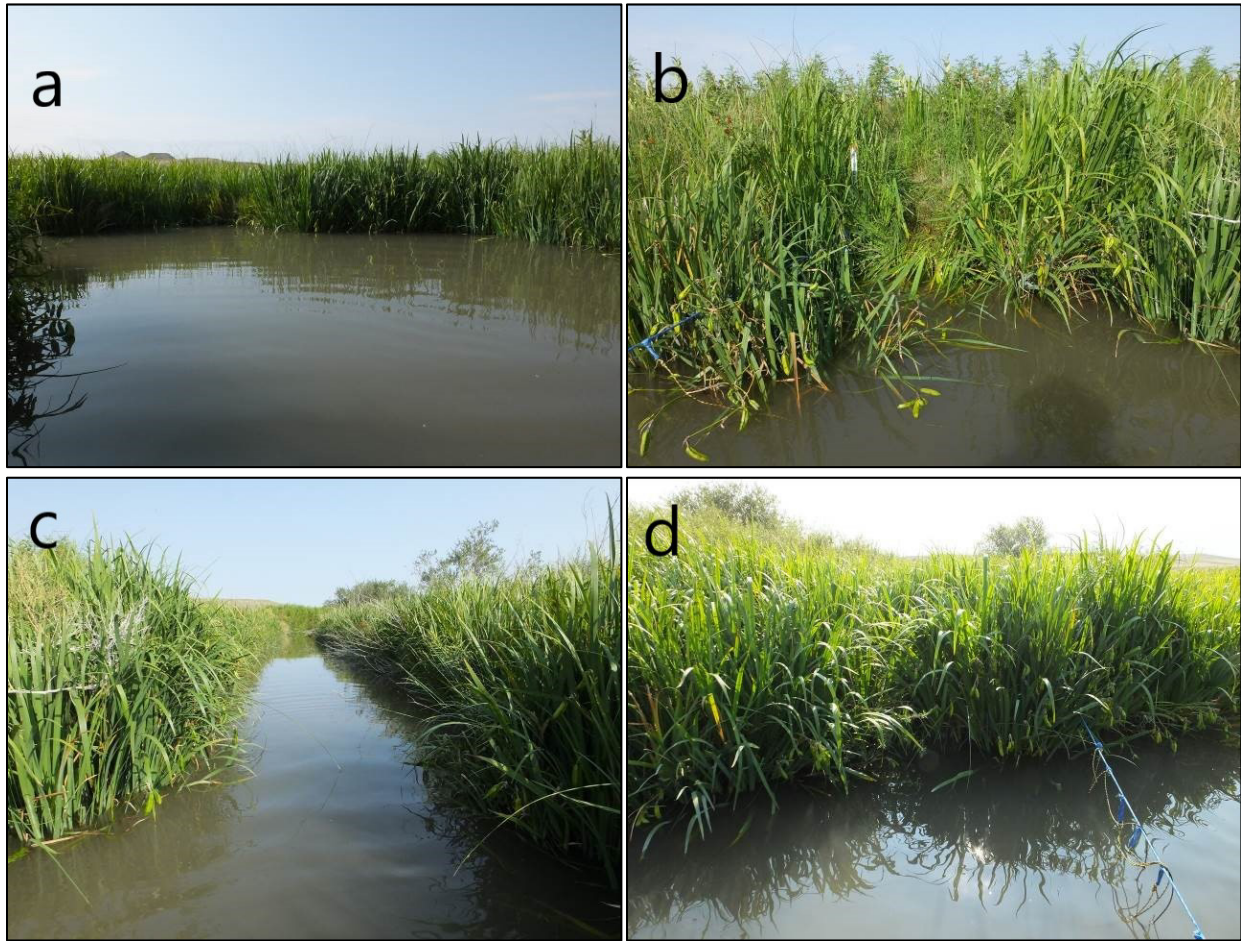


Figure E-1. (a) Photos upstream, (b) left bank, (c) downstream and (d) right bank at Agate East.

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.

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1201 Oakridge Drive, Suite 150
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