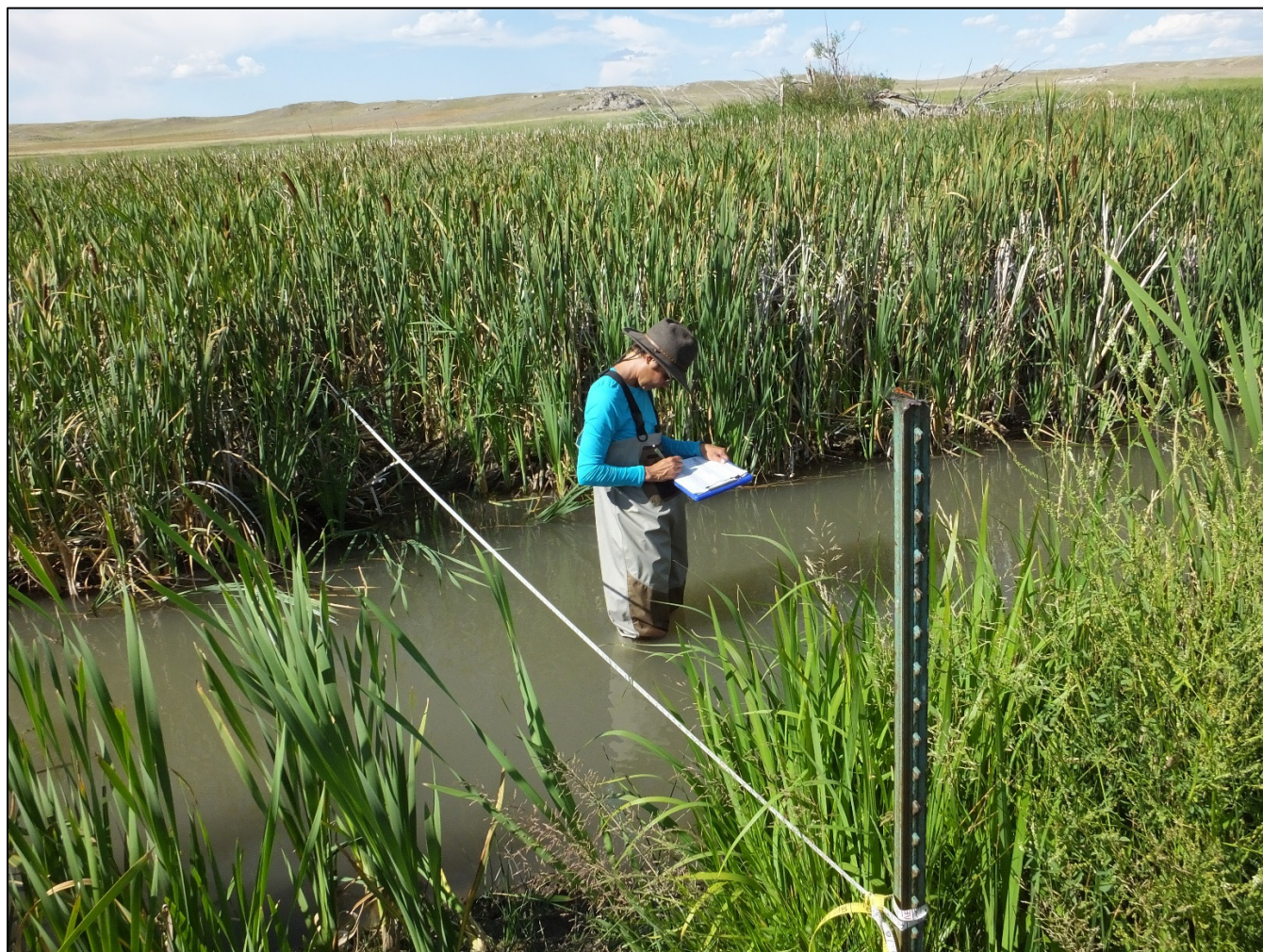




Aquatic Invertebrate Monitoring at Agate Fossil Beds National Monument

2020 Data Report

Natural Resource Data Series NPS/AGFO/NRDS—2023/1385



**ON THIS PAGE**

Bryan Tronstad collecting a Hess sample at the Agate East site along the Niobrara River at Agate Fossil Beds National Monument.

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

ON THE COVER

Lusha Tronstad recording stream characteristics at the Agate Middle site along the Niobrara River at Agate Fossil Beds National Monument.

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

Aquatic Invertebrate Monitoring at Agate Fossil Beds National Monument

2020 Data Report

Natural Resource Data Series NPS/AGFO/NRDS—2023/1385

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U.S. Department of the Interior
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Fort Collins, Colorado

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Executive Summary

Monitoring ecosystems is critical to detect trends so that managers can address perturbations. 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 and 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 perturbations differ. Aquatic invertebrates were monitored at three sites along the Niobrara River at Agate Fossil Beds National Monument in 2020 completing 24 years of data.

We identified 48 invertebrate taxa from four phyla (Annelida, Arthropoda, Mollusca, Nematoda) using Hess samplers in the Niobrara River (Appendix A). Insects (65%), Crustacea (28%), Annelida (5%) and Mollusca (2%) were the most numerous invertebrate groups in decreasing order of abundance. Of the insects, Diptera (46%) were the most abundant followed by Ephemeroptera (34%), Trichoptera (14.5%), Hemiptera (3%), Coleoptera (2%) and Odonata (<1%). Hess samples collected from the Agate East site had more invertebrates compared to Agate Middle and Agate Springs Ranch sites. Taxa richness, taxa diversity, EPT richness and the proportion of EPT were highest at the Agate East site indicating higher ecosystem quality. Hilsenhoff's Biotic Integrity was lowest at Agate Springs Ranch, which is the average tolerance value of the invertebrate assemblage. Agate Springs Ranch and Agate East had good water quality, and Agate Middle had fair water quality according to Hilsenhoff (1987).

Acknowledgments

We thank Tresize Tronstad, Joy Handley, Cymbaline Crozier and Chloe Cowen for field and laboratory assistance. We are grateful to Kara Paintner-Green, AJ Legault and Dan Morford of the National Park Service who helped with logistics and fieldwork, and gave us the opportunity to work at Agate Fossil Beds National Monument.

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 several 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 over time. 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 webs, 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).

Which invertebrate sampler to monitor ecosystem quality with 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 required also 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 a Hess sampler in 2020 to continue monitoring the river. Our specific questions were 1.) Did abiotic conditions vary among sites, 2.) To what degree did the aquatic invertebrate assemblage vary among sites and 3.) Do bioassessment metrics differ among sites? We collected five Hess samples at each of three sites to answer these questions. Data can be used to address management decisions in the park, to understand how the river changed over time and to transition to monitoring with Hess samplers.

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 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).

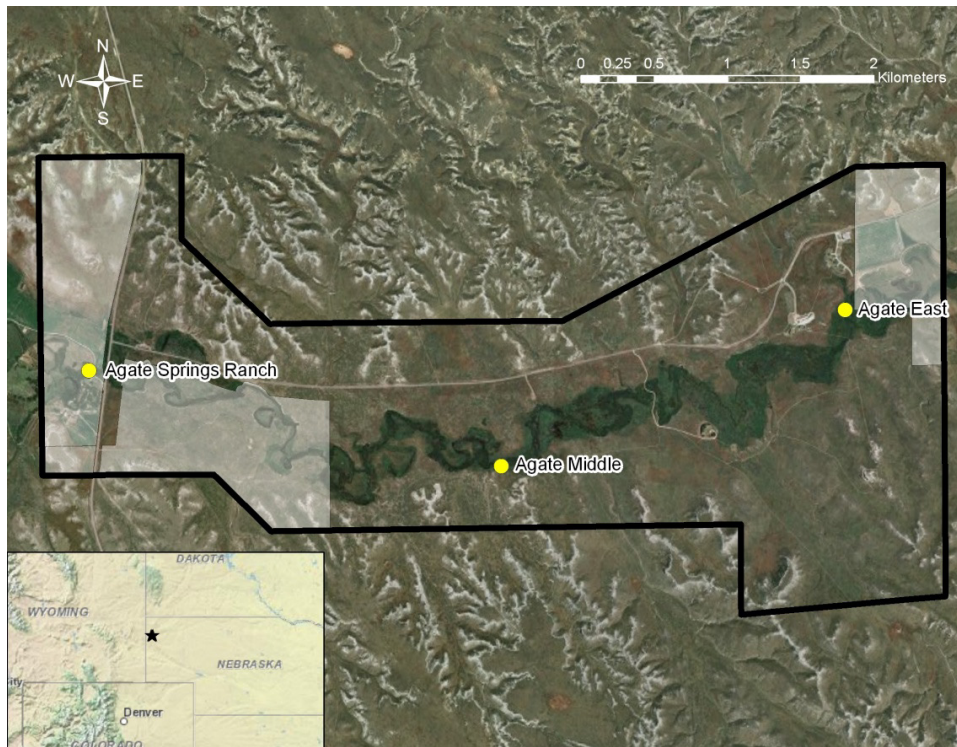


Figure 1. We sampled three sites along the Niobrara River at Agate Fossil Beds National Monuments. The black line is the Monument boundary and the transparent white areas are private land within the Monument boundary. The inset map shows the location of Agate Fossil Beds National Monument in Nebraska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

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

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). The central site, Agate Middle, lacks an overstory and has gravel substrate (Figure 2a). Both iris and cattails are abundant here. Finally, Agate East is located before the Niobrara River flows out of the park (Figure 2b). The riparian vegetation is dominated by iris with a few willows (*Salix* spp.). The river was treated in 2018 to reduce the iris growing there and riparian vegetation was recolonizing post-treatment.

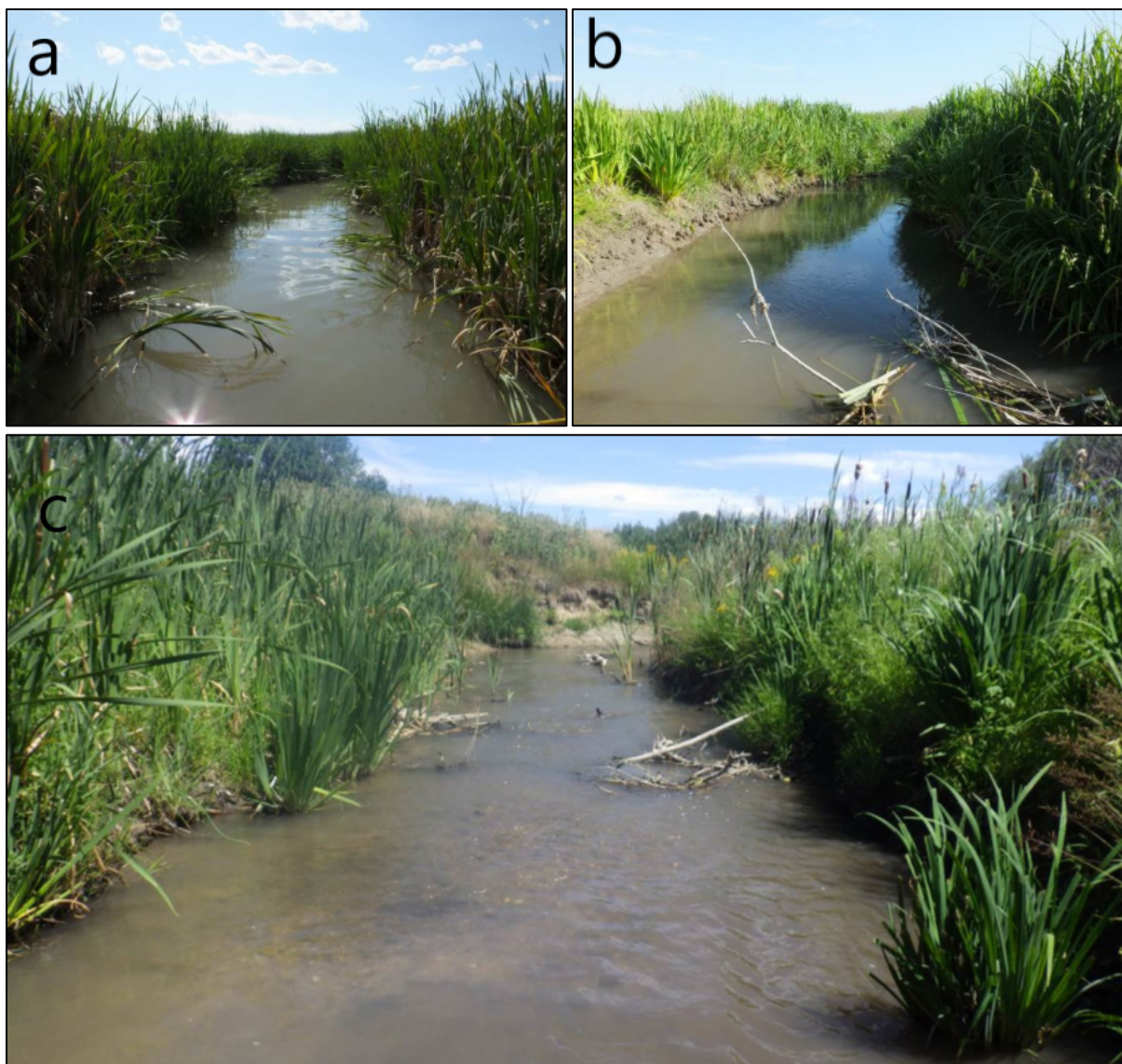


Figure 2. Photos of (a) Agate Middle, (b) Agate East and (c) Agate Springs Ranch where Hess samples are collected.

Methods

General Measurements

We measured several variables to assess the general habitat characteristics of the Niobrara River including general water quality, water clarity, sediment composition, depth and discharge. We measured dissolved oxygen concentration, pH, water temperature, specific conductivity and oxidation-reduction (ORP) potential using a Professional Plus made by Yellow Springs Instruments. The instrument was calibrated on-site before use. We measured water clarity by estimating the depth at which a Secchi disk disappeared. 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 excess 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 was firm, consolidated fine substrate. We recorded the location of each site using a global positioning system (GPS; Garmin Oregon 600, ± 5 m accuracy). 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 1 foot intervals across the stream's width (w ; ft) and summing each interval to calculate discharge using the following equation:

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

Hess Samples

We collected five Hess samples (500 μ m 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 (*Schoenoplectus tabernaemontani*) 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 a 500 μ m 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. (Merritt et al. 2019) 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 m²).

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; Hilsenhoff 1987) for each replicate sample (Bowles et al. 2013). We used generalized linear models (glm) to compare invertebrate assemblage structure (e.g., density, proportion of a taxon) and bioassessment metrics among sites. Generalized linear models can use other distributions to analyze data which is ideal because invertebrate data are usually not normally distributed. We viewed histograms of each response variable and used the package *fitdistrplus* (Delignette-Muller and Dutang 2015) to find a distribution that best matched our data, and we used the gamma distribution to analyze most response variables. We used estimated marginal means (emmeans) to calculate differences among sites (Lenth 2021). 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. Dissolved oxygen measured continuously at Agate East peaked at 8.63 mg/L during the day and the minimum values reached 7.57 mg/L at night (Figure 3a, b). Water temperature varied between 18.5 and 26.5°C during a day (Figure 3c, d). Specific conductivity and pH varied little among sites (Table 2). Reducing conditions dominated all sites (ORP; < 200 mV). The stream channel at Agate East was deepest and Agate Middle was narrowest. Discharge was highest at the Agate East. Water clarity varied little among sites (Secchi disk depths). Channel substrate was dominated by fines (sand, silt and clay) and gravel.

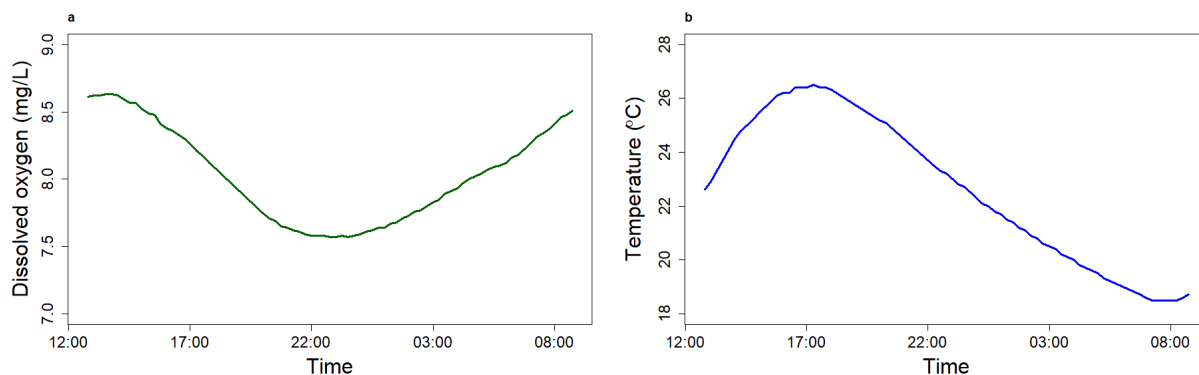


Figure 3. Dissolved oxygen (a) and water temperature (b) on 31 July to 1 August 2020..

We identified 48 invertebrate taxa from four phyla (Annelida, Arthropoda, Mollusca, Nematoda; Appendix A). Insects (65%), Crustacea (28%), Annelida (5%) and Mollusca (2%) were the most numerous invertebrate groups in decreasing order of abundance (Figure 4). Of the insects, Diptera (46%) were the most abundant followed by Ephemeroptera (34%), Trichoptera (14.5%), Hemiptera (3%), Coleoptera (2%) and Odonata (<1%; Figure 5). Hess samples from Agate East (576 ± 73 ind/sample $\pm$ standard error; 6699 ± 853 ind/m²) had more invertebrates compared to Agate Middle (178 ± 22 ind/sample; 2073 ± 256 ind/m²) and Agate Springs Ranch (196 ± 29 ind/sample; 2282 ± 340 ind/m²; $t = 2.5\text{--}2.8$, $p < 0.03$; emmeans, $p = 0.04\text{--}0.06$).

Table 2. Water quality and site characteristics at three sites along the Niobrara River. Channel width is the width of the stream channel free of emergent vegetation. Substrate is within the stream channel.

Parameter	Units	Ranch	Middle	East
Date	–	31-Jul-20	31-Jul-20	31-Jul-20
Start time	–	13:00	16:00	19:10
Water temperature	°C	21.6	22.7	25.5
Dissolved oxygen	% saturation	94.2	92.8	84.4
Dissolved oxygen	mg/L	8.26	7.98	6.9
Specific conductivity	µS/cm	380.1	384.2	382.6
pH	–	8.19	8	8.23
ORP	mV	3.7	–25.1	–27.1
Secchi disk depth	cm	36	37	35
Max depth	ft	1.3	1.75	2.1
Channel width	ft	10.3	7.8	11
Discharge	ft ³ /s	4.8	6.2	6.4
Dominant substrate	–	Sand	Gravel	Silt
Air temperatures	°C	28.9	31.1	23.9

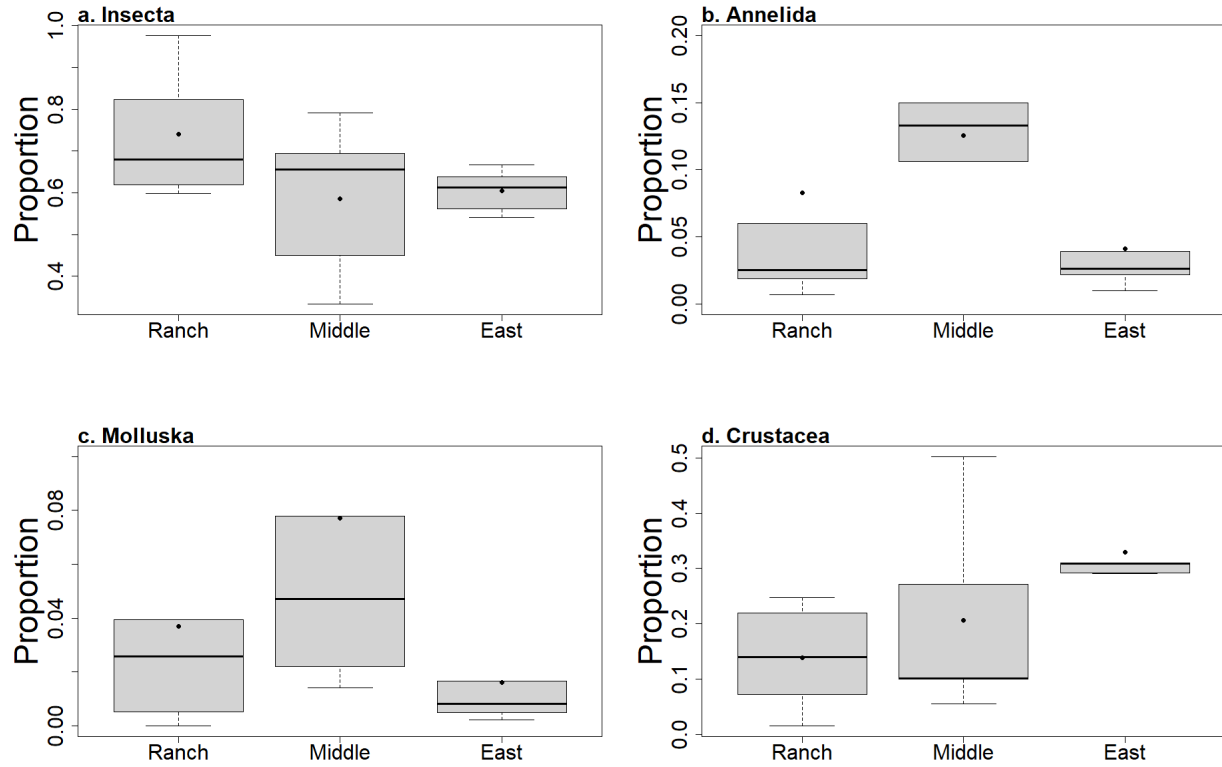


Figure 4. The proportion of (a) Insecta, (b) Annelida (c) Mollusca and (d) Crustacea collected among sites. Black circles represent mean values, bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles and whiskers indicate the lower and upper limits of the data excluding outliers. Note differing y-axis values.

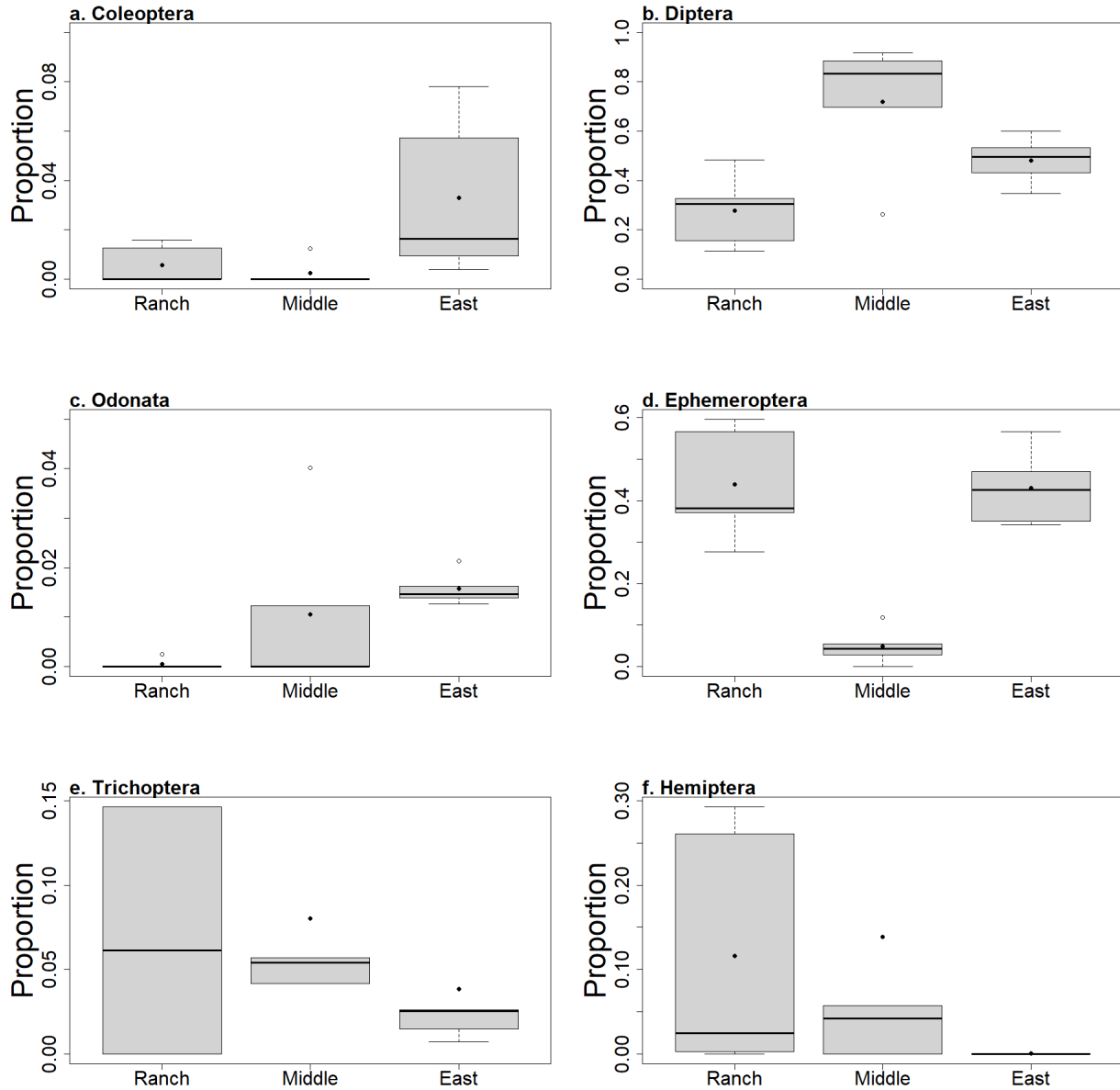


Figure 5. The proportion of (a) Coleoptera, (b) Diptera, (c) Odonata, (d) Ephemeroptera, (e) Coleoptera, Trichoptera and (f) Hemiptera collected. Black circles represent mean values, bold lines are median values, lower and upper limits of the box are the 25th and 75th percentiles and whiskers indicate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

Invertebrate bioassessment metrics often varied among sites. Taxa richness was highest at Agate East and lower at Agate Springs Ranch and Agate Middle ($t = 3.3\text{--}3.6$, $p < 0.004$; emmeans, $p < 0.03$; Table 3; Figure 6a). We calculated higher diversity at Agate East compared to Agate Middle ($t = 2.3\text{--}3.3$, $p < 0.05$; emmeans, $p = 0.02$; Figure 6b). Taxa evenness did not vary among sites ($t = 0.4\text{--}0.6$, $p = 0.60\text{--}0.68$; emmeans, $p = 0.85\text{--}0.99$; Figure 6c). Agate East had the highest richness of EPT taxa ($t = 2.4\text{--}4.3$, $p < 0.03$; emmeans, $p = 0.003\text{--}0.07$) and Agate Middle had the fewest EPT taxa (emmeans, $p = 0.07\text{--}0.20$; Figure 6d). Agate Middle had the lowest proportion of EPT taxa ($t = 0.5\text{--}$

1.8, $p = 0.10$ – 0.63 ; emmeans, $p = 0.10$ – 0.23 ; Figure 6e). The invertebrate assemblage at Agate Springs Ranch had the lowest mean tolerance value (HBI; $t = 2.5$ – 4.3 , $p < 0.03$; emmeans, $p < 0.06$; Figure 6f). According to Hilsenhoff (1987), Agate Springs Ranch and Agate East had good water quality and Agate Middle had fair water quality.

Table 3. Mean invertebrate bioassessment metrics and standard errors at each site along the Niobrara River collected Hess samplers in 2020.

Sample Type	Metric	Ranch	Middle	East
Hess samples	Taxa richness	12.0±1.10	11.2±1.28	19.6±2.38
	Taxa diversity	2.15±0.06	2.02±0.14	2.49±0.07
	Taxa evenness	0.88±0.05	0.84±0.04	0.85±0.05
	EPT richness	5.4±0.87	3.4±0.81	10.0±1.05
	Proportion EPT taxa	0.46±0.08	0.30±0.07	0.52±0.03
	HBI	4.4±0.30	6.0±0.33	5.3±0.10

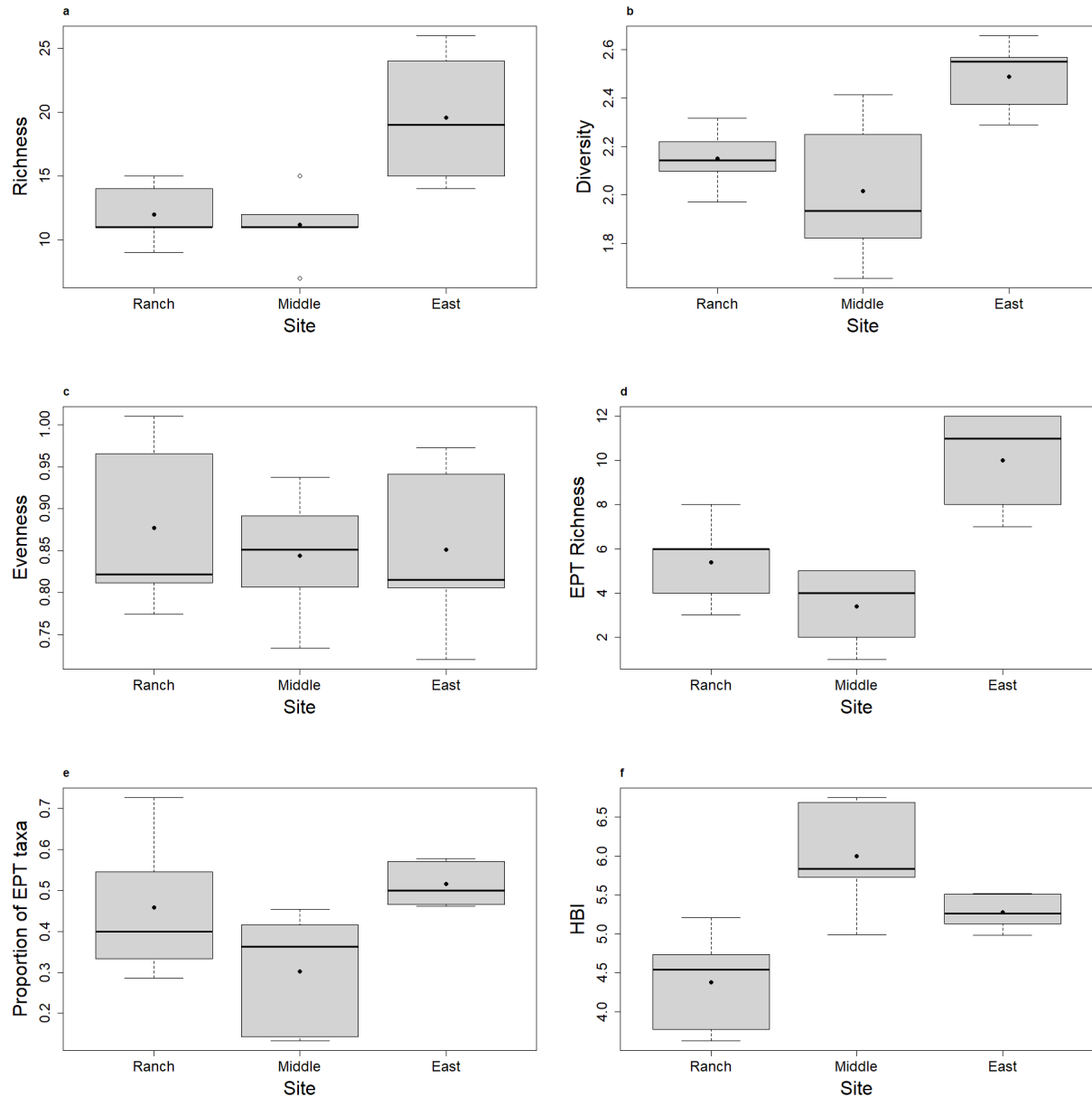


Figure 6. (a) Taxa richness, (b) taxa diversity and (c) taxa evenness calculated from Hess and Hester-Dendy samples collected from the Niobrara River in 2019. (d) Ephemeroptera, Plecoptera and Trichoptera (EPT) richness, (e) proportion of EPT taxa and, (f) Hilsenhoff's biotic index calculated from Hess samples collected from the Niobrara River in 2020. 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.

Further Analysis

This 2020 data report is intended to provide a basic review of the data collected during the invertebrate monitoring at Agate Fossil Beds National Monument. 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 Agate Fossil Beds, 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 density (ind/m²) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2020 using a Hess sampler.

Taxa Level 1	Taxa level 2	Family	Genus	East	Middle	Ranch
Annelida	–	–	–	241	184	185
Nematoda	–	–	–	86	–	12
Crustacea	Amphipoda	Gammaridae	<i>Gammarus</i>	2272	298	24
Crustacea	Anomopoda	Daphniidae	<i>Daphnia</i>	58	–	–
Crustacea	Copepoda	Harpacticoida	–	113	70	–
Crustacea	Decapoda	Cambaridae	<i>Orconectes</i>	56	79	182
Molluska	Basommatophora	Ancylidae	–	70	51	7
Molluska	Basommatophora	Physidae	–	–	47	23
Molluska	Bivalvia	Sphaeriidae	–	–	70	51
Collembola	–	–	–	–	47	–
Insecta	Coleoptera	Dytiscidae	<i>Laccobius</i>	12	–	12
Insecta	Coleoptera	Dytiscidae	<i>Nebrioporus</i>	18	–	–
Insecta	Coleoptera	Elmidae	<i>Dubiraphia</i>	15	–	12
Insecta	Coleoptera	Elmidae	<i>Stenelmis</i>	12	–	–
Insecta	Coleoptera	Gyrinidae	<i>Gyrinus</i>	87	–	–
Insecta	Coleoptera	Halipilidae	<i>Peltodytes</i>	12	–	–
Insecta	Coleoptera	Heteroceridae	–	–	12	–
Insecta	Coleoptera	Hydrophilidae	<i>Tropisternus</i>	18	–	–
Insecta	Coleoptera	Melyridae	–	–	12	–
Insecta	Diptera	Ceratopogonidae	<i>Culicoides</i>	160	–	12
Insecta	Diptera	Chironomidae	–	24	23	–
Insecta	Diptera	Empididae	<i>Hemerodromia</i>	12	23	–
Insecta	Diptera	Ephydriidae	<i>Notiphila</i>	–	–	12
Insecta	Diptera	Limoniidae	<i>Rhypholophus</i>	29	–	–
Insecta	Diptera	Simuliidae	<i>Simulium</i>	47	216	86
Insecta	Diptera	Stratiomyiidae	<i>Caloparyphus</i>	–	–	12
Insecta	Diptera	Tabanidae	<i>Chrysops</i>	12	–	23
Insecta	Diptera	Tabanidae	<i>Haematopota</i>	12	–	–

Table A-1 (continued). Mean density (ind/m²) of invertebrates collected from three sites along the Niobrara River at Agate Fossil Beds National Monument in 2020 using a Hess sampler.

Taxa Level 1	Taxa level 2	Family	Genus	East	Middle	Ranch
Insecta	Diptera	Tabanidae	<i>Silvius</i>	–	–	12
Insecta	Diptera	Tipulidae	<i>Tipula</i>	70	–	–
Insecta	Ephemeroptera	Baetidae	<i>Baetis</i>	177	58	300
Insecta	Ephemeroptera	Caenidae	<i>Caenis</i>	1042	16	81
Insecta	Ephemeroptera	Heptageniidae	<i>Heptagenia</i>	372	–	400
Insecta	Ephemeroptera	Leptophlebiidae	<i>Paraleptophlebia</i>	66	–	–
Insecta	Hemiptera	Belostomatidae	<i>Belostoma</i>	12	–	–
Insecta	Hemiptera	Corixidae	<i>Palmacorixa</i>	0	2	0
Insecta	Hemiptera	Gerridae	<i>Trepobates</i>	–	12	–
Insecta	Hemiptera	Notonectidae	<i>Notonecta</i>	–	12	–
Insecta	Hemiptera	Veliidae	<i>Rhagovelia</i>	–	–	12
Insecta	Odonata	Calopterygidae	<i>Hataerina</i>	23	–	–
Insecta	Odonata	Coenagrionidae	–	0	2	0
Insecta	Odonata	Coenagrionidae	<i>Argia</i>	0	2	0
Insecta	Odonata	Corduliidae	<i>Somatochlora</i>	16	–	–
Insecta	Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i>	154	144	654
Insecta	Trichoptera	Leptoceridae	<i>Nectopsyche</i>	–	–	12
Insecta	Trichoptera	Limnephilidae	<i>Homophylax</i>	12	–	–
Insecta	Trichoptera	Polycentropodidae	<i>Cernotina</i>	84	–	–
Insecta	Trichoptera	Polycentropodidae	<i>Polycentropus</i>	12	12	–

Appendix B



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

Appendix C

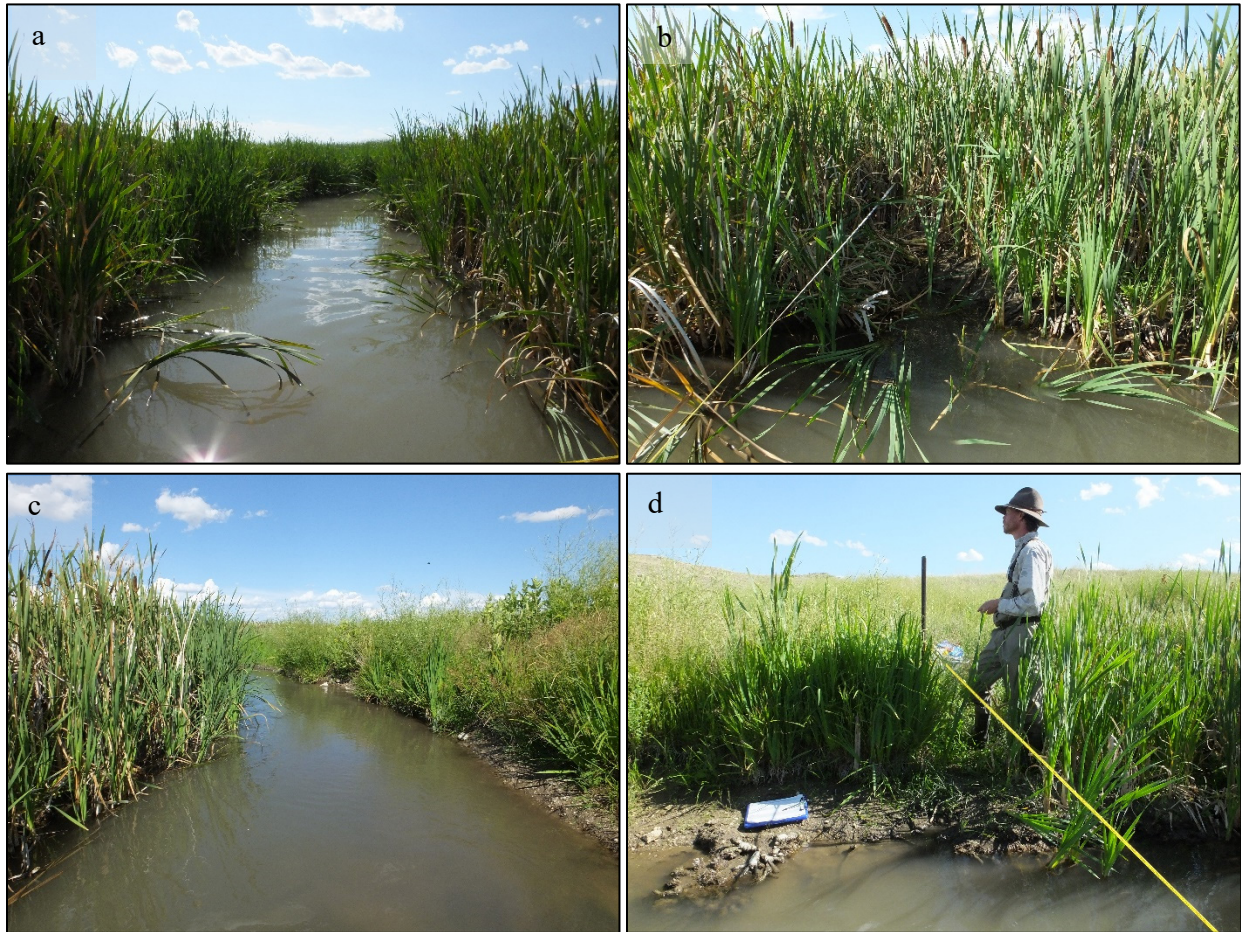


Figure C-1. Photos of (a) downstream, (b) left bank, (c) upstream and (d) right bank at Agate Middle.

Appendix D



Figure D-1. Photos of (a) downstream, (b) left bank, (c) upstream 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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National Park Service
U.S. Department of the Interior



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