

Final Report

Is the Muddy Creek food web affected by coalbed natural gas inputs?

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Abstract

The Muddy Creek watershed is a unique ecosystem because the stream contains a distinctive fish assemblage, is physically degraded, and will soon be influenced by oil and gas development. Separating the effects of multiple stressors can be challenging; however, having prior data can be very useful in determining the influence of different stressors. In our study, we attempted to separate the effects of physical degradation, and oil and gas development by sampling prior to most energy development. To extract oil and gas, groundwater must be pumped to the surface; groundwater associated with oil and gas resources can contain detectable concentrations of trace elements. The produced groundwater is often discharged into streams or ponds. Trace elements in produced water can be taken up into food resources (e.g., algae) and transferred to higher trophic levels in the food web through predation. We collected animals from each trophic level in the Muddy Creek food web above and below the current coalbed natural gas (CBNG) input, and measured their tissue for trace element concentrations and $\delta^{15}\text{N}$ (trophic position). As a result, we regressed trophic position ($\delta^{15}\text{N}$) against trace element concentration for each organism (e.g., $\mu\text{g Se/g tissue}$) to measure how trace elements moved through the food web. Al and Zn concentrations in Muddy Creek exceeded chronic standards for aquatic life. We found that only Se and Hg bioaccumulated in the Muddy Creek food web, but other trace elements bioaccumulated, peaked at intermediate trophic levels, or concentrations remained similar in the food web (Be, B, Mg, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Sr, Mo, Cd, Ba, and Pb). By understanding the Muddy Creek ecosystem now, land managers and developers can make informed decisions about management needs and potential mitigation efforts. This study will record baseline (pre-CBNG) influences on the Muddy Creek food web. We plan to repeat this study in 3-5 years after CBNG development has occurred to determine if trace metals are accumulating differently in the food web.

Objectives

1. *Are trace elements detectable in water and biota before most energy development in Muddy Creek?* We collected and analyzed water, aquatic food sources, invertebrates, birds, frogs, and fish for trace element concentrations (Be, B, Mg, Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Hg, and Pb) at 2 sites along Muddy Creek (above and below the current coalbed natural gas well).
2. *What does the food web at Muddy Creek look like?* We collected aquatic food sources, aquatic invertebrates, spiders, birds, frogs, and fish at 2 sites along Muddy Creek and analyzed these samples for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$. $\delta^{15}\text{N}$ describes trophic position of each group, because ^{15}N accumulates in biota at higher trophic levels. $\delta^{13}\text{C}$ describes carbon sources.
3. *Do trace elements bioaccumulate in the Muddy Creek food web?* To estimate how each trace element behaves in the food web, we regressed trace element concentration (e.g., $\mu\text{g Se/g tissue}$) against $\delta^{15}\text{N}$ (trophic position).

Study Sites

Muddy Creek is located south of Rawlins, Wyoming in the Colorado River Basin. Muddy Creek originates by the continental divide near the Atlantic Rim and Bridger Pass at ~2500 m elevation (Figure 1). The stream flows across high desert prairie dominated by sagebrush (*Artemisia* sp.) and greasewood (*Sarcobatus* sp.). Several tributaries empty into Muddy Creek within the watershed, including Wild Cow Creek and Dry Cow Creek. Finally, Muddy Creek joins the Little Snake River by the Colorado border near Baggs, Wyoming.

We collected samples at 2 sites along Muddy Creek. The upper site was used as a reference site, because the site was located above the current input of CBNG. The lower site was below the current CBNG input; however, produced water was not being discharged during our study. The upper and lower sites shared several characteristics, such as stream width (1-2 m wide after runoff), fine sediments, dominant vegetation, and stream morphology. One difference between sites was that the upper site had more riparian vegetations (*Salix* and *Carex*) compared to the lower site (*Salix*, *Juncus*, and *Equisetum*).

The Muddy Creek watershed is a unique ecosystem because the stream contains a distinctive fish assemblage, is physically degraded, and will soon be influenced by oil and gas development. First, Muddy Creek contains fish species not known to coexist elsewhere (Quist et al. 2006). Four fish within Muddy Creek (flannelmouth sucker (*Catostomus latipinnis*), bluehead sucker (*Catostomus discobolus*), roundtail chub (*Gila robusta robusta*), and Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*)) are considered sensitive species by Bureau of Land Management and the Wyoming Game and Fish Department. The populations of these fish have declined because of habitat degradation, dams, introduced competitors, introduced predators, and hybridization. The survival of these fish depends on intermittent flows in late summer and early fall (Bower et al. 2008). Second, Muddy Creek is on the EPA's impaired stream list due to physical degradation and steps have been taken to reduce erosion (Ellison et al. 2008). Finally, oil and gas development is beginning in the area. Currently, coalbed natural gas (CBNG) development is occurring and CBNG product water is being discharged into Muddy Creek. Therefore, Muddy Creek has multiple stressors. Because physical impairments and sensitive species are known in Muddy Creek, a prior investigation of the food web may provide developers and managers (e.g., BLM-Rawlins Field Office, Wyoming Department of Environmental Quality, and Wyoming Game and Fish) with more information about the watershed.

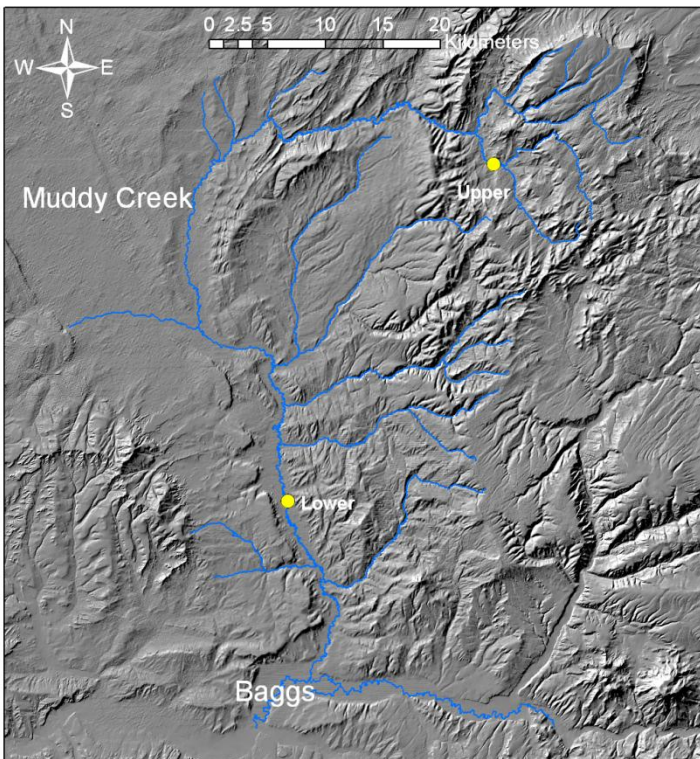


Figure 1. Map of Muddy Creek watershed located south of Rawlins, Wyoming. The locations of the upper and lower sites are shown.

Methods

To estimate how trace elements moved through the food web of Muddy Creek, we collected water, food, and biota at 2 sites along Muddy Creek, and analyzed them for trace elements (Be, B, Mg, Al, v, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, Ba, Hg, and Pb), $\delta^{15}\text{N}$ (trophic position) and $\delta^{13}\text{C}$

(carbon source). All samples were collected using trace element clean techniques with acid washed utensils and bottles. Samples were analyzed for trace element concentrations at Dartmouth Toxic Metals Superfund Research Program (inductively coupled plasma mass spectrometry), and $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ at University of Wyoming Stable Isotope Facility (Finnigan Delta Plus XP).

To measure the constituents of stream water, we collected water samples at each site between May and July 2010. Water was collected in 125 mL Nalgene HDPE bottles that were acid washed in trace element grade nitric acid. Water samples were filtered (0.45 μm) and preserved by adding trace element grade nitric acid. Additionally, we measured basic water quality parameters at both sites using a YSI Professional Plus Multiprobe.

We measured basal food resources (i.e., algae, sediment, and suspended organic matter (SOM)) for trace element concentrations to estimate how trace element moved through the food web. Stream algae was collected from tiles placed in Muddy Creek for at least 3 weeks and ambient rocks. Stream sediments were collected using a plastic corer. Finally, we collected suspended organic matter (eaten by filter feeding invertebrates) by filtering stream water through filters for analysis.

To estimate how trace elements moved through animals, we collected invertebrates, amphibians, and birds. We collected aquatic invertebrates using a dip net, and sorted invertebrates according to functional feeding groups (Merritt et al. 2008). Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies; EPT) are orders of insects that tend to be sensitive to water quality. Spiders were hand collected by searching after dark with flashlights and identified using Kaston (1953). Boreal Chorus Frogs (*Pseudacris maculate*) were collected using dip nets after locating frogs via their calls at dawn and dusk. Frogs were euthanized by immersing them in a 4g/L solution of tricaine methane sulfonate (MS-222) for 10 minutes. Although we planned to sample Boreal Chorus Frog tadpoles, none were observed at our study sites. We measured whole invertebrates and frogs (no gut clearance), because these measurements represent what a predator consumes (Farag et al. 1998).

We collected samples from riparian nesting birds to measure the concentration of trace elements in terrestrial animals adjacent to the stream. Adult birds were captured using mist nets (Bub 1995; Braun 2005; Fair et al. 2010). Mist nets were checked every 15 minutes to minimize time in nets and predation risk. We collected blood samples from adult birds by puncturing the brachial vein with a 26 gauge needle and collecting blood directly into microhematocrit capillary tubes (Fair et al. 2010). The volume of blood collected was restricted to less than 1% of the birds body mass (McGuill and Rowan 1989; Fair et al. 2010). We also collected feather samples from nestlings because feathers of chicks have been shown to reflect local contaminant concentrations (Becker et al. 1994). All sampling procedures for vertebrate species were approved by the University of Wyoming Animal Care and Use Committee.

Fish samples were provided by the United States Fish and Wildlife Service (USFWS), which is conducting a concurrent multi-year study monitoring water quality throughout the Muddy Creek drainage. We worked with the USFWS to obtain whole fish samples from white suckers and creek chubs (non-native fish with very similar feeding habits to the native suckers) and muscle plugs from roundtail chub, bluehead suckers, and flannelmouth suckers. Most fish samples are currently being analyzed for trace elements; however, all fish samples have been analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

Principle findings

Basic water quality parameters were similar between the 2 sites, except the lower site had higher conductivity (Table 1). B, Mg, Cu, Sr, and Ba had higher concentrations at the lower site, but As, Se, and Mo had higher concentrations at the upper site (2 sample t-tests, $p < 0.05$; Table 2). Al and Zn exceeded the chronic water quality standards for aquatic life at the lower site and both sites, respectively (Table 2).

Table 1. Water quality parameters at both sites along Muddy Creek in late July. We measured temperature, dissolved oxygen (DO), specific conductivity (SP conductivity), conductivity, pH, and oxidation-reduction potential (ORP) using an YSI Professional Plus Multiprobe.

Parameter	Upper	Lower
Temperature (°C)	22	25
DO (% saturation)	130	123
DO (mg/L)	8.8	8.1
SP conductivity (µS/cm)	645	1198
Conductivity (µS/cm)	611	1205
pH	8.63	8.45
ORP (mV)	227.8	202.7

Table 2. We compared trace element concentrations in water from the upper and lower sites against the EPA and Wyoming chronic water quality standards for aquatic life. NS indicates that there are no standards for the element. Six water samples were collected between May and July 2010, and the standard errors (SE) were calculated. The limit column shows if the concentrations measured in Muddy Creek were above (Y) or below (N) the aquatic life standards. Water quality standards for chromium are based on the species (III vs. VI); however, we did not determine the species of chromium in Muddy Creek.

Element	EPA Chronic (µg/L)	WY Chronic (µg/L)	Upper			Lower		
			Mean (µg/L)	SE	Limit	Mean (µg/L)	SE	Limit
Be	NS	NS	0.0	0.0		0.0	0.0	
B	NS	NS	47.0	4.6		170.0	2.2	
Mg	NS	NS	21,493.1	1433.5		44,007.2	3475.7	
Al	87	87	20.3	6.8	N	136.1	66.9	Y
V	NS	NS	1.6	0.1		2.2	0.3	
Cr	11 (VI) 74 (III)	11 (VI) 210 (III)	0.4	0.1	N	0.5	0.1	N
Mn	NS	1462	22.3	1.7	N	11.5	2.2	N
Fe	1000	1000	113.8	13.6	N	161.5	77.8	N
Ni	52	160	7.7	1.7	N	7.2	0.7	N
Cu		12	3.0	0.6	N	5.3	0.9	N
Zn	120	110	127.9	27.3	Y	125.1	13.1	Y
As	150	190	2.5	0.2	N	1.3	0.1	N
Se	5	5	3.4	0.3	N	1.0	0.1	N
Sr	NS	NS	506.3	2.6		746.5	59.9	
Mo	NS	NS	12.0	0.5		9.8	0.6	
Cd	0.25	1.1	0.1	0.0	N	0.1	0.0	N
Ba	NS	NS	39.9	0.5		50.7	0.8	
Hg	0.77	0.012	0.001	0.0	N	0.001	0.0	N
Pb	2.5	3.2	1.6	0.5	N	1.4	0.2	N

The trophic arrangement differed between upper and lower Muddy Creek, however, this may have been due, in part, to different species sampled. Although we aimed to sample the same species at both the upper and lower sites, we were not always able to do this. For example, frogs were rare in this system and we were only able to sample one Boreal Chorus Frog at the upper site. Of the species sampled at the upper site, cliff swallows, roundtail chub, and boreal chorus frogs were the top predators (Figure 2). At the lower site, mountain blue birds and creek chub were the top predators. Basal food resources also varied. SOM probably contained a higher percent of animal matter compared to soil and algae at the upper site compared to the lower, because the $\delta^{15}\text{N}$ value of SOM was higher at the upper site. Also, maximum $\delta^{15}\text{N}$ values were higher at the lower site. Filterer and predatory invertebrates had the highest $\delta^{15}\text{N}$ at the upper site, and *Ephemera* and omnivores had the lower. Conversely, spiders, crayfish, and predatory insects had the higher $\delta^{15}\text{N}$ at the lower site, and filterers, scrapers, and collector-gatherers had the lowest $\delta^{15}\text{N}$ values. The $\delta^{13}\text{C}$ values for each group were similar between sites.

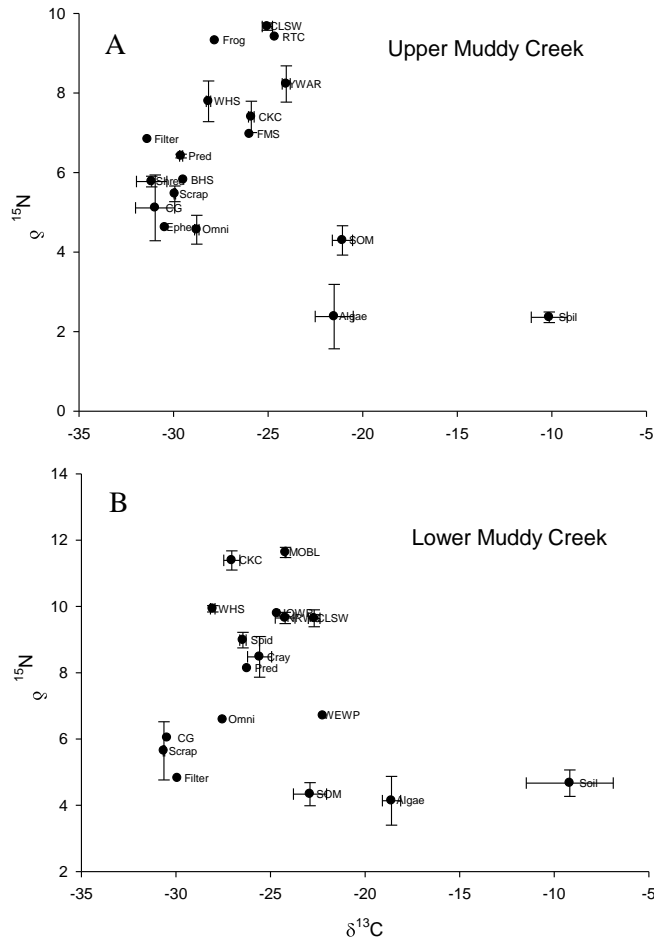


Figure 2. Plot of $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ for A. upper and B. lower Muddy Creek sites. Larger $\delta^{15}\text{N}$ values indicate a higher trophic position. $\delta^{13}\text{C}$ represents carbon sources. CLSW is cliff swallow, YWAR is yellow warbler, HOWR is house wren, MOBL is mountain blue bird, WEWP is western wood pee-wee, RTC is roundtail chub, CKC is creek chub, FMS is flannelmouth sucker, WHS is whitehead sucker, BHS is bluehead sucker, spid is spider, cray is crayfish, pred are predatory invertebrates, shred are shredding insects, filter are filtering insects, scrap are scraping insects, CG are collector-gatherer insects, Ephem is *Ephemera*, omni are omnivorous invertebrates, and SOM is suspended organic matter.

Concentrations of trace elements either biodiminished, bioaccumulated, peaked at intermediate trophic levels, or remained similar as they moved through the food web (Figure 3). Concentrations of Be, Mg, Al, V, Cr, Mn, Fe, Ni, As, Sr (upper), Mo, Ba, and Pb all biodiminished in the food web (Least Squares Regression, $p < 0.05$). Conversely, concentrations of Se (upper) and Hg (upper) bioaccumulated in the food web (Least Squares Regression, $p < 0.05$). The concentration of B, Se (lower), Sr (lower), and Hg (lower) remained similar as these elements moved through the food web (Least Squares Regression, $p > 0.05$). Finally, concentrations of Cu, Zn, and Cd were highest at intermediate trophic levels.

Aquatic food sources tended to have the highest concentrations of elements, because most elements biodiminished. Of the food sources, algae generally had the highest concentration of trace elements and SOM had the lowest concentration. One exception was that SOM had the highest B

concentration at both sites. Additionally, algae and soil often contained similar concentrations of elements at the upper site. Algae at the lower site contained the highest concentration of Be, Al, V, Fe, Ni, Ba, and Pb.

Aquatic invertebrates contained fairly high concentrations of trace elements. We collected 24 taxa of aquatic invertebrates at the upper site, but only 8 taxa at the lower site. Of these taxa 10 were EPT taxa at the upper site, and only 2 were EPT taxa at the lower site. *Ephemera*, a burrowing mayfly that is a collector-gatherer, filterer, and predator, often had high concentrations of trace elements. Insects in the functional feeding groups scraper, collector-gatherer, and omnivore often contained high concentrations of trace elements. At the upper site, snails in the family Physidae were the dominant scrapers, but the mayfly *Heptagenia* was the dominant scraper at the lower site. *Plauditus* (Baetidae), *Caenis* (Caenidae), *Sigara* (Corixidae), Elmidae, and non-Tanytopodinae chironomids were the collector-gatherers at the upper site, while *Acentrella* (Baetidae) and non-Tanytopodinae chironomids were the collector-gatherers at the lower site. Omnivores at the upper site were Amphipoda, and Amphipoda and crayfish (Decapoda) were at the lower site. Filterers contained high concentrations of Mn, Ni, Se, and Mo. *Ceratopsyche*, *Hydropsyche* (Hydropsychidae), Spheariidae, and Simuliidae were the filterers at the upper site, and *Simulium* (Simuliidae) were the only filterers we collected at the lower site. Shredders contained high concentrations of Mn, Ni, Zn, Se, Mo, and Ba. At the upper site, *Limnephilus*, *Homophylax*, *Eocosmoecus* (Limnephilidae), *Microsema* (Brachycentridae), *Lepidostoma* (Lepidostomatidae), and Tipulidae were the dominant shredders, but we did not collect shredders at the lower site. The concentrations of Mn, Zn, Se, and Cd were high in predaceous insects. *Cultus* (Perlodidae), *Ambrysus* (Naucoridae), *Anax* (Aeshnidae), *Gomphus* (Gomphidae), *Coenagrion/Enallagma* (Coenagrionidae), *Ambrysus* (Naucoridae), *Gerris* (Gerridae), *Susphisellus* (Noteridae), *Agabus* (Dytiscidae), Acari, and Hirudinidae were the predaceous insects we collected at the upper site, and *Gyrinus* and *Trepobates* (Gerridae) were the predators we collected at the lower site. Finally, spiders had high concentrations of Zn, Se, and Cd. We collected *Pachygnatha* (Tetragnathidae) and *Dictyna* (Dictynidae) at the upper site, and *Haplodrassus* (Gnaphosidae) and *Pachygnatha* (Tetragnathidae) at the lower site.

Birds and amphibians had high concentrations of some trace elements. We collected an adult boreal chorus frog at the upper site. The frog had high concentrations of Zn, Cr, Se, and Hg. Birds also had high concentrations of Se and Hg, probably because these elements bioaccumulated. Blood from Yellow warblers and cliff swallows had high concentrations of Se. Similarly, Brewer's blackbirds and cliff swallows had high concentrations of Hg. Feathers from cliff swallow hatchlings always had higher concentrations of trace elements compared to blood from adult cliff swallow, except for Fe and Se. Yellow warbler, cliff swallow (adult blood, hatchling feathers), and Brewer's blackbird had the highest concentrations of Se at the upper site in decreasing order. At the lower site, northern rough-winged swallow, cliff swallow (adult blood, hatchling feathers), western wood pee-wee, house wren, and mountain blue bird had the highest concentration of Se at the lower site in decreasing order. Brewer's blackbirds, cliff swallows (hatchling feathers), yellow warbler, and cliff swallow (adult blood) at the upper site contained the highest concentrations of Hg in decreasing order. At the lower site, cliff swallow (hatchling feathers), western wood pee-wee, northern rough-winged swallow, house wren, cliff swallow (adult blood), and mountain bluebirds had the highest Hg concentrations in decreasing order.

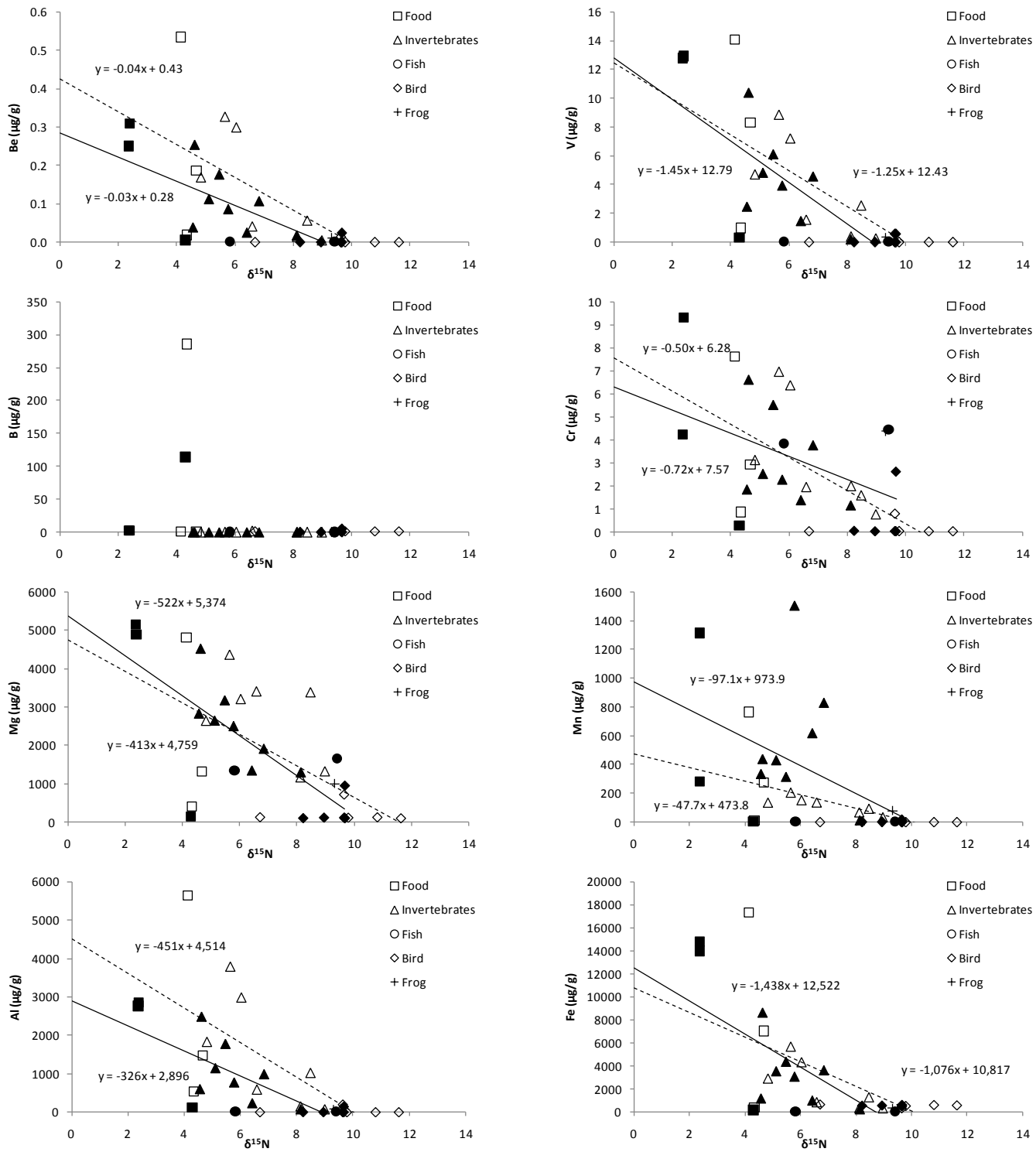


Figure 3. Elements in the Muddy Creek food web either bioaccumulated, biodiminished, peaked at intermediate trophic levels, or concentrations remained similar. Solid symbols and lines represent the upper site, and clear symbols and dotted lines represent the lower site. Aquatic food sources were algae, suspended organic matter, and sediment. Each invertebrate symbol represents a different functional feeding group or terrestrial spiders. Both adult bird blood and hatchling feathers are represented on the plots for birds. The frog was captured at the upper site.

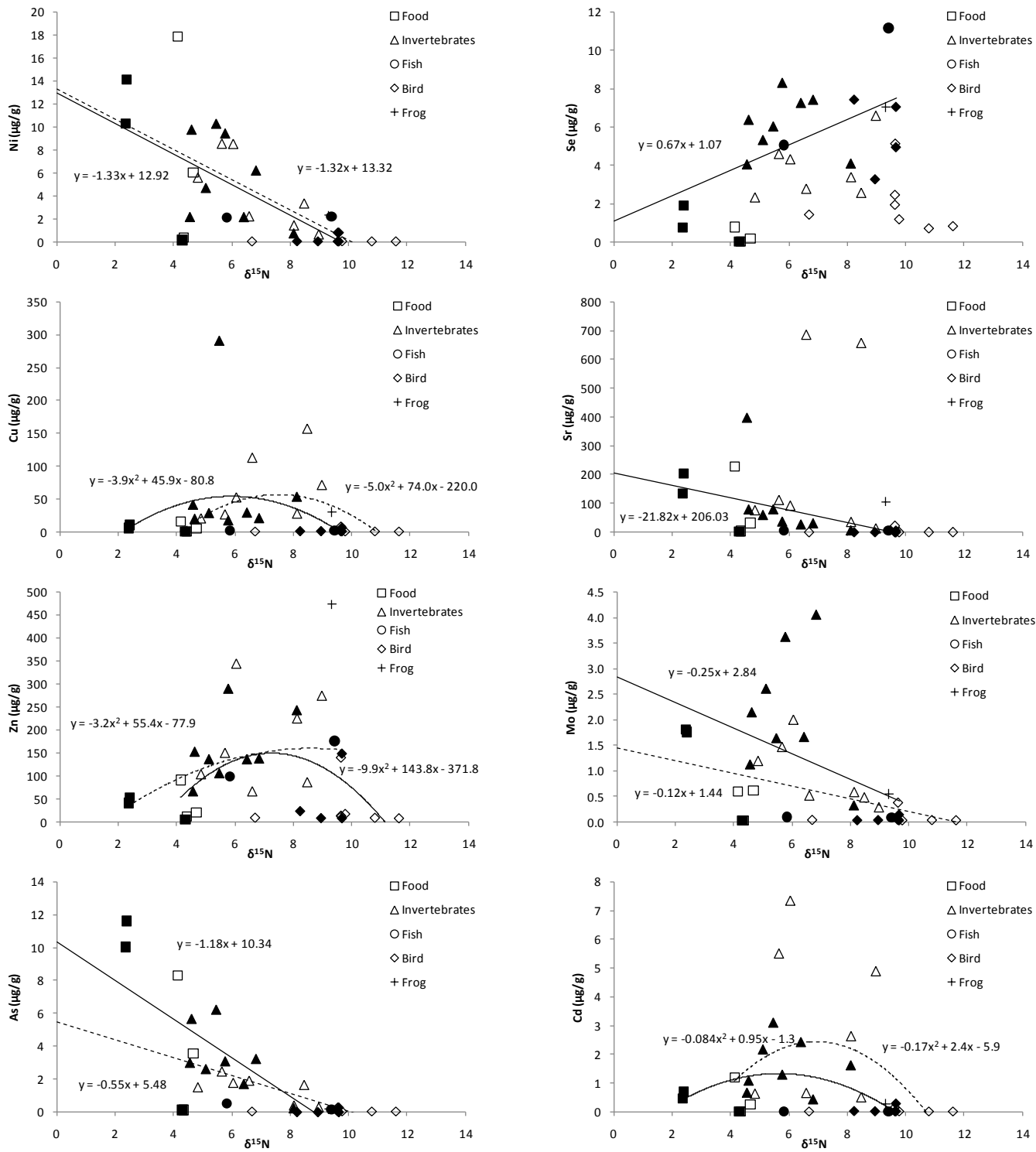


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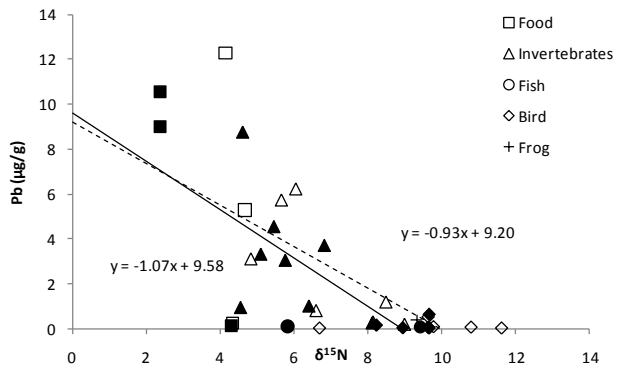
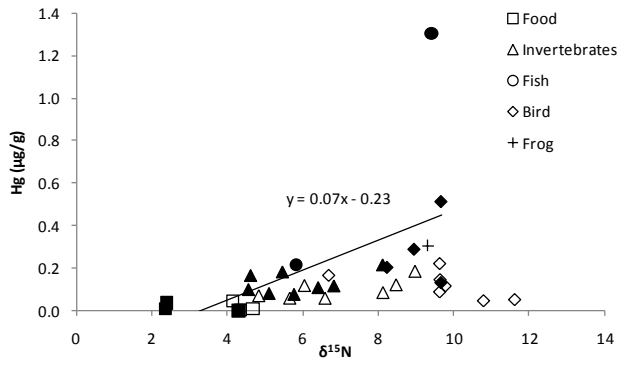
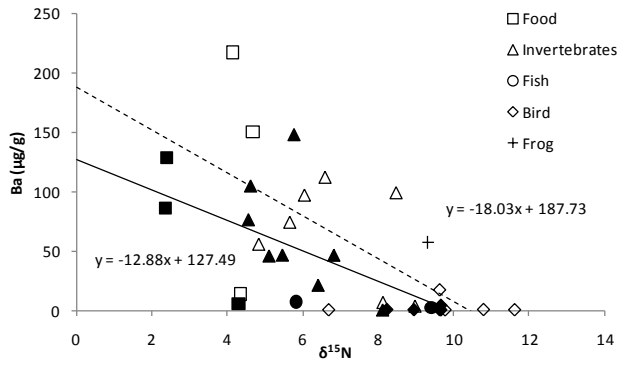


Figure 3. Continued.

Significance

Trace elements were detectable in water and biota in the Muddy Creek watershed. Trace elements in the water were taken up by algae and transferred to higher trophic levels. Algae often contained the highest concentration of trace element, because most elements bioaccumulated in the food web. Therefore, concentrations of trace elements decreased as they moved through the food web and were less likely to cause major environmental impacts. However, Hg and Se appeared to bioaccumulate at the upper Muddy Creek site. These elements have been observed to bioaccumulate in other ecosystems (e.g., Cabana and Rasmussen 1994; Ikemoto et al. 2008). According to our results, Hg and Se that originated in Muddy Creek can move to riparian animals, such as northern rough-winged swallows. Many such birds are known to feed heavily on adult insects that emerge from the stream. Trace elements can move beyond riparian habitats; Cristol et al. (2008) noted that Hg moved from a river-ecosystem to terrestrial-feeding birds and resulted in higher Hg concentrations due to bioaccumulation.

Trace elements in the environment come from both anthropogenic and natural sources. Anthropogenic activities, such as mining (e.g., Valenti et al. 2005) and manufacturing (e.g., Chen and Folt 2000) can lead to high concentrations of trace elements in the environment. Additionally, groundwater can be naturally high in certain trace elements, such as the Mekong Delta region of Vietnam, which is naturally high in arsenic, manganese, and barium (Ikemoto et al. 2008). Trace elements that we measured in the current study in the Muddy Creek watershed were probably naturally occurring; however, oil and gas development could potentially increase concentrations depending on geology. Groundwater produced in association with oil and gas development can contain trace elements and the concentration of trace elements depend on the geology of the aquifer (Rice et al. 2000). By releasing ground water into surface waters, oil and gas development can increase the concentrations of certain trace elements in rivers and streams.

The fate of trace elements in the environment is largely unknown. However, trace elements may be taken up by algae (Kiffney and Clements 1993), deposited in sediments (Lee et al. 2000), or incorporated into decaying organic matter (Sundberg et al. 2006) before being ingested by stream inhabitants. Once trace elements enter the food web, they can be transferred to higher levels through predators. The concentration of trace elements can behave differently depending on the element, the organism, and the specific conditions at the site. For example, invertebrates that eat sediment (collector-gatherers), such as burrowing mayflies, midges, or worms, can have higher trace element concentrations than invertebrates with other feeding habits (Smock 1983). Similarly, we found that collector-gatherers and *Ephemera* often contained high concentrations of trace elements. Additionally, smaller invertebrates tend to have higher concentrations (Van Hattum et al. 1991), which means juvenile fish may ingest higher concentrations of trace elements (Farag et al. 1998). We look forward to investigating how size may affect trace element concentration of fish in Muddy Creek.

Results of this project provide an understanding of baseline concentrations of trace metals in the Muddy Creek food web. These baseline data were collected before significant expansion of oil and gas development in the watershed. It is currently unknown whether water produced during oil and gas development significantly impacts local watersheds. By comparing the baseline concentrations of trace metals detected in this study to data collected in 3 to 5 years after oil and gas development has expanded in this area, we hope to assess any potential impacts energy development might have on the Muddy Creek ecosystem.

Publication and Presentation citations

We plan to present our results at for the WRP Priority and Selection Committee in July 2011. We also hope to present these results at the Wyoming-Colorado Chapter of the American Fisheries Society meetings during spring 2012. Finally, we plan to publish our results in a peer-reviewed journal.

Student support info

The project gave 2 students field and laboratory experience that will help them gain employment or enter into a graduate degree program. The project benefited the students by becoming familiar with an

array of techniques used in stream ecology, vertebrate ecology, and toxicology. One student is currently a junior in Rangeland Ecology and Watershed Management at the University of Wyoming. The other student graduated from Clemson University with a degree in Wildlife and Fisheries Biology and is currently applying to graduate programs.

Products

We plan to publish the study. However, we are waiting for results from the majority of fish samples analyzed for trace elements.

Acknowledgements

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