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## Associations Between Iron Concentration and Productivity in Montane Streams of the Black Hills, South Dakota

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**ABSTRACT** Iron is an important micronutrient found in aquatic systems that can influence nutrient availability (e.g., phosphorus) and primary productivity. In streams, high iron concentrations often are associated with low pH as a result of acid mine drainage, which is known to affect fish and invertebrate communities. Streams in the Black Hills of South Dakota are generally circumneutral in pH, yet select streams exhibit high iron concentrations associated with natural iron deposits. In this study, we examined relationships among iron concentration, periphyton biomass, macroinvertebrate abundance, and fish assemblages in four Black Hills streams. The stream with the highest iron concentration (~5 mg Fe/L) had reduced periphyton biomass, invertebrate abundance, and fish biomass compared to the three streams with lower iron levels (0.1 to 0.6 mg Fe/L). Reduced stream productivity was attributed to indirect effects of ferric iron (Fe<sup>+++</sup>), owing to iron-hydroxide precipitation that influenced habitat quality (i.e., substrate and turbidity) and food availability (periphyton and invertebrates) for higher trophic levels (e.g., fish). Additionally, reduced primary and secondary production was associated with reduced standing stocks of salmonid fishes. Our findings suggested that naturally occurring iron deposits may constrain macroinvertebrate and fish production.

**KEY WORDS** Black Hills streams, iron concentration, macroinvertebrate composition, nonnative salmonids

Iron is one of the most abundant metals found in aquatic environments and is an essential micronutrient for fauna and flora (Oborn 1960, Coughlan 1971, Lofts et al. 2008). Iron is highly reactive in freshwater and has an oxidation-reduction chemistry that has a number of significant roles in the cycling and bioavailability of other elements (Lofts et al. 2008) such as phosphorus. Leaching of ferrous iron (Fe<sup>++</sup>) from iron pyrite and subsequent precipitation as iron hydroxide, is a typical effect of acid mine drainage (AMD). Acid mine drainage and acid precipitation are known to reduce pH resulting from high iron concentrations (Tate et al. 1995, Vuori 1995, Niyogi et al. 1999) and reduced stream productivity (Vuori 1995, Butler 2009). Low primary productivity also may limit biomass at higher trophic levels. Diversity of macroinvertebrates and fishes often is reduced in streams characterized by low pH and iron hydroxide precipitation (Hynes 1970, Wellnitz et al. 1994, Vuori 1995), although some fishes (e.g., creek chub, *Semotilus atromaculatus*, brook trout, *Salvelinus fontinalis*), invertebrate taxa (e.g. water flea, *Daphnia longispina*), and colonial cyanobacteria are tolerant of reduced pH and high metal concentrations (Reash and Berra 1986, Hamilton and Reash 1988, Young and Harvey 1989, Randall et al. 1999, Hyenstrand et al. 2001).

In non-acidic streams (pH >7), Fe<sup>++</sup> quickly oxidizes to insoluble ferric iron (Fe<sup>+++</sup>; Hynes 1970, Vuori 1995). Ferric iron promotes decomposition of dissolved organic carbon in surface waters (Stumm and Morgan 1996) and forms particulate oxides and hydroxides capable of sorbing and transporting trace metals and dissolved organic matter (Dzombak and

Morel 1990). Ferric iron precipitates have had negative effects on the distribution, abundance, and diversity of periphyton, benthic invertebrates and fishes (Greenfield and Ireland 1978, McKnight and Feder 1984, Rasmussen and Lindegaard 1988, Gerhardt and Westermann 1995). Iron can limit algal growth and overall primary productivity by: 1) binding available phosphorus (Tate et al. 1995, Vuori 1995) which is an essential macronutrient required by biota (Wetzel and Ward 1996), 2) reducing substrate quality as a result of a thick covering of iron hydroxide precipitates on the substrates (Hynes 1970, McKnight and Feder 1984, Vuori 1995), 3) interfering with oxygen consumption, food consumption and mobility of aquatic animals (Gerhardt and Westermann 1995, Vuori 1995), and 4) limiting light penetration owing to high turbidity from iron precipitates (Sode 1983).

The role of iron in streams that lack confounding effects of low pH and metal toxicity has received little attention with regard to impacts on primary productivity, benthic invertebrates, and fish communities. Unlike streams affected by AMD, select streams in the Black Hills of South Dakota are influenced by natural iron deposits and generally maintain circumneutral pH (i.e., pH = 7) because exposed iron pyrite bands are fairly localized in the watershed, and limestone (CaCO<sub>3</sub>) from headwater reaches buffers acidic seeps (Webb and Chupka 2000). The lower reaches of these streams have broad, open valleys where autotrophy (i.e., periphyton) may play a substantial role in stream productivity (sensu Minshall 1978).

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A decrease in periphyton biomass because of low nutrient availability, reduced light penetration, and (or) fouling of substrates owing to iron precipitation could result in reduced stream productivity. For example, macroinvertebrate functional groups such as scrapers or collector-gatherers were less abundant in streams affected by high iron concentration. This resulted from iron precipitations on stream bottom substrates which also inhibited the growth of periphyton (McKnight and Feder 1984, Rasmussen and Lindegaard 1988). Furthermore, the thick layer of metal precipitate on the substrate can prevent the development of a stable stream community (McKnight and Feder 1984). In fishes, iron-hydroxide precipitates can cause respiratory stress and gill damage (Sykora et al. 1972, Peuranen et al. 1994, Vuori 1995, Teien et al. 2008), alter spawning habitat (Gerhardt 1992), and decrease hatching success (Smith et al. 1973, von Lukowicz 1976). Moreover, changes in macroinvertebrate abundance, combined with high turbidity, could affect feeding success for fishes.

In this study, we evaluated relationships between iron concentration and food web attributes in small, second-order streams of the Black Hills, South Dakota. We hypothesized that stream sites exhibiting high iron concentration would be characterized by lower periphyton biomass and macroinvertebrate abundance (Vuori 1995), and that these effects should cascade to higher trophic levels in the form of reduced fish biomass.

## STUDY AREA

We sampled four, second-order tributary streams in the upper Rapid Creek watershed in the Black Hills of South Da-

kota that were expected to differ in iron concentration given their underlying geology (Koth 2007; Fig. 1). The headwaters of the Rapid Creek drainage begins in the limestone plateau region of the Black Hills and flows south into the Cheyenne River, a tributary to the Missouri River. North Fork Castle Creek runs through naturally occurring ‘bog’ iron deposits and was expected to have the highest iron concentration (Holcomb 2002, Koth 2007; Fig. 2). South Fork Rapid Creek and North Fork Rapid Creek were situated in the periphery of the underlying iron band region and were expected to have intermediate iron concentrations. Castle Creek, which was situated outside of the bog iron deposits, was expected to have the lowest iron concentration (Holcomb 2002; Fig. 2).

Native cold-water fish species of the Black Hills included three cyprinids (creek chub, lake chub [*Couesius plumbeus*], and longnose dace [*Rhinichthys cataractae*]), and three catostomids (longnose sucker [*Catostomus catostomus*], mountain sucker [*C. platyrhynchus*], and white sucker [*C. commersonii*]). Common non-native salmonids included brook trout, brown trout (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*). Salmonid fishes were introduced into the Black Hills in the late 1800s to early 1900s (Barnes 2007). In our study streams, brown trout and brook trout represented naturally-reproducing populations as stocking had not occurred for at least a decade preceding this study (Cordes 2007). While rainbow trout can reproduce naturally in Castle Creek, they were annually stocked in Deerfield Reservoir and were known to move upstream (Davis 2012). Because we could not reliably differentiate between wild rainbow trout or hatchery rainbow trout, we excluded them from the analysis.

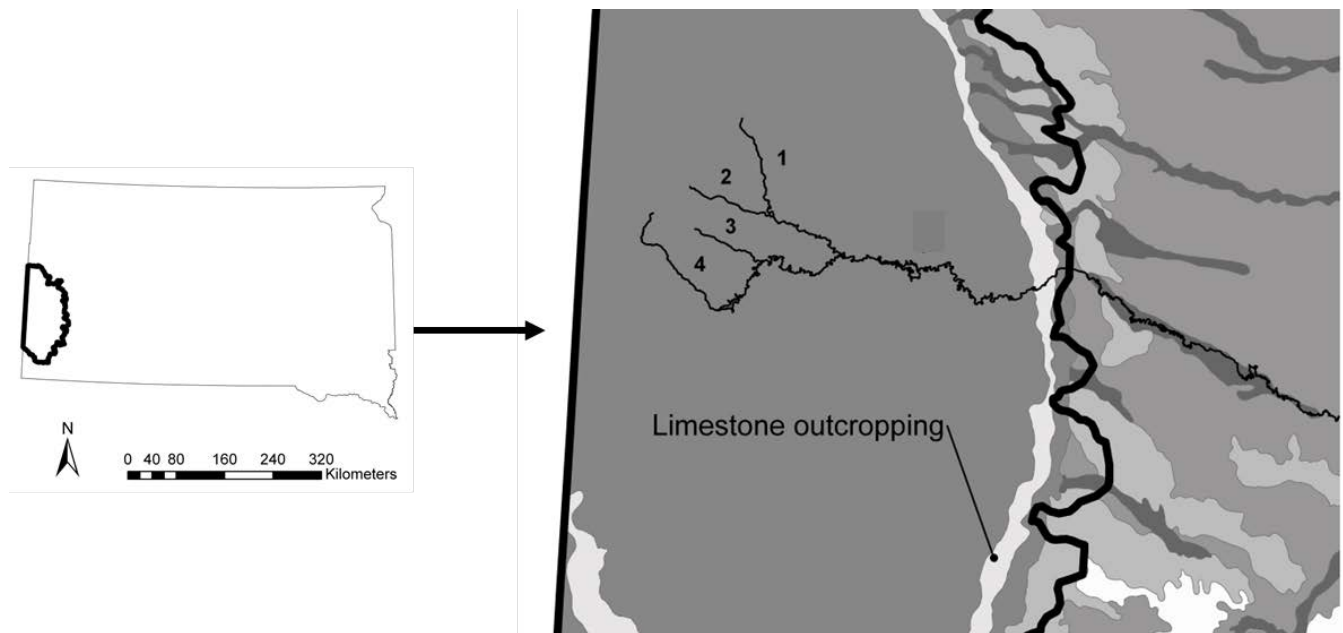


Figure 1. Black Hills region in western South Dakota showing location of the study streams: 1) North Fork Rapid, 2) South Fork Rapid, 3) North Fork Castle, and 4) Castle Creek, July 2011.



Figure 2. North Fork of Castle Creek, South Dakota (left panel) showing natural iron deposits and associated turbidity. Right panel shows the section of Castle Creek in the Black Hills, South Dakota that is not influenced by natural iron deposits, July 2011.

## METHODS

### Water quality and algae

We quantified water quality attributes and periphyton biomass in each stream by sampling three, 100-m sample reaches, spaced approximately 1.6 km apart, from 5–18 July 2001 ( $n = 12$  study sites; Table 1). At each site, we measured water temperature ( $^{\circ}$  C), pH, and turbidity (NTU) mid-channel using a YSI Data Sonde Model 650 (YSI Incorporated, Yellow Springs, Ohio, USA). We fixed water samples for iron analysis (mg Fe/L) with 5 ml of nitric acid, stored in 500-ml bottles, and analyzed for total iron content using the phenanthroline method (APHA 1998). We determined phosphorus concentration (mg/L) by collecting water samples in 250-ml Nalgene bottles; samples were stored on ice, transported to the laboratory, and analyzed using the persulfate oxidation method (APHA 1998).

We used artificial substrate samplers (15.2-cm  $\times$  15.2-cm brick tiles) to quantify periphyton biomass at each site. At each site, we placed three tiles in similar conditions (e.g., flow, depth, open canopy) and collected after two weeks. Periphyton was scraped and rinsed from each tile using a toothbrush and squirt bottle, filtered onto a GF/F filter, and frozen in aluminum foil for later analysis (United States Environmental Protection Agency 2002). We measured chlorophyll *a* from the periphyton sample using a Turner Design TD-700 fluorometer after 24-hr extraction in 90% acetone and averaged for each site (3/stream).

### Macroinvertebrate and fish sampling

We sampled benthic invertebrates in riffle habitats at each site using a 0.093-m<sup>2</sup> Surber sampler. Three samples were

collected at each site to obtain average invertebrate densities for that site. We preserved all invertebrates in 90% ETOH containing rose Bengal dye. In the laboratory, for invertebrates, we sorted, counted, identified to lowest possible taxon (family or genus), and categorized into functional feeding groups (i.e., collector-gatherer, filterer, parasite, piercer, predator, scraper, or shredder) as defined by Merritt and Cummins (2000). Functional feeding groups can reflect aquatic ecosystem attributes such as disturbance (Rawer-Jost et al. 2000) that might alter food availability (Barbour et al. 1996). For example, shredders and scrapers, which are specialized feeders, may be more sensitive to pollution, while collector-gatherers and filterers, which are generalists, are more tolerant to pollution (Barbour et al. 1996). Taxa that could not be reliably assigned to a functional feeding group were not included in the analysis.

We sampled fishes using a 3-pass sampling protocol with a backpack electrofishing unit (Smith-Root LR-20B, Smith-Root, Inc.). We blocked sampling reaches using 15-mm mesh seines at the downstream and upstream end of each site. We identified, counted and measured for total length (mm) and weight (g) all fish. Relative fish abundance (kg/100 m) was quantified for each site and expressed as a standing stock estimate (kg/ha) based on mean stream width measured for each 100 m section.

### Statistical analysis

We compared water temperature, pH, turbidity, phosphorus (mg/L), iron (mg/L), and fish biomass using analysis of variance (ANOVA) with streams as grouping factors (SAS Institute 2010). Abundance of invertebrate feeding groups was log-transformed [ $\log(\text{density}+1)$ ] and compared among streams using multivariate analysis of variance (MANOVA

Table 1. Mean stream pH, water temperature ( $^{\circ}$  C), turbidity (NTU), total iron (mg/L), total phosphorus (mg/L), and chlorophyll *a* concentration (mg/m<sup>2</sup>) measured in four Black Hills streams, July 2001. For each variable, means with the same letter are not different (Tukey's multiple comparison test,  $P > 0.05$ ). Values in parentheses represent one standard error.

Stream	N	pH	Temperature ( $^{\circ}$ C)	Turbidity (NTU)	Iron (mg/L)	Phosphorus (mg/L)	Periphyto Biomass (chl-a, mg/m <sup>2</sup> )
Castle	3	8.4 (0.03) <sup>a</sup>	17.66 (0.69) <sup>a</sup>	1.00 (0.25) <sup>a</sup>	0.18 (0.02) <sup>a</sup>	0.021 (0.002) <sup>a</sup>	19.33 (3.26) <sup>a</sup>
North Fork Castle	3	7.6 (0.15) <sup>b</sup>	15.86 (0.68) <sup>a</sup>	3.40 (0.35) <sup>b</sup>	4.95 (1.55) <sup>b</sup>	0.026 (0.011) <sup>a</sup>	4.14 (2.22) <sup>b</sup>
North Fork Rapid	3	8.4 (0.05) <sup>a</sup>	18.10 (1.34) <sup>a</sup>	0.53 (0.29) <sup>a</sup>	0.62 (0.15) <sup>a</sup>	0.014 (0.003) <sup>a</sup>	21.72 (1.60) <sup>a</sup>
South Fork Rapid	3	8.2 (0.06) <sup>a</sup>	14.50 (0.34) <sup>a</sup>	0.33 (0.24) <sup>a</sup>	0.67 (0.19) <sup>a</sup>	0.005 (0.002) <sup>a</sup>	12.14 (3.88) <sup>a, b</sup>

option; SAS Institute 2010); if the MANOVA test was significant (i.e.,  $\alpha < 0.05$ ), one-way ANOVAs were performed to compare the mean abundance of individual feeding groups among streams. For variables that differed among streams, we compared means using Tukey's multiple comparison test. We used correlation analysis to examine relationships among water variables (e.g., phosphorus, iron), and between total invertebrate abundance and periphyton biomass. We used nonlinear regression analysis to explore relationships between iron (mg/L) concentration and periphyton biomass (mg/m<sup>2</sup>), invertebrate abundance (no./m<sup>2</sup>), and fish biomass (kg/ha).

## RESULTS

Several water quality attributes varied among study streams. Stream pH ranged from 7.6 to 8.4 and was lower ( $F_{3,11} = 18.67$ ,  $P < 0.001$ ) in North Fork Castle Creek than in the other three streams (Table 1). Mean iron concentrations ranged from 0.18 to 4.95 mg/L and differed ( $F_{3,11} = 8.13$ ,  $P = 0.01$ ) among streams (Table 1). Mean iron concentration was highest (4.95 mg/L, SE = 1.55) in North Fork Castle Creek and lowest in Castle Creek at 0.18 mg/L (SE = 0.02; Table 1). Similarly, water turbidity varied among streams and was higher ( $F_{3,11} = 24.41$ ,  $P < 0.001$ ) in North Fork Castle Creek (Table 1). In contrast, we found no evidence that water temperature ( $F_{3,11} = 3.90$ ,  $P = 0.06$ ) or mean phosphorus concentration ( $F_{3,10} = 3.75$ ,  $P = 0.07$ ) differed among the four streams (Table 1). Moreover, we found no statistical relationship between total phosphorus and total iron concentration among Black Hills streams (correlation analysis,  $n = 12$ ,  $r = 0.46$ ,  $P = 0.13$ ).

Periphyton biomass, measured as chlorophyll *a* concentration, differed ( $F_{3,11} = 7.52$ ,  $P = 0.01$ ) among streams and was lowest in North Fork Castle Creek (Table 1). Although variable, chlorophyll *a* concentration (chl-a) was inversely related to total iron concentration as chl-a =  $21.31e^{-0.386(\text{Fe})}$  ( $F_{1,11} = 16.00$ ,  $P < 0.001$ ,  $r^2 = 0.61$ ; Fig. 3).

A total of 55 invertebrate taxa representing five functional feeding groups were collected from our study streams (Table 2). Total invertebrate abundance ranged from 429 to 8,458 invertebrates/m<sup>2</sup> and was lower ( $F_{3,11} = 15.53$ ,  $P = 0.001$ ) in

North Fork Castle Creek. Moreover, total abundance of invertebrates was positively correlated to periphyton biomass ( $n = 12$ ,  $r = 0.80$ ,  $P < 0.001$ ; Fig. 4), and inversely related to iron concentration (nonlinear regression analysis: total invertebrates =  $9,288.2e^{-0.608(\text{Fe})}$ ,  $F_{1,11} = 39.00$ ,  $P < 0.001$ ,  $r^2 = 0.77$ ; Fig. 3). Multivariate analysis of variance revealed that abundance of functional feeding groups varied ( $F_{15,18} = 7.65$ ,  $P < 0.001$ ) among streams. Subsequent one-way ANOVA tests showed that, except for shredders, mean abundance of all other functional feeding groups was lower in North Fork Castle Creek compared to other streams (Table 2).

We collected seven fish species from the four study streams. One or more native species, including creek chub, longnose dace, mountain sucker, and (or) white sucker were collected from all streams except South Fork Rapid Creek. However, native species represented a small proportion of total fish biomass (0 to 30%, average = 2%) compared to non-native salmonids and we found no evidence that native fish biomass varied ( $F_{3,11} = 0.86$ ,  $P = 0.50$ ) among streams (Table 3). Non-native fishes included brown trout and brook trout; rainbow trout were only collected from Castle Creek (<25% of trout biomass) and were not included in the analysis. On average, total trout biomass varied among streams and was lower ( $F_{3,11} = 15.09$ ,  $P = 0.001$ ) in North Fork Castle Creek compared to other streams (Table 3). Among streams, mean total fish biomass was positively related to total invertebrate density ( $n = 12$ ,  $r = 0.87$ ,  $P < 0.001$ ; Fig. 4) and inversely related to iron concentration (nonlinear regression analysis; fish biomass =  $352.3e^{-0.495(\text{Fe})}$ ,  $F_{1,11} = 16.38$ ,  $P = 0.002$ ,  $r^2 = 0.58$ ; Fig. 3).

## DISCUSSION

Ferric iron is generally believed to have both direct and indirect effects on aquatic plants and animals, owing to changes in habitat and (or) food resources resulting from Fe<sup>+++</sup> precipitates (Vuori 1995). A number of studies have shown that precipitation of iron hydroxide can inhibit periphyton growth and associated macroinvertebrate diversity (McKnight and Feder 1984, Niyogi et al. 2002). In Black Hills streams, periphyton biomass was negatively associated

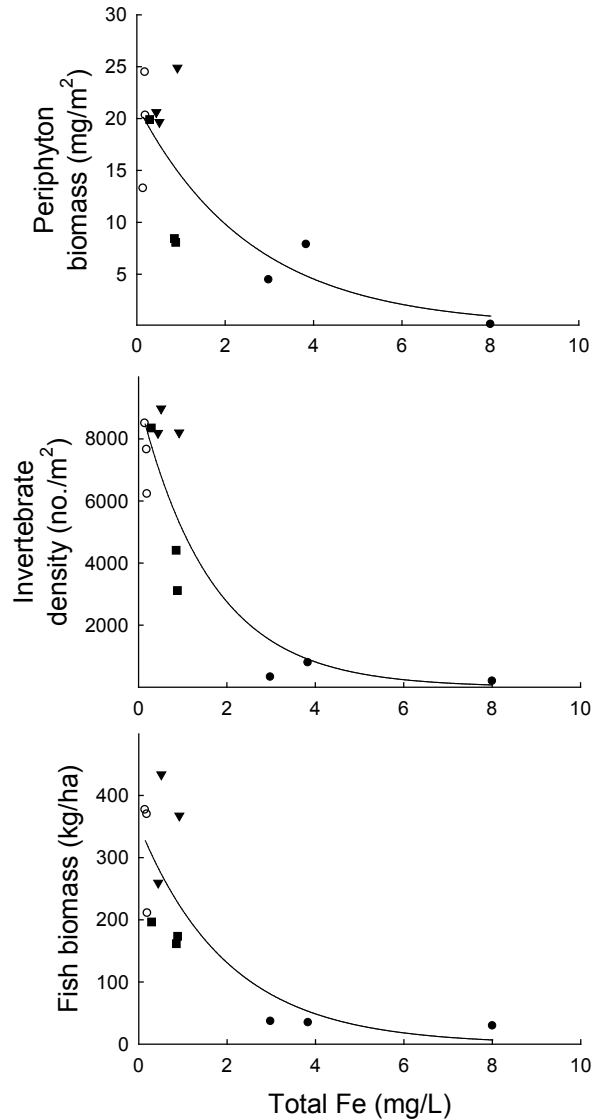


Figure 3. Relationships between chlorophyll *a* (top), invertebrate abundance (center) and fish biomass (bottom) with total iron concentration in Castle (open circles), North Fork Castle (solid circles), North Fork Rapid (solid triangles) and South Fork Rapid (solid squares) creeks, South Dakota, July 2001.

Table 2. Mean abundance (no./m<sup>2</sup>) of functional feeding groups collected in Castle, North Fork Castle, North Fork Rapid, and South Fork Rapid creeks, South Dakota, July 2001. For each feeding group, streams with the same letter are not different (Tukey's multiple comparison test,  $P > 0.05$ ). Values in parentheses represent one standard error.

Stream	Functional feeding group				
	Collector-gatherer	Filterer	Predator	Scraper	Shredder
Castle	5,004 (633) <sup>a</sup>	473 (80) <sup>a</sup>	873 (8) <sup>a</sup>	471 (222) <sup>b</sup>	633 (119) <sup>a</sup>
North Fork Castle	266 (119) <sup>b</sup>	51 (24) <sup>b</sup>	101 (45) <sup>b</sup>	7 (2) <sup>d</sup>	4 (3) <sup>b</sup>
North Fork Rapid	4,593 (417) <sup>a</sup>	613 (270) <sup>a</sup>	523 (56) <sup>a</sup>	2,613 (173) <sup>a</sup>	117 (21) <sup>a</sup>
South Fork Rapid	3,551 (1,017) <sup>a</sup>	1,266 (430) <sup>a</sup>	405 (w161) <sup>a</sup>	63 (13) <sup>c</sup>	7 (5) <sup>b</sup>

Table 3. Mean abundance of native and salmonid fishes in Castle, North Fork Castle, North Fork Rapid, and South Fork Rapid creeks, South Dakota, sampled in July 2001. Total fish abundance is given in the last column. For each column, values with the same letter are not significantly different (Tukey’s multiple comparison test,  $P > 0.05$ ). Values in parentheses represent one standard error.

Stream	Native fishes (kg/ha)	Salmonids (kg/ha)	Total (kg/ha)
Castle	1.2 (1.0) <sup>a</sup>	317.4 (53.5) <sup>a</sup>	318.6 (54.1) <sup>a</sup>
North Fork Castle	10.1 (9.4) <sup>a</sup>	23.0 (11.5) <sup>c</sup>	33.1 (2.1) <sup>c</sup>
North Fork Rapid	8.1 (4.9) <sup>a</sup>	348.3 (52.6) <sup>a</sup>	356.5 (51.2) <sup>a</sup>
South Fork Rapid	0.0	177.1 (10.2) <sup>b</sup>	177.1 (10.2) <sup>b</sup>

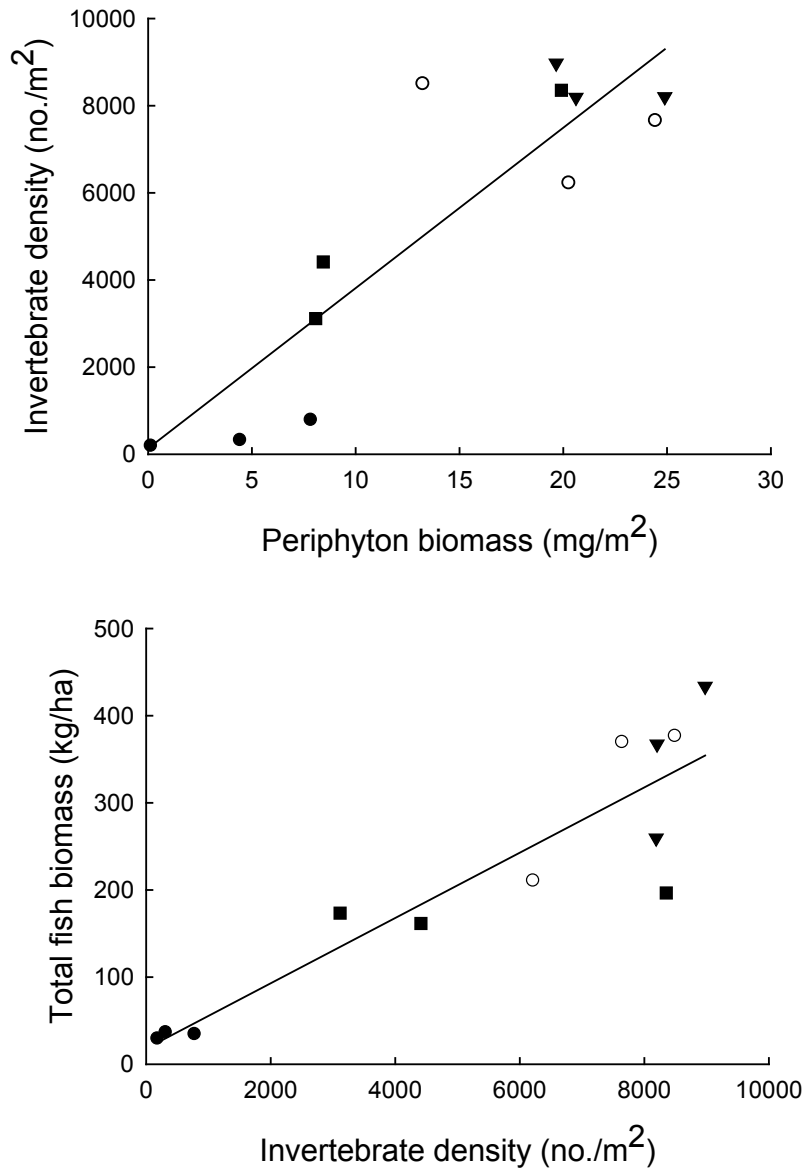


Figure 4. Relationship between periphyton biomass and invertebrate abundance (top panel) and invertebrate abundance and fish biomass (bottom panel) in Castle (open circles), North Fork Castle (solid circles), North Fork Rapid (solid triangles) and South Fork Rapid (solid squares) creeks, South Dakota, July 2001.

with iron concentration. Low algal biomass, in turn, was associated with lower invertebrate abundance, implying that bottom-up effects were important determinants of invertebrate and fish biomass. Indeed, total fish biomass in North Fork Castle Creek was appreciably lower than that observed in other streams, consistent with reduced prey availability.

Unlike fish and invertebrate abundance, we found no evidence that phosphorus concentration was reduced in the high iron stream. Tate et al. (1995) reported that in open canopy streams, photoreduction of  $\text{Fe}^{+++}$  provided sufficient phosphorus release for algal uptake and growth. Because streams in our study were characterized by open canopies and turbidity appeared to be less substantial than in North Fork Castle Creek, photoreduction may play an important role in regulating phosphorus availability.

With the exception of North Fork Castle Creek, mean iron concentration in our study streams was below 1.0 mg/L, the EPA water quality criteria for freshwater aquatic life (Vuori 1995). In a related study, Sode (1983) showed that an iron concentration as low as 1.12 mg/L had negative effects on algal community composition. Similarly, in the Snake River (Colorado), precipitation of hydrous metal oxides greatly decreased the abundance of periphyton and benthic invertebrates (McKnight and Feder 1984). Furthermore, the authors suggest that substrate fouling may have a more deleterious effect on benthic stream communities than high metal-ion activities. In streams with circumneutral or elevated pH (> 7.0), high iron concentrations have been linked to declines in invertebrate abundance and diversity. In Danish streams (pH = 6.7–8.8), invertebrate diversity and abundance declined at iron concentrations >1.0 mg/L (Rasmussen and Lindegaard 1988). Similarly, in Colorado streams (pH = 7.0–7.7), Clements et al. (2000) documented that metal concentration was the strongest predictor of invertebrate community composition and that the abundance of invertebrate collectors and predators decreased with increasing metal concentration. Thus, the observed reduced abundance of macroinvertebrates in North Fork Castle Creek may result from indirect effects of iron hydroxide precipitation that affect food availability (periphyton) and habitat conditions (Sode 1983).

Physiological impairment, reduced prey availability, and (or) increased turbidity associated with high iron concentration may limit nonnative trout production in North Fork Castle Creek. Iron accumulation on the gills of brown trout has been shown to disrupt respiratory function at extremely high iron conditions (i.e., 28–47 mg/L; Dalzell and MacFarlane 1999). Physical clogging and damage to the gills of brown trout were observed at sub-lethal exposure to iron (Dalzell and MacFarlane 1999). Similarly, in West Virginia streams, high turbidity was associated with reduced growth rates of brook trout (Sweka and Hartman 2001) and several studies have shown that trout standing stock is positively associated with prey availability (Bowlby and Roff 1986, Richardson 1993).

## MANAGEMENT IMPLICATIONS

We found that in a stream characterized by high iron concentration (>4.0 mg/L), periphyton biomass and invertebrate abundance were significantly reduced. Moreover, low abundance of primary and secondary producers was associated with reduced standing stocks of salmonid fishes. In North Fork Castle Creek, standing stock of salmonids was 89% lower than more productive areas in the Black Hills. Identifying constraints to trout production and mechanisms affecting these constraints has important implications for trout management in the Black Hills. And as shown here, naturally occurring iron deposits appear to represent an important constraint to invertebrate and fish production in North Fork Castle Creek.

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