Occurrence of chironomid species (Diptera: Chironomidae) in the high Se-78 concentrations and high pH of Fountain Creek Watershed, Colorado, USA

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ABSTRACT.—High selenium (Se) in watersheds can cause detrimental effects to many vertebrate species, but less is known regarding its effects on invertebrates comprising the basis of the food chain. This study addressed the response of a natural community of chironomid (midge) species to Se in 2 high-gradient streams that join to form a sandy-bottom front-range stream known to contain increasing downstream concentrations of Se. We identified a total of 151 species of adult male midges ($n = 714$) collected in 2007 and 2008 from 14 sites in the Fountain Creek Watershed, Colorado, USA. In the initial analysis, the midge community was assessed for possible effects of Se and pH by using binary sets of 25 variables by means of canonical correspondence analysis (CCA) procedures. Further computations revealed significant relationships between the presence of midges and spring total Se ($P = 0.0006$, unfiltered water), dissolved Se ($P = 0.0006$, filtered water), and pore water Se ($P = 0.0034$, interstitial water in coarse and fine gravels) in the spring season. In the fall, the community showed a significant response to total Se ($P = 0.0500$), a strong association with dissolved Se ($P = 0.0044$), and a weaker yet significant association with pore water Se ($P = 0.1266$). We have not found reports indicating either positive or negative responses of midges to increasing Se in field studies. Although there were no significant associations of midges with lower pH during the spring in any fraction of the water ($P = 0.7367$, 0.7367 , and 0.7469 , respectively), there were significant associations to higher pH during the fall in all fractions ($P \leq 0.0100$). Mean concentrations of dissolved Se in lower-elevation sites ranged from 2.05 to 9.69 $\mu\text{g/L}$ in the spring and from 2.67 to 18.59 $\mu\text{g/L}$ in the fall; mean pH ranged from 7.5 to 7.8 in the spring and from 8.0 to 8.1 in the fall (2007). The association of silt/clay particles with the occurrence of midges was not significant in any of the waters in the spring or fall. Since some chironomid midges have a tendency to be found in areas exhibiting increasing Se in a downstream gradient in Fountain Creek, would they be found in similar water quality profiles in other watersheds? This possibility warrants serious study in other streams, particularly in the western United States where Se has been entering aquatic food chains of fish, birds, and mammals.

RESUMEN.—El alto contenido de selenio en las cuencas hidrográficas puede causar efectos perjudiciales en muchas especies de vertebrados. Sin embargo, se sabe menos sobre los invertebrados que constituyen la base de la cadena alimenticia. Este estudio evaluó la respuesta de una comunidad natural de especies de quironómidos en dos corrientes de pendiente elevada, que se unen para formar una corriente frontal de fondo arenoso, conocida por tener concentraciones altas de selenio. Un total de 151 especies de mosquitos adultos machos ($n = 714$) colectados en 14 sitios entre 2007–2008, fueron identificados a nivel de la especie, en la cuenca Fountain Creek en Colorado, Estados Unidos. En un análisis inicial, se evaluó la comunidad de insectos para detectar posibles efectos utilizando conjuntos binarios de 25 variables mediante el Análisis de Correspondencia Canónica (CCA, por sus siglas en inglés). Cálculos posteriores revelaron una relación significativa entre la presencia de mosquitos y el selenio total, selenio disuelto y selenio en agua capilar, durante la primavera ($P = 0.0006$, $P = 0.0006$ y $P = 0.0034$ respectivamente). La comunidad mostró una respuesta significativa a la fracción total de selenio en el otoño ($P = 0.0500$), una fuerte relación con el selenio disuelto ($P = 0.0044$) y una relación débil, pero significativa, con el selenio en agua capilar ($P = 0.1266$). En los estudios de campo, no encontramos evidencia que indique respuestas positivas o negativas de los mosquitos al aumento de selenio. Aunque, no se encontró relación significativa con valores bajos de pH en la primavera, en ninguna fracción de agua ($P = 0.7367$, 0.7367 y 0.7469 , respectivamente), sí se encontraron relaciones significativas con valores altos de pH, durante el otoño en todas

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las fracciones de agua ($P \leq 0.0100$). Las concentraciones promedio de selenio disueltas en sitios de menor elevación oscilaron entre 2.05 y 9.69 $\mu\text{g/L}$ en la primavera del 2007 y entre 2.67 a 18.59 $\mu\text{g/L}$ en el otoño; el pH promedio varió de 7.5 a 7.8 en la primavera y de 8.0 a 8.1 en el otoño. La relación entre las partículas de limo/arcilla y la presencia de mosquitos no fue significativa en ninguna de las fracciones de agua durante la primavera o el otoño. Si los mosquitos quironómidos tienden a encontrarse en áreas de selenio incrementando con el gradiente de elevación en Fountain Creek, ¿podríamos encontrarlos en otras cuencas hidrográficas con perfiles de calidad de agua similares? Esta posibilidad justifica el estudio en otras corrientes, particularmente en el oeste de los Estados Unidos, donde el selenio se ha incorporado a las cadenas alimentarias acuáticas de peces, aves y mamíferos.

Selenium (Se) is an essential element for life that occurs naturally in the earth's crust. However, under some circumstances Se becomes toxic, especially in aquatic ecosystems. Chapman (2010) summarized several activities that are sources of Se contamination. Industrial sources included agricultural irrigation, animal husbandry, erosion from monoculture, and mining of hard rock, phosphate, and coal. Chapman (2010) mentioned that Se is found in organic-rich shales that are source rocks for the activities mentioned above. Lemly (2002) noted that Se is a worldwide pollution problem stemming from "coal and combustion" activities; further, the most serious impacts to aquatic life have occurred as a result of this type of contamination. Debrun and Chapman (2007) identified key issues involving Se by stating that when Se becomes available, the "element bioaccumulates, bio-transforms, and biomagnifies in some species and becomes implicated in impacts to invertebrates, fish, water birds and mammals." A concise review of the well-studied Se problem in the United States is given in Young et al. (2010) in a case history of the Kesterson Reservoir, San Joaquin Valley, California.

Due to several issues involving Se, especially in Kesterson Reservoir, Mueller et al. (1991) selected additional streams and reservoirs for study because of the high concentrations of Se in irrigation drainage, which affects water quality, bottom sediment, and biota in several western U.S. states. One location where Se has been a concern historically is the Fountain Creek Watershed in Colorado. Selenium concentrations in Fountain Creek are similar to those in several other smaller tributaries of the middle and lower Arkansas River in the vicinity. These tributaries and basins were described by Presser et al. (1994) as having several "source rock designations," with the Pierre and Carlile shales being sources of the element; the upper Arkansas River tributaries upstream are distinguished by having hard

rock substrates. The Arkansas River Valley in Colorado and Kansas has long been known for its agriculture and extensive irrigation. However, other land uses, such as increased urbanization, are recognized as affecting water quality within a watershed.

Due to the growth of Colorado Springs in El Paso County, Colorado, water quality in the Fountain Creek Watershed has become a concern. For example, Salazar (2006) wrote that "fixing the problems" of the Fountain Creek Watershed includes 2 broad issues, "flood control and water quality," and that "additional problems" include nonpoint source pollution, high water volumes, flash floods, sedimentation buildup, erosion, and "high selenium content." A "selenium-problem" in Fountain Creek will likely continue due to 3 factors: (1) the natural abundance of Se in surface bedrock and substrates of the basin, (2) the continued growth of the metropolitan area, especially in the upper watershed, and (3) sustained projected growth, if water availability allows it. Regardless, there will probably be increased loading of Se in the watershed due to increasing volumes of wastewater entering Fountain Creek in El Paso County. The expectation of increasing Se in the watershed may be reflected in recently established water quality standards for Se in Fountain Creek (David Moon, personal communication, U.S. EPA; Region 8). Further, these standards appear to mirror the increasing Se with distance of stream travel. For example, the Se standard for the upper watershed (Monument and Upper Fountain Creeks; Fig. 1) is 4.6 $\mu\text{g/L}$ in the dissolved form. The standard for the stream segment from the confluence of Monument and Fountain Creeks to the Hwy. 47 bridge in Pueblo, Colorado (includes sampling sites LF-1, LF-2, and LF-3; Fig. 1), is 4.8 $\mu\text{g/L}$ in the dissolved form, and the limit is based on the 85th percentile of the observed values. Downstream of the bridge, the standard is 28.1 $\mu\text{g/L}$ within the City of Pueblo, Colorado,

including sampling sites LF-4 and LF-5 (Fig. 1) and the stretch above the confluence with the Arkansas River. The 28.1 $\mu\text{g/L}$ limit is also the dissolved form of Se and based on the 85th percentile of the observed values.

Macroinvertebrates, fish, and plants have been used as biological indicators in Fountain Creek (von Guerard 1989a, Bruce 2002). Von Guerard (1989a) collected benthic invertebrates, including chironomids, from 5 sites in the upper watershed and found that flooding during the 30 days prior to sampling accounted for most variation in mean densities of total organisms for major taxa. Further, von Guerard (1989a, 1989b) noted that invertebrate densities were greatest at sites with larger median grain size of bed material. Later, Bruce (2002) conducted a study on the sites mentioned above but used presence-absence data of macroinvertebrate communities in the Fountain Creek Watershed that included ratios of Chironomidae to Ephemeroptera, Plecoptera, and Trichoptera groups to determine the effects of land-use activities in Upper Fountain Creek. Bruce (2002) also used cluster analysis of the number of organisms per square meter (m^2) to indicate that macroinvertebrate community structures of the tributaries were more similar to each other than they were to the mainstem sites. Bruce (2002) found canonical correspondence analysis (CCA) useful for identifying substrate particle-size gradients from site-specific species abundance data and for identifying environmental correlates that reduced the 10 study sites to 5 distinct clusters and their associated taxa. Through these efforts, we became aware that the biotic and abiotic variables of Upper Fountain Creek basin are complex. One such variable is that 2 stream segments flow into one: Upper Fountain Creek and Monument Creek form a single Y-shaped basin. Both upper basins have extremes of elevation and temperature, striking differences in lithology and vegetation, and the differing physical and biological influences discussed above. Considering this complexity, 3 key questions in the study of Fountain Creek include the following: (1) Does Se negatively affect the abundance of midges in the watershed, influencing either a subset of various species or an entire community? (2) Would the absence of chironomid midges confirm actual Se toxicity? And (3) could either the presence or absence of these insects be confounded by other physicochemical

factors in the basin? We chose midges to address these questions because Winner et al. (1980) found that the number of species of chironomids increased from 15 at the most metal-polluted site in an Ohio stream to 39 species of midge species at the least metal-polluted site.

Recent efforts have focused on possible impacts of metals, metalloids, and nutrients on a variety of biota in Fountain Creek. The availability of Se to other aquatic biota in Fountain Creek was documented by Herrmann et al. (2012), who deployed the aquatic bryophyte *Hygrohypnum ochraceum* at 14 sites in the watershed and showed bioconcentration of the metalloid by examining the ratio of Se in plants to Se in water. The analysis method used by Herrmann et al. (2012) was inductively coupled plasma mass spectrometry (ICP-MS), which also allowed evaluation of additional metals and metalloids in the same study. Nimmo et al. (2016) surveyed fish populations in the watershed and found that brown trout (*Salmo trutta*) from the upper watershed as well as 5 warm-water fish species in the lower watershed had both Se and mercury (Hg) in whole bodies and individual tissues. Later, Herrmann et al. (2016a) confirmed that Se and Hg bioaccumulated (rate of intake of a substance > rate of excretion or metabolic transformation) in 12 internal and external tissues of brown trout. The study examined stomach contents of the fish, and there was evidence that diet and not ambient water was the major source of Se and Hg in the fish. Finally, Herrmann et al. (2016b) reported on the richness, diversity, and ecology of chironomid midges in Fountain Creek. Included in the report were 2 synoptic physicochemical analyses of water (including nutrients, metals, and metalloids) and the fraction sizes of sediments in the watershed. Finding Se (and Hg) in the fish of Fountain Creek reemphasized the importance of invertebrates in the diets of fishes. Voshell (2002) noted that chironomids often comprise half of the species in an aquatic community of invertebrates and that the larvae of nonbiting species are the dominant prey consumed by many species of fish.

METHODS

Study Area

Fountain Creek is a major tributary to the middle Arkansas River west of the Colorado-Kansas boundary in southeastern Colorado,

TABLE 1. Attributes of 14 sampling sites in the Fountain Creek Watershed located in El Paso County (EPC) and Pueblo County (PC), Colorado. UF = Upper Fountain Creek, MC = Monument Creek Tributary, and LF = Lower Fountain Creek. Numbers 1 through 14 (No.) were used for site identification in the canonical correspondence analysis.

Site	No.	Latitude	Longitude	Elevation (m)	Physical description of site location
UF-1	1	38.92691	-105.004	2334.77	UF at Green Mtn. Falls (EPC)
UF-2	2	38.85948	-104.920	1940.66	UF at Manitou Springs (EPC)
UF-3	3	38.84629	-104.866	1859.89	UF at 26th St., Colorado Springs (EPC)
UF-4	4	38.83015	-104.842	1818.44	UF at 8th St., Colorado Springs (EPC)
MC-1	5	39.08271	-104.876	2097.33	MC at Mt. Herman Rd. near Monument (EPC)
MC-2	6	39.02415	-104.844	2031.80	MC at N. Entrance, U.S. Air Force Academy (EPC)
MC-3	7	38.95424	-104.834	1942.80	MC at S. Entrance, U.S. Air Force Academy (EPC)
MC-4	8	38.93322	-104.817	1924.20	MC at Woodman Road, Colorado Springs (EPC)
MC-5	9	38.84282	-104.828	1840.69	MC at Colorado College, Colorado Springs (EPC)
LF-1	10	38.81613	-104.822	1798.02	LF at Nevada St., Colorado Springs (EPC)
LF-2	11	38.60245	-104.670	1634.95	LF at S. Fountain, CO (EPC)
LF-3	12	38.42975	-104.598	1532.84	LF at Piñon Bridge (PC)
LF-4	13	38.28793	-104.602	1432.86	LF at Hwy. 50, Pueblo (PC)
LF-5	14	38.25572	-104.591	1414.88	LF upstream of confluence with Arkansas River, Pueblo (PC)

USA, and site descriptions of the area are found in Bruce (2002), Zuellig et al. (2008), and Herrmann et al. (2012). In this report, mention of Fountain Creek (along with the watershed or basin) includes the Monument Creek tributary. The watershed has a drainage area of 2398 km², with elevations ranging from 4300 m at the summit of Pike's Peak to 1432 m at the confluence with the Arkansas River in Pueblo, Colorado (Fig. 1). The Front Range of the Rocky Mountains is oriented roughly north to south and comprises about one-third of the westerly directed portion of the basin where Monument and Upper Fountain Creeks emerge from the foothills west to east. After leaving the foothills, Monument Creek turns south and, within a few kilometers, joins with Upper Fountain Creek emerging from the foothills within the city of Colorado Springs. Fountain Creek then flows south until it joins the Arkansas River in Pueblo, Colorado. Differences in gradients and substrates of the 3 segments of the watershed are stark. For example, the high-gradient streambed of Upper Fountain Creek consists of boulders and cobble with less sand and gravel, whereas the streambed of the moderate-gradient Monument Creek segment contains some cobble, gravel, and deep, coarse sand derived from eroded sandstone and mudstone of the Dawson Formation (von Guerard 1989b, Zuellig et al. 2008). Downstream from the confluence, Fountain Creek is primarily a low-gradient meandering sandy-bottom plains stream whose bed consists of varying degrees of sand, gravel, small cobble, and plate-like shale

remnants of the Carlile and Pierre deposits discussed earlier.

Study Sites

Sampling sites in the Fountain Creek Watershed have been described by Herrmann et al. (2012) and Nimmo et al. (2016) and include 4 sites in the Upper Fountain Creek segment (UF-1–4), 5 sites in Monument Creek (MC-1–5), and 5 sites in Lower Fountain Creek (LF-1–5; Fig 1, Table 1). The Fountain Creek Watershed was in a moderate to severe drought from 2002 to 2013 during the collection of insects, water samples, and substrates in this study. Therefore, there were no physical changes at any study site (i.e., the banks were stable and the sinuosity of the stream did not change). Inspection of the hydrographs for 5 calendar years (2007–2012; Site 07106500; USGS 2017) did not record any flooding. Any increasing flows reflected melting snow upstream.

Collection Methods for Adult Chironomids

Hereafter in this report, the word “midges” refers to chironomid midges. Adult chironomids were collected in the warmer months of 2007 and 2008 from 14 sites in the Fountain Creek Watershed using methods outlined in Powell (2008). Fine sweep nets (0.1 mm mesh, 28 cm in diameter, and 60 cm in length) and longwave ultraviolet (UV) lights were used to collect adult midges during late afternoon/evening at each site. Captured insects were placed in labeled jars containing 75% ethanol. The UV collections were conducted one full

hour after sunset by placing one UV lantern housing (Navy brand No. 987 with General Electric® lamp No. F6TS BLD, dimensions [L × W × H]: 27.5 × 7.5 × 7.5 cm) on an exposed shore (rocky or sandy islands) between 2 rectangular Kaiser® aluminum loaf pans (No. 408-35, dimensions [L × W × H]: 20 × 10 × 5 cm) filled with 75% ethanol. Midges collected in the loaf pans were carefully poured into a labeled jar and sorted later. Males were removed and stored in jars of 100% undenatured ethanol and appropriately labeled with date, location, and collection method.

Collections in 2007 were made at a later date in the summer than collections in 2008, but sampling during 2 consecutive years ensured collection representation and continuity of as many subfamilies and individual species as possible for each site. Data showing the number of species collected at each site for 2007 and 2008 by each collection method are available in Helland (2015) and Herrmann et al. (2016b).

The Fountain Creek Watershed microhabitats vary because of wet conditions that occur primarily during spring rains and snowmelt, but during base flows in late summer into fall and winter months, flows often become sluggish lotic sites with ponded backwater conditions. In July through mid-September, the basin receives low rainfall along with high temperatures and gentle breezes that contribute to arid conditions. Low humidity due to temperature and wind may result in desiccation of adult chironomids (Armitage 1995). During the years 2002 to 2013, Fountain Creek and the surrounding area were under moderate to severe drought; the daily mean flows at a sampling site within the City of Pueblo, Colorado, were nearly identical. At USGS station 07106500 (corresponding to site LF-4 in this study), the daily mean flows (m³/s) were as follows for 2007 and 2008, respectively: April, 2.86 and 2.86; May, 5.55 and 5.49; June, 4.11 and 4.01; July, 2.61 and 2.57; August, 3.85 and 3.91; and September, 1.56 and 1.60 (USGS 2017). Because there are no comparable permanent flowing waters in the Fountain Creek corridor, chironomid species were largely confined to lotic conditions, especially in stream segments with high banks protected from breezes. Therefore, the evening sampling of swarms along the stream banks was conducted during calm conditions

and when adults were confined at the water's edge. If winds were brisk or storms imminent, no sweeps or UV light trapping occurred. Armitage (1995) discussed how wind generally inhibited swarming and influenced landing of males. Prevailing winds in Colorado Springs were primarily from the north for all 12 months of the year, but secondary breezes from the east-southeast occur in the summer. In Pueblo, winds are from the east-southeast for 8 months of the year, including summer months (Newman 2015). Aerial translocation of midges could have occurred between sites separated by ≤1 km, but most sites are located >1.0 km apart, especially those in the lower segment of the creek.

Dissection and Identification Procedures for Adult Chironomids

Microdissections were performed on adult male specimens using variations of slide mounting procedures described by Schlee (1966), Hansen and Cook (1976), and Pinder (1989). Dissections necessary for identification were completed on male specimens by placing a drop of euparal in the center of a glass slide. Each wing was removed and placed on the upper left portion of the center of the slide. One prothoracic, one mesothoracic, and one metathoracic leg, including the trochanter, were removed from the thorax and placed in order directly below the wings. Antennae were removed and aligned to the right of the mesothoracic leg. Head, thorax, and abdomen were placed in a 12% KOH macerating solution for 24–48 h depending on specimen darkness and size to “clear” the internal anatomy. On clearing, the KOH was rinsed from the specimen with 2 changes of water followed by 2 changes of absolute ethanol to effect hydration. Another drop of euparal was added to the slide to the right of the wings, legs, and antennae, then the thorax was removed and placed to the right of the wings and the head was placed to the right of the antennae. Lastly, the abdomen was placed dorsal side up with the hypopygium directed toward the inside of the slide to prevent possible damage to the male sex organs during the placement of the cover slip. Euparal was thinned with absolute ethanol and the completed slide was placed on a slide-drying table for 2 weeks. After drying, another drop of euparal was added to the dissected specimen, which was then cover-slipped

TABLE 2. Agilent 7500ce® ICP-MS operating conditions.

Parameter	Specification
Plasma RF power	1500 W
Sample depth	8.0 mm from load coil
Carrier gas flow	0.82 L/min
Sample flow rate	400 μ L/min
Spray chamber temperature	2 °C
Nebulizer	Glass expansion micromist
Interface	Nickel sample and skimmer cones

using a 12-, 15-, or 18-mm-diameter round coverslip depending on specimen size. Completed slides were left to dry for 4 weeks before identification. Identification of chironomid males to the species level was completed by Dr. James E. Sublette (late Emeritus Professor of Biology, Colorado State University–Pueblo). After identification, all slides had a locality-collection data label placed to the left of the coverslip and an identification label to the right of the coverslip as documented by Kleinert (2008) and Powell (2008).

All slides of adult chironomids from this and other Colorado projects in the SJH collection were deposited as voucher specimens in the James E. and Mary F. Sublette Collection of Chironomidae at the University of Minnesota (UM). In addition, all UV and sweep-net collections from the Fountain Creek Watershed study were deposited at the UM.

Collection and Analysis of Selenium in Water Samples

Water samples were collected from stream transects at each of 14 sites during a 10-d period from 28 March to 7 April 2007 and again in the fall during a 10-d period extending from 24 October to 3 November 2007. During each period, duplicate samples were obtained at each of the 14 sites on day 1, day 5, and day 10. Field measurements were made at each site at the time of collections: dissolved oxygen, temperature, pH, and electrical conductivity. Total hardness was measured in the laboratory. Results of physical characteristic measurements are available in Herrmann et al. (2012). Additional variables measured in concurrent studies in Fountain Creek were included in the analysis of data discussed later. Total water Se (unfiltered) samples were obtained using a high-density polyethylene syringe. Dissolved Se water samples were filtered by passing the water through an

Acrodisc® with a pore size of 0.45 μ m. Interstitial (pore) water was obtained from each site using a vacuum-operated extractor similar to that described by Winger and Lasier (1991). The extractor sampler was constructed using a 2-m linear polypropylene tube (12 mm outer diameter, 10 mm inner diameter) coiled in a loop and secured to a maple dowel frame for strength. A 60-mL plastic syringe was cemented to the upper end of the sampling tube with silicone and used to create a vacuum to draw water into the tube. The sampling end was cut at a 45° angle to facilitate the sampling of interstitial water in coarse and fine gravels to an approximate depth of 12 cm. The water was then extruded into a 1-L low-density polyethylene Cubitainer®. All water samples were analyzed for Se (Se-78) using an Agilent® 7500ce ICP-MS, following EPA Method 200.8 (USEPA 2008). Multielement environmental external calibration standards and internal standards were purchased from Inorganic Ventures® and diluted in 1% nitric and 0.5% hydrochloric acid prior to analysis. Operating conditions of the Agilent 7500ce ICP-MS are shown in Table 2.

It should be noted that concurrent studies were in progress in the Fountain Creek Watershed. For example, as part of a study of Se in fish tissues conducted in 2007 through 2009, collections of water for analysis of Se were taken at differing seasons and locations (Nimmo et al. 2016). Therefore, we believe it pragmatic to rely on data derived from the two 10-day sampling periods of 2007 described above in which both surface and pore waters were collected. Specifically, we believe that Se in the pore waters derived from within the layers of silt and clay fractions of the sediment is an important consideration in the life history of midges because larvae and pupae are cohabitants of these sediment fractions.

Sediment Collection and Sieving Methods

Three sediment samples were collected in March 2012 across a bank-to-bank transect at each of the 14 sites when Fountain Creek was at a base flow of approximately 2.3 m³/s at site LF-4 (USGS station number 07106500) (USGS 2017). Each sample was collected in a polyethylene terephthalate (PETE) bottle measuring 122 mm in height by 64 mm in diameter, for a total volume of 280 mL. The

mouth of each bottle was forced completely into the sediment until it was full, then it was removed from the surface and sealed. Each bottle was marked by site-sample number, dated, then placed into plastic freezer bags for transport to the laboratory. Each sample was placed in enamel pans and dried at 75 °C for 48 h. After drying, each sample was sieved through 8 standardized grain-sized pans described by Wentworth (1922). Stacked sieves separated the samples into the following fractions: small cobble (65–257 mm), very coarse gravel (32–64 mm), coarse gravel (16–32 mm), medium gravel (8–16 mm), fine gravel (4–8 mm), very fine gravel (2–4 mm), coarse to fine sand (0.07–3 mm), and finally silt and clay (<62.5 µm; S/C fraction). Each fraction was placed on a plastic pan and weighed to the nearest 0.01 g using a Denver Instrument® XL-3100 balance. The final percentages of each sediment fraction were averaged for each site.

Data Analysis

Physicochemical variables selected for inclusion in the data analysis required versions or iterations of analysis using canonical correspondence (CCA) (ter Braak 1996, Ruse et al. 2000, ter Braak and Smilauer 2002). CCA is a multivariate ordination technique that extracts major gradients or components among combinations of variables in a data set, and ordination requires at least 2 or more variables, significant or not. CCA requires that the individual factors are random and independent and that variables are consistent within the sample site and error-free (McGarigal et al. 2000). One hundred and fifty-one species of midges (Table 3) and 25 physicochemical variables were used in binary analysis and CCA. Variables included substrates such as small cobble, very coarse gravels, coarse gravels, medium gravels, fine gravels, very fine gravels, coarse to fine sand, and the silt/clay (S/C) fraction (Herrmann et al. 2016b). Additional variables were elevation, temperature, dissolved oxygen, pH, electrical conductivity, total phosphorus, total Se, dissolved Se, pore water Se, log total Se, log dissolved Se, and log pore water Se. Five elements (Ca, K, Mn, Cu, and As) were also included as variables in the initial analysis because of their association with various point and nonpoint factors in the watershed.

Because of variables that were significant from the list above, the final analysis iterations included the following: log total Se, log dissolved Se, log pore water Se, and pH, again tested with the 151 species of midges. Because larval and pupal midges live in the interstitial-sedimentary matrix, the silt/clay (S/C) fraction was included in the final analysis even though it did not show significance initially. CCA analysis allows the retesting of a variable, whether it is significant or not. We included it because it seemed reasonable that pore water Se would be associated with the S/C sediment fraction.

Correspondence can be a positive or negative relationship, and in this study we have attempted to present results as being positive associations to the variables of interest. However, there could be negative or toxicological results or associations of some variables to increased Se, acidity, or some other unknown factor in the watershed.

Because the number of variables was quite large in the final analysis (151 midge species and 25 physicochemical attributes; Tables 3, 4), the evaluation of the ordination diagrams (biplots) was complicated, appearing indeterminate. An example of the complexity of one final CCA analysis is shown in Fig. 2, which shows the following data: fall total (unfiltered) Se in water, the pH, and the S/C fraction. After many of the species codes in Fig. 2 were deleted, the diagram became more easily interpreted and understood; therefore the diagram is shown again as Fig. 10. One advantage of reducing species codes is that it helps to identify key indicator species (to be discussed later) that have shown positive responses to Se. Another advantage is that the process helps to identify species codes that show negative responses toward Se. For example, the species codes TE_O and CL_C are visible in Fig. 2 and show a positive response to Se, but other species codes showing a positive response to Se are not visible because of intrusion by many other codes. Similarly, the RT_3 species code, showing a negative response to Se, is not visible in Fig. 2 but is visible in Fig. 10, along with 2 additional species codes that show a negative response to Se. The method used to reduce the number of species codes was as follows: key indicator species codes showing either positive or negative responses toward

TABLE 3. List of chironomids (including codes of reference, collecting sites in Fountain Creek, Colorado, and taxonomy) from 14 sites in the Fountain Creek Watershed. One (1) indicates species presence at a site and zero (0) indicates absence.

Code	Subfamily/tribe	Scientific binomial	MC-1	MC-2	MC-3	MC-4	MC-5	UF-1	UF-2	UF-3	UF-4	LF-1	LF-2	LF-3	LF-4	LF-5
CH_A	Chironominae	<i>Chironomus atrella</i>	0	0	0	1	0	0	0	0	0	0	1	0	0	1
CH_D	Chironominae	<i>Chironomus decorus</i>	1	1	1	1	1	1	0	1	1	1	1	1	1	1
CH_DL	Chironominae	<i>Chironomus dilutus</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0
CH_M	Chironominae	<i>Chironomus matorus</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0
CH_S	Chironominae	<i>Chironomus staegeri</i>	1	0	1	0	0	0	0	0	0	0	0	0	0	1
CH_U	Chironominae	<i>Chironomus utahensis</i>	0	1	0	0	0	0	0	0	0	0	0	0	1	0
CH_W	Chironominae	<i>Chironomus whitseli</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0
CO_CO5	Chironominae	<i>Chironomus</i> n. sp. CO-5	0	0	0	0	0	0	0	0	0	1	0	0	0	0
CH_CO10	Chironominae	<i>Chironomus</i> n. sp. CO-10	0	0	1	0	0	0	0	1	0	0	0	0	0	0
CH_ns	Chironominae	<i>Chironomus</i> n. sp.	1	0	0	0	0	0	0	1	1	0	0	0	0	0
CL_E	Chironominae	<i>Cladopelma edwardsi</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	1
CL_V	Chironominae	<i>Cladopelma viridula</i>	0	1	1	0	0	0	0	0	0	1	0	0	0	0
CY_D	Chironominae	<i>Cryptochironomus digitatus</i>	0	1	1	0	0	0	0	0	0	0	0	0	0	0
CY_F	Chironominae	<i>Cryptochironomus fulvus</i>	1	1	1	0	0	0	0	0	0	0	0	0	0	1
CY_P	Chironominae	<i>Cryptochironomus parafulvus</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0
CY_n9	Chironominae	<i>Cryptochironomus</i> n. sp. 9	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CT_E	Chironominae	<i>Cryptotendipes emorsus</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0
DT_C	Chironominae	<i>Dicrotendipes crypticus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1
DT_F	Chironominae	<i>Dicrotendipes fumidus</i>	0	0	1	0	0	1	0	1	1	1	0	0	0	1
DT_M	Chironominae	<i>Dicrotendipes modestus</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0
DT_N	Chironominae	<i>Dicrotendipes nervosus</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0
EN_N	Chironominae	<i>Endochironomus nigricans</i>	1	1	1	0	0	0	0	0	1	0	0	0	0	0
G_CO1	Chironominae	<i>Gillotia</i> n. sp. CO-1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
GL_B	Chironominae	<i>Glyptotendipes barbipes</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0
GL_L	Chironominae	<i>Glyptotendipes lobiferus</i>	1	0	0	0	0	0	0	0	0	0	1	0	0	0
PC_T	Chironominae	<i>Parachironomus tenuicaudatus</i>	0	0	0	0	1	0	0	0	0	1	1	0	0	0
PP_D	Chironominae	<i>Paracladopelma doris</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PC_ns	Chironominae	<i>Paracladopelma</i> n. sp.	0	0	1	1	0	0	0	0	0	0	1	0	0	0
PC_n1	Chironominae	<i>Paracladopelma</i> n. sp. CO-1	0	0	0	0	0	0	0	0	0	0	0	0	0	1
PT_S	Chironominae	<i>Paratendipes subaequalis</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PH_P	Chironominae	<i>Phaenopsectra profusa</i>	0	1	1	0	0	0	1	1	1	0	1	1	0	0
PP_D	Chironominae	<i>Polypedilum digitifer</i>	0	0	1	0	0	0	0	0	0	0	0	0	1	0
PP_E	Chironominae	<i>Polypedilum pedatum</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0
PP_F	Chironominae	<i>Polypedilum flavum</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0
PP_I	Chironominae	<i>Polypedilum illinoense</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	1
PP_LT	Chironominae	<i>Polypedilum laetum</i>	0	0	1	0	0	1	1	1	1	0	0	0	0	1
PP_O	Chironominae	<i>Polypedilum obtusum</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0
PP_P	Chironominae	<i>Polypedilum prolixipartum</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0
PP_SC	Chironominae	<i>Polypedilum scalaenum</i>	1	1	1	1	1	0	0	0	0	1	1	1	1	1
PP_SI	Chironominae	<i>Polypedilum simulans</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0
PP_SU	Chironominae	<i>Polypedilum sulaceps</i>	0	1	0	1	1	0	0	0	0	0	0	0	0	0
PP_n14	Chironominae	<i>Polypedilum</i> n. sp. 14	0	0	0	0	0	0	0	0	0	0	0	0	1	0
PS_R	Chironominae	<i>Pseudochironomus richardsoni</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0
RB_C	Chironominae	<i>Robackia claviger</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	0
SA_T	Chironominae	<i>Saetheria tylus</i>	0	1	1	1	1	0	0	1	1	1	1	1	1	1
SA_n1	Chironominae	<i>Saetheria</i> n. sp. 1	0	0	1	1	1	0	0	0	1	1	1	0	0	0
ST_A	Chironominae	<i>Stichtochironomus annulicrus</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0
ST_V	Chironominae	<i>Stichtochironomus varius</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0
XC_B	Chironominae	<i>Xestochironomus brunneus</i>	0	0	0	0	0	0	0	0	0	0	1	1	0	1
CL_C	Chironominae	<i>Cladotanytarsus cruscus</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	1
CL_F	Chironominae	<i>Cladotanytarsus fusiformis</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0
CL_V	Chironominae	<i>Cladotanytarsus viridiventris</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0
CL_7	Chironominae	<i>Cladotanytarsus</i> n. sp. 7	1	0	0	0	0	0	0	0	0	0	0	0	0	0
MI_L	Chironominae	<i>Micropsectra logani</i>	0	0	0	0	1	0	0	0	0	0	0	1	0	0
MI_N	Chironominae	<i>Micropsectra nigripila</i>	0	1	1	0	1	0	0	1	0	0	0	1	1	0
MI_P	Chironominae	<i>Micropsectra polita</i>	1	1	1	0	0	1	1	1	0	0	0	0	0	0
MI_R	Chironominae	<i>Micropsectra recurvata</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0
PT_3	Chironominae	<i>Paratanytarsus</i> n. sp. OH-3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
RT_3	Chironominae	<i>Rheotanytarsus</i> n. sp. 3	1	1	0	0	0	0	0	0	0	0	0	0	0	0
TT_A	Chironominae	<i>Tanytarsus acifer</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0
TT_H	Chironominae	<i>Tanytarsus hastatus</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0

TABLE 3. Continued.

Code	Subfamily/tribe	Scientific binomial	MC-1	MC-2	MC-3	MC-4	MC-5	UF-1	UF-2	UF-3	UF-4	LF-1	LF-2	LF-3	LF-4	LF-5
TH_E	Orthocladiinae	<i>Thienemanniella elana</i>	0	0	0	1	0	0	0	0	0	0	0	1	0	0
TH_X	Orthocladiinae	<i>Thienemanniella xena</i>	1	1	0	0	1	0	0	1	0	0	0	1	0	0
TH_7	Orthocladiinae	<i>Thienemanniella</i> n. sp. 7	0	0	1	0	0	0	0	0	0	0	0	0	0	0
TH_8	Orthocladiinae	<i>Thienemanniella</i> n. sp. 8	0	0	0	0	0	0	1	0	0	0	0	0	0	0
TV_P	Orthocladiinae	<i>Tvetenia paucunca</i>	0	0	0	0	1	1	1	1	1	0	0	1	0	0
TV_V	Orthocladiinae	<i>Tvetenia citracies</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0
PA_K	Podonominae	<i>Parochlus kiefferi</i>	0	0	0	0	0	1	0	0	0	0	0	0	0	0
OD_F	Prodiamesinae	<i>Odontomesa ferringtoni</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0
PR_O	Prodiamesinae	<i>Prodiamesa olivacea</i>	0	0	0	0	0	0	1	1	0	0	0	0	0	0
AB_I	Tanypodinae	<i>Ablabesmyia illinoensis</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0
AB_M	Tanypodinae	<i>Ablabesmyia mallochi</i>	0	0	1	0	0	0	0	0	0	0	0	1	0	1
AB_MO	Tanypodinae	<i>Ablabesmyia monilis</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0
AB_P	Tanypodinae	<i>Ablabesmyia pulchripennis</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0
AP_F	Tanypodinae	<i>Apsectrotanytus florens</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0
CO_C	Tanypodinae	<i>Coelotanytus concinnus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CO_P	Tanypodinae	<i>Conchapelopia pallens</i>	0	1	0	1	1	0	0	0	0	1	1	0	0	0
CP_P	Tanypodinae	<i>Conchapelopia telema</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0
CP_ns	Tanypodinae	<i>Conchapelopia</i> n. sp.	0	0	0	1	0	0	0	0	0	0	0	0	0	0
LA_L	Tanypodinae	<i>Larsia lyra</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0
PR_B	Tanypodinae	<i>Procladius bellus</i>	1	0	1	0	0	0	0	1	0	1	0	0	1	1
PR_C	Tanypodinae	<i>Procladius culciformis</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0
PR_F	Tanypodinae	<i>Procladius freemani</i>	0	0	1	1	1	0	0	1	0	1	0	0	0	0
PR_S	Tanypodinae	<i>Procladius sublettei</i>	0	0	1	0	0	0	0	1	0	0	0	0	1	0
TA_N	Tanypodinae	<i>Tanytus neopunctipennis</i>	0	0	0	0	0	0	0	0	0	1	0	0	0	0
TA_P	Tanypodinae	<i>Tanytus punctipennis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1
TA_S	Tanypodinae	<i>Tanytus stellatus</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0
TE_O	Tanypodinae	<i>Telopelopia okoboji</i>	0	0	0	0	0	0	0	0	0	0	0	0	1	1
TH-BA	Tanypodinae	<i>Thienemannimyia barberi</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0

TABLE 4. Mean selenium (Se) concentrations ($\mu\text{g/L}$), pH, and silt/clay (% fraction/dry weight) at each site used in the canonical correspondence analysis (CCA) of chironomid species (and habitat) data, 2007. Higher Se concentrations in the Lower Fountain Creek Watershed, Colorado, are shown in red.

Site ^a	Spring averages at each site ($n = 9$)					Fall averages at each site ($n = 9$)			
	DSe ^b	TSe ^b	PSe ^b	pH	Silt/clay ^c	DSe	TSe	PSe	pH
MC-1	0.21	0.22	0.71	7.4	0.49	0.18	0.21	0.38	7.9
MC-2	0.28	0.29	0.29	7.5	0.25	0.33	0.35	0.23	8.0
MC-3	0.34	0.36	0.37	7.2	0.07	0.60	0.56	1.20	8.1
MC-4	0.44	0.44	0.76	7.5	0.09	0.86	0.83	0.83	8.0
MC-5 ^d	1.86	1.87	3.69	7.4	0.06	5.28	4.68	2.44	8.1
UF-1	0.19	0.21	0.05	7.2	0.23	0.06	BDL	0.24	8.2
UF-2	0.14	0.16	0.12	7.2	0.25	0.01	0.04	0.26	8.1
UF-3	0.67	0.66	0.05	7.6	0.21	0.04	0.07	0.58	8.1
UF-4 ^d	1.32	1.34	0.19	7.6	0.89	0.34	0.36	0.56	8.1
LF-1 ^d	2.05	2.00	6.31	7.5	0.08	2.97	2.67	8.42	8.1
LF-2	2.78	2.85	3.12	7.5	0.53	3.77	3.37	3.19	8.1
LF-3	3.29	3.12	3.26	7.6	0.74	3.80	3.30	3.16	8.0
LF-4	9.69	9.46	16.14	7.7	0.0	18.59	16.04	20.27	8.1
LF-5	7.91	7.72	11.66	7.8	0.04	14.05	12.25	8.62	8.1

^aMC = Monument Creek; UF = Upper Fountain Creek; LF = Lower Fountain Creek (see Fig. 1).^bDSe = dissolved selenium; TSe = total selenium; PSe = pore water selenium.^cSingle sampling of silt/clay ($n = 3$).^dThe confluence of sites MC-5 and UF-4 is immediately above site LF-1.

Se (or pH) in the majority of the biplots were identified and retained, then the overall shape of the original midge-community clusters was preserved by eliminating many

stacked or linked codes as suggested by ter Braak and Smilauer (2002). It is important to note that the CCA analyses were still based on all 151 midge species and only the species

Fig. 2. An example of an ordination diagram that appears cluttered and possibly indeterminate when a large data set is used in the analysis. In this case, 151 midge species codes and 25 physicochemical variables were used. The diagram is shown again as Fig. 10 with many species eliminated for clarity. Both figures represent the fall total (unfiltered) Se in water, pH, and silt/clay at 14 sites in the Fountain Creek Watershed, Colorado. TE_O is an example of a species code situated directly on the “Log-Tot. Se” arrow. This species is named *Telopelopia okoboji* and was only found at sites LF-4 and LF-5. Both sites have elevated Se in the total, dissolved, and pore water fractions of the water.

Fig. 3. Reference ordination diagram showing differences in the spring total (unfiltered) Se in water, pH, and silt/clay at 14 sites in the Fountain Creek Watershed, Colorado. The multivariate analysis (CCA) suggested that sites 13 (LF-4) and 14 (LF-5) (see Fig. 1) had the highest concentrations of total Se in water (Table 4). The vector tagged “Log-Tot. Se” is the log of the total Se fraction in water.

codes were removed for easier interpretations of biplots.

The odd-numbered figures (Figs. 3, 5, 7, 9, 11, and 13) show the reference physicochemical

variables used in the analysis as red ordination axes. Sampling sites are indicated as black circles with site numbers; each can be noted in Fig. 1 with corresponding descriptions

Fig. 4. Species ordination diagram showing the relationships of chironomid species in the spring total (unfiltered) Se in water ($P = 0.0006$), pH ($P = 0.7367$), and silt/clay ($P = 0.4283$) at 14 sites in the Fountain Creek Watershed, Colorado. The vector tagged “Log-Tot. Se” is the log of the total Se fraction in water. The abbreviations TT_PA, TE_O, TA_P, and CL_C are indicated in green and represent the midge species *Tanytarsus pallidicornis*, *Telopelopia okoboji*, *Tanytarsus punctipennis*, and *Cladotanytarsus cruscus*, respectively.

Fig. 5. Reference ordination diagram showing differences in the spring dissolved (filtered) Se in water, pH, and silt/clay at 14 sites in the Fountain Creek Watershed, Colorado. The multivariate analysis (CCA) suggested that sites 13 (LF-4) and 14 (LF-5) (see Fig. 1), had the highest concentration of dissolved Se in water (Table 4). The vector tagged “Log-Diss. Se” is the log of the dissolved Se fraction in water.

in Table 1. The even-numbered figures (Figs. 4, 6, 8, 10, 12, and 14) each show midge species associated with the physicochemical variables discussed above. These data are represented by black-letter species codes overlaid on the red ordination axes. Each midge black-letter code represents a principal point associated with the 3 physicochemical variables. The first set of 6 ordination diagrams (3 physicochemical

and 3 species-association ordination diagrams) were generated from data collected in the spring, whereas the second set of 6 were generated from data collected in the fall. Our analysis approach considered the following: immature stages of the male chironomid larvae and pupae midges in Fountain Creek during their development had to be associated with Se concentrations in 3 zones of interaction:

Fig. 6. Species ordination diagram showing the relationships of chironomid species to the spring dissolved (filtered) Se in water ($P = 0.0006$), pH ($P = 0.7367$), and silt/clay ($P = 0.4276$) at 14 sites in the Fountain Creek Watershed, Colorado. The vector tagged “Log-Diss. Se” is the log of the dissolved Se fraction in water. The abbreviations TT_PA, TE_O, TA_P, and CL_C are indicated in green and represent the midge species *Tanytarsus pallidicornis*, *Telopelopia okoboji*, *Tanytus punctipennis*, and *Cladotanytarsus cruscus*, respectively.

Fig. 7. Reference ordination diagram showing differences in spring Se in pore water, pH, and silt/clay at 14 sites in the Fountain Creek Watershed, Colorado. The multivariate analysis (CCA) suggested that sites 13 (LF-4) and 14 (LF-15) (see Fig. 1) had the highest concentrations of Se in the pore water (Table 4). The vector tagged “Log-Pore Se” is the log of the Se in the pore water fraction.

(1) total Se in the water column with particulates (unfiltered), (2) dissolved Se in the water column without particulates (filtered), and (3)

pore water extracted from the S/C fraction of the sediments. The occurrence of midges also was associated with differing pH at 14 sites and at 2 different seasons—spring and fall.

RESULTS AND DISCUSSION

Chironomid Subfamilies, Genera, and Species including New Species

Based on the chironomid species found in the Fountain Creek Watershed, the stream appears to be unusually productive. A total of 714 adult male chironomid individuals were collected in this study: 309 in 2007 and 405 in 2008 for a total of 151 species (Table 3) representing 65 genera and 6 subfamilies (Chironominae, Diamesinae, Orthocladiinae, Podonominae, Prodiamesinae, and Tanypodinae [Herrmann et al. 2016b]). The Orthocladiinae and Chironominae were the dominant subfamilies at 43% and 42% of the total 151 species, respectively, followed by Tanypodinae at 13%, Prodiamesinae at 1%, Podonominae at <1%, and Diamesinae at <1%. The most new species were collected at sites MC-1 and MC-3, with a total of 16% each over 2 years; site MC-2 had 11% of the overall total of 24 new species. Species listed in Table 3 are from both UV light trap and sweep-net collections. It is

Fig. 8. Species ordination diagram showing the relationships of chironomid species to the spring Se in pore water ($P = 0.0034$), pH ($P = 0.7469$) and silt/clay ($P = 0.4017$) at 14 sites in the Fountain Creek Watershed, Colorado. The vector tagged “Log-Pore Se” is the log of the Se in the pore water fraction. The abbreviations TT_PA, TE_O, TA_P and CL_C are indicated in green and represent the midge species *Tanytarsus pallidicornis*, *Telopelopia okoboji*, *Tanytarsus punctipennis*, and *Cladotanytarsus cruscus*, respectively.

Fig. 9. Reference ordination diagram showing differences in the fall total (unfiltered) Se in water, pH, and silt/clay at 14 sites in the Fountain Creek Watershed, Colorado. The multivariate analysis (CCA) suggested that sites 13 (LF-4) and 14 (LF-5) (see Fig. 1) had the highest concentration of total Se in total (unfiltered) water (Table 4). The vector tagged “Log-Tot. Se” is the log of the total Se fraction in water.

Fig. 10. Species ordination showing the relationships of chironomid species to the fall total (unfiltered) Se in water ($P = 0.0500$), pH ($P = 0.0100$), and silt/clay ($P = 0.5465$) at 14 sites in the Fountain Creek Watershed, Colorado. The vector tagged “Log-Tot. Se” is the log of the total Se fraction in water. The abbreviations TT_PA, TE_O, TA_P, and CL_C are indicated in green and represent the midge species *Tanytarsus pallidicornis*, *Telopelopia okoboji*, *Tanytus punctipennis*, and *Cladotanytarsus cruscus*, respectively.

Fig. 11. Reference ordination diagram showing differences in the fall dissolved (filtered) Se in water, pH, and silt/clay at 14 sites in the Fountain Creek Watershed, Colorado. The multivariate analysis (CCA) suggested that sites 13 (LF-4) and 14 (LF-5) (see Fig. 1) had the highest concentrations of dissolved Se in water (Table 4). The vector tagged “Log-Diss. Se” is the log of the dissolved Se in water.

particularly noteworthy that the totals from 2 sites (LF-3 [3 Sep 2007] and MC-5 [6 Sep 2007]) for each method were different in composition with no duplication of species

(Herrmann et al. 2016b). An example of a list from a single site without duplication was site MC-5 (Table 5). If only a single method had been used to collect the insects, many species would not have been represented, suggesting that both methods were important when evaluating numbers of adult midges at specific sites and times. Based on sampling methods discussed earlier, we are confident that our adult midge collections were representative of each of the reported sites with all their individual microhabitats. We note that the collection protocols were a collaboration between coauthors JES and SJH, and the latter investigator carried out the sampling at each site in the watershed.

According to Herrmann et al. (2016b) and the species list in Table 3, there were only 21 species that occurred in all 3 stream segments of the watershed. Of the 21 species, 6 were in the family Chironominae, 12 in Orthocladinae, and 3 in Tanypodinae. *Chironomus decorus* Johannsen 1905 was present at 13 of the 14 sites (Table 3), being absent only at UF-2, a coldwater site in the Upper Fountain Creek segment. To provide perspective for the variety of chironomid species in the watershed, Herrmann et al. (2016b) observed that the species and generic richness in the watershed was greatest at the

Fig. 12. Species ordination diagram showing the relationships of chironomid species to the fall dissolved (filtered) Se in water ($P = 0.0044$), pH ($P = 0.0100$) and silt/clay ($P = 0.5083$) at 14 sites in the Fountain Creek Watershed, Colorado. The vector tagged "Log-Diss. Se" is the log of the total Se fraction in water. The abbreviations TT_PA, TE_O, TA_P, and CL_C are indicated in green and represent the midge species *Tanytarsus pallidicornis*, *Telopelopia okoboji*, *Tanytarsus punctipennis*, and *Cladotanytarsus cruscus*, respectively.

Fig. 13. Reference ordination diagram showing differences of fall Se in the pore water, pH, and silt/clay at 14 sites in the Fountain Creek Watershed, Colorado. The multivariate analysis (CCA) suggested that sites 13 (LF-4) and 14 (LF-5) (see Fig. 1) had the highest concentrations of Se in the pore water (Table 4). The vector tagged "Log-Pore Se" is the log of the Se in the pore water fraction.

uppermost Monument Creek site, MC-1 (40 species in 25 genera), and lowest in the upper segment of Fountain Creek at UF-2 (12 species in 11 genera). The Monument Creek sites (MC) had the greatest species richness and generic diversity (96 species in 45 genera), followed by the Lower Fountain Creek sites (LF) (76 species in 41 genera), and lastly the Upper Fountain Creek (UF) segment sites (53 species in 30 genera). The numbers of species at the elevational extremes of the watershed are of interest with respect to midge distribution in Fountain Creek (Table 6). For example, fewer species and genera were at the highest and lowest elevations, and most species and genera were at site MC-1 in the Monument Creek tributary, which is at the approximate elevational midpoint within the watershed. The most common group at higher elevations in the stream segments (UF-1 and MC-1) was the subfamily Orthocladinae. However, we

Fig. 14. Species ordination diagram showing the relationships of chironomid species to the fall Se in pore water ($P = 0.1266$), pH ($P = 0.0100$), and silt/clay ($P = 0.5467$) at 14 sites in the Fountain Creek Watershed, Colorado. The vector tagged “Log-Pore Se” is the log of the Se in the pore water fraction. The abbreviations TT_PA, TE_O, TA_P, and CL_C are indicated in green and represent the midge species *Tanytarsus pallidicornis*, *Telopelopia okoboji*, *Tanytus punctipennis*, and *Cladotanytarsus cruscus*, respectively.

TABLE 5. An example of differences^a in obtaining adult male chironomids using 2 collection methods, UV light and sweep netting, at site MC-5 on 6 September 2007.

Ultraviolet night lighting ^a	Sweep net collection ^a
<i>Chironomus decorus</i>	<i>Saetheria tylus</i>
<i>Polypedilum scalanum</i>	<i>Corynoneura arctica</i>
<i>Saetheria</i> n. sp. 1	<i>Cricotopus blini</i>
<i>Stichtochironomus annulicrus</i>	<i>Eudiefferiella coerulescens</i>
<i>Cricotopus infuscatus</i>	<i>Euorthocladius rivicola</i>
<i>Cricotopus trifasciatus</i>	<i>Limnophyes margaretae</i>
<i>Ablabesmyia illinoensis</i>	<i>Thienemanniella xena</i>
<i>Conchapelopia pallens</i>	<i>Micropsectra logani</i>
	<i>Micropsectra nigripila</i>

^aResults of the 2 sampling methods that resulted in totally different species composition with no duplications at the MC-5 site. MC-5 is located in Colorado Springs, Colorado.

did not find a significant correspondence or association between the elevation variable and the midges in this study. We found it interesting that Ferrington (1998), using cluster analysis, reported that chironomid genera collected in Fountain Creek at our site LF-3 (elevation 1524 m) in Colorado were also found in Wyoming at a site 8 miles east of the Devil’s Tower Monument (elevation 1372 m).

Results of Canonical Correspondence Analysis

Using the discussion of ter Braak (1996) and many of his descriptors in addition to our analysis of midges and site points, we found significant dominant patterns in midge community composition that can be explained by the environmental variable points and associated species codes (Figs. 3–14).

To our knowledge, the following data are the first to suggest that portions of midge assemblages are tolerant of or have adapted to higher Se concentrations in the lower Fountain Creek Watershed in Colorado (shown as bolded red values in Table 4). It is important to note that Se concentrations stepped up significantly in the watershed at site MC-5 (Monument Creek tributary) and continued to increase downstream at LF-1 through LF-5, the 5 sites that constitute the meandering sandy-bottom segments of the stream. Also, we found Se to be exceptionally high at sites LF-4 and LF-5, both within the City of Pueblo, Colorado (Table 4, Fig. 1). The occurrence of midges was strongly associated with the

TABLE 6. Comparison of species and genera of midges at extremes of elevation in the Fountain Creek Watershed, Colorado (Fig. 1).

Site	Elevation (m)	Species	Genera	Most common group
UF-1 Upper Fountain Creek	2335	9	9	Orthocladiinae
MC-1 Monument Creek ^a	2097	17	15	Orthocladiinae
LF-5 Lower Fountain Creek	1415	11	11	Chironominae

^aMC-1 in Monument Creek was the uppermost site in the Monument Creek tributary segment.

spring total (unfiltered) Se ($P = 0.0006$), dissolved (filtered) Se ($P = 0.0006$), and pore water Se ($P = 0.0034$) (data are represented graphically in Figs. 4, 6, and 8). In addition, the total Se fraction in the fall season was a significant factor ($P = 0.0500$); dissolved Se exhibited strong significance ($P = 0.0044$); but pore water Se was only weakly significant ($P = 0.1266$) (Figs. 10, 12, 14). It is notable that Se concentrations were similar in all 3 water fractions (Table 4), although the concentrations tended to be higher in the pore water fraction in the spring. The variability of Se measured in the pore water fractions during the spring and fall samplings could have resulted in the weaker P values at sites LF-4 and LF-5 (Table 4). We noted that Se concentrations were markedly higher at the 2 sites in the fall compared to spring, and it is important to recall that larval and pupal midges are found in the S/C fraction of the sediment surrounded by pore water.

In addition, pH was a significant factor in the fall, when pH values were generally higher (range 7.9–8.2), but not in the spring (range 7.2–7.8). The P values associated with pH—when considered in conjunction with the total, dissolved, and pore water Se fractions—were all identical ($P = 0.0100$) in the fall (Figs. 10, 12, 14), while the spring P values were similar but not significant (0.7367, 0.7367, and 0.7469, respectively) (Figs. 4, 6, 8).

The occurrence of midges was not significantly associated with the presence of silt or clay when considered with any of the 3 Se fractions in either spring ($P = 0.4283$, 0.4276, and 0.4017) (Figs. 4, 6, 8) or fall ($P = 0.5465$, 0.5083, and 0.5467) (Figs. 10, 12, 14). In addition, the CCA analysis did not reveal any association of midges to any additional physicochemical factors, such as dissolved oxygen, temperature, electrical conductivity, total hardness, or others listed earlier in the “data analysis” subsection of the methods.

Midge Species as Indicators of Selenium

The positions of 4 individual midge species, *Tanytarsus pallidicornis* (Walker) 1956 [TT_PA], *Telopelopia okoboji* (Roback) 1971 [TE_O], *Tanytus punctipennis* (Meigen) 1818 [TA_P], and *Cladotanytarsus cruscus* (Sæther) 1971 [CL_C], in all 6 ordination diagrams (Figs. 4, 6, 8, 10, 12, and 14, all bolded in green) highlight their positive relationships to Se under the physicochemical conditions discussed above. All 4 species were found only at sites in the lower segment of Fountain Creek and are clustered together in the ordination diagrams. Their positions suggest that the 4 species could serve as surrogates or “indicators” for the watershed community that corresponds to elevated Se in the spring and fall. The 4 species were found at sites in the warm-water segment of the watershed at elevations ranging from 1635 m to 1415 m (Table 1), and all were oriented to increasing concentrations of Se (Table 4).

Distributions of the Four “Indicator” Species in North America

Known distributions of the 4 indicator species in North America could provide understanding about their presence in Fountain Creek. Herrmann et al. (2016b), noted that the Holarctic *Tanytarsus pallidicornis* (TT_PA) appears to be restricted to North America, including New Mexico and Colorado (Sublette and Sublette 1979). This species occurred in Fountain Creek only at station LF-4 (Table 1), a warm-water site with high Se (Table 4) at an elevation of 1433 m. The only other report of *T. pallidicornis* in Colorado was by Ruse et al. (2000), who reported it from a coldwater site in the Arkansas River, Colorado. In contrast, the Nearctic species *Telopelopia okoboji* (TE_O), currently only known in North America, has a much broader distribution in Manitoba, Minnesota, Kansas, Iowa, Ohio, Maryland, Virginia, Texas, and New Mexico (Roback 1971, Sublette and Sublette 1979, Davis 1980, Roback

1981, Oliver et al. 1990). Ruse et al. (2000) found this species in the upper Arkansas River from one warm-water site just upstream of the Pueblo Reservoir. This midge was found in Fountain Creek only at LF-4 and LF-5 (Table 2) at elevations of 1433 m and 1415 m, respectively. The site LF-4 in Fountain Creek is separated by about 1 km from LF-5, the latter located immediately above the confluence of Fountain Creek and the Arkansas River in Pueblo, Colorado. The distribution of *Tanyptus punctipennis* (TA_P), also known only in North America, has a distribution that includes British Columbia, Saskatchewan, Minnesota, Ontario, Quebec, Florida, Georgia, Alabama, Nevada, and California (Roback 1971, 1976, Oliver et al. 1990, Caldwell et al. 1997). This Holarctic and Oriental species, found in the southeastern United States, has been collected from lakes, rivers, and streams (Caldwell et al. 1997) and was found in 8 sites by Roback (1976): 5 lotic sites, including the Potomac River, and 3 lentic sites, such as Lake Erie and a sewage lagoon in Nebraska. Roback (1976) also observed this species in a wide range of ecological conditions from “clean, clear slightly brown” streams to large, turbid rivers. In Fountain Creek, *T. punctipennis* was found only at site LF-5 (Table 1). In earlier samplings, Kleinert (2008) and Powell (2008) collected this species downstream of Pueblo Reservoir; however, Ruse et al. (2000) did not report it from the upper Arkansas River in Colorado. As opposed to the 3 midge species above, *Cladotanytarsus cruscus* (CL_C) has a more limited distribution, being found in South Dakota, New Mexico, and possibly Texas (Oliver et al. 1990). In the present study, it was found at sites LF-2 (1635 m elevation) and LF-5 (1415 m elevation). Interestingly, this species was not reported from the Arkansas River by Ruse et al. (2000), Kleinert (2008), or Powell (2008).

Other candidates for indicator species might correspond to Se in Fountain Creek. An example is *Cardiocladius obscurus* (CC_O), which can be observed in 4 ordination diagrams (Figs. 4, 6, 12, 14). Uniquely, *C. obscurus* has a broad distribution, which was noted by Herrmann et al. (2016b), who reported the species in the Ontario and Quebec provinces of Canada and the U.S. states of Pennsylvania, New York, North Carolina, South Carolina, Georgia, Florida, Mississippi, Wyoming, and Utah. This species was found in Fountain Creek only at

site LF-1, located below the confluence of the Upper Fountain and Monument Creeks, and site LF-5, immediately above the confluence with the Arkansas River. Sites LF-1 and LF-5 bracket the creek segment where Se increases substantially with stream distance (see Table 4). All 4 indicator species discussed above (as well as *C. obscurus*) might be considered “specialists” with regard to increasing Se; however, the midge *Chironomus decorus* appears to be a “facultative generalist” species with respect to the Se gradient because this midge was found at 13 of the 14 sites in the watershed.

Species Found *Only* at Sites where Selenium was Increasing Downstream

Could Se be a necessary factor for some midge autecology in the lower segment of Fountain Creek—that is, could the metalloid be primary for survival? The question became apparent when we were listing midge species that, with some exceptions, were found *only* at their respective (single) sites (Table 3) where total, dissolved, and pore water Se (Table 4) was increasing downstream at each site (Table 7). We observed that as Se began to increase at site MC-5 and continued to increase downstream to LF-5, the number of individual species unique to each site was 4; however, at site LF-5 there were 11 species that were found at no other site if *Telopelopia okoboji* and *C. cruscus* are included (Table 7). We noted that *C. cruscus* occurred at both UF-2 and LF-5, and *T. okoboji* occurred at sites LF-4 and LF-5. In summary, 28 (18.5%) of the 151 species found in our collections were from 6 sites where Se was rising in Fountain Creek. Consequently, it seems reasonable that most or all species listed in Table 7 were positively related to Se in waters with *P* values that ranged from 0.0006 to 0.0034. Four midge species identified earlier in the discussion as possible indicators of a relationship to increased Se, and in some cases a higher pH, are also listed in Table 7. Additionally, we questioned whether increasing Se and a higher pH were synergistic and positive for the midges in the lower segment of the stream in the fall. However, the association of pH with the occurrence of some midge species was significant ($P = 0.0100$). As shown in Table 4, the pH at all 14 sites in the watershed ranged from 7.2 to 7.8 in the spring season, whereas in the fall pH was ≥ 7.9 at all 14 sites in the watershed (Table 4).

TABLE 7. Chironomid midge species found **only**^{a,b} at the site listed in Fountain Creek along with average concentrations (µg/L) of dissolved (DSe), total (TSe), and pore water (PSe) selenium at that site.

Site	Spring Se averages			Fall Se averages			Species
	TSe	DSe	PSe	TSe	DSe	PSe	
MC-5	1.87	1.86	3.69	4.68	5.28	2.44	<i>Stichtochironomus annulicrus</i> ; <i>Ablabesmyla illinoensis</i>
LF-1	2.00	2.05	6.31	2.67	2.97	8.42	<i>Chironomus</i> n. sp. CO-5; <i>Halocladius</i> n. sp. CO-1; <i>Orthocladius doreus</i> ; <i>Tanytus neopunctipennis</i>
LF-2	2.85	2.78	3.12	3.37	3.77	3.39	<i>Chironomus dilutus</i> ; <i>Glyptotendipes barbipes</i> ; <i>Tanytus hastatus</i> ; <i>Ablabesmyla monilis</i> ; <i>Cladotanytarsus cruscus</i>^{a,b}
LF-3	3.12	3.29	3.26	3.80	3.30	3.16	<i>Cryptotendipes emorsus</i> ; <i>Diamesa heteropus</i> ; <i>Odontomesa ferringtoni</i> ; <i>Thienemanniella barberi</i>
LF-4	9.46	9.69	16.14	16.04	18.59	20.27	<i>Polypedilum</i> n. sp. 14; <i>Robackia claviger</i> ; <i>Tanytarsus pallidicornis</i>^b ; <i>Telopelopia okoboji</i>^{a,b}
LF-5	7.72	7.91	11.66	12.25	14.05	8.62	<i>Cryptochironomus</i> n. sp. 9; <i>Dicrotendipes crypticus</i> ; <i>Gillotia</i> n. sp. CO-1; <i>Paracaladepelma</i> n. sp. CO-1; <i>Gorygoneura artica</i> ; <i>Cricotopus annulator</i> ; <i>Thienemanniella boltoni</i> ; <i>Coelotanytus concinnus</i> ; <i>Tanytus punctipennis</i>^b ; <i>Cladotanytarsus cruscus</i>^{a,b} ; <i>Telopelopia okoboji</i>^{a,b}

^a*Cladotanytarsus cruscus* and *Telopelopia okoboji* were included at 2 sites because both species could have a positive association to increased selenium and higher pH.^bThe midge species *Cladotanytarsus cruscus*, *Tanytus pallidicornis*, *Telopelopia okoboji*, and *Tanytus punctipennis* were the 4 identified as possible indicator species (for increased selenium and higher pH) in the ordination diagrams.

Species Found in Low Concentrations of Selenium

Although the preceding narrative suggested positive associations of 5 midge species at sites with increased Se, we were not able to determine a direct opposite response using the CCA analyses, but it is possible that some midge species were negatively affected by an increasing gradient of Se. Some midge species codes (AP_F, PR_O, and RT_3) were conspicuously located away from the origins of the Se vector arrows (Log-Tot. Se, Log-Diss. Se, and Log-Pore Se) and trended in the opposite (negative) direction in the ordination diagrams. Their scientific names are *Apsectrotanytus* (*Radotanytus*) *florens*, *Prodiamesa olivacea*, and *Rheotanytarsus* n. sp. 3, respectively, with the latter being a new species. (The genus *Apsectrotanytus* has been annotated to *Radotanytus*, but we retained the code AP_F in Table 3.) It is possible that these species were negatively affected by Se because all 3 were *only* found at the sites (Table 3) where Se concentrations were low (UF-2, UF-3, MC-1, and MC-2), ranging from 0.21 to 0.71 µg/L (Table 4). We question whether the species above were affected by or avoided habitats downstream at sites with higher Se. Regardless, they were found at the headwater sites with lower Se. Additional information concerning the distributions of the 3 species above is available in Herrmann et al. (2016b) and Oliver et al. (1990).

Conversely, there were midge species widely distributed in Fountain Creek that were unexpectedly absent at other sites (Herrmann et al. 2016b). For example, we noted earlier that *Chironomus decorus* was present at 13 of the 14 sites—absent only at site UF-2, a site in Upper Fountain Creek. Another species, *Cricotopus infuscatus*, was present at 12 of 14 sites and absent at only 2 sites (UF-2 and UF-3), similar to *C. decorus*, in Upper Fountain Creek. A third species, *Saetheria tylus*, was present at 11 of 14 sites and absent at MC-1, a site in the Monument Creek tributary, and UF-1 and UF-2, in Upper Fountain Creek. These sites, UF-1, UF-2, UF-3, and MC-1, are all relatively low in Se concentrations, depending on the season (Table 4). Selenium in various fractions at the sites ranged from below detection limit (BDL) in the fall total (unfiltered) fraction at site UF-1 to 0.67 µg/L in the spring dissolved (filtered) fraction

at UF-3. Could the absence of these 3 midge species be due to a deficiency of Se during certain stages of larval or pupal development?

Historical Records of Elevated Concentrations of Selenium in Fountain Creek

There is little doubt that Chironomidae have been subjected to Se in Fountain Creek for some time, and in-stream concentrations appear to be continuing unabated. The most comprehensive monitoring was apparently begun by Bossong (2001), who reported that during the period of October 1987 to September 1997 in Fountain and Monument Creeks, Se exceeded the 5.0 $\mu\text{g/L}$ standard 49 times. In addition, during the 10 years (1987–1997) of sampling at 4 corresponding sites in Lower Fountain Creek, the dissolved Se concentrations were remarkably similar to those sampled in 2007 in the present study (Table 4). The 10-year grand mean concentrations of dissolved Se at sites LF-1, LF-2, LF-3, and LF-4 (sites in the present study) in the Bossong (2001) report were 4.45, 4.37, 4.83, and 18.90 $\mu\text{g/L}$, respectively. The grand means of Se concentrations from sampling at the same sites in 2007 were 2.05, 2.78, 3.29, and 9.69 $\mu\text{g/L}$ in the spring, respectively, and 2.97, 3.77, 3.80, and 18.59 $\mu\text{g/L}$ in the fall, respectively (Table 4). Further, Van Derveer and Canton (1997) also reported concentrations of Se in Lower Fountain Creek of 6.0 and 7.0 $\mu\text{g/L}$ at site LF-3 and 7.0 and 18 $\mu\text{g/L}$ in Pueblo, Colorado, at site LF-4. Divine and Gates (2006) found that the average concentration in Fountain Creek was approximately 3 $\mu\text{g/L}$ near Fountain, Colorado, (LF-2 in the present study) and increased downstream to approximately 12 $\mu\text{g/L}$ near Pueblo and the confluence of the Arkansas River (sites LF-4 and LF-5). Finally, Canton (2010) reported data from 12 water samples from a site corresponding to LF-5 that ranged from 7.49 to 21.1 $\mu\text{g/L}$ during January 2005 to June 2006. Note that the data of Canton (2010) are quite similar to those at site LF-5 shown in Table 4 of this study.

Negative Influences of Selenium on Midges

Although our data suggested a positive relationship between Se (and pH) and some midge taxa, a review of previous laboratory and laboratory/field studies by others suggest negative influences of Se as a physiochemical

factor. For example, Ingersoll et al. (1990) found that the emergence time of *Chironomus riparius* larvae was delayed at Se concentrations of $\geq 837 \mu\text{g/L}$. The study was conducted in laboratory-simulated test water representative of the water from the Kesterson National Wildlife Refuge, San Joaquin Valley, California, containing a 6:1 mixture of selenate to selenite. Maier and Knight (1993) reported the acute toxicities (LC50s, lethal concentrations to 50% of the test organisms) of waterborne selenate, selenite, and seleno-DL-methionine to the fourth instar *C. decorus* to be 48-h of 23.7, 48.2, and 194 mg/L Se, respectively. Comparative bioconcentration factors after 48 h in the Maier and Knight (1993) study showed that mortality of *C. decorus* larvae exposed to 25 mg Se/L as seleno-DL-methionine was greater than those exposed to 25 mg Se/L as selenate and selenite. Tsu et al. (2012) exposed *C. dilutus* to waterborne selenate, selenite, or seleno-DL-methionine in the laboratory at Se concentrations matching those of lakes receiving mine effluence in Canada. These data suggested that the selenomethionine-like substances were the most readily accumulated forms of Se, whether from diet or water. Gallego-Gallegos et al. (2012) found that elemental Se administered as nanoparticles, as waterborne dissolved Se, or as selenized algae resulted in bioaccumulation of the element at concentrations that inhibited larval growth of *C. dilutus*, regardless of the route of uptake or form of the element. Malchow et al. (1995) found that *C. decorus* individuals that were fed a diet of the seleniferous alga *Selenastrum capricornutum* showed significant reduced larval growth after 96 h. Importantly, their method of Se exposure to the midges was “natural-like” because the algae (as food for the midges) were previously exposed to selenite and selenate concentrations (levels) of 0 (control), 4, 10, and 40 $\mu\text{g Se/L}$. Total waterborne Se concentrations ranged from <1.0 to 14.8 $\mu\text{g Se/L}$. It appears that the Malchow et al. (1995) exposure regime was a combination of waterborne Se and seleniferous algae and thus a reasonable simulation of a Se-rich environment similar to that in Fountain Creek, where midges would have been exposed throughout their life cycles. Another useful comparison to the present study of midges in Fountain Creek was a “feeding study” in which *C. decorus* was

reared on contaminated *Ruppia* substrate from evaporation ponds in California (Alaimo et al. 1994). In the 14-d egg-to-prepupation exposure, the midge instars exhibited reduced growth (based on final mean weights) consistent with increasing Se in the *Ruppia* as the substrate. Furthermore, the waterborne Se in the experimental water during the 14-d exposure ranged from about ≤ 3 to ≤ 32 $\mu\text{g/L}$, bracketing the Se measured in the total, dissolved, and pore water samples from the lower segment of Fountain Creek in the present study (Table 4).

Finding chironomids positively oriented to pH in the fall might indicate the importance of acidity in chironomid communities in other environments. The first study mentioned below (Mousavi 2002) suggests that pH has a role in the abundance or paucity of midge communities in boreal lakes, and a second study (Krantzberg and Stokes 1988) shows the importance of surface adsorption and pH in metal accumulation by chironomids. Mousavi (2002), using CCA analysis and data sets from 38 North American and 43 Northern European localities, indicated that Diamesinae and Prodiamesinae subfamilies (coauthor JES separates these 2 subfamilies) were good indicators of large, deep lakes with higher pH. In contrast, Mousavi (2002) also found the subfamily Orthocladiinae to be an indicator of relatively small lakes with low pH and conductivity, while Tanytarsini occurred in lakes with relatively high pH. Krantzberg and Stokes (1988) found that midge larvae from a lake of pH 4.4 showed an initial rapid phase of accumulation of Cd, Al, Mn, Ni, Zn, and Cu when transplanted to sediments from a lake of pH 5.1. Laboratory and field study supported the hypothesis that surface adsorption contributed to total metal content in the insects and their response to pH. This observation raises the question of whether Se (not reported in the study by Krantzberg and Stokes 1988) could adsorb to the exoskeletons of the midges at a higher pH, as did the other metals mentioned in their results.

We question whether assemblages of larval or pupal midges in Fountain Creek would have accumulated Se in direct proportion to Se in lotic waters because Birkner (1978) previously found a direct relationship between Se in the tissues of chironomids and the Se levels in lentic waters from 30 ponds, lakes, and

reservoirs in Se-rich areas of Colorado and Wyoming ($R^2 = 0.91$). Certainly, adult midges collected in Fountain Creek occurred at sites where Se was at the highest concentrations. We suggest that 6 sites in the Fountain Creek Watershed (MC-5, LF-1 to LF-5) would provide a suitable Se gradient for such a study of larval or pupal stages collected there.

CONCLUSIONS

In this study, we used canonical correspondence analysis (CCA)—a statistical technique used to assess large databases—to analyze 25 variables that might be affecting 151 species of chironomid midges in a complex natural watershed. After preliminary iterations, the following variables were selected for further analysis: total, dissolved, and pore water Se concentrations; pH; and silt/clay fraction of the sediments. The outcome indicated that Se, whether as total, dissolved, or pore water Se, was a significant factor in midge assemblages along a gradient (upstream to downstream) in Fountain Creek, Colorado. And, to a lesser degree of significance, higher pH in the fall, but not in the spring, also contributed to midge assemblages. We did not find the silt/clay fraction to be a significant factor affecting the midge community. We found no reports in the literature of a negative relationship of chironomids to Se unless test concentrations in laboratory or field-controlled experiments were considerably greater than those measured in Fountain Creek, Colorado. Previous laboratory or field studies wherein the element was at higher concentrations revealed reduced growth, delayed development, or sometimes an increase in the length of time from emergence to the adult stage for midges. However, in the CCA analysis there was a hint that an overabundance of Se could have a negative effect on some midges at some sites in Fountain Creek. Still, there were signs that Se insufficiency at some sites could have been limiting to other species. Chironomid assemblages might include Se-tolerant taxa in locations where streams are underlain with soils and substrates high in Se and subjected to perturbations such as fires or deforestation (leading to flooding), unbridled urbanization, increasing agriculture, or mine extraction. Areas surrounding Fountain Creek, Colorado, have been experiencing a combination of urban

growth in a watershed underlain with ancient marine Se-bearing shales. Therefore, it would seem instructive to conduct additional studies to ascertain whether the apparent influences of Se on chironomid midges in Fountain Creek are occurring in other basins in the western United States known to have soils and substrates with elevated Se. Would an accumulation gradient of Se in midge larvae, pupae, or adult tissues parallel an increasing gradient of Se in a stream? A site in the Monument tributary and 5 sites in Fountain Creek might be an appropriate setting to address this essential question dealing with an important component of an aquatic food chain.

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