Prepared in cooperation with Colorado Springs City Engineering and Colorado Springs Utilities

Comparability among Four Invertebrate Sampling Methods, Fountain Creek Basin, Colorado, 2010–2012

Scientific Investigations Report 2014–5049

U.S. Department of the Interior
U.S. Geological Survey

COVER. Fountain Creek downstream of the Owens-Hall Diversion Dam, Colorado Springs, Colorado. Photo taken by David Walters, U.S. Geological Survey Fort Collins Science Center.

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Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
square mile (mi^2)	2.590	square kilometer (km^2)
square foot (ft^2)	0.09290	square meter (m^2)
square meter (m^2)	10.76	square foot (ft^2)

Vertical coordinate information is referenced to the North American Vertical Datum of 1988 (NAVD 88).

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Elevation, as used in this report, refers to distance above the vertical datum.

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Abstract

The U.S. Geological Survey, in cooperation with Colorado Springs City Engineering and Colorado Springs Utilities, designed a study to determine if sampling method and sample timing resulted in comparable samples and assessments of biological condition. To accomplish this task, annual invertebrate samples were collected concurrently using four sampling methods at 15 U.S. Geological Survey streamflow gages in the Fountain Creek basin from 2010 to 2012. Collectively, the four methods are used by local (U.S. Geological Survey cooperative monitoring program) and State monitoring programs (Colorado Department of Public Health and Environment) in the Fountain Creek basin to produce two distinct sample types for each program that target single-and multiple-habitats. This study found distinguishable differences between single-and multi-habitat sample types using both community similarities and multi-metric index values; while methods from each program within sample type were comparable. This indicates that the Colorado Department of Public Health and Environment methods were compatible with the cooperative monitoring program methods within multi-and single-habitat sample types. Comparisons between September and October samples found distinguishable differences based on community similarities for both sample types, whereas only differences were found for single-habitat samples when multimetric index values were considered. At one site, differences between September and October index values from single-habitat samples resulted in opposing assessments of biological condition. Direct application of the results to inform the revision of the existing Fountain Creek basin U.S. Geological Survey cooperative monitoring program are discussed.

Introduction

Invertebrate-based assessment of biological condition is often an integral component of water-quality monitoring programs (U.S. Environmental Protection Agency, 2006; Carlisle and Woodside, 2013). In 1998, the U.S. Geological Survey (USGS), in cooperation with Colorado Springs City Engineering and Colorado Springs Utilities, began sampling biological communities along with selected water-quality characteristics in the Fountain Creek basin (FCB) as partof a Municipal Separate Storm Sewer System Permit (COS-000004). During this time, the number of sites sampled on an annual basis varied from 10 to 26 and included data collection of invertebrate and fish communities, habitat, selected water chemistry, streamflow, and sediment transport. These cooperative USGS FCB studies have described patterns in surface-water hydrology (Edelmann and others, 2002; Stogner, 2000), water chemistry (Mau and others, 2007), and sediment transport (von Guerard, 1989), and have related these characteristics to biological communities and urbanization (Zuellig and others, 2007).

Additionally, Zuellig and others (2010) identified temporal change in macroinvertebrate and fish community structure in FCB, and Walters and others (2014) determined the influence of barriers on the upstream migration of flathead chub (*Platygobio gracilis*), a species of concern in Colorado. Recently (2011), the occurrence of the invasive New Zealand mud snail (*Potamopyrgus antipodarum*) was documented in the FCB (Appendix 1) for the first time as part of these cooperative monitoring efforts.

In 2010, the Colorado Department of Public Health and Environment (CDPHE) developed an invertebrate Multi-Metric Index (MMI) (Colorado Department of Public Health and Environment, Water Quality Control Division, 2010) to assess the biological condition of small to medium sized wadeable streams (drainage area < 2,700 square miles [mi^2]) in Colorado. Invertebrate data used to build the MMI were collected using various sampling methods, but the dataset was dominated by samples collected with methods typically used by CDPHE (WQCC Policy 10-1, 2010). Therefore, use of the CDPHE sampling methods should be considered to appropriately apply the MMI to new data, as the sampling method used can influence detectability of certain taxa in community samples. The MMI was also developed from data collected within a specific index period (May 1 to October 1), which ends less than 1 month before sampling typically starts in the FCB cooperative monitoring program in order to avoid the influence of typical late-summer thunderstorms and associated stormwater events. These differences in methods and timing of sampling could influence sample comparability among sample types and possibly result in opposing assessments of biological condition, especially if MMI values are near established thresholds of impairment. Therefore, Colorado Springs City Engineering and Colorado Springs Utilities are concerned about data continuity if previously used sampling methods are replaced by methods currently used by the CDPHE to make assessments of biological condition.

A better understanding of method differences and month of sampling influences on biological assessments is important to make informed adjustments to future biological sampling as part of the cooperative monitoring program in FCB. For example, if samples are found comparable between methods and month of sampling, then previously collected data can be retained or adjusted accordingly to make future trend assessments in invertebrate communities and biological condition over the entire period of record of the monitoring program. Alternatively, if samples are found to be not comparable or cannot be adjusted, then methods in use since 1998 could be replaced by CDPHE methods; abandoning previously collected data from future trend assessments unless additional resources are available to collect samples using all four methods. To address these issues, the USGS, in cooperation with Colorado Springs City Engineering and Colorado Springs Utilities, designed a study to determine if sampling method and sample timing result in comparable samples and assessments of biological condition. To accomplish this task, annual macroinvertebrate samples were collected concurrently at 15 USGS streamflow gages in the FCB from 2010 to 2012 using four sampling methods inherent to the USGS cooperative monitoring program and those recommended by CHPHE. Additionally, samples were collected using the same methods in both September and October in 2012 at 6 of the 15 sites.

Purpose and Scope

The purpose of this report is to evaluate the effects of sampling method and sampling month on the comparability of invertebrate samples and subsequent assessments of biological condition at 15 selected sites in the FCB from 2010 to 2012 (table 1, fig. 1). Data from invertebrate samples were collected concurrently using multiple- and single-habitat sampling protocols in use by the USGS cooperative program and those recommended by CDPHE.

Study Area Description

Description of the FCB and the sites included in this study were previously detailed by others (Mau and others, 2007; Zuellig and others, 2007; and Edelman and others, 2002). In general, the FCB encompasses approximately 926 mi^2 in south central Colorado draining the eastern slope of the Rocky Mountains (fig. 1). Elevation ranges from 4,700 feet (ft) at the confluence with the Arkansas River to 14,109 ft at the summit of Pikes Peak. Fountain and Monument Creeks are the two main drainages and are located in the transition of the two distinctive physiographic landforms: the Front Range and the Colorado Piedmont (Hansen and Crosby, 1982). These landforms correspond to two Level III ecoregions, the Southern Rockies and the Southwestern Tablelands (Omernik, 1987). However, the sites included in this study were restricted to the Southwestern Tablelands (fig. 1). Site elevation ranged from 4,705 to 6,620 ft (referenced to North American Vertical Datum of 1988) and drainage area ranged from 16 to 926 mi^2 (table 1).

Study Methods

The USGS cooperative FCB monitoring program specifies two-stream invertebrate sample types: a qualitative multi-habitat sample (hereafter, QMH) described in the protocols of the USGS National Water-Quality Assessment Program (NAWQA; Cuffney and others, 1993; Moulton and others, 2002), and a slightly modified NAWQA semi-quantitative richest-targeted habitat (RTH_Hess) sample (hereafter, RTH_Hess). The NAWQA richest targeted habitat (RTH) sample described in Cuffney and others (1993) was slightly modified for the USGS cooperative FCB monitoring program in order to better match methods used in the FCB prior to 1998 by Colorado Springs Utilities. The modification includes sampling less area of the stream bottom and using a Hess sampler in place of a slack sampler (Moulton and others, 2002). The USGS cooperative program has consistently used these methods since 1998 in the FCB. Similarly, CDPHE sampling methods also include two sample types: a semi-quantitative sample collected in soft-bottomed streams where the targeted habitats are woody snags, pools, and macrophyte beds (hereafter, Multi-habitat) and a semi-quantitative sample targeting riffle or run habitats in hard-bottomed streams (hereafter, Riffle-run) (WQCC Policy 10-1, 2010).

Many sites (reaches) included in this study were dominated by soft-bottomed material (sand) but often included sparse riffles containing harder substrate (gravel and sometimes larger). Strict interpretation of CDPHE methods only would require collecting a Multi-habitat sample targeting woody snags, pools, and macrophyte beds. However, in order to adequately address the objectives within the constraints of this study, all four sample types were collected at each site and visit. As a result, 204 invertebrate samples were collected from 2010 to 2012 from 15 sites where the four methods briefly outlined above were collected concurrently. Forty-eight of these samples were collected in September and October in 2012 at 6 of the 15 sites to evaluate sample similarity between the recommended index period (July 1 to October 1) and the index period when samples were typically collected since 1998 (middle of October to early November).

Table 1. Description of sites and dates where 204 multi-habitat and single-habitat invertebrate samples were collected from the Fountain Creek basin, Colorado, 2010–2012. One-hundred forty-four of the 204 samples were collected in October.

[ID, identification; USGS, U.S. Geological Survey; NAVD 88, North American Vertical Datum of 1988; ft, feet; mi^2, square miles; m, meters]

Site ID[1]	USGS station ID	USGS station name	Elevation NAVD 88 (ft)	Drainage area (mi^2)	Reach length (m)	Dates of sample collection		
						2010	2011	2012
1	07103700	Fountain Creek near Colorado Springs, Colo.	6,110	103	150	10/21/2010	10/17/2011	09/10/2012, 10/24/2012
2	07103707	Fountain Creek at 8th Street at Colorado Springs, Colo.	6,000	119	150	10/21/2010	10/17/2011	09/10/2012
3	07103960	Kettle Creek above U.S. Air Force Academy, Colo.	6,620	16	100	10/19/2010	10/13/2011	09/07/2012
4	07103970	Monument Creek above Woodmen Road at Colorado Springs, Colo.	6,270	181	150	10/19/2010	10/17/2011	09/10/2012
5	07103990	Cottonwood Creek at Mouth at Pikeview, Colo.	6,265	18.7	150	10/19/2010	10/13/2011	09/07/2012, 10/23/2012
6	385124104501301	Monument Creek Tributary 2 at Sondermann Park at Colorado Springs, Colo.	6,060	2.04	100	10/22/2010	10/13/2011	09/07/2012, 10/23/2012
7	07104905	Monument Ck at Bijou Street at Colorado Springs, Colo.	5,980	235	250	10/19/2010	10/17/2011	09/10/2012
8	384909104504401	Bear Creek above 8th Street at Colorado Springs, Colo.	6,037	9.57	150	10/22/2010	10/13/2011	09/11/2012
9	07105500	Fountain Creek at Colorado Springs, Colo.	5,900	392	175	10/21/2010	10/14/2011	09/10/2012
10	07105530	Fountain Creek below Janitell Road below Colorado Springs, Colo.	5,840	413	300	10/20/2010	10/14/2011	09/11/2012
11	07105800	Fountain Creek at Security, Colo.	5,640	495	300	10/21/2010	10/18/2011	09/11/2012, 10/24/2012
12	07105900	Jimmy Camp Creek at Fountain, Colo.	5,530	65.6	150	10/18/2010	10/18/2011	09/06/2012, 10/23/2012
13	07106000	Fountain Creek near Fountain, Colo.	5,355	681	300	10/18/2010	10/12/2011	09/11/2012
14	07106300	Fountain Creek near Pinon, Colo.	4,990	849	300	10/20/2010	10/14/2011	09/11/2012
15	07106500	Fountain Creek at Pueblo, Colo.	4,705	926	300	10/20/2010	10/12/2011	09/11/2012, 10/24/2012

[1]See figure 1.

Various combinations of these data were analyzed using multivariate and univariate tests and routines detailed below. Quality control of the invertebrate samples was provided by the USGS Biological Group (BioGroup) of the National Water Quality Laboratory (NWQL) by determining sorting effectiveness and taxonomic accuracy for a minimum of 5 percent of samples each year. Quality control results from 2010 indicated that mean sorting effectiveness was 96.9 percent, and mean taxonomic accuracy based on the Jaccard Coefficient of Community (Jaccard, 1912) and Sorensen's Coefficient of Community (Sorensen, 1948) were 87.0 and 97.7 percent, respectively. The most commonly missed taxa were immature specimens, especially aquatic naidids and mites. In 2011, the BioGroup completed a verification of the reference collection maintained by a contract laboratory (Aquatic Associates Inc., Fort Collins, Colo.). The BioGroup agreed with the original determination for 312 of the 329 taxa in the reference collection. The differences for the remaining 17 taxa included 4 nomenclature issues, 12 determinations that were taxonomic name upgrades or downgrades, and 1 misidentification. Quality control results from 2012 were not available at the time of this publication. Invertebrate data evaluated in this report are included in Appendix 1.

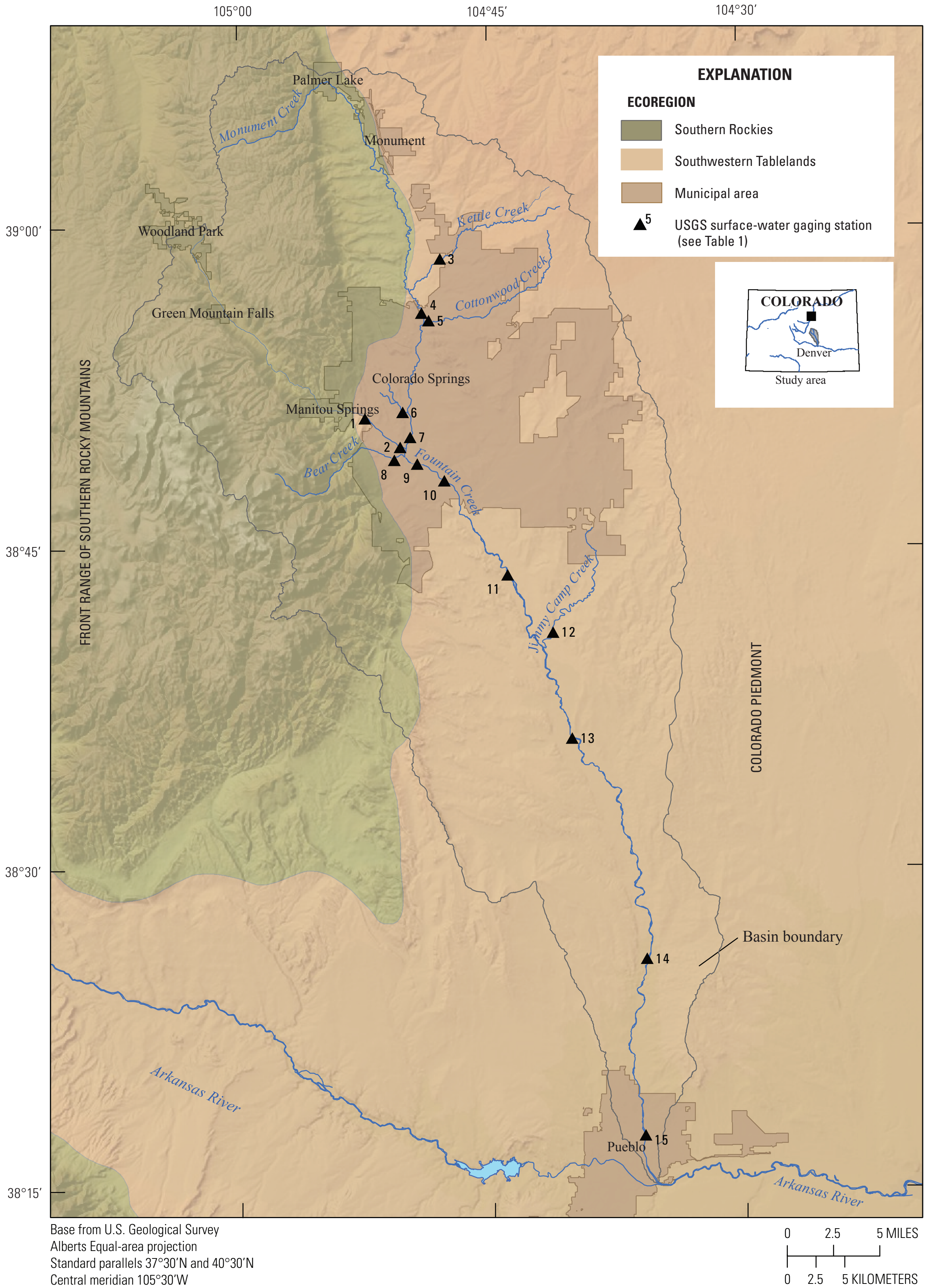

Figure 1. Map showing locations of sampling sites in the Fountain Creek basin, Colorado 2010–2012.

Data Collection

Invertebrates were sampled each year during base-flow conditions (time-of-year when streamflow is usually dominated by groundwater seepage) in September and/or October (table 1). All four sample types were collected concurrently following modified NAWQA (Cuffney and others, 1993) and CDPHE (2010) protocols during each site visit. Invertebrate sampling methods are discussed in brief below.

Multi-habitat Sampling (sample type)

USGS QMH (U.S. Geological Survey Qualitative Multi-habitat invertebrate sample method)

A D-frame kick net equipped with a 500-micrometer (µm) mesh was used to collect USGS qualitative multi-habitat (QMH) samples where organisms were collected from all available habitat types within the stream reach encompassing 1 hour (hr) (60 minutes [min]) of sampling (Cuffney and others, 1993). Different habitat types were identified and their contributing area of the reach in relative occurrence was calculated and the matching fraction of 1 hr was spent sampling each associated habitat type. For example, if the reach was 50 percent run, 25 percent pool, and 25 percent riffle, then approximately 30 min was spent sampling run habitat, 15 min pool habitat, and 15 min riffle habitat. Sampling effort (table 2) with this method did not always conform to these strict guidelines mostly due to small stream size and the lack of heterogeneous habitat and substrate typical of FCB sites. Often the entire reach and each habitat type were adequately sampled in less than 60 min.

CDPHE Multi-habitat (Colorado Department of Public Health and Environment Multi-habitat invertebrate sample method)

A kick net equipped with a 500-µm mesh was used to collect CDPHE recommended semi-quantitative multi-habitat samples from woody debris or snags, bank margins, pools, and aquatic macrophytes (WQCC Policy 10-1, 2010). Sampling effort (table 2) of this method was defined as the active collection of organisms over an area of approximately 1 square meter (m^2) (10.76 square feet [ft^2]) for a total of 60 seconds (sec). In general, the kick net was jabbed and swept through the targeted habitats by one person as another person timed the sampling effort. A maximum of four habitats were targeted for this sampling at each site. If only one habitat was identified in a reach, then it was sampled for 60 sec, and the area was restricted to the width of the net's frame and the length of the net. If multiple habitats were identified in the reach, the time and area of the sampling was reduced by the fraction of total habitats (for example, if three habitats were selected for sampling, then each was sampled for 20 sec, and the area was limited to the width of the net's frame and one-third the length of the net). These samples were always collected by two USGS personnel to ensure that the sampling effort among samples was consistent among sites. The application of this method in this study excluded hard-bottomed riffle and run habitats (if present) as this method as described by CDPHE is exclusive to soft-bottomed streams where riffles and runs are typically absent.

Table 2. Comparison of gear types and sampling effort used to collect invertebrate samples with USGS and CDPHE protocols.

[USGS, U.S. Geological Survey; CDPHE, Colorado Department of Public Health and Environment; USGS QMH, qualitative multi-habitat invertebrate sample; CDPHE Multi-habitat, soft-bottomed stream invertebrate sample; USGS RTH_Hess, richest targeted habitat invertebrate sample collected with a Hess sampler; CDPHE Riffle-run; hard-bottomed stream invertebrate sample; ~, approximately; m^2, square meter; sec, seconds; Y, yes; N, no]

Sample types and methods	Gear	Composite	Effort
Multi-habitat			
USGS QMH	D-Frame dip net	N	up to 60 minutes
CDPHE Multi-habitat	Kick net	N	~ 1m², 60 sec
Single-habitat			
USGS RTH_Hess	Hess sampler	Y	0.26 m²
CDPHE Riffle-run	Kick net	N	0.91 m², 60 sec

Single-habitat Sampling (sample type)

USGS RTH_Hess (Modified U.S. Geological Survey Richest Targeted Habitat invertebrate sample method)

In order to maintain continuity with invertebrate data collected prior to 1998 in the FCB, sampling gear, area sampled (effort), and randomization used in this method slightly departs from the NAWQA RTH protocols described in Cuffney and others (1993) and Moulton and others (2002). In general, a Hess sampler (0.086 m^2 or 0.923 ft^2) equipped with a 500-micrometer (µm) mesh was used to collect macroinvertebrates from three points within the RTH (Cuffney and others, 1993); in this case, the collections were made in riffle or runs with coarse substrate. These three discrete collections were composited and yielded a total area of 0.26 m^2 (2.80 ft^2) per sample (table 2). The USGS cooperative program in the FCB targets riffle habitat at all sites; however, when riffles are absent due to the unstable substrate of FCB sites, runs with the coarsest substrate were sampled. A random numbers table was used to determine the number of steps from the downstream boundary for the three sample locations within the length of the selected RTH (riffle or run). Randomly selected points were rejected if the point occurred outside the boundary of the RTH, and these points were replaced with alternative randomly generated points. If a sample point could not be sampled due to the substrate exceeding the limit of the sampler, the point was moved laterally or upstream one step. Attempts were made to sample the same habitat type at each site every year, but in Fountain Creek, runs and riffles are not spatially stable, due to streambed alteration caused by stormflow events and episodic utility construction activities within the channel. Samples were collected in a downstream to upstream direction. The Hess sampler was pushed firmly into the streambed and the substrate was mixed to dislodge invertebrates. When present, cobbles were scrubbed inside the sampler to further remove clinging invertebrates and inspected for remaining invertebrates. The remaining substrate within the sampler was stirred by hand or a sturdy metal utensil to a depth of approximately 0.10 meters (m) to dislodge invertebrates from smaller substrate and those dwelling within the substrate. There was no time constraint for collecting this sample as there is for other methods used in this study.

CDPHE Riffle-run (Colorado Department of Public Health and Environment Riffle-run invertebrate sample method)

A kick net equipped with a 500-µm mesh was used to collect CDPHE Riffle-run samples (WQCC Policy 10-1, 2010) in the same riffle or run as the USGS RTH_Hess samples. These samples were all collected by two USGS personnel both due to the size of the net and to ensure the sample effort limit was achieved (table 2). The area of this sample (0.91 m^2 or 9.75 ft^2) is defined as the total length of the kick net handle (1.98 m, 78 inches [in]) multiplied by the width of the net's rectangular frame (0.46 m, 18 in) (WQCC Policy 10-1, 2010). After placing the kick net firmly against the streambed, the net handle was lowered upstream horizontal over the water surface. The upstream point where the end of the kick net handle reached delineated the upstream boundary of the sample location. Next, one person held the net handle upright and timed the collection of the sample. The other person began disturbing the substrate with their boots and scrubbing cobbles in an upstream direction within the limit of the sample area until the net holder signaled the end of 60 sec. The sample was complete and the net was removed from the streambed after the plume of discolored water disappeared. These samples were collected immediately upstream, downstream, or in-between the three USGS RTH_Hess sample locations. One of these four locations was determined randomly at the beginning of each day and then at subsequent sites this position was moved one position upstream.

Sample Processing

Samples often contained a considerable amount of inorganic and organic debris that was reduced in the field by elutriating and sieving the debris (in a 500-µm mesh-metal sieve) until sample volumes were approximately 500 milliliters (mL) (about 1 pint). Samples were preserved in the field with 10-percent formalin, and stored until delivered to the contract laboratory for sample processing and taxa identification (Klemm and others, 1990). All organisms were identified to the lowest possible taxonomic resolution and enumerated. The fixed-count sub-sample target in the laboratory was 300-organisms per sample; when organisms were more numerous, a sub-sampling frame was used to randomly select organisms until the 300 fixed-count was achieved.

Data Preparation

The MMI values were calculated using a tool designed for CDPHE by Tetra Tech (Jessup, 2010) to assess the biological condition of streams in Colorado and evaluate state aquatic life use designations (WQCC Policy 10-1, 2010). The tool is packaged in a Microsoft Access® database as the Ecological Data Application System (EDAS) for Colorado. Details of how the MMI was developed are described elsewhere (Jessup, 2010; WQCC Policy 10-1, 2010). In general, EDAS for Colorado calculates MMI values using sets of metrics that best distinguished between reference and stressed sites in three designated site classes or Biotypes. These Biotypes are defined as (1) Transitional, (2) Mountains, and (3) Plains, and are distinguished by the multivariate combination of three environmental variables (ecoregion, elevation, and stream gradient). All of the FCB sites included herein were classified as Biotype 3. Biotype 3 streams in EDAS for Colorado are generally characterized by low elevation, low gradient, warm water, and a dry climate relative to the other two Biotypes. The Biotype 3 index includes six metrics; number of insect taxa, percent of non-insect taxa, percent dominant taxon, number of predator and shredder taxa combined, percent sprawler, and percent sensitive Plains families. Prior to EDAS MMI computations, taxonomic names provided by the contract laboratory were harmonized with the operational taxonomic units (OTUs) used by EDAS. This process resulted in adjusting 12 taxa names to a higher level of taxonomic resolution (for example, species identification changed to genus).

Data Analysis

A variety of analyses were used to evaluate the comparability between methods and month of sampling. Analyses used either community data or calculated MMI values and included nonparametric multivariate routines and univariate statistical analyses.

Evaluating Effects of Sampling Method and Month of Sampling

Multivariate Analysis of Similarity (two-way ANOSIM; PRIMER version 6.1, Plymouth, United Kingdom) was used to compare invertebrate communities collected using different methods and collected during two time periods (September and October) following details in Clarke and Warwick (2001) and Clarke and Gorley (2006). ANOSIM is based on a multivariate nonparametric-permutation procedure that compares the degree of separation between predefined groups of samples based on the ranks of community similarities underlying a nonparametric multidimensional scaling ordination (Clarke and Warwick, 2001). This procedure does not make assumptions about the distributional properties of the data, variance structure among groups, or about the balance of replicate samples within groups. The degree of separation among predefined groups is determined with the test statistic R, first as a global test to determine if differences among groups exist, then as pair-wise comparisons to determine which groups differ. Values of R near 0 indicate no distinguishable separation between groups, whereas values near 1 indicate complete separation. Statistical significance was determined by a general randomization procedure based on Monte Carlo significance tests described by Hope (1968). Analyses were determined statistically significant when less than 5 percent of the 9,999 permutated values were greater than the global R value.

Various combinations of samples collected in October (table 1, n = 144) were used to determine if invertebrate communities differed among sampling methods using a series of two-way ANOSIM analyses for a crossed design. The utility for two-way ANOSIM for a crossed design herein is that the analysis accounts for the effects of one factor (site) while considering the significance of the other (sampling method) (Clarke and Warwick, 2001; Clarke and Gorley, 2006). This was important because environmental differences among sites were known, making it difficult to isolate the effects of sampling method using a standard one-way ANOSIM that just tested for differences among methods without accounting for site effects. Analyses were structured to test for differences among all four methods and between similar sample types because it was expected that multi-habitat sample types (USGS QMH, CDPHE Multi-habitat) would be more similar to each other than to single-habitat sample types (USGS RTH_Hess, CDPHE Riffle-run). Likewise, single-habitat sample types were expected to be more similar to each other than to multi-habitat sample types. Similarly, two-way ANOSIM was applied to determine differences in samples collected during September and October (month of sampling) while accounting for environmental differences among sites using data collected from six sites in 2012 during both months (table 1, n = 48). Before analysis, data were presence-absence transformed and Bray Curtis similarity was calculated among samples (Bray and Curtis, 1957).

The effect of sample type (single-habitat versus multi-habitat) and month of sampling (September versus October) on MMI values also were examined using two-way factorial analysis of variance (ANOVA) in three separate analyses. The first analysis tested for the main effects of site and sample type (single-habitat versus multi-habitat), and the site by sample type interaction (SPSS version 13.0, SPSS, 2005). A significant (p less than 0.05) interaction term was interpreted as an indication that the effect of sample type was dependent upon which site was being sampled. The remaining tests were done independently using the multi-habitat and single-habitat data to test for the main effects of site and month of sampling (September versus October sampling), and the site by month of sampling interaction. A significant (p less than 0.05) interaction term was interpreted as an indication that the effect of sampling in September versus October on MMI values was dependent upon which site was being sampled. Interpretation of a significant site term was beyond the scope of this report and was expected in all ANOVA analyses given the various site characteristics and conditions included in this study; however, including a site term was important to isolate the main effects of sampling method and month of sampling while accounting for environmental differences among sites.

Effects of Sampling Method and Month on Invertebrate Community Similarities

Multivariate results of two-way ANOSIM using presence/absence transformed data indicated only marginally significant differences among the four sampling methods while accounting for environmental differences among sites (table 3; Sampling method R = 0.09; Sampling method p = 0.045). However, when samples were grouped by sample type (multi-habitat versus single-habitat), significant and weakly moderate differences were found (table 3; Sample type R = 0.33; Sample type p less than 0.001). These results indicate that multi-habitat (USGS QMH and CDPHE Multi-habitat) and single-habitat sample types (USGS RTH_Hess and CDPHE Riffle-run) capture somewhat different compilations of taxa; however, samples from within sample types were indistinguishable, suggesting CDPHE and USGS methods within sample types were compatible. Because methods within sample type were apparently compatible, samples were grouped into multi-habitat and single-habitat method types for the remaining analyses.

Although not presented herein, two-way ANOSIM using untransformed relative abundance data and fourth-root transformed raw data from this study produced similar results as described above, suggesting that samples within sample types also are comparable in terms of relative abundance and transformed taxa counts that each sample captures. Significant differences among sites also were found in all ANOSIM analyses above (table 3; Site R range = 0.59–0.67; Site p less than 0.001 in all cases); however, these results were expected because of the environmental differences among sites and additional interpretation is beyond the scope of this report.

For both multi-habitat and single-habitat sample types, two-way ANOSIM indicated moderate and significant differences between September and October samples (table 4; multi-habitat R = 0.63, p = 0.003; single-habitat R = 0.44, p = 0.025). This result was somewhat surprising because of the limited amount of time between sample collections (47 to 48 days); however, it is well known that invertebrate community structure varies among seasons in Colorado streams. As in the previous analysis, significant differences among sites were

Table 3. Results of two-way ANOSIM evaluating the influence of sampling method and site differences on invertebrate sample similarity collected from selected sites in the Fountain Creek basin, 2010–2012.

[R, ANOSIM test statistic; p, probability; USGS, U.S. Geological Survey; CDPHE, Colorado Department of Public Health and Environment; USGS QMH, qualitative multi-habitat invertebrate sample; CDPHE Multi-habitat, soft-bottomed stream invertebrate sample; USGS_RTH Hess, richest targeted habitat invertebrate sample collected with a Hess sampler; CDPHE Riffle-run; hard-bottomed stream invertebrate sample; <, less than]

Grouping	Site		Grouping	
	R	p	R	p
Method grouping				
USGS QMH, CDPHE Mulit-habitat, USGS RTH_Hess, CDPHE Riffle-run	0.59	< 0.001	0.09	0.045
USGS QMH versus CDHE Multi-habitat	0.67	< 0.001	-0.19	0.964
USGS RTH_Hess versus CDPHE Riffle-run	0.52	< 0.001	-0.14	0.920
Method grouped by sample type				
Multi-habitat (USGS QMH and CDPHE Mulit-habitat) versus Single-habitat (USGS RTH_Hess and CDPHE Riffle-run)	0.65	< 0.001	0.33	<0.001

Table 4. Results of two-way ANOSIM evaluating the influence of month of sampling (September versus October) and site differences on invertebrate sample similarity collected from selected sites in the Fountain Creek basin, 2010-2012.

[R, ANOSIM test statistic; p, probability; USGS, U.S. Geological Survey; CDPHE, Colorado Department of Public Health and Environment; USGS QMH, qualitative multi-habitat invertebrate sample; CDPHE Multi-habitat, soft-bottomed stream invertebrate sample; USGS RTH_Hess, richest targeted habitat invertebrate sample collected with a Hess sampler; CDPHE Riffle-run; hard-bottomed stream invertebrate sample; <, less than]

Sample type (and methods)	Site		Month	
	R	p	R	p
Multi-habitat (USGS QMH and CDPHE Mulit-habitat)	0.95	< 0.001	0.63	0.003
Single-habitat (USGS RTH_Hess and CDPHE Riffle-run)	0.81	< 0.001	0.44	0.025

found (table 4; multi-habitat R = 0.95, p < 0.001; single-habitat R = 0.81, p < 0.001) but were expected, and additional interpretation is beyond the scope of this report.

Effects of Sampling Method and Month of Sampling on MMI Values

Two-way factorial ANOVA indicated significant mean MMI differences (p less than 0.0001) between samples collected using single-and multi-habitat methods (fig. 2). Overall, samples collected using multi-habitat methods (mean = 55.3) were on average 11 MMI units greater than samples collected using single-habitat methods (mean = 44.4). In all but two cases (sites 2 and 10), mean MMI values remained above thresholds of attainment or impairment (Colorado Department of Public Health and Environment, Water Quality Control Division, 2010) regardless of sample type. However, this likely is an artifact of the distribution of the data evaluated in this study. Mean MMI differences of 10 units could easily fluctuate above and below thresholds if the distribution of MMI values for a given set of samples was near threshold values. The interaction term was not significant (p = 0.620), which indicated that the effect of sample type on MMI values was independent of which site was being evaluated.

The effect of sampling in September versus October on MMI values was dependent on which sample type was being evaluated (single-habitat or multi-habitat). For multi-habitat samples, neither month of sampling nor the interaction of site and month of sampling were significant (month p = 0.968; interaction p = 0.189; fig. 3.), indicating that multi-habitat samples collected in September and October, 2012 were comparable. Whereas for single-habitat samples, the interaction between site and month of sampling was significant (interaction p = 0.001; fig. 4), indicating the effect of sampling in September or October on MMI values was dependent on which site was being evaluated. In this case, the effect of collecting single-habitat samples in September or October on MMI values was unpredictable, as MMI values between months were indistinguishable. That is, September values could be greater than October values, or October values greater than September values (fig. 4).

In at least one case (site 5; fig. 4), October MMI values were below the impairment threshold; whereas September

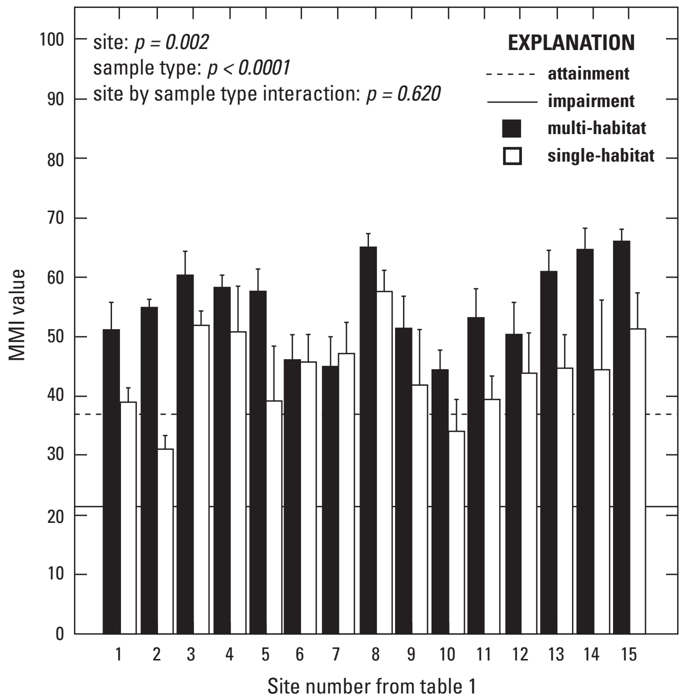

Figure 2. Mean multi-metric index (MMI) values (+1 standard error) calculated from 144 multi-and single-habitat samples collected in October, 2010–2012 from 15 sites in the Fountain Creek basin in Colorado. (p, probability; <, less than)

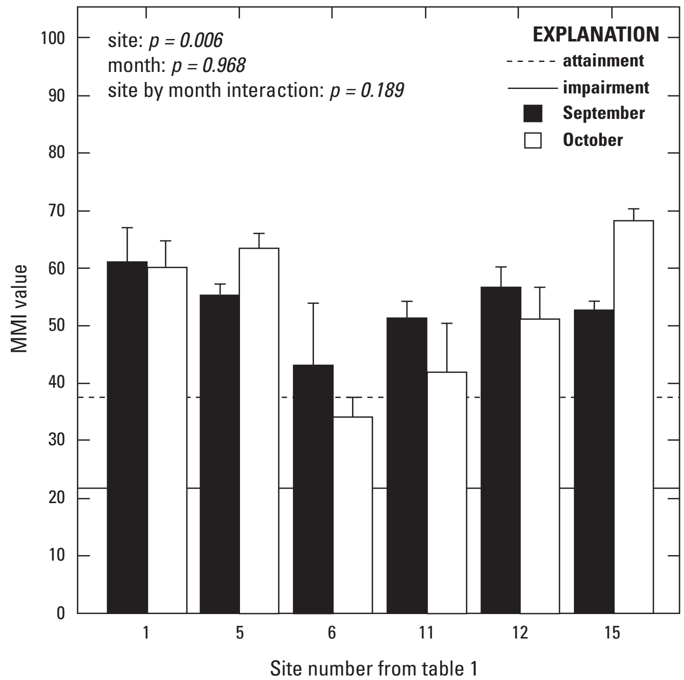

Figure 3. Mean multi-metric index (MMI) values (+1 standard error) calculated from 24 multi-habitat samples collected in September and October, 2012 from six sites in the Fountain Creek basin in Colorado.

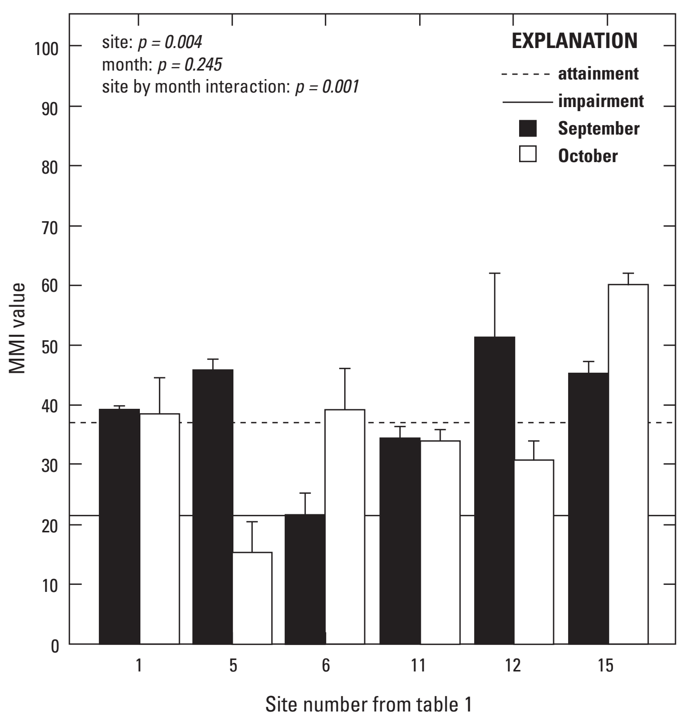

Figure 4. Mean multi-metric index (MMI) values (+1 standard error) calculated from 24 single-habitat samples collected in September and October, 2012 from six sites in the Fountain Creek basin in Colorado. (p, probability)

values at site 5 were above the attainment threshold. This result indicates that collecting single-habitat samples in either September or October could change the interpretation of MMI values and the status of biological condition at some sites in the FCB. Also, the distribution of single-habitat MMI values from 2012 were closer to threshold values than in other analyses and therefore, variability in MMI values due to month of sampling resulted in fluctuations above and below thresholds in three of six cases.

Moving Forward

Results from this study have at least two important implications for moving the biological assessment portion of the USGS cooperative FCB monitoring program forward. First, MMI values were influenced by sample type such that mean values from multi-habitat samples were on average 11 units greater than values calculated from single-habitat samples. Both hard-and soft-bottom substrates were available at 40 percent of the sites included in this study, so using either sample type may be justified at these and other FCB sites. Second, month of sampling differentially influenced sample similarity and MMI values. Multivariate analysis showed community structure was significantly distinguishable between September and October for both multi- and single-habitat samples. Discontinuing October samples in future efforts will make it difficult to incorporate previously collected data in future multivariate analysis to assess long-term trends in community similarity (see analysis of Zuellig and others, 2010).

In terms of MMI values, the influence of month of sampling was dependent on sample type. Multi-habitat samples were not affected by month of sampling, so calculating MMI values for previously and currently collected data to assess long-term trends in biological condition may be reasonable and justified. However, MMI values calculated from single-habitat samples were influenced by month of sampling, and the direction of the effect was unpredictable. Because the direction of the effect was unpredictable it will be difficult to reasonably adjust previously collected data to improve comparability in future analysis. This result makes it difficult to justify using MMI values calculated from single-habitat samples from previously collected samples and incorporate them into future assessment of long-term trends in biological condition in the FCB. Ultimately, dropping October sampling will make it difficult to incorporate previously collected invertebrate data into any future analysis using multivariate endpoints or MMI values from single-habitat samples. However, continuing October sampling of multi-habitat samples should provide comparable MMI values to those calculated from September samples and will allow continuity with previously collected data for future multivariate trend analysis or analysis of biological condition using MMI values. Furthermore, October multi-habitat samples previously were more useful in the FCB for describing relations between the environment and ecological response than single-habitat samples (see table 7 in Zuellig and others, 2007) and for detecting and explaining multivariate trends (Zuellig and others, 2010).

Summary

Colorado Springs City Engineering and Colorado Springs Utilities are concerned about data continuity if previously used sampling methods are replaced by methods currently used by the Colorado Department of Public Health and Environment (CDPHE) to make assessments of biological condition. To address this issue, the U.S. Geological Survey, in cooperation with Colorado Springs City Engineering and Colorado Springs Utilities, designed a study to determine if sampling method and sample timing influences sample comparability and assessments of biological condition. Data were analyzed using multivariate and univariate analyses. Results indicated that community similarities and multi-metric index (MMI) values from October samples are statistically distinguishable between multi-habitat and single-habitat sample types, but methods are comparable within sample types. This suggests that CDPHE methods are compatible with methods used by the cooperative monitoring program as long as samples are grouped by multi-habitat and single-habitat sample types. Multivariate analysis of community similarity found differences between September and October samples for both sample types, whereas only differences were found for single-habitat samples when MMI values were considered. At site 5, differences between September and October index values from single-habitat samples resulted in opposing assessments of biological condition. Based on the results of this study, continuation of October multi-habitat sampling should provide the most comparable MMI values to those calculated from September samples. October multi-habitat samples also provide continuity with previously collected data in future multivariate trend analysis or assessments of biological condition using MMI values.

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References Cited

Bray, J.R., and Curtis, J.T., 1957, An ordination of the upland forests communities of southern Wisconsin: Ecological Monographs, v. 27, p. 325–349.

Carlisle, D.M., and Woodside, M.D., 2013, Ecological health in the Nation’s streams: U.S. Geological Survey Fact Sheet 2013–3033, 6 p., at *http://pubs.usgs.gov/fs/2013/3033/*.

Clarke, K.R., and Gorley, R.N., 2006, Primer version 6—User manual/tutorial, Primer-E: Plymouth, United Kingdom, PRIMER-E Ltd. 190 p.

Clarke, K.R., and Warwick, R.M., 2001, Change in marine communities—An approach to statistical analysis and interpretation (1st ed.): Plymouth, United Kingdom, Plymouth Marine Laboratory, 172 p.

Colorado Department of Public Health and Environment, Water Quality Control Division, 2010, Aquatic life use attainment policy 101: Colorado Department of Public Health and Environment accessed April 8, 2013 at *http://www.colorado.gov/cs/Satellite/CDPHE-WQCC/CBON/1251590854258*.

Cuffney, T.F., Gurtz, M.E., and Meador, M.R., 1993, Methods for collecting benthic macroinvertebrate samples as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 93–406, 66 p.

Edelmann, Patrick, Ferguson, S.A., Stogner, R.W., Sr., August, M., Payne, W.F., and Bruce, J.F., 2002, Evaluation of water quality, suspended sediment, and stream morphology with an emphasis on effects of storm flow on Fountain and Monument Creek Basins, Colorado Springs and vicinity, Colorado, 1981 through 2001: U.S. Geological Survey Water-Resources Investigations Report 02–4104, 59 p.

Hansen, W.R., and Crosby, E.J., and Shroba, R.R., 1982, Environmental geology of the Front Range urban corridor and vicinity, Colorado, with a specific section on physical properties and performance characteristics of surficial deposits and rock units in the greater Denver area: U.S. Geological Survey Professional Paper 1230, 99 p.

Hope, A.C.A., 1968, A simplified Monte Carlo significance test procedure: Journal of the Royal Statistical Society, Series B, Methodological, v. 30, p. 582–598.

Jaccard, P., 1912, The distribution of flora in the alpine zone: New Phytologist, v. 11, p. 37–50.

Jessup, B.K., 2010, Recalibration of the Macroinvertebrate Multi-metric Index for Colorado: Montpelier, Vermont, Tertra Tech, Inc., Center for Ecological Sciences, variable pagination.

Klemm, D.J., Lewis, P.A., Fulk, F., and Lazorchak, J.M., 1990, Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters: U.S. Environmental Protection Agency, EPA/600/4-90/030, 256 p.

Mau, D.P., Stogner, R.W., Sr., and Edelman, Patrick, 2007, Characterization of stormflows and wastewater treatment-plant effluent discharges on water quality, suspended sediment, and channel morphology for Monument and Fountain Creek Watersheds, Colorado, 1981–2006: U.S. Geological Survey Scientific Investigations Report 07–5104, 76 p., at *http://pubs.usgs.gov/sir/2007/5104/*.

Moulton, S.R., Kennen J.G., Goldstein, R.M., and Hambrook, J.A., 2002, Revised protocols for sampling algae, invertebrate, and fish communities as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 02–150, 75 p., at *http://pubs.usgs.gov/of/2002/ofr-02-150/*.

Omernik, J.M., 1987, Ecoregions of the conterminous United States: Annals of the Association of American Geographers, v. 77, p. 118–125.

Sorensen, T.A., 1948, Method of establishing groups of equal amplitude in plant sociology based on similarity of species content, and its application to analyses of the vegetation on Danish commons: Kobenhavn, I Kommission hos Ejnar Munksgaard, Biologiske Skrifter, Kongelige Danske Videnskabernes Selskab, v. 5, p. 1–34.

SPSS, 2005, SPSS version 13.0 Brief guide: Chicago, Illinois, SPSS Inc., 231 p.

Stogner, R.W., Sr., 2000, Trends in precipitation and streamflow and changes in stream morphology in the Fountain Creek Watershed, Colorado, 1939–99: U.S. Geological Survey Water-Resources Investigations Report 00–4130, 43 p.

U.S. Environmental Protection Agency, 2006, Wadeable streams assessment—A collaborative survey of the Nation's streams: Washington, D.C., U.S. Environmental Protection Agency, Office of Research and Development and Office of Water, EPA 841-B-06-002, 113 p., accessed March 2013 at *http://www.epa.gov/owow/streamsurvey/*.

von Guerard, Paul, 1989, Sediment-transport characteristics and effects of sediment transport on benthic invertebrates in the Fountain Creek drainage basin upstream from Widefield, southeastern Colorado, 1985–88: U.S. Geological Survey Water-Resources Investigations Report 89–4161, 133 p.

Walters, D.M., Zuellig, R.E., Crockett, H.J., Bruce, J.F., Lukas, P.M., and Fitzpatrick, R.M., 2014, Barriers impede upstream spawning migration of Flathead Chub: Transactions of the American Fisheries Society, v. 143:1, p. 17–25.

Zuellig, R.E., Bruce, J.F., Evans, E.E., and Stogner, R.W., 2007, Urban-related environmental variables and their relation with patterns in biological community structure in the Fountain Creek Basin, Colorado, 2003–2005: U.S. Geological Survey Scientific Investigations Report 2007–5225, 24 p., at *http://pubs.usgs.gov/sir/2007/5225/*.

Zuellig, R.E., Bruce, J.F., and Stogner, R.W., 2010, Temporal change in biological community structure in the Fountain Creek Basin, Colorado, 2001–2008: U.S. Geological Survey Scientific Investigations Report 2017–5103, 19 p., at *http://pubs.usgs.gov/sir/2010/5103/*.

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Appendix

Appendix 1. Location, collection date, sample type, operational taxonomic unit, and number of organisms extracted from benthic samples collected at selected sites in the Fountain Creek basin from 2010–2012.

[Appendix 1 can be downloaded from *http://pubs.usgs.gov/sir2014/5049*]

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