THESIS

ESTIMATING THE POPULATION SIZE AND DISTRIBUTION OF THE ARAPAHOE SNOWFLY (*Arsapnia arapahoe*) (PLECOPTERA: CAPNIIDAE) ALONG THE NORTHERN FRONT RANGE OF COLORADO.

Submitted by

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ABSTRACT

ESTIMATING THE POPULATION SIZE AND DISTRIBUTION OF THE ARAPAHOE SNOWFLY (*Arsapnia arapahoe*) (PLECOPTERA: CAPNIIDAE) ALONG THE NORTHERN FRONT RANGE OF COLORADO.

The population size and geographic distribution of the recently described Arapahoe snowfly, *Arsapnia arapahoe* (Nelson and Kondratieff) (Plecoptera: Capniidae) is unknown. Prior to this study, *A. arapahoe* was known only from two tributaries of the Cache la Poudre River in Larimer County, Colorado, Young Gulch and Elkhorn Creek. The objectives of this study were to determine the distribution of *A. arapahoe* along the northern Front Range of the Rocky Mountains in Colorado, to estimate the abundance of adult *A. arapahoe* in Elkhorn Creek (the stream with the largest known population), and to identify co-occurring sympatric species as possible surrogate indicators of its presence. I also investigated the potential effects of wildfire and flood disturbance on known populations of the Arapahoe snowfly. To determine the distribution in other Front Range streams, I sampled 54 streams for *A. arapahoe* in 2013 and 2014, several of which were chosen based on GIS modeling for potential suitable habitats. I also conducted an intensive depletion sampling study in three 300-m segments of Elkhorn Creek (n=5 reaches per segment) using quantitative beat sheet techniques. Sampling was estimated to capture over 90% of available adult individuals, and 100% of *A. arapahoe* individuals. Thus population size estimates could be obtained. Further, adults of *A. arapahoe* were only discovered in six additional, first-order streams outside the original type localities in Cache la Poudre River Basin. New species localities were discovered in three additional drainage basins: Big Thompson

River, St. Vrain River, and Boulder Creeks. Intensive sampling efforts in Elkhorn Creek revealed substantially low abundances of *A. arapahoe* adults (only 10 individuals found in the duration of the study), justifying the designation of this species as a rare taxon. Two sympatric species, *A. decepta* (Banks) and *Capnia gracilaria* Claassen, were consistently found with *A. arapahoe*, and thus may serve as a future indicator of suitable stream habitat. Both *A. arapahoe* and associated species appeared to be adversely impacted by post-wildland fire erosion and sediment deposition; however, capniids, including *A. arapahoe*, appeared to be resilient to flooding. I present suggestions for continued monitoring of the presence, abundance and distribution of *A. arapahoe* for conservation of this rare and endemic Colorado stonefly.

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INTRODUCTION

Plecoptera (stoneflies) play an important role in rivers and streams as predators among macroinvertebrate communities, as consumers of detritus, and as prey for fish (Hynes 1970, Stewart & Stark 2002). Detritivorous stoneflies, in particular, contribute to nutrient cycling and ecosystem function by processing allochthonous inputs of leaf litter. Unfortunately, information on population size and life histories is lacking for many of the cryptic or rare stoneflies, such as the winter-emerging snowflies (Capniidae) (Stark et al. 2012). The Capniidae are the least known of all families in North America, due to the inherent difficulties of sampling streams during winter ice cover, and sampling the hyporheic zone for nymphs (Stanford & Ward 1993, Stewart & Stark 2002). More effort is needed to understand the rarity and ecological requirements of both immature and adult capniids.

Various ecological and environmental pressures threaten existing populations of Capniidae species. For example, stoneflies are generally considered one of the most sensitive indicators of disturbance in aquatic insect communities, and are one of the first taxonomic groups to disappear in streams impacted by the degradation of water quality and physical habitat (Gaufin 1973, Baumann 1979, Rosenberg & Resh 1993, Barbour et al. 1999). Stonefly nymphs are particularly susceptible to anthropogenic effects on water quality because of narrow requirements of temperature, stream size, and substrate in which they persist (Baumann 1979, Williams & Feltmate 1992). Hydrological changes such as those influenced by water storage and global climate change may pose an additional threat (Sweeney et al. 1992, Muhlfeld et al. 2011). For example, with the exception of *Capnura wanica* (Frison) and *Capnia confusa* (Claassen), most capniids have been extirpated from transitional mountains-to-plains streams in the northern

Front Range of the Rocky Mountains in eastern Colorado either due to the degradation of water quality, siltation and subsequent loss of hyporheic connections, or low flows (Zuellig et al. 2012, Stoaks & Kondratieff 2014). These disturbances may play a role in the perceived rarity of many winter stonefly species.

One species of Capniidae which appears to be extraordinarily rare is the recently described Arapahoe Snowfly, *Arsapnia arapahoe* (Nelson & Kondratieff 1988). The Arapahoe snowfly was originally described from two male specimens collected at Young Gulch on March 22, 1986 (paratype) and Elkhorn Creek on April 3, 1987 (holotype), tributaries of the Cache la Poudre River in Larimer County, Colorado. The species was initially placed in the *Capnia decepta* species group (Nelson and Kondratieff 1988, Nelson and Baumann 1989). Additional specimens were noted and the description of the previously unknown female adult was provided by Heinold and Kondratieff (2010). However, recent DNA analysis of the allotype female was inclusive to specific placement with *A. arapahoe* (Heinold et al. 2014). Currently, the specific validity of the Arapahoe snowfly is accepted by the scientific community (Nelson and Baumann 1989, Integrated Taxonomic Information System 2010). *Arsapnia arapahoe* is most closely related to the Utah snowfly (*A. utahensis* Gaufin & Jewett) found in Utah, Nevada, and California, and the Sequoia snowfly (*A. sequoia* Nelson & Baumann) found in California (Nelson & Kondratieff 1988, Murányi et al. 2014).

Efforts to document the occurrence of *A. arapahoe* at other regional sites, beyond the two type localities, were unsuccessful. Efforts to document the occurrence of *A. arapahoe* at new localities have been unsuccessful to date; however, these efforts have been limited in scope and intensity. The apparent limited distribution of *A. arapahoe* prompted a petition for listing the species under the Endangered Species Act (ESA) by a conservation advocacy organization in

July 2007 (WildEarth Guardians 2007). In 2012, the United States Fish and Wildlife Service (USFWS) found a listing warranted but precluded due to higher priority species, which resulted in the USFWS designating *A. arapahoe* as a candidate species (USFWS 2012). Factors contributing to this status included small population size and modification of habitat associated with climate-related issues such as lower snowpacks and higher risk of larger wildfires. Inadequacy of existing regulatory mechanisms to protect water quality for the species was also determined to be an issue.

Since the USFWS listing, threats to existing populations of *A. arapahoe* have included both the 2012 High Park Fire and the historic September 2013 floods in the Cache la Poudre River watershed. The High Park fire was a mixed severity wildland fire that burned roughly 35,207 hectares (87,000 acres) (Wohl 2013) including the Young Gulch watershed. In September 2013, significant flooding capable of moving accumulations of large wood and streambed sediments occurred in both Young Gulch and Elkhorn Creek. The impact of these changes on populations of Capniidae and *A. arapahoe* in these type locality streams are unknown at this time However, negative impacts of wildfire and post-fire flooding on stoneflies have been documented in many western watersheds (e.g., Mihuc et al. 1996, Minshall et al. 2001, Vieira et al. 2004, Vieira et al. 2011).

The Arapahoe snowfly warrants further study because it is a rare, endemic species with limited geographic distribution at risk to more frequent and more severe ecological disturbances in the future. Its distribution along the northern Front Range of Colorado appears to be highly restricted. However, the geographic distribution is not completely understood because extensive and exhaustive population surveys have not been conducted. Further, the abundance of this species is unknown relative to other cryptic species of winter stoneflies. My study sought to determine the distribution and abundance *A. arapahoe* in streams of the northern Front Range of Colorado, and to determine if known populations are still evident in Elkhorn Creek and Young Gulch after fire and flood disturbances.

Specifically, I investigated the following objectives: 1) to determine the extent of the distribution of the Arapahoe snowfly by sampling nearby, similar streams along the northern Front Range of Colorado, and by employing GIS tools to identify additional streams to sample that have similar habitat characteristics as Elkhorn Creek; 2) to estimate the population size of *A. arapahoe* in Elkhorn Creek by sampling population densities in pre-defined stream segments over time in 2013; 3) to determine co-occurring winter stonefly species which may be used as indicators of presence of appropriate aquatic habitats, which could be helpful for identifying critical habitat; and 4) to determine whether *A. arapahoe* is still detectable in Elkhorn Creek and Young Gulch in 2014 after fire and flooding.

METHODS AND MATERIALS

Relevant Capniid and Species Ecology

The life cycles of many capniids are considered to be univoltine, producing only one generation each year (Hynes, 1976, Dosdall & Lehmkuhl 1979, Short & Ward 1981). In late winter to early spring newly hatched nymphs move into a hyporheic zone (loose rocky substrate zone under the stream saturated with water) and diapause (physiological state of inactivity) until the late fall (Stewart & Stark 2002). Although there are no field based studies published on nymphal development or diapause (Pugsley & Hynes 1985), it was suggested by Harper & Hynes (1970, 1972) that diapause may be an evolutionary mechanism for the nymphs to avoid high temperatures during the summer. Jacobi & Cary (1996) indicated that the length of time for diapause of winter-emerging Plecoptera remains unknown. However, Snellen and Stewart (1979) suggested diapause could be more than one year.

Arsapnia adults have dark-bodies and are well adapted to the cold stream temperatures (Stark et al. 1998), which is typical of a winter stonefly species (Ward & Stanford 1982). Studies suggest that colder temperatures may be required for the development of nymphs of Capniidae leading to a late winter/early spring adult emergence period while streams may still be under ice (Shepard & Stewart 1983, Stewart & Stark 2002). Once capniid nymphs reach maturity and are ready to emerge in late winter to early spring, they crawl out on the streams edge, or onto rocks and exposed debris and molt to become winged terrestrial insects (Stewart & Stark 2002). Once the adult emerges, the average distance of travel by either crawling or flying is not well known. However, studies suggest that some species only travel several meters from the stream via crawling and, rather than disperse widely, tend to move vertically into nearby trees (Hynes 1976, Peterson et al. 1999). Illies (1965) noted that their lack of mobility provides a good understanding of historical distribution, because they are unable to cross even minor ecological barriers. Limited movement may also make capniids more amenable to depletion sampling techniques, which are used for estimating population size and are further described below.

Although the specific feeding behaviors of the nymphs of *A. arapahoe* are unknown, most species in this family shred leaf material and other available detritus and the active nymphs are commonly found in woody debris or leaf packs (Finni 1975, Stewart and Stark 2008). In addition, Bo et al. (2013) has suggested that a dietary shift takes place as the nymph matures from a collector-gatherer to a shredder. As such, nymphs can also ingest a relatively large amount of mineral matter, coarse particulate organic matter (CPOM), and fungal hyphae (Azzouz & Sánchez-Ortega 2000, Navarro-Martínez et al. 2007, Bo et al. 2013).

The adult male of *A. arapahoe* processes a slender epiproct (intromittent organ) with horns at the tip, extending from the abdomen posterior apex (Nelson & Kondratieff 1988). When observed dorsally (from above), the length of the epiproct is seven times as long as the width, and the 7th tergurm has a distinct knob (Nelson & Kondratieff 1988, Stewart & Stark 2002). Although the nymphs of *Arsapnia* and *Capnia* cannot be determined to species level, mature nymphs can be distinguished from *Capnura*, *Eucapnopsis*, *Paracapnia* and *Utacapnia* by key provided in Stewart & Stark (2002).

Known Locality Descriptions

The type localities of *A. arapahoe*, Elkhorn Creek and Young Gulch, range from 1,800m-2,300m (5,800ft-7,400ft) and are cool first-order mountain tributaries of the Cache la Poudre River watershed, which ultimately drain into the South Platte River along the northern Colorado

Front Range (Thornbury 1965, Zuellig et al. 2012). Elkhorn Creek is mostly perennial in its lower reaches, but is subject to freezing in the upper reaches (USFS 1997). The upper stream reaches include Ponderosa Pine (*Pinus ponderosa* Dougl. ex Laws.), with steep slopes and sparse riparian vegetation (Nelson & Kondratieff 1988). Young Gulch usually becomes intermittent in the late summer and early fall, and largely depends on snowmelt, summer precipitation and/or ground water for flow. The lower reaches of these two streams where Arapahoe snowfly specimens have been collected have a pebble, cobble, and bedrock substrate, with a riparian zone of cottonwood (*Populus deltoides* Bartr.), willow (*Salix alba* L.), and box elder (*Acer negundo* L.) occurring along the stream margins (Nelson & Kondratieff 1988). However, the High Park fire of June 2012 may have differentially altered the riparian zone (Wohl 2013) of Young Gulch. The hyporheic zone in these streams has not been evaluated.

These type locality streams support a variety of aquatic insect taxa including other stonefly species (Ward et al. 2002, Zuellig et al. 2006, Zuellig et al. 2012). However, before the year 1900, very few aquatic macroinvertebrate records (mostly insects) were recorded in first order tributaries of the Poudre River, so it is unclear whether current community diversity and relative abundances reflect historical levels (Zuellig et al. 2012, Stoaks and Kondratieff 2014).

Sampling Methods

Winter stoneflies were collected using beating sheets, hand-picking emergence traps, and larval rearing. Due to the emergence of adult winter stoneflies in late winter to early spring, sampling for adult winter stoneflies was conducted during late winter (February), through early spring (early May) in 2013, and February-April in 2014. A beating sheet was the primary tool at all sites for collecting adult stonefly specimens. The beating sheet (BioQuip, Rancho

Dominguez, California, catalog # 2840C) is one of the more effective methods for collecting adult Plecoptera from riparian vegetation and is often the only efficient means in the winter for sampling adult stoneflies from streamside vegetation (DeWalt et al. 2015). Individual stonefly adults dropped onto the sheet and were picked up by forceps and placed in a 20ml vial of 80% ethanol. At all additional sites, adult winter stoneflies were collected by hand (using forceps) from various substrates including streamside vegetation, exposed woody debris and surfaces of exposed rocks, snow and ice. All adult specimens collected within the boundaries of an individual sample reach in Elkhorn, or in a stream for the spatial survey, were placed together in 20ml vials of 80% ethanol.

In addition to beatsheets and hand picking, emergence traps were utilized to assess their value as an *in-situ* quantitative collection method. In 2013, three emergence traps were placed in a systematic fashion along the edge of each of three sample reaches in Elkhorn Creek (9-total were deployed). The emergent traps (25”L x 25”W x 18”H) were checked during each visit to the stream. The specimens collected in the emergent traps were included in the total number of specimens collected in that reach. Additionally, standard D-frame kick nets with 500 µm mesh (Wildco Supply Company, Yulee, Florida, Catalogue #217031) were used to collect larvae of winter stoneflies for rearing in the laboratory. This allowed the opportunity to rear adults for specific determination from streams that were not being visited as frequently. The contents of the kick net were placed into a white plastic pan and sorted in the field. Mature Capniidae larvae were transported to a laboratory at Colorado State University (Fort Collins, Colorado) and reared to adulthood for identification. Rearing adults was utilized as a sampling method for additional streams that were visited only once and the specimens collected in the rearing facility were added to the total number of specimens collected.

Distribution of Arsapnia arapahoe in Northern Front Range Streams

Adult winter stonefly sampling was completed at several streams during 2013 and 2014. In 2013, sampling was conducted in streams identified through professional judgment to be similar in stream order and habitat characteristics to type localities (Table 1). To extend our 2014 search efforts, a descriptive habitat map was created in ESRI-ArcGIS 10.2.2 to identify those streams in Larimer and Boulder counties that have similar stream basin elevation and vegetation land cover types as Elkhorn Creek and Young Gulch. Thus, a Raster Reclassification was completed, Raster to Vector (Feature) Conversion, Vector "Select", Vector "Buffer" of Streams, and Vector "Intersection" (Law & Collins 2013). The resulting area (Figure 1) was used to identify streams in Larimer and Boulder counties that are most likely to contain similar elevations and landscape characteristics to that of the known Arapahoe snowfly streams, Elkhorn Creek and Young Gulch. From February to April 2014, streams identified through GIS modelling as potential sites for the Arapahoe snowfly were sampled to verify the potential occurrence of the species. The selection method for streams to sample was based on a few general criteria: if the stream was located in the highlighted area with matching characteristics, if there was water in the stream, and legal stream access.

Population Estimation for Arsapnia arapahoe in Elkhorn Creek

To obtain population abundance estimation (number of individuals per stream length), Elkhorn Creek was repeatedly sampled within a nested spatial stratification in 2013 using a multi-pass estimation technique. Sampling began as the ice receded in March 2013 and was completed on a weekly schedule (2-3 days/week) dependent on weather conditions and stream access, for a total of 21 sample dates. Sampling in 2014 was less intensive with a total of 7 sample dates. Different stream reaches were visited at each sample date. I first stratified Elkhorn Creek into three 300m reach segments based on elevation (Lower 1983m – 1996m, Middle 2003m – 2011m, and Upper 2031m – 2042m). Each 300m reach segment was comprised of five nested and consecutive 60m reaches, providing a total of fifteen 60m reaches, representing the three elevation zones of the stream (Lower (A-E), Middle (F-J), and Upper (K-O) zones (Figure 2). During the adult emergence period (February-April), beating sheet and hand collection methods were used for each single-pass of depletion sampling of each 60m reach. Across 2013 sampling efforts, the Lower segment had n=19 total sample dates over the 2013 sample season, the Middle segment had n=19 total sample dates, and the Upper segment had only n=16 sample dates due to extended ice cover. Each stream reach was sampled 3-4 times each, albeit on different dates, as part of these totals. The area of habitat sampled varied depending on the width of the riparian habitat zone along each reach. Therefore, I expressed population size estimates as abundance on a "per stream length" basis, rather than as density on a "per area" basis. Data were analyzed using simple descriptive statistics (mean and standard errors).

The concept of depletion or removal sampling, which is more typically used for fish, is that an identified number of specimens are removed from a known area or length of habitat on each sampling occurrence, which consequently affects the number of individuals collected on the next pass (Cowx 1983). Typically, the sample reach is considered to be a closed system (e.g. with use of nets at either end, Taylor et al. 2001). However, this was not possible for free-flying adult stoneflies. Fortunately, as previously discussed, capniids, do not travel more than a few meters upon emergence thus, sampled reaches were presumed to be closed systems. The decreasing rate at which the individuals are collected can be used to directly relate the number of removed individuals (known) to the suspected true population size (unknown) (Cowx 1983).

Species Co-occurring with Arsapnia arapahoe

To address our objective of identifying the co-occurring species with *A. arapahoe*, we simply looked at the sites where we detected *A. arapahoe*, and listed all the species that were present at each positive locality. As well, we reviewed the depletion sampling data for estimating the relative abundance at Elkhorn Creek. A species list was generated from all positive site localities, and only the species that were present at all positive sites were chosen as the co-occurring species. Additional species were found at positive localities, but those that were not at all positive localities were discarded from the list.

Resilience of Capniids to Ecological Disturbances

To assess any possible effects of the historic September 2013 flooding to the Capniidae populations, Elkhorn Creek was resampled in 2014 using the same beat-sheeting and hand-collection methods employed during the 2013 field season. Thus, all individual specimens that were found during the sampling event were collected by utilizing the same procedures (beating sheet and hand collecting) as in 2013. However, during the 2013 field season there were 54 sampling events over 21 days, ranging from March 3 to May 5. During the 2014 field season there were only 32 sampling events over 7 days, from March 17 through April 16. To determine if *A. arapahoe* were still present at Young Gulch after significant disturbances, Young Gulch was re-sampled using aforementioned sampling techniques in 2014 on 4 dates.

RESULTS

Distribution of Arsapnia arapahoe in Northern Front Range Streams

During the 2013 and 2014 field seasons a total of twelve winter stonefly species and 14,492 individual adult stonefly specimens were collected from 54 streams in four Front Range drainage basins of Colorado. Only 24 adult male *A. arapahoe* were collected, representing <0.01% of the stonefly adults collected (Table 2). *Arsapnia arapahoe* were confirmed in Elkhorn Creek in both 2013 and 2014. However, no adults of *A. arapahoe* were collected from Young Gulch in either year. Overall, *A. decepta* (66%) and *C. gracilaria* (24%) made up 90% of all adult stoneflies collected, and the remaining nine species accounted for <10% of the total number of adult stoneflies collected at all 54 streams (Table 2). Of the nine remaining species collected, *Capnura wanica* made up 3%, and *Paracapnia angulata* (Hanson, 1961), *Utacapnia logana* (Nebeker & Gaufin, 1965), and *Zapada cinctipes* (Banks, 1897) each made up 2% of the total number of adult stoneflies collected. Species that made up 1% or less were *C. coloradensis* (Claassen, 1937), *C. confusa*, *Eucapnopsis brevicauda* (Claassen, 1924), *Isocapnia vedderensis* (Ricker, 1943), and *Mesocapnia frisoni* (Baumann & Gaufin, 1970) (Table 2).

Geospatial analysis identified 120,700 acres of small, lower montane watersheds along the Northern Front Range of Colorado (Figure 1). Within these areas of greatest distribution potential, 22 additional sites were sampled by beat sheeting and hand collection in 2014 (Table 3). Streams surveyed in both 2013 and 2014 fell within identified potential areas, and resulted in discovery of six new populations of *A. arapahoe* at the following first-order streams: Sheep Creek (Big Thompson Watershed); Central Gulch (St. Vrain River Watershed); and Bear Canyon Creek, Bummer’s Gulch, Martin Gulch, and Tom Davis Gulch (Boulder Creek Watershed) (see

Figure 1; see also Heinold et al. 2014). All six new positive localities for *A. arapahoe* were located in the designated area of matching characteristics for Elkhorn Creek and Young Gulch, from our GIS analysis (Figure 1). The total number of individual adult stoneflies collected in 2013 and 2014 by stream site, and number of visits per site, are listed in Table 4.

Other collection methods captured fewer winter stonefly specimens than the beat sheeting and hand collection methods. Emergent traps were not successful in collecting adult stoneflies. The total number of Capniidae specimens collected in all emergent trap sampling events, resulted in <0.02% of the total number of specimens collected at Elkhorn Creek. In 2013, *A. arapahoe* were not collected in the emergent traps and taxa collected in the traps were already represented in the samples collected by beat sheeting and hand collection techniques. Therefore, the emergent traps were discontinued during the 2014 field season. The rearing facility proved to be unsuccessful at collecting *A. arapahoe*, and otherwise inefficient at collecting adult winter stoneflies (4% of the total number of specimens collected at Elkhorn Creek), when compared to the beating sheet method and hand picking from substrate. Overall, the combined total number of Capniidae collected in both the emergent traps and the rearing facility resulted in <0.01% of all specimens collected in this study and are thus not recommended for future work on population estimation of the Arapahoe snowfly.

Arsapnia arapahoe Population Estimates (Abundance) in Elkhorn Creek

Multiple-pass depletion sampling in 2013 showed that the first pass detection was repeatedly >90% of all stoneflies and 100% of *A. arapahoe* individuals (Figure 3). Therefore, single pass depletion techniques were determined to be adequate to estimate population size and was used for Elkhorn surveys for the remainder of field sampling in 2013 and 2014.Only stoneflies collected on the first pass of the initial sampling events were used so future sample efforts would be consistent.

During the 2013 field season at Elkhorn Creek, a total of 1,605 individual stonefly adults were collected in the 15 (60m) reaches (A-O) from 3 March – 5 May 2013. Only eight *A. arapahoe* were collected over all reaches and sample dates in Elkhorn Creek in 2013. As such, the population size in Elkhorn was estimated to be 8/900m from habitats that were amenable to sampling.

The middle segment of Elkhorn Creek had greater abundances of emerging adult stoneflies, including *A. arapahoe*. The lower segment (A-E) yielded a total of 475 adult stoneflies over 19 total sampling events. The middle segment (F-J) yielded 714 adult stoneflies over 19 total sampling events. The upper segment (K-O) was only sampled 16 times, and resulted in a total of 416 adult stoneflies (Table 5). The detection of *A. arapahoe* at Elkhorn Creek was determined to be the following; an average of 0.05 individuals (SE = ±0.05) for n=19 sampling events of 60m reaches in the lower 300m segment, an average of 0.26 individuals (SE = ±0.17) for n=19 sampling events of 60m reaches in the middle 300m segment, and an average 0.13 individuals (SE = ±0.09) for n=16 sampling events of 60m reaches in the upper 300m segment (Figure 4). Arapahoe snowfly detection were thus considerably lower than total abundance of stoneflies caught per 300m in all three stream segments (Figure 5).

Species Co-occurring with Arsapnia arapahoe

Arsapnia arapahoe represented <0.01% of all specimens collected at Elkhorn Creek during the 2013 and 2014 field seasons. *Capnia gracilaria* was the most abundant species, and *A. decepta* was the second most abundant species in Elkhorn Creek. This pattern is opposite of patterns observed across all 54 streams, where *A. decepta* was the most abundant and *C. gracilaria* was the second most abundant. There were four species collected that represented >93% of all specimens collected (*C. gracilaria*, 50%; *A. decepta*, 30%; *Z. cinctipes*, 7%; and *C. wanica*, 6%) (Table 6). The remaining five species collected represented <7% of all adult stoneflies collected at Elkhorn Creek.

Two Capniidae species that consistently co-occurred with *A. arapahoe* were *A. decepta* and *C. gracilaria*. At all positive sites for *A. arapahoe, A. decepta* was the most common other capniid. The exception was Elkhorn Creek, where *C. gracilaria* was the most common capniid. Therefore, *A. decepta* and *C. gracilaria* may be useful as indicator species for identifying critical habitat for *A. arapahoe*.

There appeared to be no strong pattern of the timing of *A. arapahoe* detection, as individuals were collected in early March and later in April depending on the reach (Figures 6, 7, and 8). Sampling was most productive for *A. arapahoe* between mid-March to the third week in April. No *A. arapahoe* were collected in the last two weeks of sampling in 2013 and 2014 (Figure 9).

Arsapnia arapahoe Type Locality Populations after Wildfire and Flooding

During the course of this study, two major ecological disturbances occurred in the Cache la Poudre River watershed. Following the High Park Fire in 2012, ash and streambed sediments had accumulated in Young Gulch. Subsequently, September 2013 flooding appeared to significantly alter sediment deposits, presence of streamside vegetation, and accumulations of course woody debris in both Young Gulch and Elkhorn Creek. In Elkhorn Creek, sections of large diameter (1+m) dead-downed cottonwood trees were no longer present, large patches of willows were missing and/or significantly altered, and large wood accumulations within the stream were blown out. Due to apparent geomorphic changes following the disturbances, substrates and streamside vegetation on which to sample winter stoneflies were considerably less available in 2014 compared to 2013.   Several capniid species, including *A. arapahoe* was found in Elkhorn Creek in 2014 (2 *A. arapahoe* individuals in 2014) despite severe flood impacts. In addition, Capniids and other stoneflies were not found in Young Gulch in 2013. However, a few *A. decepta* and *C. gracilaria* individuals were collected in 2014. *Arsapnia arapahoe* were not collected in Young Gulch in 2013 or 2014.

DISCUSSION

Overall, only 24 *A. arapahoe* were discovered out of the 14,492 adult stoneflies collected at 54 streams during 2013 and 2014, which is <0.01% of the total specimens. Prior to this study *A. arapahoe* was known from two drainages in the Cache la Poudre River watershed, Young Gulch and Elkhorn Creek. My study shows that the geographic distribution of this species may be more widespread than previously understood. Currently, we now have determined that *A. arapahoe* populations occur in a total of eight mid-elevation first-order streams across 4-drainage basins of the northern Front Range of Colorado (see also Heinold et al. 2014). The six new localities are all first-order streams located outside the Cache la Poudre watershed: Sheep Creek is located in the Big Thompson watershed, Central Gulch is located in the Saint Vrain watershed, and Bear Canyon Creek, Bummer's Gulch, Martin Gulch, and Tom Davis Gulch are all located in the Boulder Creek watershed. The number of *A. arapahoe* adults collected in these streams, and in the type locality Elkhorn Creek, were consistently found in significantly lower abundances compared to the other co-occurring Capniidae species. Our findings support the classification of this species as a rare stonefly.

The sympatric Plecoptera species with *A. arapahoe* were consistent across locations where the Arapahoe snowfly was found in the northern Front Range of in Colorado. The most common sympatric species found with *A. arapahoe* were *A. decepta*, *C. gracilaria*, *C. wanica*, *C. confusa*, *U. logana*, *P. angulata*, and *Z. cinctipes*. These winter stonefly species are all common in the western United States, and the lower abundances observed in our study were likely due to the fact that some of these species are known to emerge earlier or later than the targeted timeframe of this study (Zuellig et al. 2012). The two species that were collected in the greatest abundance at each location where adult *A. arapahoe* was present were *A. decepta*, and *C. gracilaria*. *Arsapnia arapahoe* is closely related to *A. decepta* and appears to be always sympatric with this more common *Arsapnia* species. As such, *A. decepta* may be used as an indicator species for the appropriate aquatic habitats and for potential presence of *A. arapahoe* which could be helpful in identifying and protecting habitat.

Prior to this study, the population size of the Arapahoe snowfly adults at Elkhorn Creek, and its distribution along the Northern Front Range of Colorado, was unknown. *Arsapnia arapahoe* was previously known from only 14 specimens in Elkhorn Creek and Young Gulch in the Cache la Poudre watershed. From 21 separate sampling dates over 900 m of stream length in Elkhorn Creek, *A. arapahoe* represented <0.01% of the total number of adult, winter-emerging stoneflies collected in 2013. With a population size estimate of only eight *A. arapahoe* adults over 900 m of stream sampled, this species exists in only very low numbers in Elkhorn. By contrast, population estimates for the more common species of winter stoneflies were orders of magnitude higher. My detection rate for *A. arapahoe* was an average of only 0.0024 individuals across all reach-sample combinations during Elkhorn surveys in 2013. This extraordinarily low average is due to the significant number of non-detections. By contrast, mean detection of potential indicator species were higher for *A. decepta* (0.1259 individuals across all sample-date combinations) and *C. gracilaria* (0.2354 individuals across all sample-date combinations). The results found here demonstrate how the Arapahoe snowfly occurs at very low densities and is rare even in Elkhorn Creek, where it has been consistently found over several years of sampling. I also show that significant sample effort is required to detect even one individual capniid across a 900m reach of stream even when suitable habitat is available for sampling.

Ecological disturbances in Young Gulch appear to have negatively impacted known capniid and other winter stonefly presence, including *A. arapaho*. Winter stoneflies were not detected the year following the High Park fire of 2012, possibly due to substantial ash and sediment deposits in the stream bed or, perhaps the lack of available coarse particulate organic matter (CPOM) as the food source. The Arapahoe snowfly was also not found after flooding impacts, and maybe extirpated from this stream. However, it is important to note that *A. decepta* and *C. gracilaria* were recollected in relatively low abundances following wildfire and flood disturbances in 2014. In other western North American watersheds, loss of riparian subsidies and burned leaf litter retention after wildfire have been shown to cause functional shifts in benthic communities, where detritivore specialists (shredders) are replaced with generalist herbivore-detritivores (Mihuc et al. 1996, Minshall et al. 2001, Vieira et al. 2011). Once the Young Gulch watershed fully recovers from the hydro-geomorphological impacts of fire and flooding, an intensive adult stonefly survey such as our survey of Elkhorn Creek may provide a better representation of the winter stonefly species recovery following major ecological disturbances.

Interestingly, no adult females out of the 14,492 collected matched the female description of *A. arapahoe* by Heinold & Kondratieff (2010). Since no females collected in this study or in surveys conducted by Heinold et al. (2014) conformed to the description of the female, this leads one to believe that possible polymorphism, recent lineage divergence, or hybridization can not be ruled out for *A. arapahoe*. None of the presumed *A. arapahoe* females that were DNA barcoded from Heinold's previous collection(s), as well as those thought to be *A. arapahoe* females from this study, were sequenced to match *A. decepta*, as male specimens of *A. arapahoe* were (Heinold et al. 2014). One male specimen collected at Elkhorn Creek in reach-B on March 21, 2014 labeled as a Capniidae species, may be intermediate between both *A. decepta* and *A.*

arapahoe. Further analysis of the putative *A. arapahoe* female specimens, all male *A. arapahoe*, and additional genetic analysis including nuclear markers and mitochondrial investigations, are needed to better understand this species and its phylogenetic history.

Sibling or cryptic species of insects can be similar morphologically and dissimilar genetically, and vice versa (Williams et al. 2006, De Figueroa & Fochetti 2014). For example, two species in the Capniidae family, *A. coyote* and *A. decepta*, have similar male terminalia, but can be distinguished using DNA barcoding (Heinold et al. 2014). Nelson & Baumann (1987) indicated that these species are allopatric in southern California. Males of *A. arapahoe* and *A. decepta* are morphologically distinctive, but the DNA barcoding was unable to differentiate between the two species (Heinold et al. 2014). Other species have been shown to be dissimilar morphologically, yet similar genetically (Bickford et al. 2006), and this may be the case with *A. arapahoe*. These sibling or cryptic species pose challenges for determination of species status and candidacy for protection under the Endangered Species Act, and warrant further consideration regarding processes for protecting rare insect taxa.

The results from this two year study have shown that the *A. arapahoe* species apparently exists in small populations and is apparently restricted in distribution. My data suggest that the population sizes are lower in abundance and distribution than more common species of winter stoneflies. This leads to the conclusion that the Arapahoe snowfly is rare in the northeastern Front Range of the Rocky Mountains of Colorado. However, other factors such as sampling challenges may also play a role in its rarity, and these challenges have implications for assessing status and trends of this species.

Many factors could play a role in the lack of ability to collect this species in larger series, including environmental sensitivities (Fochetti & Tierno de Figueroa 2008). Variables such as sampling error related to small populations, errors in identifying time and/or spatial location of emergence, sampling limitations due to environmental conditions such as snow and wind, and temporal or spatial shifts in niche requirements can all lead to under-sampling of the population. Lack of understanding thermal requirements (Dewalt et al. 2012), topographical or geographical boundaries related to dispersal, or sensitivities to water quality and degradation (Stoaks & Kondratieff 2014), may also contribute to the limited ability to collect this species. It is unknown if *A. arapahoe* historically inhabited additional streams along the northern Front Range Mountains of Colorado making it difficult to determine if the species is capable of dispersing across nearby watersheds, or if populations discovered in my study are relict populations. Without the longer-term knowledge on the distribution of this species, we are limited to searching streams that have similar environmental features as, and are in proximity to the type localities. The specific reasons for the rarity and highly limited distributions of these capniids are ultimately unknown at this time, and further investigations are needed to identify these specific limiting factors.

Although our GIS reclassification (Law & Collins 2013) was conducted at a coarse scale in regards to environmental features (e.g., elevation, stream order), valuable information was attained on possible new stream locations that extend beyond our search efforts. Again, all eight of the known *A. arapahoe* localities were located in areas with matching landscape and elevational characteristics to Elkhorn Creek and Young Gulch. Information such as instream substrate, streamside vegetation, and basin elevations may play a key role in the spotty locations that this species inhabits. Future distribution modeling that includes a detailed Maximum Entropy Model (Merow et al. 2013) analysis of elevation data, vegetation data, precipitation data, temperature data, and climatic variables, could offer a more detailed analysis of the bioclimatic variables (Marcer et al. 2013) that might affect the limited distribution of the Arapahoe snowfly. Utilizing a species distribution model such as this would analyze all the input variables, and result in a probability of detection with the highest values being the best matched areas to extend the search efforts (Elith & Leathwick 2009). Identifying and detailing stream characteristics at both course and fine spatial scales will be key to better understand and characterize potential *A. arapahoe* streams.

Conservation of the Arapahoe snowfly will rely not only on knowledge of population size and spatial distribution, as I present here, but also on a better understanding of life-histories and habitat requirements for this species. Future analyses of microhabitat requirements related to bed substrate and connections to the hyporheic zone, preferred riparian vegetation for adult activity, availability of detrital materials and Coarse Particulate Organic Matter (CPOM) for nymphs, and thermal characteristics of these streams would provide invaluable information for species management. Such studies would characterize key habitat specific elements of the Arapahoe snowfly in the eight locations where they are currently known as well as potential streams where the Arapahoe snowfly may exist or may colonize in the future.

A thorough investigation of the population size and distribution of the Arapahoe snowfly in all eight known localities in the northern Front Range of Colorado would provide opportunity to better understand how population sizes differ between years and locations and would further elucidate potential meta-population dynamics of this species. However, a project of this magnitude would be costly, and would also result in removal of individuals from populations (for identification purposes) which are already depauperate. Therein lays the dilemma for conserving and protecting rare insect taxa. However, the information gained from such an extensive search effort in at least 1-2 more streams would allow for better predictions of the probability of success for Arapahoe snowfly populations where they are known to exist. Tracking the indicator species that I identified here, *A. decepta* and *C. gracilaria* may be a less intrusive and yet informative way to predict population dynamics of the more rare *A. arapahoe*. In summary, the Arapahoe snowfly is shown here to be rare in distribution and population size when compared to other capniids and warrants further investigation of the species' geographic distribution and limiting habitat factors to determine suitable protection and conservation measures.

LIST OF TABLES

Table 1. Complete list of streams sampled in 2013 in the Cache la Poudre River, Big Thompson River, Saint Vrain River, St. Charles River, Boulder Creek, and Saint Vrain Creek, Colorado.

Streams Sampled in 2013	
Bennett Creek (PR)	Little Thompson (BTR)
Buckhorn Creek (BTR)	Lone Pine Creek (PR)
Central Gulch (SVR)	Rabbit Creek (PR)
Dale Creek (PR)	Rabbit Creek-North Fork (PR)
Elkhorn Creek (PR)	Sand Creek (PR)
Fish Creek (PR)	Sevenmile Creek (PR)
Greenhorn Creek (SCR)	Sheep Creek (BTR)
James Creek (SVR)	Trail Creek (PR)
Keystone Gulch (BC)	Un-Named Creek (SVR)
Left Hand Creek (SVC)	Young Gulch (PR)
Little Beaver Creek (PR)	

Table 2. Total number of Plecoptera adults collected in 2013-2014, Boulder and Larimer counties, Colorado.

Plecoptera	**Total**	**Male**	**Female**	**% of Total**
Arsapnia arapahoe	24	24	0	<0.01%
Arsapnia decepta	9522	5871	3651	66%
Capnia coloradensis	65	47	18	<0.01%
Capnia confusa	85	47	38	1%
Capnia gracilaria	3455	2205	1250	24%
Capniidae sp.	9	1	7	<0.01%
Capnura wanica	385	215	170	3%
Eucapnopsis brevicauda	1	0	1	<0.01%
Isocapnia vedderensis	2	1	1	<0.01%
Mesocapnia frisoni	90	68	22	1%
Paracapnia angulata	235	79	156	2%
Unidentified	14	0	5	<0.01%
Utacapnia logana	268	160	108	2%
Zapada cinctipes	337	194	143	2%
TOTALS	**14492**	**8912**	**5570**	**100%**

Table 3. Complete list of streams identified using GIS habitat modeling and sampled in 2014 in the Cache la Poudre River, Big Thompson River, Saint Vrain River, St. Charles River, Boulder Creek, Saint Vrain Creek, South Platte River, and Clear Creek, Colorado.

Streams Sampled in 2014		
Bear Canyon Creek (BC)	Elkhorn Creek (PR)	Sheep Creek (BTR)
Boulder Creek (BC)	Greenhorn Creek (SCR)	Skunk Creek (BC)
Brush Creek (SPR)	Gregory Creek (BC)	South Creek (SCR)
Bull Creek (PR)	Hewlett Gulch (PR)	South Lone Pine Creek (PR)
Bummer's Gulch	Kennedy Gulch (SPR)	Spring Creek (PR)
Cedar Creek (BTR)	Little Thompson (BTR)	Tom Davis Gulch (BC)
Cedar Gulch (PR)	Lost Gulch (BC)	Trib Bear Gulch (BC)
Central Gulch (SVR)	Martin Gulch (BC)	Trib Bummer's Gulch (BC)
Cottonwood Creek (BTR)	Mill Creek (PR)	Trib Meadow Creek (PR)
Deadman Gulch (SVR)	Poverty Gulch (PR)	Unknown Creek (BTR)
Devil's Creek (PR)	Quillan Gulch (BTR)	Un-Named Creek (BC)
Divide Creek (PR)	Rabbit Creek-North Fork (PR)	Van Biber Creek (CC)
Dry Creek (BTR)	S. Boulder Creek (BC)	Young Gulch (PR)
Eightmile Creek (BC)	Sevenmile Creek (PR)	

Table 4. Total number of individual adult stoneflies collected by stream site for 2013 and 2014, Colorado.

Stream	Visits	Total	Stream	Visits	Total
Bear Canyon Creek - Trail S. of NCAR	2	103	Little Thompson - CR-23	3	39
Bear Canyon Creek - Bear Canyon Ck. Trail	1	13	Little Thompson - Stagecoach Trail	1	11
Bennett Creek - Bennett Creek Picnic Area (Pingree Pk Rd.)	1	10	Lone Pine Creek - Maxwell Ranch Rd.	1	25
Boulder Creek - Eben G. Fine Park	3	222	Lost Gulch - Chapman T.H.	1	14
Brush Creek - S. Platte River Rd.	1	3	Lost Gulch - Conf. with Boulder Creek	1	146
Buckhorn Creek - Buckhorn Road	5	318	Martin Gulch - Walker Ranch Loop	2	653
Bull Creek - Moen Rnch Road	1	0	Mill Creek - Mill Creek Rd.	1	0
Bummer's Gulch - Sugarloaf Rd./Millionaire Rd	2	77	Poverty Gulch - CO-14 W - mi. from U.S. 287 N	1	48
Cedar Creek - Forest Road 128	4	2298	Quillan Gulch - Rd. 122 from Pinwood Reservior	1	12
Cedar Gulch - CO-14 W - 15.5 miles from U.S. 287 N	3	73	Rabbit Creek - Rabbit Creek SWA	2	19
Central Gulch - HWY 7 (South St. Vrain Dr.)	3	483	Rabbit Creek-North Fork - Cherokee Park Rd. (CR-80C)	3	8
Cottonwood Creek - CR-18 Larimer County OS	1	19	S. Boulder Creek - Mesa T.H.	1	3
Dale Creek - Virginia Dale (HWY 287)	1	71	Sand Creek - Red Mountain OS	2	72
Deadman Gulch - HWY 7 (South St. Vrain Dr.)	3	524	Sevenmile Creek - Rustic (Manhattan Rd.)	7	270
Devil's Creek - CR-80C	1	95	Sheep Creek - Buckhorn Road	14	2908
Divide Creek - CR-67J and CR-80C	1	277	Skunk Creek - Mesa T.H.	1	3
Dry Creek - Forest Road 128	1	209	South Creek - Pueblo Mountain Park	1	52
Eightmile Creek - CR-67 Steel Bridge	1	902	South Lone Pine Creek - Magic Sky Ranch W. CR-74e	1	18
Elkhorn Creek - CO-14 W - 21 miles from U.S. 287 N	34	2876	Spring Creek - Above Horsetooth Res.	1	4
Elkhorn Creek - Boy Scouts-Ben Delatour Ranch-CR-68C	2	20	Tom Davis Gulch - Walker Rand Park	2	261
Fish Creek - FR 184 (CR-45E)	2	68	Trail Creek - Cherokee Park SWA	1	4
Greenhorn Creek - Rye Mountain Park	2	185	Trib Bear Gulch - Beaver Brook Trail	1	7
Gregory Creek - Gregory Canyon T.H.	3	260	Trib Bummer's Gulch - Sugarloaf Rd./Millionaire Rd.	1	7
Hewlett Gulch - CO-14 W - 10.5 mi. from U.S. 287 N	2	55	Trib Meadow Creek - CR-80C	1	72
James Creek - James Canyon Dr.	1	32	Unknown Creek - CR-118 S.W. of W.F. Little Thompson	1	49
Kennedy Gulch - Reynolds Park	1	11	Un-Named Creek - Sugarloaf Rd.	1	20
Keystone Gulch - 25 mi. E of Magnolia Rd.	1	147	Un-Named Creek - HWY 36 W. of Lyons	1	165
Left Hand Creek - Left Hand Canyon Dr.	1	14	Van Biber Creek - White Ranch OS	1	3
Little Beaver Creek - North CR-63E	1	132	Young Gulch - CO-14 W - 13 mi. from U.S. 287 N	8	102
			TOTAL	**148**	**14492**

Table 5. Total number of individual specimens collected of Plecoptera adults in the first pass minus totals from emergent traps, rearing, and by species and stream segment at Elkhorn Creek in Larimer County, Colorado 2013.

Plecoptera	**Lower-(A-E) n=19**	**Middle-(F-J) n=19**	**Upper-(K-O) n=16**	**SUM**
Arsapnia arapahoe	1	5	2	8
Arsapnia decepta	159	188	61	408
Capnia confusa	5	24	11	40
Capnia gracilaria	211	298	254	763
Capnia sp.	1	1	0	2
Capnura wanica	33	68	12	113
Eucapnopsis brevicauda	1	0	0	1
Paracapnia angulata	20	41	2	63
Unidentified	3	3	0	6
Zapada cinctipes	41	86	74	201
SUM	**475**	**714**	**416**	**1605**

Table 6. Percent of each species by total number of all individual specimens collected of Plecoptera adults in the first pass at Elkhorn Creek in Larimer County, Colorado 2013.

Plecoptera	**% of Each Species by Total # of Species Collected at Elkhorn Creek 2013**	**% of Each Species by Total # of Species Collected at Elkhorn Creek 2014**	**Overall % of Each Species by Total # of Species Collected at Elkhorn Creek 2013 & 2014**
Arsapnia arapahoe	<0.01%	<0.01%	<0.01%
Arsapnia decepta	28%	35%	30%
Capnia confusa	2%	1%	2%
Capnia gracilaria	44%	62%	50%
Capniidae sp.	<0.01%	<0.01%	<0.01%
Capnura wanica	9%	1%	6%
Eucapnopsis brevicauda	<0.01%	NA	<0.01%
Paracapnia angulata	5%	<0.01%	3%
Unidentified	<0.01%	<0.01%	<0.01%
Zapada cinctipes	11%	<0.01%	7%

LIST OF FIGURES

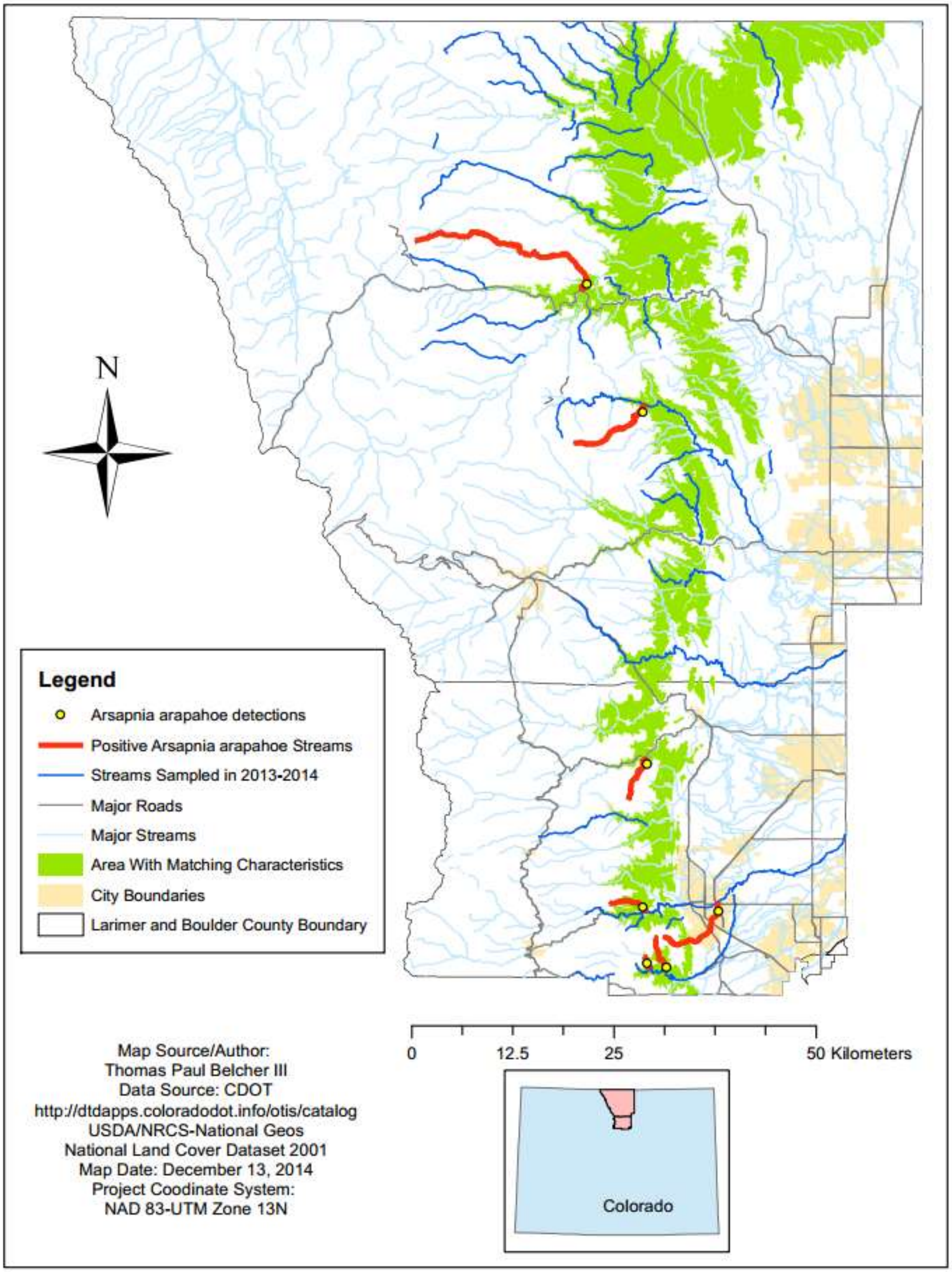

Figure 1. Map of area highlighted in green with similar elevation, vegetation, and landscape characteristics as Elkhorn Creek and Young Gulch in Larimer County, Colorado. Map also includes streams sampled in both 2013 and 2014, with the six new positive localities for *Arsapnia arapahoe* highlighted in red (Elkhorn Creek (Cache la Poudre Watershed), Sheep Creek (Big Thompson Watershed), Central Gulch (St. Vrain River Watershed), and Bear Canyon Creek, Bummer's Gulch, Martin Gulch, and Tom Davis Gulch (Boulder Creek Watershed), Colorado.

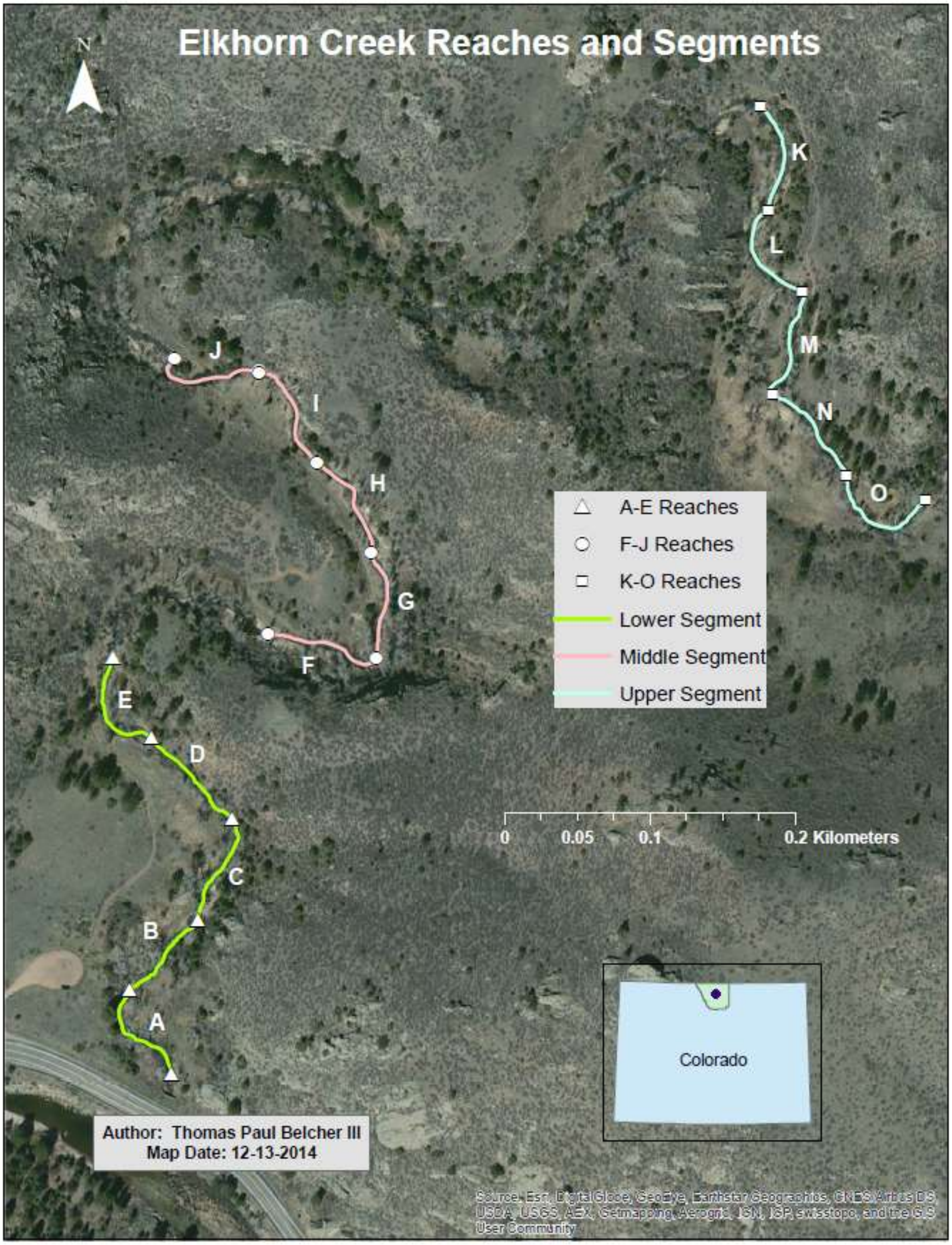

Figure 2. Map of designated reaches (A-O), and designated segments (Lower, Middle, and Upper), at Elkhorn Creek in Larimer County, Colorado.

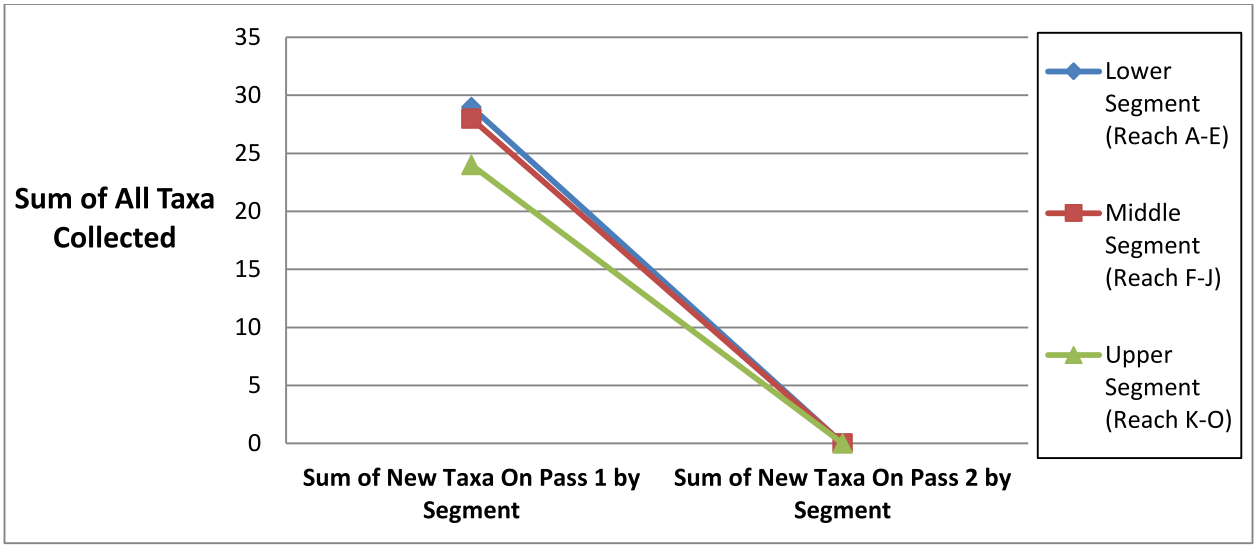

Figure 3. Total cumulative number of new Plecoptera adult taxa by pass for each 300m segment collected at Elkhorn Creek in Larimer County, Colorado 2013.

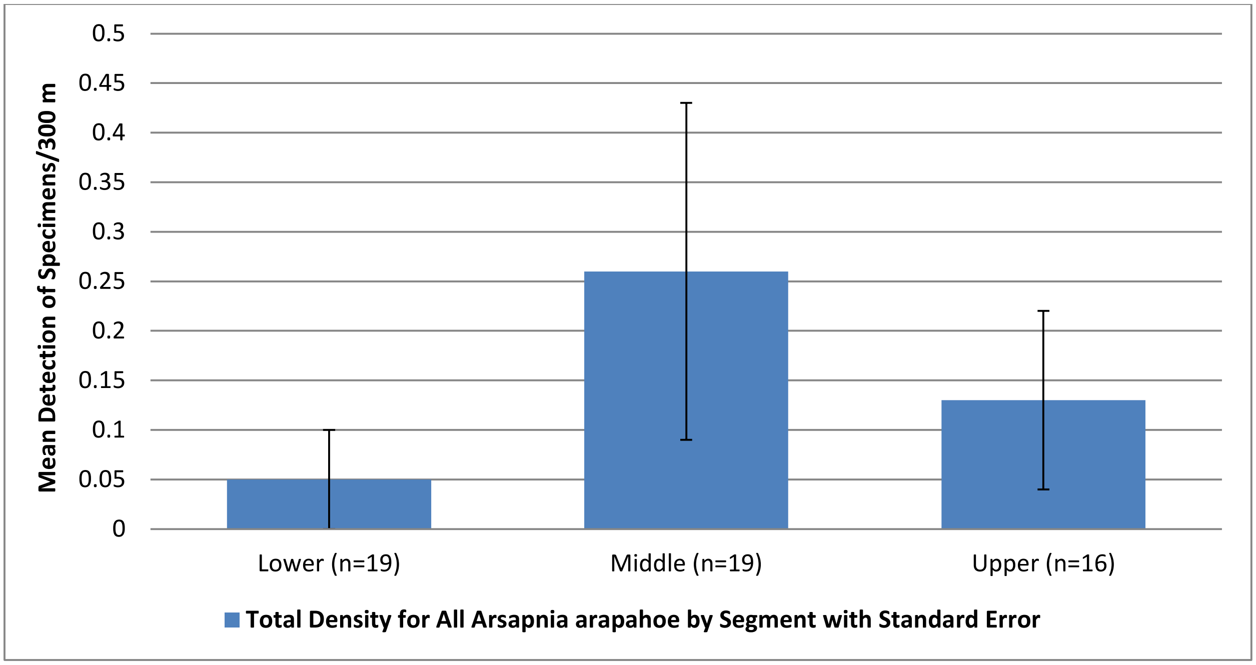

Figure 4. Total mean detection for all adult *Arsapnia arapahoe* collected by 300m segment collected at Elkhorn Creek in Larimer County, Colorado 2013.

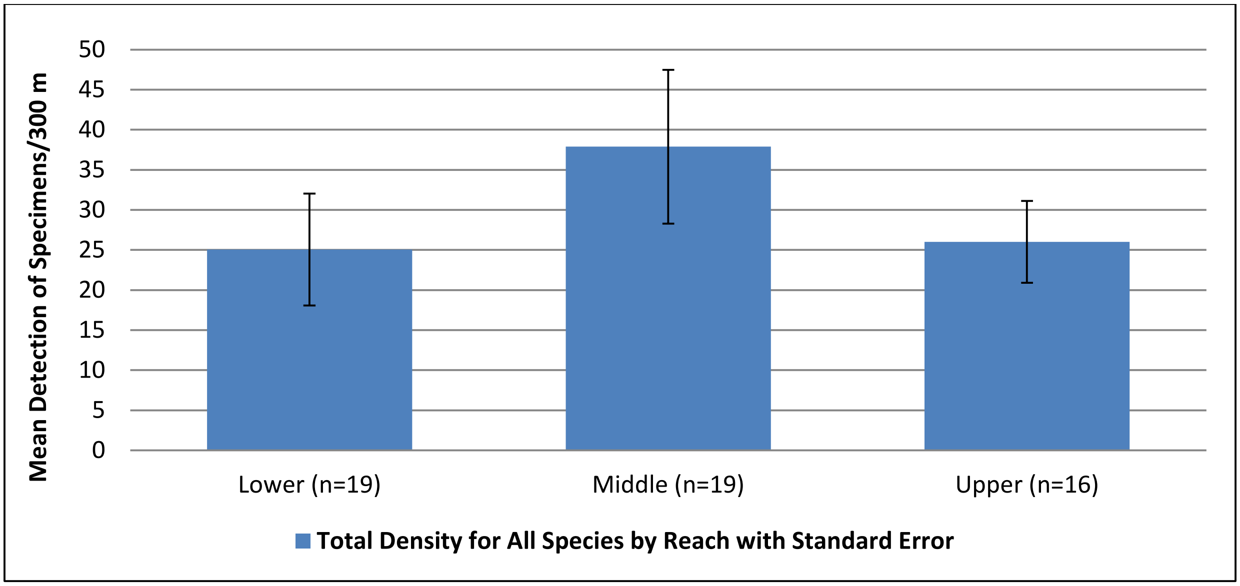

Figure 5. Total mean detection for all Plecoptera adults collected by 300m segment at Elkhorn Creek in Larimer County, Colorado 2013.

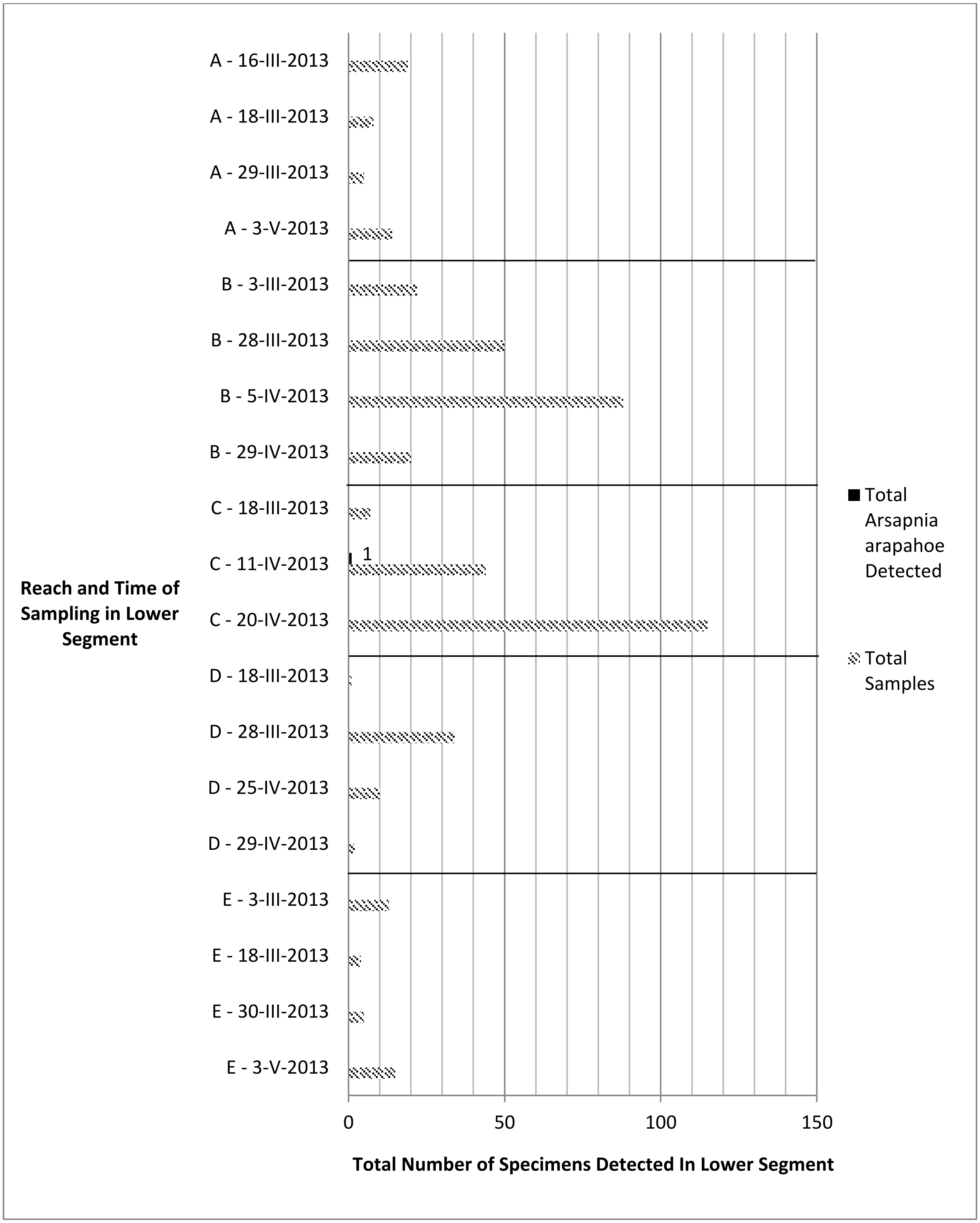

Figure 6. Cumulative number of all Plecoptera adults collected, with cumulative number of adult *Arsapnia arapahoe* collected at the lower segment (Reaches A-E) by date at Elkhorn Creek in Larimer County, Colorado 2013.

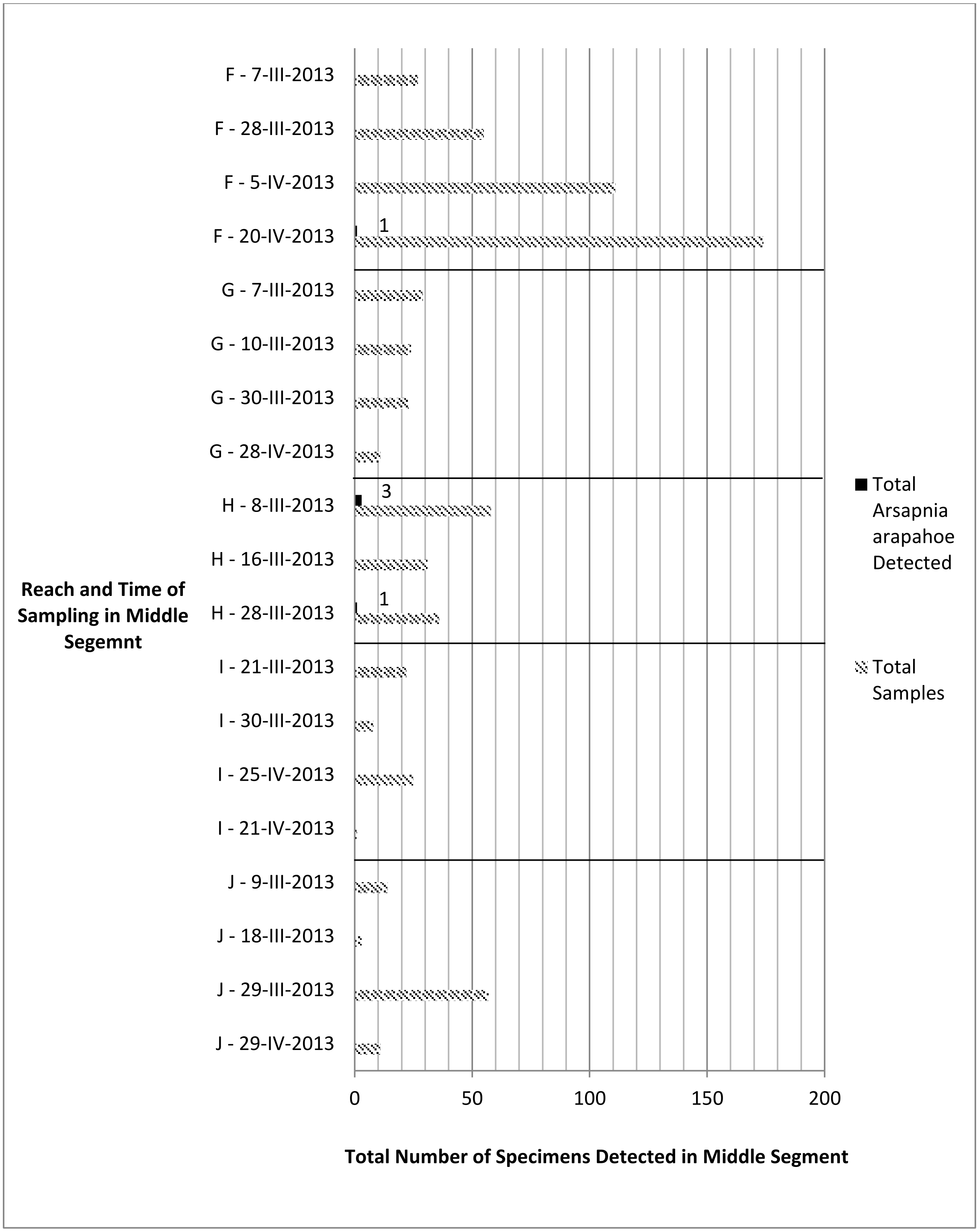

Figure 7. Cumulative number of all Plecoptera adults collected, with cumulative number of adult *Arsapnia arapahoe* collected at the middle segment (Reaches F-J) by date at Elkhorn Creek in Larimer County, Colorado 2013.

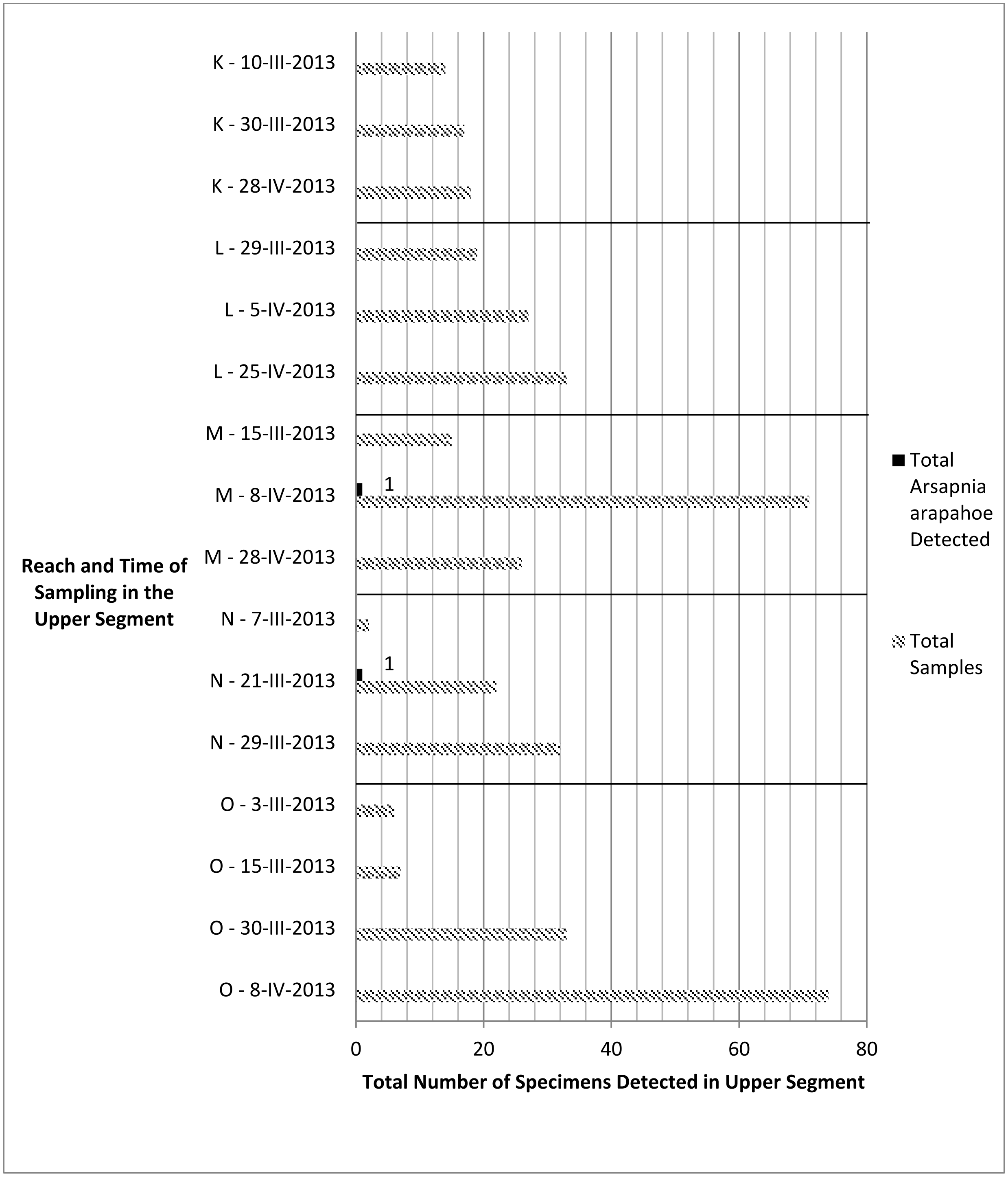

Figure 8. Cumulative number of all Plecoptera adults collected, with cumulative number of adult *Arsapnia arapahoe* collected at the upper segment (Reaches K-O) by date at Elkhorn Creek in Larimer County, Colorado 2013.

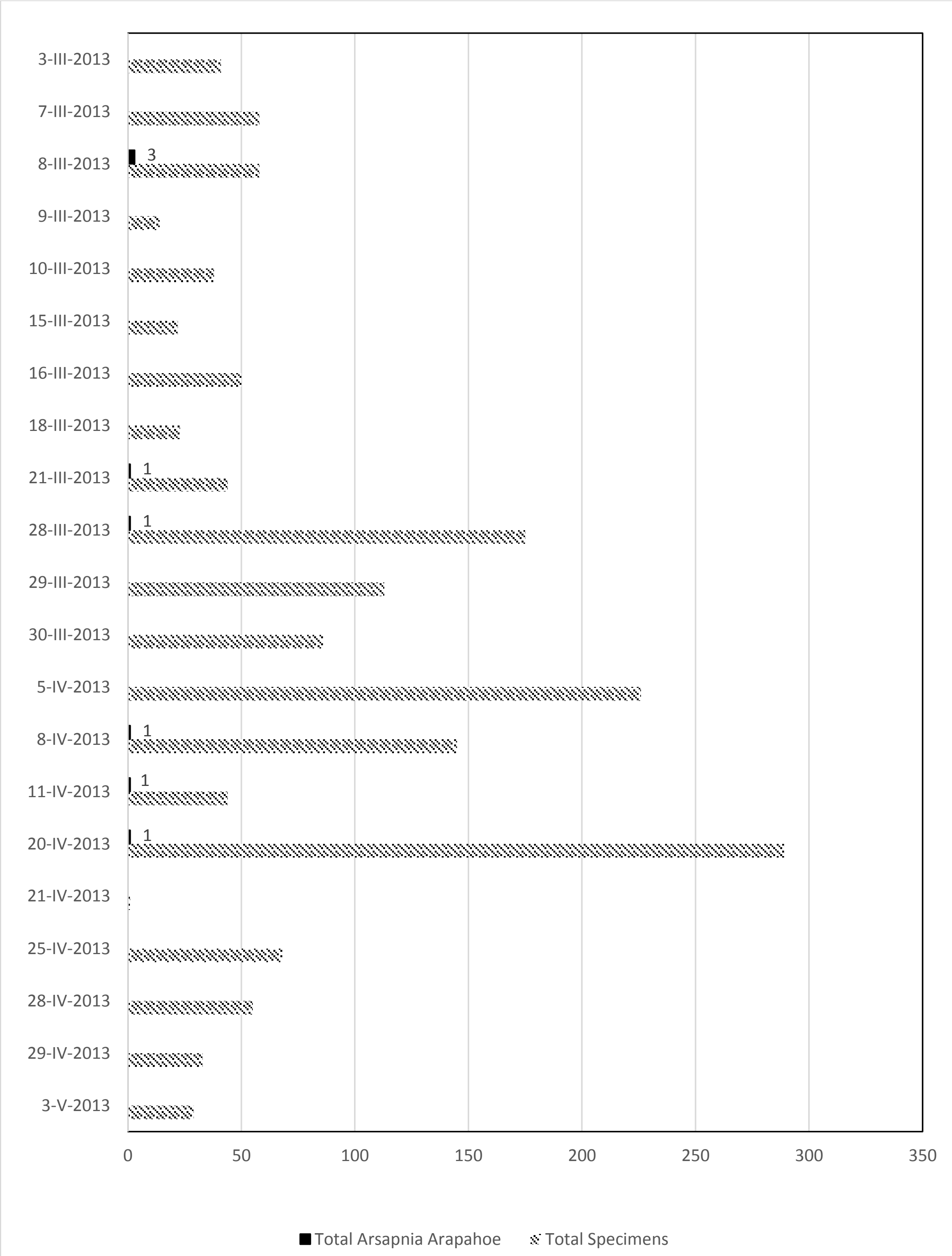

Figure 9. Cumulative number of all Plecoptera adults collected, with cumulative number of adult *Arsapnia arapahoe* collected by date at Elkhorn Creek in Larimer County, Colorado 2013.

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