

Comparison of Quantitative and Semi-Quantitative Assessments of Benthic
Macroinvertebrate Community Response to Elevated Salinity in Central Appalachian
Coalfield Streams

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ACADEMIC ABSTRACT

Anthropogenic salinization of freshwater is a global concern. In freshwater environments, elevated levels of major ions, measured as total dissolved solids (TDS) or specific conductance (SC), can cause adverse effects on aquatic ecosystem structure and function. In central Appalachia, eastern USA, studies largely rely on Rapid Bioassessment Protocols with semi-quantitative sampling to characterize benthic macroinvertebrate community response to increased salinity caused by surface coal mining. These protocols require subsampling procedures and identification of fixed numbers of individuals regardless of organism density, limiting measures of community structure. Quantitative sampling involves enumeration of all individuals collected within a defined area and typically includes larger sample sizes relative to semi-quantitative sampling, allowing expanded characterization of the benthic community. Working in central Appalachia, I evaluated quantitative and semi-quantitative methods for bioassessments in headwater streams salinized by coal mining during two time periods. I compared the two sampling methods for capability to detect SC-induced changes in the macroinvertebrate community. Quantitative sampling consistently produced higher estimates of taxonomic richness than corresponding semi-quantitative samples, and differences between sampling methods were found for community composition, functional feeding group, dominance, tolerance, and habit metrics. Quantitative methods were generally stronger predictors of benthic community-metric responses to SC and were more sensitive for detecting SC-induced changes in the macroinvertebrate community. Quantitative methods are advantageous compared to semi-quantitative sampling methods when characterizing benthic macroinvertebrate community structure because they provide more complete estimates of taxonomic richness and diversity and produce metrics that are stronger predictors of community response to elevated SC.

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PUBLIC ABSTRACT

Surface coal mining in central Appalachia, eastern USA, contributes to increased salinity of surface waters, causing adverse effects on water quality and aquatic life. Stream condition is often evaluated through sampling of benthic macroinvertebrates because they are ubiquitous in aquatic environments and differ in sensitivity to various types of pollution and environmental stressors. In central Appalachia, studies have largely relied on semi-quantitative sampling methods to characterize effects of elevated salinity on benthic macroinvertebrate communities in headwater streams. These methods are ‘semi-quantitative’ because processing of samples requires subsampling procedures and identification of a fixed number of individuals, regardless of the number of organisms that were originally collected. In contrast, quantitative sampling involves identification and counting of all collected individuals, often resulting in organism counts that are much higher than those of semi-quantitative samples. Quantitative samples are typically more time-consuming and expensive to process but allow for expanded description of the benthic macroinvertebrate community and characterization of community-wide response to an environmental stressor such as elevated salinity. Working in central Appalachian streams, I compared 1) depictions of benthic macroinvertebrate community structure; 2) benthic community response to elevated salinity; and 3) the minimum levels of salinity associated with community change between quantitative and semi-quantitative methods. Quantitative sampling methods provide many advantages over semi-quantitative methods by providing more complete enumerations of the taxa present, thus enhancing the ability to evaluate aquatic-life condition and to characterize overall benthic macroinvertebrate community response to elevated salinity caused by surface coal mining.

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CHAPTER 1. INTRODUCTION

BACKGROUND

Decades of coal extraction have transformed large areas of the Appalachian landscape. In this region known for its high biological diversity, surface coal mining and reclamation activities dominate land-use change (Saylor 2008). A net forest decline of more than 4,200 km² occurred between 1973 and 2000 (Drummond & Loveland 2010). Although the U.S. Environmental Protection Agency (USEPA) does not have current published statistics on the spatial extent of surface mining within the Appalachian region, it was predicted that by 2012, mountaintop mining would have removed approximately 5,700 km² of Appalachian forests, roughly the size of the state of Delaware (USEPA 2005). A recent study (Pericak et al. 2018) has documented mining disturbances of more than 5,900 km² in central Appalachia alone.

Surface coal mining generates large quantities of waste soil and rock (referred to as ‘spoil’) that is often disposed of in neighboring stream valleys, forming valley fills (VFs) (Palmer et al. 2010; Peng 2000). This practice exposes un-weathered rocks to water and oxygen, accelerating natural weathering processes and contributing to excess leaching of major ions including sulfate (SO₄²⁻), calcium (Ca²⁺), magnesium (Mg²⁺), and bicarbonate (HCO₃⁻), and trace elements such as manganese (Mn) and selenium (Se) in the form of dissolved salts. These constituents can be measured collectively as total dissolved solids (TDS), and can be estimated by measuring specific conductance (SC), an easily measured TDS surrogate (USEPA 2011a, 2005). Central Appalachian headwater streams un-impacted by mining operations have natural background levels of TDS that are generally < 200 mg/L (USEPA 2011b; Lindberg et al. 2011), and SC levels that are generally < 200 µS/cm (Timpano et al. 2018; Pond et al. 2014, 2008). Leaching of dissolved mineral salts from VFs and associated mines contributes to elevated levels of TDS. Headwater streams in this region may exhibit SC levels 15-100 times higher than reference conditions (USEPA 2011b; Pond et al. 2008), contributing to adverse effects on water quality and negative consequences for aquatic life that include shifts in benthic macroinvertebrate assemblages to more tolerant taxa and declines in taxonomic richness and diversity (Timpano et al. 2018, 2015; Pond et al. 2014, 2008; Cormier et al. 2013; Griffith et al. 2012; Bernhardt et al. 2012).

In central Appalachia, studies of biotic effects of mining have relied largely on widely-distributed and extensively-tested Rapid Bioassessment Protocols (RBPs) as efficient, cost-effective assessments of aquatic-life condition. Stream bioassessments typically involve use of a kick-net or D-frame net to capture benthic organisms for purposes of characterizing the macroinvertebrate community through measures of taxonomic richness, abundance, dominance, and diversity (Barbour et al. 1999; Plafkin et al. 1989). These methods are ‘semi-quantitative’ because sampling occurs over an approximated area (~0.3m²), and because collections are subsampled to obtain a fixed-count of organisms (e.g., 200±10%) for identification regardless of actual macroinvertebrate sample density. Here, I term such semi-quantitative sampling methods as conventional

because they have been employed widely to assess effects of mining in the Appalachian coalfield. Measures of taxonomic richness, abundance, and diversity are relative to the fixed-count subsample, potentially overlooking presence of rare taxa (Courtemanch 1996; Vinson & Hawkins 1996) and limiting the utility of conventional semi-quantitative sampling for characterization of macroinvertebrate community structure.

In contrast, quantitative sampling methods involve collection of organisms from a defined area of the stream bottom and require complete enumeration of all individuals within the collected sample. When sampling stream-bottom areas that yield substantially more than 200 organisms, quantitative samples are more time-consuming and expensive to process than conventional semi-quantitative samples; such samples, however, allow for more accurate estimates of taxonomic richness, abundance, and diversity, and potentially have higher power in detecting existing differences between macroinvertebrate assemblages (Courtemanch 1996; Vinson & Hawkins 1996). It is reasonable to expect that application of high-enumeration (i.e., high-organismal count) quantitative sampling procedures will allow for enhanced understanding of benthic macroinvertebrate community response to elevated SC induced by regional surface coal mining.

NEED FOR STUDY

Use of quantitative sampling to assess benthic macroinvertebrate community structure and its response to elevated salinity is rare in central Appalachian streams (Drover 2018; Hartman et al. 2005), and to my knowledge, a comparison of conventional semi-quantitative and high-enumeration quantitative techniques across a gradient of an environmental stressor such as SC in central Appalachian streams has not been reported previously. Measures of macroinvertebrate community response obtained from conventional semi-quantitative methods have been associated with high variability (Resh 1994), and thus, quantitative sampling methods may characterize stressor effects more accurately. I expect high-enumeration quantitative sampling methods will not only provide more accurate estimates of benthic community structure, but will also reveal the extent to which conventional semi-quantitative sampling methods underestimate taxonomic richness and diversity in both reference and mining-influenced streams. These issues are especially of interest given that central Appalachian rivers and streams are recognized as being among the most biologically diverse freshwater systems in North America (Chaplin et al. 2000), and new aquatic species are still being discovered throughout the central and southern Appalachians (Camp et al. 2009; Berendzen et al. 2008; Kozak et al. 2006).

Studies comparing efficiency of different aquatic macroinvertebrate sampling methods and estimates of benthic macroinvertebrate community structure derived from those methods have been conducted in other world regions (Ghani et al. 2016; Gillies et al. 2009; Stark 1993; Storey et al. 1991; Mackey et al. 1984; Hornig & Pollard 1978). However, to my knowledge, a systematic comparison of sampling methods across an environmental stressor gradient such as salinity, within central Appalachia has not been reported previously.

OBJECTIVES

The overall goal of this study was to characterize and compare benthic macroinvertebrate community response to elevated salinity, as measured by SC within mining-influenced headwater streams of central Appalachia using two sampling methods (high-enumeration quantitative and conventional semi-quantitative) for two sampling periods (spring 2014 and fall 2017). For the two biomonitoring methods, I compared: 1) metrics describing various aspects of benthic macroinvertebrate community structure; 2) benthic community response to elevated SC; and 3) minimum levels of SC associated with community change between the two sampling methods.

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CHAPTER 2. LITERATURE REVIEW

Mountaintop mining, valley fills, and alkaline mine drainage

Headwater streams are critical components of river networks, comprising 70-80% of the total surface-water stream length in central Appalachia (Leopold 1964). They represent a vital link between terrestrial and deep-water aquatic ecosystems and are essential in maintaining ecosystem structure and function within downstream reaches (Meyer et al. 2007; Gomi et al. 2002). Many central Appalachian headwater streams are impacted by surface coal-mining activities, either directly when excess mine spoil is placed in stream valleys (valley fills, VFs) or indirectly through leaching of water contaminants from VFs and associated mines.

Surface coal mining or mountaintop removal involves use of explosives to remove mountaintops and access underlying coal seams, a process that generates large quantities of waste soil and rock (spoil). Federal law requires their replacement in mined areas in order to restore approximate original contour of the landscape in most cases, but this task cannot be accomplished with precision because of the volumetric expansion of earth material that occurs as a result of mining disturbance (USEPA 2011a). Excess mine spoil is often disposed of in neighboring stream valleys, forming VFs. This practice exposes un-weathered rocks to water and oxygen, accelerating natural weathering and erosion processes. Valley Fills often permanently bury headwater streams, eliminate ephemeral, intermittent, and perennial streams, and have significant impacts on ecosystem structure and function within downstream reaches (USEPA 2011a, 2005; Fritz et al. 2010; Pond et al. 2008; Hartman et al. 2005). Recent studies have demonstrated that presence of multiple mines within a watershed can have cumulative effects on downstream water quality and aquatic communities (Bernhardt et al. 2012; Lindberg et al. 2011), and degrees of such impacts are directly related to extent and intensity of surface mining (Petty et al. 2010) and volume of VFs within contributing catchments (Ross et al. 2016).

Effects of acidic coalmine drainage and associated acid-soluble metals on downstream water chemistry and aquatic life are widely documented (Freund & Petty 2007; DeNicola & Stapleton 2002; Herlihy et al. 1990; Tomkiewicz & Dunson 1977). However, in central Appalachia (southern West Virginia, eastern Kentucky and Tennessee, and southwestern Virginia), the underlying geology consists largely of strata with alkalinity-producing minerals, including carbonates, sufficient to neutralize acidity produced from oxidation of pyrite and sulfide minerals within coal residues and mine spoils. This process is coupled with release of waters with high concentrations of major ions including sulfate (SO_4^{2-}), calcium (Ca^{2+}), magnesium (Mg^{2+}), and bicarbonate (HCO_3^-), and trace elements such as manganese (Mn) and selenium (Se) as dissolved salts, or total dissolved solids (TDS). Drainage from mines and associated VFs within this region is therefore typically alkaline and characterized by increased pH, hardness, and ionic strength relative to unmined reference streams (Cormier et al. 2013; Lindberg et al. 2011; Pond et al. 2008; Hartman et al. 2005; Bryant et al. 2002).

In headwater streams un-impacted by mining within central Appalachia, natural background levels of TDS are generally < 200 mg/L (USEPA 2011b; Lindberg et al. 2011). Leaching of dissolved mineral salts from VFs and associated mines contributes to elevated levels of TDS, in some cases at levels > 2,000 mg/L (Pond et al. 2008). The concentration of TDS in water is closely related to the water's ability to conduct an electric current, which is typically measured as electrical conductivity and expressed as specific conductance (SC), the electrical conductivity at 25°C (USEPA 2011b). Mining-influenced headwater streams in this region may exhibit SC levels 15-100 times higher than background or reference conditions (USEPA 2011b); such high SC levels adversely affect water quality and have been associated with negative consequences for aquatic life in these streams (Pond et al. 2008; Bernhardt et al. 2012; Cormier et al. 2013; Timpano et al. 2015).

Benthic macroinvertebrate response to elevated TDS and conductivity

Negative relationships between increased levels of SC, an indicator of TDS, and measures of freshwater benthic macroinvertebrate community structure have been documented widely in headwater streams of the Appalachian region. High-TDS streams are typically characterized by reduced macroinvertebrate taxon richness, changes in individual macroinvertebrate metrics and indices, and shifts in macroinvertebrate assemblages to more-tolerant taxa relative to reference conditions (Timpano et al. 2018a, 2015, 2011; Boehme et al. 2016; USEPA 2011a; Pond et al. 2008; Hartman et al. 2005). Significant losses of an especially sensitive taxon group, Ephemeroptera (mayflies) have been documented in the central Appalachian region, with taxa declines and extirpations occurring in association with increased levels of TDS and SC from mining disturbance (Timpano et al. 2018a; Pond et al. 2014, 2008; Pond 2010; Merricks et al. 2007; Hartman et al. 2005). Significant alterations of two macroinvertebrate functional feeding-group populations, scrapers and shredders, in streams with elevated SC located below VFs in West Virginia have also been documented (Pond et al. 2014; Hartman et al. 2005).

Elevated SC and major-ion concentrations may cause toxic effects to macroinvertebrate taxa, including interference with osmoregulation capabilities (Ziegler et al. 2007; Kennedy et al. 2003) and normal growth processes (Hassell et al. 2006). Salinity (estimated by measuring SC) appears to be a factor limiting biotic condition and recovery of macroinvertebrate communities within the central Appalachian region following mining (Timpano et al. 2015; Pond et al. 2014). Recent studies have reported evidence of seasonal variation of SC and seasonal variability in macroinvertebrate community structure in headwater streams (Timpano et al. 2018b; Boehme et al. 2016; Cormier et al. 2013), highlighting the importance of considering sampling time for conducting bioassessments in central Appalachian coalfield streams.

Benthic macroinvertebrates as biological indicators of environmental quality

Two key federal environmental laws regulate coal mining and reclamation activities within the US: the Surface Mining Control and Reclamation Act (SMCRA, 25 U.S.C. § 1201) and the Clean Water Act (CWA, 33 U.S.C. § 1252). The SMCRA was established to “protect society and the environment from the adverse effects of surface coal mining

operations”; the CWA was designed to “restore and maintain the chemical, physical, and biological integrity of the Nation’s waters” (CWA § 101 (a)). To ensure compliance with the CWA, states and tribes develop water-quality standards and criteria, and programs to implement and enforce the CWA. Many of these programs include use of the EPA’s Rapid Bioassessment Protocols (RBPs) as an efficient method for assessing aquatic-life conditions (Barbour et al. 1999). Rapid Bioassessment Protocols typically involve biological monitoring of benthic macroinvertebrates because they are relatively easy to sample, vary in sensitivity or tolerance to different concentrations and compositions of dissolved ions, and integrate cumulative effects of biotic stressors over time (Barbour et al. 1999).

Aquatic macroinvertebrates serve as important indicators of stream condition and environmental quality, and have been assessed using RBPs for decades (Barbour et al. 1999; Plafkin et al. 1989). Assessment techniques typically involve macroinvertebrate taxon identification at the family- or genus-level and analyses of community structure using measures of taxonomic richness and relative abundance. Many states have developed specific methods for calculating aquatic macroinvertebrate indices for use in comparing assessed streams to regional reference conditions. Merritt et al. (2008) described a method classification system based on the habitat and community being sampled, and provided a list of procedures for both qualitative (semi-quantitative) and quantitative sampling methods.

Many studies involving benthic macroinvertebrate community structure in Appalachian streams have relied on semi-quantitative methods and multimetric indices (Timpano et al. 2015, 2011; Bernhardt et al. 2012; Pond et al. 2008), such as the Virginia Stream Condition Index (VASCI) (Burton & Gerritsen 2003) and the West Virginia Stream Condition Index (WVSCI) (Tetra Tech, Inc. 2000). Semi-quantitative sampling methods typically involve use of a kick-net or D-frame net to capture benthic organisms in a small area (~0.3m²), and can be used to sample multi-habitat types including riffles, runs, pools, stream margins, and even wetlands (Merritt et al. 2008; Turner & Trexler 1997). This type of sampling, although useful for estimating benthic community structure through measures of richness and relative abundance of taxonomic and functional feeding groups in a relatively time-efficient manner, is limited in its utility for community characterization because only a fixed number of individual macroinvertebrates are characterized (e.g. 200±10%; Barbour et al. 1999) regardless of their areal density (organism #/unit-area of stream substrate within the sampled stream). Measures of taxonomic richness, abundance, and diversity are relative to the fixed-count subsample, potentially under-representing the presence of rare taxa (Courtemanch 1996; Vinson & Hawkins 1996). Hence, semi-quantitative sampling methods have limited utility for accurate characterization of macroinvertebrate community structure, especially in aquatic systems with high biodiversity such as those of central Appalachia.

In contrast, quantitative sampling of benthic macroinvertebrates typically involves use of a Surber or Hess sampler (Hess 1941; Surber 1937) to collect organisms from a strictly defined area of the stream bottom and requires complete enumeration of all individuals within the collected sample. Samples may be collected and standardized to obtain sample

replicates, from which a sample mean and variance are calculated to estimate population size and variability within the stream segment. Number of required sample replicates required for accurate characterization is a function of: (1) size of the mean, (2) degree of aggregation exhibited by the population, and (3) desired precision of the estimate (Resh 1979). A large number of sampling units may be required to assure accurate characterization of the community because of non-random distribution of most stream macroinvertebrate populations, but ultimately the number depends on specific research objectives (Merritt et al. 2008; Hauer & Resh 2006). Hence, quantitative samples are generally more time-consuming and expensive to process but allow for more accurate estimates of taxonomic richness, abundance, and diversity; and high-enumeration quantitative samples (i.e., samples with high-organismal counts) have increased power in detecting differences between macroinvertebrate assemblages when compared to conventional fixed-count semi-quantitative methods (Courtemanch 1996; Vinson & Hawkins 1996).

Comparison of sample methods used in bioassessments

Previous studies comparing efficiency of sampling methods and accuracy of estimates of benthic macroinvertebrate community structure derived from those methods have been conducted worldwide and produced variable results. Biomonitoring studies conducted within tropical Malaysian streams (Ghani et al. 2016), streams of central Brazil (Silva et al. 2005), and ephemeral aquatic systems of Ireland (O Connor et al. 2004) reported collecting samples of a richer macroinvertebrate assemblage using quantitative methods, relative to semi-quantitative methods. Storey et al. (1991) reported higher numbers of individuals but fewer taxa obtained from semi-quantitative kicknet sampling compared to quantitative Surber sampling within the same habitat types of streams in southwestern Australia. Consistently higher values of taxonomic richness were also reported with the Surber sampler, and its use was recommended in sampling for environmental-impact studies to detect rare and/or endangered taxa (Storey et al. 1991). Similarly, O Connor et al. (2004) concluded that quantitative sampling devices were more objective, specific, and reliable, especially in habitats distinguished by rare species and assemblages for which biological monitoring is driven by legislative needs. Ghani et al. (2016) compared sampling efficiency and performance of qualitative (D-frame net and square net) and quantitative (Surber) samplers in Malaysian rivers, and collected richer and more diverse macroinvertebrate assemblages with the Surber sampler compared to semi-quantitative methods, despite collecting fewer individuals using the Surber sampler.

Contrary to these findings are previous studies conducted within tributaries of the Tennessee River (Kerans et al. 1992) and streams within the Cumberland Plateau in Kentucky (Adkins & Rieske 2015). Kerans et al. (1992) collected invertebrates in three Tennessee River tributaries and recorded more taxa from semi-quantitative samples than from quantitative (Surber and Hess) samples. However, quantitative samples were only collected in riffle habitats, whereas qualitative samples were collected in multi-habitat types (riffles and pools), creating sampling inconsistencies. Despite this finding, they concluded that un-replicated, qualitative samples may not have sufficient statistical power in detecting subtle differences among stream macroinvertebrate assemblages (Kerans et al. 1992). Adkins & Rieske (2015) sampled three streams within protected

areas of the Cumberland Plateau in Kentucky and detected significant differences in total taxa richness and abundance of trophic groups among sample types, with semi-quantitative samples containing higher taxonomic richness and abundances of collector-filterers, collector-gatherers, and scrapers compared to quantitative Surber samples; however, subsampling procedures were not used in their study. In agreement with these findings are studies conducted by Mackey et al. (1984) and Hornig & Pollard (1978), in which they directly compared quantitative Surber and semi-quantitative kick-net sampling techniques in lotic systems, and found the kick-net to be superior in collecting higher taxonomic richness. Findings by Stark et al. (1993) also found higher taxa richness using qualitative hand-net sampling techniques, but did not use subsampling procedures. Instead, the hand-net samples were standardized by sampling an area of approximately 0.5m², for about 10 seconds, and all macroinvertebrates within the samples were then counted and identified regardless of their density within the sample (Stark et al. 1993).

Bias is also introduced in cases where subsampling procedures are used, because they tend to be biased in the selection of larger organisms (Gillies et al. 2009; Vinson & Hawkins 1996), which are easier to detect, and spread consistently and evenly across the subsampling grid, especially in cases where specimens are live-sorted and identified in the field. Laboratory processing of semi-quantitative samples often requires subsampling using a gridded pan method (Plafkin et al. 1989), potentially introducing sources of error, including ability to evenly distribute organisms across the grid to obtain a truly standardized, random sample. Differences in collection methods and subsampling procedures have potential to not only produce unreliable or inconsistent community structural metrics (Silva et al. 2005; Courtemanch 1996; Vinson & Hawkins 1996), but also significantly alter biomonitoring scores (Everall et al. 2017).

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CHAPTER 3. METHODS AND MATERIALS

Stream selection and stream history

Fifteen 1st-order streams (Strahler 1957) were selected for research assessing effects of saline waters discharged by surface coal-mine areas on benthic macroinvertebrates in central Appalachian headwaters, as described by Timpano et al. (2015). Streams are located within the central Appalachian coalfields of Virginia and West Virginia (Figure 3.1) and within EPA Ecoregion 69d (Level IV, Omernik 1987) (Table A-1). Rigorous stream selection (Timpano et al. 2015, 2011) ensured that all study streams were isolated from non-mining and non-salinity stressors (Table 3.1), whereas reference streams were also relatively isolated from mining effects. Mining-influenced streams had elevated concentrations of dissolved ions and trace elements relative to reference streams (Table A-2). Non-mined areas of all study-stream watersheds were predominantly forested, and all streams had intact forest in the riparian zone. Catchment areas of study-stream watersheds ranged from 0.82 to 7.21 km² (Drover 2018; Timpano 2017).

The fifteen 1st-order streams, including four reference- and eleven mining-influenced streams were selected by Drover (2018) for a comprehensive analysis of benthic macroinvertebrate community structure response along a gradient of salinity induced by surface coal mining. Quantitative and semi-quantitative samples were collected by Drover (2018) in all 15 streams during spring 2014. Sample-collection methods were repeated during fall 2017 for an approximate seasonal comparison, however one stream was removed from the study in fall 2017 because of streamside development, removal of riparian vegetation, and excessive sedimentation.

Field collection and laboratory sample processing

Conductivity data

Long-term continuous conductivity data were collected from each study stream using HOBO freshwater conductivity loggers (Onset Computer Corp., Bourne, Massachusetts) set to continuously record electrical conductivity and temperature at 30-minute intervals. Specific conductance was calculated using Onset HOBOWare Pro v.3.4.1 software. At the times of benthic macroinvertebrate sampling, water temperature, dissolved oxygen (DO), SC, and pH were measured with a handheld multi-probe meter (YSI Professional Plus; YSI Inc., Yellow Springs, Ohio) to provide “snapshot” characterization of selected water-quality parameters and to confirm that DO and pH still met selection criteria (Table 3.1). These *in situ* measurements of SC represent “snapshot” conductivity data and were used to calibrate continuous conductivity loggers and adjust for SC data drift in HOBOWare. Data from continuous conductivity loggers were calibrated similarly throughout the study period.

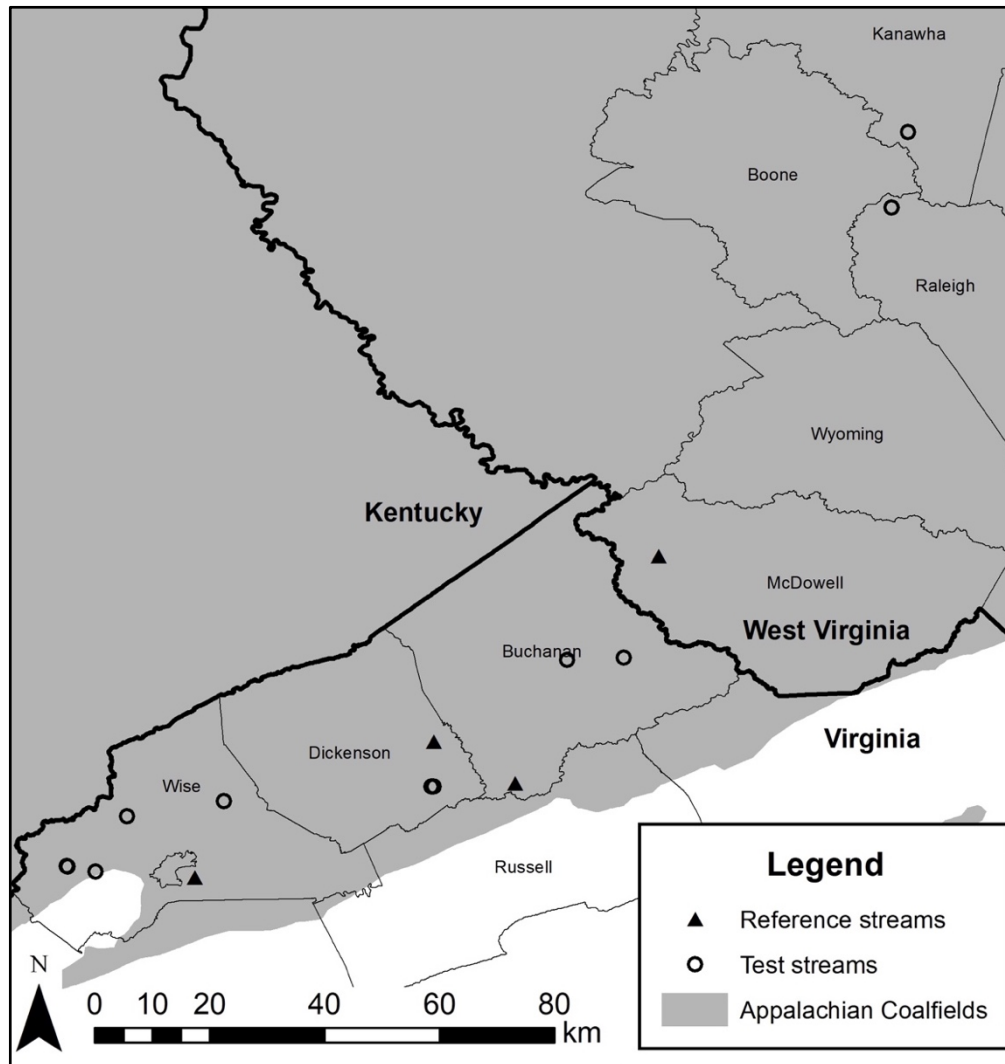


Figure 3.1. Location of headwater streams selected for study in the central Appalachian coalfields.

Table 3.1. Abiotic criteria for selection of reference and test streams (Timpano et al. 2015).

Parameter or Condition (units or range)	Selection Criterion ¹
Dissolved Oxygen (mg/L)	≥ 6.0
pH	≥ 6.0 and ≤ 9.0
Epifaunal substrate score (0-20) ²	≥ 11
Channel alteration score (0-20) ²	≥ 11
Sediment deposition score (0-20) ²	≥ 11
Bank disruptive pressure score (0-20) ²	≥ 11
Riparian vegetation zone width score, per bank (0-10) ²	≥ 6
Total RBP habitat score (0-200) ²	≥ 140
Residential land use immediately upstream	None

¹ Reference-stream selection criteria (Burton and Gerritsen 2003).

² RBP habitat assessment, high-gradient streams (Barbour et al. 1999).

Benthic macroinvertebrates: conventional semi-quantitative methods

Semi-quantitative benthic macroinvertebrate samples were collected following US EPA RBPs for the single-habitat method in high-gradient streams (Barbour et al. 1999) from April 11th – 13th, 2014 and from October 4th – 31st, 2017. Using a 0.3-m D-frame kicknet with 500- μ m mesh, six 1 x 0.3-m samples were collected at randomly-selected riffle habitats within each stream (n = 15 streams in spring 2014; n = 14 streams in fall 2017). Sampling of benthic macroinvertebrates occurred along a 100-m reach approximately centered on the location of the continuous conductivity loggers. The six kick-net samples were combined into a single composite sample (approximately 2-m²) at each study stream, for a total of 15 semi-quantitative samples for the spring 2014 sampling period and 14 semi-quantitative samples for the fall 2017 sampling period. Samples were preserved in 95% ethanol immediately upon collection in the field and returned to the laboratory for sorting and identification.

Semi-quantitative samples were sub-sampled randomly in the laboratory using a standardized gridded tray to obtain a 200 ($\pm 10\%$) organism count following Virginia Department of Environmental Quality methods (VDEQ 2008) adapted from US EPA RBPs (Barbour et al. 1999). Individuals in each sample were counted and identified to genus-level (except for individuals in family Chironomidae and sub-class Oligochaeta, which were identified at those levels) using standard keys (Merritt et al. 2008; Wiggins et al. 1996).

Benthic macroinvertebrates: high-enumeration quantitative methods

Benthic macroinvertebrates were sampled quantitatively using a 0.0929-m² Surber stream-bottom sampler (Wildco; Yulee, FL, USA) with a 500- μ m mesh, from April 25th – 29th, 2014 and from October 4th – 31st, 2017. For fall 2017 samples, semi-quantitative and quantitative samples were collected on the same date for a particular stream. At each stream, three individual quantitative samples were collected along the same 100-m reach in which semi-quantitative samples were collected. Care was taken to avoid collection of quantitative samples in the same riffle-habitat/area previously disturbed by semi-quantitative sampling. Quantitative samples were kept separate (i.e., not composited in the field), for a total of 45 quantitative samples for the spring 2014 index period and 42 quantitative samples for the fall 2017 index period. Samples were preserved in 95% ethanol upon collection in the field and returned to the laboratory for sorting, identification, and enumeration.

Laboratory analysis of quantitative samples involved sorting and identification of all individuals in each sample to genus-level (except for individuals in family Chironomidae and sub-class Oligochaeta, which were identified at those levels) using standard keys (Merritt et al. 2008; Wiggins et al. 1996). For each stream, the three quantitative samples collected during a particular sampling event were initially sorted and identified separately, but then mathematically composited for a single quantitative sample for comparison to the composited semi-quantitative sample collected at the same stream.

Data analysis

Summarizing conductivity data

Continuous conductivity data were summarized to obtain an annual SC mean value for the year preceding each spring 2014 and fall 2017 sample. If there were substantial gaps in the SC logger data (i.e., more than 15 consecutive days of data gaps) for a given stream, substitutions were made using historical continuous SC data from that same time period for the most-recent prior year with data. For example, March and April 2014 SC data were missing from continuous SC logger data for Copperhead Branch (COP), so data from March and April 2013 were used as substitutions in the calculation of an annual SC mean value in the spring 2014 analysis. Such substitutions were made because Appalachian streams salinized by surface coal mining exhibit a seasonal SC pattern with relatively consistent SC levels at a given time of the year (Timpano et al. 2018b). Maximum extents of continuous-SC data gaps ranged from 0 to 46 days among the 15 streams. All SC values used in data analyses are preceding-year means, calculated as described above.

Benthic macroinvertebrate metric selection

Thirty-seven metrics of benthic macroinvertebrate community structure were selected for analysis. These metrics are indicators of taxonomic richness, composition, functional feeding groups, dominance, diversity, tolerance, and habit of the benthic macroinvertebrate community (Table 3.2) and were selected to include those used for quantification of benthic community structure by Barbour et al. (1999), and by previous studies conducted in headwater streams of the central Appalachian coalfields (Timpano et al. 2018a; Drover 2018; Boehme et al. 2016). Family-level analogs of eight selected metrics are also included in the Virginia Stream Condition Index (VASCI), a family-level multimetric index of benthic macroinvertebrate community composition used to evaluate biological condition of non-coastal streams in Virginia (Burton and Gerritsen 2003). Pielou's evenness (J') was added as a measure of the degree of evenness in distribution of individuals among taxa of the benthic community (Pielou 1966). Functional feeding groups, tolerance values, and habits were ascribed to each genus using references by Merritt et al. (2008) and Barbour et al. (1999). Each of the thirty-seven metrics was calculated for all benthic macroinvertebrate samples, both quantitative and semi-quantitative, using R software (RStudio Team 2018).

Table 3.2. Benthic macroinvertebrate metrics by category, with abbreviations and expected response to increasing environmental disturbance.

Category	Metric	Abbrev.	Expected Response to Perturbation ¹
Taxonomic Richness	Total Taxa	<i>rTotal</i>	Decrease
	Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa	<i>rEPT</i>	Decrease
	EPT Taxa less Hydropsychidae	<i>rEPT-H</i>	Decrease
	Ephemeroptera Taxa	<i>rE</i>	Decrease
	Ephemeroptera Taxa less Baetidae	<i>rE-B</i>	Decrease
	Plecoptera Taxa	<i>rP</i>	Decrease
	Trichoptera Taxa	<i>rT</i>	Decrease
	Trichoptera Taxa less Hydropsychidae	<i>rT-H</i>	Decrease
Composition	Diptera Taxa	<i>rDip</i>	Decrease
	% EPT	<i>pEPT</i>	Decrease
	% EPT less Hydropsychidae	<i>pEPT-H</i>	Decrease
	% Ephemeroptera	<i>pE</i>	Decrease
	% Ephemeroptera less Baetidae	<i>pE-B</i>	Decrease
	% Plecoptera	<i>pP</i>	Decrease
	% Trichoptera	<i>pT</i>	Decrease
	% Trichoptera less Hydropsychidae	<i>pT-H</i>	Decrease
	% Plecoptera plus Trichoptera Taxa less Hydropsychidae	<i>pPT-H</i>	Decrease
	% Diptera	<i>pDip</i>	Increase
% Chironomidae	<i>pChi</i>	Increase	
% Oligochaeta	<i>pOligo</i>	Increase	
Functional Feeding Groups	% Collector-Gatherers	<i>pCG</i>	Decrease
	% Collector-Filterers	<i>pCF</i>	Variable
	% Predators	<i>pPR</i>	Variable
	% Scrapers	<i>pSC</i>	Decrease
	% Shredders	<i>pSH</i>	Decrease
	Scraper Taxa Richness	<i>rSC</i>	Decrease
Dominance	% 1 Dominant Taxon	<i>p1dom</i>	Increase
	% 2 Dominant Taxa	<i>p2dom</i>	Increase
	% 5 Dominant Taxa	<i>p5dom</i>	Increase
	Pielou's Evenness	<i>even</i>	Decrease
Diversity	Shannon Diversity	<i>shan</i>	Decrease
	Simpson Diversity	<i>simp</i>	Decrease
Tolerance	Intolerant Taxa Richness	<i>rINT</i>	Decrease
	% Tolerant	<i>pTOL</i>	Increase
	Hilsenhoff Biotic Index	<i>HBI</i>	Increase
Habit	% Clingers	<i>pCling</i>	Variable
	Clinger Taxa Richness	<i>rCling</i>	Variable

¹after Barbour et al. (1999).

Statistical analyses

Objective 1: Comparisons of sample methods for determining benthic community structure.

Paired-sample Wilcoxon tests (Wilcoxon signed-rank tests) were performed to assess presence of statistical differences between metrics obtained from 15 study streams in spring 2014 and 14 study streams in fall 2017 from each of the two sampling techniques:

quantitative and semi-quantitative. Exact Wilcoxon Signed Rank tests were used to meet the assumption of normality.

For purposes of graphical display, mean percent differences for each metric were calculated for each quantitative vs. semi-quantitative comparison using the following formula:

$$\text{Percent Difference} = \left[\frac{\text{Metric Value}_{\text{quantitative}} - \text{Metric Value}_{\text{semi-quantitative}}}{(\text{Metric Value}_{\text{quantitative}} + \text{Metric Value}_{\text{semi-quantitative}})/2} \right] * 100$$

Objective 2: Comparisons of sample methods for determining benthic community structure response to SC.

Spearman rank correlations were used to test for the associations between annual mean SC and 1) quantitative sample-metrics, 2) semi-quantitative sample-metrics, and 3) differences in sample metrics (quantitative minus semi-quantitative). Correlation results were evaluated to determine if the two methods characterize similarly both the direction and magnitude of community response to elevated SC. Mean values of preceding-year SC for each stream were used for these calculations. Correlations were performed using data obtained during the spring 2014 and fall 2017 sample periods.

Objective 3: Comparisons of sample methods to detect community change from reference condition.

To compare quantitative and semi-quantitative sampling methods for detection of benthic macroinvertebrate community change in response to increased SC, I constructed generalized additive models (GAM) using preceding-year mean SC as a predictor variable and benthic macroinvertebrate community metrics as response variables. The GAM is a non-parametric modification of the generalized linear model and fits a more flexible model (Quinn & Keough 2002) to represent non-linear associations, which makes it suitable for characterizing the SC-stream biota relationship (Timpano 2017). Separate models were constructed for each metric using spring 2014 and fall 2017 data and for each of the two sampling methods.

I defined ‘community change’ as deviation from reference condition, and, using similar methods described by Timpano et al. (2018a), I identified the minimum SC (‘modeled reference SC value’) associated with change in a benthic community metric from the reference condition. For each metric, a horizontal line was established at the minimum reference value (RefVal_{min}) or the maximum reference value (RefVal_{max}) depending on expected response to increasing SC. The modeled reference SC value for each metric was then derived from the intersection of the horizontal line and the GAM fitted model. Modeled reference SC values were retained for comparison if the GAM met the following three criteria for either the model based on quantitative samples or the model based on semi-quantitative samples for a specific metric and sample season:

- 1) Establishment of horizontal line at RefVal_{min} or RefVal_{max}: For a metric that is expected to decrease with (i.e., exhibit a negative response to) increasing SC,

- mining-influenced streams must have a metric value of equal or lesser value than the lowest-value reference stream. For a metric that is expected to increase with (i.e., exhibit a positive response to) increasing SC, all test streams must have a metric value of equal or greater value than the highest-value reference stream.
- 2) Model fit with associated p-values ≤ 0.05 .
 - 3) Model fit with associated R^2 values ≥ 0.50 .

For example, the horizontal line at the RefVal_{\min} for the spring 2014 quantitative model for Ephemeroptera, Plecoptera, and Trichoptera taxa richness (*rEPT*) was established at 27, which was the lowest reference value for this metric (Figure 3.2A), and the intersection of this horizontal line with the model fit produces the modeled reference SC value at 186 $\mu\text{S}/\text{cm}$ (Figure 3.2A). The same methods were used for the corresponding spring 2014 semi-quantitative model for *rEPT*, which produced a modeled reference SC value of 423 $\mu\text{S}/\text{cm}$ (Figure 3.2B). The spring 2014 semi-quantitative model for *rEPT* did not meet the RefVal_{\min} criterion because there are test streams above the established RefVal_{\min} of 13. However, because at least one of the two sampling methods (in this case, quantitative) met the RefVal_{\min} criterion (and the model-fit criteria: p -value < 0.01 and $R^2 = 0.85$), both spring 2014 models were retained for comparison of modeled reference SC values between quantitative and semi-quantitative sampling methods. Models for a particular metric indicate that SC values higher than the modeled reference SC value are unlikely to be comparable to reference streams; SC values less than the modeled reference SC value are most likely to be comparable to reference streams.

RStudio software was used to perform all statistical analyses (RStudio Team 2018). All results of statistical analyses were interpreted for significance at $\alpha = 0.05$. Paired-sample Wilcoxon tests used the package [exactRankTests] and generalized additive modeling used the package [mgcv].

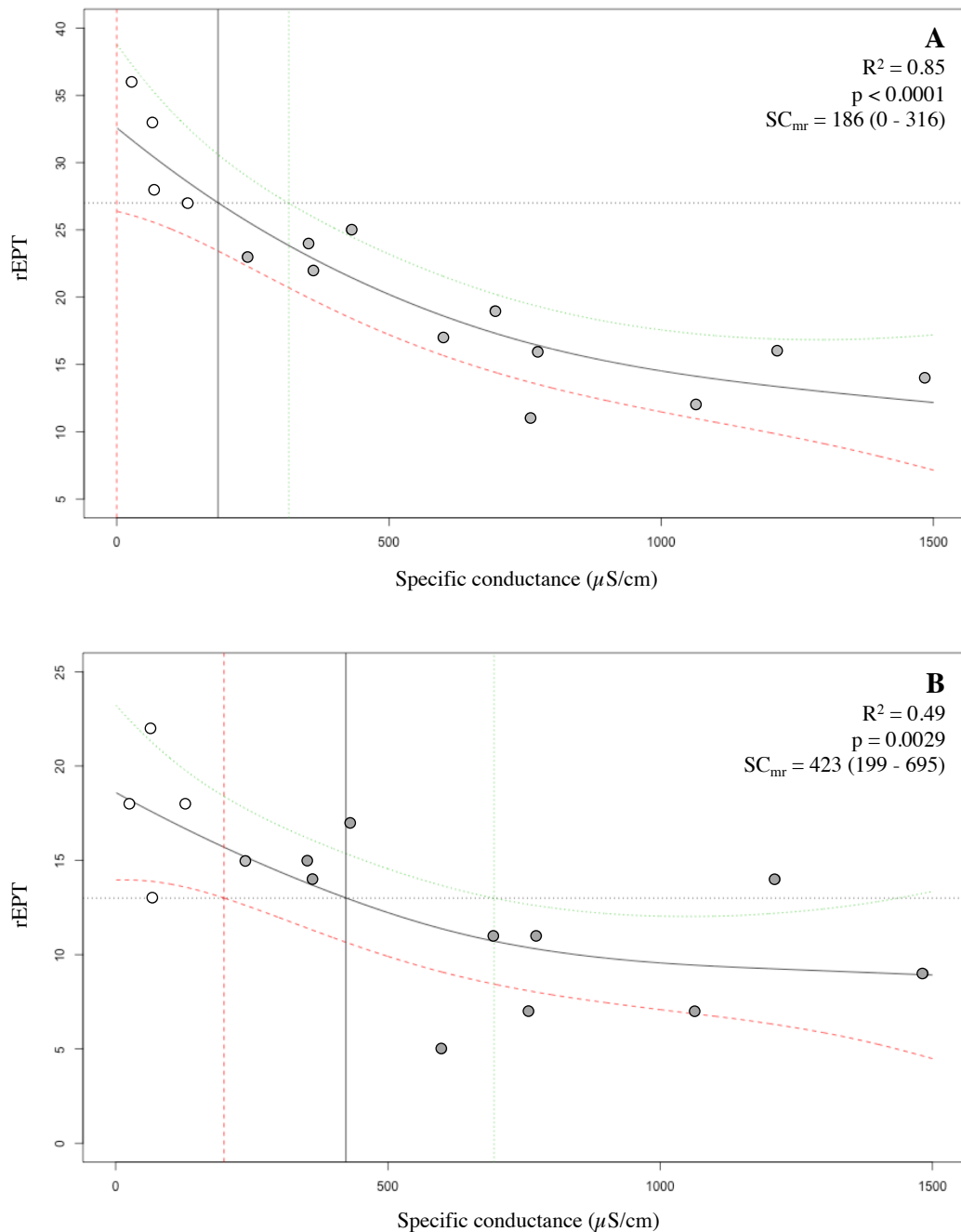


Figure 3.2. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera, Plecoptera, and Trichoptera taxa richness (*rEPT*) for spring 2014 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

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CHAPTER 4. COMPARISON OF QUANTITATIVE AND SEMI-QUANTITATIVE ASSESSMENTS OF BENTHIC MACROINVERTEBRATE COMMUNITY RESPONSE TO ELEVATED SALINITY IN CENTRAL APPALACHIAN COALFIELD STREAMS

ABSTRACT

Anthropogenic salinization of freshwater is a global concern. In freshwater environments, elevated levels of major ions, which are measured as total dissolved solids (TDS) or specific conductance (SC), can cause adverse effects on aquatic ecosystem structure and function. In central Appalachia, eastern USA, studies have largely relied on Rapid Bioassessment Protocols with semi-quantitative sampling to characterize benthic macroinvertebrate community response to increased salinity caused by surface coal mining. These protocols require subsampling procedures and identification of fixed numbers of individuals regardless of sample density, limiting measures of community structure including taxon richness, diversity, dominance, and detection of rare taxa. In contrast, quantitative sampling involves enumeration of all individuals collected within a defined area and typically includes larger sample sizes relative to semi-quantitative sampling, allowing expanded characterization of the benthic community. Working in headwater streams of central Appalachia, I evaluated high-enumeration quantitative and conventional semi-quantitative methods for bioassessments in headwater streams salinized by surface coal mining during two sample periods, spring 2014 and fall 2017. In addition, I compared the two sampling methods for capability to detect SC-induced changes in the benthic macroinvertebrate community. Quantitative samples collected during both sample periods included 1,414 organisms, on average, approximately seven times the semi-quantitative sampling organism counts. Quantitative sampling consistently produced higher estimates of taxonomic richness than corresponding semi-quantitative samples for both sample periods, and differences between sample methods were also found for composition, functional feeding group, dominance, tolerance, and habit metrics. Quantitative methods were generally stronger predictors of benthic community responses to SC and were more sensitive than semi-quantitative methods for detecting SC-induced changes in the macroinvertebrate community. Study results indicate that quantitative methods are advantageous over conventional semi-quantitative sampling methods for the purpose of characterizing benthic macroinvertebrate community structure because they provide more complete estimates of taxonomic richness and diversity, and produce metrics that are stronger predictors of community response to elevated SC.

INTRODUCTION

Decades of coal extraction have significantly transformed the Appalachian landscape. In this area known for its high biological diversity, surface coal mining and reclamation activities dominate land-use change (Saylor 2008), resulting in altered headwater stream chemistry and benthic macroinvertebrate community structure (USEPA 2011a). Effects of acid mine drainage and associated acid-soluble metals on downstream water chemistry and aquatic life are widely understood and well-documented (Freund & Petty 2007; DeNicola & Stapleton 2002; Herlihy et al. 1990; Tomkiewicz & Dunson 1977), but other water-quality effects are caused by Appalachian mining. In central Appalachia, the underlying geology consists largely of bedrock strata with sufficient alkalinity-producing minerals, including carbonates, to neutralize the acidity produced from oxidation of pyrite and sulfide minerals within coal residues and mine spoils (Clark et al. 2018; Daniels et al. 2016). This process is coupled with release of major ions including sulfate (SO_4^{2-}), calcium (Ca^{2+}), magnesium (Mg^{2+}), and bicarbonate (HCO_3^-), and trace elements such as manganese (Mn) and selenium (Se) in the form of dissolved salts, or total dissolved solids (TDS). Drainage from mines and associated valley fills (VFs) within this region is referred to as alkaline mine drainage and is characterized by elevated pH, hardness, and ionic strength relative to natural background water quality (Lindberg et al. 2011; Pond et al. 2008; Hartman et al. 2005; Bryant et al. 2002).

The dissolved-ion concentration of water is often estimated by measuring TDS. In headwater streams un-impacted by mining operations within central Appalachia, natural background levels of TDS are generally $< 200\text{mg/L}$ (USEPA 2011b; Lindberg et al. 2011). Leaching of dissolved mineral salts from VFs and associated mined lands contributes to elevated concentrations of TDS. Water concentration of TDS is closely related to electrical conductivity of the water and is often estimated by measuring specific conductance (SC; electrical conductivity at 25°C) (USEPA 2011b). Headwater streams in this region may exhibit SC levels 15-100 times higher than reference conditions (USEPA 2011b; Pond et al. 2008), contributing to adverse effects on water quality and likely negative consequences for aquatic life in these streams (Timpano et al. 2018a; Pond et al. 2014, 2008; Cormier et al. 2013; Griffith et al. 2012; Bernhardt et al. 2012).

In central Appalachia, studies of mining effects on biota have relied largely on widely-distributed and extensively-tested Rapid Bioassessment Protocols (RBPs) as efficient, cost-effective assessments of aquatic-life condition (Barbour et al. 1999). Stream bioassessments typically involve use of a kick-net or D-frame net to capture benthic organisms for purposes of characterizing the macroinvertebrate community through measures of taxonomic richness, abundance, dominance, and diversity (Barbour et al. 1999; Plafkin et al. 1989). These methods are 'semi-quantitative' because macroinvertebrate sampling occurs over an approximated area ($\sim 0.3\text{m}^2$), and because collections are composited, then subsampled to obtain a fixed-count of organisms (e.g. $200 \pm 10\%$) for identification regardless of actual macroinvertebrate sample density. Measures of taxonomic richness, abundance and diversity are derived from the fixed-count subsample, potentially overlooking presence of rare taxa if not present in the subsample (Courtemanch 1996; Vinson & Hawkins 1996). Hence, the fixed number of

organisms in the subsample may limit the utility of semi-quantitative sampling for accurate and complete characterization of macroinvertebrate community structure.

In contrast, quantitative sampling methods involve collection of organisms from a defined area of the stream bottom. For example, the Surber sampler, a traditional and commonly-used quantitative benthic sampling device, is used to collect organisms within an area of 0.0929-m². Further, quantitative sampling methods require complete enumeration of all individuals within the collected sample. Because quantitative samples typically assess benthic area inhabited by more organisms than are generally included in semi-quantitative assessments, quantitative samples are more time-consuming and expensive to process. Quantitative samples, however, also allow for more accurate estimates of taxonomic richness, abundance, and diversity, and potentially have higher power in detecting differences between macroinvertebrate assemblages relative to semi-quantitative methods (Courtemanch 1996; Vinson & Hawkins 1996). Application of quantitative sampling devices such as the Surber sampler is considered ideal for obtaining samples representative of riffle-dwelling biological communities (Davies 2001). Despite these potential advantages, application of quantitative sampling to assess macroinvertebrate community structure and response to elevated salinity is rare in central Appalachian streams (Drover 2018; Hartman et al. 2005).

To my knowledge, a comparison of conventional semi-quantitative and high-enumeration quantitative techniques across a gradient of an environmental stressor such as SC in central Appalachian streams has not been performed. I expected high-enumeration quantitative sampling would provide estimates of benthic community structure that are more reflective of actual stream assemblages, and therefore would more accurately characterize community response to elevated SC. I also expected that a comparison of high-enumeration quantitative and semi-quantitative sampling methods would reveal if and/or the extent to which semi-quantitative sampling methods underestimate taxonomic richness and diversity. These issues are especially of interest given that central Appalachian rivers and streams are recognized as among the most biologically diverse freshwater systems in North America (Chaplin et al. 2000).

Working in central Appalachia, I evaluated high-enumeration quantitative and conventional semi-quantitative methods for bioassessments in headwater streams salinized by surface coal mining. For the two biomonitoring methods, I compared: 1) metrics describing various aspects of benthic macroinvertebrate community structure; 2) benthic community response to elevated SC; and 3) minimum levels of SC associated with community change between the two sampling methods.

METHODS

Stream selection and stream history

Fifteen 1st-order streams (Strahler 1957) were selected for research assessing effects of saline waters discharged by surface coal-mine areas on benthic macroinvertebrates in central Appalachian headwaters, as described by Timpano et al. (2015). Streams are

located within the central Appalachian coalfield of Virginia and West Virginia (Figure 4.1) and within EPA Ecoregion 69d (Level IV, Omernik 1987) (Table A-1). Rigorous stream selection (Timpano et al. 2015, 2011) ensured that all study streams were isolated from non-mining and non-salinity stressors (Table 4.1), whereas reference streams were also relatively isolated from mining effects. Mining-influenced streams had elevated concentrations of dissolved ions and trace elements relative to reference streams (Table A-2). Non-mined areas of all study-stream watersheds were predominantly forested, and all streams had intact forest in the riparian zone. Catchment areas of study-stream watersheds ranged from 0.82 to 7.21 km² (Drover 2018; Timpano 2017).

The fifteen 1st-order streams, including four reference and eleven mining-influenced streams, were selected by Drover (2018) for a comprehensive analysis of benthic macroinvertebrate community structure response along a gradient of salinity induced by surface coal mining. Quantitative and semi-quantitative samples were collected by Drover (2018) in all 15 streams during spring 2014. Sample-collection methods were repeated during fall 2017 for an approximate seasonal comparison; however, one stream was removed from the study in fall 2017 because of streamside development, removal of riparian vegetation, and excessive sedimentation.

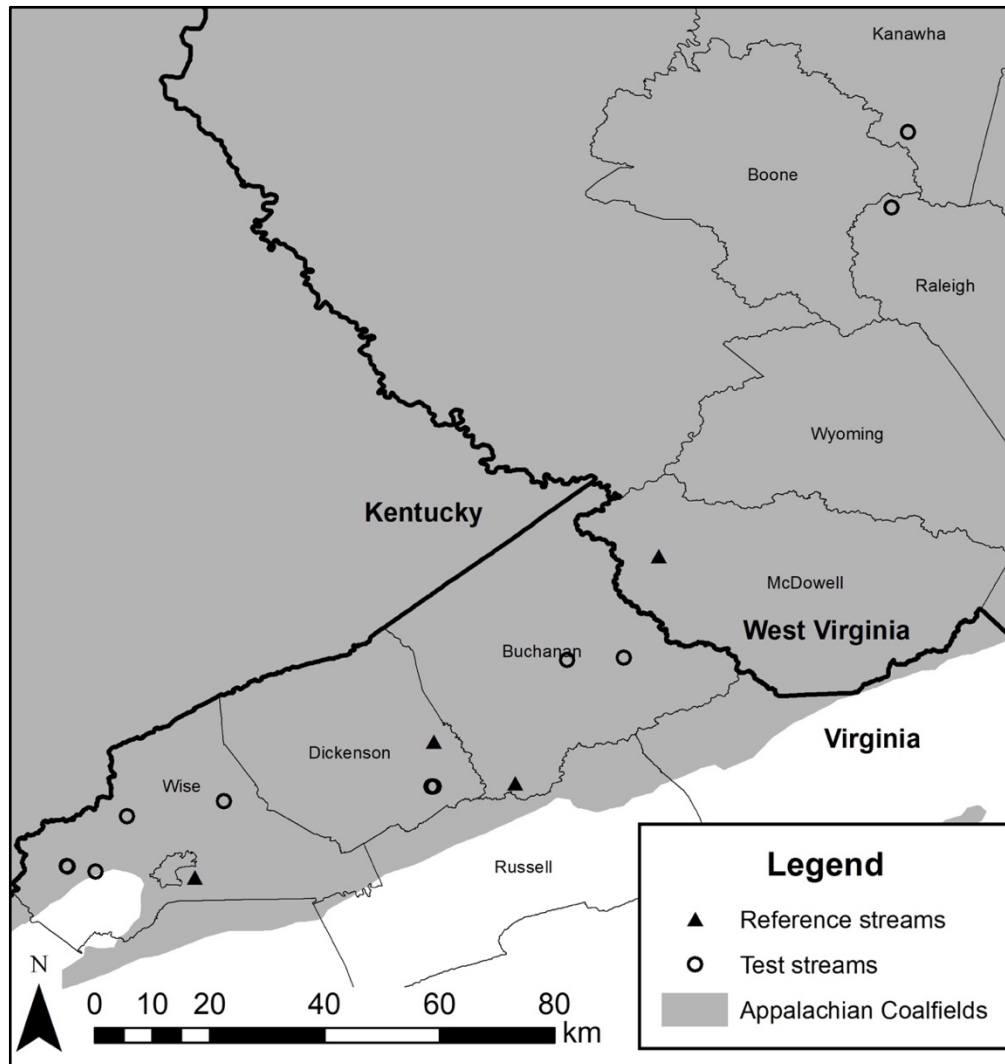


Figure 4.1. Location of headwater streams selected for study in the central Appalachian coalfields.

Table 4.1. Abiotic criteria for selection of reference and test streams (Timpano et al. 2015).

Parameter or Condition (units or range)	Selection Criterion ¹
Dissolved Oxygen (mg/L)	≥ 6.0
pH	≥ 6.0 and ≤ 9.0
Epifaunal substrate score (0-20) ²	≥ 11
Channel alteration score (0-20) ²	≥ 11
Sediment deposition score (0-20) ²	≥ 11
Bank disruptive pressure score (0-20) ²	≥ 11
Riparian vegetation zone width score, per bank (0-10) ²	≥ 6
Total RBP habitat score (0-200) ²	≥ 140
Residential land use immediately upstream	None

¹ Reference-stream selection criteria (Burton and Gerritsen 2003).

² RBP habitat assessment, high-gradient streams (Barbour et al.1999).

Field collection and laboratory sample processing

Conductivity data

Freshwater conductivity loggers (HOBO, Onset Computer Corp., Bourne, Massachusetts) measured and recorded electrical conductivity and temperature at 30-minute intervals (hereafter described as continuous) in each stream. Specific conductance was calculated using Onset HOBOWare Pro v.3.4.1 software. At the times of seasonal (spring and fall) benthic macroinvertebrate sampling, water temperature, dissolved oxygen (DO), SC, and pH were measured with a handheld multi-probe meter (YSI Professional Plus; YSI Inc., Yellow Springs, Ohio) to provide “snapshot” characterization of selected water-quality parameters and to confirm that DO and pH still met selection criteria (Table 4.1). These *in situ* measurements of SC represent “snapshot” conductivity data and were used to calibrate continuous conductivity loggers and adjust for SC data drift in HOBOWare.

Benthic macroinvertebrates: semi-quantitative methods

Semi-quantitative benthic macroinvertebrate samples were collected following US EPA RBPs for the single-habitat method in high-gradient streams (Barbour et al. 1999) from April 11th – 13th, 2014 and from October 4th – 31st, 2017. Using a 0.0929-m D-frame kicknet with 500- μ m mesh, six 1 x 0.3-m samples were collected at randomly-selected riffle habitats within each stream (n = 15 streams in spring 2014; n = 14 streams in fall 2017). Sampling of benthic macroinvertebrates occurred along a 100-m reach approximately centered on the location of the continuous conductivity loggers. The six kick-net samples were combined into a single composite sample (approximately 2-m²) at each stream, for a total of 15 semi-quantitative samples for the spring 2014 sampling period and 14 semi-quantitative samples for the fall 2017 sampling period. Samples were preserved in 95% ethanol immediately upon collection in the field and returned to the laboratory for sorting, identification, and enumeration.

Semi-quantitative samples were sub-sampled randomly in the laboratory using a standardized gridded tray to obtain a 200 ($\pm 10\%$) organism count following Virginia Department of Environmental Quality methods (VDEQ 2008) adapted from US EPA RBPs (Barbour et al. 1999). Individuals in each sample were counted and identified to genus-level (except for individuals in family Chironomidae and sub-class Oligochaeta, which were identified at those levels) using standard keys (Merritt et al. 2008; Wiggins et al. 1996).

Benthic macroinvertebrates: quantitative methods

Benthic macroinvertebrates were sampled quantitatively using a 0.0929-m² Surber stream-bottom sampler (Wildco; Yulee, FL, USA) with a 500- μ m mesh, from April 25th – 29th, 2014 and from October 4th – 31st, 2017. For fall 2017 samples, semi-quantitative and quantitative samples were collected on the same date for a particular stream. At each stream, three individual samples were collected along the same 100-m reach in which semi-quantitative samples were collected. Care was taken to avoid collection of samples in the same riffle-habitat/area previously disturbed by semi-quantitative sampling. Quantitative samples were kept separate (i.e., not composited in the field), for a total of

45 quantitative samples for the spring 2014 index period and 42 quantitative samples for the fall 2017 index period. Samples were preserved in 95% ethanol upon collection in the field and returned to the laboratory for sorting, identification, and enumeration.

Laboratory analysis of quantitative samples involved sorting and identification of all individuals in each sample to genus-level (except for individuals in family Chironomidae and sub-class Oligochaeta, which were identified at those levels) using standard keys (Merritt et al. 2008; Wiggins et al. 1996). For each stream, the three quantitative samples collected during a particular sampling event were initially sorted and identified separately, but then mathematically composited for a single quantitative sample for comparison to the semi-quantitative sample collected at the same stream.

Data analysis

Summarizing conductivity data

Continuous conductivity data were summarized to obtain an annual SC mean value for the year preceding each spring 2014 and fall 2017 sample. If there were substantial gaps in the SC logger data (i.e., more than 15 consecutive days of data gaps) for a given stream, substitutions were made using historical continuous SC data from that same time period for the most-recent prior year with data. For example, March and April 2014 SC data were missing from continuous SC logger data for Copperhead Branch (COP), so data from March and April 2013 were used as substitutions in the calculation of an annual SC mean value in the spring 2014 analysis. Such substitutions were made because Appalachian streams salinized by surface coal mining exhibit a seasonal SC pattern with relatively consistent SC levels at a given time of the year (Timpano et al. 2018b). Maximum extents of continuous-SC data gaps ranged from 0 to 46 days among the 15 streams. All SC values used in data analyses are preceding-year means, calculated as described above.

Benthic macroinvertebrate metric selection

Thirty-seven metrics of benthic macroinvertebrate community structure were selected for analysis. These metrics are indicators of taxonomic richness, composition, functional feeding groups, dominance, diversity, tolerance, and habit of the benthic macroinvertebrate community (Table 4.2) and were selected to include those used for quantification of benthic community structure by Barbour et al. (1999), and by previous studies conducted in headwater streams of the central Appalachian coalfields (Timpano et al. 2018a; Drover 2018; Boehme et al. 2016). Family-level analogs of eight selected metrics are also included in the Virginia Stream Condition Index (VASCI), a family-level multimetric index of benthic macroinvertebrate community composition used to evaluate biological condition of non-coastal streams in Virginia (Burton and Gerritsen 2003). Pielou's evenness (J') was added as a measure of the degree of evenness in distribution of individuals among taxa of the benthic community (Pielou 1966). Functional feeding groups, tolerance values, and habits were ascribed to each genus using references by Merritt et al. (2008) and Barbour et al. (1999). Each of the thirty-seven metrics was calculated for all benthic macroinvertebrate samples, both quantitative and semi-quantitative, using R software (RStudio Team 2018).

Table 4.2. Benthic macroinvertebrate metrics by category, with abbreviations and expected response to increasing environmental disturbance.

Category	Metric	Abbrev.	Expected Response to Perturbation ¹
Taxonomic Richness	Total Taxa	<i>rTotal</i>	Decrease
	Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa	<i>rEPT</i>	Decrease
	EPT Taxa less Hydropsychidae	<i>rEPT-H</i>	Decrease
	Ephemeroptera Taxa	<i>rE</i>	Decrease
	Ephemeroptera Taxa less Baetidae	<i>rE-B</i>	Decrease
	Plecoptera Taxa	<i>rP</i>	Decrease
	Trichoptera Taxa	<i>rT</i>	Decrease
	Trichoptera Taxa less Hydropsychidae	<i>rT-H</i>	Decrease
Composition	Diptera Taxa	<i>rDip</i>	Decrease
	% EPT	<i>pEPT</i>	Decrease
	% EPT less Hydropsychidae	<i>pEPT-H</i>	Decrease
	% Ephemeroptera	<i>pE</i>	Decrease
	% Ephemeroptera less Baetidae	<i>pE-B</i>	Decrease
	% Plecoptera	<i>pP</i>	Decrease
	% Trichoptera	<i>pT</i>	Decrease
	% Trichoptera less Hydropsychidae	<i>pT-H</i>	Decrease
	% Plecoptera plus Trichoptera Taxa less Hydropsychidae	<i>pPT-H</i>	Decrease
	% Diptera	<i>pDip</i>	Increase
% Chironomidae	<i>pChi</i>	Increase	
% Oligochaeta	<i>pOligo</i>	Increase	
Functional Feeding Groups	% Collector-Gatherers	<i>pCG</i>	Decrease
	% Collector-Filterers	<i>pCF</i>	Variable
	% Predators	<i>pPR</i>	Variable
	% Scrapers	<i>pSC</i>	Decrease
	% Shredders	<i>pSH</i>	Decrease
	Scraper Taxa Richness	<i>rSC</i>	Decrease
Dominance	% 1 Dominant Taxon	<i>p1dom</i>	Increase
	% 2 Dominant Taxa	<i>p2dom</i>	Increase
	% 5 Dominant Taxa	<i>p5dom</i>	Increase
	Pielou's Evenness	<i>even</i>	Decrease
Diversity	Shannon Diversity	<i>shan</i>	Decrease
	Simpson Diversity	<i>simp</i>	Decrease
Tolerance	Intolerant Taxa Richness	<i>rINT</i>	Decrease
	% Tolerant	<i>pTOL</i>	Increase
	Hilsenhoff Biotic Index	<i>HBI</i>	Increase
Habit	% Clingers	<i>pCling</i>	Variable
	Clinger Taxa Richness	<i>rCling</i>	Variable

¹after Barbour et al. (1999).

Statistical analyses

Objective 1: Comparisons of sample methods for determining benthic community structure.

Paired-sample Wilcoxon tests (Wilcoxon signed-rank tests) were performed to test for statistical differences between metrics obtained in spring 2014 and fall 2017 from each of the two sampling techniques: quantitative and semi-quantitative.

For purposes of graphical display, mean percent differences for each metric were calculated for each quantitative vs. semi-quantitative comparison using the following formula:

$$\text{Percent Difference} = \left[\frac{\text{Metric Value}_{\text{quantitative}} - \text{Metric Value}_{\text{semi-quantitative}}}{(\text{Metric Value}_{\text{quantitative}} + \text{Metric Value}_{\text{semi-quantitative}})/2} \right] * 100$$

Objective 2: Comparisons of sample methods for determining benthic community structure response to SC.

Spearman rank correlations were used to test for the associations between annual mean SC and 1) quantitative sample-metrics, 2) semi-quantitative sample-metrics, and 3) differences in sample metrics (quantitative minus semi-quantitative). Correlation results were evaluated to determine if the two methods characterize similarly both the direction and magnitude of community response to elevated SC. Mean values of preceding-year SC for each stream were used for these calculations. Correlations were performed using data obtained during the spring 2014 and fall 2017 sample periods.

Objective 3: Comparisons of sample methods to detect community change from reference condition.

To compare quantitative and semi-quantitative sampling methods for detection of benthic macroinvertebrate community change in response to increased SC, I constructed generalized additive models (GAM) using preceding-year mean SC as a predictor variable and benthic macroinvertebrate community metrics as response variables. The GAM is a non-parametric modification of the generalized linear model and fits a more flexible model (Quinn & Keough 2002) to represent non-linear associations, which makes it suitable for characterizing the SC-stream biota relationship (Timpano 2017). Separate models were constructed for each metric using spring 2014 and fall 2017 data and for each of the two sampling methods.

I defined ‘community change’ as deviation from reference condition, and, using similar methods described by Timpano et al. (2018a), I identified the minimum SC (‘modeled reference SC value’) associated with change in a benthic community metric from the reference condition. For each metric, a horizontal line was established at the minimum reference value (RefVal_{min}) or the maximum reference value (RefVal_{max}) depending on expected response to increasing SC. The modeled reference SC value for each metric was then derived from the intersection of the horizontal line and the GAM fitted model. Modeled reference SC values were retained for comparison if the GAM met the following three criteria for either the model based on quantitative samples or the model based on semi-quantitative samples for a specific metric and sample season:

- 1) Establishment of horizontal line at RefVal_{min} or RefVal_{max}: For a metric that is expected to decrease with (i.e., exhibit a negative response to) increasing SC, mining-influenced streams must have a metric value of equal or lesser value than the lowest-value reference stream. For a metric that is expected to increase with

- (i.e., exhibit a positive response to) increasing SC, all test streams must have a metric value of equal or greater value than the highest-value reference stream.
- 2) Model fit with associated p-values ≤ 0.05 .
 - 3) Model fit with associated R^2 values ≥ 0.50 .

For example, the horizontal line at the RefVal_{\min} for the spring 2014 quantitative model for Ephemeroptera, Plecoptera, and Trichoptera taxa richness (*rEPT*) was established at 27, which was the lowest reference value for this metric (Figure 4.2A), and the intersection of this horizontal line with the model fit produces the modeled reference SC value at 186 $\mu\text{S}/\text{cm}$ (Figure 4.2A). The same methods were used for the corresponding spring 2014 semi-quantitative model for *rEPT*, which produced a modeled reference SC value of 423 $\mu\text{S}/\text{cm}$ (Figure 4.2B). The spring 2014 semi-quantitative model for *rEPT* did not meet the RefVal_{\min} criterion because there are test streams above the established RefVal_{\min} of 13. However, because at least one of the two sampling methods (in this case, quantitative) met the RefVal_{\min} criterion (and the model-fit criteria: p -value < 0.01 and $R^2 = 0.85$), both spring 2014 models were retained for comparison of modeled reference SC values between quantitative and semi-quantitative sampling methods. Models for a particular metric indicate that SC values higher than the modeled reference SC value are unlikely to be comparable to reference streams; SC values less than the modeled reference SC value are most likely to be comparable to reference streams.

RStudio software was used to perform all statistical analyses (RStudio Team 2018). All results of statistical analyses were interpreted for significance at $\alpha = 0.05$. Paired-sample Wilcoxon tests used the package [exactRankTests] and generalized additive modeling used the package [mgcv].

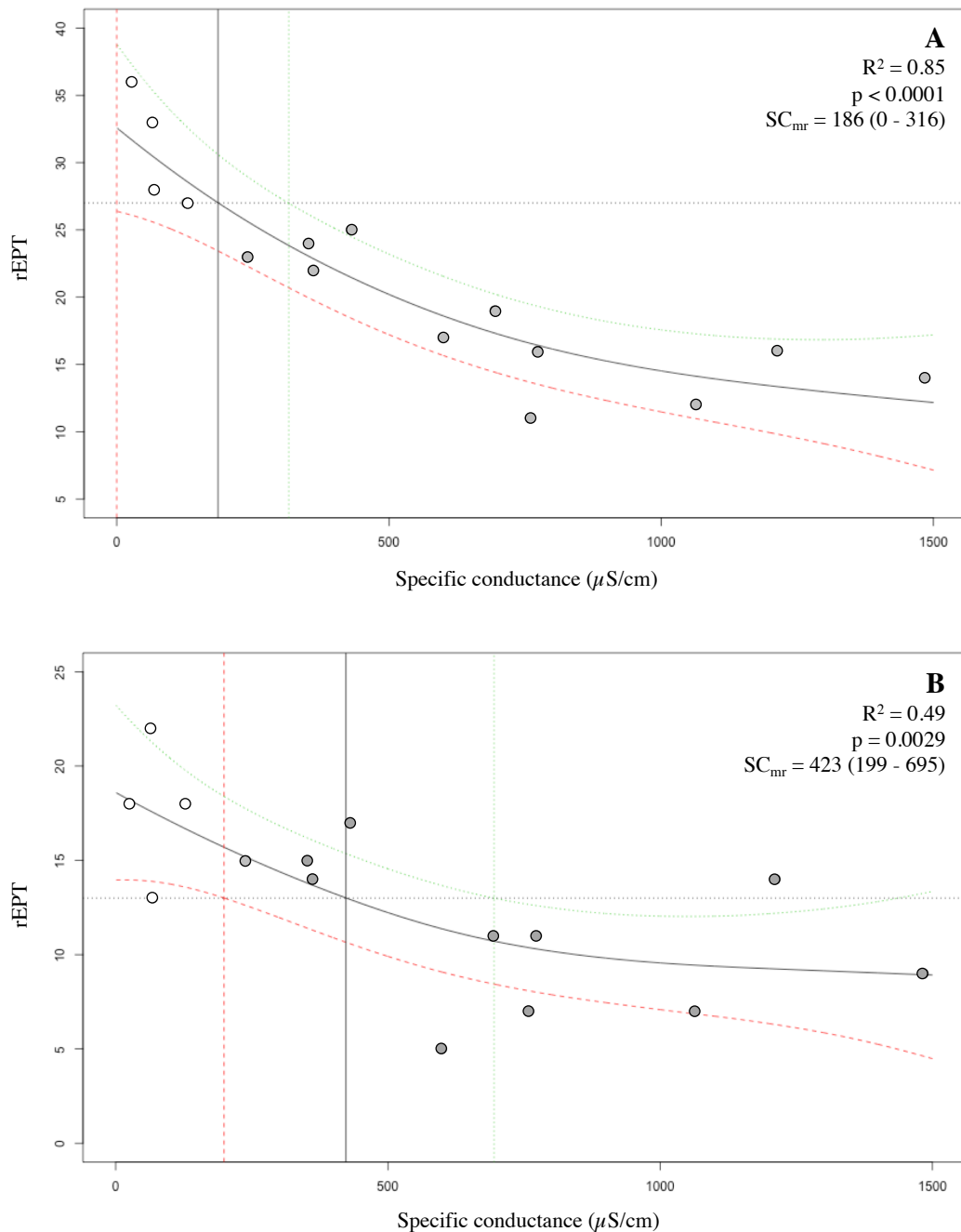


Figure 4.2. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera, Plecoptera, and Trichoptera taxa richness (*rEPT*) for spring 2014 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

RESULTS & DISCUSSION

In spring 2014, 83 total taxa from 15 quantitative samples and 64 total taxa from 15 semi-quantitative samples were identified; 30 taxa were unique to quantitative samples, whereas 11 taxa were unique to semi-quantitative samples (Table A-3). In fall 2017, 87 taxa from 14 quantitative samples and 71 taxa from 14 semi-quantitative samples were identified; 23 taxa were unique to quantitative samples, whereas 9 taxa were unique to semi-quantitative samples (Table A-3). Mean organism abundances in quantitative samples were $1,747 \pm 217$ and $1,057 \pm 160$ individuals in spring 2014 and fall 2017, respectively, whereas, by design, semi-quantitative samples were sub-sampled by collecting 200 ($\pm 10\%$) organisms (actual means, 209 ± 2 and 203 ± 5 individuals, in spring 2014 and fall 2017, respectively).

Mean SC values for reference streams ranged from 26 – 128 $\mu\text{S}/\text{cm}$ for spring 2014, whereas test-stream mean SC ranged from 239 – 1,848 $\mu\text{S}/\text{cm}$ (Table A-4). For fall 2017, mean SC ranged from 25 – 115 $\mu\text{S}/\text{cm}$ for reference streams and from 238 – 1,404 $\mu\text{S}/\text{cm}$ for test streams (Table A-5).

Objective 1: Comparisons of sampling techniques to assess macroinvertebrate community structure.

Taxonomic Richness

Quantitative sampling consistently produced higher estimates than semi-quantitative sampling for all nine taxonomic richness metrics for both spring 2014 and fall 2017. All nine measures of taxonomic richness derived from quantitative sampling differed significantly from those using semi-quantitative methods in spring 2014, and eight of the nine excepting Ephemeroptera richness less Baetidae (*rE-B*), derived from quantitative sampling differed significantly from those using semi-quantitative methods in fall 2017 (Tables A-6 & A-7). When averaged across all streams, differences in all taxonomic richness metrics ranged from 35.7 to 92.1% higher in spring 2014 quantitative samples and from 25.9 to 64.6% higher in fall 2017 quantitative samples (Figure 4.3).

These results are consistent with previous studies comparing efficiency of conventional semi-quantitative sampling and high-enumeration quantitative sampling techniques, and estimates of macroinvertebrate community structure derived from those methods. Biomonitoring studies conducted within streams of Malaysia (Ghani et al. 2016), central Brazil (Silva et al. 2005), southwestern Australia (Storey et al. 1991), and ephemeral lakes of Ireland (O Connor et al. 2004) reported a richer macroinvertebrate assemblage using quantitative methods relative to semi-quantitative fixed-count samples with fewer organisms. Storey et al. (1991) consistently found quantitative Surber samples to contain higher species richness than corresponding semi-quantitative kick-net samples taken at the same site on the same occasion in streams of southwestern Australia. Single rapid bioassessment (semi-quantitative) samples collected from a river in southeastern Australia contained only 55-63% of the total taxa collected by extensive quantitative (Surber) sampling at the same site (Gillies et al. 2009). Everall et al. (2017) collected Surber samples from rivers in the U.K. and found that they contained significantly higher

total taxa and EPT taxa richness than semi-quantitative samples collected at the same sites on the same occasion. In my study, quantitative measures of total taxa richness ($rTotal$) were 48.0% (± 4.1) higher than those from semi-quantitative samples collected in spring 2014 and 40.2% (± 4.8) higher in quantitative samples collected in fall 2017 (Figure 4.3). These findings are consistent with the well-known ecological principle that taxonomic richness tends to increase with sample size (Odum 1971; Arrhenius 1921).

My findings show that quantitative sampling methods consistently produce higher estimates of taxonomic richness, where semi-quantitative methodology can miss approximately 50% of the total taxa collected using quantitative methods. Armitage et al. (1983) suggest that a collection of at least 75% of total macroinvertebrate taxa is sufficient to provide a reliable assessment of water quality in temperate lotic systems. These results indicate that previous studies of central Appalachian headwater streams using semi-quantitative methods have likely underestimated and underrepresented benthic macroinvertebrate taxonomic richness, an important descriptor of community structure.

Community Composition

Significant differences were detected between the two sampling techniques for several community-composition metrics. Percent EPT ($pEPT$), percent EPT less Hydropsychidae ($pEPT-H$), percent Plecoptera (pP), percent Plecoptera plus Trichoptera taxa less Hydropsychidae ($pPT-H$), and percent Oligochaeta ($pOligo$) were significantly higher in quantitative samples collected in spring 2014 (Table A-6). Difference in $pOligo$ between sample methodologies was amplified (percent difference > 100%) because for many streams, Oligochaeta was not observed in the semi-quantitative (fixed-count) sample but was observed in corresponding quantitative sample, contributing to a percent difference of 100% for a given stream. Significant differences between sample methods were also detected for percent Diptera ($pDip$) in spring 2014 and percent Chironomidae ($pChi$) in fall 2017, where semi-quantitative measures exceeded quantitative measures by 23.5% (± 14.1) and 26.2% (± 10.0), respectively (Tables A-6 & A-7; Figure 4.3).

Given variation in benthic sample collection methods, as well as variation in laboratory processing and subsampling procedures, it is difficult to compare community composition metric values in this study to those found by other methodology studies. However, there is agreement over certain important aspects of semi-quantitative sample processing that tend to influence metric calculations and interpretations of bioassessment results. Laboratory processing of semi-quantitative samples often requires subsampling using a gridded pan method (Plafkin et al. 1989), potentially introducing sources of error, including ability to evenly distribute organisms across the grid to obtain a standardized, random sample. Subsampling procedures also tend to be biased in selection of larger organisms (Gillies et al. 2009; Vinson & Hawkins 1996) because they are easier to detect and spread consistently and evenly across the subsampling grid. Smaller organisms (such as individuals of the genus *Leuctra*) may be overlooked during sample processing and subsequently underrepresented in community composition metrics and analyses of community structure.

In my study, percent (%) *Leuctra* differed significantly ($p < 0.01$) between quantitative and semi-quantitative samples collected in spring 2014, and quantitative measures of % *Leuctra* exceeded semi-quantitative measures by 106.2% (± 25.3). Difference in % *Leuctra* was inflated (i.e., percent difference $> 100\%$) because *Leuctra* were not observed in semi-quantitative samples collected at two of the test streams, but comprised 4.5 and 26.1% of the two corresponding quantitative samples collected during spring 2014. At one of the reference streams, *Leuctra* were not observed in quantitative samples, but only comprised 1.0% of the corresponding semi-quantitative sample collected at the same stream in spring 2014 (data not shown). Nonetheless, these findings indicate that semi-quantitative community compositional metrics based on relative abundance (%) data are associated with a degree of sampling imprecision, potentially rendering semi-quantitative data as inconsistent and potentially unreliable for comparison of community-compositional metrics estimated from samples collected during a particular season or over multiple seasons.

Sample-type compositional differences also could be attributed to differences in field-collection protocols between semi-quantitative and quantitative methodologies. The quantitative Surber-sampling method is controlled and involves collection of organisms from a defined, fixed area, whereas the kicknet sampling method used for conventional semi-quantitative sampling does not sample a defined area and also allows organisms to scatter beyond the capture zone of the net. For example, a previous study conducted within the Cumberland Plateau of Kentucky (Adkins & Rieske 2015) is one of the only studies in which different sampling devices were used for purposes of estimating and comparing functional feeding group composition of benthic macroinvertebrate communities within two streams of differing dominant riparian vegetation. Adkins & Rieske (2015) also detected significant differences in total taxa richness and abundance of trophic groups among sample types, but found semi-quantitative kicknet samples contained higher taxonomic richness and abundances of collector-filterers, collector-gatherers, and scrapers compared to quantitative Surber samples; however, subsampling procedures were not used in their study.

Functional Feeding Groups

All functional feeding group metrics, with the exception of percent predators, *pPR*, differed significantly between sample methods for at least one of the sample periods (Tables A-6 & A-7; Figure 4.3). Semi-quantitative samples produced significantly higher measures of percent collector-filterers (*pCF*) (average difference 46.6% ± 14.8) and percent scraper (*pSC*) (average difference 55.7% ± 18.6) in spring 2014, and of percent collector-gatherers (*pCG*) in fall 2017 (average difference 30.0% ± 12.4). Percent shredders (*pSH*) was significantly higher in quantitative samples collected in spring 2014, and the average difference between the two sample methods was 22.1% (± 7.3). For both sampling periods, scraper taxa richness (*rSC*) was significantly higher in quantitative samples than corresponding semi-quantitative samples (average difference of 32.9% ± 11.2 in spring 2014 and 29.2% ± 10.1 in fall 2017) (Tables A-6 & A-7; Figure 4.3).

Differences between functional feeding group metrics were not expected, and as previously stated, it is difficult to compare community-composition metric values to those obtained by previous studies given the variation in sample collection, processing, and subsampling procedures. Functional feeding group metric comparisons to results of prior studies are especially difficult because those metrics have rarely been analyzed in studies focused on benthic sampling methods.

Dominance, Tolerance, and Habit

Pielou's evenness, (*even*), intolerant taxa richness (*rINT*), and clinger taxa richness (*rCling*) were the only metrics from the Dominance, Diversity, Tolerance, and Habit categories to display significant differences between the two sampling techniques (Tables A-6 & A-7; Figure 4.2). I did not expect to find differences in Pielou's evenness (*even*) based on previous studies (Ghani et al. 2016; Meyer et al. 2013); however, a small but statistically significant difference between sampling methods was detected in spring 2014, where semi-quantitative measures exceeded corresponding quantitative measures by 19.4% (± 4.8) (Figure 4.3; Table A-6).

Quantitative samples contained measures of intolerant taxa richness (*rINT*) that were 34.9% (± 4.5) and 43.9% (± 6.2) higher than semi-quantitative samples collected in spring 2014 and fall 2017, respectively. Clinger taxa richness (*rCling*) was also significantly higher in quantitative samples collected during both sampling periods (both cases, $p < 0.001$) (Tables A-6 & A-7; Figure 4.3). A total of 73 intolerant genera (those with pollution-tolerance values ≤ 3 , Barbour et al. 1999; Merritt et al. 2008) were observed throughout the course of this study, and more than half of these genera were also classified as clinger taxa (data not shown). I expected quantitative samples to contain a higher number of closely adherent taxa (i.e., those that maintain a relatively fixed position on firm substrate) given the more intensive nature of quantitative sampling requiring thorough disturbance of the stream-bottom. These findings are similar to those of previous studies (Everall et al. 2017; Ghani et al. 2016; Gillies et al. 2009; Storey et al. 1991; Hornig & Pollard 1978), which generally found semi-quantitative (kicknet) sampling methods to capture more easily dislodged and highly mobile taxa that were more abundant and widely distributed within the stream reach, compared to quantitative methods, such as the Surber, which tend to collect more sessile and low-occurrence taxa.

Summary of Objective 1 Findings:

Although differences between taxonomic richness measures obtained from the two sampling methods were evident, these did not translate broadly across all community composition, functional feeding group, dominance, diversity, tolerance, and habit metrics. Differences in certain community metrics indicate semi-quantitative sampling methods, which including subsampling procedures, may be biased by collection of larger organisms and taxa that are more abundant and widespread throughout the stream reach.

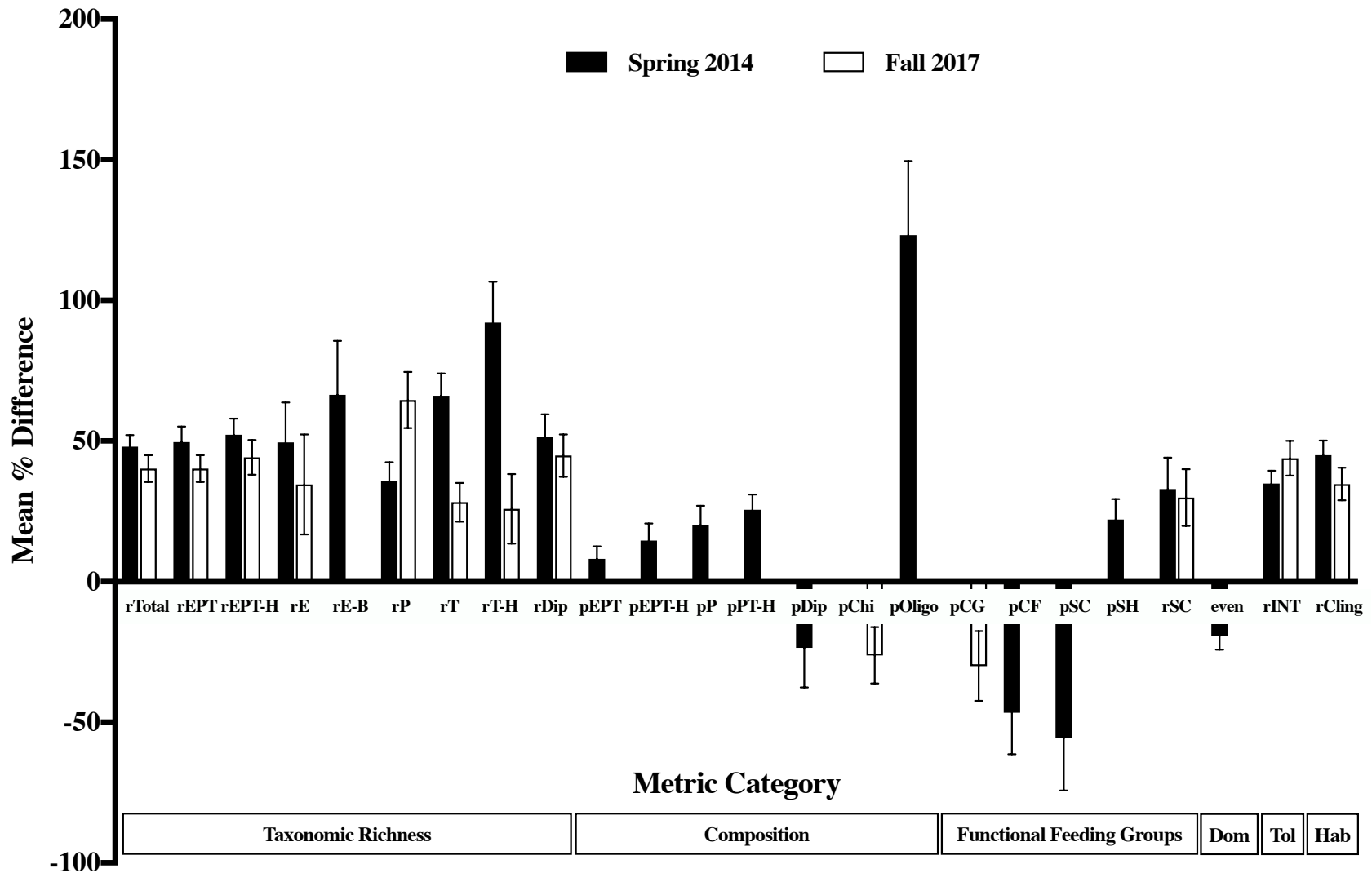


Figure 4.3. Mean metric differences (\pm SE) of macroinvertebrate community metrics between sample methodologies (Quantitative minus semi-quantitative). Differences are expressed as percent (%) differences and grouped by metric category for spring 2014 and fall 2017 sampling periods (“Dom” = Dominance; “Tol” = Tolerance; “Hab” = Habit). Only significant differences between sample types are displayed.

Objective 2 - Comparisons of benthic macroinvertebrate community response to elevated salinity using two sampling techniques.

Several benthic community metrics analyzed in this study were correlated with the SC gradient, and differences in community metrics between sample methods (quantitative metric value minus semi-quantitative metric value) were also correlated with SC. Largest differences between quantitative and semi-quantitative richness metrics were typically observed at reference streams because of higher levels of taxonomic richness present at those streams. Differences between quantitative and semi-quantitative richness metrics were reduced in high-SC streams, a function of biological response to elevated SC, as was observed, for example, with the metric Ephemeroptera, Plecoptera, and Trichoptera taxa richness (*rEPT*) in spring 2014 (Figure 4.4).

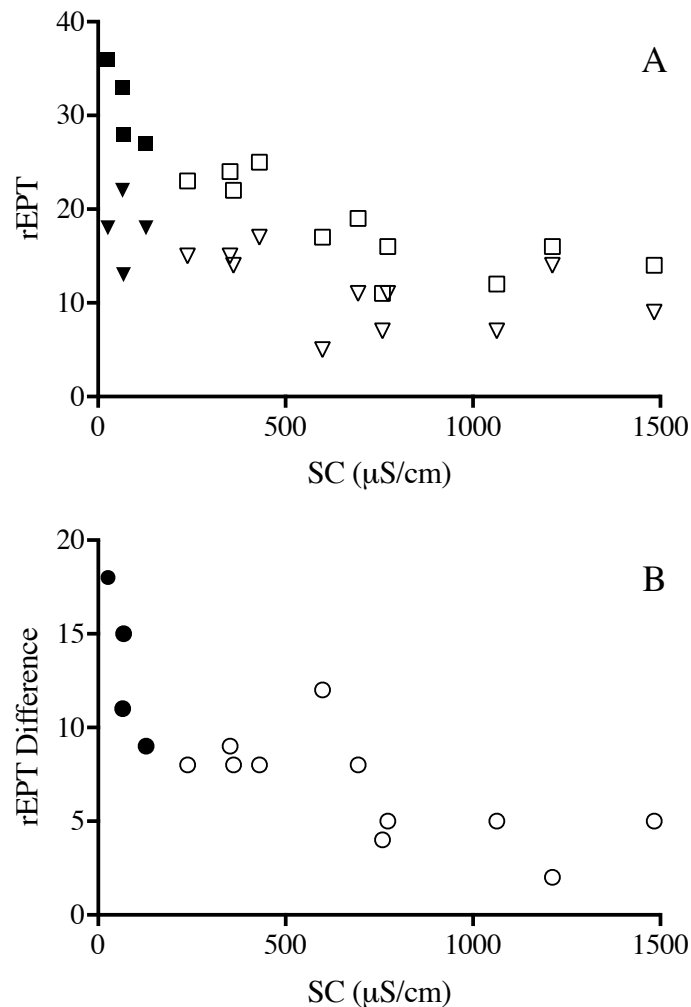


Figure 4.4. **A)** Ephemeroptera, Plecoptera, and Trichoptera taxa richness (*rEPT*) for spring 2014 quantitative and semi-quantitative methods versus specific conductance (SC). Square symbols represent quantitative samples and triangle symbols represent semi-quantitative samples. Closed symbols represent reference streams and open symbols represent test streams. **B)** Difference in *rEPT* between the two sample methods (quantitative minus semi-quantitative) versus SC. Closed symbols represent reference streams and open symbols represent test streams.

Taxonomic Richness

Most taxonomic richness metrics (r_{Total} , r_{EPT} , r_{EPT-H} , r_E , r_{E-B} , and r_P) were negatively correlated with SC for both spring 2014 and fall 2017 samples; those correlations were generally stronger for quantitative samples collected in spring 2014 (Tables 4.3 & 4.4). Overall negative responses to SC were expected for these taxonomic groups based on previous studies of macroinvertebrate community response to mining-induced freshwater salinization (Pond et al. 2014, 2008; Hartman et al. 2005). Among the pollution-sensitive EPT orders (Barbour et al. 1999), Ephemeroptera are the most sensitive to elevated SC in Appalachian coalfield streams, but members of the family Baetidae appear to be more tolerant to this type of pollution (Pond 2010). Exclusion of salt-tolerant Baetidae (Ephemeroptera taxa richness less Baetidae, r_{E-B}) generally resulted in stronger responses than the aggregate Ephemeroptera taxa richness metric (r_E) for both sampling periods, a finding that corresponds with previous studies conducted in central Appalachian coalfield streams (Timpano et al. 2018a; Pond et al. 2013). Plecoptera are also recognized as a pollution-sensitive order (Barbour et al. 1999), but relationships with SC were not as strong compared to relationships with r_E and r_{E-B} (Table 4.3 & 4.4), an observation supported by previous studies conducted in the central Appalachian coalfields (Timpano et al. 2018a; Pond et al. 2008, 2014; Pond 2010).

Negative relationships between SC and Trichoptera taxa richness (r_T), as well as between SC and Trichoptera taxa less Hydropsychidae (r_{T-H}) were also observed, but only for quantitative samples collected during spring 2014. This finding was unexpected based on previous studies conducted in my study streams, in which Trichoptera taxa generally did not exhibit a response to SC (Timpano et al. 2018a; Drover 2018). It is possible that a more intensive sampling effort improved quantification of the effects of elevated SC on Trichoptera taxa, at least in spring 2014 (Table 4.3).

Differences in taxonomic richness metrics (r_{Total} , r_{EPT} , r_{EPT-H} , r_E , r_{E-B} , and r_T) between the two sample methods were negatively correlated with SC for both spring 2014 and fall 2017, and relationships were generally stronger in spring 2014 (Tables 4.3 & 4.4). Differences in EPT taxa richness (r_{EPT}) between spring 2014 samples had the strongest relationship with SC, followed by Ephemeroptera taxa richness less Baetidae (r_{E-B}). Differences in r_E and r_{E-B} between sample methodologies quickly diminished with increasing SC because of overall Ephemeroptera taxa declines across the SC gradient with extirpations of sensitive non-Baetidae taxa occurring at high-SC streams. These differences indicate that SC-induced extirpations of sensitive taxa had likely occurred at high-SC streams, such that richness differences between the two sampling methods were smaller at high-SC streams than at reference and low-SC streams where those sensitive taxa were present.

Community Composition

Percent Ephemeroptera (p_E) and percent Ephemeroptera less Baetidae (p_{E-B}) were significantly and negatively correlated with SC for quantitative and semi-quantitative samples during both sampling periods, although in spring 2014, correlations were stronger in quantitative samples, and in fall 2017, correlations were stronger in semi-

quantitative samples (Tables 4.3 & 4.4). Previous studies have also found these metrics to be negatively correlated with SC (Drover 2018; Boehme et al. 2016; Pond et al. 2008; Hartman et al. 2005).

In spring 2014, the metrics percent Plecoptera (*pP*) and percent Plecoptera plus Trichoptera less Hydropsychidae (*PT-H*) were significantly and positively correlated with SC for both sampling methods. Additionally, in fall 2017, *pEPT* was positively correlated with SC for both sampling methods; *pP* and *pT-H* quantitative samples were negatively correlated with SC; and *pDip* and *pChi* semi-quantitative samples were negatively correlated with SC. No significant correlations of compositional sampling differences (quantitative minus semi-quantitative) with SC were noted for either sampling period (Tables 4.3 & 4.4).

Functional Feeding Groups, Dominance, and Diversity

The metrics *pCG*, *pPR*, *pSC*, and *rSC* were correlated negatively, and *pSH* was correlated positively with SC for both sampling methods in spring 2014; whereas all of these metrics except *pPR* were correlated similarly for either one or both sampling methods in fall 2017 (Tables 4.3 & 4.4). These results are comparable to those of recent studies analyzing metric response of functional feeding groups to mining-induced salinization, including those by Timpano et al. (2018a), which found a negative association between SC and scraper taxa richness based on spring samples collected over multiple years. Drover (2018) also found scraper taxa richness to be negatively correlated with SC and percent shredders to be positively correlated with SC. Correlations were generally stronger for quantitative samples. No significant correlations of sampling differences (quantitative minus semi-quantitative) with SC were noted for macroinvertebrate functional feeding groups.

All of the Dominance and Diversity metrics were correlated either positively (*p1dom*, *p2dom*, *p5dom*) or negatively (*even*, *shan*, *simp*) with SC for both sampling methods in spring 2014, and correlations were stronger for quantitative samples; whereas all were similarly correlated with SC for one or both sampling methods in fall 2017, during which correlations were observed generally for quantitative samples. However, no significant correlations of sampling differences (quantitative minus semi-quantitative) with SC were noted.

Tolerance

Intolerant taxa richness (*rINT*) was negatively correlated with SC in both spring 2014 and fall 2017, and the strongest relationship was detected for quantitative samples collected in spring 2014. In spring 2014, sample differences in *rINT* exhibited a moderate negative association with SC (Table 4.3), with larger differences occurring at reference streams and generally diminishing across the salinity gradient. These findings are supported by previous studies characterizing high-TDS streams by shifts in macroinvertebrate assemblages to more tolerant taxa relative to reference conditions (Timpano et al. 2018a; Boehme et al. 2016; Pond et al. 2008; Hartman et al. 2005).

Hilsenhoff Biotic Index (*HBI*) was negatively and moderately associated with SC, but only for quantitative samples collected during spring 2014 (Table 4.3). Although no significant differences in *HBI* were detected between sampling methods for either sampling period (Tables A-6 & A-7; Figure 4.2), the difference in *HBI* scores between sample methodologies was negatively correlated with SC in spring 2014 (Table 4.3), an association that appears to be driven by differences in both sample methods and stream types; quantitative samples typically contained higher *HBI* scores in reference streams and lower *HBI* scores in test streams when compared to corresponding semi-quantitative samples collected in spring 2014.

Habit

Spearman correlations detected a negative relationship between percent clingers (*pCling*) and SC for semi-quantitative samples collected in spring 2014, and positive relationships between *pCling* and SC for both sample types collected in fall 2017. Clinger taxa richness (*rCling*) was negatively correlated with SC for all sample types collected in spring 2014 and fall 2017 (Tables 4.3 & 4.4). Differences in *pCling* between sample types were positively correlated with SC in spring 2014 (with the largest differences between sampling methods occurring at high-SC sites), and differences in *rCling* between sample types exhibited negative relationships with SC for both sampling periods. These results indicate that although overall clinger taxa richness declines with increasing SC, certain salt-tolerant clinger taxa (including members of families Leuctridae, Hydropsychidae, and Simuliidae) are able to proliferate at high abundances in high-SC streams (Drover 2018).

Summary of Objective 2 Findings:

Numerous community metrics responded to the SC gradient in both spring and fall sample periods, and most of these responses were similar to those found by previous studies involving richness and composition metrics including Ephemeroptera taxa being especially sensitive and prone to decline with increasing SC. Several metric differences (quantitative minus semi-quantitative) also declined in response to SC, including differences for *rTotal*, *rEPT*, *rEPT-H*, *rE*, *rE-B*, *rT*, and *rCling*, all of which declined in response to increasing SC during both sample periods. These particular metrics were also among those that responded most strongly and negatively to the SC gradient. Quantitative richness values were substantially greater than corresponding semi-quantitative richness values at low-SC streams where semi-quantitative sampling methods underestimated taxonomic richness by failing to capture multiple taxa that were included in quantitative samples. Sampling-method differences were reduced at high-SC streams, as were both quantitative and semi-quantitative richness values. Semi-quantitative richness values more closely approximated quantitative richness values at high-SC streams, likely because fewer taxa were present in these streams. Correlations of community metrics with SC were generally stronger for quantitative sample-data than for corresponding semi-quantitative sample-data; this finding may have implications for how semi-quantitative biological survey data are interpreted to assess community response to gradients of environmental stressors such as SC.

Table 4.3. Mean values of community metrics for quantitative samples and semi-quantitative samples, for quantitative minus semi-quantitative (Q minus SQ) difference, and coefficients of Spearman correlation (ρ) of those measures vs. specific conductance (SC) for spring 2014.

Category	Metric	Abbrev.	Quantitative samples			Semi-quantitative samples			Q minus SQ (Difference)			
			Mean	ρ vs. SC	Sig.	Mean	ρ vs. SC	Sig.	Mean	Sig.	ρ vs. SC	Sig.
Taxonomic Richness	Total Taxa	<i>rTotal</i>	33.7	-0.91	***	20.9	-0.66	**	12.8	***	-0.82	***
	Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa	<i>rEPT</i>	21.5	-0.92	***	13.1	-0.72	**	8.5	***	-0.85	***
	EPT Taxa less Hydropsychidae	<i>rEPT-H</i>	18.3	-0.93	***	11.0	-0.78	***	7.3	***	-0.81	***
	Ephemeroptera Taxa	<i>rE</i>	6.5	-0.95	***	4.1	-0.69	**	2.4	**	-0.81	***
	Ephemeroptera Taxa less Baetidae	<i>rE-B</i>	4.9	-0.94	***	2.8	-0.86	***	2.1	***	-0.84	***
	Plecoptera Taxa	<i>rP</i>	6.7	-0.72	**	4.7	-0.61	*	1.9	***	-0.25	
	Trichoptera Taxa	<i>rT</i>	8.4	-0.68	**	4.3	0.03		4.1	***	-0.55	*
	Trichoptera Taxa less Hydropsychidae	<i>rT-H</i>	5.1	-0.58	*	2.2	-0.22		2.9	***	-0.33	
Diptera Taxa	<i>rDip</i>	7.2	0.04		4.3	-0.09		2.9	***	0.17		
Composition	% EPT	<i>pEPT</i>	80.0	0.42		74.0	-0.05		6.0	*	0.36	
	% EPT less Hydropsychidae	<i>pEPT-H</i>	73.8	0.35		64.2	-0.13		9.6	*	0.25	
	% Ephemeroptera	<i>pE</i>	16.6	-0.91	***	19.0	-0.85	***	-2.5		0.13	
	% Ephemeroptera less Baetidae	<i>pE-B</i>	8.9	-0.94	***	13.3	-0.90	***	-4.3		0.34	
	% Plecoptera	<i>pP</i>	53.3	0.83	***	42.7	0.71	**	10.5	***	0.30	
	% Trichoptera	<i>pT</i>	10.1	-0.06		12.2	0.40		-2.1		-0.34	
	% Trichoptera less Hydropsychidae	<i>pT-H</i>	4.0	-0.40		2.4	-0.25		1.5		-0.29	
	% Plecoptera plus Trichoptera Taxa less Hydropsychidae	<i>pPT-H</i>	57.2	0.84	***	45.2	0.69	**	12.1	***	0.24	
	% Diptera	<i>pDip</i>	15.4	-0.14		19.0	0.26		-3.5	*	-0.40	
	% Chironomidae	<i>pChi</i>	11.6	-0.04		9.9	0.24		1.7	**	-0.39	
% Oligochaeta	<i>pOligo</i>	1.0	0.00		0.2	0.19		0.7	**	-0.10		
Functional Feeding Groups	% Collector-Gatherers	<i>pCG</i>	24.6	-0.67	**	25.2	-0.57	*	-0.6		-0.12	
	% Collector-Filterers	<i>pCF</i>	11.0	-0.19		17.3	0.31		-6.3	*	-0.30	
	% Predators	<i>pPR</i>	6.3	-0.63	*	6.5	-0.71	**	-0.3		0.06	
	% Scrapers	<i>pSC</i>	6.8	-0.72	**	10.2	-0.80	***	-3.4	*	0.31	
	% Shredders	<i>pSH</i>	51.3	0.84	***	40.8	0.71	**	10.6	***	0.27	
	Scraper Taxa	<i>rSC</i>	5.3	-0.82	***	4.0	-0.65	**	1.3	*	-0.49	
Dominance	% 1 Dominant Taxon	<i>p1dom</i>	38.7	0.80	***	37.7	0.77	***	0.9		0.25	
	% 2 Dominant Taxa	<i>p2dom</i>	59.1	0.78	***	52.7	0.77	**	6.4		0.41	
	% 5 Dominant Taxa	<i>p5dom</i>	78.9	0.83	***	77.4	0.78	***	1.5		0.16	
	Pielou's Evenness	<i>even</i>	0.6	-0.75	**	0.7	-0.71	**	-0.1	***	-0.29	
Diversity	Shannon Diversity	<i>shan</i>	2.0	-0.84	***	2.1	-0.76	**	-0.1		-0.44	
	Simpson Diversity	<i>simp</i>	0.8	-0.81	***	0.8	-0.69	**	0.0		-0.18	
Tolerance	Intolerant Taxa	<i>rINT</i>	18.5	-0.92	***	13.3	-0.71	**	5.2	***	-0.55	*
	% Tolerant	<i>pTOL</i>	0.0	0.06		0.0	NA		0.0		0.06	
	Hilsenhoff Biotic Index	<i>HBI</i>	2.2	-0.61	*	2.5	0.12		-0.4		-0.59	*
Habit	% Clingers	<i>pCling</i>	53.6	0.00		48.5	-0.55	*	5.1		0.67	**
	Clinger Taxa	<i>rCling</i>	21.5	-0.87	***	13.9	-0.63	*	7.5	***	-0.74	**

Statistical significance (Sig.) is designated as: ***p < 0.001; **p < 0.01; *p < 0.05

Table 4.4. Mean values of community metrics for quantitative samples and semi-quantitative samples, for quantitative minus semi-quantitative (Q minus SQ) difference, and coefficients of Spearman correlation (ρ) of those measures vs. specific conductance (SC) for fall 2017.

Category	Metric	Abbrev.	Quantitative samples			Semi-quantitative samples			Q minus SQ (Difference)			
			Mean	ρ vs. SC	Sig.	Mean	ρ vs. SC	Sig.	Mean	Sig.	ρ vs. SC	Sig.
Taxonomic Richness	Total Taxa	<i>rTotal</i>	33.7	-0.75	**	22.0	-0.84	***	11.7	***	-0.61	*
	Ephemeroptera, Plecoptera, and Trichoptera (EPT) Taxa	<i>rEPT</i>	20.9	-0.78	***	13.7	-0.81	***	7.2	***	-0.60	*
	EPT Taxa less Hydropsychidae	<i>rEPT-H</i>	17.1	-0.77	**	10.8	-0.81	***	6.4	***	-0.57	*
	Ephemeroptera Taxa	<i>rE</i>	4.9	-0.89	***	3.4	-0.87	***	1.6	*	-0.58	*
	Ephemeroptera Taxa less Baetidae	<i>rE-B</i>	4.1	-0.92	***	2.8	-0.89	***	1.4		-0.60	*
	Plecoptera Taxa	<i>rP</i>	7.1	-0.51		3.8	-0.64	*	3.4	***	-0.09	
	Trichoptera Taxa	<i>rT</i>	8.9	-0.42		6.6	-0.08		2.3	**	-0.56	*
	Trichoptera Taxa less Hydropsychidae	<i>rT-H</i>	5.1	-0.33		3.9	-0.18		1.4	*	-0.38	
Diptera Taxa	<i>rDip</i>	7.2	-0.50		4.4	-0.48		2.8	***	-0.37		
Composition	% EPT	<i>pEPT</i>	74.9	0.66	*	69.0	0.65	*	5.9		-0.28	
	% EPT less Hydropsychidae	<i>pEPT-H</i>	43.2	0.01		39.5	0.02		3.7		0.02	
	% Ephemeroptera	<i>pE</i>	6.7	-0.79	**	7.1	-0.93	***	-0.5		-0.11	
	% Ephemeroptera less Baetidae	<i>pE-B</i>	6.2	-0.83	***	6.3	-0.92	***	-0.1		-0.05	
	% Plecoptera	<i>pP</i>	29.6	0.64	*	25.4	0.31		4.2		0.26	
	% Trichoptera	<i>pT</i>	38.7	0.11		36.5	0.47		2.2		-0.48	
	% Trichoptera less Hydropsychidae	<i>pT-H</i>	7.0	-0.70	**	7.0	-0.28		-0.1		-0.50	
	% Plecoptera plus Trichoptera Taxa less Hydropsychidae	<i>pPT-H</i>	36.6	0.45		32.4	0.37		4.2		-0.04	
	% Diptera	<i>pDip</i>	16.5	-0.38		19.9	-0.56	*	-3.4		0.20	
	% Chironomidae	<i>pChi</i>	11.1	-0.31		15.1	-0.54	*	-4.0		0.27	
% Oligochaeta	<i>pOligo</i>	1.6	-0.15		3.1	-0.44		-1.5	*	0.27		
Functional Feeding Groups	% Collector-Gatherers	<i>pCG</i>	16.7	-0.46		22.5	-0.73	**	-5.8	*	0.41	
	% Collector-Filterers	<i>pCF</i>	36.0	0.20		31.5	0.45		4.5		-0.48	
	% Predators	<i>pPR</i>	7.6	-0.50		7.6	-0.19		0.0		-0.49	
	% Scrapers	<i>pSC</i>	8.5	-0.79	***	10.6	-0.71	**	-2.2		-0.08	
	% Shredders	<i>pSH</i>	31.2	0.78	**	27.8	0.33		3.4		0.30	
	Scraper Taxa	<i>rSC</i>	5.9	-0.84	***	4.2	-0.73	**	1.7	*	-0.41	
Dominance	% 1 Dominant Taxon	<i>p1dom</i>	32.6	0.65	*	35.6	0.35		-3.0		0.19	
	% 2 Dominant Taxa	<i>p2dom</i>	48.1	0.76	**	51.4	0.50		-3.3		0.39	
	% 5 Dominant Taxa	<i>p5dom</i>	71.3	0.83	***	76.2	0.84	***	-4.9		0.34	
	Pielou's Evenness	<i>even</i>	0.7	-0.48		0.7	-0.55	*	0.0		-0.10	
Diversity	Shannon Diversity	<i>shan</i>	2.3	-0.79	**	2.2	-0.61	*	0.2		-0.29	
	Simpson Diversity	<i>simp</i>	0.8	-0.75	**	0.8	-0.50		0.0		-0.20	
Tolerance	Intolerant Taxa	<i>rINT</i>	19.1	-0.74	**	12.3	-0.74	**	6.8	***	-0.40	
	% Tolerant	<i>pTOL</i>	0.1	-0.10		0.0	0.45		0.1		-0.30	
	Hilsenhoff Biotic Index	<i>HBI</i>	2.4	-0.45		2.7	-0.28		-0.3		0.07	
Habit	% Clingers	<i>pCling</i>	73.9	0.60	*	70.7	0.74	**	3.1		-0.34	**
	Clinger Taxa	<i>rCling</i>	19.9	-0.81	***	13.9	-0.69	**	6.0	***	-0.66	**

Statistical significance (Sig.) is designated as: ***p < 0.001; **p < 0.01; *p < 0.05

Objective 3: Comparisons of sample-method ability to detect community change.

Of the 37 benthic community metrics analyzed for this study, 18 met the generalized additive models (GAM) selection criteria and were retained for comparison (Figure 4.5, Table A-8). Models based on quantitative samples were generally stronger predictors of community-metric responses to SC compared to models based on semi-quantitative samples, as indicated by model-fits with associated R^2 values ≥ 0.50 and p-values ≤ 0.05 . The spring 2014 community metrics generally had stronger fits with SC compared to fall 2017 models, a finding similar to that of Timpano et al. (2018a). Quantitatively-derived modeled reference-SC values were typically lower than those derived from semi-quantitative methods, regardless of season (Figure 4.5).

Taxonomic Richness

Models based on quantitative samples for total taxa richness ($rTotal$), EPT taxa richness ($rEPT$), EPT taxa richness less Hydropsychidae ($rEPT-H$), Ephemeroptera richness (rE), and Ephemeroptera taxa richness less Baetidae ($rE-B$) were stronger predictors than corresponding models based on semi-quantitative samples for both spring 2014 and fall 2017 sampling periods (Figure 4.5, Table A-8, Figures A-1 – A-10). Modeled reference SC values based on quantitative samples for taxonomic richness metrics ranged from 97 – 226 $\mu\text{S}/\text{cm}$ in spring 2014 and from 131 – 184 $\mu\text{S}/\text{cm}$ in fall 2017. Modeled reference SC values derived from models based on semi-quantitative samples ranged from 225 – 423 $\mu\text{S}/\text{cm}$ in spring 2014 and from 125 – 286 $\mu\text{S}/\text{cm}$ in fall 2017 (Figure 4.5). Values derived from models based on semi-quantitative samples were closer to the conductivity benchmark value of 300 $\mu\text{S}/\text{cm}$ set by the field-based assessment conducted by US EPA to be protective of aquatic life in central Appalachian streams (USEPA 2011b), whereas values derived from quantitative samples were consistently lower than the US EPA benchmark for all taxonomic richness models (Figure 4.5; Table A-8).

Timpano et al. (2018a) used similar methods to derive ‘critical conductivity values’ from semi-quantitative samples collected within central Appalachian coalfield streams over multiple years, for both spring and fall seasons. Spring critical conductivity values for $rTotal$, $rEPT$, rE , and $rE-B$ reported by Timpano et al. (2018a) were comparable to my modeled reference SC values based on semi-quantitative samples for the same metrics calculated in spring 2014 (Table A-9). For example, the spring critical conductivity value for Ephemeroptera taxa richness was 284 $\mu\text{S}/\text{cm}$ (Timpano et al. 2018a) compared to my modeled reference SC value of 293 $\mu\text{S}/\text{cm}$ derived from spring 2014 semi-quantitative samples (Table A-9, Figure A-7).

Community Composition

Models based on quantitative samples for percent Ephemeroptera (pE), percent Ephemeroptera less Baetidae ($pE-B$), percent Plecoptera (pP), and percent Plecoptera plus Trichoptera less Hydropsychidae ($PT-H$) were generally stronger predictors of SC-associated community change, and consistently provided modeled reference SC values lower than those from corresponding models based on semi-quantitative samples (Figure 4.5). Of the community composition metrics analyzed by this study, models of percent Ephemeroptera less Baetidae ($pE-B$) derived from quantitative samples were the strongest

predictors of community change based on model-fit criteria (Table A-8). Modeled reference SC values based on quantitative samples for *pE-B* were lower (127 $\mu\text{S}/\text{cm}$ for spring 2014 and 132 $\mu\text{S}/\text{cm}$ for fall 2017) than those derived using semi-quantitative samples (241 $\mu\text{S}/\text{cm}$ for spring 2014 and 342 $\mu\text{S}/\text{cm}$ for fall 2017). Spring 2014 values were lower than in fall 2017, and these results are comparable to those found by Timpano et al. (2018a), in which stronger relationships between stream biota and SC were observed in spring than in fall.

Functional Feeding Groups, Dominance, Diversity, Tolerance, and Habit

Models based on semi-quantitative samples for percent predators (*pPR*), percent scrapers (*pSC*), and percent shredders (*pSH*) were stronger predictors of community change than were models based on quantitative samples, but modeled reference SC values were not consistently higher for one sampling method compared with the other (Figure 4.5; Table A-8). The fall 2017 model based on quantitative samples for scraper taxa richness (*rSC*) was a stronger predictor than the model derived from semi-quantitative samples, and among all the functional feeding group models, had the lowest modeled-reference SC value, at 80 $\mu\text{S}/\text{cm}$. The scraper functional feeding group includes multiple relatively sensitive Ephemeroptera taxa, many of which were present in fall 2017 samples, including *Epeorus*, *Eurylophella*, *Habrophlebiodes*, *Heptagenia*, *Leucrocuta*, *Maccaffertium*, *Stenacron*, and *Stenonema*, which may explain why such a low modeled-reference SC value was observed for this metric.

Models based on quantitative samples for Shannon diversity (*div.shan*), intolerant taxa richness (*rINT*), and percent clingers (*pCling*) were generally better predictors of community change, but there was variation between the spring 2014 and fall 2017 sampling periods (Figure 4.5). Based on model selection criteria, modeled reference SC values for *div.shan* could only be derived in the fall, and values for *rINT* could only be derived in the spring. For *pCling*, modeled reference values were derived for both seasons, but in spring 2014, the value derived from quantitative samples was 1,455 $\mu\text{S}/\text{cm}$, which is near the maximum SC level observed in my study streams. Fall 2017 models for *pCling* were therefore stronger predictors of deviation from reference condition than spring 2014 models.

Summary of Objective 3 Findings:

When modeled for relationships with SC using GAM procedures, metrics derived from quantitative sampling typically demonstrated stronger fits relative to those derived from semi-quantitative sampling, with more consistent and clear deviation from reference condition at elevated SC. Modeled reference SC values derived from models based on semi-quantitative samples were closer to the field-based US EPA conductivity benchmark value of 300 $\mu\text{S}/\text{cm}$, and in some cases exceeded this value. Values derived from quantitative samples were typically lower than the US EPA conductivity benchmark (Figure 4.5; Table A-8); however, five of these values were unrealistically low. For example, the modeled reference SC value based on quantitative sampling for *rTotal* was 97 $\mu\text{S}/\text{cm}$ in spring 2014, and the annual SC mean value at one of the reference streams used to derive this value was 128 $\mu\text{S}/\text{cm}$ (Table A-4), which exceeds the modeled

reference SC value. This was also the case for values derived from quantitative sampling for *pE-B* and *pPT-H* in spring 2014 (127 $\mu\text{S}/\text{cm}$ and 64 $\mu\text{S}/\text{cm}$, respectively) and for *rSC* and *pCling* in fall 2017 (80 $\mu\text{S}/\text{cm}$ and 97 $\mu\text{S}/\text{cm}$, respectively) (Table A-8). The annual SC mean value at one or more reference streams exceeded these modeled reference SC values (Tables A-4 & A-5), and as such, the biological significance of these particular models is unclear.

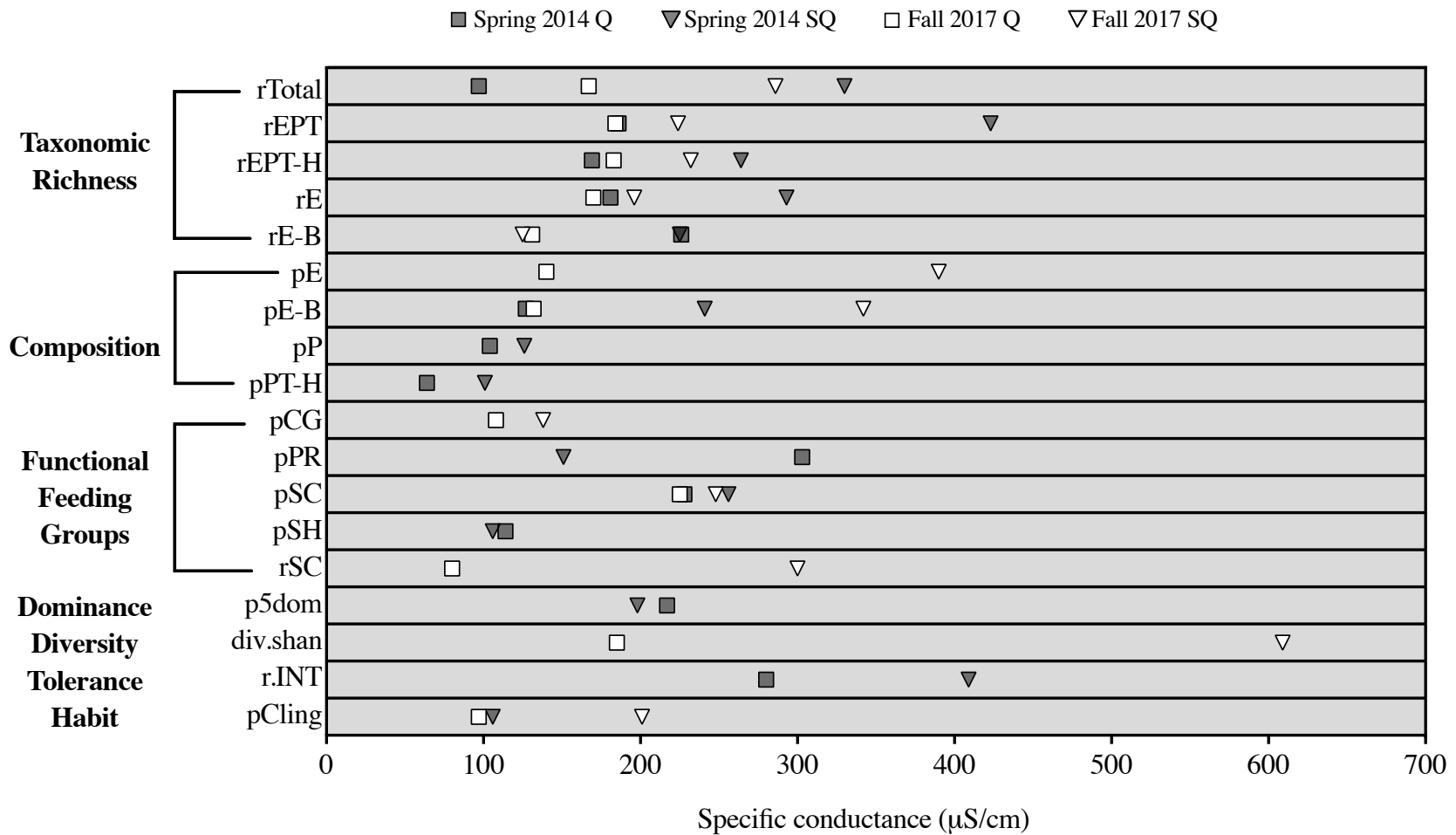


Figure 4.5. Summary of modeled reference SC values organized by metric category for both sampling techniques (quantitative and semi-quantitative) and sample periods (spring 2014 and fall 2017). Modeled reference SC values are derived from generalized additive models (GAM) of continuous conductivity (specific conductance, SC) and benthic community metrics for both of the sample periods. In the legend, “Q” indicates quantitative and “SQ” indicates semi-quantitative.

Overall Summary of Findings

Two sampling methods were used to compare metrics describing various aspects of benthic macroinvertebrate community structure and to evaluate their ability to detect community changes induced by elevated SC. Quantitative samples were collected using a Surber sampler that sampled 0.0929-m² of stream bottom. Three Surber samples were composited for each of 14 or 15 streams, providing a total sample area of 0.2787-m² and 1,414 individuals per stream, on average. Semi-quantitative samples were also collected from the same 14 or 15 streams, using the conventional kicknet (RBP) protocol commonly employed by water management agencies and in many prior scientific studies. Six D-frame kicknet samples collected organisms from a total approximate area of 1.8-m² of stream bottom in each stream and were then sub-sampled to obtain a 200 ($\pm 10\%$) organism count sample for each stream.

My data demonstrate that high-enumeration quantitative samples consistently produced higher estimates of taxonomic richness than corresponding semi-quantitative samples for both spring 2014 and fall 2017 sampling periods (Figure 4.3; Tables A-6 & and A-7). Differences between metrics obtained from the two sampling methods declined as SC increased among streams, especially for those metrics that were most sensitive to elevated SC, including richness for SC-sensitive taxa.

Quantitative samples were typically available for developing modeled-reference SC values for purposes of assessing change from reference communities. Additionally, quantitative assessments are more sensitive to SC than semi-quantitative assessments, as reflected in the modeled-reference SC values (Figure 4.5). Quantitatively-derived models were almost always stronger predictors of SC-induced changes in the biological community, and these SC values were typically lower than semi-quantitatively-derived values. Semi-quantitative models were generally less consistent in ability to predict changes in the benthic community because of higher variation in metric scores for a particular season.

Biological assessments are central to water resource management and are critical components in the enforcement of the Clean Water Act goal of “restoring and maintaining the chemical, physical, and biological integrity of the Nation’s waters” (CWA § 101 (a)). Accurate quantification of benthic macroinvertebrate community structural characteristics is important for estimating resource availability to higher trophic levels, evaluating contributions to ecosystem functions (e.g., nutrient retention and energy processing, leaf litter decomposition, consumption of periphyton), and assessing various degrees of environmental stress or degradation (Suter & Cormier 2015; Wallace & Webster 1996).

CONCLUSIONS

This study demonstrates that high-enumeration quantitative bioassessments offer advantages over semi-quantitative bioassessments of benthic macroinvertebrate communities in mining-influenced Appalachian headwater streams. Quantitative samples provide more complete enumeration of taxonomic richness, as well as identities and proportions of underrepresented macroinvertebrate taxa unaccounted for using conventional semi-quantitative sampling methods; this is especially the case for smaller organisms that are often overlooked during semi-quantitative sub-sampling procedures. Furthermore, semi-quantitative data tend to be more variable, rendering macroinvertebrate-assemblage metrics derived from such samples as less consistent and reliable for detection of community-changes induced by environmental stressors.

Quantitative sampling enhances the ability to interpret biological condition in headwater streams by characterizing community-metric responses to a stressor gradient. My data show that metrics derived from high-enumeration quantitative samples are more sensitive to SC than metrics from semi-quantitative samples, as reflected in the modeled-reference SC values. Models based on quantitative samples were typically stronger predictors of community-metric responses to elevated SC, whereas models based on semi-quantitative samples were generally less consistent for predicting changes in the benthic community because of higher variation in metric scores along the salinity gradient for a particular sampling season. Additionally, high-enumeration quantitative samples provide “background” reference-condition data for use in evaluating biodiversity effects in streams that have been subjected to mining-induced salinization. From this perspective, high-enumeration quantitative sampling also offers a more holistic view of the benthic macroinvertebrate community, including taxa that are susceptible to loss as a result of anthropogenic impacts such as mining-influenced freshwater salinization. Given the paucity of reference-condition stream sites (Burton & Gerritsen 2003) and relatively undisturbed watersheds (Sweeten et al. 2013) in the central Appalachian coalfields, there is immediate need for full characterization and quantification of available reference communities, especially those that are at-risk to degradation.

Quantitative bioassessments of benthic macroinvertebrate communities based on relatively high organism counts provide advantages over semi-quantitative sampling methods that are commonly employed in Appalachian bioassessments because they provide more accurate estimates of taxonomic richness and diversity, thereby enhancing the ability to characterize aquatic community response to environmental stressors. These advantages are of special value when conducting bioassessments of reference or well-preserved ecosystems, or when comparing slightly degraded systems to those in a reference condition. Semi-quantitative samples, with comparatively small numbers of individuals, are often able to discriminate highly-degraded systems from those in a reference condition, but may not produce accurate assessments of more subtle changes. Although semi-quantitative sampling showed stream-community sensitivity to the SC stressor evaluated in this study, the extra time and effort required for high-count quantitative sampling can provide additional insight into benthic macroinvertebrate community structure and stressor response.

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CHAPTER 5. OVERVIEW & FUTURE RESEARCH

Quantitative assessments of benthic macroinvertebrate community structure are conducted by sampling a defined area of stream bottom, and identifying all of the organisms collected. Such sampling typically identifies larger numbers of organisms than conventional semi-quantitative sampling methods, such as those that have been applied by most other studies of benthic macroinvertebrate communities in mining-influenced central Appalachian streams. High-enumeration quantitative samples offer advantages over those based on conventional semi-quantitative RBPs by providing more complete enumeration of taxonomic richness, diversity, and other important metrics of community structure. Results of this study indicate that semi-quantitative methodology did not account for approximately 50% of the total macroinvertebrate taxa collected using a high-enumeration quantitative method in central Appalachian headwater streams. This suggests that previous biological assessments based on RBPs conducted within this region may have underestimated taxonomic richness and underrepresented other metrics of community structure. If previous assessments of macroinvertebrate communities have underestimated taxonomic richness and diversity to this extent, then effects of salinity and other environmental stressors on community and biodiversity losses may have been previously underrepresented.

Although the intent of sampling is to gain insights relative to specific characteristics of a population, there is no single sampling method that can realistically capture all individuals of the macroinvertebrate community present within a given stream. However, an increased sampling effort as achieved through quantitative methods, for example, will likely provide results that are more representative of the stream community (Davies 2001). In my study, up to ten quantitative Surber samples were collected in each stream, but, because of time and resource constraints, only three Surber samples per stream were fully enumerated, analyzed, and mathematically composited for comparison to the semi-quantitative sample collected in each stream. To determine the number of required Surber samples needed to accurately reflect the macroinvertebrate community inhabiting the study streams, taxa accumulation curves should be constructed using the identification-data obtained from the 10 Surber samples. These accumulation curves, plotted with the number of taxa as a function of the number of individuals or number of samples, could then be used to estimate intensity of sampling effort required to adequately describe the benthic community for a given level of required precision and accuracy for this type of headwater stream in the central Appalachian region (Gotelli & Colwell 2001).

Once quantitative data are collected and processed, numerous metrics can be calculated and analyses performed with such data (Stark et al. 2001). Analyzing more than thirty-seven metrics seemed a bit cumbersome to address my study objectives, but inclusion of additional metrics may provide additional insight on benthic community response to elevated salinity induced by surface coal mining. This is especially the case for metrics relating to functional trait niches (Poff et al. 2006) and functional diversity (Bady et al. 2005), because community structural metric responses to elevated salinity are well-documented within the central Appalachian region (Timpano et al. 2018; Boehme et al 2016; Pond et al. 2008). Impacts of SC on stream ecosystem function could be further

analyzed by including metrics involving life history (e.g., voltinism, development, emergence characteristics, reproductive cycles and strategies), mobility, and morphology (e.g., body size and body form) (Poff et al. 2006; Bady et al. 2005). Additional metrics relating to community loss and taxonomic replacement, such as the coefficient of community loss (Courtemanch & Davies 1987), or similar, may be of special interest because there is evidence of taxonomic replacement at high-SC streams analyzed as part of this study (Drover 2018).

Quantitative sampling data, if supplemented with size measurements of each collected organism, could also be used to address impact of surface coal mining on secondary production (biomass), another important aspect of stream ecosystem function. Estimates of benthic secondary production can only be achieved through quantitative sampling and extensive analyses involving length-mass relationships (Benke et al. 1999) or direct weighing of collected individuals. These types of studies are uncommon given the intensive effort required for obtaining such dynamic assessments of stream ecosystem functions, and those conducted within the central Appalachian region have been limited to a small number of mining-influenced streams (Voss & Bernhardt 2017; Johnson et al. 2013).

High-enumeration quantitative sampling methods provide more accurate estimates of benthic macroinvertebrate community structure and response to environmental stressors, relative to conventional semi-quantitative sampling methods. Although semi-quantitative methods can be effective for sampling macroinvertebrate communities at high-SC sites of reduced taxonomic richness and diversity, quantitative sampling shows more sensitivity at the lower end of the SC gradient. The extra time and effort required for processing high-enumeration quantitative data is advantageous for assessing effects of biological stressors (including SC) at sites during critical times of initial exposure or effect, both at reference-condition streams and in pristine systems at risk to degradation. Such assessments are essential and of special interest in ecosystems of high biological diversity such as the central Appalachian region, which is recognized globally as a hotspot for biodiversity.

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APPENDIX

Table A-1. Stream attributes for selected study streams in the central Appalachian coalfield.

Stream	Stream ID	Stream Type	Stream Order	County	State	Latitude	Longitude
Copperhead Branch	COP	Reference	1	Buchanan	VA	37.06471	-82.09067
Crooked Branch	CRO	Reference	1	Dickenson	VA	37.13013	-82.21794
Eastland Creek	EAS	Reference	1	Wise	VA	36.91764	-82.59196
Fryingpan Creek	FRY	Test	1	Dickenson	VA	37.06021	-82.21774
Fryingpan Creek Right Fork*	RFF	Test	1	Dickenson	VA	37.05981	-82.22114
Grape Branch	GRA	Test	1	Buchanan	VA	37.25776	-82.00918
Hurricane Fork	HCN	Reference	1	McDowell	WV	37.42042	-81.86627
Kelly Branch	KEL	Test	1	Wise	VA	36.93472	-82.79085
Kelly Branch Unnamed Tributary	KUT	Test	1	Wise	VA	36.93575	-82.79250
Left Fork/Laurel Fork/Coal Fork	LLC	Test	1	Kanawha	WV	38.08404	-81.47592
Mill Branch West Fork	MIL	Test	1	Wise	VA	36.92717	-82.74680
Powell River	POW	Test	1	Wise	VA	37.01310	-82.69751
Rickey Branch	RIC	Test	1	Wise	VA	37.03710	-82.54583
Rockhouse Creek	ROC	Test	1	Raleigh	WV	37.96569	-81.50123
Spruce Pine Creek	SPC	Test	1	Buchanan	VA	37.26124	-81.92038

*Stream was excluded from fall 2017 analyses due to streamside development, removal of riparian vegetation, and excessive sedimentation.

Table A-2. Stream water chemistry.

Year	Season	Date	Stream	Stream Type	SC µS/cm	pH	Temp °C	Cl ⁻ mg/L	SO ₄ ²⁻ mg/L	CO ₃ ²⁻ mg/L	HCO ₃ ⁻ mg/L	Ca ²⁺ mg/L	K ⁺ mg/L	Mg ²⁺ mg/L	Na ⁺ mg/L	Al µg/L	Cu µg/L	Fe µg/L	Mn µg/L	Se µg/L	Zn µg/L
2014	Spring	4/12/14	COP	Ref	114	7.4	10.0	1.3	18.3	0.0	37.5	10.1	1.2	3.9	3.7	4.5	0.5	60.7	3.8	2.5	5.0
2014	Spring	4/12/14	CRO	Ref	55	7.1	9.9	2.3	8.9	0.0	12.3	3.1	1.1	1.9	2.3	6.6	0.5	36.0	1.6	2.5	5.0
2014	Spring	4/12/14	EAS	Ref	22	7.9	11.5	0.2	2.7	0.0	6.5	2.1	0.3	0.5	0.5	5.2	0.5	12.2	0.5	2.5	5.0
2014	Spring	4/12/14	FRY	Test	330	7.4	14.9	5.0	89.4	0.0	77.3	26.0	1.7	12.2	14.6	3.5	0.5	18.9	1.3	2.5	5.0
2014	Spring	4/11/14	GRA	Test	135		14.9	1.9	31.9	0.0	30.5	9.8	1.3	4.4	5.6	14.1	0.5	342.4	3.6	2.5	31.0
2014	Spring	4/11/14	HCN	Ref	46		14.0	1.1	9.2	0.0	7.1	2.5	1.2	1.5	2.0	8.7	0.5	54.4	2.4	2.5	5.0
2014	Spring	4/13/14	KEL	Test	795	7.5	12.5	0.9	335.1	0.0	126.3	74.0	4.1	46.0	10.7	2.6	0.5	23.7	5.4	2.5	5.0
2014	Spring	4/13/14	KUT	Test	1009	7.7	14.6		180.7	0.0	180.7	88.9	5.6	55.5	29.1	4.1	0.5	11.8	3.6	8.0	10.5
2014	Spring	4/11/14	LLC	Test	925	8.3	10.0	17.9	404.7	0.0	52.9	53.6	6.4	51.7	42.1	13.5	0.5	21.6	28.6	8.4	5.0
2014	Spring	4/13/14	MIL	Test	620	7.7	17.7	0.7	218.8	0.0	137.9	59.0	3.7	32.2	9.5	3.3	0.5	28.2	6.9	2.5	5.0
2014	Spring	4/12/14	POW	Test	795	7.2	14.1	0.4	326.5	0.0	127.9	78.6	3.4	44.8	8.2	3.5	0.5	178.8	5.0	2.5	5.0
2014	Spring	4/12/14	RFF	Test	374	7.3	12.7	3.0	77.5	0.0	131.4	24.0	1.8	9.6	32.4	4.3	0.5	11.4	1.2	2.5	5.0
2014	Spring	4/13/14	RIC	Test	1348	7.7	11.4	7.4	674.8	0.0	177.8	109.0	4.3	110.3	10.4	4.6	0.5	17.5	8.7	2.5	5.0
2014	Spring	4/11/14	ROC	Test	688	7.9	11.5	1.4	262.4	0.0	179.4	51.6	4.6	52.7	4.9	11.7	0.5	249.6	8.4	15.7	74.8
2014	Spring	4/11/14	SPC	Test	242		15.8	3.6	48.4	0.0	68.9	16.5	1.4	6.6	14.7	16.3	0.5	222.6	22.1	2.5	10.8
2017	Fall	10/30/17	COP	Ref	108	7.4	8.1	1.5	11.5	0.0	38.6	12.0	1.7	4.2	4.1	3.6	0.0	8.5	1.7	0.0	18.8
2017	Fall	10/30/17	CRO	Ref	60	7.5	7.5	2.4	7.5	0.0	16.6	4.8	1.6	2.7	2.3	9.8	0.2	39.8	2.5	0.0	17.4
2017	Fall	10/16/17	EAS	Ref	25	6.5	13.8	0.5	2.3	0.0	8.6	3.1	0.7	0.8	0.7	8.2	0.1	6.3	3.1	0.0	22.9
2017	Fall	10/30/17	FRY	Test	394	7.5	7.7	11.0	93.7	0.0	85.0	38.8	2.2	16.6	18.1	3.8	0.0	4.5	1.1	0.3	14.8
2017	Fall	10/4/17	GRA	Test	477	6.9	14.4	3.1	150.5	0.0	81.0	41.3	2.5	14.1	37.3	2.9	0.0	16.2	5.7	0.0	65.9
2017	Fall	10/31/17	HCN	Ref	63	7.4	7.5	1.0	10.0	0.0	16.7	4.7	1.6	2.4	3.9	3.9	0.0	90.6	9.6	0.1	36.7
2017	Fall	10/16/17	KEL	Test	814	7.7	12.3	2.7	346.7	0.0	129.1	90.9	5.0	53.3	12.6	1.6	0.2	21.9	8.3	2.8	19.4
2017	Fall	10/16/17	KUT	Test	1236	8.0	13.2	2.1	513.4	0.0	216.5	135.9	7.7	78.2	50.9	3.1	0.2	11.9	6.9	9.1	21.0
2017	Fall	10/20/17	LLC	Test	1944	7.5	10.7	31.7	1023.1	0.0	97.0	145.1	14.8	155.4	104.6	5.5	0.0	4.2	2.7	14.1	15.6
2017	Fall	10/30/17	MIL	Test	500	7.4	7.1	0.9	152.0	0.0	115.2	55.7	3.5	28.1	7.4	3.2	0.0	34.3	54.1	0.5	21.5
2017	Fall	10/5/17	POW	Test	1051	7.9	15.5	1.5	453.9	0.0	162.6	137.0	5.2	68.1	13.1	5.7	0.1	9.8	5.8	0.9	18.5
2017	Fall	10/16/17	RIC	Test	1725	8.0	13.5	10.9	888.9	0.0	215.6	160.8	7.4	168.6	14.7	3.1	0.1	22	14.5	0.7	19.5
2017	Fall	10/6/17	ROC	Test	932	7.8	16.1	2.5	334.3	0.0	201.5	88.5	6.4	81.5	9.7	6.4	0.4	3.1	1.5	11.3	18.4
2017	Fall	10/4/17	SPC	Test	583	7.7	12.6	6.8	82.3	0.0	227.5	34.6	2.1	12.0	86.0	5.2	0.4	18.6	2.0	0.0	89.5

Table A-3. Benthic macroinvertebrate taxon occurrence by sample period and sample type.

Taxon	Spring 2014		Fall 2017	
	SQ	Q	SQ	Q
<i>Acentrella</i>	X	X	X	X
<i>Acerpenna</i>				X
<i>Acroneuria</i>	X	X	X	X
<i>Aeshna</i>		X		
<i>Agapetus</i>	X			
<i>Agnetina</i>		X		
<i>Allocapnia</i>			X	X
<i>Alloperla</i>			X	X
<i>Ameletus</i>	X	X	X	X
<i>Amphinemura</i>	X	X		
<i>Antocha</i>	X	X	X	X
<i>Apatania</i>			X	X
<i>Atherix</i>				X
<i>Atrichopogon</i>			X	X
<i>Attenella</i>			X	X
<i>Baetis</i>	X	X	X	X
<i>Baetisca</i>				X
<i>Beloneuria</i>				
<i>Bezzia.Palpomyia</i>	X	X	X	X
<i>Brachycentrus</i>		X		
<i>Caenis</i>				X
<i>Calopteryx</i>	X		X	
<i>Ceratopsyche</i>	X	X	X	X
<i>Cernotina</i>		X		X
<i>Chelifera</i>	X	X	X	X
<i>Cheumatopsyche</i>	X	X	X	X
<i>Chimarra</i>	X		X	X
<i>Chironomidae</i>	X	X	X	X
<i>Cinygmula</i>	X	X		
<i>Clinocera</i>		X	X	
<i>Cordulegaster</i>				X
<i>Cyrnellus</i>	X	X		X
<i>Dasyhelea</i>	X			
<i>Dibusa</i>	X			
<i>Dicranota</i>	X	X		
<i>Dipheter</i>	X	X	X	X
<i>Diplectrona</i>	X	X	X	X
<i>Diploperla</i>				X
<i>Dixa</i>	X	X	X	X
<i>Dolophilodes</i>	X	X	X	X
<i>Drunella</i>	X	X		
<i>Eccoptura</i>	X	X		X
<i>Ectopria</i>	X	X	X	X
<i>Epeorus</i>	X	X	X	X
<i>Ephemera</i>	X	X	X	X
<i>Ephemerella</i>	X	X	X	X
<i>Eurylophella</i>		X	X	X
<i>Forcipomyia</i>			X	
<i>Glossosoma</i>		X	X	X
<i>Goera</i>				X
<i>Gomphus</i>				X
<i>Habrophlebiodes</i>		X	X	X
<i>Hansonoperla</i>				X
<i>Haploperla</i>	X	X		X
<i>Helichus</i>		X		
<i>Hemerodromia</i>	X	X	X	X
<i>Heptagenia</i>		X	X	X
<i>Hexatoma</i>	X	X	X	X
<i>Hydatophylax</i>			X	X
<i>Hydropsyche</i>	X	X	X	X
<i>Hydroptila</i>		X		
<i>Isonychia</i>	X	X		X

Taxon	Spring 2014		Fall 2017	
	SQ	Q	SQ	Q
<i>Isoperla</i>	X	X	X	X
<i>Lanthus</i>	X	X	X	X
<i>Lepidostoma</i>	X	X		
<i>Leucrocuta</i>	X		X	X
<i>Leuctra</i>	X	X	X	X
<i>Limnophila</i>		X		X
<i>Lype</i>	X	X	X	X
<i>Maccaffertium</i>	X	X	X	X
<i>Malirekus</i>	X			
<i>Micrasema</i>		X		
<i>Molophilus</i>		X	X	X
<i>Nemoura</i>		X		
<i>Neophylax</i>	X	X	X	X
<i>Neoplasta</i>		X		
<i>Neotrichia</i>		X	X	
<i>Nigronia</i>	X	X	X	X
<i>Ochrotrichia</i>	X			
<i>Odontomyia</i>				X
<i>Oemopteryx</i>		X		X
<i>Oligochaeta</i>	X	X	X	X
<i>Optioservus</i>	X	X	X	X
<i>Ormosia</i>				X
<i>Orthotrichia</i>		X		
<i>Ostrocerca</i>		X		X
<i>Oulimnius</i>	X	X	X	
<i>Palpomyia</i>			X	
<i>Paracapnia</i>			X	X
<i>Paraleptophlebia</i>	X	X	X	X
<i>Paraleuctra</i>			X	X
<i>Peltoperla</i>	X	X	X	X
<i>Perlesta</i>		X		
<i>Pilaria</i>			X	
<i>Polycentropus</i>	X	X	X	X
<i>Prinocerca</i>		X		
<i>Prosimulium</i>	X			
<i>Prostoia</i>	X			
<i>Psephenus</i>	X	X	X	X
<i>Pseudostenophylax</i>		X	X	X
<i>Pteronarcys</i>	X	X	X	X
<i>Pycnopsyche</i>		X	X	X
<i>Remenus</i>	X	X		
<i>Rhyacophila</i>	X	X	X	X
<i>Serratella</i>				X
<i>Sialis</i>	X	X		X
<i>Simulium</i>	X	X		X
<i>Stactobiella</i>		X	X	
<i>Stenacron</i>		X	X	X
<i>Stenelmis</i>			X	X
<i>Stenonema</i>		X	X	X
<i>Stratiomys</i>			X	
<i>Strophopteryx</i>			X	X
<i>Stylogomphus</i>		X		
<i>Suwallia</i>		X		X
<i>Sweltsa</i>	X	X	X	X
<i>Tabanus</i>		X		X
<i>Taenionema</i>				X
<i>Taeniopteryx</i>			X	X
<i>Tallaperla</i>	X	X	X	X
<i>Tipula</i>	X	X	X	X
<i>Wormaldia</i>	X		X	X
<i>Yugus</i>	X	X		

Table A-4. Summary statistics from continuous conductivity logger data expressed as specific conductance (SC), collected from 15 central Appalachian headwater streams, used in analysis of spring 2014 samples.

Stream	Stream Type	Mean SC ($\mu\text{S/cm}$)	Min. SC ($\mu\text{S/cm}$)	Max. SC ($\mu\text{S/cm}$)	Range ($\mu\text{S/cm}$)	Std. Dev.
COP	Ref	128	93	186	93	31
CRO	Ref	65	50	84	34	12
EAS	Ref	26	20	39	19	6
FRY	Test	361	248	480	231	57
GRA	Test	239	134	444	310	105
HCN	Ref	68	47	109	62	23
KEL	Test	759	687	813	126	40
KUT	Test	1064	928	1275	348	119
LLC	Test	1212	848	1788	940	334
MIL	Test	599	451	839	388	125
POW	Test	773	524	980	456	128
RFF	Test	430	188	602	414	105
RIC	Test	1484	1150	1974	824	268
ROC	Test	694	517	947	431	143
SPC	Test	352	228	567	339	110

Table A-5. Summary statistics from continuous conductivity logger data expressed as specific conductance (SC), collected from 14 central Appalachian headwater streams, used in analysis of fall 2017 samples.

Stream	Stream Type	Mean SC ($\mu\text{S/cm}$)	Min. SC ($\mu\text{S/cm}$)	Max. SC ($\mu\text{S/cm}$)	Range ($\mu\text{S/cm}$)	Std. Dev.
COP	Ref	115	89	189	100	27
CRO	Ref	57	43	125	81	22
EAS	Ref	25	18	43	24	7
FRY	Test	398	286	563	277	93
GRA	Test	238	109	387	278	112
HCN	Ref	86	44	155	111	46
KEL	Test	737	620	827	207	55
KUT	Test	1013	813	1122	310	104
LLC	Test	1132	670	1737	1067	341
MIL	Test	540	364	852	488	149
POW	Test	814	593	1149	556	133
RFF	Test	1404	964	1917	953	382
RIC	Test	667	475	856	381	146
ROC	Test	371	171	567	396	148
SPC	Test	115	89	189	100	27

Table A-6. Mean metric differences for spring 2014 samples.

Category	Metric Abbrev.	Sample Mean		Sample Difference			P-value	
		Q	SQ	Mean	SE	V		
Taxonomic Richness	<i>rTotal</i>	33.7	20.9	12.8	1.2	120	< 0.0001	***
	<i>rEPT</i>	21.5	13.1	8.5	1.1	120	< 0.0001	***
	<i>rEPT-H</i>	18.3	11.0	7.3	0.9	120	< 0.0001	***
	<i>rE</i>	6.5	4.1	2.4	0.7	76	0.0020	**
	<i>rE-B</i>	4.9	2.8	2.1	0.6	66	< 0.0010	***
	<i>rP</i>	6.7	4.7	1.9	0.3	91	0.0002	***
	<i>rT</i>	8.4	4.3	4.1	0.5	120	< 0.0001	***
	<i>rT-H</i>	5.1	2.2	2.9	0.3	120	< 0.0001	***
	<i>rDip</i>	7.2	4.3	2.9	0.4	105	0.0001	***
Composition	<i>pEPT</i>	80.0	74.0	6.0	2.9	104	0.0103	*
	<i>pEPT-H</i>	73.8	64.2	9.6	3.7	104	0.0103	*
	<i>pE</i>	16.6	19.0	-2.5	2.1	37.5	0.2130	
	<i>pE-B</i>	8.9	13.3	-4.3	2.1	18	0.0549	
	<i>pP</i>	53.3	42.7	10.5	3.3	114	0.0009	***
	<i>pT</i>	10.1	12.2	-2.1	1.8	44	0.3894	
	<i>pT-H</i>	4.0	2.4	1.5	1.2	78	0.3303	
	<i>pPT-H</i>	57.2	45.2	12.1	3.0	120	< 0.0001	***
	<i>pDip</i>	15.4	19.0	-3.5	3.0	23.5	0.0367	*
	<i>pChi</i>	11.6	9.9	1.7	2.4	680	0.0029	**
	<i>pOligo</i>	1.0	0.2	0.7	0.3	97	0.0029	**
Functional Feeding Groups	<i>pCG</i>	24.6	25.2	-0.6	2.2	52	0.6686	
	<i>pCF</i>	11.0	17.3	-6.3	2.5	16	0.0103	*
	<i>pPR</i>	6.3	6.5	-0.3	0.6	51	0.6287	
	<i>pSC</i>	6.8	10.2	-3.4	1.3	18	0.0151	*
	<i>pSH</i>	51.3	40.8	10.6	3.3	116	0.0004	***
	<i>rSC</i>	5.3	4.0	1.3	0.4	52	0.0117	*
Dominance	<i>p1dom</i>	38.7	37.7	0.9	0.4	56.5	0.8578	
	<i>p2dom</i>	59.1	52.7	6.4	3.4	90.5	0.0857	
	<i>p5dom</i>	78.9	77.4	1.5	1.6	83	0.2078	
	<i>even</i>	0.6	0.7	-0.1	0.0	4	0.0004	***
Diversity	<i>shan</i>	2.0	2.1	-0.1	0.1	56	0.8469	
	<i>simp</i>	0.8	0.8	0.0	0.0	50	0.5995	
Tolerance	<i>rINT</i>	18.5	13.3	5.2	0.7	120	< 0.0001	***
	<i>pTOL</i>	0.0	0.0	0.0	0.0	1	1	
	<i>HBI</i>	2.2	2.5	-0.4	0.2	31	0.1070	
Habit	<i>pCling</i>	53.6	48.5	5.1	4.7	78	0.3232	
	<i>rCling</i>	21.5	13.9	7.5	0.9	120	< 0.0001	***

*** p < 0.001; **p < 0.01; *p < 0.05

Table A-7. Mean metric differences for fall 2017 samples.

Category	Metric Abbrev.	Sample Mean		Sample Difference			P-value	
		Q	SQ	Mean	SE	V		
Taxonomic Richness	<i>rTotal</i>	33.7	22.0	11.7	1.8	105	0.0001	***
	<i>rEPT</i>	20.9	13.7	7.2	1.2	105	0.0001	***
	<i>rEPT-H</i>	17.1	10.8	6.4	1.2	105	0.0001	***
	<i>rE</i>	4.9	3.4	1.6	0.7	47.5	0.0430	*
	<i>rE-B</i>	4.1	2.8	1.4	0.7	52	0.1045	
	<i>rP</i>	7.1	3.8	3.4	0.5	91	0.0002	***
	<i>rT</i>	8.9	6.6	2.3	0.6	55	0.0020	**
	<i>rT-H</i>	5.1	3.9	1.4	0.6	42	0.0156	*
	<i>rDip</i>	7.2	4.4	2.8	0.5	78	0.0005	***
Composition	<i>pEPT</i>	74.9	69.0	5.9	2.6	81	0.0785	
	<i>pEPT-H</i>	43.2	39.5	3.7	3.3	69	0.3258	
	<i>pE</i>	6.7	7.1	-0.5	1.0	41	0.7729	
	<i>pE-B</i>	6.2	6.3	-0.1	0.9	35.5	0.8081	
	<i>pP</i>	29.6	25.4	4.2	3.0	69	0.3258	
	<i>pT</i>	38.7	36.5	2.2	2.9	58	0.4143	
	<i>pT-H</i>	7.0	7.0	-0.1	1.3	56	0.8552	
	<i>pPT-H</i>	36.6	32.4	4.2	3.0	72.5	0.2225	
	<i>pDip</i>	16.5	19.9	-3.4	2.2	33.5	0.2474	
	<i>pChi</i>	11.1	15.1	-4.0	2.0	42.5	0.5515	
<i>pOligo</i>	1.6	3.1	-1.5	1.3	21	0.0495	*	
Functional Feeding Groups	<i>pCG</i>	16.7	22.5	-5.8	2.4	18	0.0295	*
	<i>pCF</i>	36.0	31.5	4.5	2.6	79	0.1040	
	<i>pPR</i>	7.6	7.6	0.0	1.3	64	0.5016	
	<i>pSC</i>	8.5	10.6	-2.2	1.1	22	0.0580	
	<i>pSH</i>	31.2	27.8	3.4	3.0	70	0.2958	
	<i>rSC</i>	5.9	4.2	1.7	0.6	78	0.0222	*
Dominance	<i>p1dom</i>	32.6	35.6	-3.0	3.5	39	0.4263	
	<i>p2dom</i>	48.1	51.4	-3.3	2.9	35	0.2958	
	<i>p5dom</i>	71.3	76.2	-4.9	2.3	23	0.0676	
	<i>even</i>	0.7	0.7	0.0	0.0	32	0.2166	
Diversity	<i>shan</i>	2.3	2.2	0.2	0.1	83	0.0580	
	<i>simp</i>	0.8	0.8	0.0	0.0	69.5	0.3032	
Tolerance	<i>rINT</i>	19.1	12.3	6.8	1.0	105	0.0001	***
	<i>pTOL</i>	0.1	0.0	0.1	0.1	29	0.1484	
	<i>HBI</i>	2.4	2.7	-0.3	0.1	26	0.1040	
Habit	<i>pCling</i>	73.9	70.7	3.1	2.6	61	0.6257	
	<i>rCling</i>	19.9	13.9	6.0	1.1	105	0.0001	***

*** p < 0.001; **p < 0.01; *p < 0.05

Table A-8. Summary of generalized additive model (GAM) selection criteria and modeled-reference specific conductance (SC) values for spring 2014 and fall 2017 samples.

Category	Metric Abbrev.	Sample Type	Sample Period	Reference threshold met?	p-value \leq 0.05?		$R^2 \geq$ 0.50?		Retain for comparison ?	Modeled-Reference SC value (μ S/cm)
Taxonomic Richness	rTotal	Q	Spring 2014	Yes	Yes	<0.0001	Yes	0.80	Yes	97
	rTotal	SQ	Spring 2014	No	Yes	0.0136	No	0.42		330
	rTotal	Q	Fall 2017	Yes	Yes	<0.0001	Yes	0.69	Yes	167
	rTotal	SQ	Fall 2017	No	Yes	0.0080	Yes	0.67		286
	rEPT	Q	Spring 2014	Yes	Yes	<0.0001	Yes	0.85	Yes	186
	rEPT	SQ	Spring 2014	No	Yes	0.0029	No	0.49		423
	rEPT	Q	Fall 2017	Yes	Yes	<0.0001	Yes	0.72	Yes	184
	rEPT	SQ	Fall 2017	No	Yes	0.0038	Yes	0.67		224
	rEPT-H	Q	Spring 2014	Yes	Yes	<0.0001	Yes	0.87	Yes	169
	rEPT-H	SQ	Spring 2014	No	Yes	0.0002	Yes	0.63		264
	rEPT-H	Q	Fall 2017	Yes	Yes	<0.0001	Yes	0.71	Yes	183
	rEPT-H	SQ	Fall 2017	No	Yes	0.0006	Yes	0.68		232
	rE	Q	Spring 2014	Yes	Yes	<0.0001	Yes	0.93	Yes	181
	rE	SQ	Spring 2014	No	Yes	0.0001	Yes	0.53		293
	rE	Q	Fall 2017	Yes	Yes	<0.0001	Yes	0.80	Yes	170
	rE	SQ	Fall 2017	Yes	Yes	0.0006	Yes	0.72		196
Composition	rE-B	Q	Spring 2014	Yes	Yes	<0.0001	Yes	0.91	Yes	226
	rE-B	SQ	Spring 2014	No	Yes	0.0001	Yes	0.70		225
	rE-B	Q	Fall 2017	Yes	Yes	<0.0001	Yes	0.84	Yes	131
	rE-B	SQ	Fall 2017	Yes	Yes	0.0014	Yes	0.77		125
	pE	Q	Spring 2014	No					No	
	pE	SQ	Spring 2014	No						
	pE	Q	Fall 2017	Yes	Yes	0.0040	Yes	0.66	Yes	140
	pE	SQ	Fall 2017	Yes	Yes	0.0235	No	0.42		390
Functional Feeding Groups	pE-B	Q	Spring 2014	Yes	Yes	<0.0001	Yes	0.91	Yes	127
	pE-B	SQ	Spring 2014	No	Yes	<0.0001	Yes	0.87		241
	pE-B	Q	Fall 2017	Yes	Yes	0.0027	Yes	0.68	Yes	132
	pE-B	SQ	Fall 2017	Yes	Yes	0.0160	No	0.46		342
	pP	Q	Spring 2014	Yes	Yes	<0.0001	Yes	0.83	Yes	104
	pP	SQ	Spring 2014	Yes	Yes	<0.0001	Yes	0.9		126
	pP	Q	Fall 2017	No					No	
	pP	SQ	Fall 2017	No						
	pPT-H	Q	Spring 2014	Yes	Yes	<0.0001	Yes	0.85	Yes	64
	pPT-H	SQ	Spring 2014	Yes	Yes	<0.0001	Yes	0.88		101
	pPT-H	Q	Fall 2017	No					No	
	pPT-H	SQ	Fall 2017	No						
Dominance	pCG	Q	Spring 2014	No					No	
	pCG	SQ	Spring 2014	No						
	pCG	Q	Fall 2017	Yes	Yes	0.0103	Yes	0.76	Yes	108
	pCG	SQ	Fall 2017	No	Yes	0.0181	No	0.33		138
	pPR	Q	Spring 2014	No	Yes	0.0275	No	0.40	Yes	303
	pPR	SQ	Spring 2014	Yes	Yes	0.0011	Yes	0.68		151
	pPR	Q	Fall 2017	No					No	
	pPR	SQ	Fall 2017	No						
	pSC	Q	Spring 2014	Yes	Yes	0.0245	Yes	0.51	Yes	228
	pSC	SQ	Spring 2014	Yes	Yes	0.0017	Yes	0.65		256
	pSC	Q	Fall 2017	Yes	Yes	0.0012	Yes	0.70	Yes	225
	pSC	SQ	Fall 2017	No	Yes	0.0011	Yes	0.71		248
Diversity	pSH	Q	Spring 2014	Yes	Yes	0.0001	Yes	0.83	Yes	114
	pSH	SQ	Spring 2014	Yes	Yes	<0.0001	Yes	0.90		106
	pSH	Q	Fall 2017	No					No	
	pSH	SQ	Fall 2017	No						
	rSC	Q	Spring 2014	No					No	
	rSC	SQ	Spring 2014	No						
	rSC	Q	Fall 2017	Yes	Yes	0.0001	Yes	0.82	Yes	80
	rSC	SQ	Fall 2017	No	Yes	0.0020	Yes	0.58		300
Tolerance	p5dom	Q	Spring 2014	Yes	Yes	0.0009	Yes	0.68	Yes	217
	p5dom	SQ	Spring 2014	No	Yes	0.0003	Yes	0.74		198
	p5dom	Q	Fall 2017	No					No	
	p5dom	SQ	Fall 2017	No						
Habit	div.shan	Q	Spring 2014	No					No	
	div.shan	SQ	Spring 2014	No						
	div.shan	Q	Fall 2017	Yes	Yes	0.0081	Yes	0.55	Yes	185
	div.shan	SQ	Fall 2017	No	No	0.0523	No	0.40		609
Habit	rINT	Q	Spring 2014	Yes	Yes	0.0002	Yes	0.79	Yes	280
	rINT	SQ	Spring 2014	No	Yes	0.0015	Yes	0.57		409
	rINT	Q	Fall 2017	No					No	
	rINT	SQ	Fall 2017	No						
Habit	pCling	Q	Spring 2014	No	No	0.3599	No	0.1	Yes	1455
	pCling	SQ	Spring 2014	Yes	Yes	0.0001	Yes	0.89		106
	pCling	Q	Fall 2017	Yes	Yes	0.0032	Yes	0.84	Yes	97
	pCling	SQ	Fall 2017	No	Yes	0.0293	Yes	0.51		201

Table A-9. Modeled-reference specific conductance (SC) values obtained as part of this study and comparison to critical SC values obtained by Timpano et al. (2018a).

Metric	Season	This study Modeled-Ref SC values ($\mu\text{S}/\text{cm}$)		Timpano et al. (2018a) Critical SC value ($\mu\text{S}/\text{cm}$)
		Quantitative	Semi-quantitative	Semi-quantitative
rTotal	Spring	97	330	276
rTotal	Fall	167	286	548
rEPT	Spring	186	423	401
rEPT	Fall	184	224	605
rE	Spring	181	293	284
rE	Fall	170	196	348
rE-B	Spring	226	225	214
rE-B	Fall	131	125	236
pE	Spring	-	-	294
pE	Fall	194	390	524
pE-B	Spring	127	241	196
pE-B	Fall	132	342	348
rSC	Spring	-	-	403
rSC	Fall	80	300	-
p5dom	Spring	217	198	276
p5dom	Fall	-	-	-
div.shan	Spring	-	-	315
div.shan	Fall	185	609	-

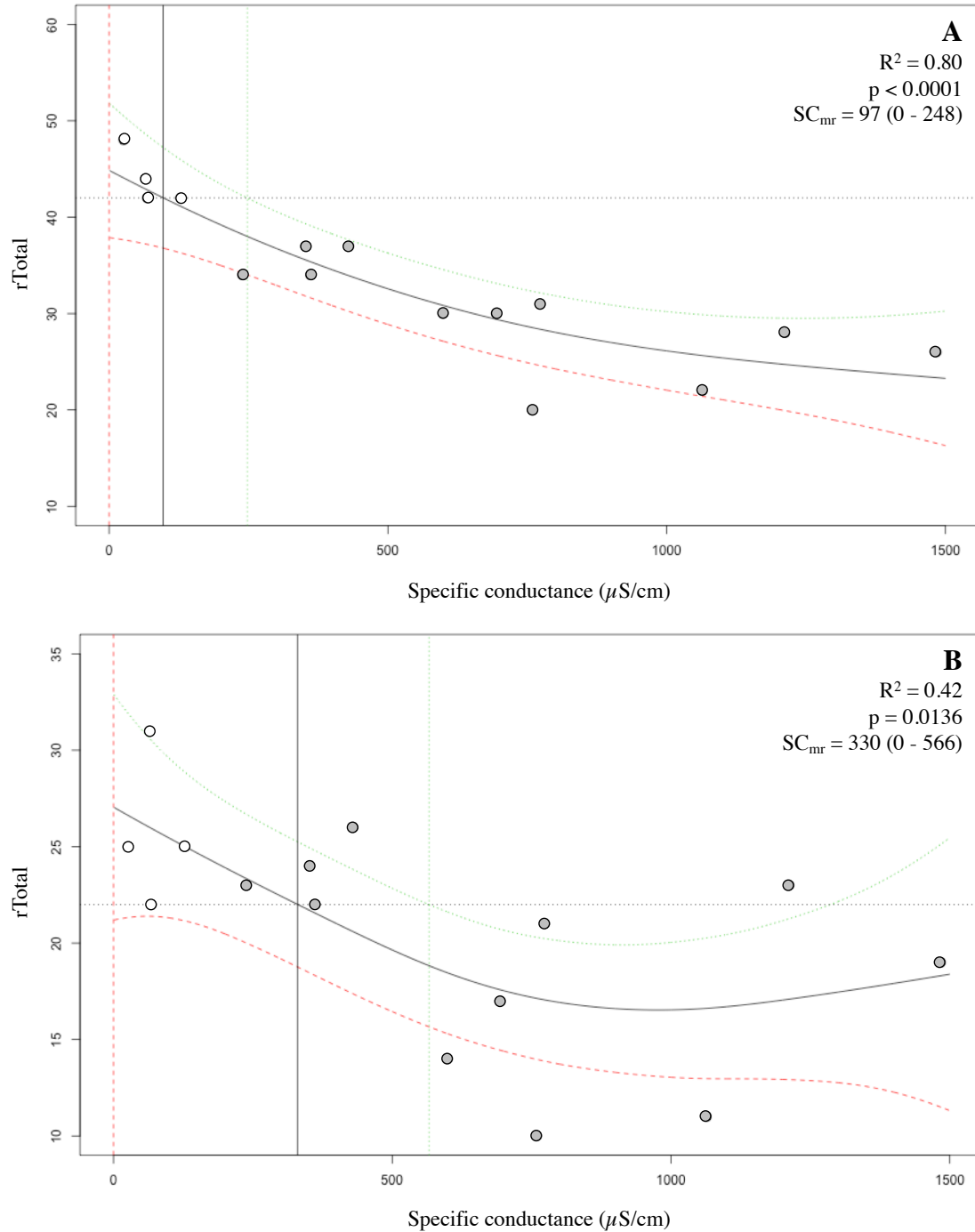


Figure A-1. Generalized additive models (GAMs) of specific conductance (SC) and total taxa richness ($rTotal$) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

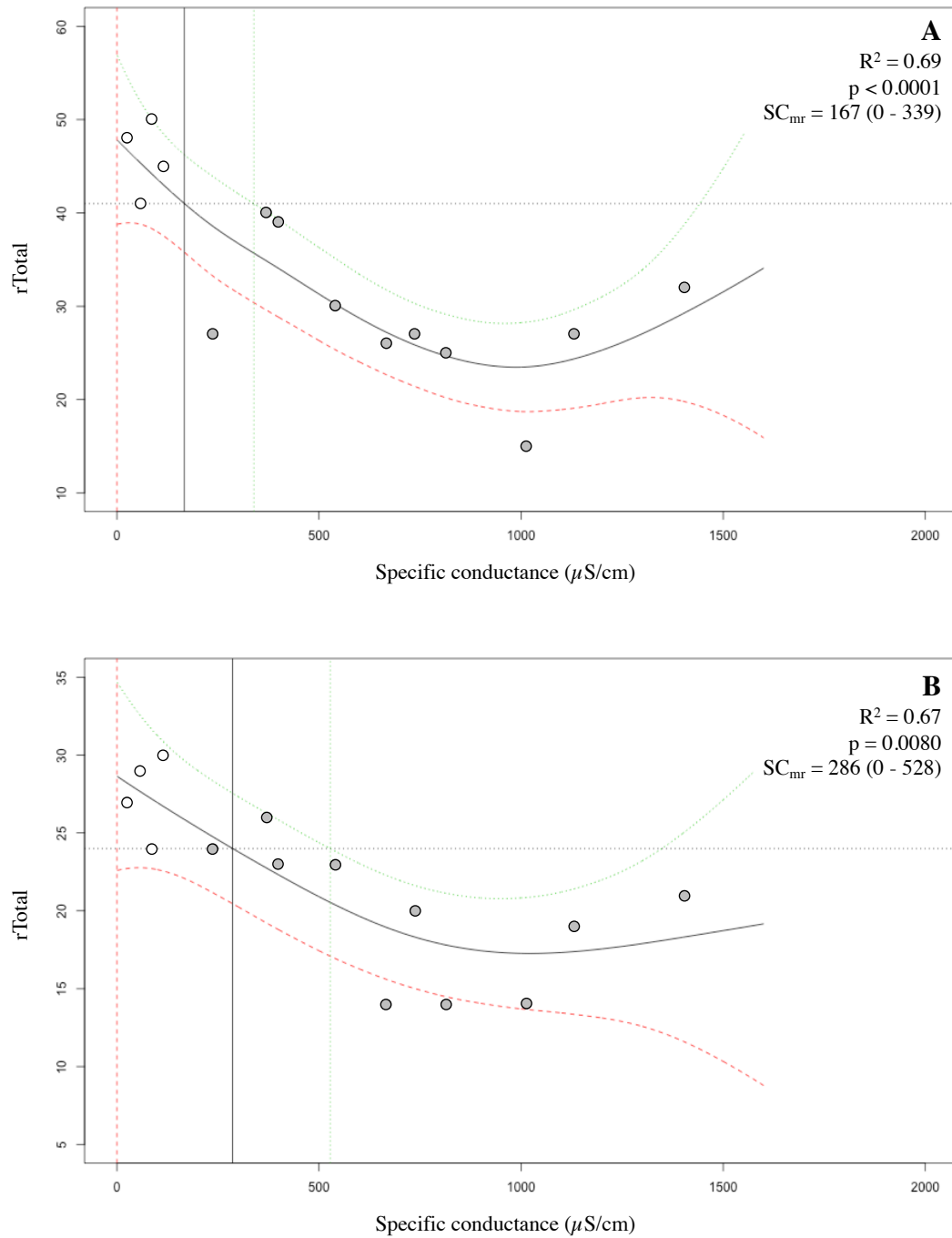


Figure A-2. Generalized additive models (GAMs) of specific conductance (SC) and total taxa richness (r_{Total}) for fall 2017 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

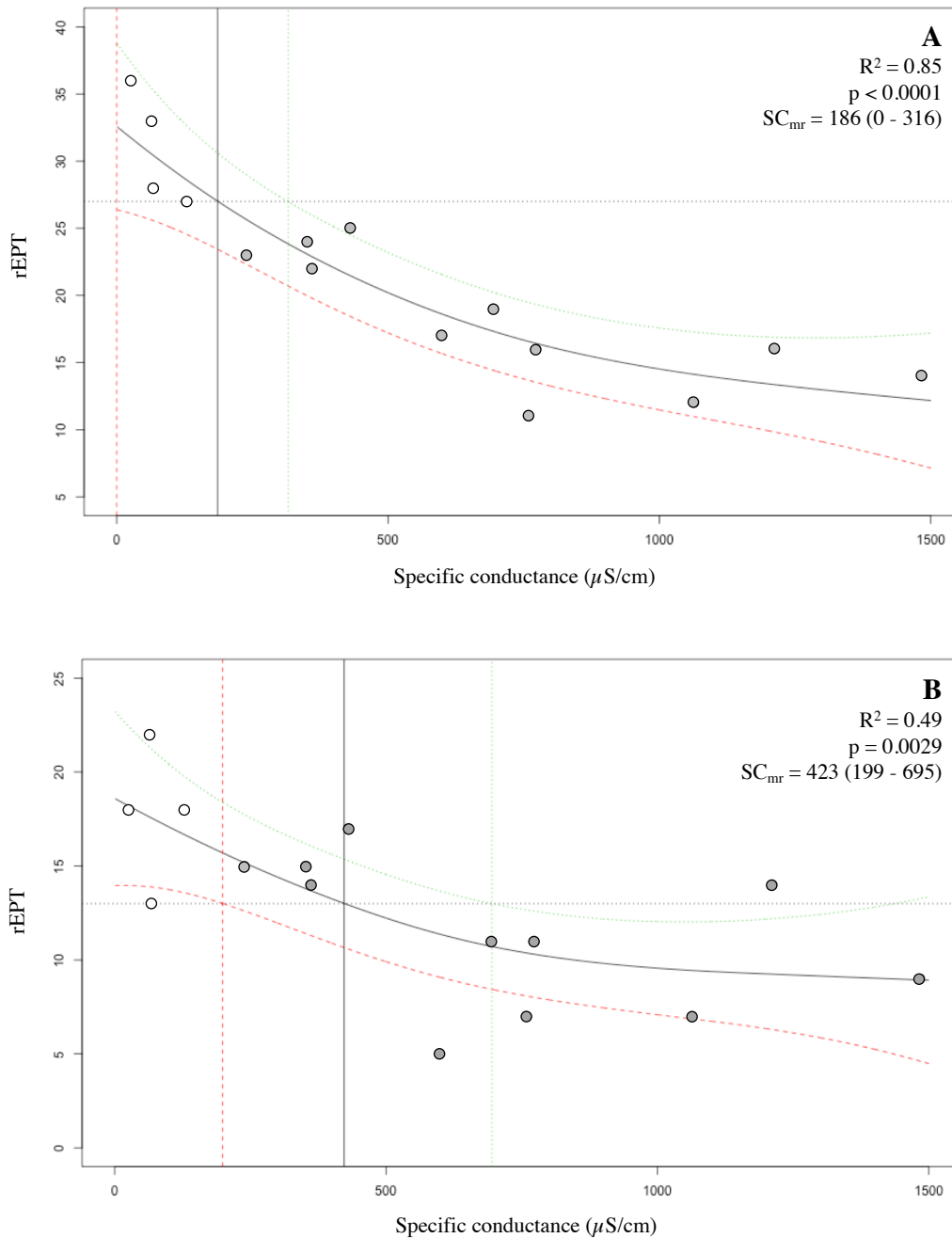


Figure A-3. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera, Plecoptera, and Trichoptera taxa richness ($rEPT$) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

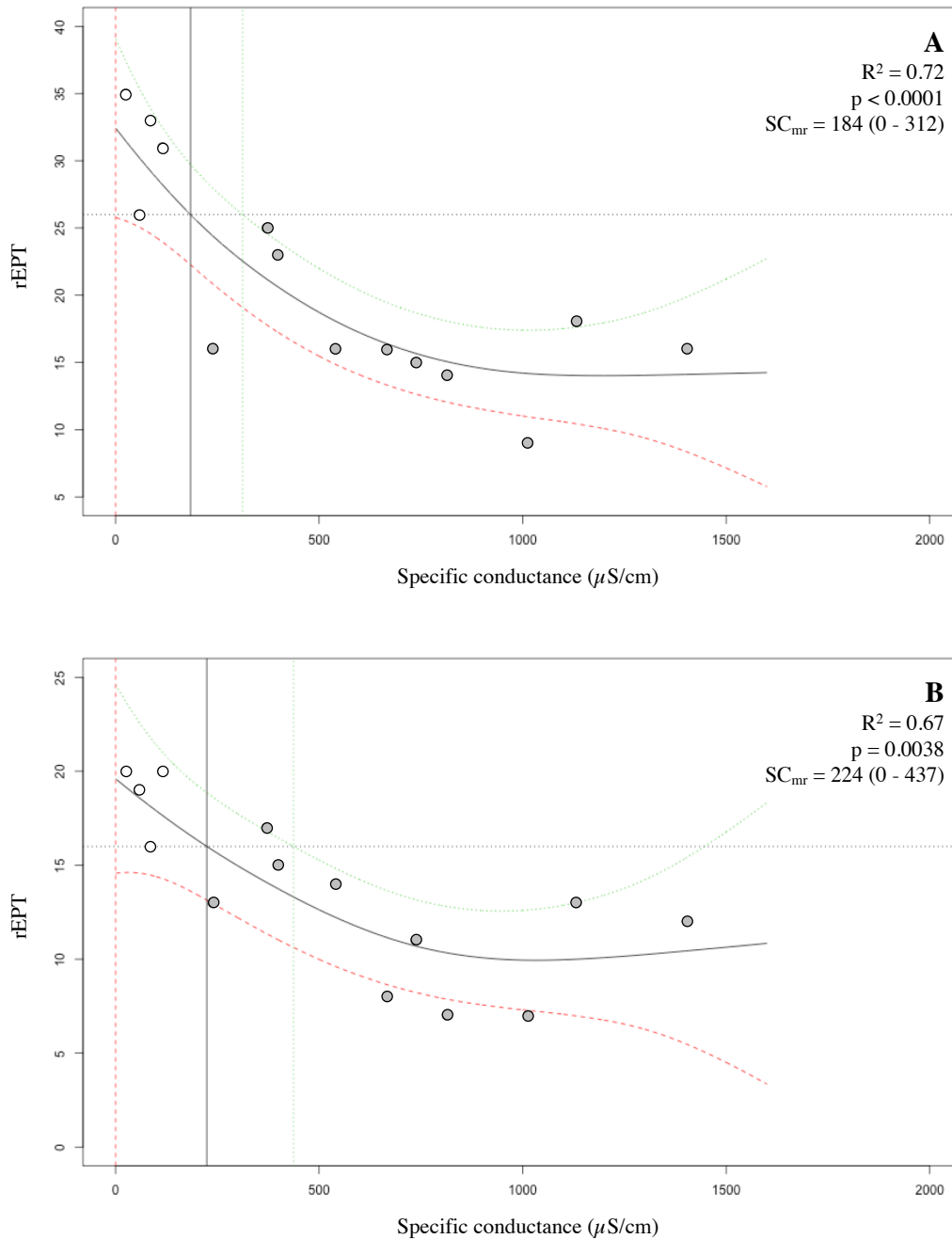


Figure A-4. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera, Plecoptera, and Trichoptera taxa richness (*rEPT*) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

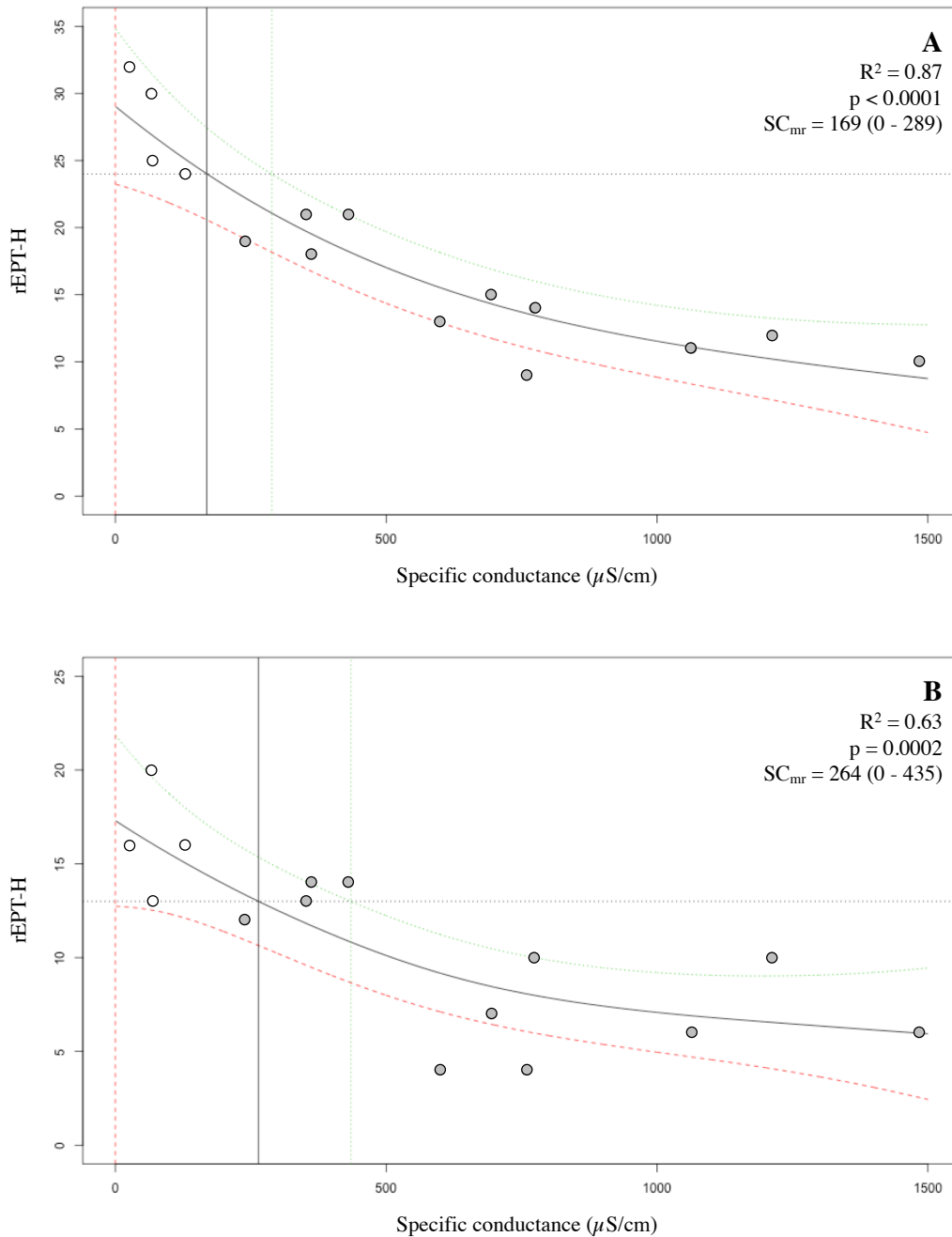


Figure A-5. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera, Plecoptera, and Trichoptera taxa richness less Hydropsychidae ($rEPT-H$) for spring 2014 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

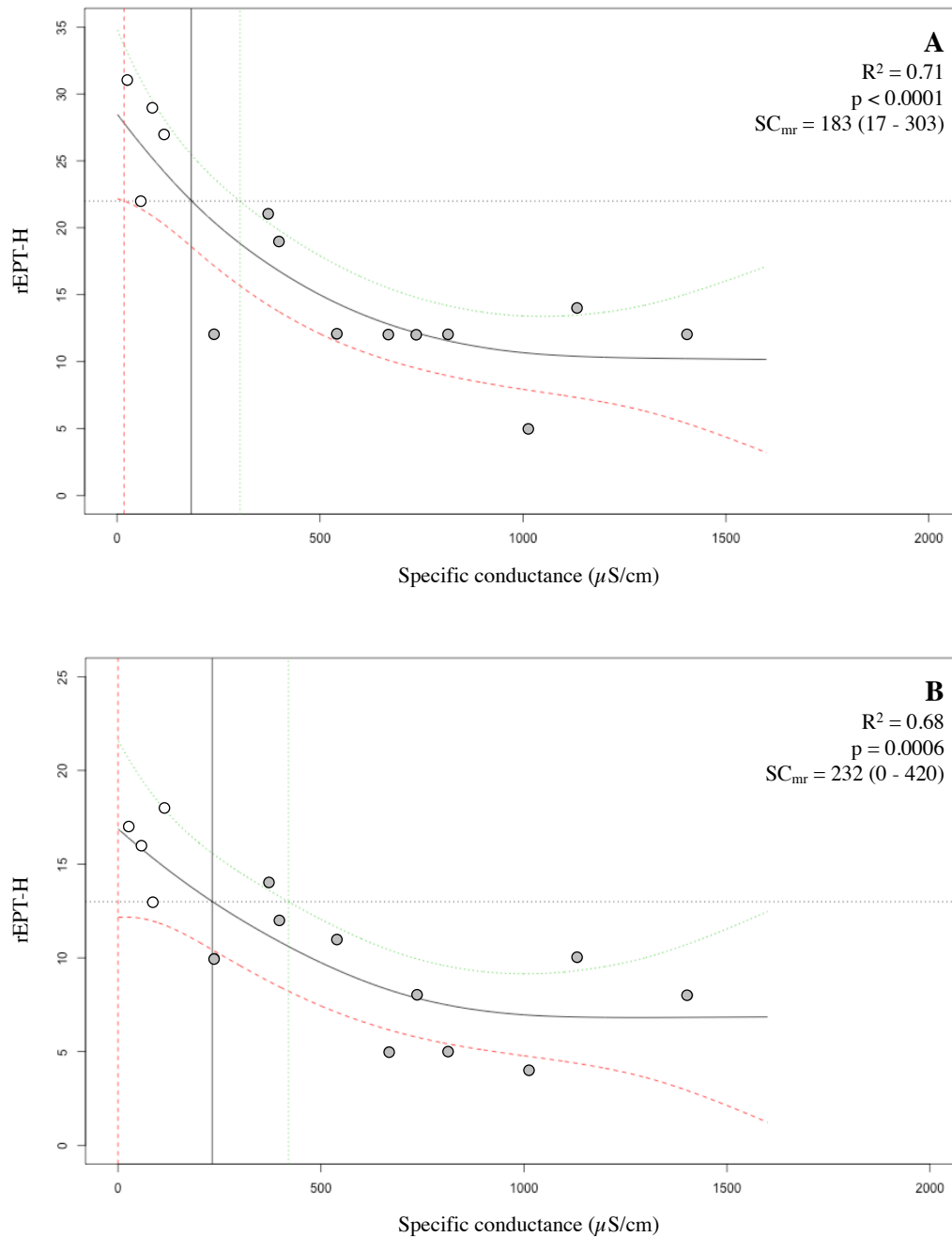


Figure A-6. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera, Plecoptera, and Trichoptera taxa richness less Hydropsychidae ($rEPT-H$) for fall 2017 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

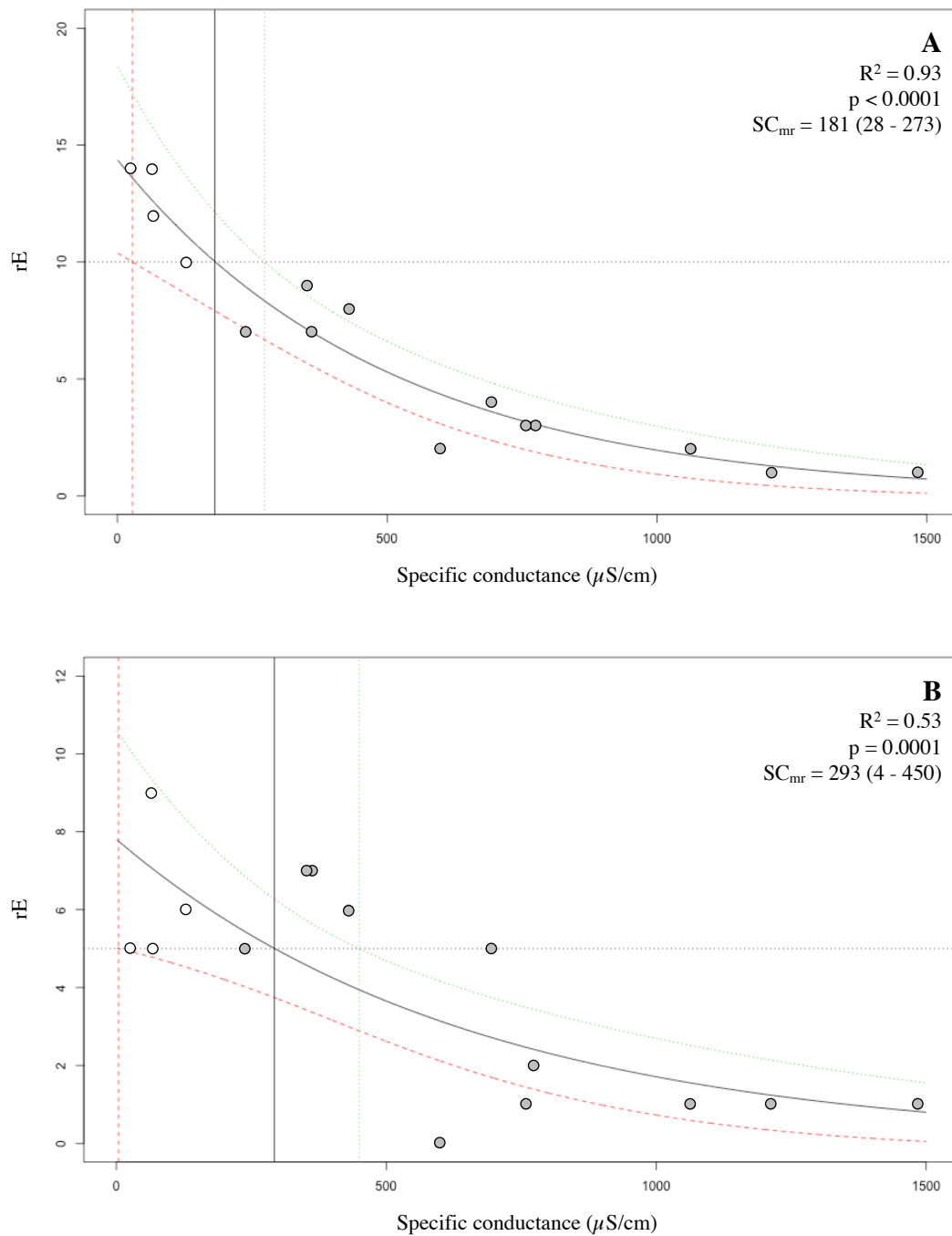


Figure A-7. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera taxa richness (rE) for spring 2014 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

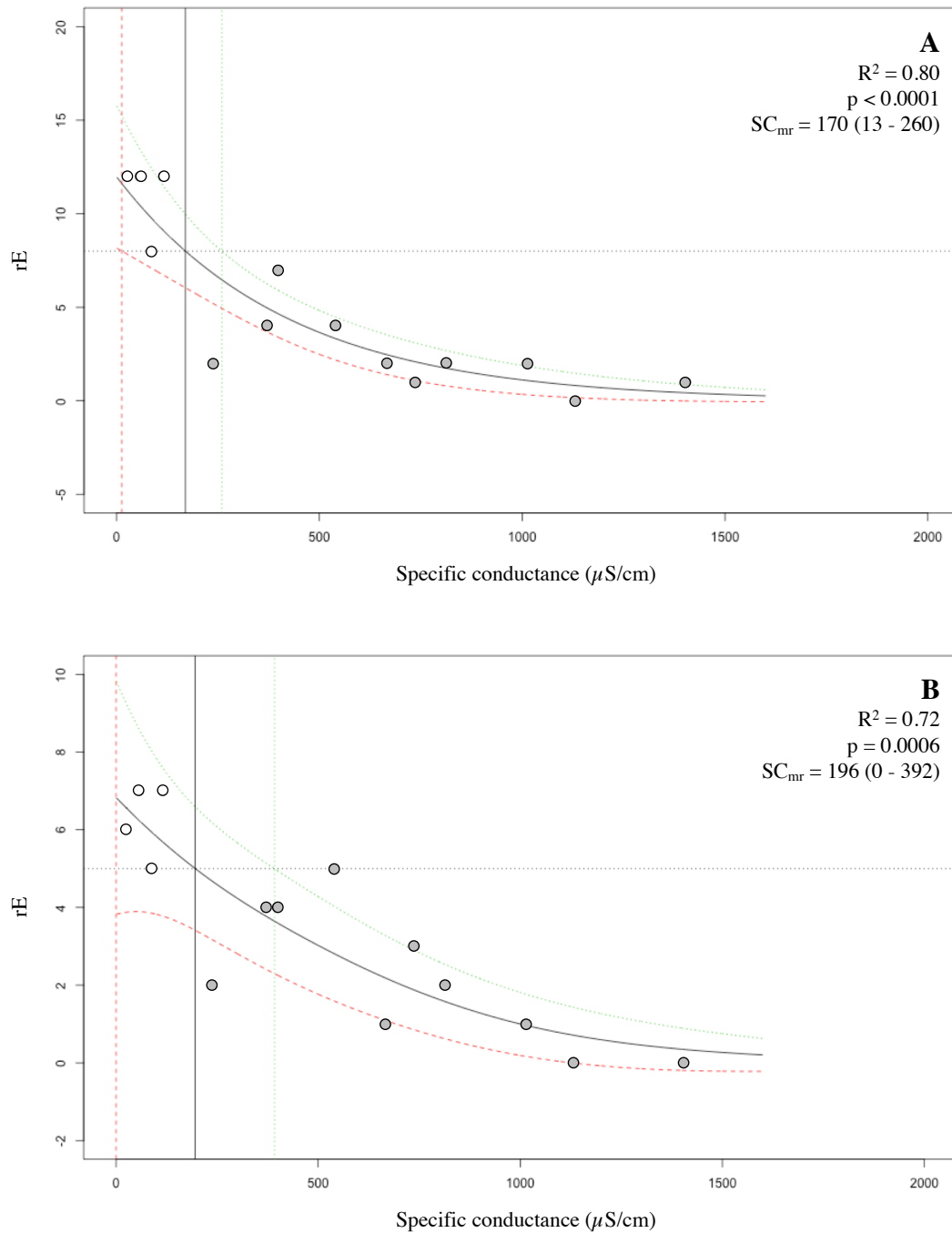


Figure A-8. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera taxa richness (rE) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

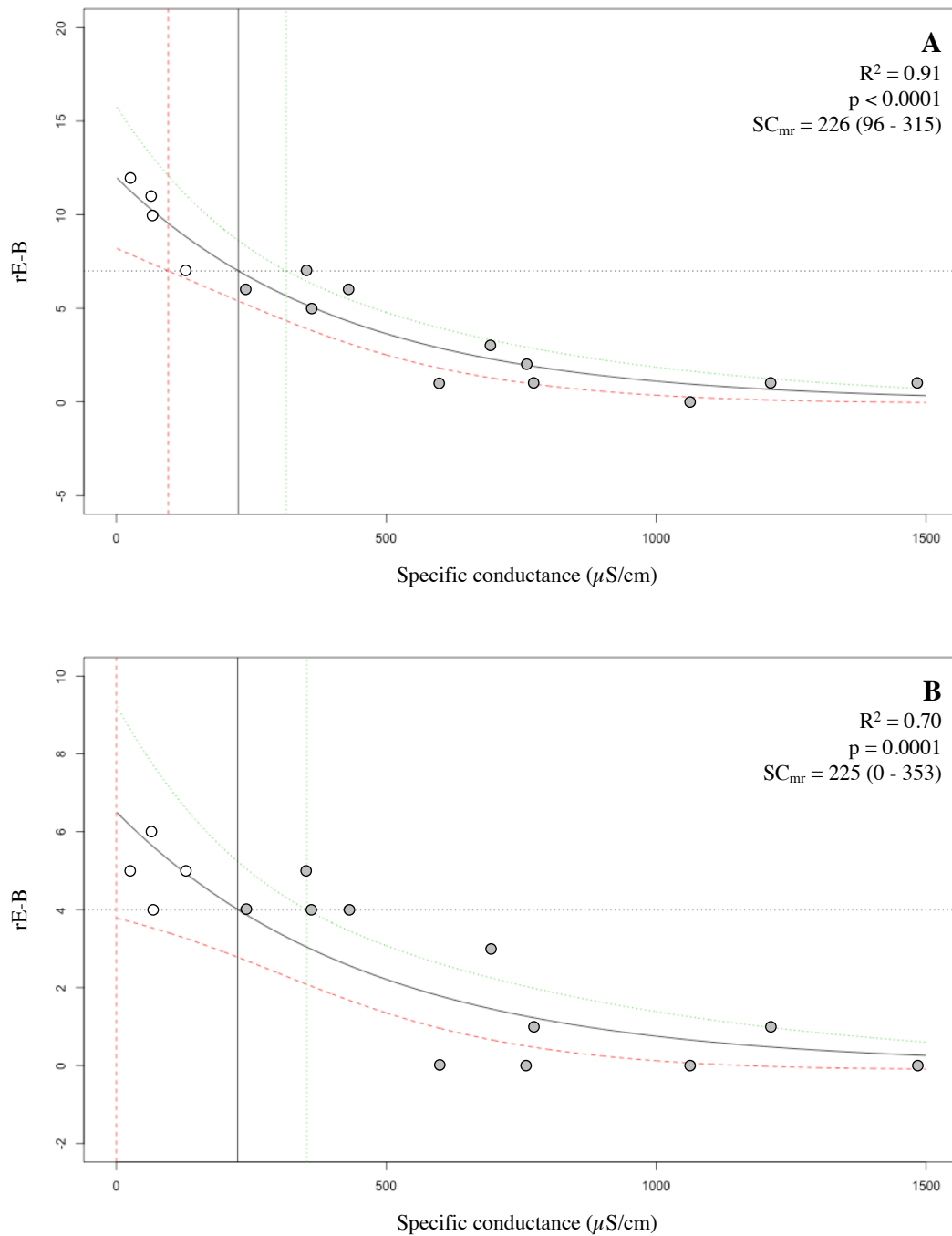


Figure A-9. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera taxa richness less Baetidae ($rE-B$) for spring 2014 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

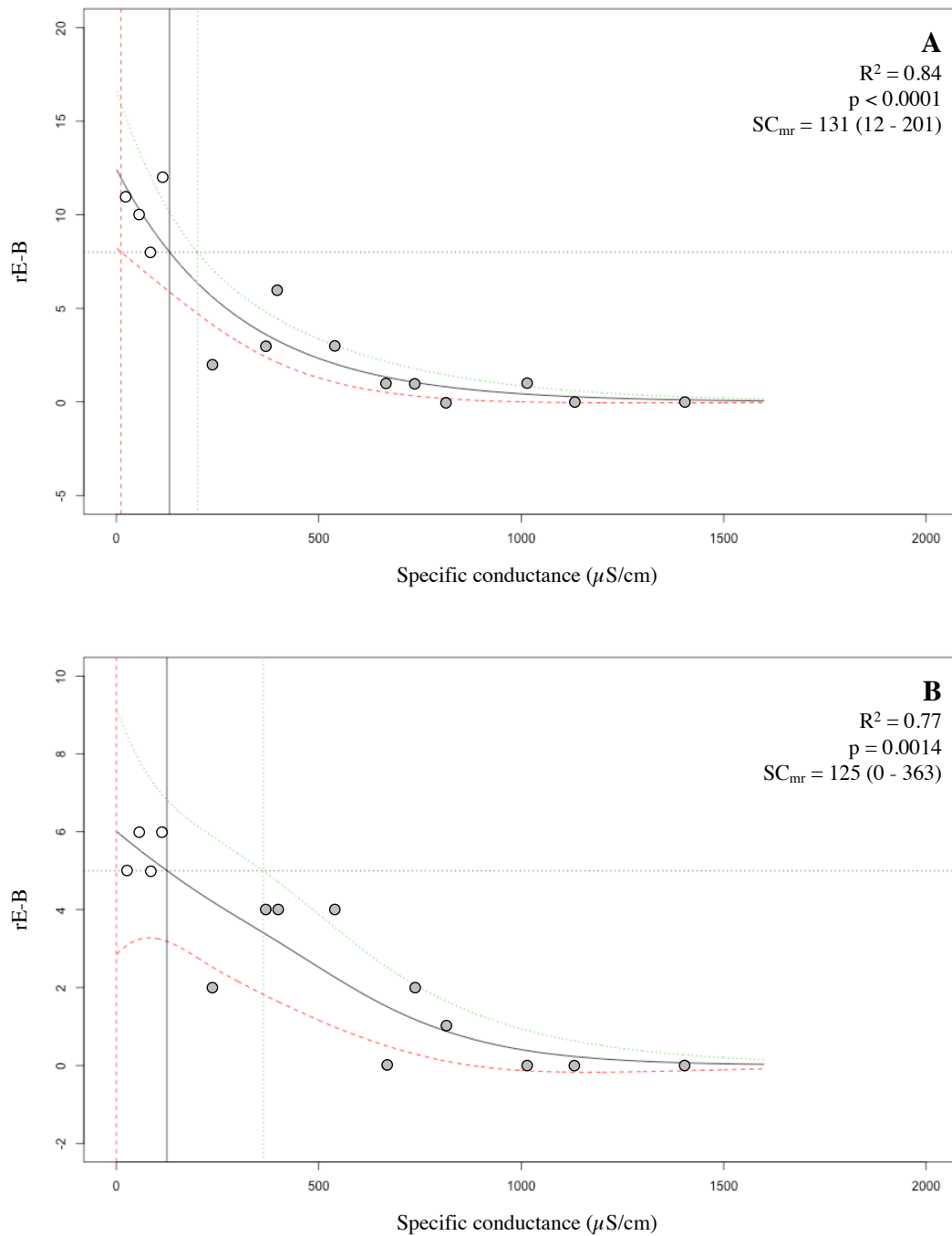


Figure A-10. Generalized additive models (GAMs) of specific conductance (SC) and Ephemeroptera taxa richness less Baetidae ($rE-B$) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

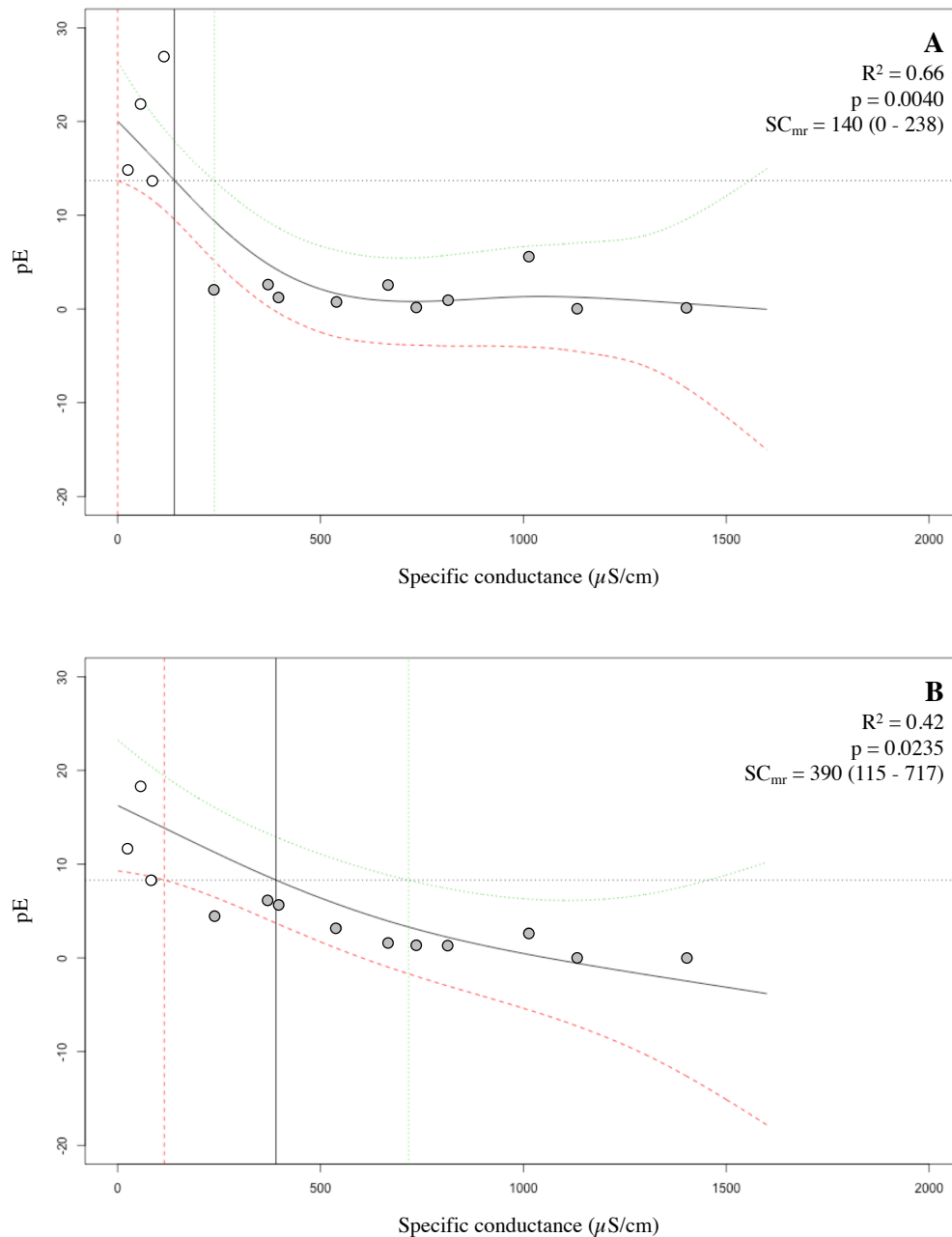


Figure A-11. Generalized additive models (GAMs) of specific conductance (SC) and percent Ephemeroptera (pE) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line. In the semi-quantitative model (**B**), one of the reference stream values (34.7) is outside the range of the y-axis.

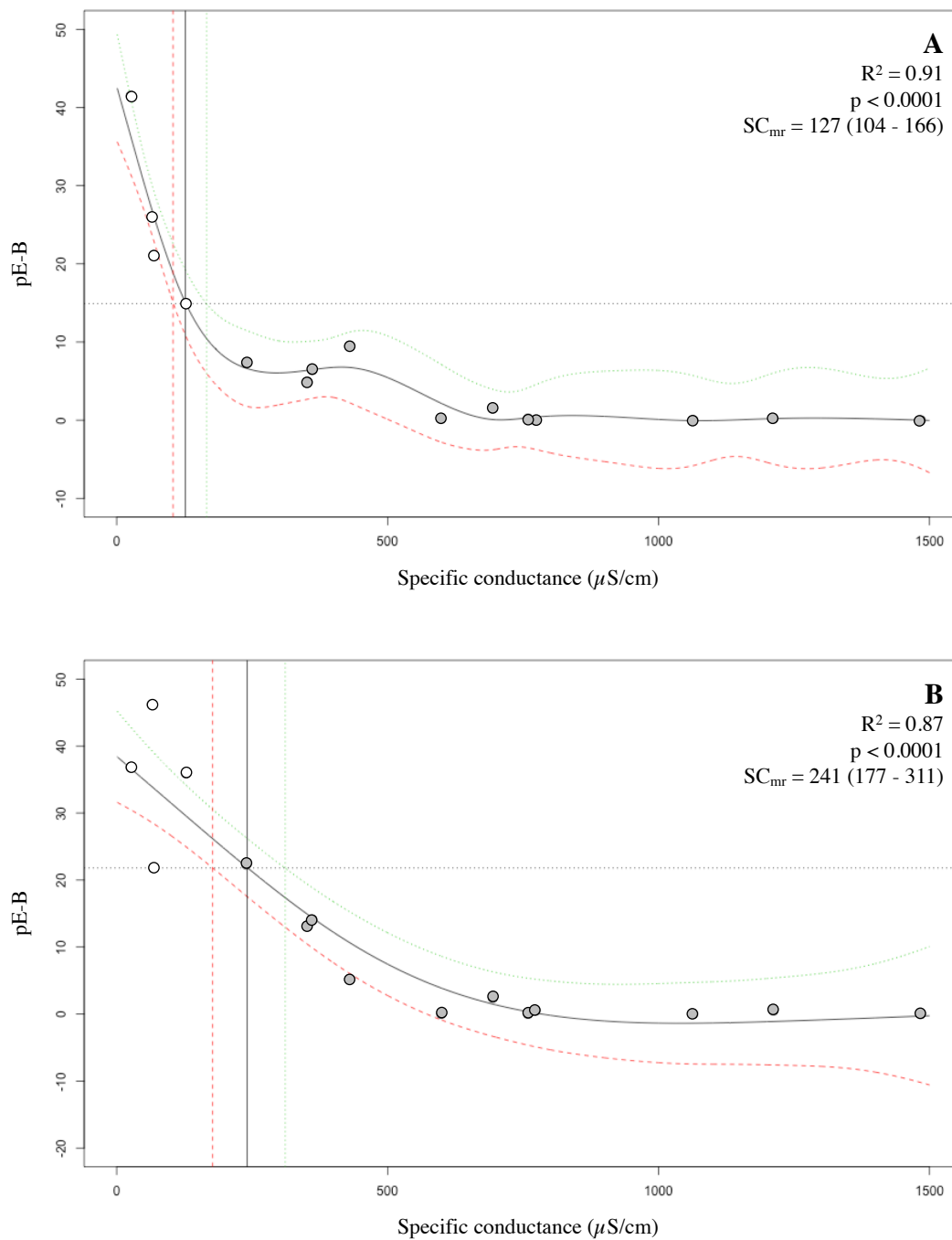


Figure A-12. Generalized additive models (GAMs) of specific conductance (SC) and percent Ephemeroptera less Baetidae (*pE-B*) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value (RefVal_{min}). The intersection of the (RefVal_{min}) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

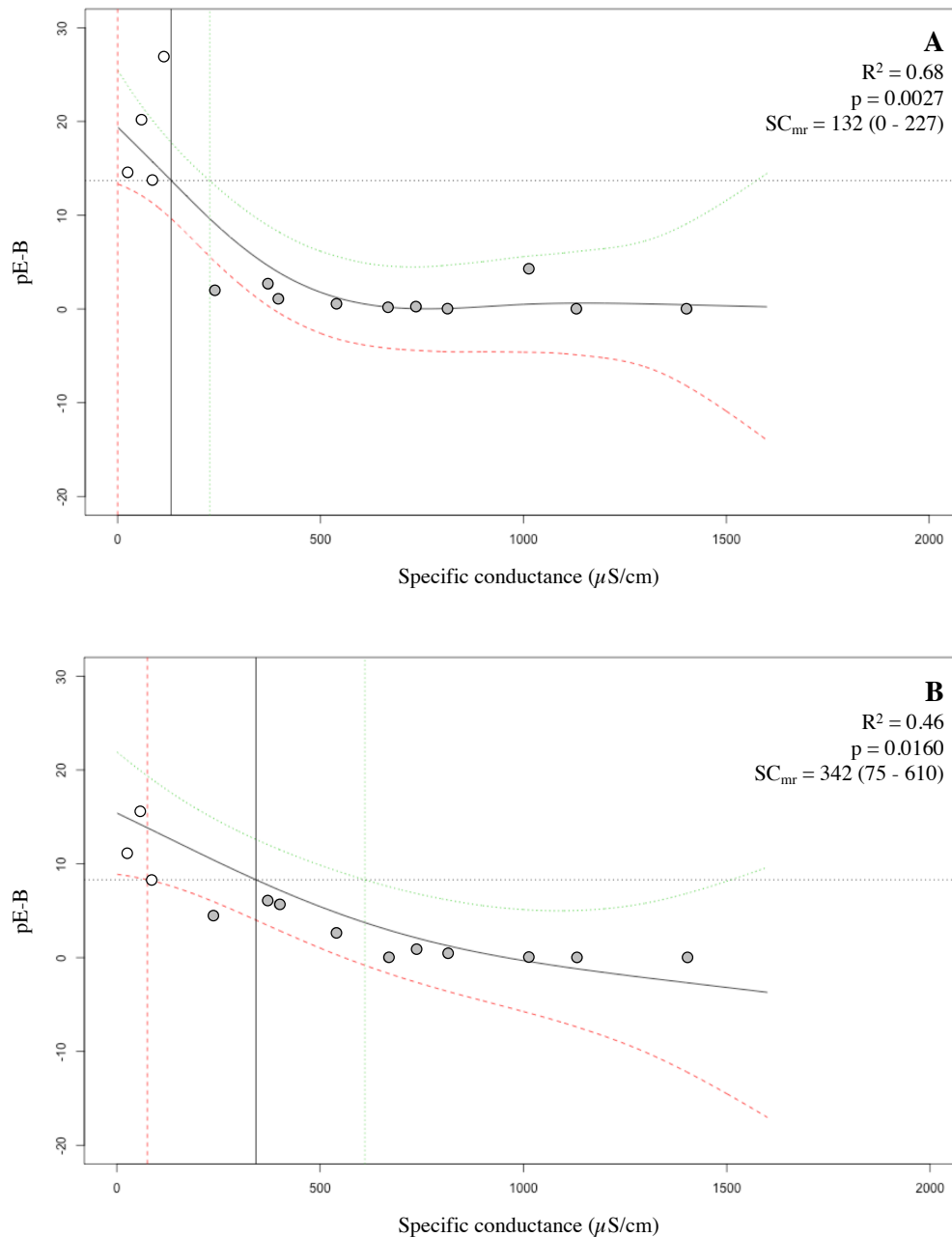


Figure A-13. Generalized additive models (GAMs) of specific conductance (SC) and percent Ephemeroptera less Baetidae ($pE-B$) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line. In the semi-quantitative model (B), one of the reference stream values (32.6) is outside the range of the y-axis.

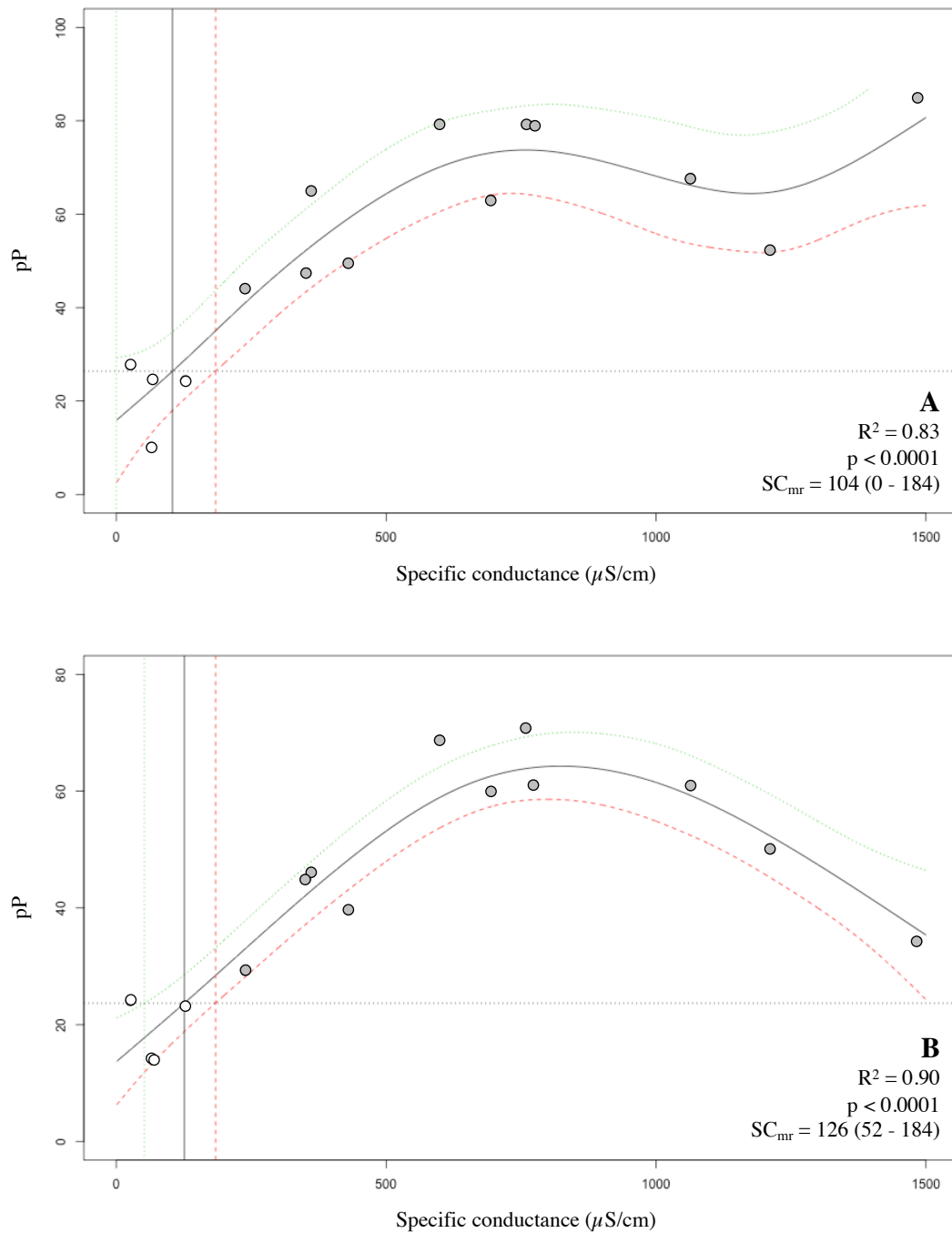


Figure A-14. Generalized additive models (GAMs) of specific conductance (SC) and percent Plecoptera (pP) for spring 2014 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the highest-reference value ($RefVal_{max}$). The intersection of the ($RefVal_{max}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

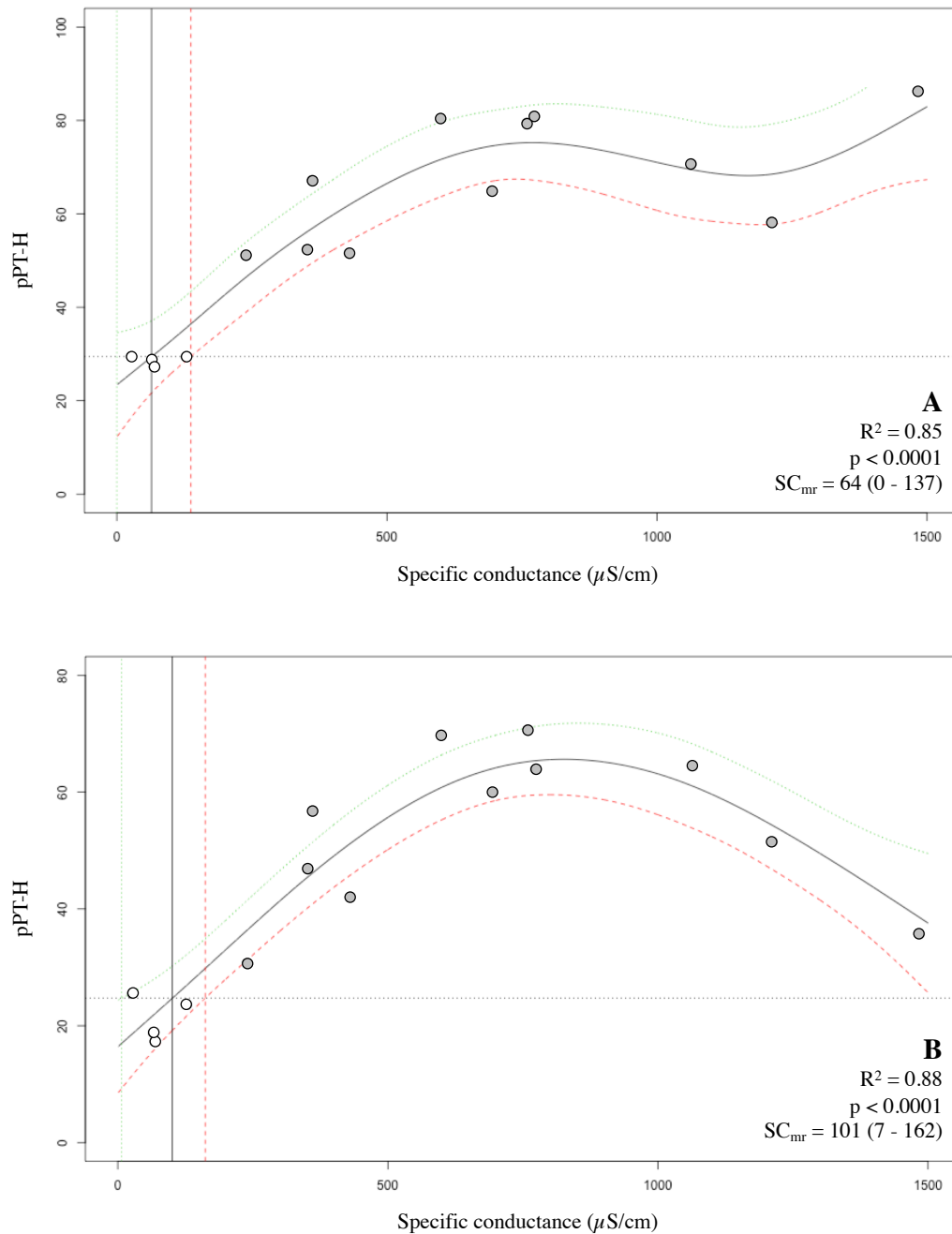


Figure A-15. Generalized additive models (GAMs) of specific conductance (SC) and percent Plecoptera plus Trichoptera less Hydropsychidae (*pPT-H*) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the highest-reference value ($RefVal_{max}$). The intersection of the ($RefVal_{max}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

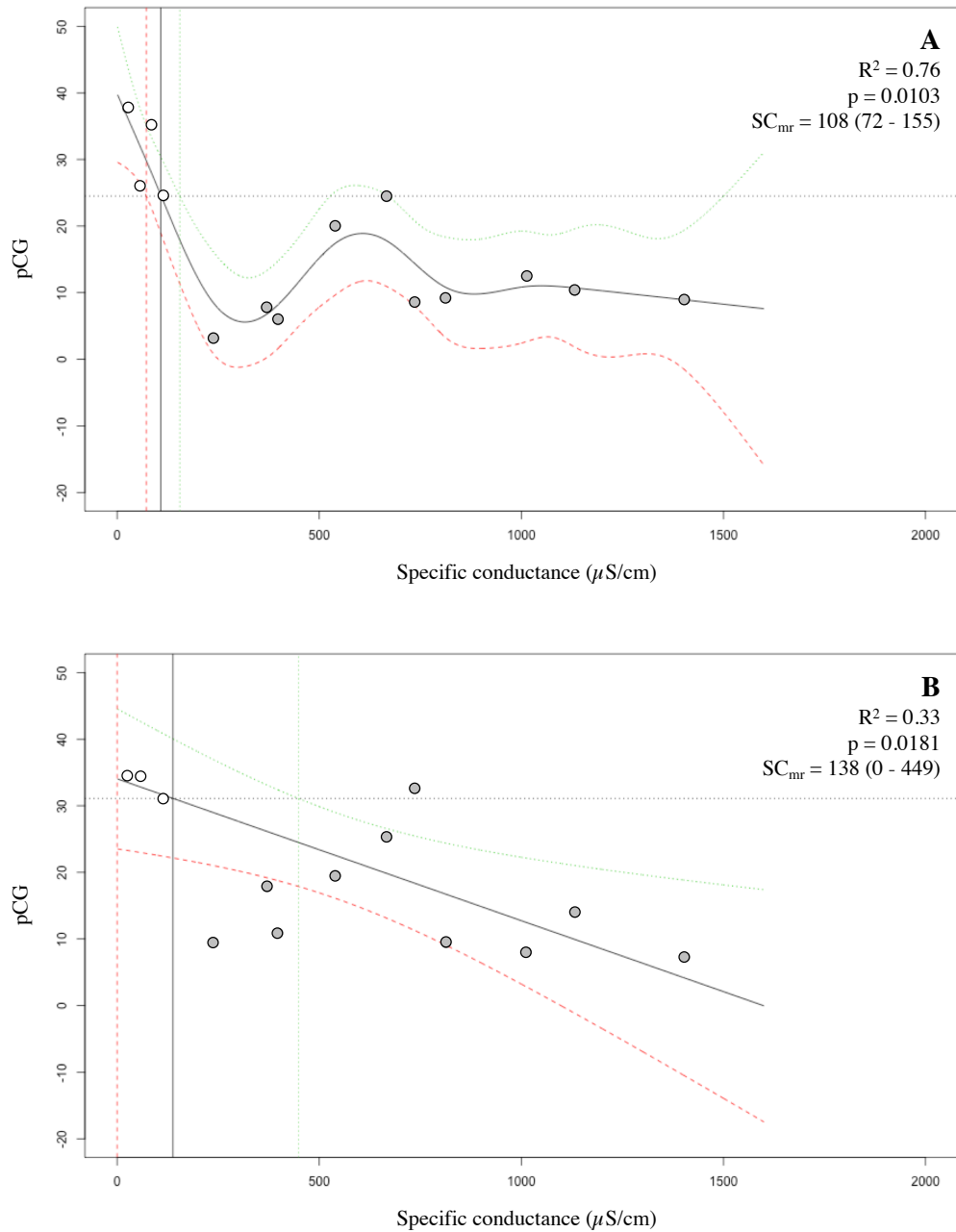


Figure A-16. Generalized additive models (GAMs) of specific conductance (SC) and percent collector-filterers (*pCG*) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line. In the semi-quantitative model (**B**), one of the reference stream values (60.6) is outside the range of the y-axis.

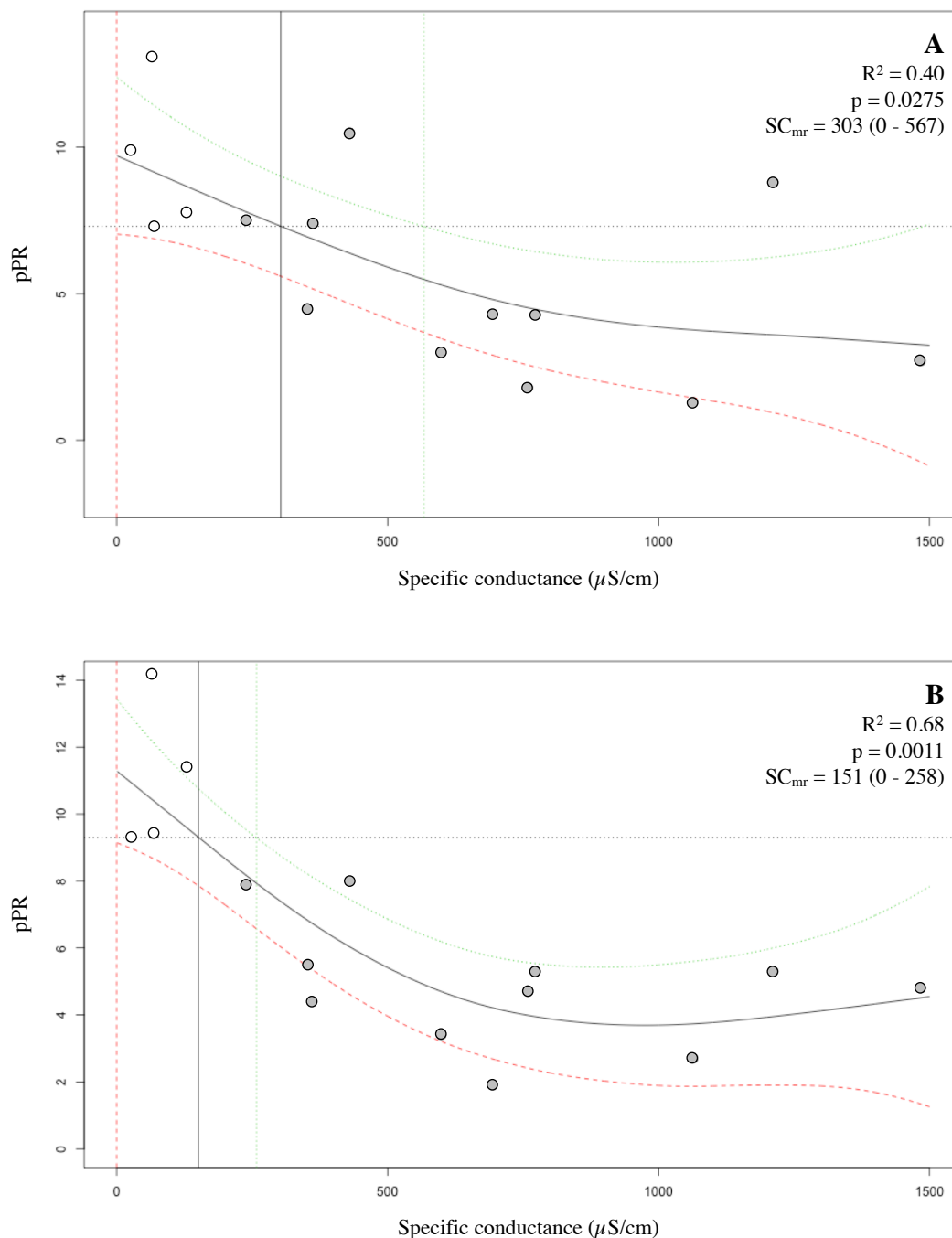


Figure A-17. Generalized additive models (GAMs) of specific conductance (SC) and percent predators (pPR) for spring 2014 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value (RefVal_{\min}). The intersection of the (RefVal_{\min}) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

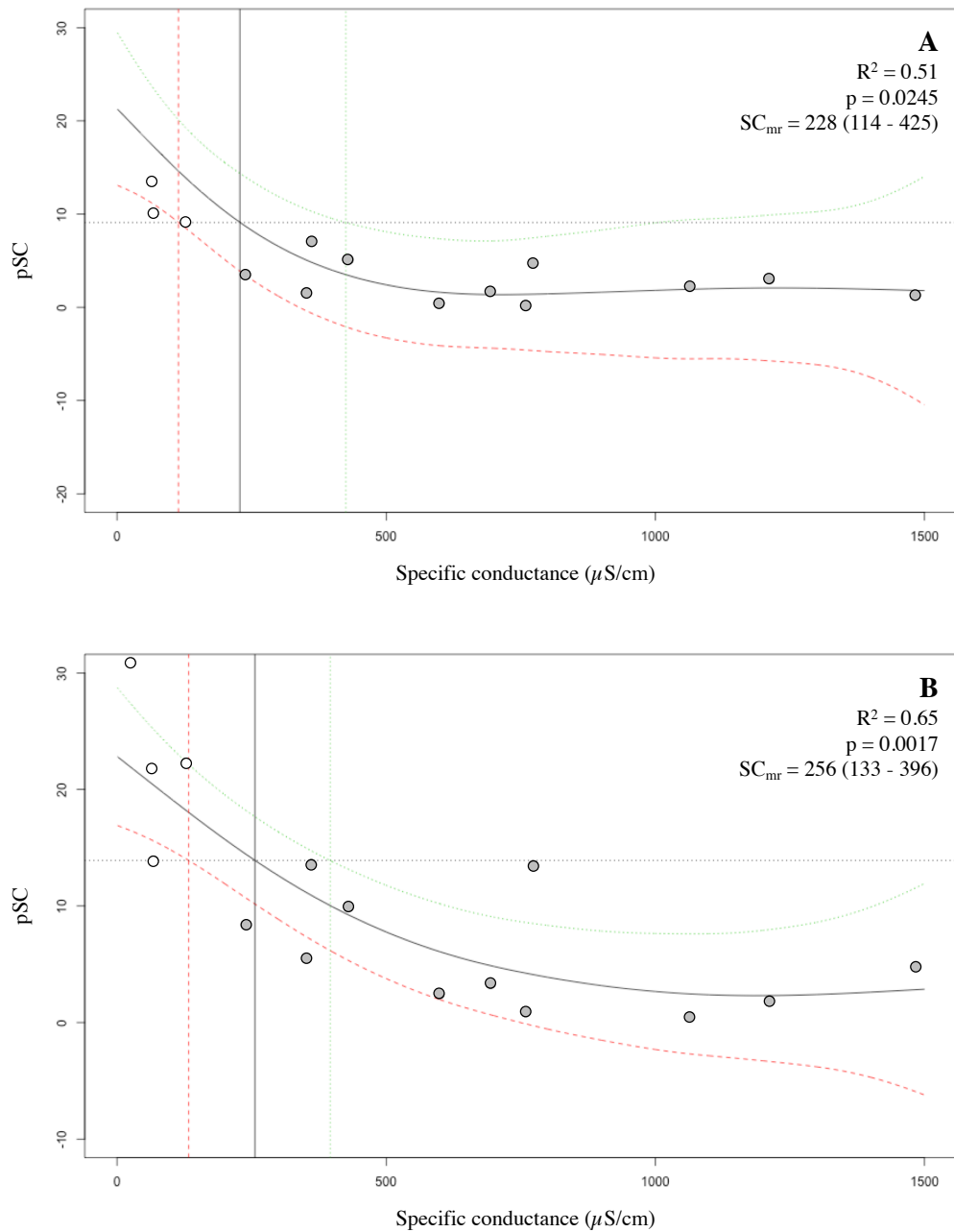


Figure A-18. Generalized additive models (GAMs) of specific conductance (SC) and percent scrapers (pSC) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($\text{RefVal}_{\text{min}}$). The intersection of the ($\text{RefVal}_{\text{min}}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

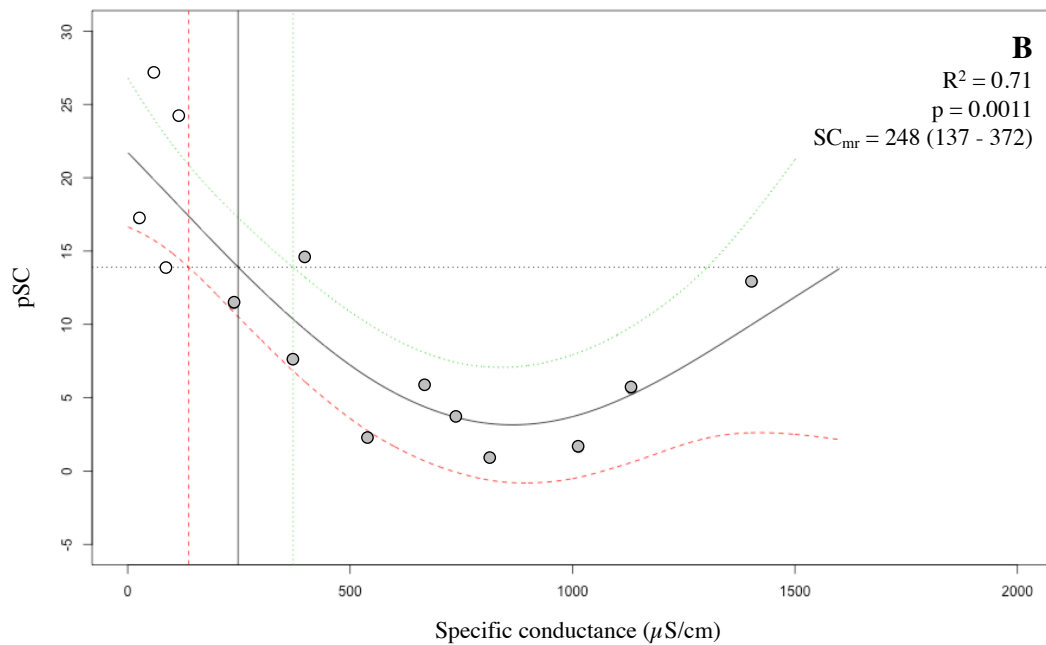
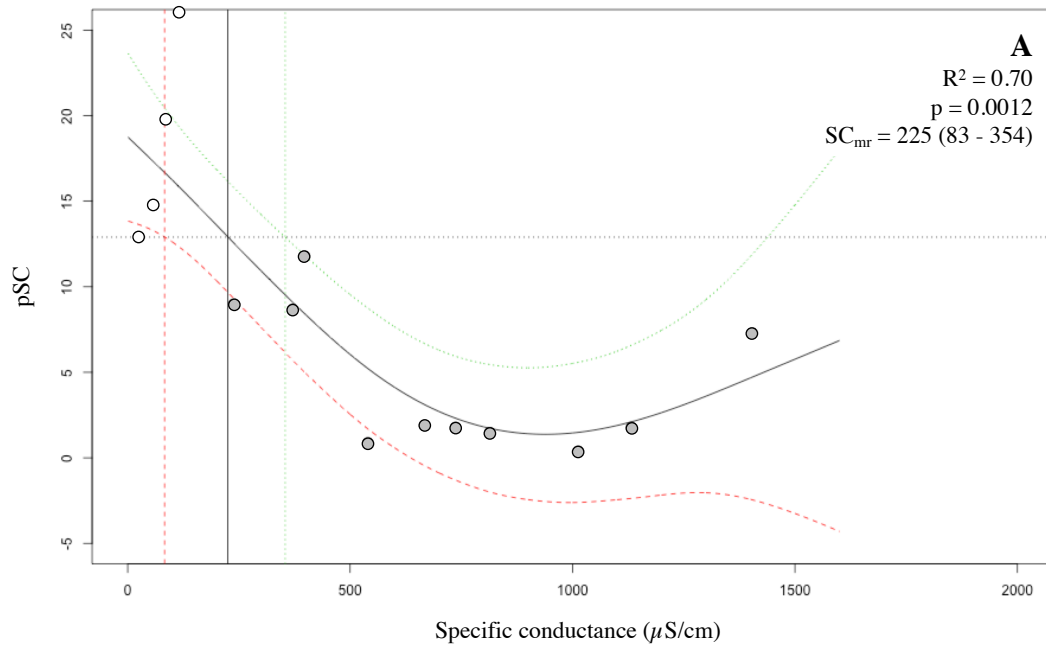


Figure A-19. Generalized additive models (GAMs) of specific conductance (SC) and percent scrapers (*pSC*) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

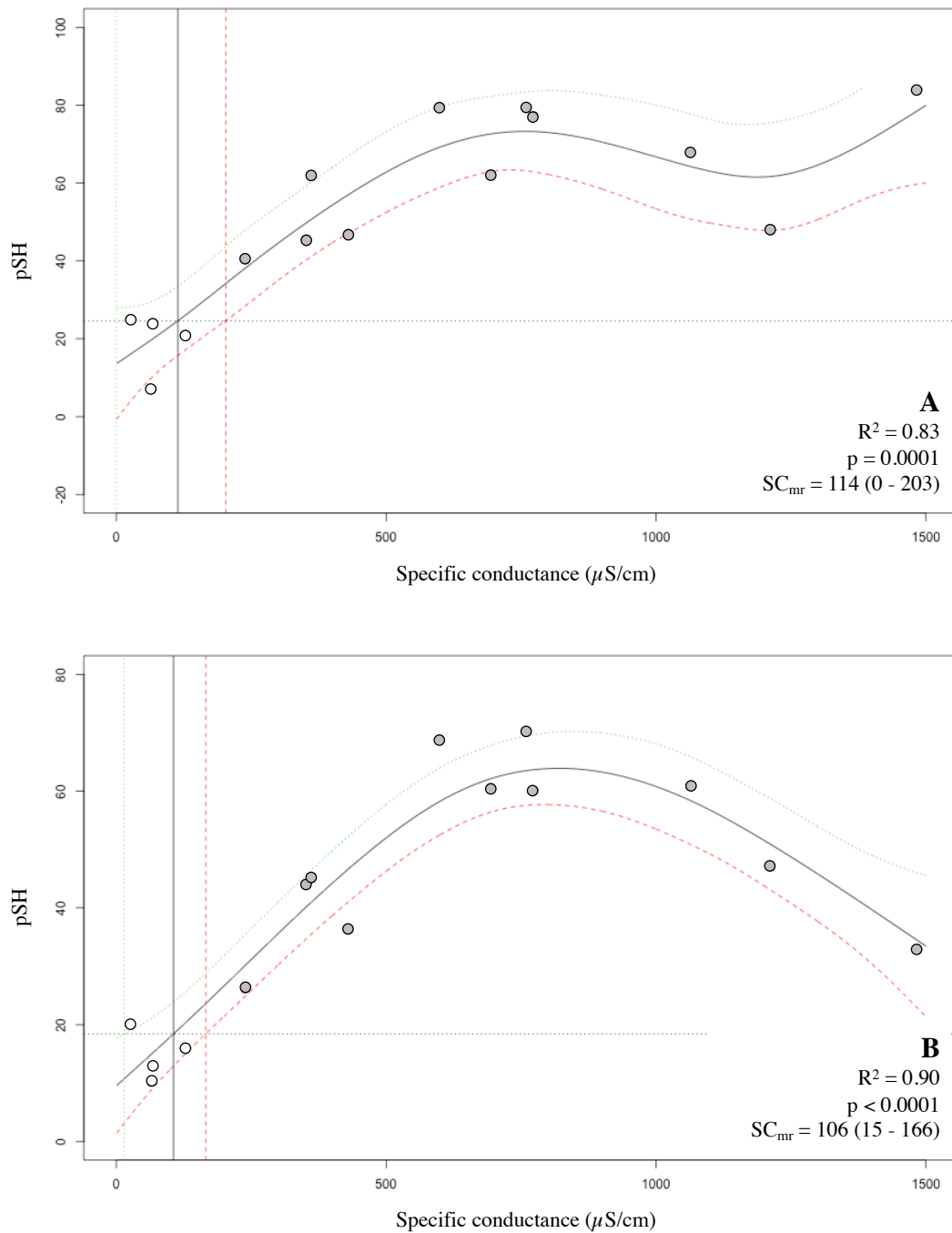


Figure A-20. Generalized additive models (GAMs) of specific conductance (SC) and percent shredders (pSH) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the highest-reference value ($RefVal_{max}$). The intersection of the ($RefVal_{max}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

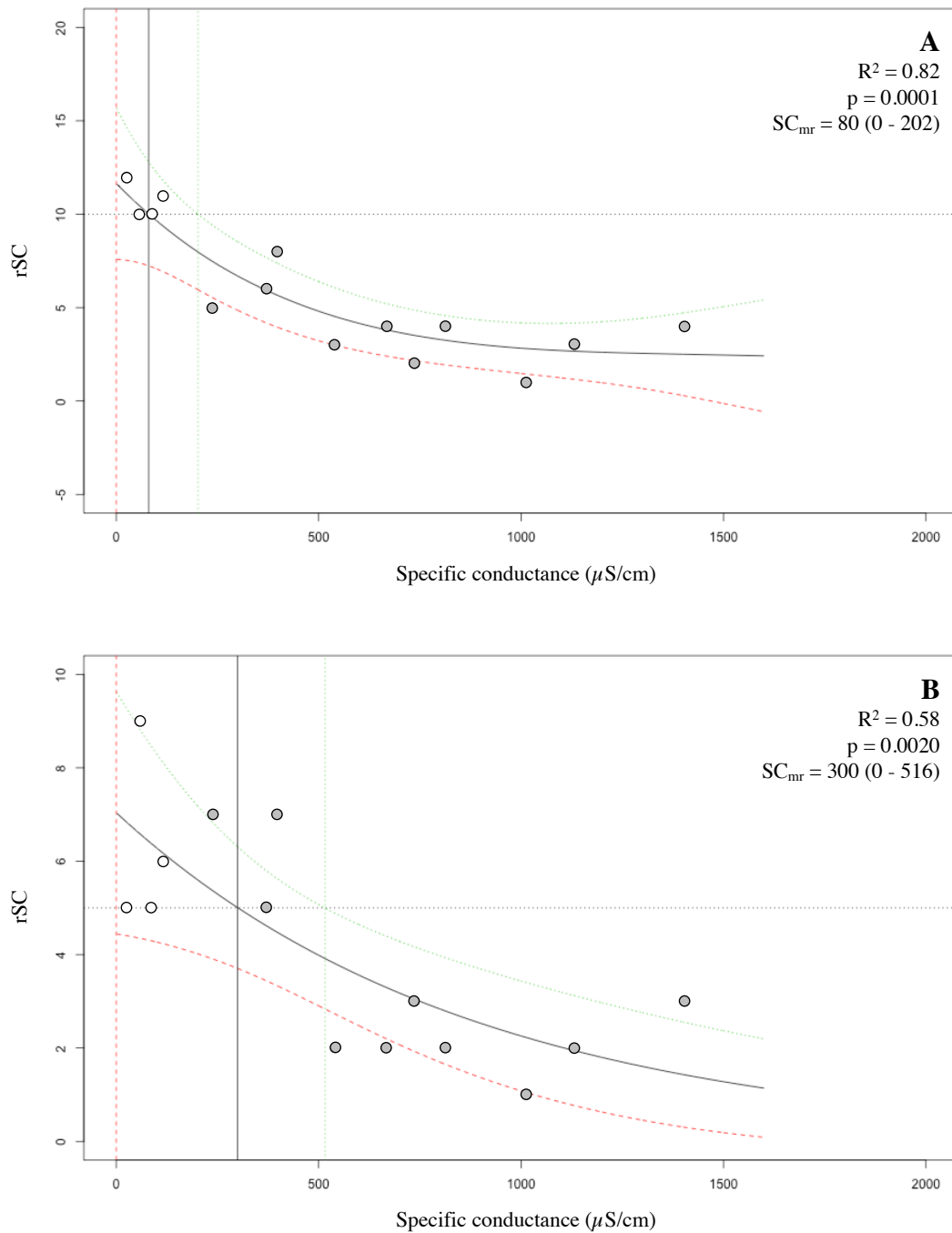


Figure A-21. Generalized additive models (GAMs) of specific conductance (SC) and scraper richness (rSC) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

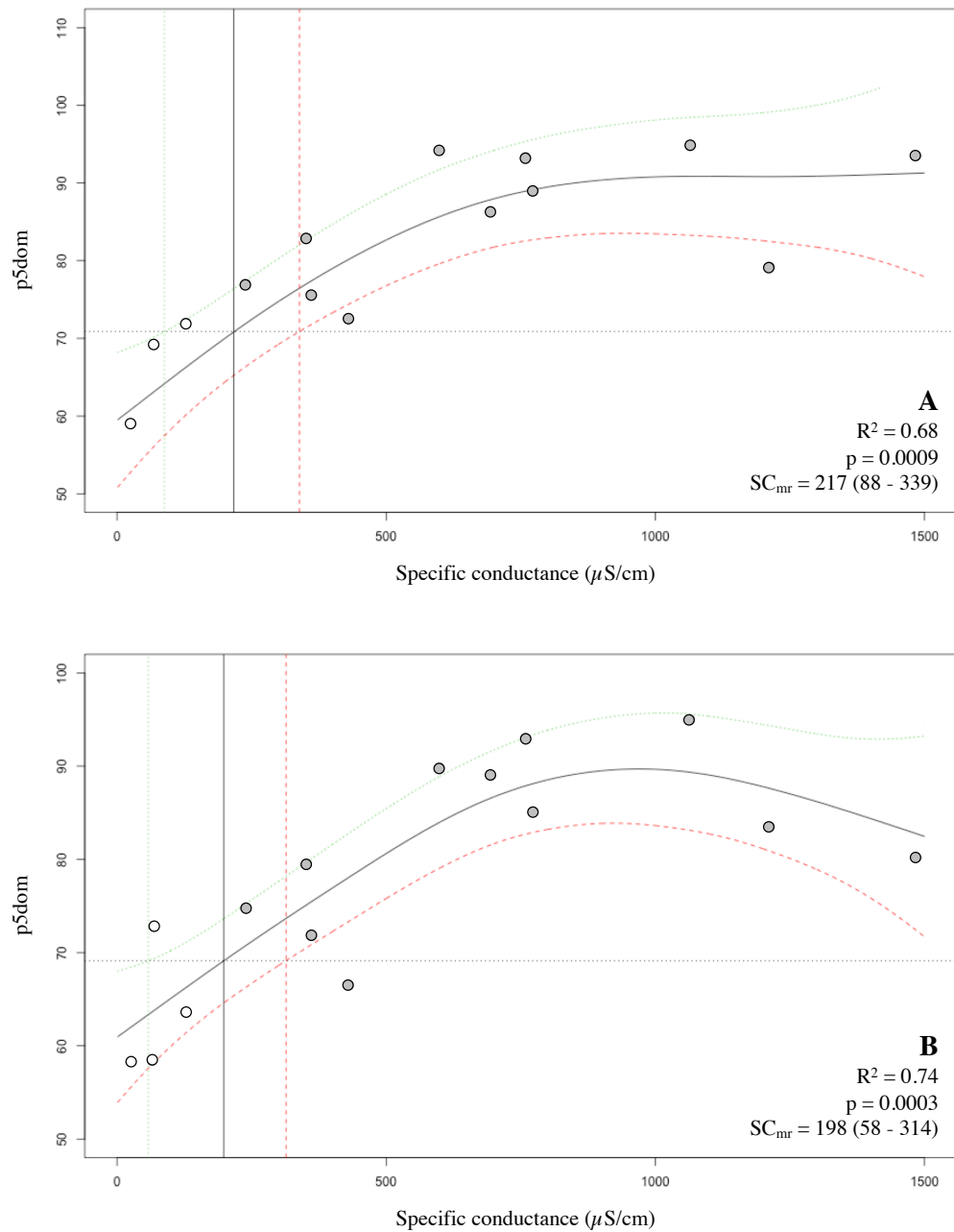


Figure A-22. Generalized additive models (GAMs) of specific conductance (SC) and percent 5 dominant taxa ($p5dom$) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the highest-reference value ($RefVal_{max}$). The intersection of the ($RefVal_{max}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line. In the quantitative model (**A**), one of the reference stream values (45.7) is outside the range of the y-axis.

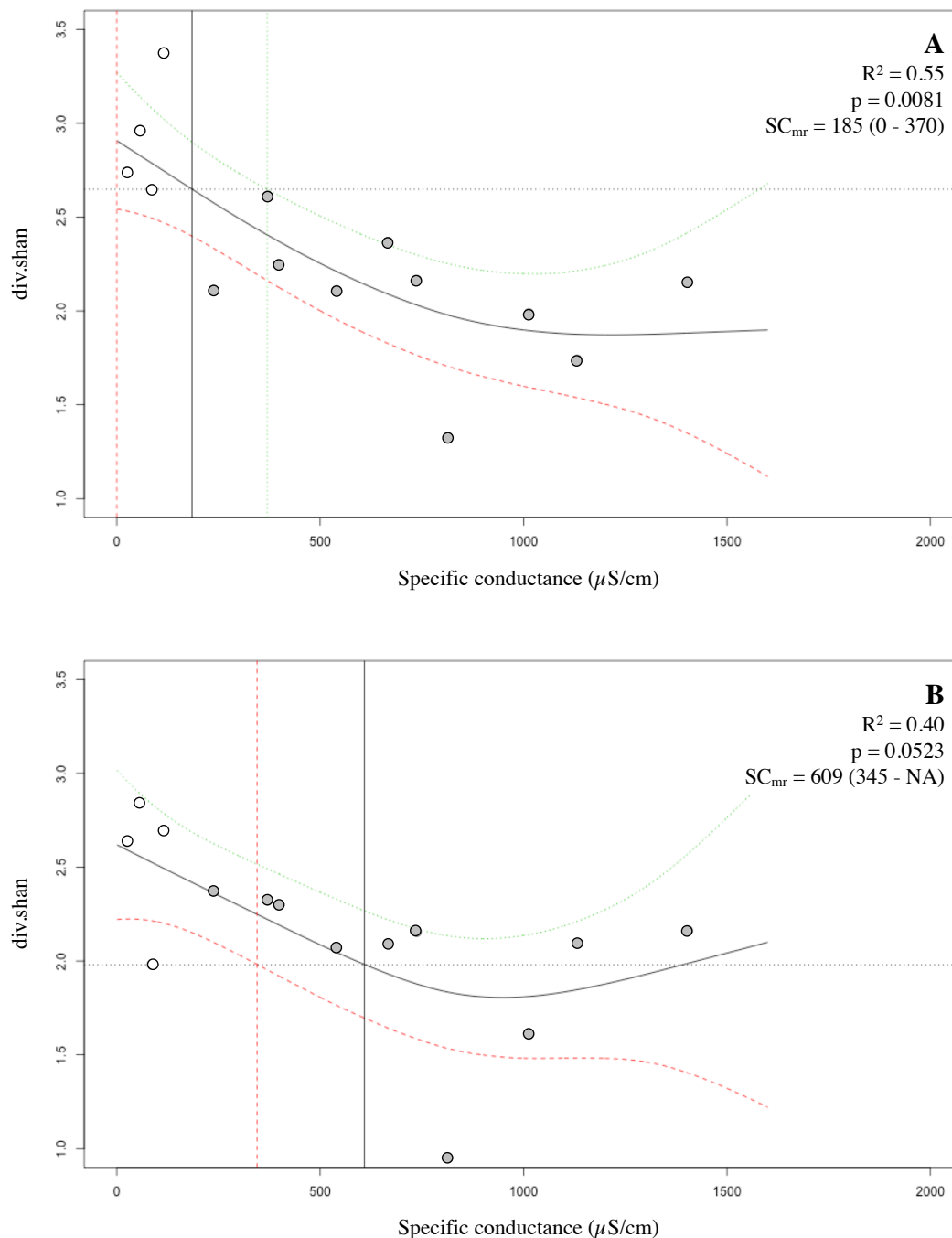


Figure A-23. Generalized additive models (GAMs) of specific conductance (SC) and Shannon diversity (*div.shan*) for fall 2017 quantitative samples (A) and semi-quantitative samples (B). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value (RefVal_{\min}). The intersection of the (RefVal_{\min}) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

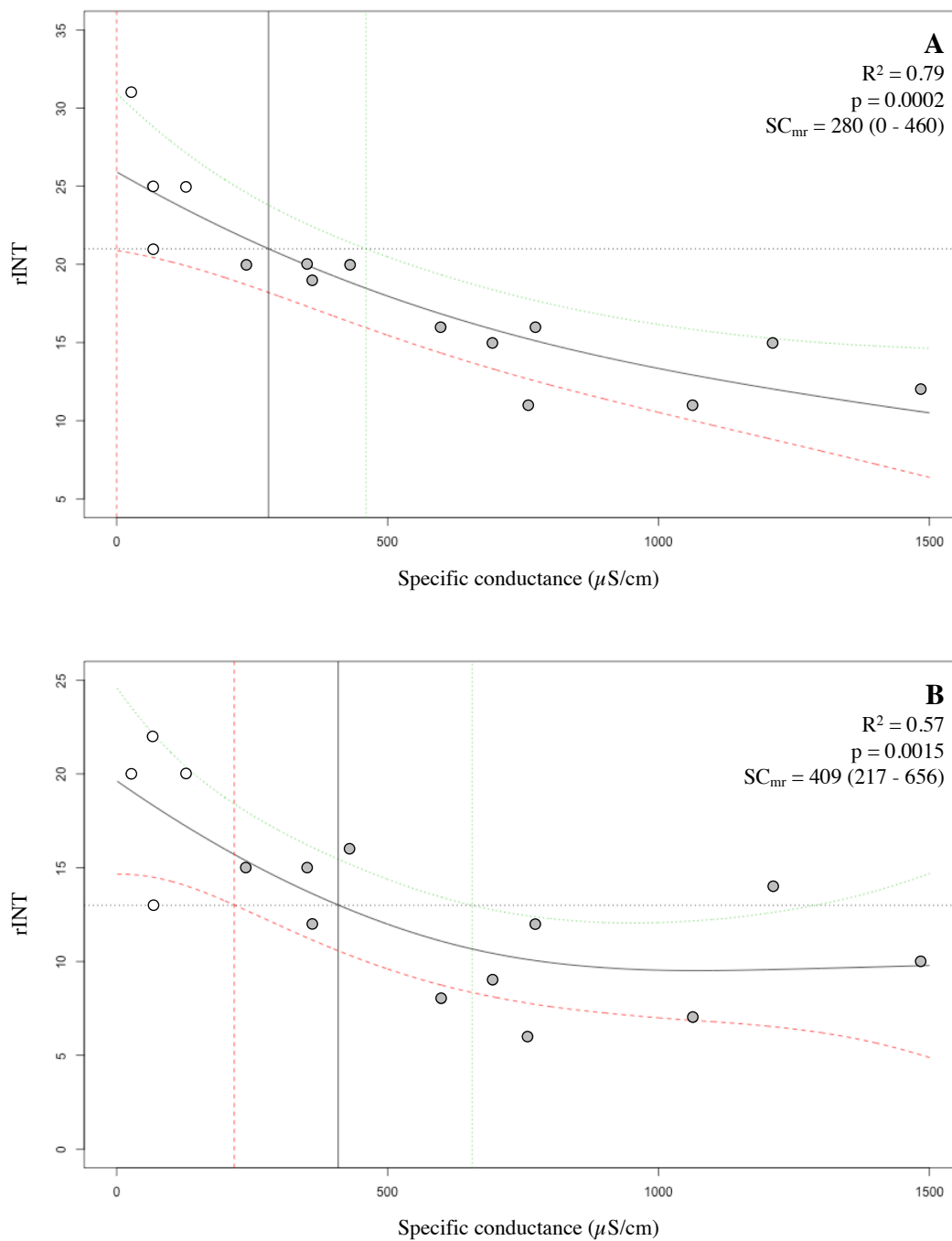


Figure A-24. Generalized additive models (GAMs) of specific conductance (SC) and intolerant taxa richness ($rINT$) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the lowest-reference value ($RefVal_{min}$). The intersection of the ($RefVal_{min}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

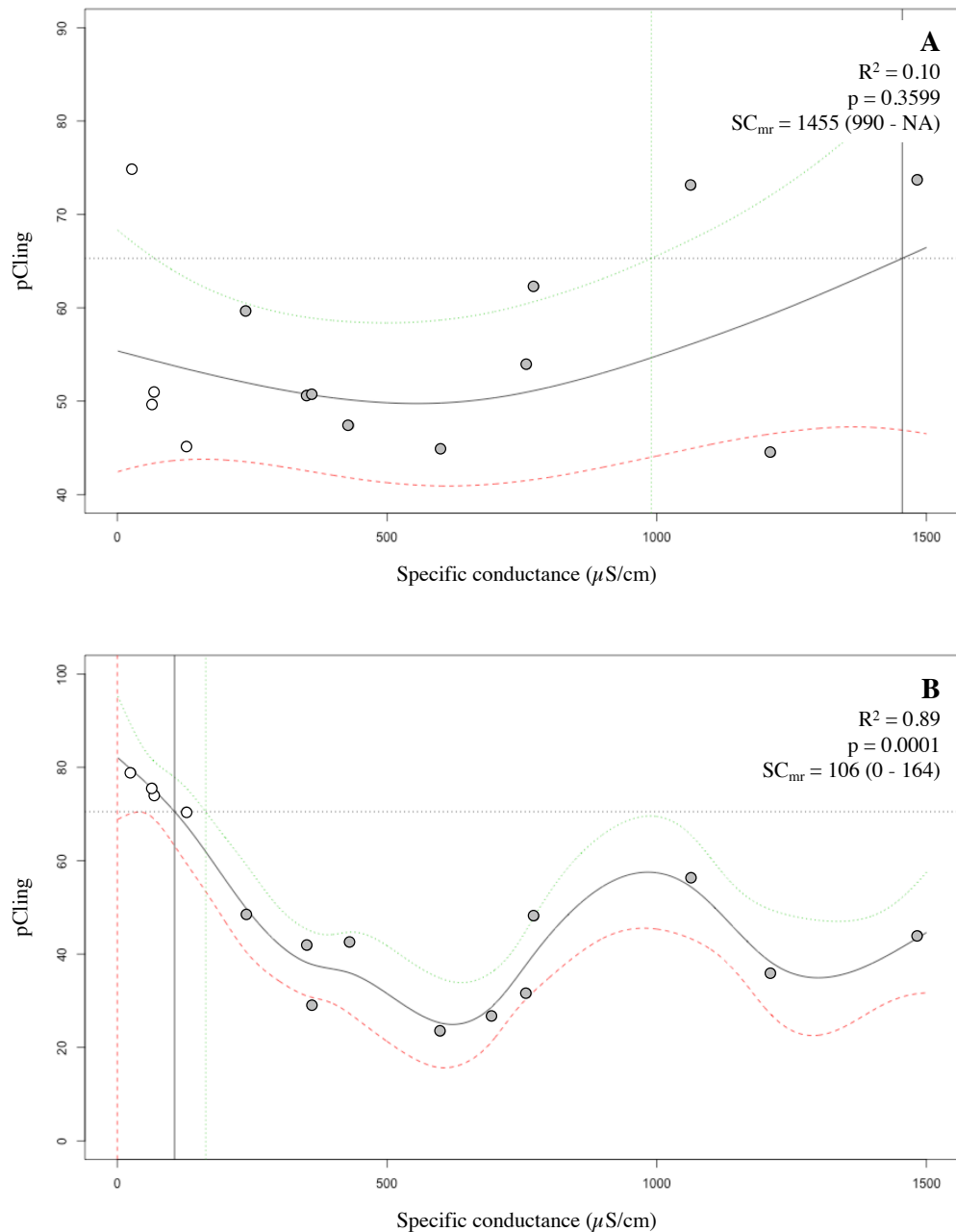


Figure A-25. Generalized additive models (GAMs) of specific conductance (SC) and percent clingers (*pCling*) for spring 2014 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the highest-reference value ($RefVal_{max}$) in (**A**) and the lowest-reference value ($RefVal_{min}$) in (**B**). The intersection of the dotted horizontal line with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line. In the quantitative model (**A**), one of the test stream values (22.7) is outside the range of the y-axis.

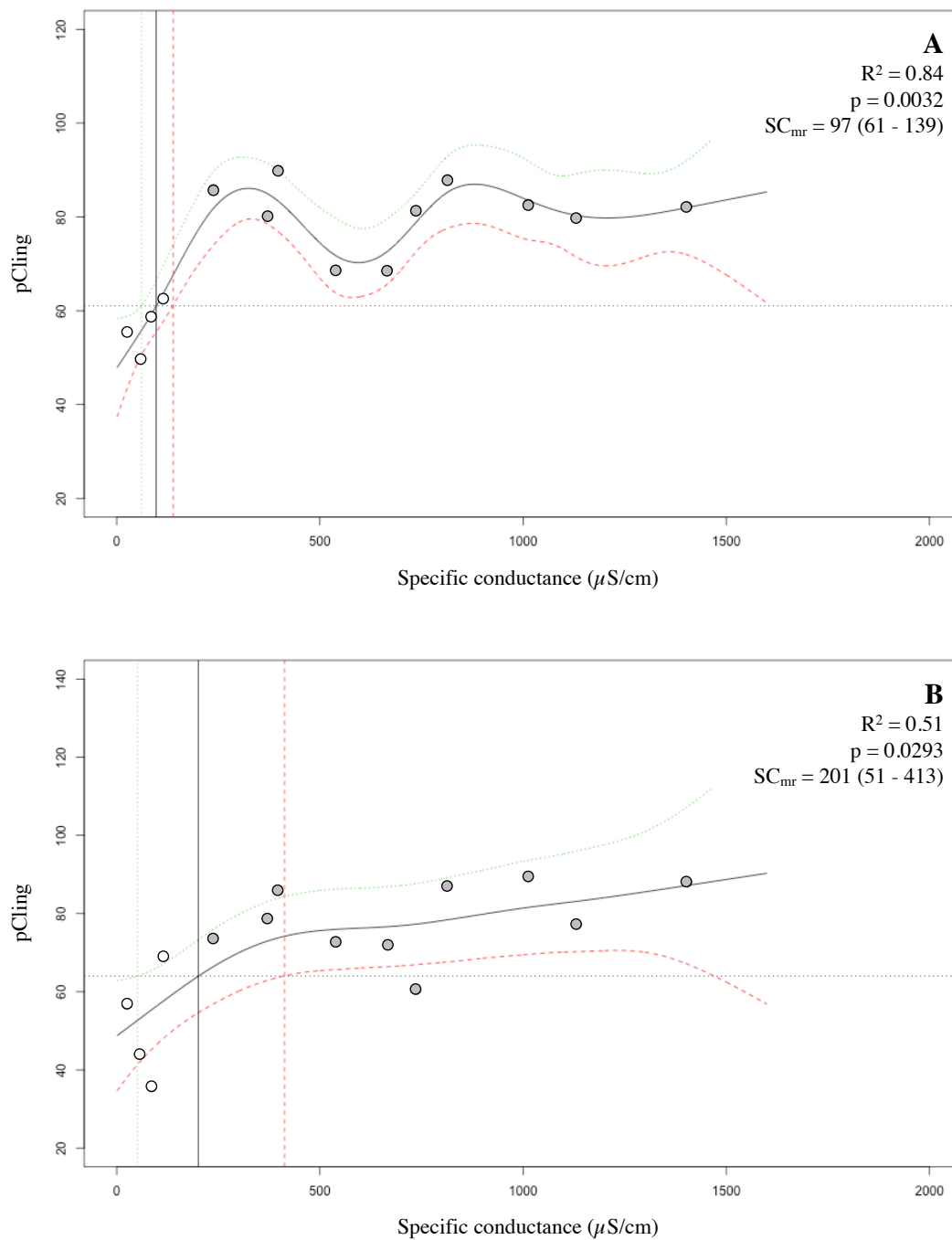


Figure A-26. Generalized additive models (GAMs) of specific conductance (SC) and percent clingers (*pCling*) for fall 2017 quantitative samples (**A**) and semi-quantitative samples (**B**). Metric values (open circles for reference streams and closed circles for test streams), smoothed GAM fit (solid line), and 95% confidence limits (dashed lines) are represented. Dotted horizontal line represents the highest-reference value ($RefVal_{max}$). The intersection of the ($RefVal_{max}$) with the smoothed GAM fit determines the model-reference SC value (SC_{mr}) on the x-axis, and is represented on the figure by the vertical solid line.

Table A-10. Benthic macroinvertebrate taxa counts by stream for spring 2014 semi-quantitative samples.

Taxon/Stream	COP	CRO	EAS	FRY	GRA	HCN	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
<i>Acentrella</i>	0	2	0	17	35	4	0	0	0	0	0	31	1	3	23
<i>Acerpenna</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acroneuria</i>	4	1	5	0	2	0	0	0	4	0	0	2	2	0	0
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agapetus</i>	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Allocapnia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alloperla</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ameletus</i>	4	7	0	2	0	0	0	0	0	0	1	0	0	0	0
<i>Amphinemura</i>	18	11	15	89	44	22	123	42	92	133	87	67	59	124	65
<i>Antocha</i>	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0
<i>Apatania</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atrichopogon</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i>	9	1	0	6	0	0	15	1	0	0	2	7	0	5	2
<i>Baetisca</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	2	1	1	0	0	0	0	0	1	0	0	1	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratopsyche</i>	2	2	5	0	1	0	0	0	21	0	0	12	7	3	0
<i>Cernotina</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chelifera</i>	0	0	1	2	0	9	0	6	1	6	6	3	3	0	1
<i>Cheumatopsyche</i>	0	0	0	0	1	0	2	0	10	0	0	8	28	1	1
<i>Chimarra</i>	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	29	16	21	0	24	9	0	46	34	10	6	13	49	18	36
<i>Cinygmula</i>	1	2	7	0	1	0	0	0	0	0	0	0	0	0	1
<i>Clinocera</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyrnellus</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Dasyhelea</i>	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0

Taxon/Stream	COP	CRO	EAS	FRY	GRA	HCN	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
<i>Dibusa</i>	0	0	0	0	0	2	0	0	1	0	0	0	0	0	0
<i>Dicranota</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dipheter</i>	0	3	0	15	0	0	0	0	0	0	0	0	0	0	0
<i>Diplectrona</i>	12	16	21	0	21	0	12	23	15	5	23	18	10	12	10
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0
<i>Dolophilodes</i>	0	0	0	9	0	0	0	0	1	0	0	0	0	0	0
<i>Drunella</i>	3	10	6	0	7	9	0	0	0	0	0	5	0	3	2
<i>Eccoptura</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Ectopria</i>	9	6	3	4	2	7	2	0	1	5	1	2	1	1	2
<i>Epeorus</i>	17	18	34	0	10	1	0	0	0	0	0	2	0	1	5
<i>Ephemera</i>	0	0	0	7	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemerella</i>	54	50	25	0	27	32	0	0	1	0	0	3	0	1	20
<i>Eurylophella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Forcipomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haploperla</i>	6	3	0	0	4	0	0	0	0	0	0	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hemerodromia</i>	0	0	0	0	0	4	0	0	2	2	2	0	1	0	0
<i>Heptagenia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	1	1	2	0	0	0	0	0	1	0	1	0	2	0	0
<i>Hydatophylax</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydropsyche</i>	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Isonychia</i>	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
<i>Isoperla</i>	1	4	0	1	0	0	1	0	1	0	0	5	0	0	2
<i>Lanthus</i>	2	0	0	0	1	0	9	0	0	2	1	0	0	0	1
<i>Lepidostoma</i>	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leucrocuta</i>	0	0	0	17	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	COP	CRO	EAS	FRY	GRA	HCN	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
<i>Leuctra</i>	13	2	13	0	8	1	28	92	4	7	35	8	9	0	31
<i>Limnophila</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Maccaffertium</i>	0	0	0	0	0	2	0	0	0	0	0	1	0	0	1
<i>Malirekus</i>	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Molophilus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neophylax</i>	0	0	1	0	1	1	0	0	0	0	1	1	0	0	0
<i>Neoplasta</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nigronia</i>	0	0	0	0	0	0	0	0	0	1	0	1	1	0	3
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oemopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oligochaeta</i>	0	1	0	0	0	1	0	1	0	2	1	0	1	0	0
<i>Optioservus</i>	0	0	0	0	1	2	0	0	1	0	0	4	4	5	0
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oulimnius</i>	22	15	18	5	2	13	0	0	1	0	26	8	5	0	2
<i>Palpomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i>	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Peltoperla</i>	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Perlesta</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i>	0	1	0	0	0	0	0	0	0	0	1	0	0	0	2
<i>Prinocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	2	5	0	2	71	0	0	0	0	0	0	0	0	1
<i>Prostoia</i>	0	0	0	0	0	0	0	0	1	1	0	0	0	2	0

Taxon/Stream	COP	CRO	EAS	FRY	GRA	HCN	KEL	KUT	LLC	MIL	POW	RFF	RIC	ROC	SPC
<i>Psephenus</i>	0	0	0	2	0	0	0	0	0	0	0	3	0	0	1
<i>Pseudostenophylax</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pteronarcys</i>	0	6	5	4	0	3	0	0	0	0	0	2	0	0	0
<i>Pycnopsyche</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Remenus</i>	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
<i>Rhyacophila</i>	1	5	1	3	2	4	0	6	1	2	3	3	3	0	2
<i>Serratella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
<i>Simulium</i>	1	2	0	1	4	0	22	1	10	28	4	1	20	28	5
<i>Stactobiella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenacron</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenelmis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenonema</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stratiomys</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Suwallia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sweltsa</i>	2	0	1	0	0	1	0	0	1	0	0	0	1	0	0
<i>Tabanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taenionema</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tallaperla</i>	2	0	7	0	1	0	0	0	0	0	2	0	0	0	1
<i>Tipula</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
<i>Wormaldia</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Yugus</i>	3	1	0	0	0	0	0	0	0	0	3	0	0	0	0

Table A-11. Benthic macroinvertebrate taxa counts by stream for spring 2014 quantitative samples. The replicate number for individual quantitative samples is in parentheses.

Taxon/Stream	COP (1)	COP (2)	COP (3)	CRO (1)	CRO (2)	CRO (3)	EAS (1)	EAS (2)	EAS (3)	FRY (1)	FRY (2)	FRY (3)
<i>Acentrella</i>	4	4	2	23	66	40	0	0	0	52	42	41
<i>Acerpenna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acroneuria</i>	0	0	0	3	12	15	1	1	12	5	13	6
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agapetus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Allocapnia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alloperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ameletus</i>	0	0	1	1	2	10	1	1	4	0	0	1
<i>Amphinemura</i>	10	31	54	8	22	56	4	17	19	194	236	210
<i>Antocha</i>	0	0	1	0	0	0	0	0	4	9	5	4
<i>Apatania</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atrichopogon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i>	0	4	22	1	57	43	0	2	3	15	11	18
<i>Baetisca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	0	1	12	0	0	4	0	3	0	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>Caenis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratopsyche</i>	0	0	0	0	0	0	0	3	0	13	13	3
<i>Cernotina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chelifera</i>	0	0	7	1	5	0	0	0	0	4	3	0
<i>Cheumatopsyche</i>	0	0	2	0	0	4	0	0	1	2	1	1
<i>Chimarra</i>	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	97	107	262	17	124	68	15	46	76	12	19	18
<i>Cinygmula</i>	0	0	0	0	5	3	22	0	44	0	0	0
<i>Clinocera</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>Cordulegaster</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	COP (1)	COP (2)	COP (3)	CRO (1)	CRO (2)	CRO (3)	EAS (1)	EAS (2)	EAS (3)	FRY (1)	FRY (2)	FRY (3)
<i>Cynellus</i>	10	0	0	24	0	0	1	0	0	0	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dibusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dicranota</i>	0	0	2	0	0	0	0	0	23	0	0	0
<i>Dipheter</i>	1	1	0	2	57	20	1	0	0	0	0	0
<i>Diplectrona</i>	4	12	16	8	40	41	13	13	0	19	37	26
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dolophilodes</i>	0	1	29	0	161	124	0	0	2	1	4	12
<i>Drunella</i>	1	0	0	5	9	15	10	11	8	10	20	17
<i>Eccopectura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ectopria</i>	4	12	6	1	4	1	2	4	30	2	0	4
<i>Epeorus</i>	21	9	15	11	36	21	92	74	44	9	11	15
<i>Ephemera</i>	0	0	0	0	0	1	0	0	1	0	0	0
<i>Ephemerella</i>	38	25	55	15	41	80	7	29	46	5	9	2
<i>Eurylophella</i>	0	0	0	0	0	3	0	0	0	0	0	0
<i>Forcipomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	0	0	0	15	13	20	1	5	19	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haploperla</i>	4	4	10	0	7	3	0	4	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hemerodromia</i>	0	0	0	0	2	1	0	1	1	0	0	0
<i>Heptagenia</i>	0	0	0	4	40	23	0	1	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	0	0	2	0	0	0	3	0	1	0	0	0
<i>Hydatophylax</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydropsyche</i>	3	16	0	2	7	1	5	0	3	0	5	3
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Isonychia</i>	2	0	3	7	48	51	0	0	0	0	0	0

Taxon/Stream	COP (1)	COP (2)	COP (3)	CRO (1)	CRO (2)	CRO (3)	EAS (1)	EAS (2)	EAS (3)	FRY (1)	FRY (2)	FRY (3)
<i>Isoperla</i>	6	8	10	2	18	7	1	4	4	4	13	8
<i>Lanthus</i>	0	1	3	8	57	43	0	0	1	0	1	0
<i>Lepidostoma</i>	0	0	0	0	0	0	0	2	0	0	0	0
<i>Leucrocuta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leuctra</i>	17	37	80	0	0	0	23	47	79	147	183	147
<i>Limnophila</i>	0	0	1	0	0	0	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Maccaffertium</i>	1	0	0	0	0	0	1	1	0	0	0	0
<i>Malirekus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	3	5	2	1	11	7	0	0	0	1	0	0
<i>Molophilus</i>	0	0	1	0	0	0	0	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neophylax</i>	0	0	0	0	3	0	1	0	2	0	0	0
<i>Neoplasta</i>	0	4	4	0	1	0	0	0	3	0	4	0
<i>Neotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nigronia</i>	0	0	1	0	0	0	0	0	0	0	1	1
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oemopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oligochaeta</i>	0	3	0	1	37	5	0	0	0	0	3	0
<i>Optioservus</i>	2	13	25	2	21	23	11	15	29	15	15	10
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	0	0	0	0	12	0	0	0	0
<i>Oulimnius</i>	0	0	0	0	0	0	0	0	0	2	0	0
<i>Palpomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i>	0	3	4	3	3	9	0	3	7	3	15	1
<i>Paraleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Peltoperla</i>	0	1	0	0	0	2	0	3	1	0	0	0

Taxon/Stream	COP (1)	COP (2)	COP (3)	CRO (1)	CRO (2)	CRO (3)	EAS (1)	EAS (2)	EAS (3)	FRY (1)	FRY (2)	FRY (3)
<i>Perlesta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i>	1	0	0	5	1	1	1	0	1	0	1	0
<i>Prinocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Psephenus</i>	0	0	0	3	2	3	0	0	0	11	10	26
<i>Pseudostenophylax</i>	0	0	0	0	2	0	0	0	0	0	0	0
<i>Pteronarcys</i>	1	2	3	2	12	10	4	1	3	4	3	6
<i>Pycnopsyche</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Remenus</i>	0	0	0	0	0	10	0	0	0	0	0	0
<i>Rhyacophila</i>	2	1	4	0	6	10	1	1	8	5	7	8
<i>Serratella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Simulium</i>	1	0	0	0	4	4	1	2	0	0	0	0
<i>Stactobiella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenacron</i>	0	0	0	0	0	0	0	7	8	0	0	0
<i>Stenelmis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenonema</i>	0	0	0	0	0	0	0	6	0	0	0	0
<i>Stratiomys</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Suwallia</i>	0	0	0	0	0	3	0	0	0	0	0	0
<i>Sweltsa</i>	0	6	0	0	0	0	1	2	0	0	0	0
<i>Tabanus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taenionema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tallaperla</i>	0	2	1	0	0	1	4	22	31	0	0	1
<i>Tipula</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>Wormaldia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Yugus</i>	0	3	2	0	0	0	0	0	0	11	0	0

Taxon/Stream	GRA (1)	GRA (2)	GRA (3)	HCN (1)	HCN (2)	HCN (3)	KEL (1)	KEL (2)	KEL (3)	KUT (1)	KUT (2)	KUT (3)
<i>Acentrella</i>	115	99	89	12	32	105	0	0	0	0	12	2
<i>Acerpenna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acroneuria</i>	7	6	10	0	0	0	0	0	0	0	0	0
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>Agapetus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Allocapnia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alloperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ameletus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphinemura</i>	26	19	72	3	19	197	173	111	148	12	12	24
<i>Antocha</i>	0	2	1	3	0	1	0	0	0	0	0	0
<i>Apatania</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atrichopogon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i>	0	0	0	0	0	1	21	13	30	9	8	0
<i>Baetisca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	0	0	0	4	0	1	0	0	0	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratopsyche</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Cernotina</i>	0	0	0	0	0	0	0	0	0	3	0	0
<i>Chelifera</i>	1	0	0	5	0	5	0	0	2	3	1	3
<i>Cheumatopsyche</i>	5	2	1	8	3	6	3	2	1	0	0	0
<i>Chimarra</i>	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	12	10	16	77	64	70	16	5	29	31	164	392
<i>Cinygmula</i>	2	1	1	3	1	2	0	0	0	0	0	0
<i>Clinocera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	GRA (1)	GRA (2)	GRA (3)	HCN (1)	HCN (2)	HCN (3)	KEL (1)	KEL (2)	KEL (3)	KUT (1)	KUT (2)	KUT (3)
<i>Cynellus</i>	28	12	12	3	2	0	0	0	0	0	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dibusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dicranota</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dipheter</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diplectrona</i>	26	27	46	2	1	0	13	13	12	29	21	53
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dolophilodes</i>	0	0	9	0	1	12	0	0	0	0	0	0
<i>Drunella</i>	9	8	4	10	6	13	0	0	0	0	0	0
<i>Eccoptura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ectopria</i>	2	2	3	12	1	3	0	1	1	1	1	3
<i>Epeorus</i>	4	9	11	0	1	5	1	0	0	0	0	0
<i>Ephemera</i>	0	0	0	1	0	0	0	0	0	0	0	0
<i>Ephemerella</i>	8	6	16	22	37	133	0	0	1	0	0	0
<i>Eurylophella</i>	0	0	0	9	0	5	0	0	0	0	0	0
<i>Forcipomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	0	0	0	4	0	0	0	0	0	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haploperla</i>	8	5	5	0	0	0	0	0	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	1	0	0	0	0	0	0
<i>Hemerodromia</i>	0	1	0	5	2	13	0	0	0	0	0	1
<i>Heptagenia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydatophylax</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydropsyche</i>	2	7	1	0	0	1	0	0	0	0	0	0
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	0	0	11	0
<i>Isonychia</i>	0	0	0	1	0	1	0	0	0	0	0	0

Taxon/Stream	GRA (1)	GRA (2)	GRA (3)	HCN (1)	HCN (2)	HCN (3)	KEL (1)	KEL (2)	KEL (3)	KUT (1)	KUT (2)	KUT (3)
<i>Isoperla</i>	0	0	1	2	3	5	0	0	0	0	0	0
<i>Lanthus</i>	0	2	0	4	1	1	0	11	0	1	0	7
<i>Lepidostoma</i>	0	0	0	0	0	0	1	0	0	0	1	0
<i>Leucrocuta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leuctra</i>	108	78	154	40	18	35	116	114	318	441	399	949
<i>Limnophila</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Maccaffertium</i>	1	0	0	24	1	2	0	0	0	0	0	0
<i>Malirekus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	1	0	0	0	0	0	0	2
<i>Molophilus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neophylax</i>	1	0	0	0	0	4	0	0	0	0	0	0
<i>Neoplasta</i>	0	0	0	0	5	7	0	1	0	0	2	0
<i>Neotrichia</i>	0	0	0	0	0	0	0	0	0	0	3	0
<i>Nigronia</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oemopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oligochaeta</i>	6	1	2	16	2	0	4	0	0	1	12	29
<i>Optioservus</i>	1	1	0	32	10	14	0	0	0	0	0	0
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	40
<i>Ostrocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oulimnius</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Palpomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i>	1	0	5	2	0	0	0	0	0	0	0	0
<i>Paraleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Peltoperla</i>	0	0	0	0	0	0	2	0	0	0	0	0

Taxon/Stream	GRA (1)	GRA (2)	GRA (3)	HCN (1)	HCN (2)	HCN (3)	KEL (1)	KEL (2)	KEL (3)	KUT (1)	KUT (2)	KUT (3)
<i>Perlesta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i>	0	0	11	8	0	0	0	0	0	0	0	0
<i>Prinocerca</i>	0	0	0	2	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Psephenus</i>	1	0	1	1	0	1	0	0	0	0	0	0
<i>Pseudostenophylax</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pteronarcys</i>	0	0	0	0	0	5	0	0	0	0	0	0
<i>Pycnopsyche</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Remenus</i>	0	0	0	3	0	0	0	0	0	0	0	0
<i>Rhyacophila</i>	5	2	3	0	2	2	1	1	0	1	3	15
<i>Serratella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Simulium</i>	1	1	3	0	92	67	19	21	26	4	2	1
<i>Stactobiella</i>	0	0	0	0	0	1	0	0	0	0	0	0
<i>Stenacron</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenelmis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenonema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stratiomys</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	2	0	6	0	0	0
<i>Suwallia</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>Sweltsa</i>	0	0	0	0	0	1	0	0	0	0	0	0
<i>Tabanus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taenionema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tallaperla</i>	4	3	15	0	0	0	0	0	3	0	0	0
<i>Tipula</i>	0	0	0	0	0	0	0	0	1	0	0	1
<i>Wormaldia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Yugus</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	LLC (1)	LLC (2)	LLC (3)	MIL (1)	MIL (2)	MIL (3)	POW (1)	POW (2)	POW (3)	RFF (1)	RFF (2)	RFF (3)
<i>Acentrella</i>	0	0	0	1	0	0	0	0	1	68	183	52
<i>Acerpenna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acroneuria</i>	1	1	3	0	1	2	2	0	0	1	3	17
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agapetus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Allocapnia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Alloperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ameletus</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>Amphinemura</i>	38	61	88	214	335	367	326	225	112	66	102	292
<i>Antocha</i>	0	0	0	0	0	0	0	1	0	2	1	0
<i>Apatania</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atrichopogon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i>	0	0	0	0	0	0	3	8	0	12	8	10
<i>Baetisca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratopsyche</i>	11	9	28	0	1	0	0	0	0	20	17	15
<i>Cernotina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chelifera</i>	0	0	1	6	0	6	26	15	16	4	0	0
<i>Cheumatopsyche</i>	2	4	11	1	8	3	0	1	0	4	5	8
<i>Chimarra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chironomidae</i>	32	11	38	23	44	30	53	1	30	8	26	9
<i>Cinygmula</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>Clinocera</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	LLC (1)	LLC (2)	LLC (3)	MIL (1)	MIL (2)	MIL (3)	POW (1)	POW (2)	POW (3)	RFF (1)	RFF (2)	RFF (3)
<i>Cynellus</i>	7	5	0	0	0	0	0	0	0	5	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dibusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dicranota</i>	0	0	1	0	0	0	1	1	2	0	0	0
<i>Dipheter</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diplectrona</i>	14	3	13	9	12	8	52	44	27	12	30	42
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	0	0	0	0	1	1	0	1	0	0	0	0
<i>Dolophilodes</i>	0	0	0	2	0	0	3	2	7	0	6	6
<i>Drunella</i>	0	0	0	0	0	0	0	0	0	25	27	31
<i>Eccoptura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ectopria</i>	0	0	1	0	1	2	4	2	4	1	2	3
<i>Epeorus</i>	0	0	1	0	0	0	0	0	0	11	9	9
<i>Ephemera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemerella</i>	0	0	0	1	2	1	0	0	0	10	11	24
<i>Eurylophella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Forcipomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	0	0	0	0	0	0	2
<i>Hemerodromia</i>	0	1	0	3	4	8	0	1	0	0	1	1
<i>Heptagenia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	0	0	1	0	0	0	0	1	1	0	0	0
<i>Hydatophylax</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hydropsyche</i>	0	2	0	0	1	0	0	0	0	0	6	12
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>Isonychia</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	LLC (1)	LLC (2)	LLC (3)	MIL (1)	MIL (2)	MIL (3)	POW (1)	POW (2)	POW (3)	RFF (1)	RFF (2)	RFF (3)
<i>Isoperla</i>	0	4	0	0	0	0	16	24	7	3	0	13
<i>Lanthus</i>	0	0	0	4	4	2	4	2	0	0	0	0
<i>Lepidostoma</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leucrocuta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leuctra</i>	21	5	23	103	362	171	516	300	243	64	143	102
<i>Limnophila</i>	0	0	2	0	0	0	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	2	0	0	0	0	0	0	0
<i>Maccaffertium</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>Malirekus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	0	2	0	0	0	0	0	0
<i>Molophilus</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Neophylax</i>	3	0	0	0	0	0	0	0	0	0	0	0
<i>Neoplasta</i>	0	0	0	1	0	9	2	6	0	3	7	8
<i>Neotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nigronia</i>	0	0	0	0	0	0	1	0	0	4	2	4
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oemopteryx</i>	0	0	0	3	0	0	0	0	0	0	0	0
<i>Oligochaeta</i>	0	0	2	0	3	0	2	1	11	3	4	15
<i>Optioservus</i>	1	1	8	0	0	1	48	33	15	16	16	12
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oulimnius</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Palpomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i>	0	0	0	0	0	0	0	0	0	2	1	0
<i>Paraleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Peltoperla</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	LLC (1)	LLC (2)	LLC (3)	MIL (1)	MIL (2)	MIL (3)	POW (1)	POW (2)	POW (3)	RFF (1)	RFF (2)	RFF (3)
<i>Perlesta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i>	5	1	0	0	0	0	4	0	1	3	0	0
<i>Prinocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Psephenus</i>	0	0	0	0	0	0	0	0	0	1	5	1
<i>Pseudostenophylax</i>	0	0	0	0	0	0	0	0	0	13	0	0
<i>Pteronarcys</i>	0	0	0	0	1	0	0	0	0	4	1	11
<i>Pycnopsyche</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Remenus</i>	4	0	0	0	0	0	0	0	0	0	15	0
<i>Rhyacophila</i>	2	3	4	1	3	8	10	6	6	3	0	0
<i>Serratella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Simulium</i>	1	7	0	23	129	20	5	3	0	0	0	0
<i>Stactobiella</i>	0	0	0	0	0	0	0	3	0	0	0	0
<i>Stenacron</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenelmis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenonema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stratiomys</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Suwallia</i>	0	0	0	0	0	0	0	1	0	1	2	5
<i>Sweltsa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabanus</i>	0	0	0	0	8	0	0	0	0	0	0	0
<i>Taenionema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tallaperla</i>	1	1	0	0	0	0	22	4	1	0	1	0
<i>Tipula</i>	0	0	0	1	1	1	1	2	1	0	0	1
<i>Wormaldia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Yugus</i>	0	1	9	0	0	0	0	0	0	0	0	0

Taxon/Stream	RIC (1)	RIC (2)	RIC (3)	ROC (1)	ROC (2)	ROC (3)	SPC (1)	SPC (2)	SPC (3)
<i>Acentrella</i>	0	0	0	17	15	1	222	217	268
<i>Acerpenna</i>	0	0	0	0	0	0	0	0	0
<i>Acroneuria</i>	2	1	1	0	0	0	19	16	0
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0
<i>Agapetus</i>	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	0	0	0
<i>Allocapnia</i>	0	0	0	0	0	0	0	0	0
<i>Alloperla</i>	0	0	0	0	0	0	0	0	0
<i>Ameletus</i>	0	0	0	0	0	0	0	0	0
<i>Amphinemura</i>	156	116	171	151	126	271	241	45	73
<i>Antocha</i>	0	0	0	2	1	0	0	0	0
<i>Apatania</i>	0	0	0	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	0	0	0	0	0	0
<i>Atrichopogon</i>	0	0	0	0	0	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0	0	0	0
<i>Baetis</i>	0	0	0	0	0	0	16	1	0
<i>Baetisca</i>	0	0	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	0	1	8	0	0	0	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0	0	0	0
<i>Ceratopsyche</i>	14	2	8	3	9	4	0	0	0
<i>Cernotina</i>	0	0	0	0	0	0	0	0	0
<i>Chelifera</i>	1	0	4	0	1	1	40	19	2
<i>Cheumatopsyche</i>	17	5	0	4	5	6	6	2	1
<i>Chimarra</i>	0	0	0	0	0	0	0	0	0
<i>Chironomidae</i>	78	13	65	54	58	31	249	144	204
<i>Cinygmula</i>	0	0	0	0	1	0	4	0	0
<i>Clinocera</i>	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i>	0	0	0	0	0	0	0	0	0

Taxon/Stream	RIC (1)	RIC (2)	RIC (3)	ROC (1)	ROC (2)	ROC (3)	SPC (1)	SPC (2)	SPC (3)
<i>Cynellus</i>	4	0	0	2	0	0	0	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0	0	0	0
<i>Dibusa</i>	0	0	0	0	0	0	0	0	0
<i>Dicranota</i>	0	1	0	0	0	0	0	0	0
<i>Dipheter</i>	0	0	0	0	0	0	0	0	0
<i>Diplectrona</i>	15	5	27	16	17	30	28	9	12
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	0	0	0	0	0	0	0	0	0
<i>Dolophilodes</i>	0	0	0	0	1	0	115	14	19
<i>Drunella</i>	0	0	0	3	5	3	9	10	7
<i>Eccopectura</i>	0	0	0	0	0	0	2	0	0
<i>Ectopria</i>	6	1	6	1	1	0	10	0	1
<i>Epeorus</i>	0	0	1	0	0	0	14	3	0
<i>Ephemera</i>	0	0	0	0	0	0	0	0	0
<i>Ephemerella</i>	0	0	0	0	0	0	72	34	27
<i>Eurylophella</i>	0	0	0	0	0	0	1	0	2
<i>Forcipomyia</i>	0	0	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	0	0	0	0	0	0	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0	0	0	0
<i>Haploperla</i>	0	0	0	1	0	0	0	1	0
<i>Helichus</i>	0	0	0	0	0	0	0	0	0
<i>Hemerodromia</i>	1	0	0	1	0	1	10	0	2
<i>Heptagenia</i>	0	0	0	0	0	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	0	0	0	0	0	0	0	0	0
<i>Hydatophylax</i>	0	0	0	0	0	0	0	0	0
<i>Hydropsyche</i>	1	1	0	5	3	5	2	0	0
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	0
<i>Isonychia</i>	0	0	0	0	0	0	0	0	0

Taxon/Stream	RIC (1)	RIC (2)	RIC (3)	ROC (1)	ROC (2)	ROC (3)	SPC (1)	SPC (2)	SPC (3)
<i>Isoperla</i>	0	0	0	8	0	2	10	9	24
<i>Lanthus</i>	0	0	0	1	0	0	2	0	0
<i>Lepidostoma</i>	0	0	0	0	0	0	0	0	0
<i>Leucrocuta</i>	0	0	0	0	0	0	0	0	0
<i>Leuctra</i>	358	632	623	23	7	13	809	262	321
<i>Limnophila</i>	0	0	0	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	0	0	0	0	0
<i>Maccaffertium</i>	0	0	0	0	0	0	2	0	2
<i>Malirekus</i>	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	0	0	0	2	1
<i>Molophilus</i>	0	0	0	0	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	0	0	0	0	0
<i>Neophylax</i>	0	0	0	0	0	0	0	0	0
<i>Neoplasta</i>	1	0	0	0	0	0	6	0	2
<i>Neotrichia</i>	0	0	0	0	0	0	0	0	0
<i>Nigronia</i>	0	1	1	0	0	1	4	0	1
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	0	0	0	0	0	0
<i>Oemopteryx</i>	0	0	0	0	0	0	0	0	0
<i>Oligochaeta</i>	3	6	11	7	2	0	104	12	22
<i>Optioservus</i>	15	4	0	8	3	2	15	1	2
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	0	0	0	0	0	0
<i>Oulimnius</i>	0	0	0	0	0	0	0	0	0
<i>Palpomyia</i>	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	0	0	0	0	0	0	0	0	0
<i>Paraleptophlebia</i>	0	0	0	1	0	1	0	1	0
<i>Paraleuctra</i>	0	0	0	0	0	0	0	0	0
<i>Peltoperla</i>	0	0	0	0	0	0	0	0	0

Taxon/Stream	RIC (1)	RIC (2)	RIC (3)	ROC (1)	ROC (2)	ROC (3)	SPC (1)	SPC (2)	SPC (3)
<i>Perlesta</i>	0	0	8	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i>	1	0	0	1	2	0	12	0	1
<i>Prinocerca</i>	0	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0	0	0	0
<i>Psephenus</i>	0	0	0	0	0	0	5	0	1
<i>Pseudostenophylax</i>	0	0	0	0	1	0	0	0	1
<i>Pteronarcys</i>	0	0	0	0	0	0	0	0	0
<i>Pycnopsyche</i>	0	1	0	0	0	0	0	0	0
<i>Remenus</i>	0	0	0	0	0	0	0	0	0
<i>Rhyacophila</i>	5	3	19	5	2	2	17	6	4
<i>Serratella</i>	0	0	0	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0	1	0	0
<i>Simulium</i>	6	0	1	1	4	0	4	0	1
<i>Stactobiella</i>	0	0	0	0	0	0	0	0	0
<i>Stenacron</i>	0	0	0	0	0	0	0	0	0
<i>Stenelmis</i>	0	0	0	0	0	0	0	0	0
<i>Stenonema</i>	0	0	0	0	0	0	0	0	0
<i>Stratiomys</i>	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	0	0	0	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	0	0	0
<i>Suwallia</i>	0	0	0	3	0	0	0	0	0
<i>Sweltsa</i>	0	0	0	0	0	0	0	0	0
<i>Tabanus</i>	0	0	0	0	0	0	0	0	0
<i>Taenionema</i>	0	0	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	0	0	0	0	0	0
<i>Tallaperla</i>	0	0	0	1	0	2	1	0	0
<i>Tipula</i>	0	2	1	0	0	3	0	0	1
<i>Wormaldia</i>	0	0	0	0	0	0	0	0	0
<i>Yugus</i>	12	0	0	0	0	0	0	0	0

Table A-12. Benthic macroinvertebrate taxa counts by stream for fall 2017 semi-quantitative samples.

Taxon/Stream	COP	CRO	EAS	FRY	GRA	HCN	KEL	KUT	LLC	MIL	POW	RIC	ROC	SPC
<i>Acentrella</i>	0	0	0	0	0	0	0	0	0	0	0	0	3	0
<i>Acerpenna</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acroneuria</i>	1	1	2	0	2	0	0	0	1	0	0	0	2	2
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agapetus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Allocapnia</i>	2	4	10	65	20	17	7	18	58	90	173	72	41	49
<i>Alloperla</i>	1	0	0	1	0	0	0	0	0	1	0	0	0	0
<i>Ameletus</i>	0	0	0	0	0	0	0	0	0	2	1	0	0	0
<i>Amphinemura</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Antocha</i>	0	0	4	1	0	3	0	0	0	0	0	0	0	0
<i>Apatania</i>	0	7	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atrichopogon</i>	1	0	0	0	1	0	1	0	0	1	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	2
<i>Baetis</i>	4	0	1	0	0	0	1	5	0	1	2	0	0	0
<i>Baetisca</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	3	0	5	0	0	0	0	0	0	1	0	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Ceratopsyche</i>	34	10	32	25	75	8	74	100	50	30	1	39	23	54
<i>Cernotina</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chelifera</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Cheumatopsyche</i>	0	1	3	24	5	6	5	5	31	8	0	42	23	1
<i>Chimarra</i>	0	0	0	0	0	1	0	0	2	0	0	1	0	2
Chironomidae	21	38	41	14	6	112	26	7	30	32	16	16	40	29
<i>Cinygmula</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Clinocera</i>	0	0	0	0	1	0	0	0	0	0	0	2	1	2
<i>Cordulegaster</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyrnellus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	COP	CRO	EAS	FRY	GRA	HCN	KEL	KUT	LLC	MIL	POW	RIC	ROC	SPC
<i>Dibusa</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dicranota</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dipheter</i>	0	5	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diplectrona</i>	10	7	2	10	7	0	14	8	2	12	16	2	28	4
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	1	0	0	0	0	0	3	2	0	0	2	0	0	2
<i>Dolophilodes</i>	0	0	6	0	4	3	3	1	3	0	1	1	0	3
<i>Drunella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eccoptura</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ectopria</i>	4	3	3	0	3	0	6	3	1	4	1	4	0	0
<i>Epeorus</i>	25	2	4	0	0	0	1	0	0	0	0	0	0	0
<i>Ephemera</i>	1	1	0	2	0	1	0	0	0	0	0	0	0	0
<i>Ephemerella</i>	28	0	1	0	0	0	1	0	0	2	0	0	0	0
<i>Eurylophella</i>	0	1	1	1	0	1	0	0	0	0	0	0	0	0
<i>Forcipomyia</i>	0	0	0	0	1	0	0	0	0	0	0	0	1	0
<i>Glossosoma</i>	0	4	0	0	0	0	0	0	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	2	5	2	0	0	0	0	0	0	0	0	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hemerodromia</i>	0	0	0	0	0	1	1	2	2	1	0	0	0	0
<i>Heptagenia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	2	2	3	0	0	2	0	0	1	0	0	4	0	0
<i>Hydatophylax</i>	0	0	1	2	0	0	0	0	0	1	0	0	0	0
<i>Hydropsyche</i>	0	0	0	0	0	1	0	0	0	0	0	2	0	0
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Isonychia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Isoperla</i>	0	0	1	0	0	1	0	0	0	0	0	0	0	0
<i>Lanthus</i>	2	6	0	1	8	0	2	1	0	12	1	4	0	2
<i>Lepidostoma</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leucrocuta</i>	4	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	COP	CRO	EAS	FRY	GRA	HCN	KEL	KUT	LLC	MIL	POW	RIC	ROC	SPC
<i>Leuctra</i>	0	1	0	0	0	0	0	0	2	0	0	2	0	4
<i>Limnophila</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Maccaffertium</i>	0	4	0	1	4	13	0	0	0	0	0	0	0	5
<i>Malirekus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Molophilus</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neophylax</i>	2	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Neoplasta</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neotrichia</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Nigronia</i>	0	0	0	1	0	0	0	0	0	2	0	0	0	0
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oemopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oligochaeta</i>	2	3	3	4	13	13	39	1	0	5	0	1	4	1
<i>Optioservus</i>	9	15	21	10	6	13	1	0	11	0	1	20	10	2
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oulimnius</i>	0	0	0	0	0	0	0	0	0	0	0	6	0	0
<i>Palpomyia</i>	0	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	15	22	6	0	2	1	0	0	4	0	0	0	0	0
<i>Paraleptophlebia</i>	2	15	12	0	0	2	0	0	0	1	0	0	0	0
<i>Paraleuctra</i>	0	0	0	0	5	0	0	0	0	0	0	0	0	3
<i>Peltoperla</i>	1	0	0	0	0	0	2	0	0	0	0	0	0	0
<i>Perlesta</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Polycentropus</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Prinocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	COP	CRO	EAS	FRY	GRA	HCN	KEL	KUT	LLC	MIL	POW	RIC	ROC	SPC
<i>Psephenus</i>	0	8	0	7	3	0	0	0	0	0	0	0	0	3
<i>Pseudostenophylax</i>	0	0	4	0	0	0	0	0	0	0	0	0	0	0
<i>Pteronarcys</i>	1	3	3	2	0	0	0	0	0	0	0	0	0	0
<i>Pycnopsyche</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Remenus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila</i>	6	4	7	8	1	4	12	33	2	8	1	8	3	10
<i>Serratella</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Simulium</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stactobiella</i>	0	0	0	0	0	0	0	0	2	0	0	0	3	0
<i>Stenacron</i>	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>Stenelmis</i>	0	0	0	0	0	2	0	0	0	0	0	0	0	0
<i>Stenonema</i>	0	0	0	7	5	0	0	0	0	0	0	0	0	4
<i>Stratiomys</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Suwallia</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sweltsa</i>	1	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Tabanus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taenionema</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	0	0	2	0	0	6	2	0	2	0	3
<i>Tallaperla</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Tipula</i>	3	1	0	3	22	5	13	2	6	2	6	0	4	4
<i>Wormaldia</i>	1	4	1	0	5	3	3	0	1	1	0	3	0	3
<i>Yugus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A-13. Benthic macroinvertebrate taxa counts by stream for fall 2017 quantitative samples. The replicate number for individual quantitative samples is in parentheses.

Taxon/Stream	COP (1)	COP (2)	COP (3)	CRO (1)	CRO (2)	CRO (3)	EAS (1)	EAS (2)	EAS (3)	FRY (1)	FRY (2)	FRY (3)
<i>Acentrella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acerpenna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acroneuria</i>	1	0	0	5	7	2	3	10	14	6	0	1
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agapetus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Allocapnia</i>	3	3	2	49	15	3	67	15	30	57	59	560
<i>Alloperla</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>Ameletus</i>	7	1	2	1	5	2	2	0	0	2	0	1
<i>Amphinemura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Antocha</i>	0	0	0	3	0	0	0	0	0	3	0	0
<i>Apatania</i>	6	0	11	0	0	0	4	0	11	0	0	0
<i>Atherix</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>Atrichopogon</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Attenella</i>	0	15	0	0	0	3	0	0	0	0	0	0
<i>Baetis</i>	0	0	0	0	7	0	0	2	2	0	0	2
<i>Baetisca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	4	1	4	3	1	0	43	7	1	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratopsyche</i>	4	12	9	45	29	14	24	117	44	242	45	144
<i>Cernotina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chelifera</i>	0	0	0	0	0	0	0	2	0	0	0	0
<i>Cheumatopsyche</i>	0	0	1	1	0	0	0	3	2	78	9	143
<i>Chimarra</i>	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	16	9	4	61	29	10	333	81	92	33	0	56
<i>Cinygmula</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Clinocera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	COP (1)	COP (2)	COP (3)	CRO (1)	CRO (2)	CRO (3)	EAS (1)	EAS (2)	EAS (3)	FRY (1)	FRY (2)	FRY (3)
<i>Cynellus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dibusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dicranota</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dipheter</i>	0	0	0	6	0	0	0	0	0	0	0	0
<i>Diplectrona</i>	11	12	3	18	44	6	68	24	8	3	10	21
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	0	2	0	2	0	0	0	2	0	0	0	1
<i>Dolophilodes</i>	2	4	0	14	1	4	54	39	9	43	1	29
<i>Drunella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eccoptura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ectopria</i>	3	1	7	4	2	1	8	6	4	2	0	0
<i>Epeorus</i>	9	12	10	0	4	0	10	24	14	2	0	0
<i>Ephemera</i>	1	1	0	2	3	0	2	0	10	1	0	2
<i>Ephemerella</i>	5	0	0	0	0	0	0	0	0	0	0	0
<i>Eurylophella</i>	1	0	5	2	4	0	4	1	0	2	0	7
<i>Forcipomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	3	0	1	6	42	1	2	4	10	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0	4	0	0	0	0	0
<i>Haploperla</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hemerodromia</i>	0	0	0	1	0	0	2	0	0	0	0	4
<i>Heptagenia</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	2	6	2	9	3	12	2	8	1	1	0	0
<i>Hydatophylax</i>	0	0	0	2	2	1	0	2	0	0	0	4
<i>Hydropsyche</i>	3	0	0	1	0	0	10	0	0	63	0	4
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Isonychia</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	COP (1)	COP (2)	COP (3)	CRO (1)	CRO (2)	CRO (3)	EAS (1)	EAS (2)	EAS (3)	FRY (1)	FRY (2)	FRY (3)
<i>Isoperla</i>	3	2	0	4	0	0	2	0	2	0	0	0
<i>Lanthus</i>	1	0	0	7	13	2	3	1	0	8	1	2
<i>Lepidostoma</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leucrocuta</i>	2	0	0	7	4	0	13	2	0	0	0	0
<i>Leuctra</i>	2	0	0	0	0	0	77	29	0	0	0	0
<i>Limnophila</i>	0	0	1	1	0	0	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Maccaffertium</i>	1	0	3	0	1	0	0	1	0	2	1	0
<i>Malirekus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Molophilus</i>	0	0	4	0	0	0	16	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neophylax</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neoplasta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nigronia</i>	0	0	0	0	0	0	0	0	0	0	1	3
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Odontomyia</i>	2	0	0	0	0	0	0	0	0	1	0	1
<i>Oemopteryx</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>Oligochaeta</i>	6	0	3	2	2	0	0	2	1	14	2	0
<i>Optioservus</i>	7	3	4	10	4	4	57	5	21	102	4	11
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>Oulimnius</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Palpomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	3	8	13	0	71	24	0	9	9	6	1	3
<i>Paraleptophlebia</i>	14	1	0	33	27	11	98	39	17	0	0	0
<i>Paraleuctra</i>	0	0	0	0	0	0	0	0	0	2	0	0
<i>Peltoperla</i>	1	6	1	0	0	0	1	1	1	0	0	0

Taxon/Stream	COP (1)	COP (2)	COP (3)	CRO (1)	CRO (2)	CRO (3)	EAS (1)	EAS (2)	EAS (3)	FRY (1)	FRY (2)	FRY (3)
<i>Perlesta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prinocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Psephenus</i>	0	0	0	5	3	2	0	0	0	14	4	17
<i>Pseudostenophylax</i>	0	0	0	0	0	0	0	3	0	0	0	0
<i>Pteronarcys</i>	1	5	0	4	1	0	1	6	3	8	4	3
<i>Pycnopsyche</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>Remenus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila</i>	7	2	1	9	10	2	20	35	17	11	2	23
<i>Serratella</i>	0	0	0	0	0	0	0	3	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Simulium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stactobiella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenacron</i>	0	0	0	3	2	0	0	0	6	0	0	0
<i>Stenelmis</i>	2	0	0	5	3	0	24	0	1	67	0	2
<i>Stenonema</i>	1	0	2	0	0	0	0	0	3	2	0	1
<i>Stratiomys</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Suwallia</i>	0	2	2	6	0	1	0	0	0	0	0	0
<i>Sweltsa</i>	0	0	1	0	0	0	1	0	0	0	0	1
<i>Tabanus</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>Taenionema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tallaperla</i>	0	0	0	0	0	0	0	0	5	0	0	0
<i>Tipula</i>	4	7	0	0	1	1	2	0	1	4	7	44
<i>Wormaldia</i>	8	2	1	15	6	6	37	3	1	13	0	3
<i>Yugus</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	GRA (1)	GRA (2)	GRA (3)	HCN (1)	HCN (2)	HCN (3)	KEL (1)	KEL (2)	KEL (3)	KUT (1)	KUT (2)	KUT (3)
<i>Acentrella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acerpenna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acroneuria</i>	3	2	1	0	3	1	1	0	0	0	0	0
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agapetus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Allocaupnia</i>	25	4	13	14	63	158	9	73	32	33	5	1
<i>Alloperla</i>	0	0	0	0	0	3	0	0	0	0	0	0
<i>Ameletus</i>	0	0	0	0	0	0	0	0	0	10	0	0
<i>Amphinemura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Antocha</i>	0	0	0	0	4	3	0	0	0	0	0	0
<i>Apatania</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atrichopogon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i>	0	0	0	0	0	0	0	0	0	0	1	2
<i>Baetisca</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	0	0	0	0	1	2	0	0	0	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratopsyche</i>	231	22	109	0	14	4	117	41	37	6	47	23
<i>Cernotina</i>	0	0	0	3	0	0	0	0	0	0	0	0
<i>Chelifera</i>	0	0	0	0	0	3	0	0	1	0	0	0
<i>Cheumatopsyche</i>	2	0	35	1	154	14	12	3	13	3	0	4
<i>Chimarra</i>	0	0	0	0	1	5	0	0	0	0	0	0
Chironomidae	11	1	5	45	110	235	8	5	22	8	2	3
<i>Cinygmula</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Clinocera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i>	0	0	0	0	0	0	0	0	1	0	0	0

Taxon/Stream	GRA (1)	GRA (2)	GRA (3)	HCN (1)	HCN (2)	HCN (3)	KEL (1)	KEL (2)	KEL (3)	KUT (1)	KUT (2)	KUT (3)
<i>Cynellus</i>	0	0	0	1	0	0	0	0	0	0	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dibusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dicranota</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dipheter</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diplectrona</i>	9	12	15	1	1	3	1	14	7	31	14	6
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	0	2	1	0	0	0	0	0	1	0	3	0
<i>Dolophilodes</i>	1	0	16	0	32	5	0	1	1	0	0	0
<i>Drunella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eccoptura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ectopria</i>	14	10	2	0	9	5	7	2	0	1	0	0
<i>Epeorus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera</i>	0	0	0	1	6	3	0	0	0	0	0	0
<i>Ephemerella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eurylophella</i>	0	0	0	0	1	2	0	0	0	0	0	0
<i>Forcipomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	0	0	0	1	2	10	0	0	0	0	0	0
<i>Hansonoperla</i>	0	0	0	1	0	0	0	0	0	0	0	0
<i>Haploperla</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hemerodromia</i>	2	0	0	0	2	4	0	0	3	0	0	0
<i>Heptagenia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	0	0	0	2	10	6	0	0	0	0	0	0
<i>Hydatophylax</i>	0	0	1	0	0	2	0	0	0	0	0	0
<i>Hydropsyche</i>	42	0	0	1	3	1	0	0	0	1	0	0
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Isonychia</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	GRA (1)	GRA (2)	GRA (3)	HCN (1)	HCN (2)	HCN (3)	KEL (1)	KEL (2)	KEL (3)	KUT (1)	KUT (2)	KUT (3)
<i>Isoperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lanthus</i>	2	1	4	1	2	4	8	0	6	1	1	0
<i>Lepidostoma</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leucrocuta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Limnophila</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Maccaffertium</i>	7	0	4	10	35	43	0	1	0	0	0	0
<i>Malirekus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Molophilus</i>	0	0	0	0	0	1	0	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neophylax</i>	0	0	0	0	7	0	0	0	0	0	0	0
<i>Neoplasta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nigronia</i>	1	0	0	0	0	1	0	0	0	0	0	0
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Oemopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oligochaeta</i>	4	0	0	0	2	63	7	0	3	0	0	0
<i>Optioservus</i>	3	0	0	4	20	72	0	0	0	0	0	0
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	1	0	0	0	0	0	0	0	0
<i>Oulimnius</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Palpomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	6	4	5	0	1	8	0	1	0	0	0	0
<i>Paraleptophlebia</i>	0	0	0	8	18	15	0	0	0	0	0	0
<i>Paraleuctra</i>	4	3	6	0	0	0	2	1	0	0	0	2
<i>Peltoperla</i>	0	0	0	0	0	0	0	1	0	0	0	0

Taxon/Stream	GRA (1)	GRA (2)	GRA (3)	HCN (1)	HCN (2)	HCN (3)	KEL (1)	KEL (2)	KEL (3)	KUT (1)	KUT (2)	KUT (3)
<i>Perlesta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i>	0	0	0	7	0	0	0	0	0	0	0	0
<i>Prinocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Psephenus</i>	19	2	2	0	1	0	0	0	0	0	0	0
<i>Pseudostenophylax</i>	0	0	0	0	0	1	0	0	0	0	0	0
<i>Pteronarcys</i>	0	0	0	0	2	1	0	0	0	0	0	0
<i>Pycnopsyche</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Remenus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila</i>	4	3	1	1	24	40	17	5	8	3	4	3
<i>Serratella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>Simulium</i>	0	1	0	0	0	0	2	27	2	4	0	1
<i>Stactobiella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenacron</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Stenelmis</i>	0	0	0	8	12	5	0	0	0	0	0	0
<i>Stenonema</i>	0	0	4	1	40	3	0	0	0	0	0	0
<i>Stratiomys</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	0	0	0	2	0	0	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Suwallia</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Sweltsa</i>	3	0	0	0	0	1	0	0	0	0	0	0
<i>Tabanus</i>	0	0	0	0	2	0	0	0	1	0	0	0
<i>Taenionema</i>	0	0	0	0	4	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	0	4	10	0	11	0	0	0	0
<i>Tallaperla</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Tipula</i>	20	8	28	2	3	8	4	13	6	7	0	2
<i>Wormaldia</i>	4	0	1	0	13	2	2	2	0	0	0	0
<i>Yugus</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	LLC (1)	LLC (2)	LLC (3)	MIL (1)	MIL (2)	MIL (3)	POW (1)	POW (2)	POW (3)	RIC (1)	RIC (2)	RIC (3)
<i>Acentrella</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>Acerpenna</i>	0	0	0	0	0	0	2	0	0	0	0	0
<i>Acroneuria</i>	0	1	0	0	0	0	0	0	0	0	1	1
<i>Aeshna</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agapetus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Allocapnia</i>	105	43	250	56	29	83	639	448	190	189	91	250
<i>Alloperla</i>	0	0	2	0	0	0	0	0	0	0	0	0
<i>Ameletus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Amphinemura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Antocha</i>	0	0	0	4	0	0	0	0	0	1	0	0
<i>Apatania</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	3	0	0	0	0	0	12	0	0
<i>Atrichopogon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Baetis</i>	0	0	0	0	1	0	5	7	3	0	0	0
<i>Baetisca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Boyeria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	1	0	0	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceratopsyche</i>	18	40	50	47	17	62	5	83	49	90	51	73
<i>Cernotina</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chelifera</i>	0	0	0	0	0	1	0	0	0	0	0	0
<i>Cheumatopsyche</i>	6	8	27	7	2	13	0	0	0	45	38	65
<i>Chimarra</i>	0	0	4	0	1	0	0	0	0	1	1	1
<i>Chironomidae</i>	14	24	37	46	29	10	76	16	37	71	17	14
<i>Cinygmula</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Clinocera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cordulegaster</i>	0	0	0	1	0	0	0	0	0	0	0	0

Taxon/Stream	LLC (1)	LLC (2)	LLC (3)	MIL (1)	MIL (2)	MIL (3)	POW (1)	POW (2)	POW (3)	RIC (1)	RIC (2)	RIC (3)
<i>Cynellus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dibusa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dicranota</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dipheter</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diplectrona</i>	5	2	1	0	9	26	69	78	23	0	7	6
<i>Diploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Dixa</i>	0	0	0	0	1	0	7	12	5	0	0	1
<i>Dolophilodes</i>	1	1	4	1	0	0	3	0	0	9	2	4
<i>Drunella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eccoptura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ectopria</i>	1	0	0	2	0	0	4	0	1	6	1	3
<i>Epeorus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemera</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ephemerella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eurylophella</i>	0	0	0	0	1	0	0	0	0	0	0	0
<i>Forcipomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	1	0	0	0	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Habrophlebiodes</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Haploperla</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hemerodromia</i>	0	0	2	0	1	0	3	0	0	0	0	0
<i>Heptagenia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexatoma</i>	0	0	0	0	0	0	0	0	0	9	0	5
<i>Hydatophylax</i>	0	0	0	1	0	0	0	2	0	0	0	1
<i>Hydropsyche</i>	9	0	2	0	0	1	0	0	0	11	3	0
<i>Hydroptila</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Isonychia</i>	0	0	0	0	0	1	0	0	0	0	0	0

Taxon/Stream	LLC (1)	LLC (2)	LLC (3)	MIL (1)	MIL (2)	MIL (3)	POW (1)	POW (2)	POW (3)	RIC (1)	RIC (2)	RIC (3)
<i>Isoperla</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Lanthus</i>	1	0	1	27	20	0	1	8	6	4	6	9
<i>Lepidostoma</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leucrocuta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leuctra</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Limnophila</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>Limonia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lype</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Maccaffertium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Malirekus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Molophilus</i>	0	0	0	0	0	0	0	0	0	1	0	1
<i>Nemoura</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neophylax</i>	0	0	0	0	2	0	0	0	0	0	0	0
<i>Neoplasta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Nigronia</i>	0	0	0	1	0	0	0	0	0	0	0	0
<i>Ochrotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>Oemopteryx</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>Oligochaeta</i>	3	0	0	10	9	0	5	0	2	7	5	0
<i>Optioservus</i>	2	7	3	0	0	0	2	2	11	50	1	7
<i>Ormosia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Oulimnius</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Palpomyia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paracapnia</i>	0	0	50	0	0	7	0	0	9	0	0	27
<i>Paraleptophlebia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Paraleuctra</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>Peltoperla</i>	0	0	0	0	0	0	0	0	0	0	1	0

Taxon/Stream	LLC (1)	LLC (2)	LLC (3)	MIL (1)	MIL (2)	MIL (3)	POW (1)	POW (2)	POW (3)	RIC (1)	RIC (2)	RIC (3)
<i>Perlesta</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polycentropus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prinocerca</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Psephenus</i>	0	0	0	0	0	0	0	0	0	2	0	0
<i>Pseudostenophylax</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pteronarcys</i>	0	1	1	0	0	0	0	0	0	0	0	0
<i>Pycnopsyche</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Remenus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Rhyacophila</i>	1	4	3	10	0	9	6	1	8	14	7	10
<i>Serratella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	1	0	0	1	0	1	0	0
<i>Simulium</i>	0	0	0	0	0	2	14	14	4	0	0	0
<i>Stactobiella</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenacron</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stenelmis</i>	0	0	0	0	0	0	4	3	1	17	9	1
<i>Stenonema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Stratiomys</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Strophopteryx</i>	5	0	0	0	0	0	0	0	0	15	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Suwallia</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Sweltsa</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tabanus</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>Taenionema</i>	0	0	0	0	0	0	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	8	0	0	1	0	0	0	0	5	9
<i>Tallaperla</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>Tipula</i>	0	1	4	0	3	2	9	9	3	7	0	5
<i>Wormaldia</i>	0	0	1	0	0	0	0	1	0	5	1	13
<i>Yugus</i>	0	0	0	0	0	0	0	0	0	0	0	0

Taxon/Stream	ROC (1)	ROC (2)	ROC (3)	SPC (1)	SPC (2)	SPC (3)
<i>Acentrella</i>	0	0	0	0	0	0
<i>Acerpenna</i>	0	0	0	0	0	0
<i>Acroneuria</i>	3	1	2	4	3	1
<i>Aeshna</i>	0	0	0	0	0	0
<i>Agapetus</i>	0	0	0	0	0	0
<i>Agnetina</i>	0	0	0	0	0	0
<i>Allocaupnia</i>	45	123	29	45	163	10
<i>Alloperla</i>	0	0	0	0	0	1
<i>Ameletus</i>	0	0	0	0	0	0
<i>Amphinemura</i>	0	0	0	0	0	0
<i>Antocha</i>	0	0	0	0	0	0
<i>Apatania</i>	0	0	0	0	0	0
<i>Atherix</i>	0	0	0	0	0	0
<i>Atrichopogon</i>	0	0	0	0	0	0
<i>Attenella</i>	0	0	0	0	0	0
<i>Baetis</i>	0	10	12	0	0	1
<i>Baetisca</i>	0	0	0	0	0	0
<i>Beloneuria</i>	0	0	0	0	0	0
<i>Bezzia.Palpomyia</i>	0	1	0	1	0	0
<i>Boyeria</i>	0	0	0	0	0	0
<i>Brachycentrus</i>	0	0	0	0	0	0
<i>Caenis</i>	0	0	0	0	0	0
<i>Calopteryx</i>	0	0	0	0	0	0
<i>Ceratopsyche</i>	18	120	57	56	129	75
<i>Cernotina</i>	0	0	0	0	0	0
<i>Chelifera</i>	0	0	0	0	0	0
<i>Cheumatopsyche</i>	10	8	30	2	4	18
<i>Chimarra</i>	1	0	0	74	16	0
Chironomidae	53	97	14	38	20	10
<i>Cinygmula</i>	0	0	0	0	0	0
<i>Clinocera</i>	0	0	0	0	0	0
<i>Cordulegaster</i>	0	0	0	0	0	0

Taxon/Stream	ROC (1)	ROC (2)	ROC (3)	SPC (1)	SPC (2)	SPC (3)
<i>Cyrnellus</i>	0	0	0	1	0	0
<i>Dasyhelea</i>	0	0	0	0	0	0
<i>Dibusa</i>	0	0	0	0	0	0
<i>Dicranota</i>	0	0	0	0	0	0
<i>Diphetor</i>	0	0	0	0	0	0
<i>Diplectrona</i>	39	21	13	128	52	10
<i>Diploperla</i>	1	0	0	0	0	0
<i>Dixa</i>	0	0	0	0	2	0
<i>Dolophilodes</i>	34	2	2	5	2	2
<i>Drunella</i>	0	0	0	0	0	0
<i>Eccoptura</i>	0	0	0	7	0	0
<i>Ectopria</i>	0	1	1	6	1	0
<i>Epeorus</i>	2	0	0	1	0	0
<i>Ephemera</i>	0	0	0	0	0	0
<i>Ephemerella</i>	0	0	0	0	0	0
<i>Eurylophella</i>	0	0	0	0	0	0
<i>Forcipomyia</i>	0	0	0	0	0	0
<i>Glossosoma</i>	0	0	0	0	0	0
<i>Goera</i>	0	0	0	0	0	0
<i>Gomphus</i>	0	0	0	0	0	1
<i>Habrophlebiodes</i>	0	0	0	0	0	0
<i>Hansonoperla</i>	0	0	0	0	0	0
<i>Haploperla</i>	0	0	0	0	0	0
<i>Helichus</i>	0	0	0	0	0	0
<i>Hemerodromia</i>	4	16	4	0	1	0
<i>Heptagenia</i>	0	0	0	0	0	0
<i>Heterocloeon</i>	0	0	0	0	0	0
<i>Hexatoma</i>	0	0	0	0	0	0
<i>Hydatophylax</i>	0	0	0	0	0	1
<i>Hydropsyche</i>	20	15	0	6	2	2
<i>Hydroptila</i>	0	0	0	0	0	0
<i>Isonychia</i>	0	0	0	0	0	0

Taxon/Stream	ROC (1)	ROC (2)	ROC (3)	SPC (1)	SPC (2)	SPC (3)
<i>Isoperla</i>	0	0	0	0	0	0
<i>Lanthus</i>	1	1	0	89	10	0
<i>Lepidostoma</i>	0	0	0	0	0	0
<i>Leucrocuta</i>	0	0	0	0	0	0
<i>Leuctra</i>	0	0	0	2	0	0
<i>Limnophila</i>	0	0	0	0	0	0
<i>Limonia</i>	0	0	0	0	0	0
<i>Lype</i>	2	0	0	0	0	0
<i>Maccaffertium</i>	0	0	0	7	15	4
<i>Malirekus</i>	0	0	0	0	0	0
<i>Megaleuctra</i>	0	0	0	0	0	0
<i>Micrasema</i>	0	0	0	0	0	0
<i>Molophilus</i>	0	0	0	0	0	0
<i>Nemoura</i>	0	0	0	0	0	0
<i>Neophylax</i>	0	0	0	0	0	0
<i>Neoplasta</i>	0	0	0	0	0	0
<i>Neotrichia</i>	0	0	0	0	0	0
<i>Nigronia</i>	0	0	1	1	6	1
<i>Ochrotrichia</i>	0	0	0	0	0	0
<i>Odontomyia</i>	0	0	0	1	0	0
<i>Oemopteryx</i>	0	0	0	0	0	0
<i>Oligochaeta</i>	26	19	0	19	8	0
<i>Optioservus</i>	3	6	3	18	10	3
<i>Ormosia</i>	0	0	0	0	0	0
<i>Orthotrichia</i>	0	0	0	0	0	0
<i>Ostrocerca</i>	0	0	0	0	5	0
<i>Oulimnius</i>	0	0	0	0	0	0
<i>Palpomyia</i>	0	0	0	0	0	0
<i>Paracapnia</i>	0	0	0	18	3	8
<i>Paraleptophlebia</i>	0	0	0	0	0	0
<i>Paraleuctra</i>	0	0	0	21	4	0
<i>Peltoperla</i>	3	6	0	1	0	0

Taxon/Stream	ROC (1)	ROC (2)	ROC (3)	SPC (1)	SPC (2)	SPC (3)
<i>Perlesta</i>	0	0	0	0	0	0
<i>Pilaria</i>	0	0	0	0	0	0
<i>Polycentropus</i>	0	0	0	0	0	0
<i>Prinocerca</i>	0	0	0	0	0	0
<i>Prosimulium</i>	0	0	0	0	0	0
<i>Prostoia</i>	0	0	0	0	0	0
<i>Psephenus</i>	0	0	0	20	12	9
<i>Pseudostenophylax</i>	0	0	0	0	0	0
<i>Pteronarcys</i>	0	0	0	0	0	0
<i>Pycnopsyche</i>	0	0	0	0	0	0
<i>Remenus</i>	0	0	0	0	0	0
<i>Rhyacophila</i>	2	6	2	35	2	16
<i>Serratella</i>	0	0	0	0	0	0
<i>Sialis</i>	0	0	0	0	0	0
<i>Simulium</i>	3	3	3	0	4	2
<i>Stactobiella</i>	0	0	0	0	0	0
<i>Stenacron</i>	0	0	0	0	0	0
<i>Stenelmis</i>	0	0	0	0	0	0
<i>Stenonema</i>	0	0	0	6	0	0
<i>Stratiomys</i>	0	0	0	0	0	0
<i>Strophopteryx</i>	0	0	0	0	0	0
<i>Stylogomphus</i>	0	0	0	0	0	0
<i>Suwallia</i>	0	0	0	0	1	0
<i>Sweltsa</i>	1	0	2	0	0	0
<i>Tabanus</i>	0	0	0	3	0	0
<i>Taenionema</i>	0	0	0	0	0	0
<i>Taeniopteryx</i>	0	0	0	4	0	0
<i>Tallaperla</i>	0	0	0	0	0	0
<i>Tipula</i>	13	26	0	8	2	2
<i>Wormaldia</i>	3	3	0	1	3	0
<i>Yugus</i>	0	0	0	0	0	0