Quantifying changes in macroinvertebrate community composition, biomass, and emergence in response to mining-induced salinization in central Appalachian streams

Aryanna Lee James

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science
In
Entomology

Committee Chair:
Sally Entrekin

Committee Members:
Aaron Gross
Gregory Pond
Stephen Schoenholtz
Carl Zipper

10 May 2021
Blacksburg, Virginia

Keywords: conductivity, resource extraction, biodiversity, biomass, insect emergence, central Appalachia, headwaters

Quantifying changes in macroinvertebrate community composition, biomass, and emergence in response to mining-induced salinization in central Appalachian streams

Aryanna Lee James

ACADEMIC ABSTRACT

Many ecosystems are losing biodiversity, raising concern for the services they provide. However, the extent of loss is uncertain, especially for insects that use freshwater during their life. Further study is needed to assess freshwater insect abundances and diversity. In Central Appalachian streams, macroinvertebrate diversity declines in response to mining-induced salinization and resulting changes to ecosystem processes remain largely unknown, such as how the availability and movement of macroinvertebrate biomass is altered in stream food webs. However, taxa observed are dependent upon sampling effort that could bias diversity-process interpretation. Taxon sampling curves can be used to estimate sampling effort that maximizes the probability of complete community characterization. We sampled six streams in the Central Appalachian region for benthic macroinvertebrates and explored the number of samples needed to capture taxonomic richness in salinized streams. Sampling effort did not differ between reference and salinized streams, though more uneven distributions of macroinvertebrates in salinized streams seemed to necessitate greater sampling effort relative to reference streams. We also used taxon and trait-based sampling curves to expand our understanding of biodiversity and functional responses to environmental change. Because macroinvertebrate biomass and emergence can assess the movement and changes in organic material and energy in response to a salinization gradient, we added them as additional metrics. Macroinvertebrates may have varied responses to a stressor dependent upon life stage, suggesting that assessments relying only on immatures may not fully characterize the effects of salinization. We sampled benthic macroinvertebrate biomass and emergent insect biomass from six streams in the Central Appalachian region to be representative of a salinization gradient. We predicted benthic biomass would either decrease, be maintained by greater density and biomass of salt-tolerant taxa, or increase from a salt subsidy effect, while emergent biomass would decrease disproportionately relative to benthic biomass due to late instar and pupae succumbing to stress. Our results suggest that total benthic macroinvertebrate biomass is maintained along a salinization gradient despite the loss of salt-sensitive mayflies due to compensation by salt-tolerant taxa that experience a subsidizing effect. Emergent biomass was variable among streams with peak emergence occurring in spring, with no apparent negative response to increasing conductivity. The present study can help to further develop metrics of stream ecosystem processes in response to a disturbance gradient.

Quantifying changes in macroinvertebrate community composition, biomass, and emergence in response to mining-induced salinization in central Appalachian streams

Aryanna Lee James

PUBLIC ABSTRACT

Freshwater salinization is a growing, global concern. Pollution and accelerated weathering of rock, caused by human activities, introduce salts to streams and other freshwaters. Surface coal mining is a common land use in the Central Appalachian region and increases leaching of sulfate (SO_4^{2-}) and other major ions that increase stream salinity, leading to losses of aquatic insect species. Aquatic insects are important to stream processes, such as providing food to other animals, and they can serve as the bioassessments when impacts are suspected. For example, the impacts of salinization on streams are not fully understood despite bioassessments. We sampled aquatic insects from six Appalachian streams with varying levels of salinity. We estimated the sampling effort needed to characterize aquatic larval insect communities in streams with low salinity compared to streams with high salinity. We found that about six samples captured 80 percent of estimated total taxa and that insect communities with greater unevenness required more sampling effort. Such comparisons will allow us to make more informed decisions when sampling aquatic insects and assessing the effects of salts on streams. We also estimated insect biomass in streams using two life stages, larvae and adults, to determine if these life stages would respond differently to salinization. As we expected, total larval biomass slightly increased as the concentration of salt increased, but mayfly biomass decreased. Mayflies are an important and diverse group of insects in Appalachian streams and decreases in their biomass can have consequences for insect communities and stream food webs. Even though emergent insect biomass was found to represent only a small proportion of the larval biomass observed in streams, they represent critical food for terrestrial animals. Estimates of benthic and emergent

biomass could be considered to refine bioassessments that support future management and policy regarding surface mining and the rising issue of freshwater salinization.

DEDICATION

To my loving family
(this is probably the only page they will read in its entirety)

ACKNOWLEDGEMENTS

I would like to thank the following people:

The Virginia Tech Entomology Department and the Fralin Life Sciences Institute for their financial support.

My advisor, Sally Entrekin, for her encouragement throughout my program.

My other committee members, Aaron Gross, Gregory Pond, Stephen Schoenholtz, and Carl Zipper for their knowledge and enthusiasm.

My work wife, Kelly McIntyre, for her assistance in the lab, field, and R as well as her support and comradery.

Joseph Girgente, Chris Lucy, Shawn Johnson, Seth Ramsay, Marlaina Marvin, Emily Bess, Grant Schiermeyer, Jared Dyer, Hannah Angel, Jyoti Thapa, Abigail Belvin, Sarah Turner, and Taylore Sydnor for their instrumental assistance in the field and laboratory.

Dr. Tony Timpano and Tommy Cianciolo for introducing me to the study streams. I would also like to thank Dr. Timpano for his initial selection and characterization of Appalachian headwater streams from which my study streams are a subset.

Rachel Pence for her prior work in these streams and collection of macroinvertebrate samples that helped to inspire Chapter 2 of this thesis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Introduction

Freshwater salinization

Salinization is a growing concern as increasing levels of salts enter freshwaters on a global scale (Cañedo-Argüelles et al. 2013). In the contiguous U.S., changes in salinization have affected the majority of drainage area over the past century (Kaushal et al. 2018). Salinity is a product of the total concentrations of dissolved inorganic ions in water and soil (Williams and Sherwood 1994) and is a naturally occurring attribute of aquatic systems that is tied to the weathering of geologic materials in a catchment and sea salts in coastal areas. However, human activities that pollute or accelerate weathering, such as road de-icing, agriculture, wastewater, and resource extraction, introduce elevated levels of salts to freshwater systems that surpass natural background concentrations. This anthropogenic salinization (also referred to as secondary salinization) has repercussions for human health and economies as well as for ecosystems and the services they can provide (Cañedo-Argüelles et al. 2013, Kaushal 2016). Central to freshwater ecosystems are aquatic macroinvertebrates that serve as important food resources to other trophic levels, aid in nutrient cycling, and the translocation of carbon (Vannote et al. 1980, Wallace and Webster 1996, Baxter et al. 2005). Thus, salt-mediated changes to biota can have cascading effects on downstream and riparian linkages and processes, such as biomass production and carbon cycling (Berger et al. 2019).

Surface coal mining in Central Appalachia

Resource extraction, or more particularly surface coal mining, is the dominant land use change in the central Appalachian region of the United States, covering more than 5,900 km^2, (Townsend et al. 2009, Pericak et al. 2018). Central Appalachia has a long history of mining that is reflected in its landscape with mines of varying ages and practices that contribute historic and on-going influences on water quality (Haering et al. 2004). Early mining practices were predominantly underground, but technological advancements have allowed for coal seams to be accessed through surface mining (Skousen and Zipper 2014). This process requires the removal of overburden or mine spoil, which is all rock and earthen materials overlying the coal seam. Explosives and heavy machinery are often used to expedite this process. In mountain top removal and valley fill operations, as much as 300 meters (1,000 feet) of overburden are displaced (USEPA 2011).

The fracturing of rock generates a greater volume of spoil that exceeds the placement of its original contour and is typically placed into adjacent valleys where streams are buried (Bernhardt and Palmer 2011). This disturbance and movement of the natural geology occurs on a relatively large scale, and the exposure of new rock surfaces to air and water accelerates weathering and the leaching of ions into effluent. Pyrite minerals, originating in coal, produce sulfuric acid (Singer and Stumm 1970) that is neutralized by the carbonate matrix of bedrock. As a result of pyrite dissolution, elevated levels of sulfate ions (SO_4^{2-}) accompanied by other major ions such as calcium, magnesium, and bicarbonate (Ca^{2+}, Mg^{2+}, HCO_3^-) are associated with mining discharge (Griffith et al. 2012). The ionic composition of salinity in mine-water discharge may change with time or age of the mine as well. Sulfate occurs in greater concentrations in initial leachates while bicarbonate is the dominant anion found in later leachates (Orndorff et al. 2015, Clark et al. 2018). The mine drainage can be further characterized as being alkaline with elevated pH (Timpano et al. 2015). The weathering of spoil material is a source of elevated ions to streams (USEPA 2011, Griffith et al. 2012, Timpano et al. 2015). Ion concentrations are often measured as specific conductance (SC), a proxy for total dissolved solids (TDS). Relatively undisturbed streams in the region are comparatively dilute with conductivities generally between 40 and 100 µS/cm (Pond et al. 2008, USEPA 2011).

Surface mining has been shown to cause physical, hydrological, and chemical changes to catchments and the streams that drain them (Palmer et al. 2010) that are long-lasting and slow to recover (Pond et al. 2014, Cianciolo et al. 2020). Ross et al. (2016) found that in southern West Virginia alone, over six km^3 of bedrock has been disturbed and deposited into valley fills, lowering the median slope of the landscape by about 10 degrees and increasing the average elevation by three meters. Altered topography, creation of mine spoil, compaction, and the removal of vegetation in the landscape lead to altered hydrologic patterns in mined catchments relative to that of unmined, often characterized by increased peak flows and base flows (Evans et al. 2015). Increased summer baseflows have been attributed to the retention of stormflows within spoil material, which also contribute disproportionately more ions to streams (Nippgen et al. 2017). Seasonal or intra-annual fluctuation of conductivity in Appalachian streams has been characterized as having a sinusoidal pattern with maximum conductivities occurring in late summer and minimum conductivities occurring in late winter because of seasonal dilution and catchment evapotranspiration (Timpano et al. 2018).

Regulation of surface mining

There are both federal and state regulations concerning surface mining in the United States. The major regulation governing surface mining is the Surface Mining Control and Reclamation Act (SMCRA, 25 U.S.C. § 1201) of 1977, under which 600,000 ha of land have been mined in Appalachia (Zipper et al. 2011). The Office of Surface Mining Reclamation and Enforcement (OSMRE) was created in concert with the SMCRA. OSMRE is a bureau within the U.S. Department of Interior that has an array of responsibilities all relating to mining and stemming from the SMCRA. In addition to the SMCRA, the environmental impacts of surface mining can be regulated through the Clean Water Act (CWA, 33 U.S.C. § 1252). The narrative criteria of the CWA states that it "is to restore and maintain the chemical, physical, and biological integrity of the Nation's waters," (33 U.S.C. § 1362(7)). Two sections of the CWA are relevant to the permitting of surface mining operations. Section 404 regulates the dredging and filling of material, such as the construction of sediment ponds below mining operations. Section 402, National Pollution Discharge Elimination System (NPDES), regulates the discharge of pollutants from a point source. These permits are regulated by the U.S. Army Corps of Engineers and the U.S. Environmental Protection Agency (EPA), respectively.

Biological effects of surface mining in streams

Ensuant of the catchment-scale changes associated with surface mining are biological impacts to streams. Field studies are valuable in that they measure actual responses of communities to the combined and interacting stressors present in the environment. However, causal relationships are often difficult to isolate in field studies because of the combined nature of stressors. Cormier et al. (2013) addressed this issue of causality using an epidemiological, weight-of-evidence methodology, finding that available evidence supports a causal relationship between ions (HCO_3^-, SO_4^-, Ca_2^+, and Mg_2^+) in ecoregions 68, 69, and 70 and biological impairments, such as the extirpation of benthic macroinvertebrates. Aquatic macroinvertebrates are often used in the bioassessment of regional waters, including mine-influenced streams. Because of their important role in ecosystem function and diversity of life histories, diets, and adaptations, aquatic macroinvertebrates are informative indicators of stream health (Merritt et al. 2008). Their obligation to water to complete their life cycle renders aquatic macroinvertebrates relatively stationary and reliable indicators of long-term water quality and land use changes. As the valley rules the stream (Hynes 1975), so does it the aquatic macroinvertebrate communities that live there.

The EPA recognizes the potential detrimental effects of conductivity to aquatic organisms. In response to the concentration of coal mining activity in the Appalachian region and associated increases in stream conductivities, the EPA developed a field-based benchmark for conductivity to protect aquatic life (Cormier et al. 2011). A benchmark value of 300µS/cm was derived from biological field surveys to be applicable to Ecoregions 68, 69, and 70 and to protect 95% of native species from local extirpation. This water-quality benchmark is based on the fifth centile of a species sensitivity distribution (SSD), which represents the response of aquatic life with respect to exposure. The SSD is based on extirpation concentrations (XCs) from field data, in contrast to median lethal concentrations (LC_{50}s), which are concentrations required to kill 50% of individuals in toxicological studies. The field-based methodology of the conductivity benchmark ensures its relevance to the targeted region. Though, its protection is not inclusive of all genera. That is, the 5% of genera under the fifth centile of the SSD (or every 1 out of 20) may be expected to face extirpation. These genera are likely to be the most salt-sensitive organisms, such as mayflies (USEPA 2011, Kefford 2018). Additionally, the benchmark does not account for reductions in the abundances of genera, only their presence or absence. Most aquatic diversity may be maintained under the suggested benchmark and, with the assumption that diversity is informative of stream function, the benchmark will help ensure that these waterways can provide their designated services and uphold CWA standards. Yet, little is known regarding the functional responses of streams that are mediated by aquatic life in response to mining-induced salinization (Fritz et al. 2010).

Elevated ion concentrations have led to losses of macroinvertebrate richness and diversity and shifts in community composition to more tolerant taxa in streams influenced by coal mining in central Appalachia (Merricks et al. 2007, Pond et al. 2008, Cormier et al. 2013, Johnson et al. 2013, Timpano et al. 2015, Boehme et al. 2016, Timpano et al. 2018, Drover et al. 2019, Vander Vorste et al. 2019). Responses across taxonomic groups vary such that some macroinvertebrates are tolerant while others are more sensitive and occur in lower abundances. Salinity tolerances vary across taxonomic units (e.g. Order, Family, Genera, or Species), such that aggregate metrics can mask the responses of sensitive taxa via the inclusion of tolerant taxa (Boehme et al. 2016, Castillo et al. 2018). Baetidae, Hydropsychidae, and Leuctridae in the orders Ephemeroptera, Trichoptera, and Plecoptera (EPT), respectively, have been shown to be relatively salt-tolerant (Pond 2010, Pond 2012, Boehme et al. 2016), despite the general sensitivities of these EPT orders (Barbour et al. 1999).

Notable is the loss of salt-sensitive Ephemeroptera downstream of mined catchments (Merricks et al. 2007, Pond et al. 2008, Pond 2010, Boehme et al. 2016). Timpano et al. (2018) observed declines in relative abundances and richness of non-Baetidae Ephemeroptera at SC as low as 200 μS/cm. In fact, many Ephemeroptera are extirpated at lower ion concentrations than many other taxonomic groups (Cormier et al. 2013, Kefford 2018), rendering the mayfly an ecologically significant test organism for toxicity testing and the development of criteria protective of aquatic life (Kennedy et al. 2004, Echols et al. 2010, Soucek and Dickinson 2015).

The toxic, lethal and sublethal effects that salts of varying concentrations and mixtures have on aquatic macroinvertebrates are evidenced as decreased growth rates, delayed development and emergence, and decreased survivorship (Kennedy et al. 2004, Kunz et al. 2013, Soucek and Dickinson 2015, Buchwalter et al. 2019). When subjected to simulated coal mining effluent with a range of specific conductivities (240 µS/cm to > 9,000 µS/cm), the mayfly *Isonychia* experienced greater mortality and less frequent molts (measured as the number of exuviae) with increasing conductivities (Kennedy et al. 2004). The mayfly *Neocloeon triangulifer* exhibited reduced survival (as percent emerged) and delayed development when reared in a blend of $CaSO_4$ and $MgSO_4$ (5 to 1500 mg/L SO4; Buchwalter et al. 2019). In the same study, total body sulfur content was used to infer sulfate content and changed little in the subimagos of *N. triangulifer,* despite increasing uptake rates of sulfate with increasing exposure concentration. These results suggest that the stress of ion regulation (i.e. osmoregulation) in maintaining homeostasis is the driver affecting the survival and performance of *N. triangulifer*. However, it is plausible that sulfur or sulfate is differentially compartmentalized within the body and shed with exuviae of transitional life stages, such as molts between late instar and subimagos or imagos. The mechanism through which ions negatively affect the physiology of aquatic macroinvertebrates is still an area of active research (Kefford 2018).

Differential salinity tolerances of macroinvertebrates are evident among functional feeding groups as well. Functional feeding groups reflect processes such as trophic interaction, production, and resource availability (Barbour et al. 1999). A quantitative review of published LC_{50} values (an estimate of salinity tolerance) found variability in NaCl salinity tolerances across aquatic insect taxa, functional feeding groups, and climatic regions (Castillo et al. 2018). The functional feeding groups scrapers, gatherers, and filterers exhibited consistently lower tolerances to elevated NaCl. Changes to functional feeding groups among macroinvertebrates have been observed downstream of Appalachian, mined catchments with elevated sulfate concentrations, such as declines in scraper richness and relative abundance (Pond et al. 2014, Timpano et al. 2018). Vander Vorste et al. (2019) found that overall, reach-wide macroinvertebrate taxa richness decreased with increasing SC, but shredder taxa richness and abundance within leaf-packs did not. Changes among functional feeding groups could alter trophic diversity and interactions in Appalachian streams with uncertain consequences for ecosystem processes (Castillo et al. 2018).

The importance of headwater streams

First to third order streams are headwaters that cumulatively comprise 60 to 80 percent of stream length and riparian interface in river networks (Benda et al. 2005, Downing et al. 2012), support biodiversity, and sustain connectivity among downstream and terrestrial ecosystems (Meyer et al. 2007). Headwater streams are dominated by allochthonous inputs, such as dead leaves, (Wallace et al. 1999), that are processed by resident, aquatic macroinvertebrates that play a crucial role in the function of streams by aiding in nutrient cycling and the translocation of carbon (Vannote et al. 1980, Wallace and Webster 1996). Insects that emerge from streams and enter riparian habitats serve as a primary food resource to other trophic levels (Baxter et al. 2005), including other arthropods (Paetzold and Tockner 2005). A subsidy can be defined as a moveable resource in the form of organisms, energy, and nutrients that flow between and connect ecosystems (Polis et al. 1997, Marcarelli et al. 2011). In addition to carbon, many essential polyunsaturated fatty acids are produced mainly in aquatic systems and are transferred across water-land interfaces through emergent insects (Gladyshev et al. 2019). Alterations in macroinvertebrate-mediated processes could have cascading effects on local and downstream ecosystems, and alterations in emergence could have potential consequences for the food quantity and quality available to terrestrial consumers. In headwater streams, where terrestrial-to-aquatic fluxes often exceed aquatic-to-terrestrial fluxes (Bartels et al. 2012), emergent insects are important in returning reciprocal flows, transporting aquatic carbon into riparian systems

(Murakami and Nakano 2001). The centrality and interconnection of macroinvertebrates in stream food webs renders them an appropriate and suitable subject of study in addressing ecosystem-level questions (Wallace and Webster 1996).

Though functionally important, headwaters and their biota are vulnerable to disturbances (Creed et al. 2017, Reid et al. 2019). Biodiversity facilitates the function of an ecosystem, where the presence of more and different taxa equivalate to increasing function (Peterson et al. 1998, Cardinale et al. 2006). However, many ecosystems are currently undergoing a loss of biodiversity due to anthropogenic activities, raising concern for the services that these systems can provide (Ehrlich and Wilson 1991). Recent concern has developed for the defaunation of insects (Jarvis 2018). Despite the media attention, there is still ambiguity surrounding the status and trends of insects around the world, and further study is needed to assess insect abundances, biomass, species richness, diversity, and their functions (Thomas et al. 2019), including that of our freshwater systems (Darwall et al. 2008, Castillo et al. 2018). The need for metrics that express communicable quantities and that are comparable across ecosystem contexts are evident to address global-scale issues. For instance, concurrent trends of salinization and alkalinization have been occurring over the majority of drainage area in the contiguous United States during the past century (Kaushal et al. 2018), but correspondent large-scale trends of aquatic organisms, either increasing or decreasing in number or biomass, are largely unknown.

Conceptual models in predicting biological responses

Generalized conceptual models can be used to explain or predict biological response to a stressor such as salinity. The simplest model would be linear where an incremental increase in salinity results in a predictable incremental biological response. In contrast, a subsidy-stress response (Odum et al. 1979) shows an initial increase in productivity (positive biological response) at low concentrations of the stressor followed by a decrease in productivity (negative biological response) at higher concentrations of the stressor, producing a “hump-shaped” curve. Salts are essential elements required for life. At low, sublethal concentrations, salts may subsidize organisms via more than one pathway (Entrekin et al. 2018). For instance, salt-mediated alterations to microbial decomposer communities may alter food quality available to macroinvertebrate detritivores.

Osmoregulation is another pathway through which salts may cause subsidy or stress. Aquatic macroinvertebrates are hyper-osmotic regulators, meaning that they maintain their internal fluids at higher concentrations than the external water (Merritt et al. 2008). It has been inferred that changes in osmolarity between organisms and their environment can alter osmoregulatory energetics such that salinity increases approaching an isosmotic point lessen the energy expenditure associated with osmoregulation (Kefford 2018). When exposed to a range of sublethal, chronic NaCl salinities (0-20 mS/cm), the percent survival of two mayfly species, *Cloeon* sp. and *Centroptilum* sp., and one midge species, *Chironomus* sp., exhibited hump-shaped responses with greatest survival occurring at intermediate salinities (Hassell et al. 2006). These results suggest that these three species experienced subsidy and stress. However, low salinity increases below the isosmotic point do not always impart a subsidy effect to aquatic taxa, such as the case with many salt-sensitive mayfly species, that experience mortality at osmolalities below that of their hemolymph (Kefford 2018). Therefore, the conventional model of osmoregulation is not a total explanation for the biological and subsidy responses observed to salts. The mechanisms by which salts subsidize, stress, or cause mortality among aquatic taxa are not fully understood, but additional hypotheses such as localized toxicity effects and the energetic costs of ion uptake and turnover have been proposed (Kefford 2018). Thus, salts have the potential to be considered either a usable input or a toxic input.

Yet, a compensation scenario is another conceptual framework to understand biological response where variability over time among groups of organisms (e.g. taxa or functional groups) manifests as stability for the community as a whole (Micheli et al. 1999). In the case of mining-influenced, salinized streams, taxonomic replacement may occur as salt-sensitive taxa are extirpated but are replaced by salt-tolerant taxa (Vander Vorste et al. 2019). Across a salinity gradient of 24-1437µS/cm, Drover et al. (2019) found that overall macroinvertebrate densities did not respond to salinization despite decreases in densities of salt-sensitive taxa, such as Ephemeroptera, that were compensated for by increasing densities of other taxa. Johnson et al. (2013) found no significant difference in total secondary production estimates, measured by emergent adult insect biomass, between mined and forested catchments, but there were significant differences among EPT metrics. Forested catchments had greater EPT production while in mined catchments chironomids accounted for more than 80% of production. Such significant differences in taxonomic composition despite insignificant differences between total metrics suggests compensation.

Sampling effort and characterizing biodiversity

Sampling methods can impart differing levels of accuracy in characterizing communities as well. For instance, the use of more traditional semi-quantitative sampling methods that are often employed with kick-nets by regulatory agencies (e.g., Barbour et al. 1999) versus high-enumerative quantitative sampling methods employing different capture strategies can return different values for the same metrics within a habitat (Pence 2019).

Biodiversity assessment is integral to species conservation (Darwall et al. 2008). Ecologists often need to know the number of species (species richness) present in a given area to characterize a community. Species richness is an important measure of biodiversity at the habitat level (Colwell and Coddington 1994). However, it may be difficult or unfeasible to enumerate all species directly. Therefore, sampling is necessary. Species diversity in sites or habitats at a local scale (alpha diversity) is dependent on sample size. Ecologists are often constrained by resources, so arises the issue of sampling effort required to obtain a reliable estimate of taxa richness. One way of addressing this issue is with the use of taxa accumulation curves that graphically display the number of observed taxa as a function of the sampling effort deployed (Colwell et al. 2004). Unlike species-area relationships that are concerned with the number of species in areas of differing size, the species accumulation curve is concerned with the rates at which new species are collected with additional samples of fixed area (Ugland et al. 2003). Species accumulation curves consider the number and identity of species within a given sampling unit but do not require input regarding their distribution (Ugland et al. 2003). This relationship between sampling effort and the expected number of species can be used for efficient planning and sampling protocol (Soberón and Llorente 1993).

Macroinvertebrate function in response to salinization

Less is known about the macroinvertebrate functional responses to elevated salts in Central Appalachian headwater systems. This gap in understanding is founded in part by the relatively infrequent use of quantitative sampling methods, which can inform densities, biomass, and secondary production of aquatic macroinvertebrates. Quantitative sampling methods employ a pre-determined area in which organisms are collected and enumerated, allowing for calculations such as individuals/meter (i.e., density), grams of biomass/meter (i.e., biomass), and grams of biomass/meter/year (i.e., production). Biomass is an amount of mass or matter that is in a living organism(s), and it can be measured as a community response, taxon-specific group, or functional group. The advantage of quantitative methods is that these values convey amounts that are comparable across studies, informative of ecological processes such as the flow of organic matter and energy, and in turn, tell of stream ecosystem processes. Therefore, quantitative measures provide a common link between population and ecosystem level processes (Benke 1993).

Few studies have explored functional metrics of macroinvertebrate communities (e.g. secondary production) to assess the effects of mining-induced salinization and even fewer include the emergent life stage. To my knowledge, no study has quantified the response of *both* larval and emergent insect biomass along a salinization gradient. Johnson et al. (2013) measured seasonal emergent insect density (indiv. $m^{-2}d^{-1}$) as a proxy for larval biomass and to compare estimates of benthic secondary production between streams draining mined and forested catchments (49−2513 µS/ cm) in West Virginia. No treatment effect was found on emergent insect abundance, biomass, or estimated annual secondary production. It is possible that emergence measures alone are not a suitable predictor of benthic production due to differential tolerances across macroinvertebrate life stages and a decoupling of life stage responses (Wesner et al. 2014, Johnson et al. 2015). Schmidt et al. (2013) explored differential responses of life stages by measuring and comparing both larval and emergent adult densities along a gradient of stream metal contamination in the Central Colorado Rocky Mountains, finding that emergence declined disproportionately to larval density. These findings show that adult emergence cannot always be assumed a constant proportion of larval density and highlight the need for studies inclusive of macroinvertebrate life stages to assess the effects of a stressor. Sole reliance on the assessment of larval life stages may underestimate the cumulative impacts of a stressor. Juxtaposed responses of both larval and adult aquatic insects can be considered to gain a more complete understanding of salt impacts among insect life stages and stream processes.

Through structural metrics, changes in macroinvertebrate communities in response to mining-induced salinization have been documented (Merricks et al. 2007, Pond et al. 2008, Cormier et al. 2013, Boehme et al. 2016, Timpano et al. 2018, Drover et al. 2019). However, persistent uncertainty regarding the propensity of these changes and their relevance to ecosystem function and services warrants further investigation (e.g., Johnson et al. 2013). Comparisons of insect biomass across a stressor gradient can provide insight into functional stream-level processes and allows for inferences regarding the stressor-mediated changes. Additionally, most studies have focused primarily on the larval life stage of aquatic insects, while few have considered their emergence (Johnson et al. 2013), a potentially more sensitive indicator to a stressor (Schmidt et al. 2013, Wesner et al. 2014). I aim to improve our understanding of how salts impact lotic, freshwater systems and how these changes are propagated across life stages of aquatic macroinvertebrates that may differ in response to salinization in terms of community composition and biomass.

In the following chapter, I will discuss the sampling effort (number of Surber samples with stream-bottom area of 0.09 m^2) necessary to get an accurate representation of the macroinvertebrate community at a given site with a single-habitat approach using riffle habitat. An inventory of taxa occurring in streams precedes the determination of other metrics and the impacts of stressors. This relationship between taxa richness and sampling effort can be explored using taxa accumulation curves (Colwell and Coddington 1994). This exercise has both practical applications for sampling approach and conceptual applications. For instance, taxonomic data can be categorized into functional feeding groups to explore process-oriented ecological questions, such as the Biodiversity-Ecosystem Function relationship (BEF) (Cummins 1974, Palmer et al. 2016, Daam et al. 2019). Related to the BEF is the concept of response diversity (also known as ecological redundancy or the portfolio effect) (Elmqvist et al. 2003, Palmer et al. 2016). Having more taxa and a greater diversity of responses to a stressor within a functional group that contribute to the same ecosystem function imparts greater ecosystem resilience (Elmqvist et al. 2003). The loss of taxa within functional groups decreases response diversity and increases the vulnerability of a functional group to be lost entirely following a stressor such as salinization (Suárez et al. 2017). Therefore, taxa accumulation curves via functional (feeding) groups can identify vulnerable ecosystem processes.

In Chapter 3, I will discuss how salinization resulting from surface coal mining alters macroinvertebrate community composition, biomass, and emergence to then infer impacts to ecosystem functions of the streams in which they live. As larvae and nymphs, benthic macroinvertebrates process organic matter. In the later stages of their life histories, many aquatic insects molt into winged adults that enter adjacent riparian systems, serving as a subsidy in terrestrial food webs (Baxter et al. 2005). The impacts of freshwater salinization on this aquatic-terrestrial linkage are not fully understood. The quantification of insect emergence can tell the magnitude in which this linkage is altered. Across all life stages, aquatic insects perform important functions, whether it be through their acquisition of food (e.g., detritus processing) or serving as food for other organisms (e.g., consumption of larvae or emergent insects), that collectively influence stream processes.

References

Barbour, M. T., J. Gerritsen, B. D. Snyder and J. B. Stribling (1999). Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, Benthic Macroinvertebrates and

Fish, Second Edition. EPA 841-B-99-002. O. o. Water. Washington, D.C., U.S. Environmental Protection Agency.

Bartels, P., J. Cucherousset, K. Steger, P. Eklöv, L. J. Tranvik and H. Hillebrand (2012). "Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics." Ecology **93**(5): 1173-1182.

Baxter, C. V., K. D. Fausch and W. Carl Saunders (2005). "Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones." Freshwater biology **50**(2): 201-220.

Benda, L., M. A. Hassan, M. Church and C. L. May (2005). "Geomorphology of steepland headwaters: the transition from hillslopes to channels." Journal of the American Water Resources Association **41**(4): 835-851.

Benke, A. C. (1993). "Concepts and patterns of invertebrate production in running waters." Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen **25**(1): 15-38.

Berger, E., O. Frör and R. B. Schäfer (2019). "Salinity impacts on river ecosystem processes: a critical mini-review." Philosophical Transactions of the Royal Society B **374**(1764): 20180010.

Bernhardt, E. S. and M. A. Palmer (2011). "The environmental costs of mountaintop mining valley fill operations for aquatic ecosystems of the Central Appalachians." Annals of the New york Academy of Sciences **1223**(1): 39-57.

Boehme, E. A., C. E. Zipper, S. H. Schoenholtz, D. J. Soucek and A. J. Timpano (2016). "Temporal dynamics of benthic macroinvertebrate communities and their response to elevated specific conductance in Appalachian coalfield headwater streams." Ecological Indicators **64**: 171-180.

Buchwalter, D., S. Scheibener, H. Chou, D. Soucek and J. Elphick (2019). "Are sulfate effects in the mayfly Neocloeon triangulifer driven by the cost of ion regulation?" Philosophical Transactions of the Royal Society B **374**(1764): 20180013.

Cañedo-Argüelles, M., B. J. Kefford, C. Piscart, N. Prat, R. B. Schäfer and C.-J. Schulz (2013). "Salinisation of rivers: an urgent ecological issue." Environmental pollution **173**: 157-167.

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran and C. Jouseau (2006). "Effects of biodiversity on the functioning of trophic groups and ecosystems." Nature **443**(7114): 989.

Castillo, A. M., D. M. Sharpe, C. K. Ghalambor and L. F. De León (2018). "Exploring the effects of salinization on trophic diversity in freshwater ecosystems: a quantitative review." Hydrobiologia **807**(1): 1-17.

Cianciolo, T. R., D. L. McLaughlin, C. E. Zipper, A. J. Timpano, D. J. Soucek and S. H. Schoenholtz (2020). "Impacts to water quality and biota persist in mining-influenced Appalachian streams." Science of The Total Environment **717**: 137216.

Clark, E. V., W. L. Daniels, C. E. Zipper and K. Eriksson (2018). "Mineralogical influences on water quality from weathering of surface coal mine spoils." Applied Geochemistry **91**: 97-106.

Colwell, R. K. and J. A. Coddington (1994). "Estimating terrestrial biodiversity through extrapolation." Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **345**(1311): 101-118.

Colwell, R. K., C. X. Mao and J. Chang (2004). "Interpolating, extrapolating, and comparing incidence-based species accumulation curves." Ecology **85**(10): 2717-2727.

Cormier, S., G. Suter, L. Yuan and L. Zheng (2011). A field-based aquatic life benchmark for conductivity in central Appalachian streams, EPA/600/R-10/023F National Center for Environmental Assessment, Office of ….

Cormier, S. M., G. W. Suter and L. Zheng (2013). "Derivation of a benchmark for freshwater ionic strength." Environmental Toxicology Chemistry **32**(2): 263-271.

Cormier, S. M., G. W. Suter, L. Zheng and G. J. Pond (2013). "Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions." Environmental Toxicology Chemistry **32**(2): 277-287.

Creed, I. F., C. R. Lane, J. N. Serran, L. C. Alexander, N. B. Basu, A. J. Calhoun, J. R. Christensen, M. J. Cohen, C. Craft and E. D'Amico (2017). "Enhancing protection for vulnerable waters." Nature geoscience **10**(11): 809-815.

Cummins, K. W. (1974). "Structure and function of stream ecosystems." BioScience **24**(11): 631-641.

Daam, M. A., H. Teixeira, A. I. Lillebø and A. J. Nogueira (2019). "Establishing causal links between aquatic biodiversity and ecosystem functioning: Status and research needs." Science of the Total Environment **656**: 1145-1156.

Darwall, W., K. Smith, D. Allen, M. Seddon, G. Mc Gregor Reid, V. Clausnitzer and V. J. T. Kalkman (2008). "Freshwater biodiversity–a hidden resource under threat." 126-232.

Downing, J. A., J. J. Cole, C. Duarte, J. J. Middelburg, J. M. Melack, Y. T. Prairie, P. Kortelainen, R. G. Striegl, W. H. McDowell and L. J. Tranvik (2012). "Global abundance and size distribution of streams and rivers." Inland waters **2**(4): 229-236.

Drover, D. R., C. E. Zipper, D. J. Soucek and S. H. Schoenholtz (2019). "Using density, dissimilarity, and taxonomic replacement to characterize mining-influenced benthic macroinvertebrate community alterations in central Appalachia." Ecological Indicators **106**: 105535.

Echols, B. S., R. J. Currie and D. S. Cherry (2010). "Preliminary results of laboratory toxicity tests with the mayfly, Isonychia bicolor (Ephemeroptera: Isonychiidae) for development as a standard test organism for evaluating streams in the Appalachian coalfields of Virginia and West Virginia." Environmental monitoring assessment **169**(1-4): 487-500.

Ehrlich, P. R. and E. O. Wilson (1991). "Biodiversity studies: science and policy." Science **253**(5021): 758-762.

Elmqvist, T., C. Folke, M. Nyström, G. Peterson, J. Bengtsson, B. Walker and J. Norberg (2003). "Response diversity, ecosystem change, and resilience." Frontiers in Ecology the Environment **1**(9): 488-494.

Entrekin, S. A., N. A. Clay, A. Mogilevski, B. Howard-Parker and M. A. Evans-White (2018). "Multiple riparian–stream connections are predicted to change in response to salinization." Philosophical Transactions of the Royal Society B **374**(1764): 20180042.

Evans, D. M., C. E. Zipper, E. T. Hester and S. H. Schoenholtz (2015). "Hydrologic effects of surface coal mining in Appalachia (US)." Journal of the American Water Resources Association **51**(5): 1436-1452.

Fritz, K. M., S. Fulton, B. R. Johnson, C. D. Barton, J. D. Jack, D. A. Word and R. A. Burke (2010). "Structural and functional characteristics of natural and constructed channels draining a reclaimed mountaintop removal and valley fill coal mine." Journal of the North American Benthological Society **29**(2): 673-689.

Gladyshev, M. I., E. E. Gladysheva and N. N. Sushchik (2019). "Preliminary estimation of the export of omega-3 polyunsaturated fatty acids from aquatic to terrestrial ecosystems in biomes via emergent insects." Ecological Complexity **38**: 140-145.

Griffith, M. B., S. B. Norton, L. C. Alexander, A. I. Pollard and S. D. LeDuc (2012). "The effects of mountaintop mines and valley fills on the physicochemical quality of stream ecosystems in the central Appalachians: a review." Science of the Total Environment **417**: 1-12.

Haering, K. C., W. L. Daniels and J. M. Galbraith (2004). "Appalachian mine soil morphology and properties." Soil Science Society of America Journal **68**(4): 1315-1325.

Hassell, K. L., B. J. Kefford and D. Nugegoda (2006). "Sub-lethal and chronic salinity tolerances of three freshwater insects: Cloeon sp. and Centroptilum sp.(Ephemeroptera: Baetidae) and Chironomus sp.(Diptera: Chironomidae)." Journal of Experimental Biology **209**(20): 4024-4032.

Hynes, H. B. N. (1975). "The stream and its valley." Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen **19**(1): 1-15.

Jarvis, B. (2018). The insect apocalypse is here. The New York Times. New York City, New York, A.G. Sulzberger.

Johnson, B. R., K. M. Fritz and R. Price (2013). "Estimating benthic secondary production from aquatic insect emergence in streams affected by mountaintop removal coal mining, West

Virginia, USA." Fundamental Applied Limnology/Archiv für Hydrobiologie **182**(3): 191-204.

Johnson, B. R., P. C. Weaver, C. T. Nietch, J. M. Lazorchak, K. A. Struewing and D. H. Funk (2015). "Elevated major ion concentrations inhibit larval mayfly growth and development." Environmental toxicology chemistry **34**(1): 167-172.

Kaushal, S. S. (2016). "Increased Salinization Decreases Safe Drinking Water." Environmental Science & Technology **50**(6): 2765-2766.

Kaushal, S. S., G. E. Likens, M. L. Pace, R. M. Utz, S. Haq, J. Gorman and M. Grese (2018). "Freshwater salinization syndrome on a continental scale." Proceedings of the National Academy of Sciences **115**(4): E574-E583.

Kefford, B. J. (2018). "Why are mayflies (Ephemeroptera) lost following small increases in salinity? Three conceptual osmophysiological hypotheses." Philosophical Transactions of the Royal Society B **374**(1764): 20180021.

Kennedy, A., D. Cherry and R. Currie (2004). "Evaluation of ecologically relevant bioassays for a lotic system impacted by a coal-mine effluent, using Isonychia." Environmental Monitoring Assessment **95**(1-3): 37-55.

Kunz, J. L., J. M. Conley, D. B. Buchwalter, T. J. Norberg-King, N. E. Kemble, N. Wang and C. G. Ingersoll (2013). "Use of reconstituted waters to evaluate effects of elevated major ions associated with mountaintop coal mining on freshwater invertebrates." Environmental Toxicology Chemistry **32**(12): 2826-2835.

Marcarelli, A. M., C. V. Baxter, M. M. Mineau and R. O. Hall Jr (2011). "Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters." Ecology **92**(6): 1215-1225.

Merricks, T. C., D. S. Cherry, C. E. Zipper, R. J. Currie and T. W. Valenti (2007). "Coal-mine hollow fill and settling pond influences on headwater streams in southern West Virginia, USA." Environmental Monitoring Assessment **129**(1-3): 359-378.

Merritt, R., K. Cummins and M. Berg (2008). An Introduction to the Aquatic Insects of North America. Dubuque, Iowa, USA, Kendall Hunt Publishing.

Meyer, J. L., D. L. Strayer, J. B. Wallace, S. L. Eggert, G. S. Helfman and N. E. Leonard (2007). "The Contribution of Headwater Streams to Biodiversity in River Networks 1." Journal of the American Water Resources Association **43**(1): 86-103.

Micheli, F., K. L. Cottingham, J. Bascompte, O. N. Bjørnstad, G. L. Eckert, J. M. Fischer, T. H. Keitt, B. E. Kendall, J. L. Klug and J. A. Rusak (1999). "The dual nature of community variability." Oikos: 161-169.

Murakami, M. and S. Nakano (2001). "Species-specific foraging behavior of birds in a riparian forest." Ecological Research **16**(5): 913-923.

Nippgen, F., M. R. Ross, E. S. Bernhardt and B. L. McGlynn (2017). "Creating a more perennial problem? Mountaintop removal coal mining enhances and sustains saline baseflows of Appalachian watersheds." Environmental science technology **51**(15): 8324-8334.

Odum, E. P., J. T. Finn and E. H. Franz (1979). "Perturbation theory and the subsidy-stress gradient." Bioscience **29**(6): 349-352.

Orndorff, Z. W., W. L. Daniels, C. E. Zipper, M. Eick and M. Beck (2015). "A column evaluation of Appalachian coal mine spoils' temporal leaching behavior." Environmental Pollution **204**: 39-47.

Paetzold, A. and K. Tockner (2005). "Effects of riparian arthropod predation on the biomass and abundance of aquatic insect emergence." Journal of the North American Benthological Society **24**(2): 395-402.

Palmer, M. A., E. S. Bernhardt, W. H. Schlesinger, K. N. Eshleman, E. Foufoula-Georgiou, M. S. Hendryx, A. D. Lemly, G. E. Likens, O. L. Loucks and M. E. Power (2010). "Mountaintop mining consequences." Science **327**(5962): 148-149.

Palmer, M. A., J. B. Zedler and D. A. Falk (2016). Foundations of restoration ecology. Washington, D.C., Island Press.

Pence, R. A. (2019). Comparison of Quantitative and Semi-Quantitative Assessments of Benthic Macroinvertebrate Community Response to Elevated Salinity in central Appalachian Coalfield Streams, Virginia Tech.

Pericak, A. A., C. J. Thomas, D. A. Kroodsma, M. F. Wasson, M. R. Ross, N. E. Clinton, D. J. Campagna, Y. Franklin, E. S. Bernhardt and J. F. Amos (2018). "Mapping the yearly extent of surface coal mining in Central Appalachia using Landsat and Google Earth Engine." PloS one **13**(7): e0197758.

Peterson, G., C. R. Allen and C. S. Holling (1998). "Ecological resilience, biodiversity, and scale." Ecosystems **1**(1): 6-18.

Polis, G. A., W. B. Anderson and R. D. Holt (1997). "Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs." Annual review of ecology systematics **28**(1): 289-316.

Pond, G. (2012). "Biodiversity loss in Appalachian headwater streams (Kentucky, USA): Plecoptera and Trichoptera communities." Hydrobiologia **679**(1): 97-117.

Pond, G. J. (2010). "Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA)." Hydrobiologia **641**(1): 185-201.

Pond, G. J., M. E. Passmore, F. A. Borsuk, L. Reynolds and C. J. Rose (2008). "Downstream effects of mountaintop coal mining: comparing biological conditions using family-and genus-level macroinvertebrate bioassessment tools." Journal of the North American Benthological Society **27**(3): 717-737.

Pond, G. J., M. E. Passmore, N. D. Pointon, J. K. Felbinger, C. A. Walker, K. J. Krock, J. B. Fulton and W. L. Nash (2014). "Long-term impacts on macroinvertebrates downstream of reclaimed mountaintop mining valley fills in central Appalachia." Environmental management **54**(4): 919-933.

Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden and S. J. Ormerod (2019). "Emerging threats and persistent conservation challenges for freshwater biodiversity." Biological Reviews **94**(3): 849-873.

Ross, M. R., B. L. McGlynn and E. S. Bernhardt (2016). "Deep impact: effects of mountaintop mining on surface topography, bedrock structure, and downstream waters." Environmental science technology **50**(4): 2064-2074.

Schmidt, T. S., J. M. Kraus, D. M. Walters and R. B. Wanty (2013). "Emergence flux declines disproportionately to larval density along a stream metals gradient." Environmental science technology **47**(15): 8784-8792.

Singer, P. C. and W. Stumm (1970). "Acidic mine drainage: the rate-determining step." Science **167**(3921): 1121-1123.

Skousen, J. and C. E. Zipper (2014). "Post-mining policies and practices in the Eastern USA coal region." International journal of coal science technology **1**(2): 135-151.

Soberón, J. and J. Llorente (1993). "The use of species accumulation functions for the prediction of species richness." Conservation biology **7**(3): 480-488.

Soucek, D. J. and A. Dickinson (2015). "Full-life chronic toxicity of sodium salts to the mayfly Neocloeon triangulifer in tests with laboratory cultured food." Environmental toxicology chemistry **34**(9): 2126-2137.

Suárez, M., M. Sánchez-Montoya, R. Gómez, M. Arce, R. Del Campo and M. Vidal-Abarca (2017). "Functional response of aquatic invertebrate communities along two natural stress gradients (water salinity and flow intermittence) in Mediterranean streams." Aquatic Sciences **79**(1): 1-12.

Thomas, C. D., T. H. Jones and S. E. Hartley (2019). "“Insectageddon”: A call for more robust data and rigorous analyses." Global change biology **25**(6): 1891-1892.

Timpano, A. J., S. H. Schoenholtz, D. J. Soucek and C. E. Zipper (2015). "Salinity as a limiting factor for biological condition in mining-influenced central Appalachian headwater streams." Journal of the American Water Resources Association **51**(1): 240-250.

Timpano, A. J., S. H. Schoenholtz, D. J. Soucek and C. E. Zipper (2018). "Benthic macroinvertebrate community response to salinization in headwater streams in Appalachia USA over multiple years." Ecological indicators **91**: 645-656.

Timpano, A. J., C. E. Zipper, D. J. Soucek and S. H. Schoenholtz (2018). "Seasonal pattern of anthropogenic salinization in temperate forested headwater streams." Water research **133**: 8-18.

Townsend, P. A., D. P. Helmers, C. C. Kingdon, B. E. McNeil, K. M. de Beurs and K. N. Eshleman (2009). "Changes in the extent of surface mining and reclamation in the

Central Appalachians detected using a 1976–2006 Landsat time series." Remote Sensing of Environment **113**(1): 62-72.

Ugland, K. I., J. S. Gray and K. E. Ellingsen (2003). "The species–accumulation curve and estimation of species richness." Journal of Animal Ecology **72**(5): 888-897.

USEPA (2011). The effects of mountaintop mines and valley fills on aquatic ecosystems of the central Appalachian coalfields.

Vander Vorste, R., A. J. Timpano, C. Cappellin, B. D. Badgley, C. E. Zipper and S. H. Schoenholtz (2019). "Microbial and macroinvertebrate communities, but not leaf decomposition, change along a mining-induced salinity gradient." Freshwater biology **64**(4): 671-684.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing (1980). "The river continuum concept." Canadian journal of fisheries aquatic sciences **37**(1): 130-137.

Wallace, J. B., S. Eggert, J. L. Meyer and J. Webster (1999). "Effects of resource limitation on a detrital-based ecosystem." Ecological monographs **69**(4): 409-442.

Wallace, J. B. and J. R. Webster (1996). "The role of macroinvertebrates in stream ecosystem function." Annual review of entomology **41**(1): 115-139.

Wesner, J. S., J. M. Kraus, T. S. Schmidt, D. M. Walters and W. H. Clements (2014). "Metamorphosis enhances the effects of metal exposure on the mayfly, Centroptilum triangulifer." Environmental science technology **48**(17): 10415-10422.

Williams, W. and J. Sherwood (1994). "Definition and measurement of salinity in salt lakes." International Journal of Salt Lake Research **3**(1): 53-63.

Zipper, C. E., J. A. Burger, J. G. Skousen, P. N. Angel, C. D. Barton, V. Davis and J. A. Franklin (2011). "Restoring forests and associated ecosystem services on Appalachian coal surface mines." Environmental Management **47**(5): 751-765.

CHAPTER 2

Taxon and trait-based sampling curves expand our understanding: A case study in the salinized headwaters of Central Appalachia

Abstract

Many ecosystems are losing biodiversity, raising concern for the services they provide. However, the extent of loss is uncertain, especially for hyperdiverse insects, because of ambiguity associated with measures of diversity. Further study is needed to assess freshwater insect abundances, taxa richness, and diversity because some species are declining at alarming rates while some are increasing. In the central Appalachian region of the U.S., aquatic insect communities have lost diversity in streams with elevated salinity following mining activities. However, the taxa observed are dependent upon sampling effort that could bias diversity-function interpretation. Taxon sampling curves can be used to estimate sampling effort that maximizes the probability of accurate community characterization. We sampled six, first-order streams in the central Appalachian region for benthic macroinvertebrates where we predicted: (1) macroinvertebrate taxa richness, diversity, evenness, and density to be lower in salinized compared to reference streams, thus, predicting (2) our sampling effort needed to capture taxonomic richness in salinized streams would be lower than in reference streams, and (3) the loss of taxa richness in salinized streams would also reduce response diversity as fewer taxa represent select traits or attributes. Taxa richness, diversity, evenness and density did not differ significantly between reference and salinized streams though numerical trends suggest lower taxa richness, evenness, and diversity despite maintained or increasing densities in salinized streams. Sampling effort required to fully characterize macroinvertebrate communities also did not differ between reference and salinized streams, though patchier distributions of macroinvertebrates seem to necessitate greater sampling effort in Appalachian headwaters. Approximately six Surber samples captured 80 percent of theoretical total taxa in each stream. Greater densities and richness of small-bodied, shorter-lived, and fast-developing taxa tended to be sampled from salinized streams. Response diversity depended on the function. Shredder densities were greater in salinized streams but with lower response diversity with only about five shredder taxa predicted in salinized streams relative to eight shredder taxa in reference streams. However, greater sampling efforts may be needed to accurately assess response diversity of select traits, such as for herbivores where a reference model did not perform well, and salinized models suggest that 17 samples were required to characterize 80 percent of the herbivore taxa richness. Taxon and trait-based sampling curves expand our understanding of biodiversity and functional responses to environmental change in these headwater streams.

Introduction

Biodiversity contributes to the function of ecosystems as biomass production, resource use and recycling, decomposition, and modulation of disease (Civitello et al. 2015). Freshwater ecosystems are considered hotspots of biodiversity, supporting 9.5% of Earth’s described species despite covering only 2.3% of the Earth’s surface, but they are disproportionately impacted by anthropogenic activities (Darwall et al. 2008, Reid et al. 2019, Outhwaite et al. 2020). Biodiversity-ecosystem function relationships (BEFs) are changing as ecosystems continue to lose species, raising concern for the services that these systems can provide (Ehrlich and Wilson 1991, Chapin Iii et al. 2000, Daam et al. 2019). There is recent concern for the defaunation of insects with measurably fewer species and individuals encountered than in the past, and phrases such as “Insect Apocalypse” and “Insectageddon” appear in news headlines (Jarvis 2018). Despite the media hype, there is still ambiguity surrounding the status and trends of insects around the world, and further study is needed to assess insect abundances, taxa richness, diversity, and their functions (Thomas et al. 2019).

The enumeration of all taxa within a community directly, especially for hyperdiverse groups such as insects, is not feasible. Taxa are often unevenly distributed and reflect the heterogeneity of their habitats (Downes et al. 2002), necessitating the need for sufficient sampling effort to accurately represent the whole community. Taxa richness is a measure of biodiversity at the habitat level (Colwell and Coddington 1994) and is the most universal community metric used to evaluate environmental change (Courtemanch 1996). However, the measure of taxonomic richness is often underestimated and highly dependent on sample size or sampling effort. This relationship between richness and sample size is rarely considered in biomonitoring (Ramos-Merchante and Prenda 2017). Subsampling can be used with statistical methods to estimate sampling coverage and total richness. Taxon sampling curves that represent richness-sampling effort relationships can provide more comprehensive community characterization and trends in biodiversity by interpolating and sometimes extrapolating taxonomic richness beyond actual sampling effort.

Taxon sampling curves (also known as species accumulation curves or collector's curves) take into account the number and identity of taxa but do not require information on the distribution of individuals among taxa, making the taxon sampling model versatile in handling both abundance-based and incidence-based data input (Colwell and Coddington 1994, Ugland et al. 2003). Sample-based taxon sampling curves that plot the number of samples collected are better equipped in estimating richness of communities with patchier distributions of individuals than individual-based curves that plot the number of individuals and ignore heterogeneity between samples and may overestimate the number of taxa (Ugland et al. 2003). Additionally, sample-based taxon sampling curves relate richness estimates directly to sampling effort units (e.g., the number of Surber samples) allowing for straightforward comparison, interpretation, and applicability to future sampling efforts within the study area, given that the same sampling approach and equipment are used. Comparisons among assemblages, regardless of fixed-count or whole-sample processing, can be misleading without the accompaniment of richness-sampling effort curves to account for differences in unobserved taxa, relative abundances, or the number of individuals counted (Gotelli and Colwell 2001).

Because taxon sampling curves are based on richness measures, they can also be used to measure trait richness or selected traits of taxa, like feeding mode, habit, or other life history characteristics (Bonada et al. 2007). Trait-based approaches characterize the functional composition of macroinvertebrate community responses to environmental change (Poff et al. 2006). Poff et al. (2006) described 59 trait states, including attributes like univoltine, strong swimming ability, gill respiration, clinging habit, etc., belonging to one of four main trait categories: life history, morphology, mobility, and ecology. Having more taxa should result in a greater diversity of community responses to a stressor within a functional group that contribute to the same ecosystem functions (Elmqvist et al. 2003). Therefore, trait-based taxon sampling curves can inform response diversity (also known as ecological redundancy or the portfolio effect) and identify vulnerable ecosystem functions that may be lost entirely with the loss of a functional group (Elmqvist et al. 2003, Palmer et al. 2016). Thus, taxon sampling curves can be used to bridge trends between taxonomic biodiversity and functions using trait designations.

In the contiguous U.S., salinization has impacted 37% of drainage area over the past century with the fastest rates of dissolved-salt increases occurring in the humid, eastern U.S. (Kaushal et al. 2018). Such rises in dissolved ion concentrations have led to losses of aquatic biodiversity (Cañedo-Argüelles et al. 2013). This is the case in the central Appalachian region where surface coal mining is the dominant land use change (Townsend et al. 2009, Pericak et al. 2018), and ion concentrations represented as specific conductance (SC) have been associated with losses of benthic macroinvertebrate richness and diversity and shifts in community composition to more salt-tolerant taxa (Merricks et al. 2007, Pond et al. 2008, Cormier et al. 2013, Johnson et al. 2013, Timpano et al. 2015, Boehme et al. 2016, Timpano et al. 2018, Drover et al. 2019, Vander Vorste et al. 2019). Despite numerous studies documenting changes in aquatic macroinvertebrate communities, the extent of biodiversity loss and its impacts on stream functions are not fully understood.

We aimed to improve the understanding of how macroinvertebrate richness – sampling effort relationships may differ in streams experiencing land use change to assess diversity and functional loss used as stream assessments. We modelled the relationship between sampling effort and the characterization of macroinvertebrate biodiversity and function. To do this, we selected the central Appalachian coalfield region as a case study where mining-induced salinization is a persistent water quality concern (Pond et al. 2014, Cianciolo et al. 2020). We predicted that (1) non-extrapolated or actual macroinvertebrate taxa richness, diversity, evenness, and density to be lower in salinized streams compared to reference streams; (2) sampling effort needed to capture taxonomic richness in salinized streams would be lower than in reference streams; and (3) the reduced taxa richness in salinized streams would result in reduced response diversity as fewer taxa would represent select traits.

Materials and methods

Study area

A high density of first- and second-order streams dissect the mountainous topography and coalfields within the Appalachian Plateaus of Virginia and West Virginia (Fenneman 1938, Strahler 1957) (Figure 2.1). Coal mining is a common land use in the region that causes the accelerated weathering of rock and spoil, leading to elevated levels of ions and conductivities downstream (Timpano et al. 2015). The geology is comprised of Pennsylvanian-age sandstones, siltstones, shales, conglomerate, and many coal beds (Wanless 1946). The headwater stream sites we selected exhibit alkaline mine drainage with elevated SO_4^{2-}, Ca^{2+}, and other associated major ions (Pond et al. 2008, Timpano et al. 2010, Timpano et al. 2018). Streams are comprised of cobble and boulder substrates and are shaded primarily by temperate deciduous or mixed deciduous/coniferous forests. Precipitation occurs throughout the year with annual accumulations around 1200 mm with humid, warm summers and cold winters (Timpano et al. 2018). The sites for this study (Table 2.1) are a subset of streams previously selected by Timpano et al. (2015) where they aimed to isolate salinity as the exposure variable by minimizing confounding effects of other covariates (e.g., good habitat conditions, canopy cover, no residences upstream). All sampling sites were predominantly forested, with upstream watersheds subjected to minimal anthropogenic influence other than coal mining in parts of the watersheds of the salinized streams (Cianciolo et al. 2020). Previous measurements of dissolved Selenium concentrations in streams are reported as means from April 2014 and Summer 2015 (Whitmore et al. 2018; Table 2.1).

Macroinvertebrate richness, density, evenness, and diversity in reference and salinized streams

To characterize macroinvertebrate community richness, density, evenness, and diversity; benthic macroinvertebrates were sampled quantitatively using a 0.09-m^2 Surber stream-bottom sampler (Wildco; Yulee, FL, USA) with a 500-µm mesh, from May 16th, 2017 to June 1st, 2017 by Rachel Pence, a former graduate student of Virginia Tech. Five to ten Surber sample replicates were collected from riffle habitat within a 100-meter reach from each of three reference sites (annual mean SC 31-73 µS/cm) and three salinized sites (annual mean SC ≥1000µS/cm). Only five samples were collected from one stream site (i.e. 1340µS/cm) due to drought and low flow conditions that made sample collection difficult (correspondence with Rachel Pence). Samples were preserved in 95% ethanol in the field and were later decanted and sieved through a 500 µm mesh. Macroinvertebrates were identified to genus or the lowest practicable level using standard identification keys (Merritt et al. 2008) with the exception of Chironomidae (family) and Oligochaeta (subclass).

Taxonomic ambiguity within the community density dataset was addressed using the "distribute parents among children" (DPAC) method adapted from Cuffney et al. (2007). Taxon counts that were less than 15 were not redistributed to children because (1) it is roughly equivalent to ≤10% of individuals in a sample and (2) it lessens the expenditure of time processing numerous small values. Non-distributed, parent classifications were then excluded from analyses as to not bias or inflate taxonomic richness counts.

To test our first prediction, we compared taxa richness, density, evenness and diversity of salinized and reference streams as an initial step in characterizing community differences associated with salinization. Shapiro-Wilk tests showed richness was the only normally distributed variable. Therefore, we performed an ANOVA on richness and Kruskal-Wallis tests on the other community metrics to compare sample means among streams. We compared community metric means of reference streams versus salinized streams using a Student's t-test for richness and Mann-Whitney-Wilcoxon tests for the other non-normally distributed community metrics.

To further characterize differences between stream communities in addressing our first prediction, we performed an indicator species analysis (ISA) that combines two kinds of information: the abundance of a taxon within a group (exclusiveness) and the occurrence of the taxon within a group (faithfulness) (McCune and Grace 2002).

Estimating total taxonomic richness and sampling effort between reference and salinized streams

We used richness-sampling effort relationships to extrapolate a theoretical total taxa richness and sampling effort required to collect nominal percentages, 80 and 90 percent, of theoretical taxa richness (Figure 2.2). The number of samples collected were used as the metric of sampling effort. The abundance-based Chao1 estimator from the R vegan package was used for each sample to estimate the number of novel taxa accumulated with successive sampling effort (Oksanen et al. 2019).

We found that the mechanistic growth model, originally developed for physiological processes, fit our data well and sometimes better than the Michaelis-Menten model, based on low Akaike information criterion (AIC) (Akaike 1974). We fit mechanistic growth models (equation 1) to the Chao estimates for each stream site. An asymptote value was extracted and representative of the theoretical total taxa (observed + unobserved) present within the study site. From the model parameters, we also estimated the number of samples needed to optimize our observations of different taxa in stream riffle habitat. We calculated nominal percentages, 80 and 90 percent, of the asymptote value to represent 80 and 90 percent of the theoretical total taxa within a given study site. Sampling effort estimates were partitioned by stream condition and a

Student’s t-test ($\alpha=0.05$) was used to test for differences between reference and salinized streams.

Mechanistic Growth model: $Y = a * (1-b * e^{(-c * x)})$ (equation 1)

where a is the asymptote (theoretical total taxonomic richness), b is the scale (y-intercept determined by first sample), c is the growth rate (rate at which new, unobserved taxa are encountered in samples), and x refers to the replicate sample number.

We found that we had uneven numbers of viable sample replicates for each of our six study streams ranging from five to ten samples. To address this, we ran two suites of models for comparison purposes among our six streams. One set of models was informed with all possible number of samples per stream (n=5-10) and will henceforth be referred to as the unequal sampling effort models. The other set of models was constrained and informed by only five samples per stream (n=5) and will henceforth be referred to as the equal sampling effort models. The five samples chosen to run equal sampling effort models were selected randomly using a random number generator.

Assessing response diversity in reference and salinized streams

To identify taxa and trait states responding to salinization, we performed non-metric multidimensional scaling (NMDS) using a Bray-Curtis dissimilarity matrix based on non-transformed, taxonomic densities. Similarly, we performed a second NMDS using trait-based macroinvertebrate densities to infer functional changes associated with salinization. Trait designations for macroinvertebrates were based on Poff et al. (2006) nomenclature. NMDS analysis was performed using the R vegan package with the metaMDS function that runs NMDS several times with random starting configurations, scales and rotates the solution, and adds species scores to the configuration as weighted averages (Oksanen et al. 2019).

The NMDS assisted in the selection of trait states on which we tested our third prediction. We created taxon sampling models for herbivore, shredder, and small body size taxa to explore potential differences in theoretical total taxa richness within these traits and infer differences in response diversity among streams. To do this, we parsed out taxa belonging to each trait state for each sample and re-ran the same model methods used to address theoretical total taxa richness. We also compared theoretical total taxa from within trait states to the actual number of taxa belonging to each trait state. We calculated actual total taxa within trait states by averaging counts of taxa belonging to each trait within samples to produce stream mean values. Herbivores were of interest because they include Ephemeroptera taxa that are known to be especially salt-sensitive (Hartman et al. 2005, Pond et al. 2008). We selected for shredder taxa because they are important to the breakdown of leaflitter in headwater stream systems (Vannote et al. 1980), and it has been suggested that they respond to mining-induced salinization (Vander Vorste et al. 2019). We selected for small body size because it is a trait expected to have better resilience after disturbances and vary on disturbance gradients (Bonada et al. 2007, Townsend and Thompson 2007)

Results

Macroinvertebrate richness, density, evenness, and diversity in reference and salinized streams

We processed the total number of samples available for each site: 10 samples for the 31µS/cm, 72µS/cm, and 1061µS/cm streams, nine samples for the 73µS/cm stream, seven samples for the 1045µS/cm stream, and five samples for the 1340µS/cm stream, totaling 50 macroinvertebrate samples for all study streams combined. A total of 95 unique taxa were identified with 16 taxa unique to salinized streams and 31 taxa unique to reference streams. As we predicted, macroinvertebrate richness tended to be lower in salinized streams (maximum = 27 taxa) compared with reference streams (maximum = 34 taxa), but that difference was nominal as mean richness values did not differ statistically between reference and salinized streams (Table 2.2). However, salinized streams had about five fewer taxa than reference streams on average (Table 2.2). Macroinvertebrate densities varied among the six streams (p=0.002; Figure 2.3) and suggested nominally greater macroinvertebrate densities by 60% on average in salinized streams (Table 2.2). Pielou's evenness differed among streams (p<0.001; Figure 2.3) and showed nominally less evenness by about 38% on average in salinized streams relative to reference streams (p=0.1; Table 2.2). Shannon's diversity varied among streams (p<0.001; Figure 2.3) and was nominally less by 43% on average in salinized streams (Table 2.2). Bimodal distributions were apparent for evenness, diversity, and to a lesser extent, total taxa richness (Figure 2.3).

Indicator species analysis identified several taxa that were only sampled in reference streams: *Isonychia, Maccaffertium, Ephemera, Acentrella,* and *Antocha* and only one taxon, *Atherix*, exclusive to salinized streams (A = 1.0; Table 2.3). No taxon was 100 percent faithful to either reference or salinized streams. The most frequently sampled taxon among reference stream samples was *Hexatoma* (B = 0.76), and for salinized stream samples, *Clinocera* (B = 0.62).

Estimating total taxonomic richness and sampling effort between reference and salinized streams

Chao estimates were obtained for each sample and accumulated for each stream (Table 2-A1). Mechanistic growth models were fit to the Chao estimates for both unequal (n=5-10; Figure 2.4) and equal (n=5; Figure 2.5) sampling effort models, and an asymptote value that represents theoretical total taxa richness was found for each stream (Table 2-A2). Theoretical taxa richness ranged from 35 to 63 taxa across sites for the unequal sampling effort models and 26 to 103 for the equal sampling effort models (Table 2-A2). Surprisingly, both the richness minimum and maximum values for theoretical total taxa occurred in salinized stream models for unequal sampling effort models, but this did not hold true for the equal sampling effort models where the maximum value for theoretical total taxa instead occurred for a reference stream (Table 2-A2).

When comparing equal sampling effort models to unequal sampling effort models, we found that theoretical taxa richness estimates (i.e., the asymptotes) differed for most sites (Table 2-A2). For instance, when models were constrained to standardize sample effort input among sites, the asymptotes became greater for two sites (i.e., 31µS/cm and 72µS/cm), lower for two sites (i.e., 73µS/cm and 1045µS/cm), and stayed about the same for the remaining two sites (i.e., 1061µS/cm and 1340µS/cm). The standard error increased if the asymptote increased and the standard error decreased if the asymptote decreased. We concluded that the actual sampling effort input to a model may influence estimates and that the direction of change, increase or decrease, in estimates may not be consistent for sites. To account for the influence associated with unequal sampling effort, we chose to continue our analyses using only the equal sampling effort models.

Using the model parameters, we also estimated the number of samples needed to collect nominal percentages 80% and 90% of different taxa (Table 2.4). On average, reference streams required 6.3 (±3.8 SD) samples while salinized streams required 5.6 (±2.1 SD) samples to collect 80% of theoretical total taxa (Table 2.4). To collect 90% of theoretical taxa in reference streams, 9.2 (±5.6 SD) samples were needed, while salinized streams required 8.6 (±3.3 SD) samples (Table 2.4). We found no difference in sampling effort for attaining either 80% (Student’s t-test; t=0.28, df=3.1, p=0.8) or 90% (Student’s t-test; t=0.16, df=3.3, p=0.9) of theoretical total taxa

(Figure 2.5). Though, on average, reference streams required slightly more sampling effort, nominally, than salinized stream by almost one additional sample.

Assessing response diversity in reference and salinized streams

NMDS revealed compositional differences between macroinvertebrate densities in reference and salinized streams suggesting potential for changes in response diversity (PERMANOVA; df=1, F=7.17, p=0.001; Figure 2.6a). Taxa primarily responsible for this separation were greater densities of *Pteronarcys*, *Oulimnius*, *Psephenus*, and *Drunella* in reference streams and more *Atrichopogon, Leuctra, Clinocera, Optioservus, Cheumatopsyche*, and *Ceratopsyche* in salinized streams (Table 2.5). The ISA also returned taxa in common with our NMDS analysis, including more *Pteronarcys*, *Drunella*, and *Psephenus* in reference streams and more *Ceratopsyche*, *Clinocera*, *Cheumatopsyche*, and *Atrichopogon* in salinized streams.

NMDS also revealed different trait-based macroinvertebrate densities between reference and salinized streams (PERMANOVA; df=1, F=4.17, p=0.013; Figure 2.6b). Shredders were the most highly correlated to streams with greater SC (Table 2.5). Short lifespan, fast seasonal development, and small body size also correlated with streams having the greatest SC (Figure 2.6b). Strong flight, some sessile, and collector-filterer trait states were more closely associated with the community belonging to the intermediate SC (1061µS/cm).

To assess response diversity, we produced taxon sampling models for our streams based on herbivore, shredder, and small-bodied taxa. We wanted to see if the number of taxa within each trait state differed for salinized and reference streams as this might suggest changes in response diversity and, therefore, changes in stream function. Estimates of theoretical taxa richness were based on model asymptote parameters that consisted of accumulated values that were always greater than the respective mean actual taxa richness values (Table 2.6). In two instances, models did not converge on an asymptote, resulting in erroneous parameter values that disallowed for further calculations of sampling effort (i.e., herbivore model for 72µS/cm and shredder model for 1340µS/cm; Table 2.6).

Actual taxa richness within trait states between salinized and reference sites showed no significant differences for any trait state (herbivore: p=0.1, shredder: p=0.1, small body size: p=0.9). Likewise, theoretical taxa richness within trait states between salinized and reference sites also did not differ (herbivore: p=0.4, shredder: p=0.2, small body size: p=0.4). Theoretical taxa richness ranged from about five to 20 for herbivore taxa, four to 13 shredder taxa, and 10 to 36 small-bodied taxa among streams (Table 2.6). On average, about three herbivore and three shredder taxa were collected in reference samples, while about two taxa of each were collected in salinized samples. On average, nearly identical numbers of small-bodied taxa (i.e., about eight small-bodied taxa) were collected in samples, regardless of which streams they were taken from. Estimates of theoretical taxa richness within each trait increased two- to three-fold for reference samples when compared to respective stream means of actual taxa richness. Theoretical richness increased two-fold for shredders, about three-fold for small-bodied taxa, and seven-fold for herbivore taxa in salinized samples when compared to mean actual taxa richness.

It is important to note the greater confidence intervals evident in the salinized taxon sampling models, which was especially pronounced for the herbivore trait state (Figure 2.7). The greater confidence intervals were due to poor model fit, and the resultant estimates of richness and sampling effort were less reliable. However, the poor fit of the model itself was indicative that more sampling effort is needed to produce a more confident model. Thus, we infer that more than 80 percent of total taxa should be characterized to accurately assess the response diversity of trait states nested within the total taxa.

When we calculated the difference between theoretical taxa richness and mean actual richness, we found that there was a greater difference for herbivores and small body size in salinized sites than in reference sites (Table 2.6). That is, models suggested that there were potentially greater number of unobserved herbivore and small-bodied taxa present in salinized sites relative to reference sites. The reverse was true of shredders where a greater difference was observed for reference sites, suggesting that greater sampling effort may return more unobserved shredder taxa. In addition, the estimated sampling effort required to capture nominal percentages of taxa within each trait state was always greater in salinized sites when compared to reference sites (Table 2.6).

Discussion

Our goals were to evaluate macroinvertebrate community responses to salinization in headwater streams and how to characterize community changes with varying sampling effort. We evaluated how freshwater salinization can influence macroinvertebrate communities, the level of sampling effort appropriate for characterizing communities under different levels of salinization, and the potential for differences in macroinvertebrate response diversity among levels of freshwater salinization. We discuss our findings for salinized and reference stream reaches and richness-sampling effort models to examine the efficacy of extensive field sampling.

Macroinvertebrate richness, diversity, and evenness decreased in salinized streams

Salts can have lethal and sublethal effects on macroinvertebrates evidenced by decreased growth rates, delayed development and emergence, and increased mortality (Kennedy et al. 2004, Kunz et al. 2013, Soucek and Dickinson 2015, Buchwalter et al. 2019). In support of our 1st prediction, our findings suggest lower macroinvertebrate richness, evenness, and diversity following mining-induced salinization that manifest from these known physiological responses. Fewer taxa were found in salinized streams, about five fewer on average, and we observed 43% lower Shannon's diversity. Our work, and other studies within the central Appalachians, have documented negative correlations between benthic macroinvertebrate metrics and SC (Pond et al. 2008, Pond et al. 2014, Timpano et al. 2018, Cianciolo et al. 2020). In our same six Appalachian streams, Cianciolo et al. (2020) saw an average richness reduction of nine taxa and a 25% decrease in Shannon's diversity in salinized streams relative to reference streams, based on an eight-year semi-quantitative dataset.

Macroinvertebrate communities experiencing an environmental change may respond less predictably over time as the perturbation increases (Odum et al. 1979). We noted greater variability in macroinvertebrate metrics for our salinized streams relative to reference streams. Pond et al. (2008) found that mining-influenced, salinized streams showed more temporal variability than reference streams in total taxa, Ephemeroptera, Plecoptera, and Trichoptera richness in a study where six West Virginia streams were sampled over a six-to-seven-year period to assess post-mining recovery. Our salinized stream with an annual mean SC of 1061µS/cm was an outlier within our salinized stream group by having taxa richness and diversity greater than other salinized streams and comparable to reference streams, resulting in greater variability in the average taxonomic richness observed among salinized streams (range: 12.3-19.6) compared to the reference streams (20.3-21.7). The assemblage at the 1061µS/cm site had greater diversity than the other two salinized streams and had greater densities of strong flying insects. We speculate the community at the 1061µS/cm site may be maintained by source populations from proximally located tributaries with lower salinity upstream and downstream of our reach that could contribute colonist individuals (e.g., Pond et al. 2014).

Despite the evidenced and supposed physiological and energetic costs of elevated ion concentrations to some aquatic taxa, other taxa are found to be salt-tolerant, persist, and even increase in abundance at some elevated conductivities (Timpano et al. 2018). Because taxa respond differently to ionic strength, they can exhibit a decrease, increase, or hump-shaped optimum in response to increasing conductivity (Cormier et al. 2013). For instance, we observed an average 60% greater macroinvertebrate density in salinized streams relative to reference streams due to greater abundances of *Leuctra, Clinocera, Optioservus, Cheumatopsyche,* and *Ceratopsyche* that have been shown to be salt-tolerant and can be found in other mining-influenced, salinized streams with SCs well over 2,000µS/cm (Cormier et al. 2013). The bimodal response observed in our community metrics may be attributable to the varied and combined responses of macroinvertebrate taxa that make up the community (Figure 2.3). Across a SC gradient of 24-1437µS/cm, Drover et al. (2019) found that overall macroinvertebrate densities did not respond to salinization despite decreases in densities of salt-sensitive taxa, such as Ephemeroptera, that were compensated for by increased densities of other taxa. Similarly to our findings, Drover et al. (2019) found *Leuctra* to increase in density with increasing SC and could comprise up to 85% of sample densities seasonally in high-SC streams. Other indirect salinization effects, such as competitive release, altered food quality, and altered predator-prey and host-pathogen relationships may influence the presence and abundance of macroinvertebrate taxa, such as *Leuctra* proliferation, observed in field studies (Kefford et al. 2004, Entrekin et al. 2018, Timpano et al. 2018, Drover et al. 2019).

Differences in taxonomic composition suggests compensation or bimodal responses (Micheli et al. 1999, Cormier et al. 2013) despite insignificant differences between total metrics. In a study of 24 streams from which our six study sites are subset, Vander Vorste et al. (2019)

found that differences in community composition along their SC gradient of 25–1,383µS/cm was due to taxonomic replacement where salt-tolerant taxa replaced sensitive taxa. Vander Vorste et al. (2019) also found *Leuctra* and *Cheumatopsyche* along with *Diplectrona,* Taeniopterygidae, *Hydropsyche, Allocapnia*, *Rhyacophila*, and *Optioservus* to increase in relative abundance along a mining-induced SC gradient. Plecoptera are often considered to be sensitive to perturbation (Barbour et al. 1999), but a suite of studies have shown the stoneflies *Leuctra* and *Amphinemura* as being relatively salt-tolerant as they can be prevalent in samples from these and other mining-influenced streams (Pond et al. 2008, Cormier et al. 2013, Pond et al. 2014, Timpano et al. 2018). *Amphinemura* were present in all of our streams with the exception of one reference stream (i.e., site of mean SC 72µS/cm), and there were slightly greater (though not significant; t=-0.5, df=3.5, p=0.6) densities of *Amphinemura* in salinized streams (mean: 18 ind./m^2) than in reference streams (11 ind./m^2). The drought conditions observed at the 1340 µS/cm stream may have influenced the availability of riffle habitat and the macroinvertebrate densities and taxa richness present in riffle and pool habitat (Boulton 2003). Data combined from daily summary observations produced by the National Oceanic and Atmospheric Administration (NOAA) showed that Wise county, Virginia, the county in which the 1340 µS/cm stream resides, received 1.36 inches of precipitation four days prior to sampling and 16.55 inches of precipitation during the preceding three months prior to sampling.

Mining-induced salinization is a threat to aquatic biodiversity in central Appalachia (Cormier et al. 2013). The U.S. Environmental Protection Agency (USEPA) developed a field-based benchmark of 300µS/cm to protect 95% of native species for Ecoregions 68, 69, and 70 (USEPA 2011). Three of our study streams fell below this SC benchmark and the other three mining-influenced streams exceeded the SC benchmark by three-fold. The conductivity benchmark was developed using a species sensitivity distribution (SSD) model (Cormier et al. 2013), and we used the SSD to assess the relative efficacy of our actual and theoretical values of total taxa richness (all of our streams are in Ecoregion 69). At approximately 1,150 µS/cm (the average annual mean SC for our three salinized streams), the SSD estimates about 30 percent of macroinvertebrate taxa are likely lost. We calculated the proportion of taxa 'lost' in our salinized streams to be 22 percent when based on average actual taxa richness and 37 percent when based on theoretical total taxa estimates. Therefore, our actual sampling efforts may have underestimated the loss of macroinvertebrate taxa and the impact of salinization on headwaters. Alternatively, the theoretical estimates may provide an upper bound for the number of taxa that may be present or lost. Some other factors that may contribute to differing results between our study and that of Cormier et al. (2013) include their use of many more stream communities (2,210 stream samples) covering a larger study region of West Virginia and their use of semi-quantitative sampling methodologies. Using taxon sampling curves, we were able to provide a measure of uncertainty for assessing biodiversity against a salinization gradient and pre-established models set forth by the USEPA.

Estimated sampling effort needed to capture all taxa did not differ by stream type

Streams where macroinvertebrate communities are characterized by lower evenness will require greater sampling effort to obtain more complete inventories of biodiversity (Thompson and Withers 2003). Contrary to our 2nd prediction, losses of taxa in salinized streams did not result in great differences in estimated sampling effort needed to capture 80% or 90% of theoretical total taxa relative to reference streams. We speculate the reason that the estimated sampling effort to capture nominal percentages of theoretical total taxa did not differ significantly between streams was because of within- and across-stream variability in community evenness. Taxon sampling curves summarize the relationship of both richness and evenness, two aspects of diversity (Olszewski 2004). Our Pielou's evenness results suggested a decreasing trend with increasing SC and greater among-stream variability, which likely influenced the overall shape of taxon sampling curves. While it is intuitive to think that greater taxa richness dictates greater sampling effort to characterize a given community, other metrics such as evenness may be more informative. For example, increased abundances or the dominance of salt-tolerant *Cheumatopsyche, Leuctra*, or *Optioservus* (Cormier et al. 2013) in our salinized sites would increase their probability of being collected in samples. Thus, samples are more likely to contain common taxa that have already been observed and less likely to contain novel taxa.

It might be that salt-sensitive taxa are rarer in streams, occurring in very low abundances, and require more effort to encounter. Li et al. (2001) found that the presence of rare genera (and Chironomidae to tribe) in Oregon streams resulted in taxon sampling curves that did not asymptote even after 50-60 Surber samples. However, we found no difference in the number of singletons (i.e., rare taxa with abundances of one individual) occurring in either reference or salinized stream samples (p=0.7, df=3, t=0.4273), suggesting that unevenness among samples plays a larger role in driving models than the presence of rare taxa occurring in low abundances.

Many macroinvertebrate indices and community metrics are reliant on measures of taxonomic richness, and richness metrics are used widely to assess the integrity of waters in accordance with clean water policies (e.g., the Clean Water Act) and for inferring the functional capacity of waters (e.g., resource availability and decomposition) (Merritt et al. 2008). However, bioassessment for biotic integrity does not necessarily translate into species inventories. Our findings suggest that not all taxa are collected and represented by our actual sampling efforts. On average, one Surber sample collected between 20-47 percent of theoretical total taxa. Headwater streams alone comprise 60 to 80 percent of cumulative stream length and riparian interface in river networks (Benda et al. 2005, Downing et al. 2012). Therefore, stemming from a combination of fiscal responsibility and policy-driven objectives, quantitative inventories of biodiversity are not a direct objective of many study designs.

Response diversity requires more sampling effort than total taxonomic assessments

Changes or shifts in the composition of macroinvertebrate body sizes within stream communities may be indicative of biological response to perturbation and metabolic functions at the individual to ecosystem levels (Brown et al. 2004). Our models suggested greater numbers of unobserved small-bodied taxa may be present in salinized streams (i.e., seven more taxa than reference), although actual small-body-size richness did not differ between the two stream types. Thus, small-bodied macroinvertebrates may be greater in both abundance (Figure 2.6B) and richness (Table 2.6) within salinized streams. These findings support our prediction that small-bodied taxa would be better adapted to salinized or disturbed conditions. The metabolic theory of ecology predicts that body size is related to the bioenergetic variables of organisms and how material and energy are processed at the stream ecosystem level (Brown et al. 2004). Aside from water temperature, body size is one of the most influential factors determining rates of biomass turnover in macroinvertebrate communities, and smaller-bodied macroinvertebrates can result in higher rates of biomass turnover in streams (Huryn and Benke 2007). Despite the important contribution of small-bodied taxa to macroinvertebrate production and food web linkages, small-bodied taxa are often overlooked due to their small size. The use of taxon sampling curves combined with trait-based approaches may aid researchers in addressing questions regarding stream function.

Greater taxa richness should result in a greater diversity of community responses to a stressor within a functional group that contributes to the same ecosystem functions (Elmqvist et al. 2003). While small-bodied taxa impart higher rates of biomass turnover, shredder and herbivore taxa are important to the breakdown and availability of basal resources, such as leaflitter and algae, to other trophic levels (Vannote et al. 1980). There were not large differences in actual numbers of herbivore, shredder, or small-bodied taxa collected in salinized and reference streams but there were in theoretical estimates. Contrary to our 3rd prediction, whether response diversity was greater in salinized or reference streams was dependent on the function in question. For example, our models suggested greater theoretical richness of shredders and, therefore, greater response diversity of macroinvertebrate shredding processes in reference streams. In contrast, our models suggested a greater theoretical richness of herbivore taxa and greater response diversity of herbivory processes in salinized streams despite lower total taxa richness. Herbivore taxon sampling models for salinized streams were produced with less confidence (larger confidence intervals), suggesting that greater actual sampling effort is needed and that herbivores may be characterized by greater unevenness in salinized streams. The soundness of these inferences from our models regarding response diversity are questionable since two trait-based stream models did not successfully converge on an asymptote. It is likely that greater sampling efforts (i.e., more than five Surber samples in this case) are needed to assess the response diversity of stream functions, since trait states are a smaller subset of the community dataset.

Characterizing the taxon sampling model: assumptions and limitations

The taxon sampling model operates under two main assumptions that serve as caveats. First, the Chao estimator has the assumption that taxa collected with abundances of one or two individuals are, in fact, representative of rare taxa, which then informs the estimation of unobserved taxa within a community. The Chao definition of rare taxa is reliant on the low abundances of taxa. Therefore, the presence of vagrant species, - individuals that venture or drift into atypical habitat - , could influence estimates of total taxa. Second, there is the assumption that samples are collected from a community that is closed with unchanging number of species and abundances. Of course, communities tend not to be closed and are, instead, metacommunities that share the movement of species and their distributions (Brown et al. 2011). For instance, macroinvertebrates may drift downstream, travel upstream, or emerge and fly to other stream reaches. The timeframe of sampling is an additional consideration since the temporal occurrences of macroinvertebrate taxa varies and can lead to low abundances and increased rarity of some taxa throughout the year (Resh et al 2005). Macroinvertebrate community response to SC has been shown to vary by sampling month (Drover et al 2019). Over an eight-year timeframe, Cianciolo et al. (2020) found macroinvertebrate metrics to be significantly, negatively correlated with SC for all metrics and all years but found the same correlations to be weaker or not significant for scraper richness and Shannon Diversity in the fall. Therefore, richness-sampling effort relationships may also vary through time. That is, a taxon sampling curve developed from fall samples may differ in size and shape from that of our spring sampling curves presented. Taxon sampling curves should be constructed from samples collected concurrently or within a timeframe of relatively unvarying environmental conditions within a study site to answer the question of “who is here now?” (O'hara 2005).

There are biological and computational factors that influence the shape and output of our sample-based taxon sampling models. Contributing biological factors include the number of taxa and their abundances or densities within the community, since both factors influence the probability of collecting novel taxa (Gotelli & Colwell 2011). Samples or regions with greater taxa richness will result in models with greater asymptotes and estimates of theoretical taxa richness. However, the steepness of the curve or how quickly the curve reaches the asymptote is influenced by the distribution of taxa. For instance, taxa abundances that are evenly distributed result in curves that rise rapidly. Conversely, taxa abundances that are uneven (e.g., prevalence of a few dominant taxa with many rare taxa) result in slowly rising curves since collections will contain common taxa that have already been observed. Contributing computational factors include artifacts of the randomization methods used to obtain mean Chao estimates used to inform the taxon sampling models. Streams with more rare taxa and uneven communities will have greater heterogeneity among samples, resulting in greater variability when Monte Carlo techniques are used for permutations of accumulation models.

Our comparisons among stream assemblages using sample-based taxon sampling models does not directly control for differences in overall abundance between sets of samples. Gotelli and Colwell (2011) describe this discrepancy as a pitfall to avoid in using sample-based curves to compare richness between sample sets, since samples with a greater number of individuals are thought to have a greater number of taxa. One way to address this potential for bias is to re-plot the sample-based curve against an x-axis representing the number of individuals (Gotelli & Colwell 2001). For our study streams, macroinvertebrate densities did vary among assemblages, with slightly greater values observed among salinized streams, though they were not significantly different between reference and salinized stream groups. It is possible that streams from which lower densities of macroinvertebrates were collected were biased towards having lower taxa counts relative to streams from which greater densities and therefore greater number of taxa could be collected. Despite the potential for density-based bias in comparisons among richness counts, there is value in retaining the sample-based x-axis opposed to the individual-based axis since model parameters are related back to sample number, the operational unit of sampling effort.

The taxon sampling models should be interpreted in the context of sampling methodologies elsewhere. There is no one-size-fits-all sampling model for every dataset just as there is no one sampling method well suited for all habitats or study systems. Often, the choice of taxon sampling model or the decision to produce one altogether will be dependent upon context, study objectives, and cost motives. In fact, investigators may test and compare multiple estimators of taxa richness (Melo & Froehlich 2001). Different richness estimators and curve-fitting models will predict different estimates of taxa richness (Melo & Froehlich 2001, Thompson et al 2003). Additionally, in instances where taxa richness is not a reliable indicator of environmental change (e.g., taxonomic replacement occurs), then richness-sampling effort models can be used in conjunction with trait-based approaches to infer response diversity of selected traits and the resiliency of ecosystem processes that are mediated by aquatic macroinvertebrates.

Conclusions

The Appalachian region is revered as a biodiversity hotspot (Collen et al. 2014). However, its legacy of coal mining will remain for decades with streams continuing to exhibit elevated conductivities accompanied by uncertain biotic recovery (Lindberg et al. 2011, Pond et al. 2014, Cianciolo et al. 2020). Land use change threatens aquatic macroinvertebrate biodiversity and abundances, having potential cascading effects on local and downstream ecosystem processes and services. Our results demonstrate that richness-sampling effort models can be used to estimate site-specific taxonomic richness, sampling effort, and the level of community characterization associated with environmental change. However, we faced challenges in applying our taxon sampling models to trait states to assess the response diversity of stream functions because models required greater initial sampling effort. While five samples of initial sampling effort was sufficient in exploring total taxonomic richness of stream macroinvertebrate communities using taxon sampling models, the same level of sampling effort was insufficient for assessing subsets of taxa belonging to functional groups. Taxon sampling models may provide greater resolution towards assessing impairment or recovery following perturbation for macroinvertebrate communities. Effective sampling and estimation procedures will allow for useful biodiversity inventories of aquatic insects as well as declining terrestrial insect populations that better inform the conservation and management of this hyperdiverse group of organisms and the ecosystem processes to which they contribute (Colwell and Coddington 1994). This case study provides further resolution for local and global freshwater biodiversity trends and the role of land use in driving these trends.

References

Akaike, H. (1974). "A new look at the statistical model identification." IEEE transactions on automatic control **19**(6): 716-723.

Benda, L., M. A. Hassan, M. Church and C. L. May (2005). "Geomorphology of steepland headwaters: the transition from hillslopes to channels." Journal of the American Water Resources Association **41**(4): 835-851.

Boehme, E. A., C. E. Zipper, S. H. Schoenholtz, D. J. Soucek and A. J. Timpano (2016). "Temporal dynamics of benthic macroinvertebrate communities and their response to elevated specific conductance in Appalachian coalfield headwater streams." Ecological Indicators **64**: 171-180.

Bonada, N., S. Doledec and B. Statzner (2007). "Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios." Global Change Biology **13**(8): 1658-1671.

Boulton, A. J. (2003). "Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages." Freshwater Biology **48**(7): 1173-1185.

Brown, B. L., C. M. Swan, D. A. Auerbach, E. H. Campbell Grant, N. P. Hitt, K. O. Maloney and C. Patrick (2011). "Metacommunity theory as a multispecies, multiscale framework for studying the influence of river network structure on riverine communities and ecosystems." Journal of the North American Benthological Society **30**(1): 310-327.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage and G. B. West (2004). "Toward a metabolic theory of ecology." Ecology **85**(7): 1771-1789.

Buchwalter, D., S. Scheibener, H. Chou, D. Soucek and J. Elphick (2019). "Are sulfate effects in the mayfly Neocloeon triangulifer driven by the cost of ion regulation?" Philosophical Transactions of the Royal Society B **374**(1764): 20180013.

Cañedo-Argüelles, M., B. J. Kefford, C. Piscart, N. Prat, R. B. Schäfer and C.-J. Schulz (2013). "Salinisation of rivers: an urgent ecological issue." Environmental pollution **173**: 157-167.

Chapin Iii, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala and S. E. Hobbie (2000). "Consequences of changing biodiversity." Nature **405**(6783): 234-242.

Cianciolo, T. R., D. L. McLaughlin, C. E. Zipper, A. J. Timpano, D. J. Soucek and S. H. Schoenholtz (2020). "Impacts to water quality and biota persist in mining-influenced Appalachian streams." Science of The Total Environment **717**: 137216.

Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal and S. Young (2015). "Biodiversity inhibits parasites: broad evidence for the dilution effect." Proceedings of the National Academy of Sciences

112(28): 8667-8671.

Collen, B., F. Whitton, E. E. Dyer, J. E. Baillie, N. Cumberlidge, W. R. Darwall, C. Pollock, N. I. Richman, A. M. Soulsby and M. Böhm (2014). "Global patterns of freshwater species diversity, threat and endemism." Global ecology Biogeography **23**(1): 40-51.

Colwell, R. K. and J. A. Coddington (1994). "Estimating terrestrial biodiversity through extrapolation." Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **345**(1311): 101-118.

Cormier, S. M., G. W. Suter and L. Zheng (2013). "Derivation of a benchmark for freshwater ionic strength." Environmental Toxicology Chemistry **32**(2): 263-271.

Cormier, S. M., G. W. Suter, L. Zheng and G. J. Pond (2013). "Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions." Environmental Toxicology Chemistry **32**(2): 277-287.

Courtemanch, D. L. (1996). "Commentary on the subsampling procedures used for rapid bioassessments." Journal of the North American Benthological Society **15**(3): 381-385.

Cuffney, T., M. D. Bilger and A. Haigler (2007). "Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages." Journal of the North American Benthological Society **26**(2): 286-307.

Daam, M. A., H. Teixeira, A. I. Lillebø and A. J. Nogueira (2019). "Establishing causal links between aquatic biodiversity and ecosystem functioning: Status and research needs." Science of the Total Environment **656**: 1145-1156.

Darwall, W., K. Smith, D. Allen, M. Seddon, G. Mc Gregor Reid, V. Clausnitzer and V. J. T. Kalkman (2008). "Freshwater biodiversity–a hidden resource under threat." 126-232.

Downes, B. J., L. A. Barmuta, P. G. Fairweather, D. P. Faith, M. J. Keough, P. S. Lake, B. D. Mapstone and G. P. Quinn (2002). Monitoring Ecological Impacts: Concepts and Practice in Flowing Waters. New York, New York, USA, Cambridge University Press.

Downing, J. A., J. J. Cole, C. Duarte, J. J. Middelburg, J. M. Melack, Y. T. Prairie, P. Kortelainen, R. G. Striegl, W. H. McDowell and L. J. Tranvik (2012). "Global abundance and size distribution of streams and rivers." Inland waters **2**(4): 229-236.

Drover, D. R., C. E. Zipper, D. J. Soucek and S. H. Schoenholtz (2019). "Using density, dissimilarity, and taxonomic replacement to characterize mining-influenced benthic

macroinvertebrate community alterations in central Appalachia." Ecological Indicators **106**: 105535.

Ehrlich, P. R. and E. O. Wilson (1991). "Biodiversity studies: science and policy." Science **253**(5021): 758-762.

Elmqvist, T., C. Folke, M. Nyström, G. Peterson, J. Bengtsson, B. Walker and J. Norberg (2003). "Response diversity, ecosystem change, and resilience." Frontiers in Ecology the Environment **1**(9): 488-494.

Entrekin, S. A., N. A. Clay, A. Mogilevski, B. Howard-Parker and M. A. Evans-White (2018). "Multiple riparian–stream connections are predicted to change in response to salinization." Philosophical Transactions of the Royal Society B **374**(1764): 20180042.

Fenneman, N. M. (1938). Physiography of Eastern United States. McGraw-Hill, New York.

Gotelli, N. J. and R. K. Colwell (2001). "Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness." Ecology letters **4**(4): 379-391.

Hartman, K. J., M. D. Kaller, J. W. Howell and J. A. Sweka (2005). "How much do valley fills influence headwater streams?" Hydrobiologia **532**(1-3): 91.

Huryn, A. D. and A. C. Benke (2007). Relationship between biomass turnover and body size for stream communities. Body size: the structure and function of aquatic ecosystems. A. G. Hildrew, D. G. Raffaelli and R. Edmunds-Brown. Cambridge, New York, Cambridge University Press**:** 55-76.

Jarvis, B. (2018). The insect apocalypse is here. The New York Times. New York City, New York, A.G. Sulzberger.

Johnson, B. R., K. M. Fritz and R. Price (2013). "Estimating benthic secondary production from aquatic insect emergence in streams affected by mountaintop removal coal mining, West

Virginia, USA." Fundamental Applied Limnology/Archiv für Hydrobiologie **182**(3): 191-204.

Kaushal, S. S., G. E. Likens, M. L. Pace, R. M. Utz, S. Haq, J. Gorman and M. Grese (2018). "Freshwater salinization syndrome on a continental scale." Proceedings of the National Academy of Sciences **115**(4): E574-E583.

Kefford, B. J., P. J. Papas, L. Metzeling and D. Nugegoda (2004). "Do laboratory salinity tolerances of freshwater animals correspond with their field salinity?" Environmental Pollution **129**(3): 355-362.

Kennedy, A., D. Cherry and R. Currie (2004). "Evaluation of ecologically relevant bioassays for a lotic system impacted by a coal-mine effluent, using Isonychia." Environmental Monitoring Assessment **95**(1-3): 37-55.

Kunz, J. L., J. M. Conley, D. B. Buchwalter, T. J. Norberg-King, N. E. Kemble, N. Wang and C. G. Ingersoll (2013). "Use of reconstituted waters to evaluate effects of elevated major ions associated with mountaintop coal mining on freshwater invertebrates." Environmental Toxicology Chemistry **32**(12): 2826-2835.

Li, J., A. Herlihy, W. Gerth, P. Kaufmann, S. Gregory, S. Urquhart and D. P. Larsen (2001). "Variability in stream macroinvertebrates at multiple spatial scales." Freshwater Biology **46**(1): 87-97.

Lindberg, T. T., E. S. Bernhardt, R. Bier, A. Helton, R. B. Merola, A. Vengosh and R. T. Di Giulio (2011). "Cumulative impacts of mountaintop mining on an Appalachian watershed." Proceedings of the National Academy of Sciences **108**(52): 20929-20934.

McCune, B. and J. B. Grace (2002). Analysis of ecological communities. Gleneden Beach, Oregon, MjM Software Design.

Merricks, T. C., D. S. Cherry, C. E. Zipper, R. J. Currie and T. W. Valenti (2007). "Coal-mine hollow fill and settling pond influences on headwater streams in southern West Virginia, USA." Environmental Monitoring Assessment **129**(1-3): 359-378.

Merritt, R., K. Cummins and M. Berg (2008). An Introduction to the Aquatic Insects of North America. Dubuque, Iowa, USA, Kendall Hunt Publishing.

Micheli, F., K. L. Cottingham, J. Bascompte, O. N. Bjørnstad, G. L. Eckert, J. M. Fischer, T. H. Keitt, B. E. Kendall, J. L. Klug and J. A. Rusak (1999). "The dual nature of community variability." Oikos: 161-169.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs and H. Wagne (2019). vegan: Community Ecology Package.

Olszewski, T. D. (2004). "A unified mathematical framework for the measurement of richness and evenness within and among multiple communities." Oikos **104**(2): 377-387.

Outhwaite, C. L., R. D. Gregory, R. E. Chandler, B. Collen and N. J. Isaac (2020). "Complex long-term biodiversity change among invertebrates, bryophytes and lichens." Nature Ecology Evolution **4**(3): 384-392.

Palmer, M. A., J. B. Zedler and D. A. Falk (2016). Foundations of restoration ecology. Washington, D.C., Island Press.

Pericak, A. A., C. J. Thomas, D. A. Kroodsma, M. F. Wasson, M. R. Ross, N. E. Clinton, D. J. Campagna, Y. Franklin, E. S. Bernhardt and J. F. Amos (2018). "Mapping the yearly extent of surface coal mining in Central Appalachia using Landsat and Google Earth Engine." PloS one **13**(7): e0197758.

Poff, N. L., J. D. Olden, N. K. Vieira, D. S. Finn, M. P. Simmons and B. C. Kondratieff (2006). "Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships." Journal of the North American Benthological Society

25(4): 730-755.

Pond, G. J., M. E. Passmore, F. A. Borsuk, L. Reynolds and C. J. Rose (2008). "Downstream effects of mountaintop coal mining: comparing biological conditions using family-and genus-level macroinvertebrate bioassessment tools." Journal of the North American Benthological Society **27**(3): 717-737.

Pond, G. J., M. E. Passmore, N. D. Pointon, J. K. Felbinger, C. A. Walker, K. J. Krock, J. B. Fulton and W. L. Nash (2014). "Long-term impacts on macroinvertebrates downstream of reclaimed mountaintop mining valley fills in central Appalachia." Environmental management **54**(4): 919-933.

Ramos-Merchante, A. and J. Prenda (2017). "Macroinvertebrate taxa richness uncertainty and kick sampling in the establishment of Mediterranean rivers ecological status." Ecological Indicators **72**: 1-12.

Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden and S. J. Ormerod (2019). "Emerging threats and persistent conservation challenges for freshwater biodiversity." Biological Reviews **94**(3): 849-873.

Soucek, D. J. and A. Dickinson (2015). "Full-life chronic toxicity of sodium salts to the mayfly Neocloeon triangulifer in tests with laboratory cultured food." Environmental toxicology chemistry **34**(9): 2126-2137.

Strahler, A. N. (1957). "Quantitative analysis of watershed geomorphology." Eos, Transactions American Geophysical Union **38**(6): 913-920.

Thomas, C. D., T. H. Jones and S. E. Hartley (2019). "“Insectageddon”: A call for more robust data and rigorous analyses." Global change biology **25**(6): 1891-1892.

Thompson, G. G. and P. C. Withers (2003). "Effect of species richness and relative abundance on the shape of the species accumulation curve." Austral Ecology **28**(4): 355-360.

Timpano, A. J., S. H. Schoenholtz, D. J. Soucek and C. E. Zipper (2015). "Salinity as a limiting factor for biological condition in mining-influenced central Appalachian headwater streams." Journal of the American Water Resources Association **51**(1): 240-250.

Timpano, A. J., S. H. Schoenholtz, D. J. Soucek and C. E. Zipper (2018). "Benthic macroinvertebrate community response to salinization in headwater streams in Appalachia USA over multiple years." Ecological indicators **91**: 645-656.

Timpano, A. J., S. H. Schoenholtz, C. E. Zipper and D. J. Soucek (2010). Isolating effects of total dissolved solids on aquatic life in central Appalachian coalfield streams. Proc. 2010 national meeting of the american society of mining and reclamation, pittsburgh, PA.

Timpano, A. J., C. E. Zipper, D. J. Soucek and S. H. Schoenholtz (2018). "Seasonal pattern of anthropogenic salinization in temperate forested headwater streams." Water research **133**: 8-18.

Townsend, C. R. and R. M. Thompson (2007). Body size in streams: macroinvertebrate community size composition along natural and human-induced environmental gradients. Body size: The structure function of aquatic ecosystems. A. G. Hildrew, D. G. Raffaelli and R. Edmonds-Brown. Cambridge, New York, Cambridge University Press**:** 77-97.

Townsend, P. A., D. P. Helmers, C. C. Kingdon, B. E. McNeil, K. M. de Beurs and K. N. Eshleman (2009). "Changes in the extent of surface mining and reclamation in the Central Appalachians detected using a 1976–2006 Landsat time series." Remote Sensing of Environment **113**(1): 62-72.

Ugland, K. I., J. S. Gray and K. E. Ellingsen (2003). "The species–accumulation curve and estimation of species richness." Journal of Animal Ecology **72**(5): 888-897.

USEPA (2011). The effects of mountaintop mines and valley fills on aquatic ecosystems of the central Appalachian coalfields.

Vander Vorste, R., A. J. Timpano, C. Cappellin, B. D. Badgley, C. E. Zipper and S. H. Schoenholtz (2019). "Microbial and macroinvertebrate communities, but not leaf decomposition, change along a mining-induced salinity gradient." Freshwater biology **64**(4): 671-684.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing (1980). "The river continuum concept." Canadian journal of fisheries aquatic sciences **37**(1): 130-137.

Wanless, H. R. (1946). Pennsylvanian geology of a part of the southern Appalachian coal field, Geological Society of America.

Whitmore, K. M., S. H. Schoenholtz, D. J. Soucek, W. A. Hopkins and C. E. Zipper (2018). "Selenium dynamics in headwater streams of the central Appalachian coalfield." Environmental toxicology chemistry **37**(10): 2714-2726.

Figures and Tables

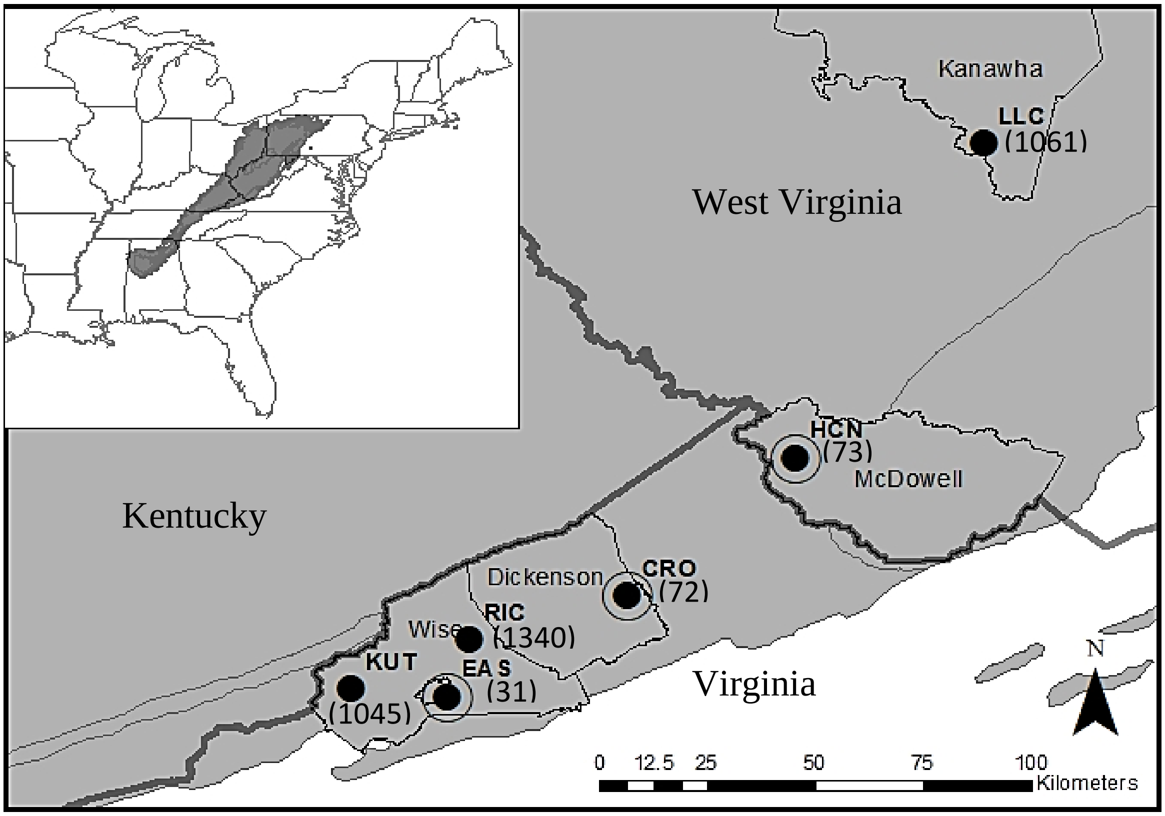
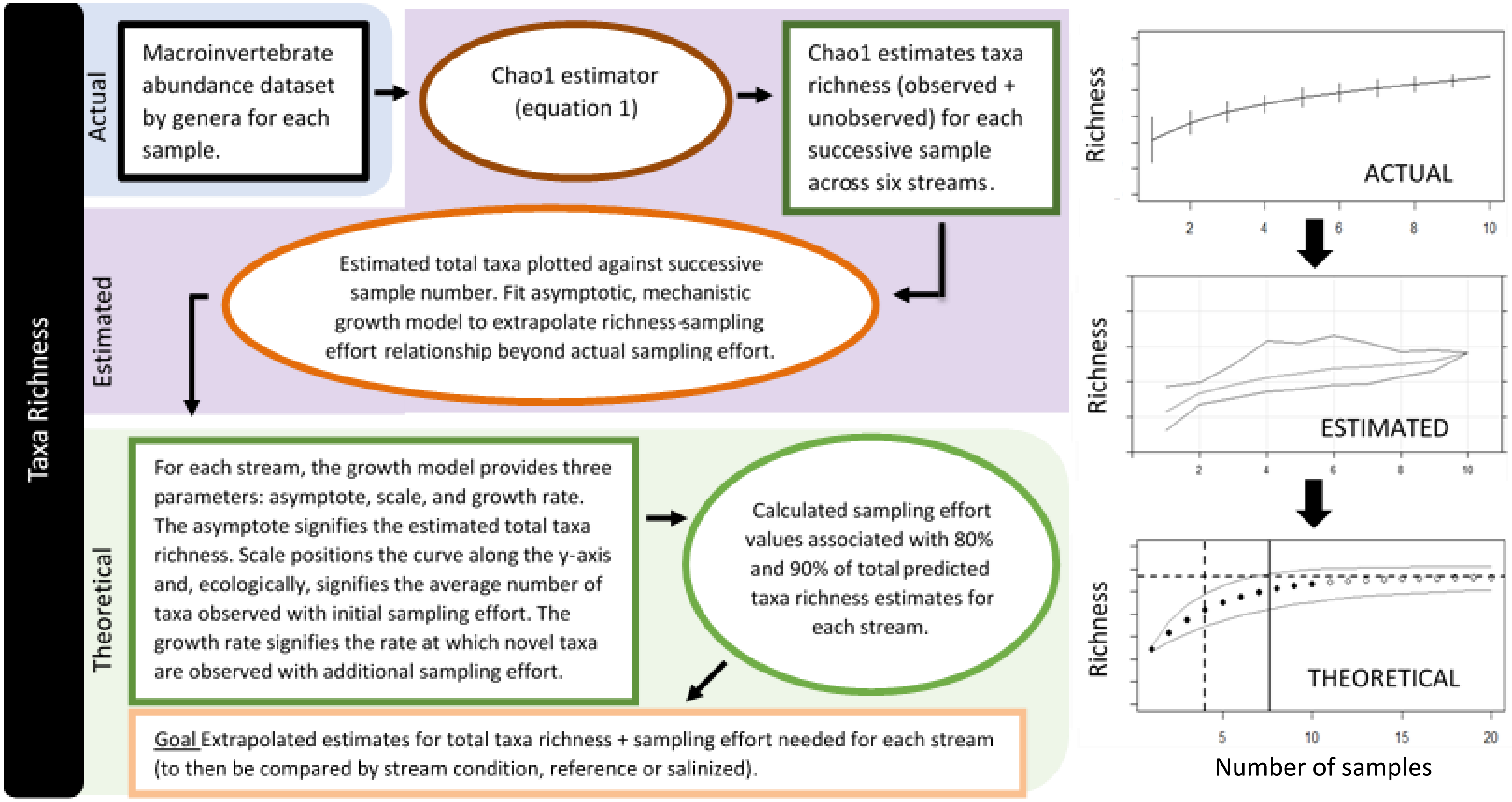


Figure 2. 1. Location of six headwater streams selected for study spanning a conductance gradient from surface mining in southwestern Virginia and southern West Virginia. Annual mean SC is shown in parentheses. Streams with the lowest conductance and without past mining in their catchment are circled. The Appalachian coalfield region is indicated by the gray shading. State boundaries are indicated by thick lines and county boundaries are indicated by thin lines.



Macroinvertebrate abundance dataset by genera for each sample.

Chao1 estimator (equation 1)

Chao1 estimates taxa richness (observed + unobserved) for each successive sample across six streams.

Estimated total taxa plotted against successive sample number. Fit asymptotic, mechanistic growth model to extrapolate richness-sampling effort relationship beyond actual sampling effort.

For each stream, the growth model provides three parameters: asymptote, scale, and growth rate. The asymptote signifies the estimated total taxa richness. Scale positions the curve along the y-axis and, ecologically, signifies the average number of taxa observed with initial sampling effort. The growth rate signifies the rate at which novel taxa are observed with additional sampling effort.

Calculated sampling effort values associated with 80% and 90% of total predicted taxa richness estimates for each stream.

Goal Extrapolated estimates for total taxa richness + sampling effort needed for each stream (to then be compared by stream condition, reference or salinized).

Figure 2. 2. The process used to produce taxon sampling curves in determining the expected sampling effort required to collect theoretical total taxonomic richness in streams. Squares indicate text describing a set of values or dataframe, while circles indicate data transformations. Actual taxa richness was recorded from actual sampling effort. Estimated taxa richness was produced from the Chao estimator that relies on taxa abundances within samples to estimate the number of observed + unobserved taxa given actual sampling effort. Theoretical taxa richness relies on the Mechanistic Growth model to extrapolate taxa richness estimates beyond actual sampling effort based on Chao richness mean estimates. Within the theoretical model (bottom, right), the theoretical total taxa value (horizontal line), sampling effort corresponding with 80% of theoretical total taxa (dashed, vertical line), and sampling effort corresponding with 90% of theoretical total taxa (solid, vertical line) are shown.

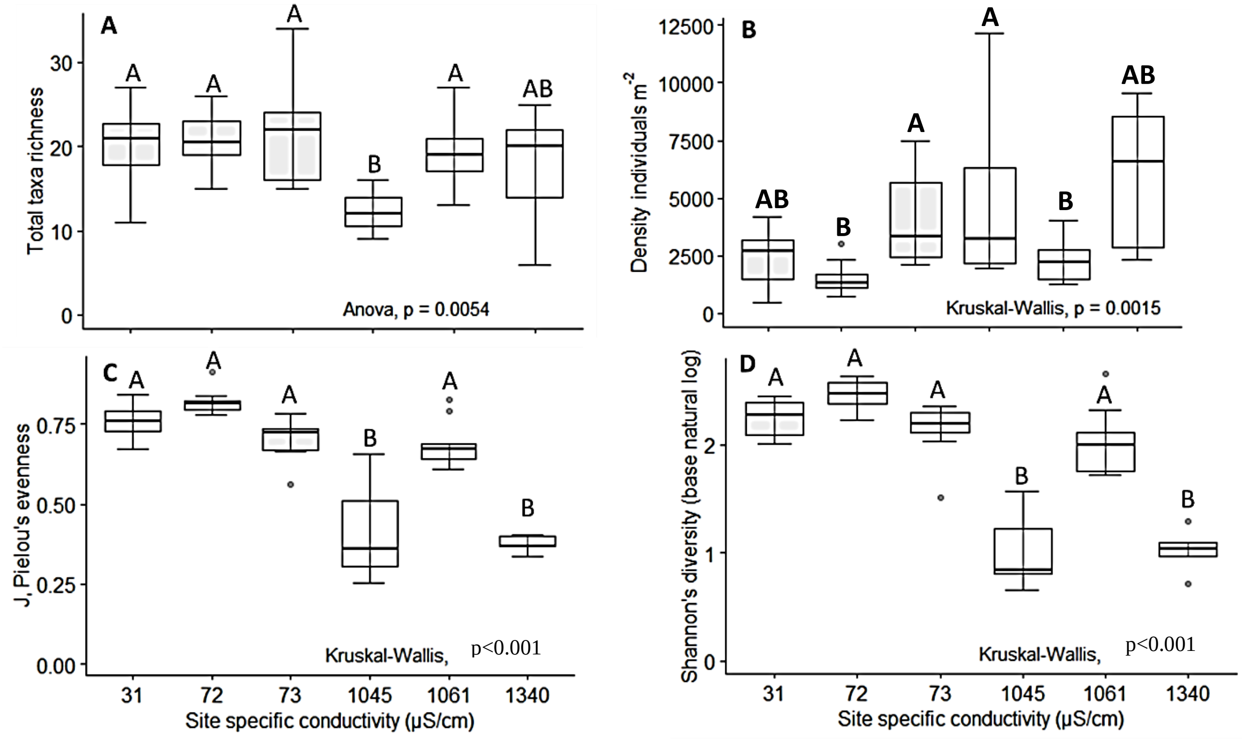


Figure 2. 3. Box plots of A) total taxa richness, B) density, C) evenness, and D) diversity for six streams using all samples per stream (n=5-10). Lower and upper box boundaries are 25th and 75th percentiles, line inside box is median, lower and upper whiskers are 10th and 90th percentiles, and filled circles are data points outside the 10^{th} and 90th percentiles. Streams with different letters above the boxplot are significantly different from one another ($p < 0.05$; pairwise Student's t-test/Mann-Whitney Wilcoxon with Holm–Bonferroni correction).

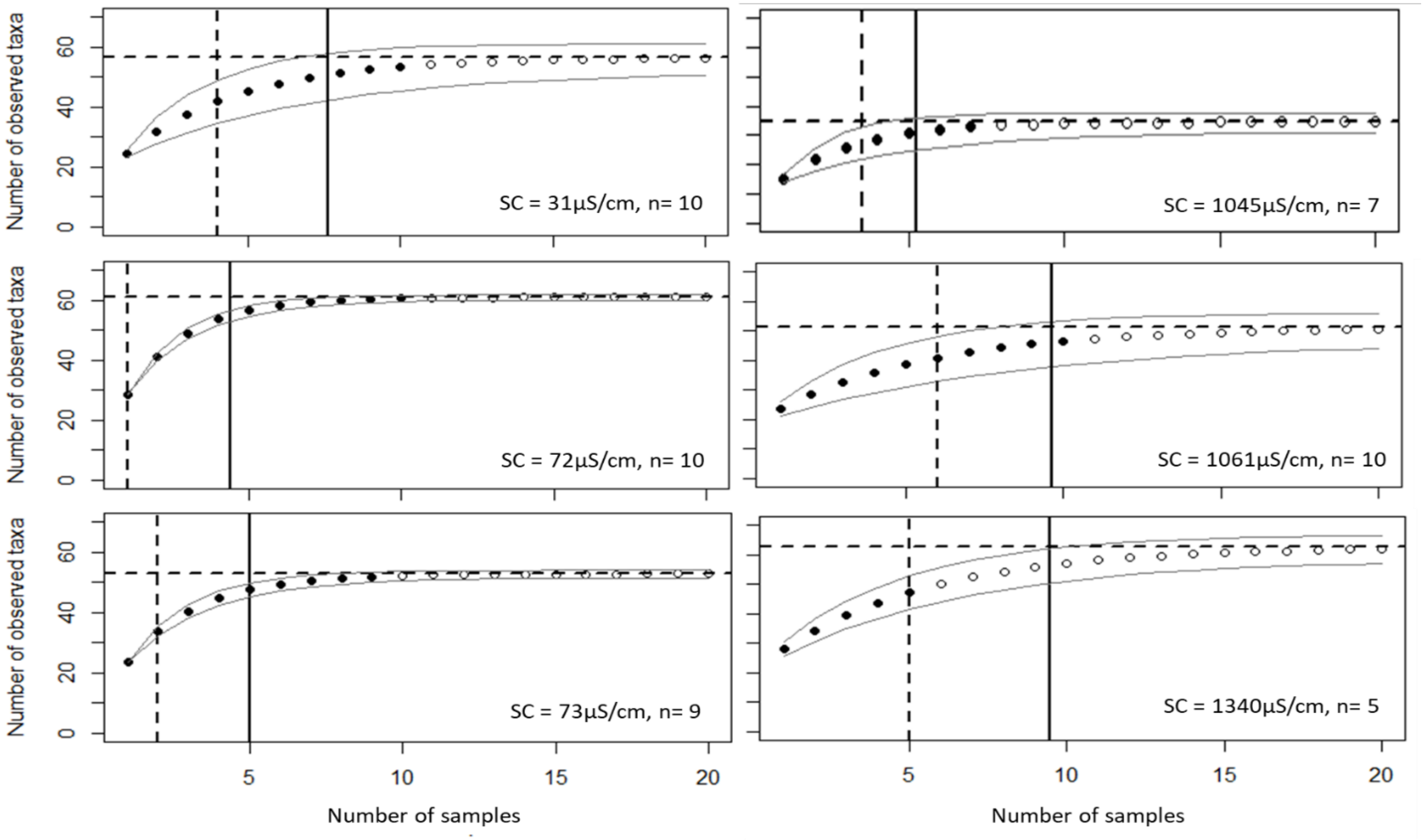


Figure 2. 4. Taxon sampling curves showing the projected number of taxa to be observed given the number of samples collected. Mechanistic growth models were fitted to Chao estimates for each stream. Black points indicate the level of actual sampling effort while empty points indicate the extrapolated theoretical sampling effort. The horizontal dashed line indicates the asymptote representing the theoretical total taxa. Vertical lines indicate the extrapolated number of samples to capture nominal percentages 80% (dashed line) and 90% (solid line) of the theoretical total taxa within a given stream. Gray lines indicate lower and upper 95% confidence intervals of model. Estimated sampling effort did not differ significantly for reference and salinized streams for either 80% (p=0.26, t=-1.34, df=3.6) or 90% estimates (p=0.25, t=-1.39, df=3.5).

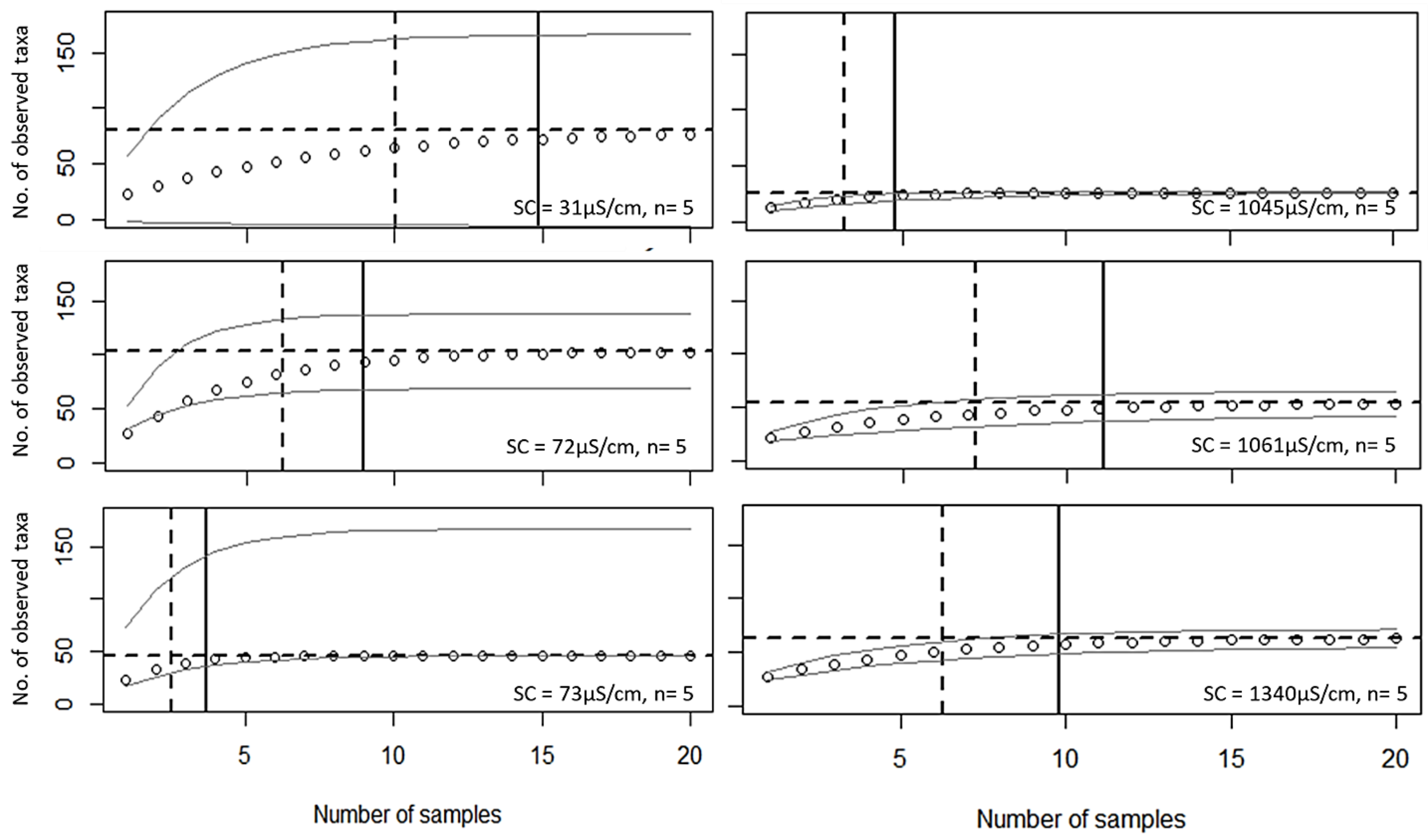


Figure 2. 5. Equal sampling effort taxon sampling models constrained to five replicate samples for each stream, showing extrapolated theoretical taxa richness and associated sampling effort. The horizontal dashed line is the asymptote representing the theoretical total taxa. Vertical lines indicate the extrapolated number of samples to capture nominal percentages 80% (dashed line) and 90% (solid line) of the theoretical total taxa within a given stream. Gray lines indicate lower and upper 95% confidence intervals of model. Estimated sampling effort did not differ significantly for reference and salinized streams for either 80% (p=0.8, t=0.28, df=3.1) or 90% estimates (p=0.9, t=0.16, df=3.3). Note that y-axis values differ from those of Figure 4.

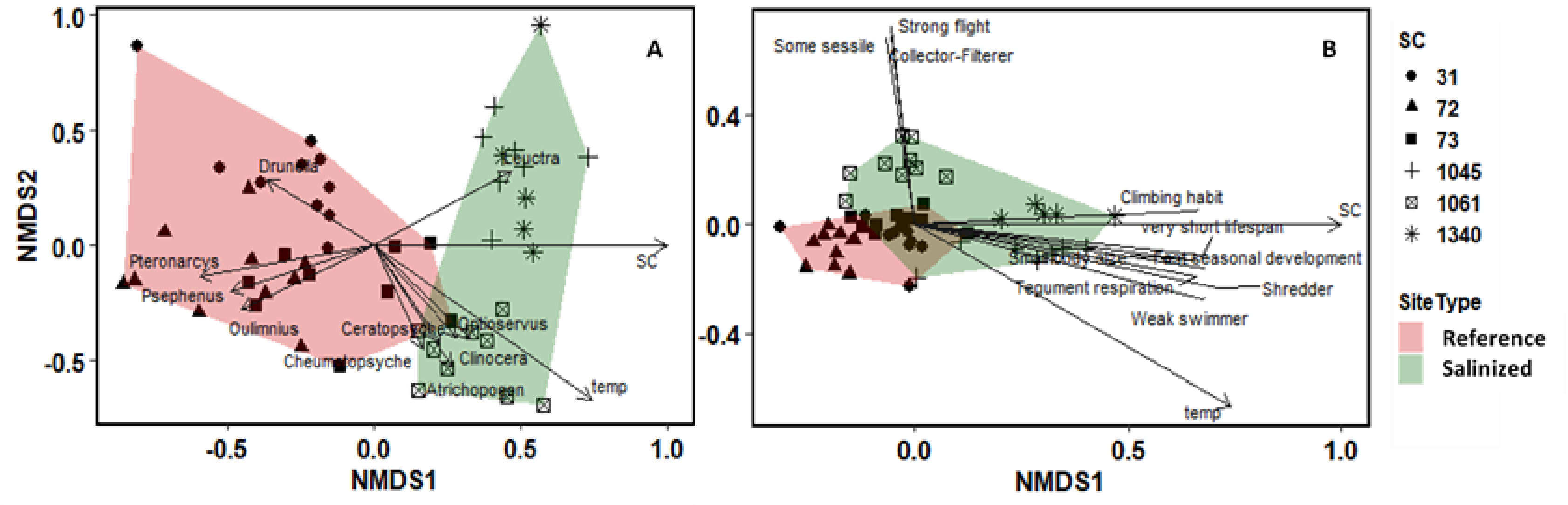


Figure 2. 6. Non-metric multidimensional scaling (NMDS) ordination using Bray-Curtis dissimilarity matrix of (a) macroinvertebrate taxonomic density (3 dimensions, stress level = 0.16) and (b) macroinvertebrate trait density (2 dimensions, stress level = 0.12). Taxonomic density composition was significantly different between reference and salinized streams (PERMANOVA; df=1, F=7.17, R^2=0.13, p=0.001). Trait density composition differed significantly between reference and salinized streams (PERMANOVA; df=1, F=4.17, R^2=0.08, p=0.013). Each point is a sample collected in Spring 2017. Top ten taxa with highest r squared (highest correlation) with ordination are overlain with axes. Mean SC (r^2=0.71, p=0.001) and mean temperature (r^2=0.28, p=0.001) environmental vectors, calculated from daily averages over the course of the preceding year, are overlain to show correlation with community composition.

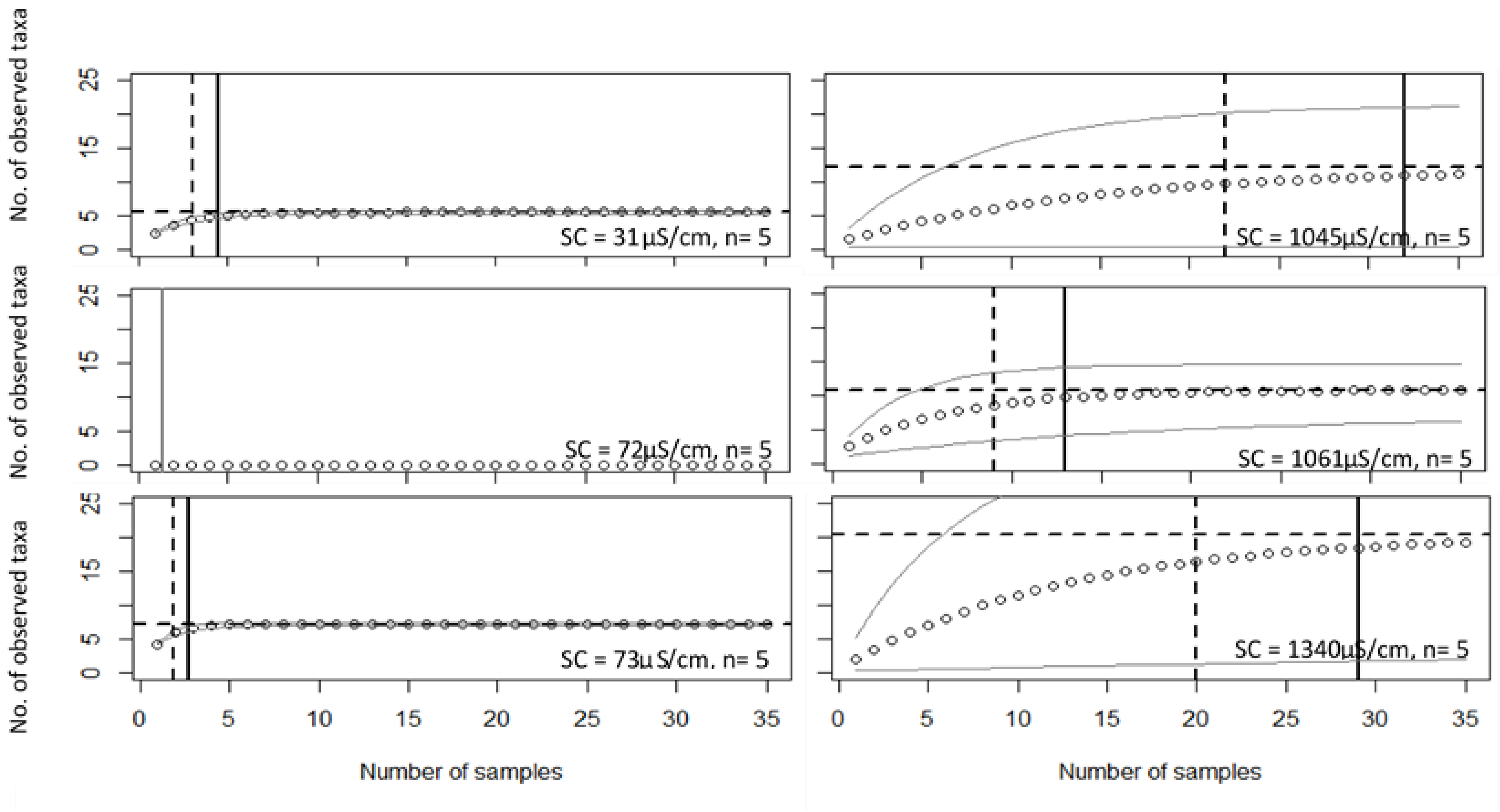


Figure 2. 7. Taxon sampling models for herbivores for each stream as well as the estimated sampling effort required to obtain herbivore richness. Mechanistic growth models were fitted to Chao estimates for each stream. The horizontal dashed line is the asymptote representing the estimated total taxa. Vertical lines indicate the extrapolated number of samples to capture nominal percentages 80% (dashed line) and 90% (solid line) of the estimated total taxa within a given stream. Gray lines indicate lower and upper 95% confidence intervals of model. Note that the model for one stream (72 µS/cm) did not converge, resulting in a displayed asymptote of zero.

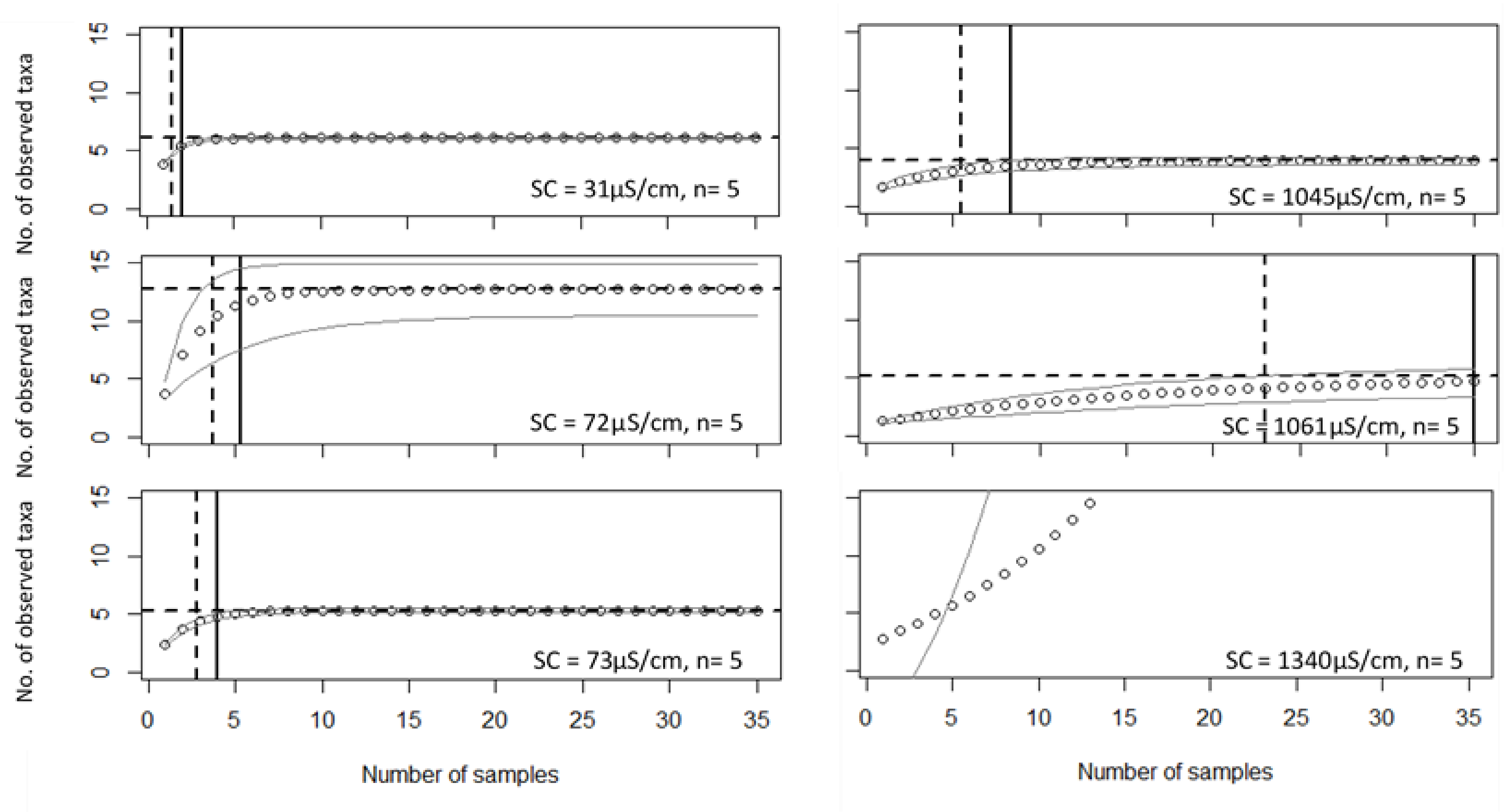


Figure 2. 8. Taxon sampling models for shredder taxa showing theoretical total taxa richness or, in other words, the response diversity of the shredder trait state within each stream as well as the estimated sampling effort required to obtain shredder richness. Mechanistic growth models were fitted to Chao estimates for each stream. The horizontal dashed line is the asymptote representing the estimated total taxa. Vertical lines indicate the extrapolated number of samples to capture nominal percentages 80% (dashed line) and 90% (solid line) of the estimated total taxa within a given stream. Gray lines indicate lower and upper 95% confidence intervals of model.

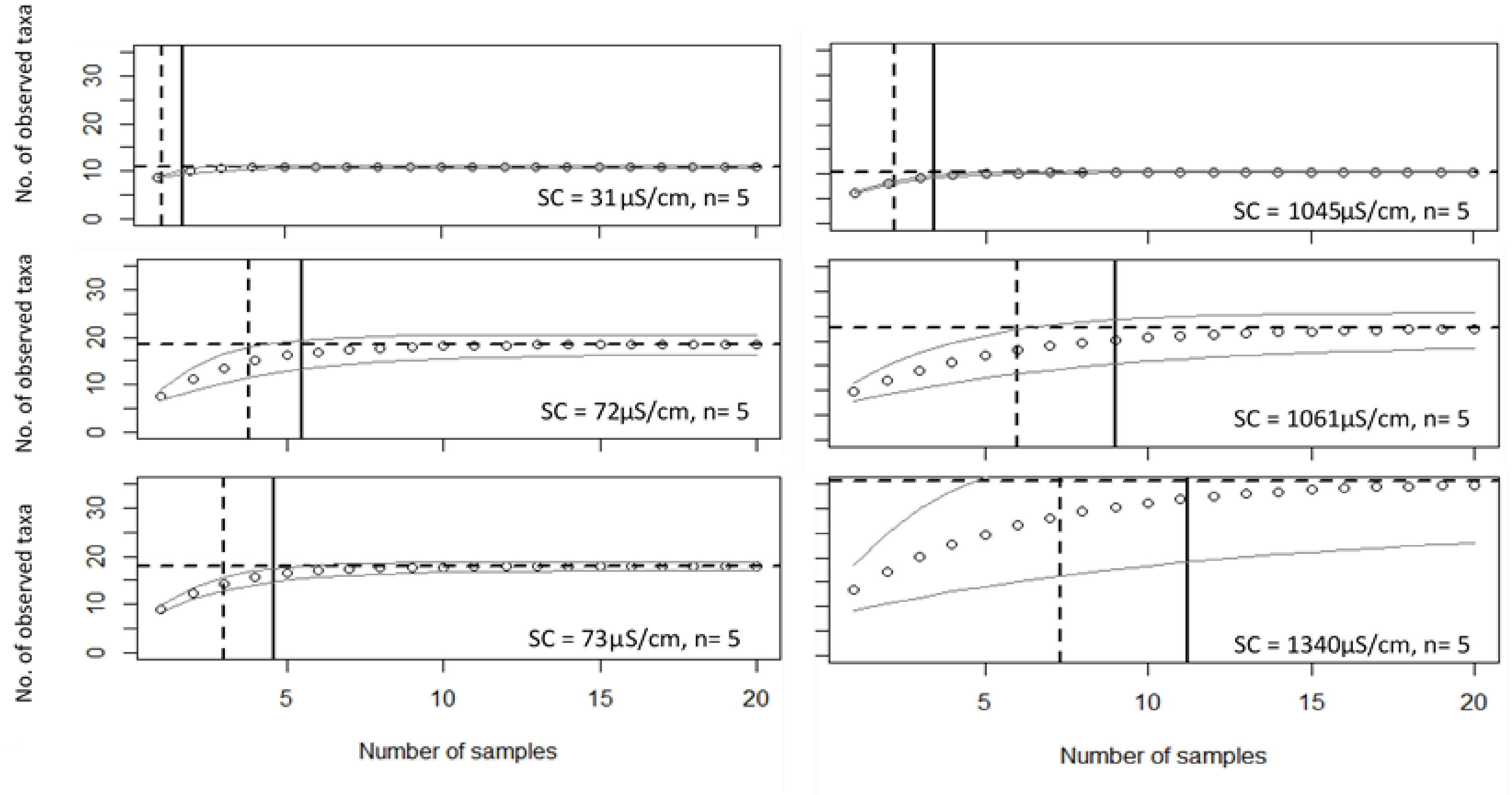


Figure 2. 9. Taxon sampling models for small-bodied taxa showing theoretical total taxa richness or, in other words, the response diversity of the small body size trait state within each stream as well as the estimated sampling effort required to obtain richness of small-bodied taxa. Mechanistic growth models were fitted to Chao estimates for each stream. The horizontal dashed line is the asymptote representing the estimated total taxa. Vertical lines indicate the extrapolated number of samples to capture nominal percentages 80% (dashed line) and 90% (solid line) of the estimated total taxa within a given stream. Gray lines indicate lower and upper 95% confidence intervals of model.

Table 2. 1. Stream site attributes. Annual mean specific conductance (SC) and annual mean stream temperature were calculated using daily means from one year prior to macroinvertebrate sampling and are followed by standard deviation. Also shown are water column selenium concentrations and watershed area. Selenium (Se) data are means of April 2014 and Summer 2015 Se concentrations in stream water. R = reference. T = treatment (i.e. salinized).

Stream	Site code	Stream type	Lat (N)	Long (W)	SC (µS/cm)	Temp (C)	Se (µg/L)	Watershed area (km^2)
Eastland Creek	EAS	R	36.91766	-82.592	31 (±20.4)	13.1 (±4.1)	<2.5	2.38
Crooked Branch	CRO	R	37.13019	-82.2179	72 (±23.9)	13.1 (±5.8)	<2.5	2.27
Hurricane Fork	HCN	R	37.42064	-81.8663	73 (±33.1)	12.5 (±5.3)	<2.5	5.93
Kelly Branch - Unnamed Tributary	KUT	T	36.93574	-82.7926	1045 (±131.5)	12.9 (±2.1)	8.4	1.09
Left Fork/Laurel Fork/Coal Fork	LLC	T	38.08411	-81.476	1061 (±324.4)	14.3 (±5.8)	14.5	4.17
Rickey Branch	RIC	T	37.0373	-82.5458	1340 (±354.7)	13.8 (±5.5)	<2.5	4.22

Table 2. 2. Mean macroinvertebrate taxonomic richness, density, Shannon diversity, and Pielou's evenness values summarized by stream site and stream type. Mean values are followed by standard deviation and range in parenthesis. The number of replicate samples used to calculate stream means are designated by n. SC= annual mean specific conductance. No significant differences are present between salinized and reference stream types (Student's t-test/ Mann-Whitney-Wilcoxon; p>0.05).

SC (µS cm^{-1})	**n**	**Stream type**	**Taxonomic richness**	**Density (indiv. m^{-2})**	**Shannon diversity**	**Pielou's evenness**
31	10	reference	20.3 ±4.7 (11 -27)	2424.0 ±1157.9 (452 - 4187)	2.3 ±0.2 (2.0 - 2.5)	0.8 ±0.05 (0.7 - 0.8)
72	10	reference	20.8 ±3.3 (15 - 26)	1516.5 ±692.7 (764 - 3014)	2.5 ±0.1 (2.2 - 2.6)	0.8 ±0.04 (0.8 - 0.9)
73	9	reference	21.7 ±6.1 (15 - 34)	4280.6 ±2100.6 (2131 - 7481)	2.1 ±0.3 (1.5 - 2.4)	0.7 ±0.07 (0.6 - 0.8)
		reference	20.9 ±0.7	2740.4 ±1408.9	2.3 ±0.2	0.8 ±0.06
1045	7	salinized	12.3 ±2.6 (9 - 16)	4891.7 ± 3804.7 (1970 - 12153)	1.0 ±0.4 (0.7 - 1.6)	0.4 ±0.2 (0.3 - 0.7)
1061	10	salinized	19.6 ±4.5 (13 - 27)	2271.1 ±917.4 (1281 - 4004)	2.0 ±0.3 (1.7 - 2.7)	0.7 ±0.07 (0.6 - 0.8)
1340	5	salinized	17.4 ±7.5 (6 - 25)	5963.4 ±3268.5 (2314 - 9559)	1.0 ±0.2 (0.7 - 1.3)	0.4 ±0.03 (0.3 - 0.4)
		salinized	16.4 ±3.7	4375.4 ±1899.5	1.3 ±0.6	0.5 ±0.2

Table 2.3. Indicator taxa for reference and salinized stream groups. Component A is the specificity of a taxa as an indicator to a group. Component B is the fidelity of a taxa as an indicator to a group; indicator value (IV) statistic is √(A x B). For example, *Isonychia* is a good indicator of reference streams because it only occurs in reference streams (i.e. A=1.0), although not all reference sites include *Isonychia* (i.e. B=0.552). Significance of indicator values obtained from randomization Monte Carlo technique.

Taxa	**Group**	**A**	**B**	**IV stat**	**p-value**
Hexatoma	Reference	0.96	0.76	0.855	0.005
Epeorus	Reference	0.99	0.69	0.826	0.005
Drunella	Reference	0.98	0.59	0.756	0.005
Pteronarcys	Reference	0.95	0.59	0.746	0.005
Isonychia	Reference	1	0.55	0.743	0.005
Ephemerella	Reference	0.98	0.55	0.736	0.005
Psephenus	Reference	0.98	0.45	0.664	0.005
Maccaffertium	Reference	1	0.41	0.643	0.005
Ephemera	Reference	1	0.35	0.587	0.01
Peltoperla	Reference	0.98	0.31	0.553	0.025
Acentrella	Reference	1	0.24	0.491	0.035
Antocha	Reference	1	0.24	0.491	0.035
Clinocera	Salinized	0.81	0.62	0.708	0.005
Cheumatopsyche	Salinized	0.73	0.57	0.647	0.045
Atrichopogon	Salinized	0.93	0.43	0.63	0.005
Perlesta	Salinized	0.97	0.24	0.481	0.03
Ceratopsyche	Salinized	0.92	0.24	0.468	0.04
Atherix	Salinized	1	0.19	0.436	0.02

Table 2.4. The estimated sampling effort needed to collect 80% and 90% of theoretical taxonomic richness in each stream site using either unequal, actual sampling effort among streams (n=5-10) and equal, actual sampling effort among streams (constrained to n=5 for each). Mean values are followed by standard error.

Model	Stream	SC	Stream type	Actual richness	Theoretical richness	80% taxa	90% taxa
Unequal	EAS	31	reference	20.3 ±1.5	56.4 ±2.4	5.0	7.6
Unequal	CRO	72	reference	20.8 ±1.0	60.9 ±0.5	3.0	4.4
Unequal	HCN	73	reference	21.7 ±2.0	52.7 ±0.6	3.4	5.0
	mean		reference	20.9 ±0.4	56.6 ±2.4	3.8 ±1.1	5.7 ±1.7
Unequal	KUT	1045	salinized	12.3 ±1.0	34.5 ±1.7	3.5	5.2
Unequal	LLC	1061	salinized	19.6 ±1.4	51.2 ±2.5	6.1	9.6
Unequal	RIC	1340	salinized	17.4 ±3.4	62.6 ±2.2	6.0	9.5
	mean		salinized	16.4 ±2.1	49.4 ±8.2	5.2 ±1.5	8.1 ±2.5
Equal	EAS	31	reference		80.4 ±44.2	10.0	14.9
Equal	CRO	72	reference		103.5 ±17.7	6.2	9.0
Equal	HCN	73	reference		45.9 ±0.1	2.5	3.7
	mean		reference		76.6 ±16.7	6.3 ±3.8	9.2 ±5.6
Equal	KUT	1045	salinized		25.9 ±0.3	3.2	4.8
Equal	LLC	1061	salinized		54.5 ±5.3	7.2	11.1
Equal	RIC	1340	salinized		63.4 ±4.1	6.3	9.8
	mean		salinized		47.9 ±11.3	5.6 ±2.1	8.6 ±3.3

Table 2.5. Top ten taxa and traits with highest, significant squared correlations (r^2) with density ordination axes shown in Figure 2.6. These taxa or traits are indicative of compositional changes in macroinvertebrate densities associated with mining-induced salinization. FFG=functional feeding group.

Taxon or Trait type	Trait level	NMDS1	NMDS2	p	r^2
Pteronarcys	Na	-0.60	-0.13	0.001	0.371
Atrichopogon	Na	0.27	-0.53	0.001	0.350
Leuctra	Na	0.47	0.32	0.001	0.324
Oulimnius	Na	-0.45	-0.28	0.001	0.283
Psephenus	Na	-0.49	-0.20	0.002	0.277
Clinocera	Na	0.33	-0.41	0.001	0.273
Optioservus	Na	0.28	-0.40	0.002	0.237
Cheumatopsyche	Na	0.17	-0.45	0.004	0.227
Ceratopsyche	Na	0.22	-0.42	0.004	0.225
Drunella	Na	-0.37	0.29	0.004	0.217
FFG	shredder	0.71	0.23	0.001	0.564
flightstrength	strong flight	-0.06	-0.73	0.001	0.544
swim	weak swimmer	0.68	0.27	0.001	0.539
attachment	some sessile	-0.07	-0.70	0.001	0.502
FFG	collector-filterer	-0.06	-0.70	0.001	0.496
development	Fast seasonal development	0.68	0.15	0.001	0.490
respiration	Tegument respiration	0.66	0.19	0.001	0.473
rheophily	erosional rheophily	-0.12	-0.67	0.001	0.467
lifespan	very short lifespan	0.67	0.11	0.001	0.465
habit	climbing habit	0.66	-0.06	0.001	0.443

Table 2.6. Actual and theoretical taxa richness within the herbivore, shredder, and small body trait states for reference and salinized streams. Actual richness is presented as stream means calculated from the number of taxa belonging to the relevant trait state within each sample. Theoretical richness was obtained from the asymptotes of trait-based taxon sampling models. * In two instances the models failed to converge on an asymptote, resulting in erroneous values for theoretical richness and disallowed further calculations of difference or sampling effort. Negative richness values are excluded from calculations for means.

Trait	**SC**	**n**	**Actual richness**	**Theoretical richness**	**Difference (Theoretical – Actual)**	**Sampling effort 80% taxa**	**Sampling effort 90% taxa**
Herbivore	31	5	2.1 (±0.9)	5.45	3.35	3.1	4.5
Herbivore	72	5	3.1 (±1.0)	*	---	---	---
Herbivore	73	5	3.9 (±1.5)	7.18	3.28	1.9	2.7
Herbivore	reference mean		3.0	6.32	3.32	2.5	3.6
Herbivore	1045	5	1.3 (±0.8)	12.11	10.81	22.1	32.0
Herbivore	1061	5	2.3 (±1.0)	10.75	8.45	8.9	13.0
Herbivore	1340	5	2.0 (±1.7)	20.47	18.47	19.9	28.5
Herbivore	Salinized mean		1.9	14.44	12.58	17.0	24.5
Shredder	31	5	3.4 (±1.2)	6.05	2.65	1.5	2.1
Shredder	72	5	3.2 (±0.9)	12.68	9.48	3.7	5.3
Shredder	73	5	2.3 (±0.7)	5.31	3.01	2.8	4.0
Shredder	reference mean		3.0	8.01	5.05	2.7	3.8
Shredder	1045	5	2.0 (±1.2)	3.89	1.89	5.5	8.4
Shredder	1061	5	2.0 (±1.4)	5.16	3.16	23.3	34.9
Shredder	1340	5	2.6 (±0.6)	*	NA	N	---
Shredder	Salinized mean		2.2	4.53	2.53	14.4	21.7
Small body size	31	5	8.0 (±2.0)	10.96	2.96	1.1	1.8
Small body size	72	5	7.8 (±2.1)	18.43	10.63	3.8	5.5
Small body size	73	5	7.8 (±2.8)	17.87	10.07	3.0	4.6
Small body size	reference mean		7.9	15.75	7.89	2.6	4.0
Small body size	1045	5	5.9 (±2.0)	10.37	4.47	2.2	3.4
Small body size	1061	5	8.8 (±2.2)	22.64	13.84	6.0	9.3
Small body size	1340	5	9.0 (±3.8)	35.64	26.64	7.3	11.2
Small body size	Salinized mean		7.9	22.88	14.98	5.2	8.0

CHAPTER 3

Aquatic insect biomass and emergence were sustained despite mayfly declines in mining-induced salinized Appalachian headwaters.

Abstract

Secondary salinization is a growing concern as salts enter freshwaters through anthropogenic activities. In central Appalachian headwaters, macroinvertebrate diversity declines have been observed in response to mining-induced salinization. However, the effects of salinization on ecosystem processes remains largely unknown. Moreover, macroinvertebrates may have varied responses to a stressor dependent upon taxonomic identity and life stage, suggesting that assessments relying on immatures may not fully characterize the effects of salinization. We predicted benthic macroinvertebrate biomass would either decrease, be maintained via compensation by salt-tolerant taxa, or increase from a salt subsidy effect, whereas emergent insect biomass would decrease disproportionately relative to benthic biomass due to late instar and pupae succumbing to cumulative stress resulting from oxygen deprivation, ion regulation, or diet alteration. To test our predictions, we sampled six streams in central Appalachia that represent a salinization gradient (25-1422 µS/cm). We deployed emergence traps for 1-3 weeks and collected insects. Our results suggest that total benthic macroinvertebrate biomass was maintained along a salinization gradient despite the loss of salt-sensitive mayflies. Other salt-tolerant taxa, like flies, experienced a subsidizing effect. Emergent biomass showed high variability among streams with peak emergence occurring in spring, though a strong, negative response to increasing conductivity was not apparent. We used macroinvertebrate biomass and emergence as metrics to assess movement and changes in organic material and energy in response to a salinization gradient. Although taxa richness declines following salinization, biomass may be sustained but by different taxa that can shift the allocation and movement of

energy through aquatic food webs. As mayfly biomass is reduced in salinized, Appalachian headwaters, we can infer that food subsidies to riparian consumers are altered by either quantity, quality, or phenological timing.

Introduction

Salinization is a growing concern as increasing levels of salts enter freshwaters on a global scale (Cañedo-Argüelles et al. 2013). Salinity is a product of the total concentrations of dissolved inorganic ions in water and soil (Williams and Sherwood 1994) and is a naturally occurring attribute of aquatic systems that is tied to the weathering of geologic materials in a catchment and sea salts in coastal areas. However, human activities that pollute or accelerate weathering, such as road salt application, agriculture, wastewater, and resource extraction, introduce elevated levels of salts to freshwater systems that surpass natural background concentrations. This anthropogenic salinization (also referred to as secondary salinization) has repercussions for human health and ecosystems services (Cañedo-Argüelles et al. 2013, Kaushal 2016).

Resource extraction, or more particularly surface coal mining, is the dominant land use change in the central Appalachian region of the United States, covering more than 5,900 km^2, (Townsend et al. 2009, Pericak et al. 2018). Surface mining has been shown to cause physical, hydrological, chemical, and biological changes to catchments and the streams that drain them that are long-lasting and slow to recover (Pond et al. 2014, Cianciolo et al. 2020). As a result of pyrite dissolution and the carbonate matrix of bedrock, elevated levels of sulfate ions (SO_4^{2-}) accompanied by other major ions such as calcium, magnesium, and bicarbonate (Ca^{2+}, Mg^{2+}, HCO_3^-) are associated with mining discharge (Griffith et al. 2012).

Elevated ion concentrations have led to losses of macroinvertebrate richness and diversity and shifts in community composition to more salt-tolerant taxa in central Appalachian headwaters (Merricks et al. 2007, Pond et al. 2008, Cormier et al. 2013, Johnson et al. 2013, Boehme et al. 2016, Timpano et al. 2018, Drover et al. 2019, Vander Vorste et al. 2019). There are a number of hypothesized mechanisms by which elevated ion concentrations impact the survival and performance of macroinvertebrates, including ion regulation, osmoregulation, and localized toxicity in specific cells, tissues, or organs (Kefford 2018). Buchwalter et al. (2019) reared the mayfly *Neocloeon triangulifer* in a blend of $CaSO_4$ and $MgSO_4$ (5 to 1500 mg L^{-1} SO_4) and found that individuals exhibited delayed development, decreased survival to emergence, and little change in the total body sulfur content of the mayfly subimagos, despite increasing uptake rates of sulfate with increasing exposure concentration. These results suggest that the stress of ion regulation is the driver affecting the survival and performance of *N. triangulifer*.

Less is known about how stream processes respond to salinization following the physiological and community level changes that are observed in macroinvertebrates (Berger et al. 2019). Headwaters cumulatively comprise 60 to 80 percent of stream length and riparian interface in river networks that support biodiversity and sustain connectivity among downstream and terrestrial ecosystems (Benda et al. 2005, Meyer et al. 2007, Downing et al. 2012). Allochthonous inputs, such as dead leaves are consumed by aquatic macroinvertebrates that play a crucial role in the function of headwater streams by aiding in nutrient cycling and the translocation of carbon (Vannote et al. 1980, Wallace and Webster 1996). Insects that emerge from streams serve as an important food resource to other trophic levels in adjacent terrestrial habitats (Baxter et al. 2005, Paetzold and Tockner 2005). Many essential polyunsaturated fatty acids are produced mainly in aquatic systems and are transferred from water to land by emergent insects (Gladyshev et al. 2011). Alterations in macroinvertebrate-mediated processes can have cascading effects on local and downstream ecosystems, and changes in insect emergence can have consequences for the food quantity and quality available to consumers like fish, birds, bats, and spiders (Baxter et al. 2005).

Changes in the biomass produced by aquatic macroinvertebrates and insects in response to mining-induced salinization is less studied and inconclusive. For instance, Voss and Bernhardt (2017) found that total secondary production by benthic macroinvertebrates was depressed in mining-influenced streams as a result of decreased Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa. In contrast, Johnson et al. (2013) found that total secondary production measured through emergent adult insects was maintained in mining-influenced streams despite losses of EPT taxa as a result of compensation by salt-tolerant taxa. The sustained insect biomass observed by Johnson et al. (2013) despite increased salinization was largely attributed to increases in Chironomidae emergence where they comprised more than 80 percent of emergent biomass in mining-influenced streams. The subsidy-stress response provides a possible explanation for observed increases of insect biomass in response to salinization where some taxa may respond positively at intermediate levels of ion concentrations or at some optimum range of conductivity (Odum et al. 1979, Cormier et al. 2013). Although these two studies converge on the well-documented loss of salt-sensitive taxa such as mayflies, they differ in their findings for the response of total macroinvertebrate secondary production to salinization and the insect life stages that were measured.

Different life stages of an insect can exhibit differing responses to the same stressor and result in decreased aquatic insect emergence (Schmidt et al. 2013). Late instars and pupae may succumb to the cumulative stress associated with salinization and the physiological demand of emergence (e.g., oxygen deprivation). Schmidt et al. (2013) explored differential responses of aquatic insect life stages by measuring and comparing both larval and emergent adult densities along a gradient of stream metal contamination in the Central Colorado Rocky Mountains, finding that emergence declined disproportionately to larval density. These findings show that adult emergence cannot always be assumed a constant proportion of larval density and highlight the need for studies inclusive of macroinvertebrate life stages to assess the effects of a stressor. Juxtaposed responses of both larval and adult aquatic insects can be considered to gain a more complete understanding of salinization impacts among insect life stages and stream processes.

We aimed to compare macroinvertebrate biomass and emergence for salinized streams relative to non-salinized, reference streams and across a salinization gradient to improve our understanding of how freshwater salinization may be affecting life stages of insects differentially and, ultimately, altering stream processes. To do this, we sampled benthic macroinvertebrates and emergent insects from six, first-order streams in the central Appalachian coalfield region where mining-induced salinization is a persistent water quality concern and a contributor of elevated conductivities downstream (Pond et al. 2014, Cianciolo et al. 2020). We predicted that total benthic macroinvertebrate biomass would exhibit one of three responses: 1) a compensation scenario of community variability (Micheli et al. 1999) where individual taxa exhibit variable responses to the salt stressor, but biomass remains constant for the benthic community as a whole, 2) a decrease with increased salinity due to local extirpation (Pond 2010, Timpano et al. 2018) and altered individual performance of salt-sensitive taxa (Buchwalter et al. 2019), or 3) a subsidy-stress response along a salinization gradient due to some or most taxa having an optimum range of conductivity (Odum et al. 1979, Cormier et al. 2013). We expected that total emergent biomass would always be less than the total larval biomass observed in streams, because we would not expect 100 percent survival of individuals, and that emergent biomass would decrease disproportionately to larval biomass in response to the same salinization gradient because late instars and pupae do not successfully develop into imagos. We therefore expect a greater number of late instars and pupae will succumb to increases in salinity relative to early instars. Additionally, we expected a decrease in both larval and emergent biomass of more salt-sensitive taxa regardless of the overall community response to salinization.

Materials and methods

Site selection

Six forested, first-order streams were selected within the Appalachian coalfield region based on physical and chemical parameters used by Timpano et al. (2015) and with the additional criteria of seclusion from human traffic to protect emergence traps from intervention. Three streams without mining influence were selected to be reference condition with annual mean specific conductance (SC) ranging 25-127µS cm^{-1}. Three streams with a history of mining activity within their catchments and varying levels of annual mean SC (562-1422µS cm^{-1}) were selected to represent a gradient of salinization (Figure 3.1).

Chemical and physical parameters

Water temperature and SC were measured at 30-minute intervals by automated dataloggers placed in each of the streams (HOBO Freshwater Conductivity Data Logger, model U24-001, Onset Computer Corp., Bourne, Massachusetts). We calculated annual mean SC and annual mean water temperature using daily means for 365 days prior to sampling dates (Table 3.1). Dissolved selenium data are means of April 2014 and Summer 2015 Se concentrations in stream water filtered through a 0.45-mm pore membrane (Whitmore et al. 2018). Stream substrate is a primary physical determinant of benthic macroinvertebrate communities (Cobb et al. 1992). Therefore, we characterized streambed substrates within one 100-meter study reach in each stream. To do this, we conducted substrate point counts following the protocol outlined by Wofford et al. (2018) across ten transects, each 10 meters apart and perpendicular to stream flow. Then, to obtain a measure of substrate heterogeneity for each stream, we applied the Shannon's diversity index to each transect and averaged across transects for each stream (Boyero 2003).

Benthic macroinvertebrate biomass

To characterize benthic macroinvertebrate biomass, macroinvertebrates were sampled quantitatively using a 0.09-m^2 Surber stream-bottom sampler (Wildco; Yulee, FL, USA) with a 250-µm mesh, in November 2019 and April 2020. Five Surber sample replicates were collected from riffle habitat within a 100-meter reach from each of three reference sites and three salinized sites. Samples were preserved in 95% ethanol and later decanted and sieved through a 250 µm mesh. Macroinvertebrates were identified to genus or the lowest practicable level using standard identification keys (Merritt et al. 2008) with the exception of Chironomidae (Tanypodinae or Non-Tanypodinae), Acari (subclass), Nematoda (subclass), and Oligochaeta (subclass). Length measurements of specimens were recorded to the nearest 1.0 mm using a printed 1mm x 1mm grid paper under a dissecting microscope. Length measurements were used in combination with published length-dry mass regressions (e.g., Benke et al. 1999) to estimate dry mass values.

To test our first set of predictions regarding the response of benthic macroinvertebrate biomass to salinization, we performed Kruskal-Wallis tests to compare biomass metrics (i.e., total biomass, taxonomic groupings, and trait groups) between salinized streams and reference streams. Additionally, we plotted total benthic biomass against annual mean SC to assess their relationship. Benthic biomass data were ln(x+1) transformed to improve homogeneity of variance and normality. We fit a linear mixed effect model to the benthic biomass data with stream input as a random effect. A smoothing spline was fit to data to observe nonlinear responses. Other covariates were not considered in this modeling as previous analysis demonstrated the salinization gradient was sufficient to describe mean invertebrate responses and few other factors were strongly correlated with the salinization gradient (Timpano et al. 2015).

To identify taxa and trait states responding to salinization, we performed non-metric multidimensional scaling (NMDS) using a Bray-Curtis dissimilarity matrix based on macroinvertebrate biomass. Trait designations for macroinvertebrates were based on Poff et al. (2006) nomenclature. NMDS analysis was performed using the R vegan package with the metaMDS function that runs NMDS several times with random starting configurations, scales and rotates the solution, and adds species scores to the configuration as weighted averages (Oksanen et al. 2019).

Emergent insect biomass

To characterize insect emergent biomass, we deployed four quantitative net traps with a basal sampling area of $0.24m^2$ and mesh size of 250µm over randomly selected riffles in each stream reach (Johnson et al. 2013). We sampled emergent insects for one-to-three-week periods in September 2019, November 2019, and April 2020, concurrent with fall and spring sampling dates for benthic macroinvertebrates. Emergence sampling periods were identical at all streams for the summer and fall seasons with traps deployed for one week. For the spring sampling period, net traps were deployed for differing numbers of cumulative days ranging 7-27 days among streams because of field conditions (e.g., high stream flow). Emergent insects were stored in 70% ethanol. Ephemeroptera, Plecoptera, and Trichoptera were identified to family while the remaining insects were identified to order. Length measurements of specimens were recorded to the nearest 1.0 mm using a printed 1mm x 1mm grid paper under a dissecting microscope. Length measurements were used in combination with published length-dry mass regressions (e.g., Sabo et al. 2002) to obtain dry mass values.

To test our second set of predictions regarding the response of emergent biomass to salinization, we visually assessed the emergent biomass using boxplots and tested for differences among streams using Kruskal-Wallis tests. We were not able to achieve normality by ln(x+1) transformation for the emergent biomass data in preparation for regression analyses comparable to that of the benthic biomass.

Comparing benthic and emergent biomass

To test our third prediction regarding how insect emergence and benthic macroinvertebrates respond to salinization relative to one another, we first calculated seasonal estimates of biomass (mg m^{-2} $season^{-1}$) for fall and spring by multiplying the daily emergent (mg m^{-2} day^{-1}) and benthic biomass (mg m^{-2}) by the number of days in the season (Johnson et al. 2013). Then, we divided the seasonal emergent biomass by the seasonal benthic emergent biomass and multiplied by 100 to obtain the percent emerged biomass relative to the benthic biomass present in each stream for both seasons.

Results

Benthic macroinvertebrate biomass

We collected a total of 60 benthic macroinvertebrate samples from six streams with annual mean SCs ranging from 25-1,422 μS cm^{-1}. We identified and measured a total of 15,885 macroinvertebrates for the fall and 19,512 macroinvertebrates for the spring. Total macroinvertebrate biomass calculated for samples ranged from 1,141-7,804 mg m^{-2} (Table 3.2).

Based on stream means, macroinvertebrate biomass was 26% greater in the spring than in the fall. Non-metric multidimensional scaling (NMDS) analyses revealed compositional differences between macroinvertebrate biomass in spring and fall, suggesting seasonality in macroinvertebrate community composition (PERMANOVA; Sum of squares=2.051, F=9.017, R2=0.135, P=0.001; Figure 3.2). Taxa primarily responsible for this separation were greater *Amphinemura*, *Leuctra*, and *Diplectrona* biomass in the spring and greater *Allocapnia*, early instar winter stoneflies (Capniidae or Leuctridae), and *Cheumatopsyche* biomass in the fall (Table 3.3). We chose to assess spring and fall macroinvertebrate biomass responses to SC separately.

We performed season-specific NMDS ordinations to explore taxonomic compositional differences in macroinvertebrate biomass associated with SC for the fall and spring. We found that the taxonomic composition of macroinvertebrate biomass showed greater differences between salinized and reference assemblages in the spring (Figure 3.3A; PERMANOVA, df=1, Sum of sqs=1.3472, F=7.2882, R^2=0.2065, p=0.001) than in the fall (Figure 3.3B; PERMANOVA, df=1, Sum of sqs=0.4212, F=2.335, R^2=0.07697, p=0.078). For the fall, some taxa primarily associated with greater SC were *Cheumatopsyche*, *Rhyacophila*, and non-Tanypodinae, while some of the taxa primarily associated with low SC were Leptophlebiidae, early instar Ephemeroptera, and *Oulimnius* (Table 3.4). For the spring, some taxa primarily associated with greater SC were *Leuctra*, *Amphinemura*, and early instar predatory stoneflies (Perlidae and Perlodidae), while some of the taxa primarily associated with low SC were *Drunella*, Leptophlebiidae, and *Ephemerella* (Table 3.5). Out of the three environmental vectors (i.e. annual mean SC, annual mean temperature, and substrate heterogeneity) overlain on the ordinations, SC was the best and most strongly correlated vector to the fall and spring ordinations ($r^2 > 0.45$, $p = 0.001$).

We performed season-specific NMDS ordinations to explore trait compositional differences in macroinvertebrate biomass associated with SC for the fall and spring. Trait composition of macroinvertebrate biomass did not differ greatly for low and high SC assemblages in either fall (Figure 3.4; PERMANOVA, df=1, Sum of sqs=0.05566, F=0.7239, $R^2 = 0.0252$, $p = 0.5$) or spring (PERMANOVA, df=1, Sum of sqs=0.06743, F=1.567, $R^2 = 0.053$, $p = 0.18$). Of the three environmental variables assessed, SC correlated well with the spring trait ordination ($r^2 = 0.592$, $p = 0.001$) but did not correlate well with the fall trait ordination ($r^2 = 0.122$, $p = 0.16$). Instead, the substrate heterogeneity vector correlated best with the fall trait ordination ($r^2 = 0.2573$, $p = 0.024$). For the fall, some of the trait biomass primarily associated with high SC assemblages were strong swimming ability, drift abundant, and bi- or multivoltine (>1 gen yr^{-1}) (Table 3.6). For the spring, some of the trait biomass primarily associated with high SC assemblages were long adult lifespan, clinging habit, and heavy sclerotization (Table 3.7).

We compared benthic macroinvertebrate biomass between salinized streams and reference streams, and Kruskal-Wallis tests revealed no significant differences between stream types for any of the biomass metrics we tested (Figure 3.5). However, nominal differences were observed in biomass metrics between stream types. For instance, we observed on average 125% greater total benthic macroinvertebrate biomass in salinized streams relative to reference streams for the fall and 85% greater biomass in salinized streams for the spring. Some macroinvertebrate groups appeared to have greater biomass in salinized streams relative to reference streams for both fall and spring, including Chironomidae, collector-filterers, predators, and detritivores (Figure 3.5). In contrast, we observed 66% less larval mayfly biomass in salinized streams relative to reference streams for the fall and 60% less larval mayfly biomass in salinized streams for the spring. The exclusion of the salt-tolerant family Baetidae from analysis further strengthened this negative trend for mayfly biomass (Figure 3.5).

We assessed the responses of macroinvertebrate biomass to a gradient of SC using linear mixed effect models (Table 3.8). Effect sizes, constants, and other model output values are presented in ln(x+1) scale (Table 3.8). However, effect size values are largely unchanged when back-transformed. Hence, we describe effect sizes here in linear scale. Of the 14 biomass metrics regressed, four metrics had significant effect sizes in the fall, while seven were significant in the spring. In the fall, total macroinvertebrate biomass exhibited a slight increase in response to SC with about a 0.001 mg increase for every one unit increase in SC, while Ephemeroptera minus Baetidae exhibited a 0.003 mg decrease for one unit increase in SC ($p<0.05$; Table 3.8). Detritivores showed increases of about 0.002 mg with increases in SC in the fall. Other fall biomass metrics showed nominal increases in response to SC except for Ephemeroptera and herbivores that showed decreasing trends in their biomass. In the spring, total macroinvertebrate biomass showed a nominally small increasing response to SC. Chironomidae and collector-filterer biomass both showed increasing trends of about 0.002 mg per one unit increase in SC for the spring ($p<0.05$; Table 3.8). Ephemeroptera biomass metrics showed stronger responses to SC in the spring than in the fall with a 0.002 mg decrease in Ephemeroptera biomass and a 0.004 mg decrease in non-Baetidae Ephemeroptera biomass for every one unit increase in SC. For both fall and spring regressions, the exclusion of Baetidae biomass strengthened the decreasing response of Ephemeroptera biomass to the SC gradient.

Emergent insect biomass

Of the 24 traps deployed during each season, 21 traps were recovered in the summer (88%), 16 in the fall (67%), and 17 in the spring (71%) (Table 3.9). Emergent biomass is not presented for one of our streams for the fall and two streams for the spring due to high stream flows and demonic intrusions (e.g., human or animal disturbance of traps) that prevented data collection during those sampling periods. We collected a total of 286 insects from the summer, 45 insects from the fall, and 503 insects from the spring. Mean daily emergent insect biomass calculated for samples ranged from 0.2-53 mg m^{-2} day^{-1} (Table 3.9). On average, daily emergent biomass was greatest in the spring with about 28 mg m^{-2} day^{-1}, followed by summer with 14 mg m^{-2} day^{-1} and fall with 6 mg m^{-2} day^{-1}. Emergence was often highly variable among streams (Figure 3.6).

Streams did not differ significantly in total emergent biomass for any season (Figure 3.7). However, total emergent biomass tended to be greater and more variable in salinized streams for the fall and spring. The two streams with the greatest annual mean SC also had the greatest mean and maximum emergent biomass in fall and spring (Table 3.9). Total emergent biomass exhibited an increasing trend with SC in the summer as well except that the stream with the lowest SC had the greatest and most variable emergent biomass (Figure 3.7A).

Trends for EPT emergent biomass appeared to closely follow that of total emergent biomass for all three seasons (Figure 3.7B). The highest SC stream in the fall had significantly greater EPT emergent biomass (range: 3.3-64.1 mg m^{-2} day^{-1}) than the other streams (0-3.2 mg m^{-2} day^{-1}) (Table 3.9). Ephemeroptera emergent biomass differed significantly among streams for the fall and spring (Figure 3.7C). For the fall, the only stream that had emergent Ephemeroptera was the highest SC stream, and the Ephemeroptera belonged to the family Baetidae. For the spring, only three of the four streams had emergent Ephemeroptera, and one of the low SC streams had the greatest mean Ephemeroptera emergent biomass of about 5 mg m^{-2} day^{-1}, followed by a high SC stream with 3 mg m^{-2} day^{-1} (Table 3.9). Streams did not differ for summer Ephemeroptera emergent biomass, but low SC streams generally had greater Ephemeroptera emergent biomass (Figure 3.7C).

Streams did not differ significantly in Diptera emergent biomass for any season (Figure 3.7D). For the summer, the highest SC stream had the greatest Diptera emergent biomass with a mean of about 6 mg m^{-2} day^{-1}, while the other streams had lesser and more comparable values (range: 0.5-1.3 mg m^{-2} day^{-1}) (Table 3.9). Very little Diptera emergent biomass was observed in any stream for the fall (≤1 mg m^{-2} day^{-1}) (Table 3.9). Generally, greater Diptera emergent biomass was observed in low SC streams for the spring (Figure 3.7D).

Comparing benthic and emergent biomass

From our stream means for daily emergent biomass, we estimated seasonal values of benthic and emergent biomass for the fall and spring. From these seasonal values, we calculated the percent emerged biomass from the benthic biomass where data were available for both benthic and emergent biomass in a stream (Table 3.10). As expected, emergent biomass values were always less than benthic biomass values. For the fall, the highest SC stream had the greatest percent emerged biomass of about 2 percent, while the other four streams had only between 0.04 to 0.2 percent. For the spring, the two low SC streams had greater percent emerged biomass with values of about 3 and 4 percent, while the two high SC streams had only about 1 and 2 percent emerged biomass.

Discussion

Benthic macroinvertebrate biomass was maintained along a salinization gradient

The subsidy-stress response can provide an explanatory framework for observed increases in a biological response to intermediate levels of a stressor, such as salinization (Odum et al 1979). In support of our first prediction, our findings suggest that total benthic biomass of macroinvertebrates was maintained and even increased in response to a gradient of salinization ranging 25-1,422 μS cm^{-1}. This finding is similar to that of Johnson et al (2013) showing that total secondary production was maintained in mining-influenced streams (1170-2500 μS cm^{-1}) relative to unmined, forested streams (41-82 μS cm^{-1}). Our finding of increasing Chironomidae biomass with increasing SC corroborates with previous findings as well (Johnson et al 2013, Voss & Bernhardt 2017). For instance, Johnson et al (2013) found that Chironomidae production comprised up to 87% of total production in mining-influenced, Appalachian streams versus ≤54% in unmined streams. Chironomidae are a diverse group of insects that persist in many environments and are relatively tolerant to salinization with some Chironomid taxa able to inhabit Appalachian streams with conductivities over 11,000μS cm^{-1} (Cormier et al 2013a).

There are a number of pathways through which salinization might alter macroinvertebrate biomass and their composition. Additionally, macroinvertebrate taxa respond differently to ionic strength by exhibiting differences at which the populations decrease, increase, or show an optimum to increasing conductivity (Cormier et al 2013a). Therefore, depending on the SC gradient, some macroinvertebrate taxa may experience a subsidizing effect while others simultaneously experience stress. Salts are essential micronutrients for life that, when elevated in stream and riparian habitats, have the potential to subsidize salt-limited microbes, macroinvertebrates, and other consumers (Entrekin et al 2018). Elevated sulfate, bicarbonate, carbonate, magnesium, and other associated ions in Appalachian streams may alter and supplement the diet of some macroinvertebrate taxa through either direct consumption of salts or by the alteration of the microbial communities that are also consumed by macroinvertebrates. In a study of 24 streams (SC gradient of 25–1,383µS/cm) from which our six study streams are a subset, Vander Vorste et al (2019) showed that microbial communities changed in their composition along with macroinvertebrate communities. Aside from altering food quality, elevated ions may impart a subsidy to some macroinvertebrates by alleviating hyperosmotic osmoregulatory demands by changing ambient solute concentrations (e.g., Hassell et al 2006).

However, elevated salts are well known to have lethal and sublethal effects on macroinvertebrates of some taxa demonstrated by decreased growth rates and delayed development (Buchwalter et al 2019, Kennedy et al 2004, Kunz et al 2013, Soucek & Dickinson 2015) with decreased relative abundances, densities, and biomass of salt-sensitive macroinvertebrate taxa observed in Appalachian streams (Drover et al 2019, Timpano et al 2018a, Voss & Bernhardt 2017). A commonly accepted hypothesis is that salts energetically tax aquatic organisms in maintaining their ion- and osmo-regulatory processes, in which Ephemeroptera are notably susceptible due to their mitochondrion-rich, epithelial chloride cells that depend on the passive regulation of HCO^{3-}/Cl^{-} antiport (Cormier et al 2013b, Kefford 2018). The loss of Ephemeroptera biomass we observed with increases in SC supports previous studies in the Appalachian coalfield region finding decreases in their relative abundances (Cianciolo et al 2020, Pond 2010, Pond et al 2008, Pond et al 2014, Timpano et al 2018a), densities (Drover et al 2019, Pence 2019), and secondary production (Johnson et al 2013, Voss & Bernhardt 2017). The loss of Ephemeroptera from stream communities likely has ecological consequences. In the spring, Ephemeroptera can comprise 25-50% of macroinvertebrate abundances and represent about 20% of taxonomic diversity in relatively undisturbed Appalachian headwaters (Pond 2010). Additionally, Ephemeroptera have synchronous emergences in the spring that can serve as an important food resource subsidy to other trophic levels (Baxter et al. 2005), including to other arthropods (Paetzold and Tockner 2005). Alterations in emergence by Ephemeroptera alone could alter the food quantity and quality available to terrestrial consumers. In headwater streams, emergent insects are important in returning reciprocal flows, transporting aquatic carbon into riparian systems (Bartels et al. 2012, Nakano and Murakami 2001).

Season exhibited a strong influence on the taxonomic composition of macroinvertebrate biomass, similarly to what has been shown previously for macroinvertebrate densities (Drover et al 2019). A number of stonefly taxa drove seasonal separations of macroinvertebrate biomass, such as *Amphinemura*, *Leuctra*, and early instar winter stoneflies occurring in high SC assemblages in the spring. For the fall, *Allocapnia*, early instar winter stoneflies, and *Taeniopteryx* in high SC assemblages and *Paracapnia* in low SC assemblages contributed to seasonal separation of macroinvertebrate biomass. Drover et al. (2019) found *Leuctra* and *Amphinemura* densities to increase in response to SC with greatest densities in late-spring to mid-summer for *Leuctra* and April-May for *Amphinemura*. We found that mayfly taxa also influenced seasonal separations of macroinvertebrate biomass, especially in spring when *Drunella*, Leptophlebiidae, *Ephemerella*, and *Epeorus* occurred in low SC assemblages. Despite the greater difference in macroinvertebrate community composition in spring compared to fall, macroinvertebrate biomass was more positively related to SC in fall where we estimated a 0.001mg increase in macroinvertebrate biomass for a one unit increase in SC. The weaker, positive response for total benthic biomass in the spring may be attributable to the decreased larval biomass of mayflies in high SC assemblages. Also, contributing to fall trends of increasing total macroinvertebrate biomass with increasing SC were *Cheumatopsyche*, a finding similar to Drover et al. (2019) where *Cheumatopsyche* densities dominated November samples.

Ordination analysis of macroinvertebrate biomass revealed that taxonomic- and trait-based approaches performed differently. Macroinvertebrate biomass represented by taxonomic groupings showed separation of low SC and high SC assemblages, but trait groupings did not. Instead, stream assemblages were more similar in the macroinvertebrate traits than the taxa represented along the salinization gradient. This finding differs from that of Szöcs et al (2014) where trait-based ordination better discriminated between saline and non-saline sites in the Werra River, Germany. However, Szöcs et al (2014) assessed macroinvertebrate trait responses to a salinization gradient of about 500-7,500µS cm^{-1}, a range of conductivity much larger than that of the present study. Perhaps the lack of separation observed for our trait biomass between low SC and high SC assemblages is because of the smaller range of SC assessed. In addition, the salinization gradient in the Werra River is a result of potash industry and is comprised of differing ion identities from that of mining-influenced, Appalachian streams, and biological responses may differ depending upon ion identity. Another hypothesis is that the trait assignments used in our analysis did not encompass the physiological or life history adaptations relevant to macroinvertebrate response to SC. For instance, a trait providing greater resolution to macroinvertebrate respiratory strategies, such as describing the amount of epithelial exchange surfaces possessed by a gilled macroinvertebrate, could potentially better discriminate macroinvertebrate biomass of low SC and high SC communities (Buchwalter et al 2003).

Substrate heterogeneity appeared to play a more influential role in determining trait biomass than SC in the spring. This may be attributable to intra-annual variability of SC where peak SC typically occurs in late summer when evapotranspiration is high and ions are more concentrated in the stream (Timpano et al 2018b). Therefore, the effects of SC on macroinvertebrate trait biomass may be less pronounced during the spring when streams are more diluted with precipitation and the absence of evapotranspiration with leaf-off of trees.

Emergent insect biomass was highly variable among streams

Insect emergence was highly variable among streams, an observation also made by Johnson et al. (2013) in their emergence study of mining-influenced Appalachian streams. Contrary to our second prediction, we did not observe strong, decreasing trends for emergent biomass in response to increasing SC. Our mean daily emergent biomass estimates were comparable to those of Johnson et al. (2013), though peak emergence was observed for different seasons. For instance, they reported peak summer averages of 22.9 mg m^{-2} d^{-1} for forested streams and 34.4 mg m^{-2} d^{-1} for mining-influenced streams. We observed peak spring averages of 18.7 mg m^{-2} d^{-1} for low SC streams and 36.4 mg m^{-2} d^{-1} for high SC streams. Unlike Johnson et al (2013), we did not observe large amounts of Diptera biomass compensating for losses of Ephemeroptera biomass in high SC streams. Instead, we generally observed greater Plecoptera and Trichoptera emergent biomass in our high SC streams.

Comparing benthic and emergent biomass

We calculated relatively low percentages of season-specific emerged biomass (range: 0.04-3.5%) relative to other studies of stream insect emergence that report annual ratios of emergence to secondary production ranging 4-57% (Jackson and Fisher 1986). We reported our highest values for percent emerged from two low SC streams in spring. This observation may provide evidence to support our third prediction that there is a deficit of emergence in high SC streams relative to low SC streams because of a greater number of late instars and pupae succumbing to increases in salinity relative to their younger cohorts. However, a reverse trend was observed for the fall where the highest percent emerged occurred from a high SC stream. It is possible that our emergence traps did not sufficiently characterize seasonal emergence among streams due to the spatial and temporal variability of insect emergence, which may also explain our low values for percent emerged.

Conclusions

To our knowledge, the present study is the first to conduct field measurements of *both* larval and emergent insect biomass along a mining-induced salinization gradient. Notable and concerning is our finding of decreased larval mayfly biomass in salinized streams, a trend that tracks what has been widely documented for relative abundances, densities, and secondary production of this order in other Appalachian stream studies (e.g., Pond 2010, Voss and Bernhardt 2017, Drover et al. 2019). The advantage of biomass metrics is that these values convey amounts that are comparable across studies, informative of ecological processes such as the flow of organic matter and energy, and in turn, tell of stream ecosystem processes. As mayfly biomass is reduced in salinized, Appalachian headwaters, we can infer that food subsidies to riparian consumers are altered by either quantity, quality, or phenological timing. Macroinvertebrate biomass measures provide a common link between population and ecosystem level processes (Benke 1993).

References

Baxter, C. V., K. D. Fausch and W. Carl Saunders (2005). "Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones." Freshwater biology **50**(2): 201-220.

Benda, L., M. A. Hassan, M. Church and C. L. May (2005). "Geomorphology of steepland headwaters: the transition from hillslopes to channels." Journal of the American Water Resources Association **41**(4): 835-851.

Benke, A. C. (1993). "Concepts and patterns of invertebrate production in running waters." Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen **25**(1): 15-38.

Benke, A. C., A. D. Huryn, L. A. Smock and J. B. Wallace (1999). "Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States." Journal of the North American Benthological Society **18**(3): 308-343.

Berger, E., O. Frör and R. B. Schäfer (2019). "Salinity impacts on river ecosystem processes: a critical mini-review." Philosophical Transactions of the Royal Society B **374**(1764): 20180010.

Boehme, E. A., C. E. Zipper, S. H. Schoenholtz, D. J. Soucek and A. J. Timpano (2016). "Temporal dynamics of benthic macroinvertebrate communities and their response to elevated specific conductance in Appalachian coalfield headwater streams." Ecological Indicators **64**: 171-180.

Boyero, L. (2003). "The quantification of local substrate heterogeneity in streams and its significance for macroinvertebrate assemblages." Hydrobiologia **499**(1): 161-168.

Buchwalter, D., S. Scheibener, H. Chou, D. Soucek and J. Elphick (2019). "Are sulfate effects in the mayfly Neocloeon triangulifer driven by the cost of ion regulation?" Philosophical Transactions of the Royal Society B **374**(1764): 20180013.

Cañedo-Argüelles, M., B. J. Kefford, C. Piscart, N. Prat, R. B. Schäfer and C.-J. Schulz (2013). "Salinisation of rivers: an urgent ecological issue." Environmental pollution **173**: 157-167.

Cianciolo, T. R., D. L. McLaughlin, C. E. Zipper, A. J. Timpano, D. J. Soucek and S. H. Schoenholtz (2020). "Impacts to water quality and biota persist in mining-influenced Appalachian streams." Science of The Total Environment **717**: 137216.

Cobb, D., T. Galloway and J. Flannagan (1992). "Effects of discharge and substrate stability on density and species composition of stream insects." Canadian Journal of Fisheries Aquatic Sciences **49**(9): 1788-1795.

Cormier, S. M., G. W. Suter and L. Zheng (2013). "Derivation of a benchmark for freshwater ionic strength." Environmental Toxicology Chemistry **32**(2): 263-271.

Cormier, S. M., G. W. Suter, L. Zheng and G. J. Pond (2013). "Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions." Environmental Toxicology Chemistry **32**(2): 277-287.

Downing, J. A., J. J. Cole, C. Duarte, J. J. Middelburg, J. M. Melack, Y. T. Prairie, P. Kortelainen, R. G. Striegl, W. H. McDowell and L. J. Tranvik (2012). "Global abundance and size distribution of streams and rivers." Inland waters **2**(4): 229-236.

Drover, D. R., C. E. Zipper, D. J. Soucek and S. H. Schoenholtz (2019). "Using density, dissimilarity, and taxonomic replacement to characterize mining-influenced benthic

macroinvertebrate community alterations in central Appalachia." Ecological Indicators **106**: 105535.

Gladyshev, M., A. Y. Kharitonov, O. Popova, N. Sushchik, O. Makhutova and G. Kalacheva (2011). Quantitative estimation of dragonfly role in transfer of essential polyunsaturated fatty acids from aquatic to terrestrial ecosystems. Doklady. Biochemistry and biophysics, Springer Science & Business Media.

Griffith, M. B., S. B. Norton, L. C. Alexander, A. I. Pollard and S. D. LeDuc (2012). "The effects of mountaintop mines and valley fills on the physicochemical quality of stream ecosystems in the central Appalachians: a review." Science of the Total Environment **417**: 1-12.

Jackson, J. K. and S. G. Fisher (1986). "Secondary production, emergence, and export of aquatic insects of a Sonoran Desert stream." Ecology **67**(3): 629-638.

Johnson, B. R., K. M. Fritz and R. Price (2013). "Estimating benthic secondary production from aquatic insect emergence in streams affected by mountaintop removal coal mining, West Virginia, USA." Fundamental Applied Limnology/Archiv für Hydrobiologie **182**(3): 191-204.

Kaushal, S. S. (2016). "Increased Salinization Decreases Safe Drinking Water." Environmental Science & Technology **50**(6): 2765-2766.

Kefford, B. J. (2018). "Why are mayflies (Ephemeroptera) lost following small increases in salinity? Three conceptual osmophysiological hypotheses." Philosophical Transactions of the Royal Society B **374**(1764): 20180021.

Merricks, T. C., D. S. Cherry, C. E. Zipper, R. J. Currie and T. W. Valenti (2007). "Coal-mine hollow fill and settling pond influences on headwater streams in southern West Virginia, USA." Environmental Monitoring Assessment **129**(1-3): 359-378.

Merritt, R., K. Cummins and M. Berg (2008). An Introduction to the Aquatic Insects of North America. Dubuque, Iowa, USA, Kendall Hunt Publishing.

Meyer, J. L., D. L. Strayer, J. B. Wallace, S. L. Eggert, G. S. Helfman and N. E. Leonard (2007). "The Contribution of Headwater Streams to Biodiversity in River Networks 1." Journal of the American Water Resources Association **43**(1): 86-103.

Micheli, F., K. L. Cottingham, J. Bascompte, O. N. Bjørnstad, G. L. Eckert, J. M. Fischer, T. H. Keitt, B. E. Kendall, J. L. Klug and J. A. Rusak (1999). "The dual nature of community variability." Oikos: 161-169.

Odum, E. P., J. T. Finn and E. H. Franz (1979). "Perturbation theory and the subsidy-stress gradient." Bioscience **29**(6): 349-352.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs and H. Wagne (2019). vegan: Community Ecology Package.

Paetzold, A. and K. Tockner (2005). "Effects of riparian arthropod predation on the biomass and abundance of aquatic insect emergence." Journal of the North American Benthological Society **24**(2): 395-402.

Pericak, A. A., C. J. Thomas, D. A. Kroodsma, M. F. Wasson, M. R. Ross, N. E. Clinton, D. J. Campagna, Y. Franklin, E. S. Bernhardt and J. F. Amos (2018). "Mapping the yearly extent of surface coal mining in Central Appalachia using Landsat and Google Earth Engine." PloS one **13**(7): e0197758.

Poff, N. L., J. D. Olden, N. K. Vieira, D. S. Finn, M. P. Simmons and B. C. Kondratieff (2006). "Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships." Journal of the North American Benthological Society

25(4): 730-755.

Pond, G. J. (2010). "Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA)." Hydrobiologia **641**(1): 185-201.

Pond, G. J., M. E. Passmore, F. A. Borsuk, L. Reynolds and C. J. Rose (2008). "Downstream effects of mountaintop coal mining: comparing biological conditions using family-and genus-level macroinvertebrate bioassessment tools." Journal of the North American Benthological Society **27**(3): 717-737.

Pond, G. J., M. E. Passmore, N. D. Pointon, J. K. Felbinger, C. A. Walker, K. J. Krock, J. B. Fulton and W. L. Nash (2014). "Long-term impacts on macroinvertebrates downstream of reclaimed mountaintop mining valley fills in central Appalachia." Environmental management **54**(4): 919-933.

Sabo, J. L., J. L. Bastow and M. E. Power (2002). "Length–mass relationships for adult aquatic and terrestrial invertebrates in a California watershed." Journal of the North American Benthological Society **21**(2): 336-343.

Schmidt, T. S., J. M. Kraus, D. M. Walters and R. B. Wanty (2013). "Emergence flux declines disproportionately to larval density along a stream metals gradient." Environmental science technology **47**(15): 8784-8792.

Timpano, A. J., S. H. Schoenholtz, D. J. Soucek and C. E. Zipper (2015). "Salinity as a limiting factor for biological condition in mining-influenced central Appalachian headwater streams." Journal of the American Water Resources Association **51**(1): 240-250.

Timpano, A. J., S. H. Schoenholtz, D. J. Soucek and C. E. Zipper (2018). "Benthic macroinvertebrate community response to salinization in headwater streams in Appalachia USA over multiple years." Ecological indicators **91**: 645-656.

Townsend, P. A., D. P. Helmers, C. C. Kingdon, B. E. McNeil, K. M. de Beurs and K. N. Eshleman (2009). "Changes in the extent of surface mining and reclamation in the Central Appalachians detected using a 1976–2006 Landsat time series." Remote Sensing of Environment **113**(1): 62-72.

Vander Vorste, R., A. J. Timpano, C. Cappellin, B. D. Badgley, C. E. Zipper and S. H. Schoenholtz (2019). "Microbial and macroinvertebrate communities, but not leaf decomposition, change along a mining-induced salinity gradient." Freshwater biology **64**(4): 671-684.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing (1980). "The river continuum concept." Canadian journal of fisheries aquatic sciences **37**(1): 130-137.

Voss, K. A. and E. S. Bernhardt (2017). "Effects of mountaintop removal coal mining on the diversity and secondary productivity of Appalachian rivers." Limnology Oceanography **62**(4): 1754-1770.

Wallace, J. B. and J. R. Webster (1996). "The role of macroinvertebrates in stream ecosystem function." Annual review of entomology **41**(1): 115-139.

Whitmore, K. M., S. H. Schoenholtz, D. J. Soucek, W. A. Hopkins and C. E. Zipper (2018). "Selenium dynamics in headwater streams of the central Appalachian coalfield." Environmental toxicology chemistry **37**(10): 2714-2726.

Williams, W. and J. Sherwood (1994). "Definition and measurement of salinity in salt lakes." International Journal of Salt Lake Research **3**(1): 53-63.

Wofford, J. E. B., E. D. Demarest and J. R. V. Jr. (2018). Shenandoah National Park Benthic Macroinvertebrate Monitoring Protocol, National Park Service.

Figures and Tables

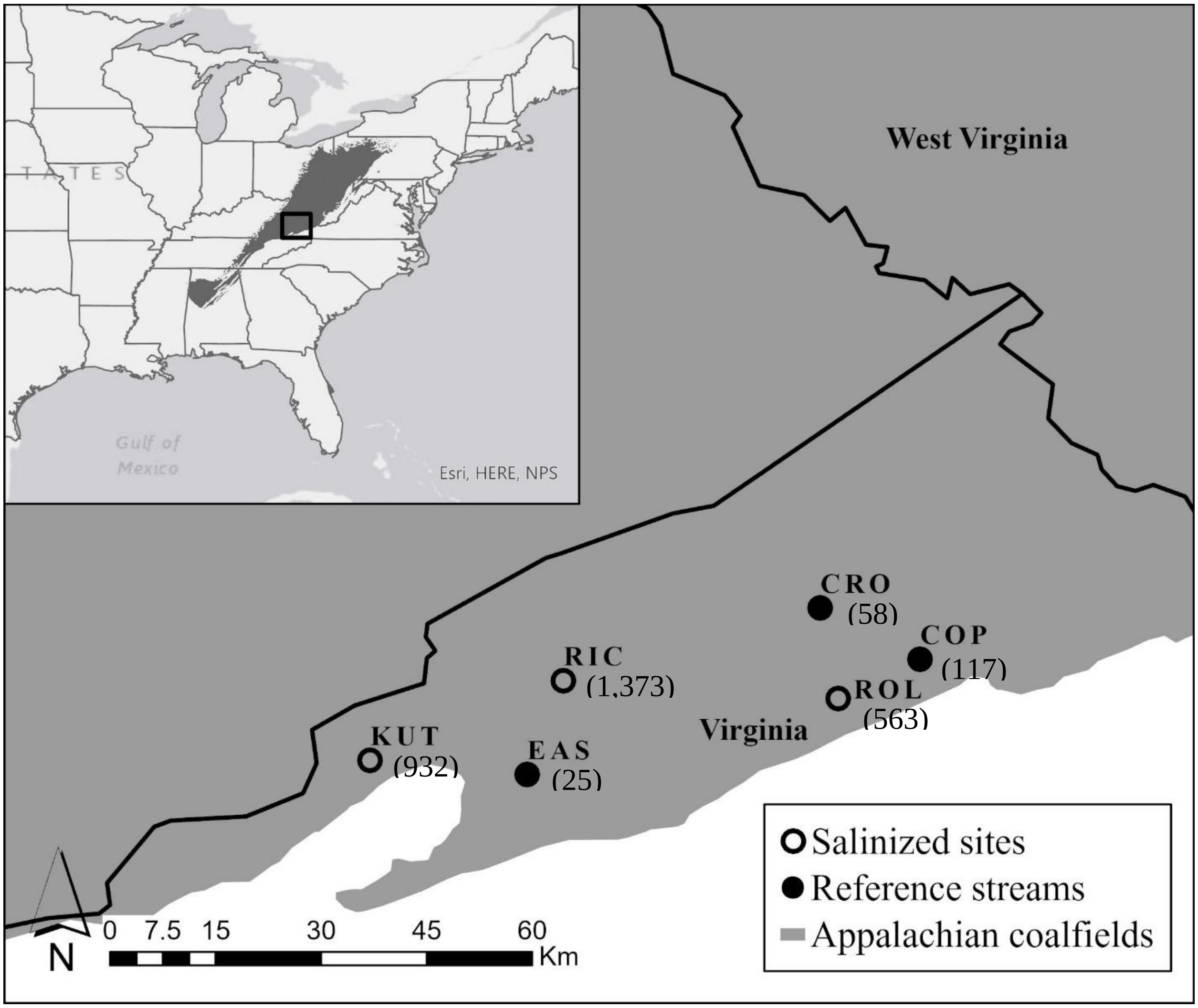


Figure 3. 1. Location of study streams within the Appalachian coalfields of Virginia, U.S. Three-season average of annual mean SC from summer 2019, fall 2019, and spring 2020 shown in parentheses.

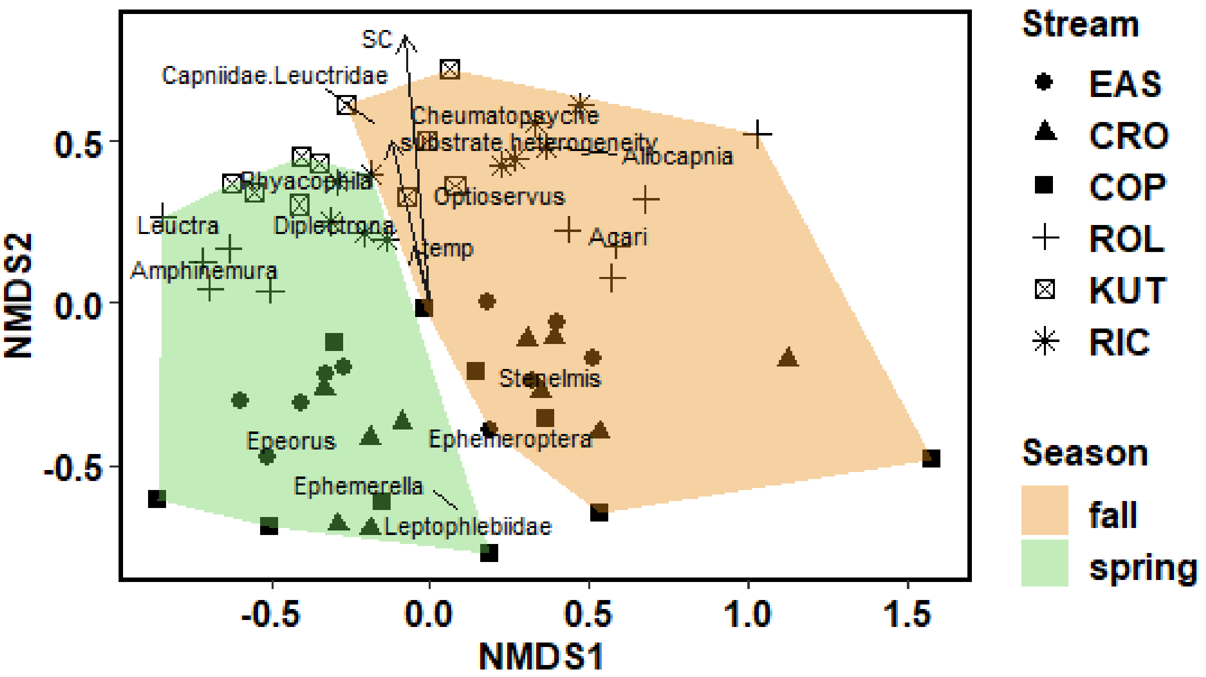


Figure 3. 2. NMDS ordination (dimensions=2, stress level=0.2269) using Bray-Curtis dissimilarity matrix of macroinvertebrate biomass. Biomass values were ln(x+1) transformed. Annual mean SC, annual mean temperature, and substrate heterogeneity environmental vectors overlain. Fourteen significantly correlated taxa ($r^2>0.25$, $p<0.5$) are shown. SC ($r^2=0.6887$, $p=0.001$), season ($r^2=0.3969$, $p=0.001$), and substrate heterogeneity ($r^2=0.2691$, $p=0.001$) environmental vectors correlated well with ordination points, while temperature ($r^2=0.0343$, $p=0.365$) did not. PERMANOVA showed macroinvertebrate community composition differed between spring and fall seasons (df=1, Sum of sqs=2.0511, F=9.0172, $R^2=0.1346$, $p=0.001$), but season only explains a small amount of the variance.

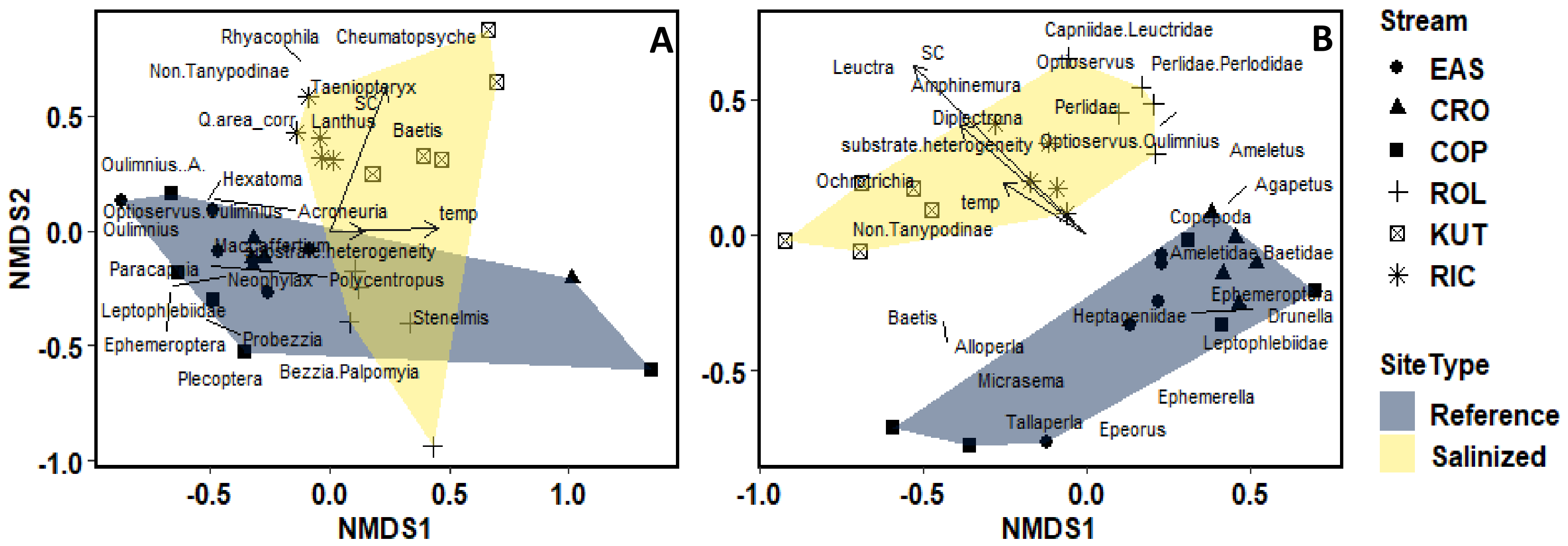


Figure 3. 3. (A) NMDS ordination (dimensions=2, stress level=0.1693) using Bray-Curtis dissimilarity of Fall 2019 taxonomic biomass that is ln(x+1) transformed. Annual mean SC, annual mean temperature, and substrate heterogeneity environmental vectors overlain. Twenty-two significantly correlated taxa (r^2>0.25, p<0.5) are shown. SC (r^2=0.4540, p=0.001) and temperature (r^2=0.2084, p=0.043) environmental vectors correlated well with ordination points but substrate heterogeneity did not (r^2=0.0208, p=0.741). PERMANOVA showed macroinvertebrate community composition did not differ between salinized and references streams (df=1, Sum of sqs=0.4212, F=2.335, R^2=0.07697, p=0.078). (B) NMDS ordination (dimensions=2, stress level=0.1719) using Bray-Curtis dissimilarity of Spring 2020 taxonomic biomass that is ln(x+1) transformed. Annual mean SC, annual mean temperature, and substrate heterogeneity environmental vectors overlain. Twenty-eight significantly correlated taxa (r^2>0.25, p<0.5) are shown. SC (r^2=0.6710, p=0.001) and substrate heterogeneity (r^2=0.3158, p=0.009) correlated well with ordination points while temperature (r^2=0.1030, p=0.239) did not. PERMANOVA showed macroinvertebrate community composition differs between salinized and reference streams (df=1, Sum of sqs=1.3472, F=7.2882, R^2=0.2065, p=0.001).

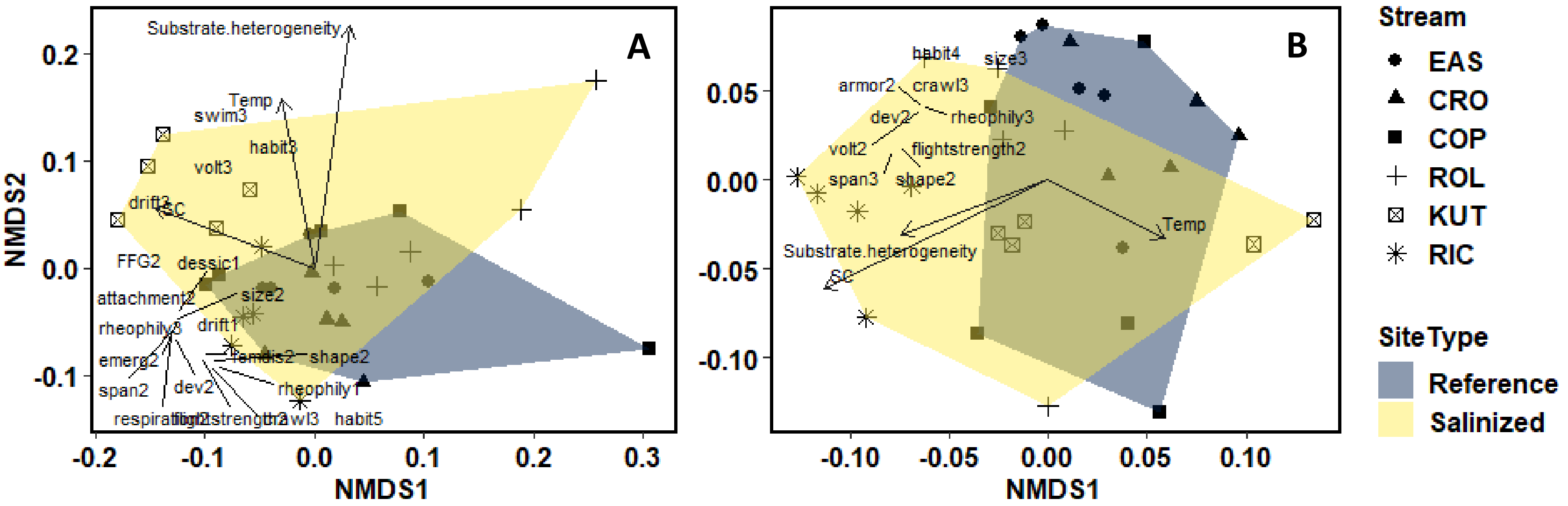


Figure 3. 4. (A) Trait-based NMDS ordination (dimensions=2, stress level=0.1408) using Bray-Curtis dissimilarity of fall 2019 macroinvertebrate biomass that is ln(x+1) transformed. Annual mean SC, annual mean temperature, and substrate heterogeneity environmental vectors overlain. Twenty significantly correlated trait states (r^2>0.4, p<0.5) are shown. Substrate heterogeneity (r^2=0.2573, p=0.024) environmental vector correlated well with ordination points while SC (r^2=0.1220, p=0.159) and temperature (r^2=0.1277, p=0.153) did not. PERMANOVA showed macroinvertebrate trait biomass composition did not differ between salinized and references streams (df=1, Sum of sqs=0.05566, F=0.7239, R^2=0.0252, p=0.503). (B) Trait-based NMDS ordination (dimensions=2, stress level=0.2025) using Bray-Curtis dissimilarity of spring 2020 macroinvertebrate biomass that is ln(x+1) transformed. Annual mean SC, annual mean temperature, and substrate heterogeneity environmental vectors overlain. Sixteen significantly correlated trait states (r^2>0.55, p<0.5) are shown. SC (r^2=0.5922, p=0.001) correlated well with ordination points while temperature (r^2=0.1345, p=0.154), and substrate heterogeneity (r^2=0.1980, p=0.059) did not correlate well with ordination points. PERMANOVA showed macroinvertebrate trait biomass composition did not differ between salinized and references streams (df=1, Sum of sqs=0.06743, F=1.567, R^2=0.053, p=0.176).

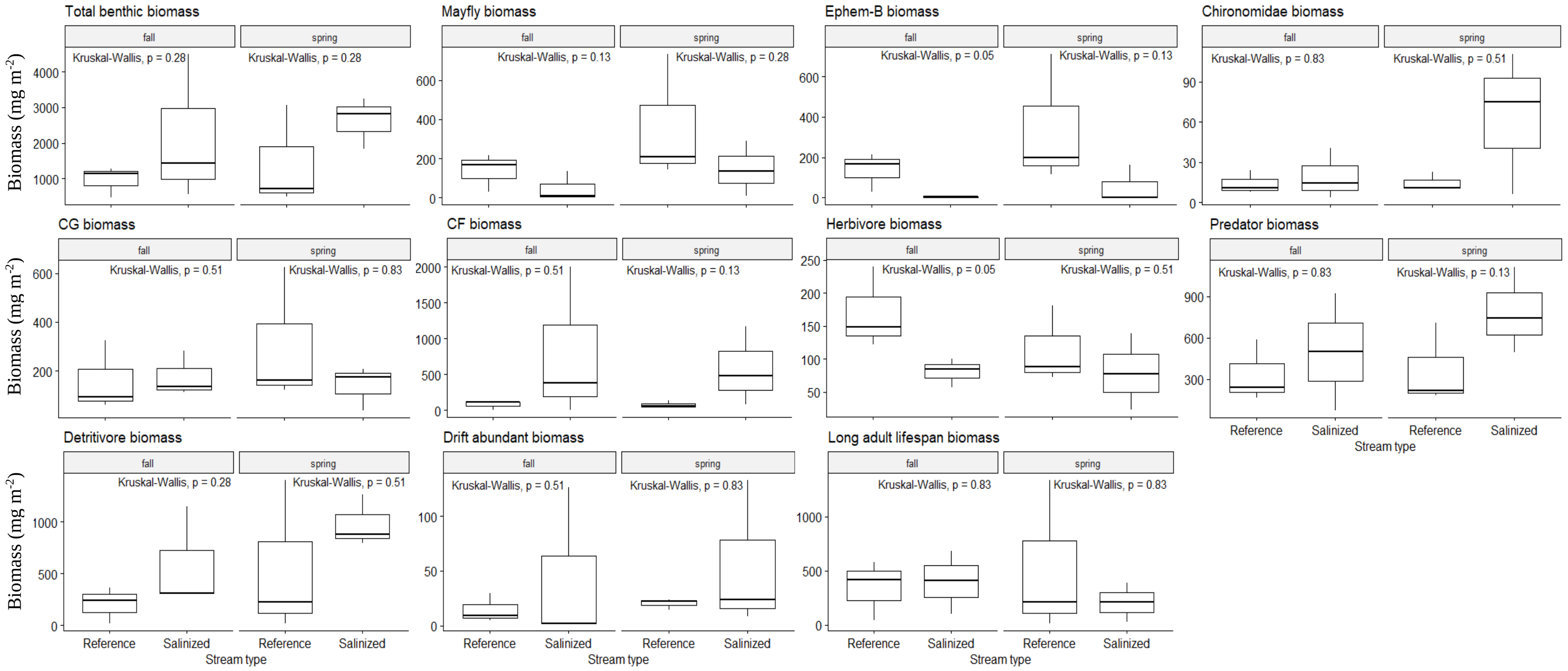


Figure 3. 5. Comparison of benthic macroinvertebrate biomass between salinized streams and reference streams for total biomass and select taxonomic and trait groups. Lower and upper box boundaries are 25th and 75th percentiles, line inside box is median, lower and upper whiskers are 10th and 90th percentiles, filled circles are data points outside the 10th and 90th percentiles. Ephem-B = Ephemeroptera excluding Baetidae. CG = collector-gatherer. CF = collector-filterer.

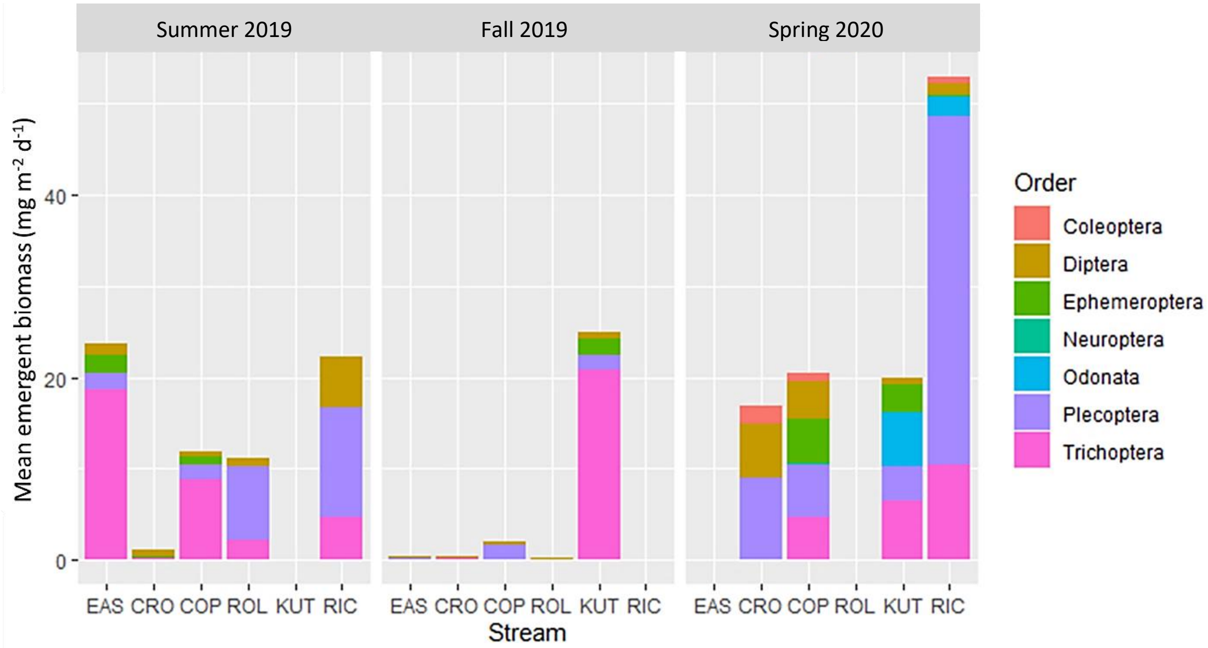


Figure 3.6. Seasonal contribution of insect Orders to mean daily emergent biomass by stream. Note that KUT in the summer of 2019, RIC in the fall of 2019, and EAS and ROL in the spring of 2020 do not have data and are not indicative of zero biomass.

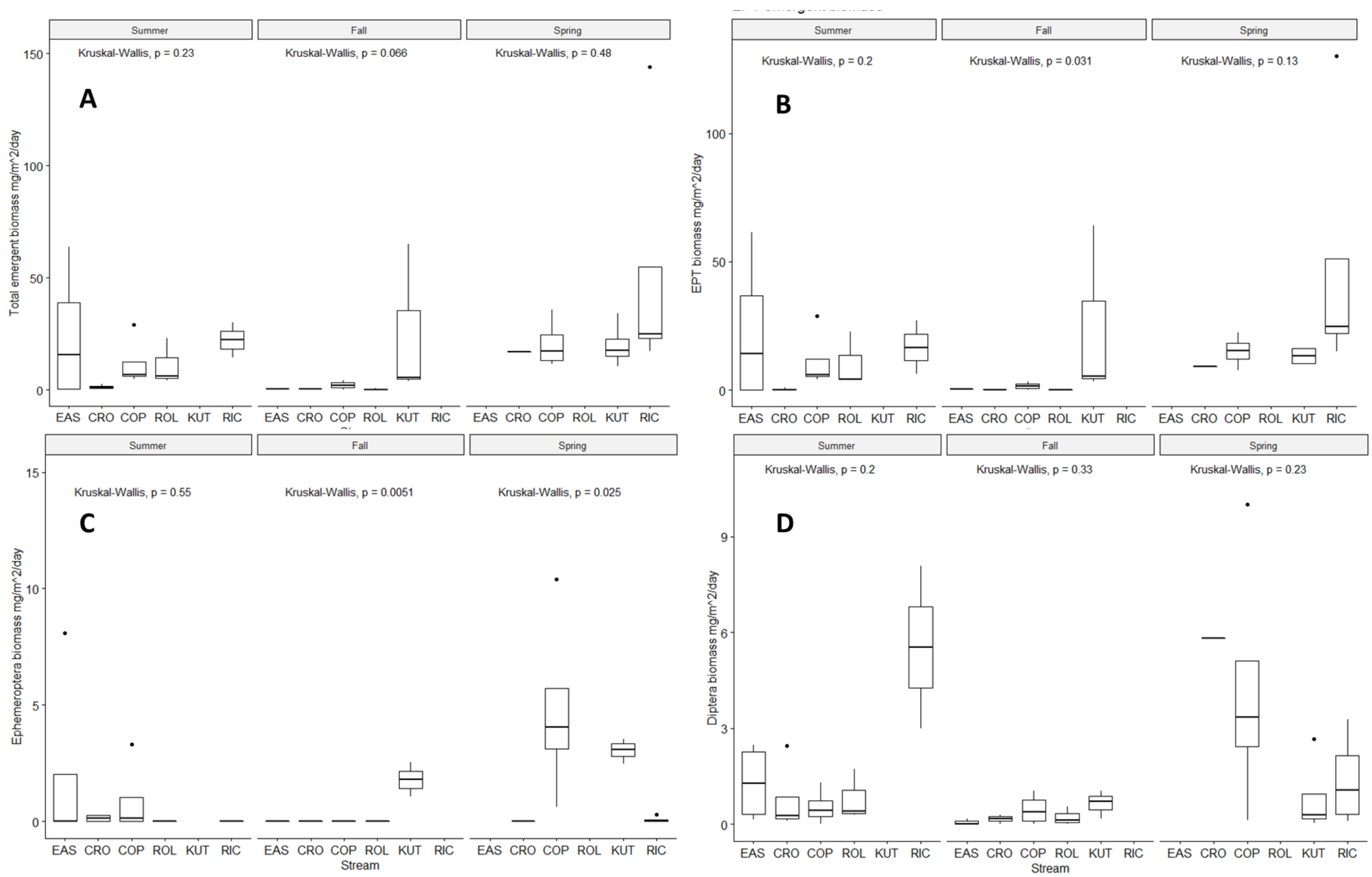


Figure 3.7. Daily emergent biomass for (A) total, (B) Ephemeroptera, Plecoptera, and Trichoptera (EPT), (C) Ephemeroptera, and (D) Diptera by season. Streams without box and whiskers do not have data collected due to weather or demonic intrusion. Kruskal-Wallis shows that streams do not differ in emergent biomass for any season.

Table 3. 1. Stream site attributes that include annual mean specific conductance (SC) values, annual mean water temperature, water column selenium concentrations, and watershed area. Annual means are followed by standard deviations in parentheses. R = reference. T = treatment (i.e., salinized).

Stream	Site code	Stream type	Lat (N)	Long (W)	SC (μS cm^{-1})			Temperature (°C)			Se (μg L^{-1})	Watershed area (km^2)
					Summer 2019	Fall 2019	Spring 2020	Summer 2019	Fall 2019	Spring 2020		
Eastland Creek	EAS	R	36.917	-82.592	24 (±2.9)	25 (±3.7)	25 (±4.4)	11.3 (±4.9)	11.3 (±4.9)	11.8 (±4.4)	<2.5	2.38
Crooked Branch	CRO	R	37.130	-82.218	56 (±11.9)	58 (±13.8)	60 (±13.9)	13.5 (±5.9)	13.3 (±6)	13.4 (±5.9)	<2.5	2.27
Copperhead Branch	COP	R	37.065	-82.091	101 (±24.5)	123 (±41.9)	127 (±39.1)	12.2 (±5)	12.1 (±5)	12.4 (±4.7)	<2.5	0.81
Rollpone Branch	ROL	T	37.015	-82.195	508 (±127.5)	562 (±142.1)	619 (±105.3)	13.8 (±4.1)	12.8 (±4.5)	12.4 (±4.7)	<2.5	1.3
Kelly Branch - Unnamed Tributary	KUT	T	36.936	-82.793	933 (±124.9)	920 (±108.9)	944 (±106.9)	13.7 (±2)	13.6 (±2.1)	13.5 (±2.3)	8.4	1.09
Rickey Branch	RIC	T	37.037	-82.546	1330 (±249.6)	1422 (±289.5)	1366 (±316.4)	10.4 (±6.5)	11.4 (±6.6)	12.6 (±5.3)	<2.5	4.22

Table 3. 2. Mean, standard deviation (sd), minimum, and maximum benthic macroinvertebrate biomass for streams of varying annual mean SC for fall and spring. SC = annual mean specific conductance.

Stream	SC (µS cm^{-1})	Season	Year	n (samples)	Mean benthic biomass (mg m^{-2})	sd	min	max
EAS	25	fall	2019	5	1135.2	785	146.2	2272.3
CRO	58	fall	2019	5	456	385	246.7	1141.6
COP	123	fall	2019	5	1282.5	1801.7	35.4	4320.5
ROL	562	fall	2019	5	546.9	428.3	116	1219.1
KUT	920	fall	2019	5	1434.1	843.4	614.5	2859.6
RIC	1422	fall	2019	5	4496.8	1906.8	1554.1	6614.9
EAS	25	spring	2020	5	3073.3	2734.4	1197.4	7803.5
CRO	60	spring	2020	5	479.1	494.3	195.1	1358.6
COP	127	spring	2020	5	719.4	1163.4	131.4	2795.3
ROL	619	spring	2020	5	1822.3	661.5	864.6	2490.3
KUT	944	spring	2020	5	2824.8	2339	734.7	6367
RIC	1366	spring	2020	5	3235.7	2207.3	452.8	6633.7

Table 3. 3. Significantly correlated taxa from all data (fall and spring seasons) NMDS.

Taxon	**NMDS1**	**NMDS2**	**pval**	**r^2**
Amphinemura	-0.6745	0.1790	0.001	0.49
Leuctra	-0.6182	0.2649	0.001	0.45
Allocapnia	0.4216	0.4850	0.001	0.41
Diplectrona	-0.5248	0.2609	0.001	0.34
Ephemerella	-0.3202	-0.4898	0.001	0.34
Epeorus	-0.3357	-0.4791	0.001	0.34
Capniidae.Leuctridae	-0.1812	0.5548	0.001	0.34
Leptophlebiidae	0.0146	-0.5786	0.001	0.34
Rhyacophila	-0.2827	0.4559	0.001	0.29
Cheumatopsyche	0.0430	0.5228	0.001	0.28
Optioservus	0.3200	0.4058	0.001	0.27
Acari	0.4890	0.1530	0.001	0.26
Stenelmis	0.4798	-0.1585	0.002	0.26
Ephemeroptera	0.1598	-0.4739	0.001	0.25
Tipula	-0.1197	0.4757	0.003	0.24
Baetidae	-0.4895	-0.0160	0.002	0.24
Bezzia.Palpomyia	0.1427	-0.4665	0.003	0.24
Baetis	-0.4176	0.2135	0.001	0.22
Drunella	-0.2098	-0.4139	0.002	0.22
Probezzia	0.3491	-0.2944	0.003	0.21
Perlidae.Perlodidae	-0.4397	0.1023	0.002	0.20
Ameletus	-0.3910	-0.2219	0.004	0.20
Tallaperla	-0.2772	-0.3476	0.002	0.20
Lanthus	-0.0750	0.4363	0.004	0.20
Wormaldia	-0.3247	0.2900	0.003	0.19
Paraleptophlebia	0.3000	-0.3070	0.002	0.18
Taeniopteryx	0.1466	0.4017	0.008	0.18
Paracapnia	0.3751	-0.2044	0.001	0.18
Non.Tanypodinae	-0.2117	0.3563	0.005	0.17
Isoperla	-0.4085	-0.0162	0.005	0.17
Heptageniidae	-0.0867	-0.3978	0.004	0.17
Optioservus..A.	0.1030	0.3924	0.008	0.16
Agapetus	-0.2851	-0.2818	0.007	0.16
Dicranota	-0.1102	-0.3753	0.011	0.15
Hydropsychidae	0.1678	0.3274	0.015	0.14
Ochrotrichia	-0.2389	0.2750	0.016	0.13
Yugus	-0.3075	-0.1857	0.024	0.13
Simulium	-0.3568	-0.0378	0.019	0.13
Ceratopogon	0.1576	-0.3035	0.035	0.12
Heterocloeon	-0.3302	0.0796	0.024	0.12
Peltoperlidae	-0.2684	-0.2079	0.03	0.12
Hemerodromia	-0.0625	0.3321	0.03	0.11

Ephemera	0.0264	-0.3339	0.032	0.11
Perlodidae	-0.0849	-0.3212	0.033	0.11
Alloperla	-0.1590	-0.2829	0.029	0.11
Acentrella	-0.3216	-0.0030	0.04	0.10
Chironomidae..P.	-0.0227	0.3192	0.047	0.10
Neoplasta	-0.1967	0.2520	0.047	0.10
Chimarra	0.0806	0.3031	0.046	0.10
Glossosoma	0.0946	0.2923	0.045	0.09
Nemoura	0.2937	-0.0695	0.042	0.09

Table 3. 4. The 28 significantly correlated taxa for the Fall 2019 taxonomic NMDS of macroinvertebrate biomass.

Taxon	**NMDS1**	**NMDS2**	**pval**	**r^2**
Cheumatopsyche	0.0596	0.7834	0.001	0.62
Leptophlebiidae	-0.7131	-0.2576	0.001	0.57
Rhyacophila	-0.1278	0.7453	0.001	0.57
Ephemeroptera	-0.6672	-0.2667	0.001	0.52
Oulimnius	-0.7092	-0.0548	0.001	0.51
Oulimnius..A.	-0.6638	0.2249	0.001	0.49
Non.Tanypodinae	-0.2873	0.6371	0.001	0.49
Neophylax	-0.6541	-0.2341	0.001	0.48
Stenelmis	0.5998	-0.2907	0.005	0.44
Plecoptera	-0.3929	-0.5311	0.002	0.44
Probezzia	-0.5174	-0.3877	0.001	0.42
Paracapnia	-0.6411	-0.0783	0.001	0.42
Bezzia.Palpomyia	-0.2570	-0.5459	0.004	0.36
Optioservus.Oulimnius	-0.5875	0.0275	0.005	0.35
Baetis	0.2671	0.5232	0.006	0.35
Taeniopteryx	0.0405	0.5598	0.005	0.32
Lanthus	0.0242	0.5586	0.008	0.31
Maccaffertium	-0.5488	-0.0420	0.01	0.30
Hexatoma	-0.5117	0.1419	0.014	0.28
Polycentropus	-0.4927	-0.1513	0.015	0.27
Acroneuria	-0.4777	0.1388	0.028	0.25
Ephemerellidae	-0.4850	-0.1014	0.038	0.25
Ceratopsyche	-0.1418	0.4668	0.026	0.24
Tanypodinae	-0.4858	0.0364	0.028	0.24
Hemerodromia	-0.1307	0.4660	0.037	0.23
Ceratopogon	-0.3141	-0.3614	0.038	0.23
Tipula	0.0830	0.4667	0.039	0.22
Ameletidae.Baetidae	-0.1433	-0.4462	0.027	0.22
Chloroperlidae	-0.3320	-0.2968	0.041	0.20

Table 3. 5. The 41 significantly correlated taxa for the Spring 2020 taxonomic NMDS of macroinvertebrate biomass.

Taxon	NMDS1	NMDS2	pval	r^2
Leuctra	-0.5915	0.5690	0.001	0.67
Capniidae.Leuctridae	-0.1505	0.7549	0.001	0.59
Drunella	0.6720	-0.3479	0.001	0.57
Leptophlebiidae	0.5262	-0.4481	0.001	0.48
Ephemerella	0.4348	-0.5252	0.001	0.46
Amphinemura	-0.2236	0.6372	0.001	0.46
Epeorus	0.1478	-0.6157	0.001	0.40
Perlidae.Perlodidae	0.1769	0.6056	0.002	0.40
Tallaperla	-0.0518	-0.6212	0.003	0.39
Ochrotrichia	-0.6042	0.1462	0.004	0.39
Ameletus	0.4880	0.3848	0.003	0.39
Ameletidae.Baetidae	0.5862	-0.1212	0.004	0.36
Micrasema	-0.1483	-0.5744	0.005	0.35
Baetis	-0.4289	-0.3893	0.008	0.34
Ephemeroptera	0.5522	-0.1573	0.006	0.33
Heptageniidae	0.5053	-0.2716	0.007	0.33
Optioservus	0.0515	0.5707	0.007	0.33
Non.Tanypodinae	-0.5647	0.0932	0.002	0.33
Diplectrona	-0.2350	0.5020	0.006	0.31
Agapetus	0.5101	0.1982	0.01	0.30
Perlidae	0.1238	0.5227	0.009	0.29
Alloperla	-0.2504	-0.4650	0.003	0.28
Optioservus.Oulimnius	0.2697	0.4492	0.013	0.27
Copepoda	0.4870	0.1780	0.012	0.27
Heterocloeon	0.1436	0.4710	0.022	0.24
Dicranota	0.0000	-0.4882	0.019	0.24
Perlodidae	0.4612	-0.1350	0.025	0.23
Neophylax	0.4577	0.1410	0.029	0.23
Bezzia.Palpomyia	0.3219	-0.3541	0.022	0.23
Isonychia	0.4682	-0.0945	0.025	0.23
Tipula	-0.2826	0.3698	0.042	0.22
Ephemerellidae	0.4127	-0.1904	0.046	0.21
Simuliidae..P.	0.0424	0.4253	0.039	0.18

Table 3. 6. The 46 significantly correlated trait states for the fall 2019 trait NMDS of macroinvertebrate biomass.

Trait state	**Trait description**	**NMDS1**	**NMDS2**	**pval**	**r^2**
attachment2	some sessile	-0.7342	-0.0580	0.001	0.54
respiration2	gill respiration	-0.6565	-0.3149	0.001	0.53
swim3	strong swimming ability	-0.3301	0.6489	0.001	0.53
rheophily3	erosional rheophily	-0.6795	-0.2356	0.001	0.52
dev2	slow seasonal development	-0.6331	-0.3363	0.001	0.51
span2	short adult lifespan (<1 mo)	-0.6379	-0.2804	0.001	0.49
drift3	drift abundant	-0.6495	0.2423	0.001	0.48
emerg2	well synchronized emergence	-0.6361	-0.2573	0.001	0.47
habit5	swim habit	0.0577	-0.6778	0.001	0.46
size2	medium size at maturity (9-16 mm)	-0.6260	-0.2368	0.001	0.45
flightstrength2	strong flight	-0.5079	-0.4291	0.001	0.44
crawl3	high crawl speed (>100 cm/hr)	-0.4675	-0.4716	0.003	0.44
FFG2	collector-filterer	-0.6625	-0.0340	0.001	0.44
habit3	sprawl habit	-0.3404	0.5612	0.001	0.43
volt3	Bi- or Multivoltine (>1 gen/yr)	-0.3483	0.5555	0.001	0.43
dessic1	no dessication resistance	-0.6142	-0.1998	0.003	0.42
drift1	rare drift occurrence (catastrophic only)	-0.5448	-0.3317	0.003	0.41
femdis2	high female dispersal (>1 km flight before laying eggs)	-0.4977	-0.3987	0.001	0.41
rheophily1	depositional rheophily	-0.4300	-0.4622	0.002	0.40
shape2	not Streamlined (cylindrical, round, or bluff)	-0.4578	-0.4309	0.003	0.40
armor1	no armoring (soft-bodied forms)	-0.6042	-0.1633	0.001	0.39
exit2	adult exit ability	-0.3432	-0.5131	0.004	0.38
swim1	no swimming ability	-0.4368	-0.4346	0.002	0.38
FFG4	predator (piercer and engulfer)	-0.3342	-0.5120	0.006	0.37
thermophily2	Cool/Warm Eurythermal	-0.4493	-0.4062	0.003	0.37
armor2	poor armoring (heavily sclerotized)	-0.2602	-0.5451	0.004	0.36
thermophily1	Cold Stenothermal or Cool Eurythermal	-0.5762	-0.1422	0.004	0.35
habit2	climb habit	-0.5329	-0.2426	0.006	0.34

exit1	adult exit ability absent (not including emergence)	-0.5119	-0.2734	0.008	0.34
volt2	univoltine (1 gen/yr)	-0.4208	-0.3972	0.005	0.33
femdis1	low female dispersal (<1 km flight befory laying eggs)	-0.4791	-0.3068	0.008	0.32
habit1	burrow habit	-0.2112	-0.5233	0.007	0.32
crawl2	low crawl speed (<100 cm/hr)	-0.4131	-0.3520	0.01	0.29
span3	long adult lifespan (>1 mo)	-0.1520	-0.5144	0.013	0.29
attachment1	no attachment (free-ranging)	-0.3427	-0.4030	0.011	0.28
dessic2	dessication resistance present	-0.2035	-0.4832	0.016	0.27
flightstrength1	weak flight (e.g. cannot fly into light breeze)	-0.4456	-0.2726	0.017	0.27
size3	large size at maturity (>16 mm)	-0.2309	-0.4668	0.016	0.27
volt1	semivoltine (<1 gen/yr)	-0.4517	-0.2531	0.019	0.27
crawl1	very low crawl speed (<10 cm/h)	-0.5164	-0.0244	0.017	0.27
swim2	weak swimming ability	-0.3862	-0.3058	0.03	0.24
size1	small size at maturity (<9 mm)	-0.2789	-0.3814	0.034	0.22
drift2	drift occurrence common	-0.2660	-0.3780	0.044	0.21
habit4	cling habit	-0.2862	-0.3549	0.044	0.21
dev3	nonseasonal development	0.0212	-0.4546	0.048	0.21
rheophily2	depositional and erosional rheophily	-0.3054	-0.3363	0.047	0.21

Table 3. 7. The 52 significantly correlated trait states for the spring 2020 NMDS of macroinvertebrate biomass.

Trait state	**Trait description**	**NMDS1**	**NMDS2**	**pval**	**r^2**
span3	long adult lifespan (>1 mo)	-0.7920	0.1392	0.001	0.65
habit4	cling habit	-0.4511	0.6300	0.001	0.60
armor2	poor armoring (heavily sclerotized)	-0.6480	0.4239	0.001	0.60
flightstrength2	strong flight	-0.7327	0.1995	0.001	0.58
volt2	Univoltine (1 gen/yr)	-0.6552	0.3768	0.001	0.57
shape2	not Streamlined (cylindrical, round, or bluff)	-0.7321	0.1744	0.001	0.57
dev2	slow seasonal development	-0.6466	0.3844	0.001	0.57
crawl3	high crawl speed (>100 cm/hr)	-0.6069	0.4432	0.001	0.56
size3	large size at maturity (>16 mm)	-0.4124	0.6282	0.001	0.56
rheophily3	erosional rheophily	-0.6210	0.4051	0.001	0.55
shape1	streamlined (flat, fusiform)	0.0190	0.7344	0.001	0.54
FFG3	Herbivore (Scraper, Piercer, and Shedder)	0.1225	0.7221	0.001	0.54
drift1	rare drift occurrence (catastrophic only)	-0.5075	0.5271	0.001	0.54
span2	short adult lifespan (<1 mo)	-0.5896	0.4302	0.001	0.53
thermophily1	Cold Stenothermal or Cool Eurythermal	-0.3084	0.6610	0.001	0.53
rheophily1	depositional rheophily	-0.6068	-0.4007	0.001	0.53
exit1	adult exit ability absent (not including emergence)	-0.4890	0.5334	0.001	0.52
emerg2	well synchronized emergence	-0.5949	0.4061	0.001	0.52
FFG4	predator (Piercer and Engulfer)	-0.6337	0.3325	0.001	0.51
dessic1	no dessication resistance	-0.4764	0.5293	0.001	0.51
attachment1	no attachment (free-ranging)	-0.4737	0.5243	0.001	0.50
drift2	drift occurrence common	-0.6372	0.3036	0.001	0.50
respiration2	gill respiration	-0.4502	0.5420	0.001	0.50
thermophily2	Cool/Warm Eurythermal	-0.6503	0.2610	0.001	0.49
dev3	nonseasonal development	-0.7006	-0.0090	0.001	0.49
armor3	good armoring (e.g. some cased caddisflies)	-0.1070	0.6896	0.001	0.49
swim1	no swimming ability	-0.6863	0.0887	0.001	0.48

femdis2	high female dispersal (>1 km flight before laying eggs)	-0.6466	0.2285	0.001	0.47
habit1	burrow habit	-0.6325	0.2618	0.001	0.47
volt3	bi- or multivoltine (>1 gen/yr)	0.2900	0.6167	0.001	0.46
exit2	adult exit ability	-0.6796	-0.0251	0.001	0.46
crawl2	low crawl speed (<100 cm/hr)	-0.4828	0.4691	0.002	0.45
emerg1	poorly synchronized emergence	-0.4559	0.4879	0.002	0.45
FFG2	collector-filterer	-0.6635	0.0630	0.001	0.44
armor1	no armoring (soft-bodied forms)	-0.5707	0.3423	0.002	0.44
femdis1	low female dispersal (<1 km flight befory laying eggs)	-0.4354	0.4988	0.001	0.44
respiration1	tegument respiration	-0.6130	0.2439	0.001	0.44
swim2	weak swimming ability	-0.2706	0.5992	0.001	0.43
habit2	climb habit	-0.5220	0.3964	0.003	0.43
dessic2	dessication resistance present	-0.6363	-0.0941	0.002	0.41
flightstrength1	weak flight (e.g. cannot fly into light breeze)	-0.3832	0.5089	0.002	0.41
FFG5	detritivore (shredder)	-0.6256	0.1037	0.002	0.40
size1	small size at maturity (<9 mm)	-0.5563	0.2687	0.002	0.38
dev1	fast seasonal development	-0.2862	0.5437	0.004	0.38
size2	medium size at maturity (9-16 mm)	-0.4799	0.3569	0.002	0.36
attachment2	some sessile	-0.5595	0.1886	0.002	0.35
rheophily2	depositional and erosional rheophily	-0.3455	0.4787	0.005	0.35
span1	very short life span (<1 wk)	-0.3384	0.4821	0.003	0.35
thermophily3	Warm Eurythermal	0.3977	0.4056	0.007	0.32
volt1	semivoltine (<1 gen/yr)	-0.5437	0.1460	0.006	0.32
swim3	strong swimming ability	0.1122	0.5088	0.014	0.27
FFG1	collector-gatherer	-0.0079	0.4984	0.026	0.25

Table 3. 8. Linear mixed effect model parameter estimates for benthic macroinvertebrate biomass in response to SC with stream as a random effect. Standard deviation in parentheses. Note: values are on ln(x+1) scale. Ephem-B = Ephemeroptera-Baetidae. CG = collector-gatherer. CF = collector-filterer.

	fall			*spring*		
Model	**SC effect size**	**Constant**	**sd(Stream)**	**SC effect size**	**Constant**	**sd(Stream)**
total	0.001** (0.001)	5.816*** (0.488)	0.682	0.001 (0.001)	6.470*** (0.507)	0.759
Ephemeroptera	-0.002 (0.001)	3.796*** (0.935)	1.499	-0.002** (0.001)	5.865*** (0.647)	1.027
Ephem -B	-0.003*** (0.001)	3.683*** (0.457)	0.515	-0.004*** (0.001)	5.686*** (0.779)	1.274
Chironomidae	0.001 (0.001)	2.139*** (0.511)	0.825	0.002** (0.001)	2.220*** (0.503)	0.806
Hydropsychidae	0.003*** (0.002)	1.506 (1.196)	1.913	0.002** (0.001)	3.244*** (0.719)	1.062
Leuctra	--	--	--	0.003*** (0.001)	1.841*** (0.6)	0.922
Amphinemura	--	--	--	0.002** (0.001)	1.986** (0.834)	1.34
drift abundant	0.0002 (0.001)	1.780** (0.883)	1.436	0.0004 (0.001)	2.716*** (0.711)	1.142
long adult lifespan	0.001 (0.001)	4.063*** (0.496)	0.399	0.001 (0.001)	3.093*** (0.617)	0.579
CG	0.001* (0.0004)	4.236*** (0.303)	0.273	-0.001* (0.001)	5.477*** (0.458)	0.709
CF	0.003* (0.002)	1.962 (1.214)	1.96	0.002** (0.001)	3.381*** (0.65)	0.891
herbivore	-0.0003 (0.001)	4.111*** (0.454)	0	-0.001 (0.001)	4.335*** (0.529)	0.644
predator	0.001 (0.001)	4.424*** (0.704)	1.017	0.001 (0.001)	4.853*** (0.636)	0.921
detritivore	0.002*** (0.001)	3.351*** (0.463)	0.447	0.002* (0.001)	4.237*** (0.773)	1.158
Note:	*p<0.1; **p<0.05; ***p<0.01					

Table 3. 9. Daily mean emergent biomass for total, Ephemeroptera, Plecoptera, and Trichoptera (EPT), Ephemeroptera, and Diptera.

Year	Season	Stream	n (samples)	Mean total emergent (mg m^{-2} d^{-})	Mean emergent EPT (mg m^{-2} d^{-})	Mean emergent Ephem (mg m^{-2} d^{-})	Mean emergent Diptera (mg m^{-2} d^{-})
2019	Summer	EAS	4	23.7 ± 30.3 (0.1 – 63.8)	22.4 ± 29.2 (0 – 61.3)	2 ± 4 (0 – 8.1)	1.3 ± 1.2 (0.1 – 2.5)
2019	Summer	CRO	4	1.1 ± 1 (0.2 – 2.4)	0.3 ± 0.5 (0 – 1.1)	0.1 ± 0.2 (0 – 0.3)	0.8 ± 1.1 (0.1 – 2.4)
2019	Summer	COP	4	11.8 ± 11.5 (4.6 – 29)	11.2 ± 11.7 (4.1 – 28.7)	0.9 ± 1.6 (0 – 3.3)	0.5 ± 0.5 (0 – 1.3)
2019	Summer	ROL	3	11.1 ± 10.3 (4.2 – 22.9)	10.3 ± 10.6 (4 – 22.5)	0	0.8 ± 0.8 (0.3 – 1.7)
2019	Summer	RIC	2	22.2 ± 11.1 (14.3 – 30.1)	16.6 ± 14.7 (6.2 – 27.1)	0	5.5 ± 3.6 (3 – 8.1)
2019	Fall	EAS	3	0.5 ± 0 (0.5 – 0.5)	0.3 ± 0.3 (0 – 0.5)	0	0.1 ± 0.1 (0 – 0.2)
2019	Fall	CRO	3	0.3 ± 0.2 (0.1 – 0.6)	0.2 ± 0.2 (0 – 0.4)	0	0.2 ± 0.1 (0 – 0.3)
2019	Fall	COP	4	2 ± 1.8 (0.1 – 4.2)	1.6 ± 1.4 (0 – 3.2)	0	0.4 ± 0.5 (0 – 1)
2019	Fall	ROL	3	0.2 ± 0.3 (0 – 0.5)	0	0	0.2 ± 0.3 (0 – 0.5)
2019	Fall	KUT	3	24.9 ± 34.9 (4 – 65.1)	24.3 ± 34.5 (3.3 – 64.1)	1.8 ± 1.7 (1.1 – 2.5)	0.6 ± 0.4 (0.2 – 1)
2020	Spring	CRO	1	16.9 ± NA (16.9 – 16.9)	9.1 ± NA (9.1 – 9.1)	0	5.8 ± NA (5.8 – 5.8)
2020	Spring	COP	4	20.4 ± 11 (11.4 – 35.9)	15.2 ± 6.1 (7.7 – 22.3)	4.8 ± 4.1 (0.6 – 10.4)	4.2 ± 4.2 (0.1 – 10)
2020	Spring	KUT	4	20 ± 10.1 (10.6 – 34.2)	13.2 ± 3.4 (10.3 – 16.2)	3 ± 0.5 (2.5 – 3.5)	0.8 ± 1.2 (0 – 2.7)
2020	Spring	RIC	4	52.8 ± 61 (17.2 – 144.2)	48.6 ± 54.5 (15.1 – 130.1)	0.1 ± 0.1 (0 – 0.3)	1.4 ± 1.5 (0.1 – 3.3)

Table 3. 10. Seasonal estimates of benthic biomass and emergent biomass used in calculation of percent emerged biomass by stream. * Data unavailable for some streams in some seasons due to high stream flows or demonic intrusion.

Stream	SC	Season	Year	benthic biomass whole season (mg m^{-2} season-^{1})	emergent biomass whole season (mg m^{-2} season-^{1})	Percent emerged biomass from benthic biomass
EAS	25	fall	2019	103,303.2	45.5	0.04
CRO	58	fall	2019	41,496	27.3	0.07
COP	123	fall	2019	116,707.5	182	0.16
ROL	562	fall	2019	49,767.9	18.2	0.04
KUT	920	fall	2019	130,503.1	2,265.9	1.74
RIC	1422	fall	2019	409,208.8	*--	--
EAS	25	spring	2020	279,670.3	--	--
CRO	60	spring	2020	435,98.1	1,537.9	3.53
COP	127	spring	2020	65,465.4	1,856.4	2.84
ROL	619	spring	2020	165,829.3	--	--
KUT	944	spring	2020	257,056.8	1,820	0.71
RIC	1366	spring	2020	294,448.7	4,804.8	1.63

CHAPTER 4

Conclusions

The Appalachian region is revered as a biodiversity hotspot (Collen et al. 2014). However, its legacy of coal mining will remain for decades with streams continuing to exhibit elevated conductivities accompanied by uncertain biotic recovery (Lindberg et al. 2011, Pond et al. 2014, Cianciolo et al. 2020). Land use threatens aquatic macroinvertebrate biodiversity and abundances, having potential cascading effects on local and downstream ecosystem processes and services. Our results demonstrate that richness-sampling effort models can be used to estimate site-specific taxonomic richness, sampling effort, and the level of community characterization associated with environmental change. However, we faced challenges in applying our taxon sampling models to trait states to assess the response diversity of stream functions because models required greater initial sampling effort. While five samples were sufficient in exploring total taxonomic richness of stream macroinvertebrate communities, the same level of sampling effort was insufficient for assessing subsets of taxa belonging to functional groups. Taxon sampling models may provide greater resolution towards assessing impairment or recovery following perturbation for macroinvertebrate communities. Effective sampling and estimation procedures will support more comprehensive biodiversity inventories of aquatic insects as well as declining terrestrial insect populations that better inform the conservation and management of this hyperdiverse group of organisms and the ecosystem processes that they contribute (Colwell and Coddington 1994).

To our knowledge, the present study is the first to conduct field measurements of *both* larval and emergent insect biomass along a mining-induced salinization gradient. Notable and concerning is our finding of decreased larval mayfly biomass in salinized streams, a trend that tracks what has been widely documented for relative abundances, densities, and secondary production of this order in other Appalachian stream studies (e.g., Pond 2010, Voss and Bernhardt 2017, Drover et al. 2019). Because macroinvertebrate biomass measures provide a common link between population and ecosystem level processes (Benke 1993), these values convey amounts that are comparable across studies, are informative of ecological processes such as the flow of organic matter and energy, and in turn, tell of stream ecosystem processes. As mayfly biomass is reduced in salinized, Appalachian headwaters, we can infer that food subsidies to riparian consumers are altered by either quantity, quality, or phenological timing.

Future directions

Streams and other freshwaters are increasingly imperiled by human activities and land use. Aquatic macroinvertebrates will continue to serve as important indicators of the ecological processes that give rise to stream condition. The combined lack of historical and quantitative data regarding insect biodiversity and abundances plus the uncertainty surrounding the ecological and economic impact of biodiversity loss have culminated in a general interest for the Biodiversity-Ecosystem Function relationship among researchers (Daam et al. 2019). Taxon sampling curves are suited to explore such ecological questions. In particular, additional study is needed to assess the relationship more accurately between sampling effort and macroinvertebrate response diversity. More than five Surber samples are needed to explore the application of taxon sampling models to macroinvertebrate traits in these Central Appalachian headwater streams.

We measured and compared the biomass of two aquatic insect life stages in response to a disturbance gradient. While we only compared larval and adult life stages, future studies may benefit from the inclusion of other life stages, such as eggs, or the differentiation of larval instars. Future studies may also consider assessing biomass metrics against multiple stressors to explore the potential for synergistic effects and to better reflect other conditions occurring in streams. Additionally, more intensive spatial and temporal sampling regimes could be conducted for emergence in an attempt to better characterize the composition of emergent biomass. A study of the phenological timing of insect emergence and its variability across Appalachian streams would help to fill current knowledge gaps and help to calibrate future efforts in measuring insect emergence to disturbance gradients in central Appalachia.

References

Benke, A. C. (1993). "Concepts and patterns of invertebrate production in running waters." Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen **25**(1): 15-38.

Cianciolo, T. R., D. L. McLaughlin, C. E. Zipper, A. J. Timpano, D. J. Soucek and S. H. Schoenholtz (2020). "Impacts to water quality and biota persist in mining-influenced Appalachian streams." Science of The Total Environment **717**: 137216.

Collen, B., F. Whitton, E. E. Dyer, J. E. Baillie, N. Cumberlidge, W. R. Darwall, C. Pollock, N. I. Richman, A. M. Soulsby and M. Böhm (2014). "Global patterns of freshwater species diversity, threat and endemism." Global ecology Biogeography **23**(1): 40-51.

Colwell, R. K. and J. A. Coddington (1994). "Estimating terrestrial biodiversity through extrapolation." Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **345**(1311): 101-118.

Daam, M. A., H. Teixeira, A. I. Lillebø and A. J. Nogueira (2019). "Establishing causal links between aquatic biodiversity and ecosystem functioning: Status and research needs." Science of the Total Environment **656**: 1145-1156.

Drover, D. R., C. E. Zipper, D. J. Soucek and S. H. Schoenholtz (2019). "Using density, dissimilarity, and taxonomic replacement to characterize mining-influenced benthic macroinvertebrate community alterations in central Appalachia." Ecological Indicators **106**: 105535.

Lindberg, T. T., E. S. Bernhardt, R. Bier, A. Helton, R. B. Merola, A. Vengosh and R. T. Di Giulio (2011). "Cumulative impacts of mountaintop mining on an Appalachian watershed." Proceedings of the National Academy of Sciences **108**(52): 20929-20934.

Pond, G. J. (2010). "Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA)." Hydrobiologia **641**(1): 185-201.

Pond, G. J., M. E. Passmore, N. D. Pointon, J. K. Felbinger, C. A. Walker, K. J. Krock, J. B. Fulton and W. L. Nash (2014). "Long-term impacts on macroinvertebrates downstream of reclaimed mountaintop mining valley fills in central Appalachia." Environmental management **54**(4): 919-933.

Voss, K. A. and E. S. Bernhardt (2017). "Effects of mountaintop removal coal mining on the diversity and secondary productivity of Appalachian rivers." Limnology Oceanography **62**(4): 1754-1770.

REFERENCES

Akaike, H. (1974). "A new look at the statistical model identification." IEEE transactions on automatic control **19**(6): 716-723.

Barbour, M. T., J. Gerritsen, B. D. Snyder and J. B. Stribling (1999). Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, Benthic Macroinvertebrates and

Fish, Second Edition. EPA 841-B-99-002. O. o. Water. Washington, D.C., U.S. Environmental Protection Agency.

Bartels, P., J. Cucherousset, K. Steger, P. Eklöv, L. J. Tranvik and H. Hillebrand (2012). "Reciprocal subsidies between freshwater and terrestrial ecosystems structure consumer resource dynamics." Ecology **93**(5): 1173-1182.

Baxter, C. V., K. D. Fausch and W. Carl Saunders (2005). "Tangled webs: reciprocal flows of invertebrate prey link streams and riparian zones." Freshwater biology **50**(2): 201-220.

Benda, L., M. A. Hassan, M. Church and C. L. May (2005). "Geomorphology of steepland headwaters: the transition from hillslopes to channels." Journal of the American Water Resources Association **41**(4): 835-851.

Benke, A. C. (1993). "Concepts and patterns of invertebrate production in running waters." Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen **25**(1): 15-38.

Benke, A. C., A. D. Huryn, L. A. Smock and J. B. Wallace (1999). "Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States." Journal of the North American Benthological Society **18**(3): 308-343.

Berger, E., O. Frör and R. B. Schäfer (2019). "Salinity impacts on river ecosystem processes: a critical mini-review." Philosophical Transactions of the Royal Society B **374**(1764): 20180010.

Bernhardt, E. S. and M. A. Palmer (2011). "The environmental costs of mountaintop mining valley fill operations for aquatic ecosystems of the Central Appalachians." Annals of the New york Academy of Sciences **1223**(1): 39-57.

Boehme, E. A., C. E. Zipper, S. H. Schoenholtz, D. J. Soucek and A. J. Timpano (2016). "Temporal dynamics of benthic macroinvertebrate communities and their response to elevated specific conductance in Appalachian coalfield headwater streams." Ecological Indicators **64**: 171-180.

Bonada, N., S. Doledec and B. Statzner (2007). "Taxonomic and biological trait differences of stream macroinvertebrate communities between mediterranean and temperate regions: implications for future climatic scenarios." Global Change Biology **13**(8): 1658-1671.

Boulton, A. J. (2003). "Parallels and contrasts in the effects of drought on stream macroinvertebrate assemblages." Freshwater Biology **48**(7): 1173-1185.

Boyero, L. (2003). "The quantification of local substrate heterogeneity in streams and its significance for macroinvertebrate assemblages." Hydrobiologia **499**(1): 161-168.

Brown, B. L., C. M. Swan, D. A. Auerbach, E. H. Campbell Grant, N. P. Hitt, K. O. Maloney and C. Patrick (2011). "Metacommunity theory as a multispecies, multiscale framework for studying the influence of river network structure on riverine communities and ecosystems." Journal of the North American Benthological Society **30**(1): 310-327.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage and G. B. West (2004). "Toward a metabolic theory of ecology." Ecology **85**(7): 1771-1789.

Buchwalter, D., S. Scheibener, H. Chou, D. Soucek and J. Elphick (2019). "Are sulfate effects in the mayfly Neocloeon triangulifer driven by the cost of ion regulation?" Philosophical Transactions of the Royal Society B **374**(1764): 20180013.

Cañedo-Argüelles, M., B. J. Kefford, C. Piscart, N. Prat, R. B. Schäfer and C.-J. Schulz (2013). "Salinisation of rivers: an urgent ecological issue." Environmental pollution **173**: 157-167.

Cardinale, B. J., D. S. Srivastava, J. E. Duffy, J. P. Wright, A. L. Downing, M. Sankaran and C. Jouseau (2006). "Effects of biodiversity on the functioning of trophic groups and ecosystems." Nature **443**(7114): 989.

Castillo, A. M., D. M. Sharpe, C. K. Ghalambor and L. F. De León (2018). "Exploring the effects of salinization on trophic diversity in freshwater ecosystems: a quantitative review." Hydrobiologia **807**(1): 1-17.

Chapin Iii, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala and S. E. Hobbie (2000). "Consequences of changing biodiversity." Nature **405**(6783): 234-242.

Cianciolo, T. R., D. L. McLaughlin, C. E. Zipper, A. J. Timpano, D. J. Soucek and S. H. Schoenholtz (2020). "Impacts to water quality and biota persist in mining-influenced Appalachian streams." Science of The Total Environment **717**: 137216.

Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal and S. Young (2015). "Biodiversity inhibits parasites: broad evidence for the dilution effect." Proceedings of the National Academy of Sciences **112**(28): 8667-8671.

Clark, E. V., W. L. Daniels, C. E. Zipper and K. Eriksson (2018). "Mineralogical influences on water quality from weathering of surface coal mine spoils." Applied Geochemistry **91**: 97-106.

Cobb, D., T. Galloway and J. Flannagan (1992). "Effects of discharge and substrate stability on density and species composition of stream insects." Canadian Journal of Fisheries Aquatic Sciences **49**(9): 1788-1795.

Collen, B., F. Whitton, E. E. Dyer, J. E. Baillie, N. Cumberlidge, W. R. Darwall, C. Pollock, N. I. Richman, A. M. Soulsby and M. Böhm (2014). "Global patterns of freshwater species diversity, threat and endemism." Global ecology Biogeography **23**(1): 40-51.

Colwell, R. K. and J. A. Coddington (1994). "Estimating terrestrial biodiversity through extrapolation." Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences **345**(1311): 101-118.

Colwell, R. K., C. X. Mao and J. Chang (2004). "Interpolating, extrapolating, and comparing incidence-based species accumulation curves." Ecology **85**(10): 2717-2727.

Cormier, S., G. Suter, L. Yuan and L. Zheng (2011). A field-based aquatic life benchmark for conductivity in central Appalachian streams, EPA/600/R-10/023F National Center for Environmental Assessment, Office of ….

Cormier, S. M., G. W. Suter and L. Zheng (2013). "Derivation of a benchmark for freshwater ionic strength." Environmental Toxicology Chemistry **32**(2): 263-271.

Cormier, S. M., G. W. Suter, L. Zheng and G. J. Pond (2013). "Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions." Environmental Toxicology Chemistry **32**(2): 277-287.

Courtemanch, D. L. (1996). "Commentary on the subsampling procedures used for rapid bioassessments." Journal of the North American Benthological Society **15**(3): 381-385.

Creed, I. F., C. R. Lane, J. N. Serran, L. C. Alexander, N. B. Basu, A. J. Calhoun, J. R. Christensen, M. J. Cohen, C. Craft and E. D'Amico (2017). "Enhancing protection for vulnerable waters." Nature geoscience **10**(11): 809-815.

Cuffney, T., M. D. Bilger and A. Haigler (2007). "Ambiguous taxa: effects on the characterization and interpretation of invertebrate assemblages." Journal of the North American Benthological Society **26**(2): 286-307.

Cummins, K. W. (1974). "Structure and function of stream ecosystems." BioScience **24**(11): 631-641.

Daam, M. A., H. Teixeira, A. I. Lillebø and A. J. Nogueira (2019). "Establishing causal links between aquatic biodiversity and ecosystem functioning: Status and research needs." Science of the Total Environment **656**: 1145-1156.

Darwall, W., K. Smith, D. Allen, M. Seddon, G. Mc Gregor Reid, V. Clausnitzer and V. J. T. Kalkman (2008). "Freshwater biodiversity–a hidden resource under threat." 126-232.

Downes, B. J., L. A. Barmuta, P. G. Fairweather, D. P. Faith, M. J. Keough, P. S. Lake, B. D. Mapstone and G. P. Quinn (2002). Monitoring Ecological Impacts: Concepts and Practice in Flowing Waters. New York, New York, USA, Cambridge University Press.

Downing, J. A., J. J. Cole, C. Duarte, J. J. Middelburg, J. M. Melack, Y. T. Prairie, P. Kortelainen, R. G. Striegl, W. H. McDowell and L. J. Tranvik (2012). "Global abundance and size distribution of streams and rivers." Inland waters **2**(4): 229-236.

Drover, D. R., C. E. Zipper, D. J. Soucek and S. H. Schoenholtz (2019). "Using density, dissimilarity, and taxonomic replacement to characterize mining-influenced benthic

macroinvertebrate community alterations in central Appalachia." Ecological Indicators **106**: 105535.

Echols, B. S., R. J. Currie and D. S. Cherry (2010). "Preliminary results of laboratory toxicity tests with the mayfly, Isonychia bicolor (Ephemeroptera: Isonychiidae) for development as a standard test organism for evaluating streams in the Appalachian coalfields of Virginia and West Virginia." Environmental monitoring assessment **169**(1-4): 487-500.

Ehrlich, P. R. and E. O. Wilson (1991). "Biodiversity studies: science and policy." Science **253**(5021): 758-762.

Elmqvist, T., C. Folke, M. Nyström, G. Peterson, J. Bengtsson, B. Walker and J. Norberg (2003). "Response diversity, ecosystem change, and resilience." Frontiers in Ecology the Environment **1**(9): 488-494.

Entrekin, S. A., N. A. Clay, A. Mogilevski, B. Howard-Parker and M. A. Evans-White (2018). "Multiple riparian–stream connections are predicted to change in response to salinization." Philosophical Transactions of the Royal Society B **374**(1764): 20180042.

Evans, D. M., C. E. Zipper, E. T. Hester and S. H. Schoenholtz (2015). "Hydrologic effects of surface coal mining in Appalachia (US)." Journal of the American Water Resources Association **51**(5): 1436-1452.

Fenneman, N. M. (1938). Physiography of Eastern United States. McGraw-Hill, New York.

Fritz, K. M., S. Fulton, B. R. Johnson, C. D. Barton, J. D. Jack, D. A. Word and R. A. Burke (2010). "Structural and functional characteristics of natural and constructed channels draining a reclaimed mountaintop removal and valley fill coal mine." Journal of the North American Benthological Society **29**(2): 673-689.

Gladyshev, M., A. Y. Kharitonov, O. Popova, N. Sushchik, O. Makhutova and G. Kalacheva (2011). Quantitative estimation of dragonfly role in transfer of essential polyunsaturated fatty acids from aquatic to terrestrial ecosystems. Doklady. Biochemistry and biophysics, Springer Science & Business Media.

Gladyshev, M. I., E. E. Gladysheva and N. N. Sushchik (2019). "Preliminary estimation of the export of omega-3 polyunsaturated fatty acids from aquatic to terrestrial ecosystems in biomes via emergent insects." Ecological Complexity **38**: 140-145.

Gotelli, N. J. and R. K. Colwell (2001). "Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness." Ecology letters **4**(4): 379-391.

Griffith, M. B., S. B. Norton, L. C. Alexander, A. I. Pollard and S. D. LeDuc (2012). "The effects of mountaintop mines and valley fills on the physicochemical quality of stream ecosystems in the central Appalachians: a review." Science of the Total Environment **417**: 1-12.

Haering, K. C., W. L. Daniels and J. M. Galbraith (2004). "Appalachian mine soil morphology and properties." Soil Science Society of America Journal **68**(4): 1315-1325.

Hartman, K. J., M. D. Kaller, J. W. Howell and J. A. Sweka (2005). "How much do valley fills influence headwater streams?" Hydrobiologia **532**(1-3): 91.

Hassell, K. L., B. J. Kefford and D. Nugegoda (2006). "Sub-lethal and chronic salinity tolerances of three freshwater insects: Cloeon sp. and Centroptilum sp.(Ephemeroptera: Baetidae) and Chironomus sp.(Diptera: Chironomidae)." Journal of Experimental Biology **209**(20): 4024-4032.

Huryn, A. D. and A. C. Benke (2007). Relationship between biomass turnover and body size for stream communities. Body size: the structure and function of aquatic ecosystems. A. G.

Hildrew, D. G. Raffaelli and R. Edmunds-Brown. Cambridge, New York, Cambridge University Press**:** 55-76.

Hynes, H. B. N. (1975). "The stream and its valley." Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen **19**(1): 1-15.

Jackson, J. K. and S. G. Fisher (1986). "Secondary production, emergence, and export of aquatic insects of a Sonoran Desert stream." Ecology **67**(3): 629-638.

Jarvis, B. (2018). The insect apocalypse is here. The New York Times. New York City, New York, A.G. Sulzberger.

Johnson, B. R., K. M. Fritz and R. Price (2013). "Estimating benthic secondary production from aquatic insect emergence in streams affected by mountaintop removal coal mining, West Virginia, USA." Fundamental Applied Limnology/Archiv für Hydrobiologie **182**(3): 191-204.

Johnson, B. R., P. C. Weaver, C. T. Nietch, J. M. Lazorchak, K. A. Struewing and D. H. Funk (2015). "Elevated major ion concentrations inhibit larval mayfly growth and development." Environmental toxicology chemistry **34**(1): 167-172.

Kaushal, S. S. (2016). "Increased Salinization Decreases Safe Drinking Water." Environmental Science & Technology **50**(6): 2765-2766.

Kaushal, S. S., G. E. Likens, M. L. Pace, R. M. Utz, S. Haq, J. Gorman and M. Grese (2018). "Freshwater salinization syndrome on a continental scale." Proceedings of the National Academy of Sciences **115**(4): E574-E583.

Kefford, B. J. (2018). "Why are mayflies (Ephemeroptera) lost following small increases in salinity? Three conceptual osmophysiological hypotheses." Philosophical Transactions of the Royal Society B **374**(1764): 20180021.

Kefford, B. J., P. J. Papas, L. Metzeling and D. Nugegoda (2004). "Do laboratory salinity tolerances of freshwater animals correspond with their field salinity?" Environmental Pollution **129**(3): 355-362.

Kennedy, A., D. Cherry and R. Currie (2004). "Evaluation of ecologically relevant bioassays for a lotic system impacted by a coal-mine effluent, using Isonychia." Environmental Monitoring Assessment **95**(1-3): 37-55.

Kunz, J. L., J. M. Conley, D. B. Buchwalter, T. J. Norberg-King, N. E. Kemble, N. Wang and C. G. Ingersoll (2013). "Use of reconstituted waters to evaluate effects of elevated major ions associated with mountaintop coal mining on freshwater invertebrates." Environmental Toxicology Chemistry **32**(12): 2826-2835.

Li, J., A. Herlihy, W. Gerth, P. Kaufmann, S. Gregory, S. Urquhart and D. P. Larsen (2001). "Variability in stream macroinvertebrates at multiple spatial scales." Freshwater Biology **46**(1): 87-97.

Lindberg, T. T., E. S. Bernhardt, R. Bier, A. Helton, R. B. Merola, A. Vengosh and R. T. Di Giulio (2011). "Cumulative impacts of mountaintop mining on an Appalachian watershed." Proceedings of the National Academy of Sciences **108**(52): 20929-20934.

Marcarelli, A. M., C. V. Baxter, M. M. Mineau and R. O. Hall Jr (2011). "Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters." Ecology **92**(6): 1215-1225.

McCune, B. and J. B. Grace (2002). Analysis of ecological communities. Gleneden Beach, Oregon, MjM Software Design.

Merricks, T. C., D. S. Cherry, C. E. Zipper, R. J. Currie and T. W. Valenti (2007). "Coal-mine hollow fill and settling pond influences on headwater streams in southern West Virginia, USA." Environmental Monitoring Assessment **129**(1-3): 359-378.

Merritt, R., K. Cummins and M. Berg (2008). An Introduction to the Aquatic Insects of North America. Dubuque, Iowa, USA, Kendall Hunt Publishing.

Meyer, J. L., D. L. Strayer, J. B. Wallace, S. L. Eggert, G. S. Helfman and N. E. Leonard (2007). "The Contribution of Headwater Streams to Biodiversity in River Networks 1." Journal of the American Water Resources Association **43**(1): 86-103.

Micheli, F., K. L. Cottingham, J. Bascompte, O. N. Bjørnstad, G. L. Eckert, J. M. Fischer, T. H. Keitt, B. E. Kendall, J. L. Klug and J. A. Rusak (1999). "The dual nature of community variability." Oikos: 161-169.

Murakami, M. and S. Nakano (2001). "Species-specific foraging behavior of birds in a riparian forest." Ecological Research **16**(5): 913-923.

Nippgen, F., M. R. Ross, E. S. Bernhardt and B. L. McGlynn (2017). "Creating a more perennial problem? Mountaintop removal coal mining enhances and sustains saline baseflows of Appalachian watersheds." Environmental science technology **51**(15): 8324-8334.

Odum, E. P., J. T. Finn and E. H. Franz (1979). "Perturbation theory and the subsidy-stress gradient." Bioscience **29**(6): 349-352.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. O'Hara, G. L. Simpson, P. Solymos, M. Henry, H. Stevens, E. Szoecs and H. Wagne (2019). vegan: Community Ecology Package.

Olszewski, T. D. (2004). "A unified mathematical framework for the measurement of richness and evenness within and among multiple communities." Oikos **104**(2): 377-387.

Orndorff, Z. W., W. L. Daniels, C. E. Zipper, M. Eick and M. Beck (2015). "A column evaluation of Appalachian coal mine spoils' temporal leaching behavior." Environmental Pollution **204**: 39-47.

Outhwaite, C. L., R. D. Gregory, R. E. Chandler, B. Collen and N. J. Isaac (2020). "Complex long-term biodiversity change among invertebrates, bryophytes and lichens." Nature Ecology Evolution **4**(3): 384-392.

Paetzold, A. and K. Tockner (2005). "Effects of riparian arthropod predation on the biomass and abundance of aquatic insect emergence." Journal of the North American Benthological Society **24**(2): 395-402.

Palmer, M. A., E. S. Bernhardt, W. H. Schlesinger, K. N. Eshleman, E. Foufoula-Georgiou, M. S. Hendryx, A. D. Lemly, G. E. Likens, O. L. Loucks and M. E. Power (2010). "Mountaintop mining consequences." Science **327**(5962): 148-149.

Palmer, M. A., J. B. Zedler and D. A. Falk (2016). Foundations of restoration ecology. Washington, D.C., Island Press.

Pence, R. A. (2019). Comparison of Quantitative and Semi-Quantitative Assessments of Benthic Macroinvertebrate Community Response to Elevated Salinity in central Appalachian Coalfield Streams, Virginia Tech.

Pericak, A. A., C. J. Thomas, D. A. Kroodsma, M. F. Wasson, M. R. Ross, N. E. Clinton, D. J. Campagna, Y. Franklin, E. S. Bernhardt and J. F. Amos (2018). "Mapping the yearly extent of surface coal mining in Central Appalachia using Landsat and Google Earth Engine." PloS one **13**(7): e0197758.

Peterson, G., C. R. Allen and C. S. Holling (1998). "Ecological resilience, biodiversity, and scale." Ecosystems **1**(1): 6-18.

Poff, N. L., J. D. Olden, N. K. Vieira, D. S. Finn, M. P. Simmons and B. C. Kondratieff (2006). "Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships." Journal of the North American Benthological Society

25(4): 730-755.

Polis, G. A., W. B. Anderson and R. D. Holt (1997). "Toward an integration of landscape and food web ecology: the dynamics of spatially subsidized food webs." Annual review of ecology systematics **28**(1): 289-316.

Pond, G. (2012). "Biodiversity loss in Appalachian headwater streams (Kentucky, USA): Plecoptera and Trichoptera communities." Hydrobiologia **679**(1): 97-117.

Pond, G. J. (2010). "Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA)." Hydrobiologia **641**(1): 185-201.

Pond, G. J., M. E. Passmore, F. A. Borsuk, L. Reynolds and C. J. Rose (2008). "Downstream effects of mountaintop coal mining: comparing biological conditions using family-and genus-level macroinvertebrate bioassessment tools." Journal of the North American Benthological Society **27**(3): 717-737.

Pond, G. J., M. E. Passmore, N. D. Pointon, J. K. Felbinger, C. A. Walker, K. J. Krock, J. B. Fulton and W. L. Nash (2014). "Long-term impacts on macroinvertebrates downstream of reclaimed mountaintop mining valley fills in central Appalachia." Environmental management **54**(4): 919-933.

Ramos-Merchante, A. and J. Prenda (2017). "Macroinvertebrate taxa richness uncertainty and kick sampling in the establishment of Mediterranean rivers ecological status." Ecological Indicators **72**: 1-12.

Reid, A. J., A. K. Carlson, I. F. Creed, E. J. Eliason, P. A. Gell, P. T. Johnson, K. A. Kidd, T. J. MacCormack, J. D. Olden and S. J. Ormerod (2019). "Emerging threats and persistent conservation challenges for freshwater biodiversity." Biological Reviews **94**(3): 849-873.

Ross, M. R., B. L. McGlynn and E. S. Bernhardt (2016). "Deep impact: effects of mountaintop mining on surface topography, bedrock structure, and downstream waters." Environmental science technology **50**(4): 2064-2074.

Sabo, J. L., J. L. Bastow and M. E. Power (2002). "Length–mass relationships for adult aquatic and terrestrial invertebrates in a California watershed." Journal of the North American Benthological Society **21**(2): 336-343.

Schmidt, T. S., J. M. Kraus, D. M. Walters and R. B. Wanty (2013). "Emergence flux declines disproportionately to larval density along a stream metals gradient." Environmental science technology **47**(15): 8784-8792.

Singer, P. C. and W. Stumm (1970). "Acidic mine drainage: the rate-determining step." Science **167**(3921): 1121-1123.

Skousen, J. and C. E. Zipper (2014). "Post-mining policies and practices in the Eastern USA coal region." International journal of coal science technology **1**(2): 135-151.

Soberón, J. and J. Llorente (1993). "The use of species accumulation functions for the prediction of species richness." Conservation biology **7**(3): 480-488.

Soucek, D. J. and A. Dickinson (2015). "Full-life chronic toxicity of sodium salts to the mayfly Neocloeon triangulifer in tests with laboratory cultured food." Environmental toxicology chemistry **34**(9): 2126-2137.

Strahler, A. N. (1957). "Quantitative analysis of watershed geomorphology." Eos, Transactions American Geophysical Union **38**(6): 913-920.

Suárez, M., M. Sánchez-Montoya, R. Gómez, M. Arce, R. Del Campo and M. Vidal-Abarca (2017). "Functional response of aquatic invertebrate communities along two natural stress gradients (water salinity and flow intermittence) in Mediterranean streams." Aquatic Sciences **79**(1): 1-12.

Thomas, C. D., T. H. Jones and S. E. Hartley (2019). "“Insectageddon”: A call for more robust data and rigorous analyses." Global change biology **25**(6): 1891-1892.

Thompson, G. G. and P. C. Withers (2003). "Effect of species richness and relative abundance on the shape of the species accumulation curve." Austral Ecology **28**(4): 355-360.

Timpano, A. J., S. H. Schoenholtz, D. J. Soucek and C. E. Zipper (2015). "Salinity as a limiting factor for biological condition in mining-influenced central Appalachian headwater streams." Journal of the American Water Resources Association **51**(1): 240-250.

Timpano, A. J., S. H. Schoenholtz, D. J. Soucek and C. E. Zipper (2018). "Benthic macroinvertebrate community response to salinization in headwater streams in Appalachia USA over multiple years." Ecological indicators **91**: 645-656.

Timpano, A. J., S. H. Schoenholtz, C. E. Zipper and D. J. Soucek (2010). Isolating effects of total dissolved solids on aquatic life in central Appalachian coalfield streams. Proc. 2010 national meeting of the american society of mining and reclamation, pittsburgh, PA.

Timpano, A. J., C. E. Zipper, D. J. Soucek and S. H. Schoenholtz (2018). "Seasonal pattern of anthropogenic salinization in temperate forested headwater streams." Water research **133**: 8-18.

Townsend, C. R. and R. M. Thompson (2007). Body size in streams: macroinvertebrate community size composition along natural and human-induced environmental gradients.

Body size: The structure function of aquatic ecosystems. A. G. Hildrew, D. G. Raffaelli and R. Edmonds-Brown. Cambridge, New York, Cambridge University Press**:** 77-97.

Townsend, P. A., D. P. Helmers, C. C. Kingdon, B. E. McNeil, K. M. de Beurs and K. N. Eshleman (2009). "Changes in the extent of surface mining and reclamation in the Central Appalachians detected using a 1976–2006 Landsat time series." Remote Sensing of Environment **113**(1): 62-72.

Ugland, K. I., J. S. Gray and K. E. Ellingsen (2003). "The species–accumulation curve and estimation of species richness." Journal of Animal Ecology **72**(5): 888-897.

USEPA (2011). The effects of mountaintop mines and valley fills on aquatic ecosystems of the central Appalachian coalfields.

Vander Vorste, R., A. J. Timpano, C. Cappellin, B. D. Badgley, C. E. Zipper and S. H. Schoenholtz (2019). "Microbial and macroinvertebrate communities, but not leaf decomposition, change along a mining-induced salinity gradient." Freshwater biology **64**(4): 671-684.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell and C. E. Cushing (1980). "The river continuum concept." Canadian journal of fisheries aquatic sciences **37**(1): 130-137.

Voss, K. A. and E. S. Bernhardt (2017). "Effects of mountaintop removal coal mining on the diversity and secondary productivity of Appalachian rivers." Limnology Oceanography **62**(4): 1754-1770.

Wallace, J. B., S. Eggert, J. L. Meyer and J. Webster (1999). "Effects of resource limitation on a detrital-based ecosystem." Ecological monographs **69**(4): 409-442.

Wallace, J. B. and J. R. Webster (1996). "The role of macroinvertebrates in stream ecosystem function." Annual review of entomology **41**(1): 115-139.

Wanless, H. R. (1946). Pennsylvanian geology of a part of the southern Appalachian coal field, Geological Society of America.

Wesner, J. S., J. M. Kraus, T. S. Schmidt, D. M. Walters and W. H. Clements (2014). "Metamorphosis enhances the effects of metal exposure on the mayfly, Centroptilum triangulifer." Environmental science technology **48**(17): 10415-10422.

Whitmore, K. M., S. H. Schoenholtz, D. J. Soucek, W. A. Hopkins and C. E. Zipper (2018). "Selenium dynamics in headwater streams of the central Appalachian coalfield." Environmental toxicology chemistry **37**(10): 2714-2726.

Williams, W. and J. Sherwood (1994). "Definition and measurement of salinity in salt lakes." International Journal of Salt Lake Research **3**(1): 53-63.

Wofford, J. E. B., E. D. Demarest and J. R. V. Jr. (2018). Shenandoah National Park Benthic Macroinvertebrate Monitoring Protocol, National Park Service.

Zipper, C. E., J. A. Burger, J. G. Skousen, P. N. Angel, C. D. Barton, V. Davis and J. A. Franklin (2011). "Restoring forests and associated ecosystem services on Appalachian coal surface mines." Environmental Management **47**(5): 751-765.

APPENDIX

Table 2-A 1. Taxonomic richness based on Chao1 estimates accumulated with successive sample number. Chao1 estimates are means presented with standard deviation and 2.5% and 97.5% quantiles based on 100 permutations. Model column specifies whether estimates are based on unequal sampling design (n=5-10 samples among streams), equal sampling design (n=5 for all streams), or trait-based design where taxa counts are limited to only those belonging to the trait state of interest. SC is annual mean calculated from daily averages in µS/cm.

Model	Site SC	n	Chao	2.50%	97.50%	Std.Dev
Unequal	31	1	23.3	12.5	37	6.1
Unequal	31	2	33	27	39.5	3.6
Unequal	31	3	38	30.5	49.2	4.8
Unequal	31	4	42.4	34.1	63	7.6
Unequal	31	5	44.8	35.7	61.8	6.7
Unequal	31	6	47.6	37.7	66	7.6
Unequal	31	7	48.6	38.5	62.2	6.1
Unequal	31	8	49.7	42.5	57.1	4.6
Unequal	31	9	51.5	46.2	57.8	3.2
Unequal	31	10	56.3	56.3	56.3	0
Unequal	72	1	28.6	21	39	5.9
Unequal	72	2	40.7	30	72.5	9.6
Unequal	72	3	48.8	33.7	82.4	13
Unequal	72	4	54.1	38.7	87.5	12.7
Unequal	72	5	56.5	43.6	92.6	11.6
Unequal	72	6	59.2	46	97.1	12.6
Unequal	72	7	57.9	49.6	68.9	8
Unequal	72	8	59.7	51.1	71.2	5.8
Unequal	72	9	60.4	53.8	67	4.5
Unequal	72	10	60.9	60.9	60.9	0
Unequal	73	1	23.1	16.3	37.3	7
Unequal	73	2	34.8	23.5	55	7
Unequal	73	3	40.7	30	55.4	6.1
Unequal	73	4	44.3	36.6	52.8	4.5
Unequal	73	5	46.9	40.6	52.6	3.5
Unequal	73	6	48.7	43	52.8	2.8
Unequal	73	7	50.3	46.1	53	1.8
Unequal	73	8	51.6	49.1	53	1
Unequal	73	9	52.5	52.5	52.5	0
Unequal	1045	1	14.9	9.3	25.3	5.5
Unequal	1045	2	21.2	12.3	56	9.1
Unequal	1045	3	26.4	18.7	57	10.4
Unequal	1045	4	29.3	20.5	67	12.4

Unequal	1045	5	29.9	22.3	60	8.9
Unequal	1045	6	30.7	23.8	33	2.8
Unequal	1045	7	34	34	34	0
Unequal	1061	1	22.6	15.5	34	6
Unequal	1061	2	29.5	21.2	41.5	6.4
Unequal	1061	3	32.9	23.2	42.5	5.5
Unequal	1061	4	35.8	27	42.6	4.5
Unequal	1061	5	38.2	28.2	44.3	4
Unequal	1061	6	40	31.2	44.4	3.1
Unequal	1061	7	42.1	36.3	45.8	2.2
Unequal	1061	8	44.3	41.1	46.1	1.4
Unequal	1061	9	46.1	46.1	46.1	0
Unequal	1340	1	27.8	6	47.5	13.9
Unequal	1340	2	34.4	17.8	51.5	11.5
Unequal	1340	3	39.3	26.5	52.5	10.1
Unequal	1340	4	43.7	28	52.5	8.4
Unequal	1340	5	47.2	47.2	47.2	0
Equal	31	1	21.0	12.5	29.5	5.7
Equal	31	2	31.0	27	34.2	2.1
Equal	31	3	36.3	35	42	1.9
Equal	31	4	40.5	39	43	1.6
Equal	31	5	48.3	48.25	48.25	0.0
Equal	73	1	26.9	21.0	37	5.8
Equal	73	2	43.8	34.2	72.5	11.4
Equal	73	3	56.2	41.0	81.5	13.3
Equal	73	4	70.0	49.3	108	22.4
Equal	73	5	74.3	74.3	74.3	0.0
Equal	72	1	22.9	16.4	25.5	3.4
Equal	72	2	33.2	28.4	39.0	4.1
Equal	72	3	39.2	31.3	48.3	4.7
Equal	72	4	41.8	38.2	44.2	2.2
Equal	72	5	43.9	43.9	43.9	0.0
Equal	1045	1	12.3	9.3	17.5	2.9
Equal	1045	2	17.2	12.3	20.0	2.3
Equal	1045	3	20.2	16.8	22.0	1.5
Equal	1045	4	22.3	21.2	24.3	1.1
Equal	1045	5	23.5	23.5	23.5	0.0
Equal	1061	1	21.6	19.5	25.9	2.2
Equal	1061	2	27.3	23.0	32.7	3.2
Equal	1061	3	31.3	27.8	35.7	2.8
Equal	1061	4	35.0	30.5	37.5	2.5
Equal	1061	5	38.5	38.5	38.5	0.0
Equal	1340	1	27.5	6.0	47.5	13.8

Equal	1340	2	33.7	17.8	51.5	11.1
Equal	1340	3	39.0	26.5	52.5	10.2
Equal	1340	4	43.0	28.0	52.5	8.7
Equal	1340	5	47.2	47.2	47.2	0.0
herbivore	31	1	2.4	1.0	5.0	1.3
herbivore	31	2	3.5	2.0	6.0	1.4
herbivore	31	3	4.32	3.0	6.0	1.1
herbivore	31	4	4.8	4.0	6.0	0.8
herbivore	31	5	5	5.0	5.0	0.0
herbivore	72	1	3.54	2.0	5.0	1.0
herbivore	72	2	5.36	4.0	7.0	0.9
herbivore	72	3	6.73	5.0	10.0	1.6
herbivore	72	4	8.23	7.0	10.0	1.5
herbivore	72	5	10	10.0	10.0	0.0
herbivore	73	1	4.24	2.0	10.0	2.9
herbivore	73	2	5.88	3.0	10.0	2.8
herbivore	73	3	6.63	4.0	10.0	2.0
herbivore	73	4	7.05	5.0	8.0	1.1
herbivore	73	5	7	7.0	7.0	0.0
herbivore	1045	1	1.5	1.0	3.5	1.0
herbivore	1045	2	2.23	1.0	4.0	1.5
herbivore	1045	3	2.8	1.0	4.0	1.5
herbivore	1045	4	3.46	1.0	4.0	1.2
herbivore	1045	5	4	4.0	4.0	0.0
herbivore	1061	1	2.4	0.0	4.0	1.6
herbivore	1061	2	3.8	1.0	5.0	1.2
herbivore	1061	3	4.74	3.0	6.5	0.9
herbivore	1061	4	5.615	5.0	6.5	0.7
herbivore	1061	5	6.5	6.5	6.5	0.0
herbivore	1340	1	1.87	0	7	2.7
herbivore	1340	2	3.36	0	7	3.1
herbivore	1340	3	4.66	1	7	2.9
herbivore	1340	4	5.74	1	7	2.5
herbivore	1340	5	7	7	7	0
shredder	31	1	3.9	2.0	5.0	1.5
shredder	31	2	5.3	4.0	6.0	0.7
shredder	31	3	5.9	5.0	6.5	0.4
shredder	31	4	6.0	6.0	6.0	0.0
shredder	31	5	6.0	6.0	6.0	0.0
shredder	72	1	3.8	3.0	4.0	0.4
shredder	72	2	6.8	6.0	11.0	1.9
shredder	72	3	9.2	6.3	13.0	2.5
shredder	72	4	10.8	8.0	18.0	3.5

shredder	72	5	11.0	11.0	11.0	0.0
shredder	73	1	2.4	2.0	3.0	0.5
shredder	73	2	3.6	2.0	4.5	0.8
shredder	73	3	4.4	3.0	5.5	0.8
shredder	73	4	4.8	4.0	5.0	0.4
shredder	73	5	5.0	5.0	5.0	0.0
shredder	1045	1	1.6	1.0	3.0	0.8
shredder	1045	2	2.1	1.0	3.0	0.8
shredder	1045	3	2.5	1.0	3.0	0.7
shredder	1045	4	2.8	2.0	3.0	0.4
shredder	1045	5	3.0	3.0	3.0	0.0
shredder	1061	1	1.2	1.0	2.0	0.4
shredder	1061	2	1.4	1.0	2.0	0.5
shredder	1061	3	1.6	1.0	2.0	0.5
shredder	1061	4	1.8	1.0	2.0	0.4
shredder	1061	5	2.0	2.0	2.0	0.0
shredder	1340	1	2.8	2.0	4.0	0.7
shredder	1340	2	3.3	2.0	5.0	0.8
shredder	1340	3	4.0	3.0	5.5	0.8
shredder	1340	4	4.8	4.0	5.5	0.7
shredder	1340	5	5.5	5.5	5.5	0.0
small body size	31	1	8.5	4.0	13.0	2.8
small body size	31	2	10.2	9.0	11.0	0.8
small body size	31	3	10.5	9.0	11.0	0.7
small body size	31	4	10.8	10.0	11.0	0.4
small body size	31	5	11.0	11.0	11.0	0.0
small body size	72	1	7.7	6.0	13.0	2.5
small body size	72	2	11.0	8.0	15.0	2.7
small body size	72	3	13.4	9.0	17.0	3.0
small body size	72	4	15.4	11.0	18.0	2.6
small body size	72	5	16.0	16.0	16.0	0.0
small body size	73	1	9.0	6.0	12.0	2.0
small body size	73	2	12.4	11.0	15.0	1.3

small body size	73	3	14.2	13.0	15.5	0.8
small body size	73	4	15.5	15.0	16.5	0.5
small body size	73	5	16.5	16.5	16.5	0.0
small body size	1045	1	6.2	4.0	8.3	1.5
small body size	1045	2	8.1	6.0	9.0	1.0
small body size	1045	3	9.1	8.0	10.0	0.5
small body size	1045	4	9.6	9.0	10.0	0.5
small body size	1045	5	10.0	10.0	10.0	0.0
small body size	1061	1	9.6	8.0	13.0	1.9
small body size	1061	2	12.2	10.0	15.3	2.1
small body size	1061	3	14.0	11.0	16.0	1.8
small body size	1061	4	15.5	13.0	17.0	1.4
small body size	1061	5	17.0	17.0	17.0	0.0
small body size	1340	1	13.6	4.0	21.0	6.5
small body size	1340	2	16.9	6.5	26.5	5.6
small body size	1340	3	20.6	13.3	26.5	5.3
small body size	1340	4	22.8	13.3	26.5	4.9
small body size	1340	5	25.0	25.0	25.0	0.0

Table 2-A 2. Theoretical total taxonomic richness based on mechanistic growth model parameter estimates for each stream with unequal, actual sampling effort among streams (n=5-10) and equal, actual sampling effort among streams (constrained to n=5 for each). The asymptote value signifies the point at which sampling effort no longer returns unobserved taxa for the given study area and is therefore the estimate for total taxonomic richness. The scale parameter determines the starting y-value of the model or the taxonomic richness with initial sampling effort. The growth rate determines the rate at which new taxa are observed and therefore the initial steepness of the sampling curve.

Model	SC	Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Unequal	31	Asymptote	56.44	2.35	575.94	<0.0001	51.83	61.05
	31	Scale	0.74	0.05	265.61	<0.0001	0.65	0.83
	31	Growth Rate	0.26	0.05	28.07	<0.0001	0.16	0.36
	72	Asymptote	60.92	0.45	18006.80	<0.0001	60.03	61.81
Unequal	72	Scale	0.88	0.03	670.00	<0.0001	0.81	0.94
	72	Growth Rate	0.50	0.03	313.17	<0.0001	0.44	0.55
	73	Asymptote	52.73	0.63	6904.74	<0.0001	51.48	53.97
Unequal	73	Scale	0.85	0.03	595.05	<0.0001	0.78	0.92
	73	Growth Rate	0.43	0.03	186.93	<0.0001	0.37	0.49
	1045	Asymptote	34.47	1.66	429.54	<0.0001	31.21	37.73
Unequal	1045	Scale	0.85	0.08	105.08	<0.0001	0.69	1.02
	1045	Growth Rate	0.41	0.09	20.02	<0.0001	0.23	0.59
	1061	Asymptote	51.18	2.50	418.30	<0.0001	46.28	56.09
Unequal	1061	Scale	0.66	0.02	1219.26	<0.0001	0.62	0.70
	1061	Growth Rate	0.20	0.04	31.34	<0.0001	0.13	0.26
	1340	Asymptote	62.62	2.15	847.83	<0.0001	58.40	66.83
Unequal	1340	Scale	0.68	0.01	11350.32	<0.0001	0.67	0.69
	1340	Growth Rate	0.20	0.02	122.68	<0.0001	0.17	0.24
	31	Asymptote	80.37	44.19	3.31	0.07	-6.24	166.99
Equal	31	Scale	0.84	0.05	263.97	<0.0001	0.74	0.94
	31	Growth Rate	0.14	0.14	1.02	0.31	-0.14	0.42
	72	Asymptote	103.47	17.69	34.19	<0.0001	68.78	138.15
Equal	72	Scale	0.96	0.05	333.69	<0.0001	0.86	1.06
	72	Growth Rate	0.25	0.09	7.58	0.01	0.07	0.43
	73	Asymptote	45.94	0.10	196781.23	<0.0001	45.74	46.14
Equal	73	Scale	0.91	0.01	24633.40	<0.0001	0.90	0.92

	73	Growth Rate	0.60	0.01	7398.49	<0.0001	0.58	0.61
Equal	1045	Asymptote	25.85	0.29	8032.52	<0.0001	25.29	26.42
	1045	Scale	0.81	0.01	4622.82	<0.0001	0.79	0.83
	1045	Growth Rate	0.44	0.02	501.58	<0.0001	0.40	0.47
Equal	1061	Asymptote	54.48	5.33	104.56	<0.0001	44.03	64.92
	1061	Scale	0.72	0.02	1768.58	<0.0001	0.68	0.75
	1061	Growth Rate	0.18	0.04	19.35	<0.0001	0.10	0.26
Equal	1340	Asymptote	63.39	4.14	234.61	<0.0001	55.28	71.50
	1340	Scale	0.69	0.01	3344.05	<0.0001	0.67	0.71
	1340	Growth Rate	0.20	0.03	36.10	<0.0001	0.13	0.26

Table 2-A 3. Mechanistic model growth parameters for traits herbivore, shredder, and small body size.

Model	SC	Trait	Parameter	Estimate	Std Error	Wald ChiSquare	Prob > ChiSquare	Lower 95%	Upper 95%
Unequal	31	herbivore	Asymptote	5.06	0.06	6818.35	<0.0001	4.94	5.18
Unequal	31	herbivore	Scale	0.79	0.03	853.51	<0.0001	0.74	0.84
Unequal	31	herbivore	Growth Rate	0.35	0.02	203.71	<0.0001	0.31	0.40
Unequal	72	herbivore	Asymptote	8.21	0.05	24294.22	<0.0001	8.10	8.31
Unequal	72	herbivore	Scale	0.99	0.03	1166.83	<0.0001	0.93	1.04
Unequal	72	herbivore	Growth Rate	0.49	0.02	547.95	<0.0001	0.45	0.53
Unequal	73	herbivore	Asymptote	9.53	0.16	3544.95	<0.0001	9.21	9.84
Unequal	73	herbivore	Scale	0.77	0.02	1133.98	<0.0001	0.72	0.81
Unequal	73	herbivore	Growth Rate	0.31	0.02	158.29	<0.0001	0.26	0.36
Unequal	1045	herbivore	Asymptote	9.25	3.09	8.97	0.00	3.19	15.30
Unequal	1045	herbivore	Scale	0.94	0.01	5230.23	<0.0001	0.91	0.96
Unequal	1045	herbivore	Growth Rate	0.07	0.03	4.45	0.03	0.01	0.14
Unequal	1061	herbivore	Asymptote	34.74	23.00	2.28	0.13	-10.35	79.82
Unequal	1061	herbivore	Scale	0.95	0.03	753.87	<0.0001	0.88	1.01
Unequal	1061	herbivore	Growth Rate	0.02	0.01	1.73	0.19	-0.01	0.04
Unequal	1340	herbivore	Asymptote	20.47	7.41	7.63	0.01	5.95	35.00
Unequal	1340	herbivore	Scale	0.98	0.01	29971.63	<0.0001	0.97	0.99
Unequal	1340	herbivore	Growth Rate	0.08	0.04	4.61	0.03	0.01	0.15
Unequal	31	shredder	Asymptote	6.05	0.04	21611.93	<0.0001	5.97	6.13
Unequal	31	shredder	Scale	0.63	0.04	323.19	<0.0001	0.56	0.70
Unequal	31	shredder	Growth Rate	0.54	0.04	162.93	<0.0001	0.45	0.62
Unequal	72	shredder	Asymptote	14.03	0.51	742.88	<0.0001	13.02	15.04
Unequal	72	shredder	Scale	1.08	0.09	148.60	<0.0001	0.90	1.25
Unequal	72	shredder	Growth Rate	0.36	0.06	41.18	<0.0001	0.25	0.46
Unequal	73	shredder	Asymptote	7.74	0.19	1691.61	<0.0001	7.38	8.11
Unequal	73	shredder	Scale	0.87	0.02	2638.94	<0.0001	0.84	0.90
Unequal	73	shredder	Growth Rate	0.23	0.02	169.53	<0.0001	0.20	0.26
Unequal	1045	shredder	Asymptote	4.14	0.06	5179.99	<0.0001	4.02	4.25
Unequal	1045	shredder	Scale	0.86	0.03	726.51	<0.0001	0.80	0.92
Unequal	1045	shredder	Growth Rate	0.46	0.03	186.95	<0.0001	0.40	0.53
Unequal	1061	shredder	Asymptote	12.09	0.96	157.73	<0.0001	10.20	13.98
Unequal	1061	shredder	Scale	0.94	0.01	5186.57	<0.0001	0.92	0.97
Unequal	1061	shredder	Growth Rate	0.12	0.02	44.26	<0.0001	0.09	0.16
Unequal	1340	shredder	Asymptote	-6.16	6.12	1.01	0.31	-18.16	5.85
Unequal	1340	shredder	Scale	1.35	0.36	13.87	0.00	0.64	2.06
Unequal	1340	shredder	Growth Rate	-0.07	0.04	2.79	0.10	-0.15	0.01

Unequal	31	small.body.size	Asymptote	13.20	0.10	18664.46	<0.0001	13.01	13.39
Unequal	31	small.body.size	Scale	0.61	0.03	404.31	<0.0001	0.55	0.67
Unequal	31	small.body.size	Growth Rate	0.48	0.04	167.76	<0.0001	0.41	0.55
Unequal	72	small.body.size	Asymptote	18.24	0.11	28816.47	<0.0001	18.02	18.45
Unequal	72	small.body.size	Scale	0.83	0.02	1234.49	<0.0001	0.78	0.87
Unequal	72	small.body.size	Growth Rate	0.47	0.02	514.17	<0.0001	0.43	0.51
Unequal	73	small.body.size	Asymptote	16.06	0.07	47306.30	<0.0001	15.92	16.21
Unequal	73	small.body.size	Scale	0.75	0.01	2751.61	<0.0001	0.72	0.78
Unequal	73	small.body.size	Growth Rate	0.45	0.01	912.23	<0.0001	0.42	0.48
Unequal	1045	small.body.size	Asymptote	14.48	0.28	2638.01	<0.0001	13.93	15.04
Unequal	1045	small.body.size	Scale	0.79	0.03	514.42	<0.0001	0.72	0.85
Unequal	1045	small.body.size	Growth Rate	0.42	0.04	99.00	<0.0001	0.34	0.50
Unequal	1061	small.body.size	Asymptote	22.20	1.16	363.25	<0.0001	19.92	24.49
Unequal	1061	small.body.size	Scale	0.66	0.01	3071.77	<0.0001	0.64	0.68
Unequal	1061	small.body.size	Growth Rate	0.14	0.02	42.60	<0.0001	0.10	0.18
Unequal	1340	small.body.size	Asymptote	35.64	4.51	62.45	<0.0001	26.80	44.48
Unequal	1340	small.body.size	Scale	0.75	0.02	1670.92	<0.0001	0.71	0.78
Unequal	1340	small.body.size	Growth Rate	0.18	0.05	12.12	0.00	0.08	0.29
Equal	31	herbivore	Asymptote	5.45	0.12	2228.31	<0.0001	5.23	5.68
Equal	31	herbivore	Scale	0.94	0.03	731.72	<0.0001	0.87	1.01
Equal	31	herbivore	Growth Rate	0.50	0.04	139.67	<0.0001	0.42	0.58
Equal	72	herbivore	Asymptote	-544.68	10772.21	0.00	0.96	-21657.82	20568.46
Equal	72	herbivore	Scale	1.00	0.08	178.97	<0.0001	0.86	1.15
Equal	72	herbivore	Growth Rate	0.00	0.06	0.00	0.96	-0.11	0.11
Equal	73	herbivore	Asymptote	7.18	0.11	4053.74	<0.0001	6.96	7.40
Equal	73	herbivore	Scale	0.95	0.10	93.88	<0.0001	0.76	1.15
Equal	73	herbivore	Growth Rate	0.84	0.11	63.82	<0.0001	0.64	1.05
Equal	1045	herbivore	Asymptote	12.11	4.73	6.56	0.01	2.85	21.38

Equal	1045	herbivore	Scale	0.94	0.02	2471.94	<0.0001	0.90	0.97
Equal	1045	herbivore	Growth Rate	0.07	0.03	3.87	0.05	0.00	0.13
Equal	1061	herbivore	Asymptote	10.75	1.94	30.60	<0.0001	6.94	14.56
Equal	1061	herbivore	Scale	0.91	0.01	4492.89	<0.0001	0.89	0.94
Equal	1061	herbivore	Growth Rate	0.17	0.05	9.80	0.00	0.06	0.27
Equal	1340	herbivore	Asymptote	20.47	7.41	7.63	0.01	5.95	35.00
Equal	1340	herbivore	Scale	0.98	0.01	29971.63	<0.0001	0.97	0.99
Equal	1340	herbivore	Growth Rate	0.08	0.04	4.61	0.03	0.01	0.15
Equal	31	shredder	Asymptote	6.05	0.04	27982.13	<0.0001	5.98	6.13
Equal	31	shredder	Scale	1.15	0.09	164.06	<0.0001	0.97	1.33
Equal	31	shredder	Growth Rate	1.15	0.08	216.53	<0.0001	1.00	1.31
Equal	72	shredder	Asymptote	12.68	1.15	121.60	<0.0001	10.43	14.93
Equal	72	shredder	Scale	1.12	0.14	67.88	<0.0001	0.85	1.39
Equal	72	shredder	Growth Rate	0.46	0.13	12.80	0.00	0.21	0.72
Equal	73	shredder	Asymptote	5.31	0.09	3458.98	<0.0001	5.14	5.49
Equal	73	shredder	Scale	0.99	0.04	569.68	<0.0001	0.91	1.07
Equal	73	shredder	Growth Rate	0.58	0.04	166.29	<0.0001	0.49	0.67
Equal	1045	shredder	Asymptote	3.89	0.17	515.59	<0.0001	3.55	4.22
Equal	1045	shredder	Scale	0.75	0.01	10136.38	<0.0001	0.73	0.76
Equal	1045	shredder	Growth Rate	0.24	0.03	73.97	<0.0001	0.18	0.29
Equal	1061	shredder	Asymptote	5.16	0.53	95.09	<0.0001	4.12	6.20
Equal	1061	shredder	Scale	0.81	0.02	2089.42	<0.0001	0.78	0.85
Equal	1061	shredder	Growth Rate	0.06	0.01	45.02	<0.0001	0.04	0.07
Equal	1340	shredder	Asymptote	-6.16	6.12	1.01	0.31	-18.16	5.85
Equal	1340	shredder	Scale	1.35	0.36	13.87	0.00	0.64	2.06
Equal	1340	shredder	Growth Rate	-0.07	0.04	2.79	0.10	-0.15	0.01
Equal	31	small.body.size	Asymptote	10.96	0.13	7364.54	<0.0001	10.71	11.21
Equal	31	small.body.size	Scale	0.59	0.11	29.20	<0.0001	0.37	0.80
Equal	31	small.body.size	Growth Rate	0.98	0.19	26.25	<0.0001	0.61	1.36
Equal	72	small.body.size	Asymptote	18.43	1.03	317.18	<0.0001	16.40	20.45
Equal	72	small.body.size	Scale	0.87	0.05	338.11	<0.0001	0.77	0.96
Equal	72	small.body.size	Growth Rate	0.39	0.07	28.03	<0.0001	0.25	0.54
Equal	73	small.body.size	Asymptote	17.87	0.45	1585.59	<0.0001	16.99	18.75
Equal	73	small.body.size	Scale	0.78	0.03	708.83	<0.0001	0.72	0.84
Equal	73	small.body.size	Growth Rate	0.45	0.05	84.77	<0.0001	0.36	0.55
Equal	1045	small.body.size	Asymptote	10.37	0.07	25132.87	<0.0001	10.24	10.50

Equal	1045	small.body.size	Scale	0.73	0.02	2373.85	<0.0001	0.70	0.76
Equal	1045	small.body.size	Growth Rate	0.59	0.02	631.99	<0.0001	0.55	0.64
Equal	1061	small.body.size	Asymptote	22.64	1.59	201.63	<0.0001	19.51	25.76
Equal	1061	small.body.size	Scale	0.71	0.01	3770.24	<0.0001	0.69	0.73
Equal	1061	small.body.size	Growth Rate	0.21	0.04	30.95	<0.0001	0.13	0.28
Equal	1340	small.body.size	Asymptote	35.64	4.51	62.45	<0.0001	26.80	44.48
Equal	1340	small.body.size	Scale	0.75	0.02	1670.92	<0.0001	0.71	0.78
Equal	1340	small.body.size	Growth Rate	0.18	0.05	12.12	0.00	0.08	0.29

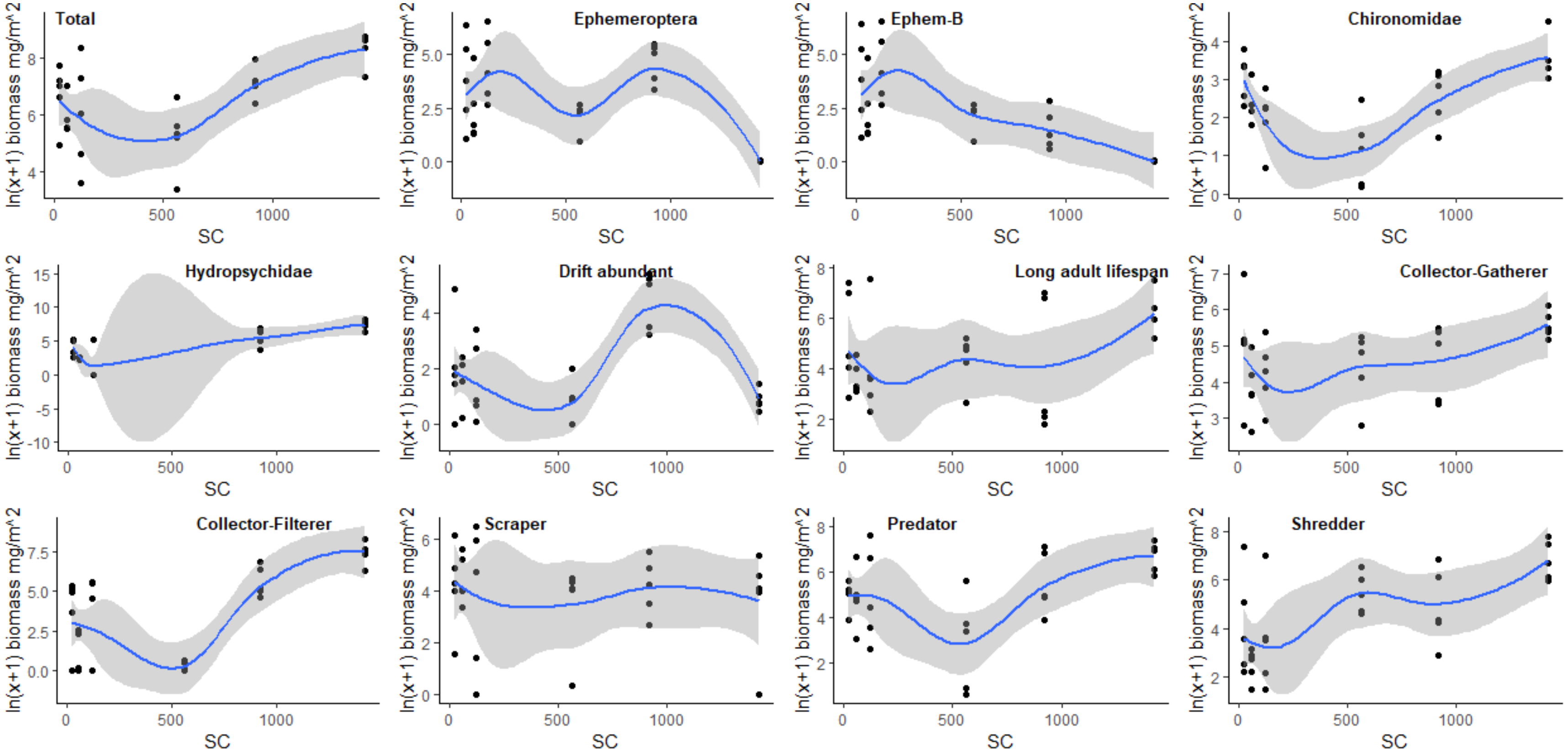


Figure 3-A 1. Response of benthic macroinvertebrate biomass (ln(x+1) transformed) to a gradient of salinization comprised of six headwater streams for the fall of 2019.

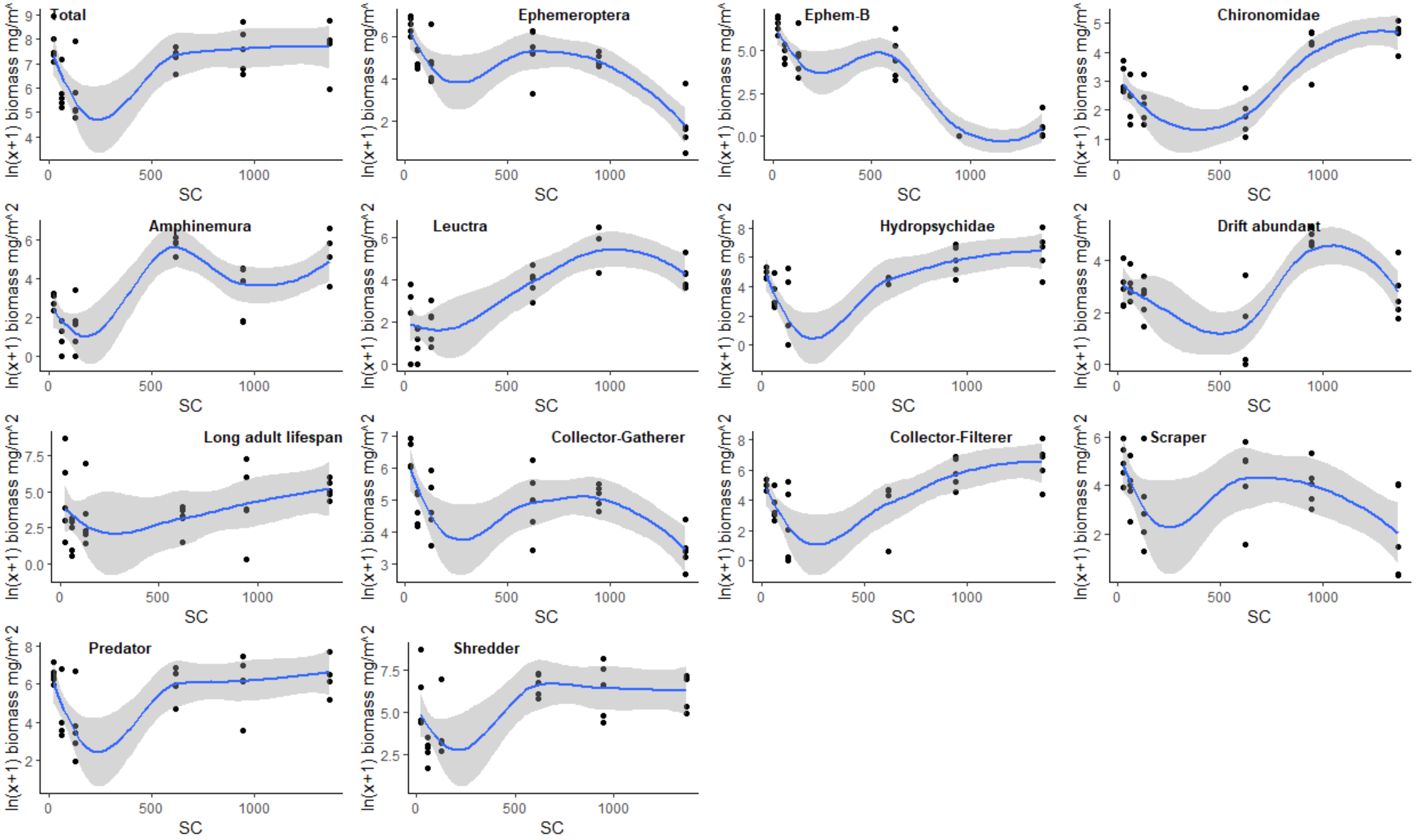


Figure 3-A 2. Response of benthic macroinvertebrate biomass (ln(x+1) transformed) to a gradient of salinization comprised of six headwater streams for the spring of 2020.