THE EFFECT OF HABITAT AND *DIDYMOSPHENIA GEMINATA* ON MACROINVERTEBRATE COMMUNITY STRUCTURE IN THE SOUTH HOLSTON RIVER TAILWATER

A Thesis
by
MATTHEW WILLIAM GREEN

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Abstract

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In the South Fork Holston River, near Bristol, Tennessee, we examined benthic macroinvertebrate communities in three river reaches along a tailwater river thermal gradient and in one control site located upstream of the reservoir. Directly downstream of the dam, the South Fork Holston Tailwater has a very narrow annual thermal range (11.83°C) and few cumulative growing degree days (GDD=243); abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) was as much as 80% lower at this site than at the two downstream sites, which had higher annual thermal ranges (19.39°C, 21.54°C respectively) and more degree growing days (GDD=438, 522 respectively). Species richness at the control site (Mean *S*=20) was 2–3× higher than any of the tailwater sites, although there was little variation in richness between tailwater river reaches (Mean *S*=7–11). Partial least squares regression and NMDS joint plots indicated that conductivity, surface flow rate, and benthic flow rate were significant predictors of observed variation in benthic macroinvertebrate diversity among sites. Our data and analyses suggest that macroinvertebrate communities in hypolimnetic tailwater systems are strongly influenced by changes to thermal range, conductivity and flow rate in downstream reaches. These shifts may be attributable to the exclusion of certain species from parts of thermal gradients. Tailwaters in particular may not meet the thermal niche requirements of key traits involved in the life history and development of the excluded species. We establish and elaborate upon the term "diapause termination temperature thresholds" to explain the diversity patterns seen in our own data.

Additionally, *Didymosphenia geminata*, a invasive nuisance diatom, and macroinvertebrate collections were made seasonally between January 2015 and March 2016 in riffle and run mesohabitats at the previously mentioned sampling locations. We found that *D. geminata* ash-free dry mass (AFDM) and dry mass differed significantly between mesohabitats with significantly higher *D. geminata* AFDM and dry mass in run habitats. Partial least squares regression (PLS) revealed that surface flow, benthic flow, dissolved oxygen, conductivity, and turbidity were significant variables of importance for predicting the observed variation in *D. geminata* AFDM and accounted for 45.2% of the total variation in *D. geminata* AFDM between sites. A second PLS regression found that surface flow, benthic flow, temperature, conductivity, turbidity, and *D. geminata* AFDM were significant variables of importance for predicting Shannon diversity between sites and accounted for 51.4% of the variation in *H'*. Nonmetric multidimensional scaling (NMDS) ordination plots indicated three distinct communities present, with joint plots and Pearson and Kendall correlation coefficients confirming PLS results except for the exclusion of temperature as a variable of importance. Relative abundance of specific taxonomic groups differed between mesohabitats, which is attributable to the significant difference in *D. geminata* between riffles and runs. Riffle habitats had increased Ephemeroptera (especially *Ephemerella* and

Baetis spp.), EPT (Ephemeroptera + Plecoptera + Trichoptera) taxa in general, and Amphipoda. Run habitats saw increases in Oligochaeta, Turbellaria, and Isopoda. Diptera and Chironomidae increased only at runs furthest downstream, where *D. geminata* AFDM and dry mass were statistically different between mesohabitats. Our results indicate that surface and benthic flow rate, specifically low flow rates, along with dissolved oxygen, conductivity, and turbidity influence *D. geminata* AFDM, which in turn affects macroinvertebrate abundance and diversity. Our research suggests that federal and state resource managers should consider increasing minimum tailwater base flows, the frequency of high discharge pulses, and the maximum discharge during power generation, to minimize the effects of low flows on *D. geminata* growth and macroinvertebrates.

Acknowledgments

The author would like to extend his deepest gratitude to the committee chair, Dr. Shea Tuberty, for giving him the opportunity to succeed and follow his dream to become a professional aquatic entomologist and to other committee members, Dr. Michael Gangloff, for advice concerning field methodology and statistics and, Dr. Luke Jacobus, for professional direction and instruction on the biology and ecology of Ephemeroptera. The author is especially grateful for his various funding sources and sponsors including Mr. Frank Smith, Hunter Banks Fly Fishing Company, Sigma Xi Grants in Aid of Research, The International Federation of Fly Fishers, The Edgemont Club, LLC., Appalachian State University office of student research, and the Cratis D. Williams Graduate School. Without these sponsors, a large portion of the work performed might not have been possible. The author is appreciative of contributions made by the following individuals to insure the timely completion of the thesis. Megan Maloney for her creation of study site maps, Drs. Ray Williams and Michael Madritch for their instruction in multivariate statistical procedures used in the analysis of the author's data, Dr. Robert Creed for assistance in sample preparation for ash-free dry mass (AFDM) calculations, Dr. Michael Hambourger for his technical assistance in regard to ion chromatography, and Stephanie Sellers for her assistance in the collection of field data and laboratory processing of macroinvertebrates. Lastly, the author would like to thank his significant other for her understanding and compassion during the writing of this manuscript and frequent overnight, multi-day collection trips.

Dedication

I would like to dedicate this thesis to my parents, Chuck and Claudia Green, and my Aunt and Uncle, Julia Moore and David Schultz (deceased). Without your support, my life dreams would have never come true.

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Foreword

Chapters 1 and 2 of this thesis manuscript will be submitted to the journal, *Freshwater Science*. The thesis has been formatted according to the style guide for this journal for rapid acceptance with minimal revisions.

Introduction

Aquatic insects have evolved life history strategies that are tied to natural variation in riverine thermal and flow regimes (Ward and Stanford 1982). Aquatic insects respond both to discrete and cumulative thermal cues. Stream temperature patterns affect larval development and emergence, dissolved oxygen tolerance and other behavioral traits (Ward and Stanford 1982). The construction of dams may dramatically affect these deeply rooted phenological relationships by altering thermal and discharge regimes. A dam that forms a reservoir may result in either an epilimnetic (warm water release) or hypolimnetic-release (cold water release) tailwater. Hypolimnetic-releases produce characteristically constant thermal regimes in tailwaters that may result in higher winter temperatures and cooler summer temperatures, depending on management operations, compared to unaffected reaches (Kondratieff and Voshell 1980). Epilimnetic tailwaters are known to have higher productivity than their hypolimnetic counterparts from reservoir epilimnion contributions of zooplankton and phytoplankton. Consequently, coarse (CPOM) and fine (FPOM) particulate organic matter are broken down by aerobic bacteria in the reservoir hypolimnion, making hypolimnetic tailwater discharges extremely anoxic (Ward and Stanford 1979, Ward and Stanford 1983, Ward and Stanford 1995). Many federal and state regulatory agencies have implemented various technologies, such as direct oxygen injection systems and aeration weirs, to help combat biological and ecological problems associated with low tailwater dissolved oxygen (DO) (Adams and Brock 1993). Hypolimnetic and epilimnetic tailwaters also differ greatly in operational use and function. In the Tennessee River system, for example, hypolimnion discharges are utilized more often for hydroelectric power generation than releases from the epilimnion (Bednarek and Hart 2005). Variation in diel flow velocity of hypolimnetic-releases is also much greater than epilimnetic releases, which typically produce higher flow discharges, but at much lower velocities (Ward and Stanford 1983). The resultant flooding, scouring, desiccation, substrate instability, and localized variations in water velocity caused by extreme annual and diel variation in hypolimnetic tailwater flow rate lead to spatial and temporal variation in invertebrate community structure at these sites (Bunn and Arthington 2002).

Dams on mid-order streams may create dramatic thermal serial discontinuities and affect the diel and annual temperature range for a substantial distance downstream (Ward and Stanford 1979, Ward and Stanford 1983, Ward and Stanford 1995). The serial discontinuity concept builds upon the river continuum concept by hypothesizing that dams may have dramatic consequences to the hydraulic, thermal, and energetic equilibria of streams. Interestingly, modifications to the natural thermal and flow regimes of mid order streams have resulted in significant changes to aquatic invertebrate biodiversity across much of the southeastern USA (Bednarek and Hart 2005). Many tailwater communities are dominated by insects, crustaceans, oligochaetes and turbellarians with life history strategies that tolerate these radical shifts in habitat conditions.

Many investigators have argued that water temperature is the single most important driver of aquatic insect life history and reproductive events (Rader and Ward 1989, Brown et al. 2010). Many aquatic insects grow more rapidly and mature sooner in warmer temperatures, but they usually achieve smaller adult body sizes as a result (Markarian 1980, Giberson and Rosenberg 1992, Hogg and Williams 1996, McKee and Atkinson 2000). Consequently, at colder temperatures, this relationship is reversed, resulting in slower development but greater adult body size following emergence (Sweeny and Vannote 1978, Markarian 1980). Slight changes in development rate may then cause aquatic insects to emerge earlier or later and with a smaller or larger body size relative to water temperature and cumulative growing degree day accumulation. Given that fecundity is directly related to adult body size, and that most aquatic insect taxa have brief adult stages, changes in emergence timing and maturation size may have strong consequences for maintaining reproductive success, adequate fitness, and survivorship (Sweeny and Vannote 1978). Sweeny and Vannote (1978) proposed that aquatic insects have a narrow temperature range within which adult body size and fecundity are optimized, and they hypothesized that changes of only 2–3° C could lead to population extirpations at the limits of species' geographic ranges. Aquatic insects that are specialists with narrow thermal niche requirements are particularly prone to extirpation by sudden shifts in temperature.

The homogenous environmental conditions typical of tailwater systems, especially the homogenization of temperature along an latitudinal gradient, can lead to losses in biodiversity and the exclusion of species ill-adapted to these dam-modified thermal regimes. Natural environmental heterogeneity and disturbance in unregulated rivers constitutes a physical habitat template that dictates the types of species adaptations necessary for local persistence (Poff and Ward 1990, Huryn and Wallace 2000). In addition, a lack of exposure to extreme thermal diversity may exclude a large percentage of taxa from colonizing habitats directly downstream of dams because requisite thermal heterogeneity is not met (Lehmkuhl 1972, Lehmkuhl 1974, Kraft and Mundahl 1984, Mundahl and Kraft 1988, Saltveit et al. 1994). Species currently occupying tailwaters are presumably better adapted to low and relatively constant temperatures relative to those species in unregulated sections upstream of the reservoir. However, these tailwater species may also be poor competitors that are only able to compete successfully in depauperate communities.

The construction of dams to regulate the flow of rivers for drinking water storage, flood control, and power generation has resulted in numerous social and economic benefits (Lessard et al. 2013). However, the altered flow regimes of regulated rivers promote the establishment of invasive species. Invasive species are usually non-native or exotic species that threaten the diversity and abundance of native species and the ecological integrity of infested habitats. Most invasive species become introduced outside of their native range with the help of humans and result in environmental (changes to trophic system energy dynamics, nutrient cycling, extinction of native species, etc.), economic (~$120 billion a year in damages), and social (negative public perception) consequences that demand management action (Pimental et al. 2005). Few non-native immigrant species actually become truly invasive following their introduction to new habitats. Invasion success is typically demonstrated by the Tens rule of Williamson, which states that for every ~10% of species that become introduced outside of their native range, ~10% of these species become invasive (Williamson 1996). Some have challenged the Williamson rule citing original research that of vertebrate introductions to North America and Europe, approximately 50% of introduced species have become invasive (Jeschke and Strayer 2005). Currently, similar research has not been undertaken to determine the invasive success of non-native aquatic species in North America, specifically periphyton and aquatic emergent and submergent plants.

Invasive species can also become nuisance species or a species that poses an adverse effect on and to humans. These effects can be direct (a species that causes direct physical harm) or indirect (a species that destroys habitats that humans find enjoyable for recreation, vacation, and leisure) (Colautti and MacIsaac 2004). *Didymosphenia geminata* has become affiliated with indirect negative social connotations from recreational fisherman and outdoor enthusiasts, which validate its classification as an invasive, nuisance species. In the southeastern United States, the species is also considered cryptogenic or a species with unknown origins (Carlton 1996). Some investigators contend that *D. geminata* is an invasive species originally native to Vancouver Island, British Columbia and western North America (Bothwell et al. 2009), whereas others suggest that *D. geminata* is a native nuisance species (Taylor and Bothwell 2014). However, regardless of *D. geminata*'s status as a cryptogenic, invasive, or nuisance species, its extensive bloom forming behavior and the resultant effects on aquatic macroinvertebrate communities pose a threat to aquatic life in southeastern USA tailwaters.

Algal blooms of the diatom *D. geminata* were first reported in the South Holston River, Bristol, Tennessee, USA, downstream of South Holston Reservoir in 2005 by Tennessee Valley Authority (TVA) limnologist Tyler Baker. Invasive diatom species like *D. geminata* flourish in tailwater rivers because these regulated rivers typically have homogeneous flow regimes with little or no variation in flow rate, with extended periods of below average discharge relative to pre-dam levels (Segura et al. 2010, Lessard et al. 2013). Kirkwood et al. (2007) found that discharge on the Bow and Red Deer rivers in Manitoba is inversely correlated with *D. geminata* biomass. Additionally, Richardson et al. (2014) found that high mean flow rates result in lower *D. geminata* biomass and that low flows promote *D. geminata* blooms. Substrate mobility has also been shown to affect *D. geminata* biomass (Miller et al. 2009, Cullis et al. 2013). Miller et al. (2009) found that *D. geminata* biomass in regulated streams along the Colorado Front Range was higher in streams with less bed movement compared to less stable channels. Because regulated river tailwaters are typically sediment-starved and do not experience large magnitude, substrate-mobilizing floods, benthic algal assemblages are frequently dominated by mat-forming diatoms like *D. geminata* (Lessard et al. 2013).

Unlike nuisance green algae (e.g., *Cladophora* spp.) that form large mats in eutrophic lakes and rivers, *D. geminata* prefers the oligotrophic conditions of high-elevation and tailwater streams (Ellwood and Whitton 2007, Kirkwood et al. 2007, Kirkwood et al. 2009, Miller et al. 2009, Bothwell and Kilroy 2011, Kilroy and Bothwell 2011, Kilroy and Bothwell 2012, Richardson et al. 2014, Reid and Torres 2014, Taylor and Bothwell 2014). In addition, turbidity is important for *D. geminata* proliferation and stalk establishment because river systems with low turbidity have greater light penetration, and cell division is more rapid (Kirkwood et al. 2007, Kirkwood et. 2009, Bothwell and Kilroy 2011, James et al. 2014).

In the southeastern USA*, D. geminata* only occurs in tailwater rivers with regulated flows and thermal regimes that are artificially cooled by hypolimnetic releases from large upstream reservoirs. *Didymosphenia geminata* populations have been reported from Kentucky, Maryland, North Carolina, Tennessee and Virginia (Spaulding and Elwell 2007). *Didymosphenia geminata* may prefer tailwaters because these habitats have stable, homogeneous flows, low water temperatures, low levels of dissolved inorganic phosphorus and low turbidity (Taylor and Bothwell 2014). These conditions are also associated with significant changes to macroinvertebrate community structure relative to unimpacted sites. Changes include reduced species richness and increased dominance of disturbance-tolerant taxa such as oligochaetes, crustaceans and some Chironomidae (Larson 2007, Kilroy et al. 2009, Gillis and Chalifour 2010, James et al. 2010, Richardson et al. 2014).

No studies have investigated the effects of *D. geminata* stalk and cell biomass on macroinvertebrate diversity in tailwaters of the southeastern USA. However, studies from elsewhere suggest that *D. geminata* blooms may negatively affect benthic community structure due to proliferation of mucopolysaccharide stalk and algal cell biomass (Larson 2007, Kilroy and Bothwell 2009, Gillis and Chalifour 2010, James et al. 2010, Richardson et al. 2014). Specifically, *D. geminata* blooms are associated with reductions in Ephemeroptera, Plecoptera, and Trichoptera (EPT) abundance and diversity and increases in more tolerant species including Chironomidae and oligochaetes (Larson 2007, Kilroy et al. 2009, James et al. 2010, Gillis and Chalifour 2010, Richardson et al. 2014).

Changes to macroinvertebrate community structure likely reflect larger community-wide shifts in riverine trophic interactions and habitat integrity (Gillis and Chalifour 2010). Explanations for observed shifts in community structure following *D. geminata* introductions and nuisance bloom presence include the trapping of fine sediment within interstitial spaces typically inhabited by invertebrates (Larson 2007); the creation of novel, patchy mixed *D. geminata* benthic habitat that supports an abundant and diverse Dipteran fauna (Gillis and Chalifour 2010); and the proliferation of *D. geminata* coverage during base flow conditions (Richardson et al. 2014).

Methods

Study area

Downstream of South Holston Reservoir, the South Fork Holston River is managed by the Tennessee Valley Authority (TVA) as a ~20 km long hydroelectric and recreational cool-water tailwater river with an average base flow of 2.5 $m^3 \bullet s^{-1}$. However, the tailwater picks up considerable flow volume from first- and second-order tributaries that increase base flow by approximately ~2.8 $m^3{\bullet}s^{-1}$. During hydropeaking, base flow can increase to 70.8 $m^3{\bullet}s^{-1}$and remain so until power generation ceases. In addition to hydropeaking, the TVA often uses an hour long "pulse" of approximately 42.8 $m^3{\bullet}s^{-1}$ (Habera et al. 2005) during the summer months to mitigate the negative effects of seasonal warming on the recreational trout fishery. Upstream of the reservoir, the control site is best characterized as an unregulated, fourth-order Blue Ridge Mountain stream with moderate to large cobble and boulder substrate with natural thermal and flow conditions. The bedrock layer for both tailwater and control sites is mostly comprised of dolomite ridges from the Knox Dolomite Formation in the lower Great Appalachian Valley (Oder 1934, Davis 1990), In the tailwater, numerous species of aquatic macrophytes flourish including *Fontinalis antipyretica* (common river moss), Bryaceae, *Chara* spp. (musk grasses), *Elodea canadensis* (water weed), *Potamogeton crispus* (curly leaf pondweed), and *Potamogeton* spp. (pondweeds), *Ludwigia* sp. (water primrose), and *Nasturtium officinale* (watercress). Field surveys also identified dominant populations of *Gomphonema* diatoms at control sites and small, scattered distributions of *Rhizoclonium* green algae at tailwater sites.

It is important to note that *D. geminata* blooms were never confirmed from control sites upstream of South Holston Reservoir. Microscopic evaluation of periphyton collected from the control site yielded spring and summer populations of *Gomphonema* spp., which are similar to *D. geminata* in cell morphology, mat coloration, and stalk structure.

Fieldwork was conducted between January 2015 and March 2016. We established four study sites; three sites were located downstream from the dam and a fourth site was located upstream of the dam, which served as a control site under a natural temperature and flow regime (see discussion above). Field collections were made in winter (January/February), spring (April/May), summer (August/September) 2015 and late winter (late January-early March) of 2016. Mesohabitats with different flow rates were sampled (e.g., riffles and runs) from three different tailwater sites [upper (1), middle (2), and lower (3)], increasing in distance from the dam (Figure 1, Table 1). Riffles (mean depth=0.32 m) had shallow, turbulent fast-flowing water and cobble/gravel substrates, whereas runs (mean depth=0.41 m) had more laminar and slow-moving water and sandy substrates interspersed with occasional cobbles and bedrock outcroppings. Surface and benthic flow rates were measured under baseflow conditions in the absence of scheduled dam water-releases and were significantly different between mesohabitats at all sites (Figure 2).

Macroinvertebrate and **Didymosphenia geminata** *collection and processing*

For collecting *D. geminata*, we placed 5 cm x 25 cm x 4 cm artificial substrates of Tennessee grey flagstone (n=6) at each sampling site with *D. geminata* harvested approximately every 2-3 months. Three subsamples of *D. geminata* were pooled and randomly selected from artificial substrates using a random number generator and grid and harvested using a 5 cm wide PVC coupling and a scalpel. Harvested algae were transferred into a 150 mL solution of 8% lugols iodine and deionized water (Smayda 1978) for preservation and further processing.

At each artificial substrate, aquatic macroinvertebrates were harvested from Surber samplers (30.5 cm^2; 500-µm-mesh) to characterize benthic community structure (Hynes 1971). Sampling units were modified to accommodate a wooden pole that was fastened to the metal rim of the Surber sampler to serve as a handle. Surber samples were collected adjacent to artificial tiles and substrate was sampled for three minutes. All specimens were preserved in 80% ethanol. Benthic samples were vortexed and a 200 mL subsample was selected using a random number generator. Macroinvertebrates (except Coleoptera and Chironomidae) were identified to the genus level using taxonomic keys (Merritt et al. 2008) and a stereomicroscope (Olympus SZ61 zoom, Center Valley, PA, USA), and the abundance of all taxa within each sample was recorded.

Didymosphenia geminata samples with minimal mat biomass were filtered by vacuum using a Corning PYREX 47 mm microfiltration glass assembly and filter flask onto pre-weighed 55 mm borosilicate glass microfiber filters (Whatman 934-AH, 1 μm nominal pore size). Many collection jars contained suspended algal cells and much greater mat biomass, proving difficult to vacuum filter. Therefore, these samples were sieved using a #230 (63 μm-mesh-size; 20.32 x 5.08 cm diameter) Standard Testing Sieve (Dual Manufacturing Co., Inc., Franklin Park, IL, USA). Algal cells and mat biomass not retrieved by the sieve were collected with a funnel and Erlenmeyer flask and centrifuged in nalgene bottles at 5000 rpm for 15 minutes using a Sorvall RC 6+ centrifuge and FIBERlite F14-6x250y rotor (Thermo Fisher Scientific, Waltham, MA, USA). All filtered, sieved, and centrifuged samples were transferred to pre-weighed aluminum weigh boats and dried in a drying oven (Quincy Lab, Inc. 40GC, Chicago, IL, USA) for 7 days at 60° C to a constant mass. Dried samples were then re-weighed and placed into a muffle furnace (Sybron Thermolyne 1500, Milwaukee, WI, USA) for 1 hr at 550°C using metal racks. Once samples had cooled to room temperature, the mass of the ash product was subtracted from the mass of the dried algae to calculate the ash-free dry mass (AFDM) of algae in the sample and scaled to 0.15 m^2 of total habitat sampled accounting for all three *D geminata* subsamples. It should be noted that due to the thickness and density of *D. geminata* mats, muffled samples were ground with a mortar and pestle every 30 minutes to insure homogeneous Carbon oxidation during ashing.

Habitat, physiochemical, and nutrient data collection

In order to characterize the effect of habitat on *D. geminata* blooms, we measured flow rate, dissolved oxygen (DO), pH, conductivity, temperature, turbidity, orthophosphate (PO_4^{3-}) and nitrate (NO_3^-) concentrations during macroinvertebrate and *D. geminata* collection. Flow rate ($m \bullet s^{-1}$) was measured using a hand-held flow meter (Global Water Instrumentation, College Station, TX, USA) at the surface and substrate level. DO (mg L^{-1}), pH, conductivity (µs cm^{-1}), and temperature were measured with a multi-parameter water chemistry meter (Thermo Fisher Scientific Orion, Waltham, MA, USA) and turbidity (NTU) with a portable turbidimeter (HF Scientific DRT-15CE, Fort Myers, FL, USA)**.** Ion-exchange Chromatography (Dionex ICS-3000, Sunnyvale, CA, USA) was used to calculate the concentration of NO_3^- (mg L^{-1}) at each site. Orthophosphate (µg L^{-1}) concentration was determined via the molybdate blue colorimetric method (Murphy and Riley 1962, Stainton et al. 1974) by measuring absorbance with a spectrophotometer (Bruker Vector 22 FT-IR, Billerica, MA, USA) at 885 nm using a 5 cm quartz cell. All water samples for N and P analysis were acidified within 12 hours of collection with 4% conc. H_2SO_4 by volume for a ~30 day maximum holding time.

Statistical analysis

HOBOware software (HOBOware Pro/3.7, Onset Computer Corp., Bourne, MA, USA) was used to collect, log, and calculate cumulative growing degree-days (GDD) for all water temperature data from data loggers mounted to dolomite boulders. We calculated the number of cumulative annual growing degree-days for each river reach by using the equation: Cumulative GDD = $\sum [(\frac{T_{max}+T_{min}}{2}) - T_{base}]$, where T_{base}=10°C. Shapiro-Wilk and quick frequency distribution tests were conducted to assess normality and detected significant right and left skewed data. We calculated Shannon (*H'*) and Simpson (D) Diversity Index values and richness (S) and evenness (*J'*) using the macroinvertebrate abundances for each Surber subsample. Pearson correlation matrices determined that *D. geminata* dry mass (0.718; $p<0.001$) and inorganic mass (0.663; $p<0.001$) were significantly and positively correlated to *D. geminata* AFDM; Simpson Index (0.927; $p<0.001$), richness (0.787; $p<0.001$), and evenness (0.762; $p<0.001$) values were significantly and positively correlated to Shannon Index values. Therefore, *D. geminata* AFDM and Shannon Index values were used in all analyses as dependent variables. To determine whether *D. geminata*, benthic invertebrate communities, and environmental parameters were different between sites and mesohabitats, SPSS software was used to perform 2-way ANOVAs with *D. geminata* AFDM and Shannon Index as mutually exclusive dependent variables and site and mesohabitat as fixed factors (SPSS/23, IBM Corp., Armonk, NY, USA). We used Student's t-tests to determine whether *D. geminata* AFDM and dry mass and surface and benthic flow rates differed between mesohabitats.

Nonmetric multidimensional scaling (NMDS) was conducted (PC-ORD/6, Gleneden Beach, OR, USA) to visualize differences in macroinvertebrate community structure using

Sorenson (Bray-Curtis) distance measures and site as a grouping variable. Multi-response permutation procedure (MRPP) analysis was then used to determine whether macroinvertebrate community metrics were significantly different among sites using Sorenson (Bray-Curtis) distances. Joint plots were fitted to the NMDS ordination plot showing the relationship of environmental variables to the NMDS structure and Pearson's r and Kendall's tau correlation coefficients were calculated to quantify the relationship and "fit" of environmental variables to the macroinvertebrate ordination along NMDS axes 1 and 2.

In order to determine the effect of habitat, physiochemical data, and nutrients on *D. geminata* AFDM and *D. geminata* effects on Shannon diversity, We conducted partial least squares regression analyses (PLS, JMP/10 Pro, SAS Institute, Cary, NC, USA) by utilizing the NIPALS method with k-fold cross validation (k=7). *Didymosphenia geminata* AFDM and Shannon Index values were each selected as dependent response variables whereas surface flow rate, benthic flow rate, DO, pH, conductivity, temperature, turbidity, orthophosphate, and nitrate were selected as independent predictor variables. In addition, we included *D. geminata* AFDM as an independent variable in the PLS model to predict Shannon Index to determine if *D. geminata* contributed to the overall variation in *H'* between sites. PLS is based on a projection of the predictor (x) and response (y) variables into a set of latent, uncorrelated predictor variables (or PLS factors) and corresponding scores, which minimizes potential collinearity between individual predictors and maximizes predictor and response covariance (Carrascal et al. 2009). Prior to performing a PLSR with the data, a multiple linear regression found our independent variables to exhibit moderate collinearity

(Table 4), and therefore we chose to negate these effects by choosing partial least squares regression (PLS).

Summary of findings

In the South Fork Holston River, near Bristol, Tennessee, we examined benthic macroinvertebrate communities in three river reaches along a tailwater river thermal gradient and in one control site located upstream of the reservoir. Directly downstream of the dam, the South Fork Holston Tailwater has a very narrow annual thermal range (11.83°C) and few cumulative growing degree days (GDD=243); abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) was as much as 80% lower at this site than at the two downstream sites, which had higher annual thermal ranges (19.39°C, 21.54°C respectively) and more degree growing days (GDD=438, 522 respectively). Species richness at the control site (Mean *S*=20) was 2–3× higher than any of the tailwater sites, although there was little variation in richness between tailwater river reaches (Mean *S*=7–11). Partial least squares regression and NMDS joint plots indicated that conductivity, surface flow rate, and benthic flow rate were significant predictors of observed variation in benthic macroinvertebrate diversity among sites. Our data and analyses suggest that macroinvertebrate communities in hypolimnetic tailwater systems are strongly influenced by changes to thermal range, conductivity and flow rate in downstream reaches. These shifts may be attributable to the exclusion of certain species from parts of thermal gradients. Tailwaters in particular may not meet the thermal niche requirements of key traits involved in the life history and development of the excluded species. We establish and elaborate upon the term “diapause termination temperature thresholds” to explain the diversity patterns seen in our own data.

Additionally, we conducted a large-scale field experiment in the South Fork Holston River near Bristol, Tennessee to understand how *Didymosphenia geminata* affects tailwater macroinvertebrate communities. *Didymosphenia geminata* and macroinvertebrate collections were made seasonally between January 2015 and March 2016 in riffle and run mesohabitats at three different tailwater reaches at increasing distance from the dam and at a single control site upstream of South Holston Reservoir. We found that *D. geminata* ash-free dry mass (AFDM) and dry mass differed significantly between mesohabitats with significantly higher *D. geminata* AFDM and dry mass in run habitats. Partial least squares regression (PLS) revealed that surface flow, benthic flow, dissolved oxygen, conductivity, and turbidity were significant variables of importance for predicting the observed variation in *D. geminata* AFDM and accounted for 45.2% of the total variation in *D. geminata* AFDM between sites. A second PLS regression found that surface flow, benthic flow, temperature, conductivity, turbidity, and *D. geminata* AFDM were significant variables of importance for predicting Shannon diversity between sites and accounted for 51.4% of the variation in *H'*. Nonmetric multidimensional scaling (NMDS) ordination plots indicated three distinct communities present, with joint plots and Pearson and Kendall correlation coefficients confirming PLS results except for the exclusion of temperature as a variable of importance. Relative abundance of specific taxonomic groups differed between mesohabitats, which is attributable to the significant difference in *D. geminata* between riffles and runs. Riffle habitats had increased Ephemeroptera (especially *Ephemerella* and *Baetis* spp.), EPT (Ephemeroptera + Plecoptera + Trichoptera) taxa in general, and Amphipoda. Run habitats saw increases in Oligochaeta, Turbellaria, and Isopoda. Diptera and Chironomidae increased only at runs furthest downstream, where *D. geminata* AFDM and dry mass were statistically different between mesohabitats. Our results indicate that surface and benthic flow rate, specifically low flow rates, along with dissolved oxygen, conductivity, and turbidity influence *D. geminata* AFDM, which in turn affects macroinvertebrate abundance and diversity. Our research suggests that federal and state resource managers should consider increasing minimum tailwater base flows, the frequency of high discharge pulses, and the maximum discharge during power generation, to minimize the effects of low flows on *D. geminata* growth and macroinvertebrates.

Future Directions: Management Recommendations For *D. geminata*

Our research on *D. geminata* in the South Holston tailwaters is the first of its kind in the southeastern USA and builds upon previous research, while also adding new information that can be applied to management. Above, we provided statistical evidence that *D. geminata* AFDM increases with decreasing flow rate and that high water temperatures are associated with reduced *D. geminata* AFDM. Specifically, we attributed decreases in *D. geminata* biomass to a cumulative increase in water temperature at sites two and three during the spring and summer (Figure 3). This is consistent with what is known of *D. geminata*'s biology and ecology, as reviewed above. Thus, river operation managers may face an interesting paradox: increased power generation frequency and discharge may mitigate *D. geminata* blooms through increased shear stress, but these increase could also create more favorable thermal conditions for continued bloom survivorship by lowering tailwater water temperatures.

Here exists a no-win situation if management plans to mitigate blooms are not implemented properly. Given that *D. geminata* AFDM is lowest during the summer, a series of "flood pulse discharges" during the spring and summer months may scour algal mats while warm temperatures help to mitigate bloom formation. If initiated in proper time intervals (e.g. early morning and late evening), these flood pulses should flush algal mats downstream in between periods of short growth (during hydropeaking) and widespread die off (during the day) due to natural warming. Consideration in the timing of water releases should reflect the need for mat removal in specific river reaches. For example, water released from the dam takes an average of 4–5 hours to reach lower river reaches. If morning hours are target periods for flushing to allow adequate die off during the day, then water must be released from the dam during the night to insure an early morning water arrival time and fulfillment of desired management objectives. Significant deviations from the latter may result in undesired effects or wasted management effort to mitigate bloom formation. Lastly, modification to existing dam infrastructure to allow surface releases from the epilimnion may prove to be the ultimate mitigation solution for *D. geminata* blooms, but it may not be considered feasible by federal and state agencies due to high cost and potential negative effects to the tailwater's recreational trout fishery, which has had an historical annual economic value of ~1 million dollars (Williams and Bettoli 2003).

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Thermal and conductivity effects on aquatic macroinvertebrate communities in a hypolimnetic tailwater

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Abstract

Hypolimnetic tailwaters have great potential as model systems for furthering our understanding of thermal and other constraints on diversity in freshwater systems. In the South Fork Holston River, near Bristol, Tennessee, we examined benthic macroinvertebrate communities in three river reaches along a tailwater river thermal gradient and in one control site located upstream of the reservoir. Directly downstream of the dam, the South Fork Holston Tailwater has a very narrow annual thermal range (11.83°C) and few cumulative growing degree days (GDD=243); abundance of Ephemeroptera, Plecoptera and Trichoptera (EPT) was as much as 80% lower at this site than at the two downstream sites, which had higher annual thermal ranges (19.39°C, 21.54°C respectively) and more degree growing days (GDD=438, 522 respectively). Species richness at the control site (Mean *S*=20) was 2–3× higher than any of the tailwater sites, although there was little variation in richness between tailwater river reaches (Mean *S*=7–11). Partial least squares regression and NMDS joint plots indicated that conductivity, surface flow rate, and benthic flow rate were significant predictors of observed variation in benthic macroinvertebrate diversity among sites. Our data and analyses suggest that macroinvertebrate communities in hypolimnetic tailwater systems are strongly influenced by changes to thermal range, conductivity and flow rate in downstream reaches. These shifts may be attributable to the exclusion of certain species from parts of thermal gradients. Tailwaters in particular may not meet the thermal niche requirements of key traits involved in the life history and development of the excluded species. We establish and elaborate upon the term “diapause termination temperature thresholds” to explain the diversity patterns seen in our own data.

Keywords: diapause termination temperature threshold, Shannon diversity, flow rate, partial least squares regression, NMDS, South Fork Holston River Tailwater

Introduction

Aquatic insects have evolved life history strategies that are tied to natural variation in riverine thermal and flow regimes (Ward and Stanford 1982). Aquatic insects respond both to discrete and cumulative thermal cues. Stream temperature patterns affect larval development and emergence, dissolved oxygen tolerance and other behavioral traits (Ward and Stanford 1982). The construction of dams may dramatically affect these deeply rooted phenological relationships by altering thermal and discharge regimes. A dam that forms a reservoir may result in either an epilimnetic (warm water release) or hypolimnetic-release (cold water release) tailwater. Hypolimnetic-releases produce characteristically constant thermal regimes in tailwaters that may result in higher winter temperatures and cooler summer temperatures, depending on management operations, compared to unaffected reaches (Kondratieff and Voshell 1980). Epilimnetic tailwaters are known to have higher productivity than their hypolimnetic counterparts from reservoir epilimnion contributions of zooplankton and phytoplankton. Consequently, coarse (CPOM) and fine (FPOM) particulate organic matter are broken down by aerobic bacteria in the reservoir hypolimnion, making hypolimnetic tailwater discharges extremely anoxic (Ward and Stanford 1979, Ward and Stanford 1983, Ward and Stanford 1995). Many federal and state regulatory agencies have implemented various technologies, such as direct oxygen injection systems and aeration weirs, to help combat biological and ecological problems associated with low tailwater dissolved oxygen (DO) (Adams and Brock 1993). Hypolimnetic and epilimnetic tailwaters also differ greatly in operational use and function. In the Tennessee River system, for example, hypolimnion discharges are utilized more often for hydroelectric power generation than releases from the epilimnion (Bednarek and Hart 2005). Variation in diel flow velocity of hypolimnetic-releases is also much greater than epilimnetic releases, which typically produce higher flow discharges, but at much lower velocities (Ward and Stanford 1983). The resultant flooding, scouring, desiccation, substrate instability, and localized variations in water velocity caused by extreme annual and diel variation in hypolimnetic tailwater flow rate lead to spatial and temporal variation in invertebrate community structure at these sites (Bunn and Arthington 2002).

Dams on mid-order streams may create dramatic thermal serial discontinuities and affect the diel and annual temperature range for a substantial distance downstream (Ward and Stanford 1979, Ward and Stanford 1983, Ward and Stanford 1995). The serial discontinuity concept builds upon the river continuum concept by hypothesizing that dams may have dramatic consequences to the hydraulic, thermal, and energetic equilibria of streams. Interestingly, modifications to the natural thermal and flow regimes of mid order streams have resulted in significant changes to aquatic invertebrate biodiversity across much of the southeastern USA (Bednarek and Hart 2005). Many tailwater communities are dominated by insects, crustaceans, oligochaetes and turbellarians with life history strategies that tolerate these radical shifts in habitat conditions.

Many investigators have argued that water temperature is the single most important driver of aquatic insect life history and reproductive events (Rader and Ward 1989, Brown et al. 2010). Many aquatic insects grow more rapidly and mature sooner in warmer temperatures, but they usually achieve smaller adult body sizes as a result (Markarian 1980, Giberson and Rosenberg 1992, Hogg and Williams 1996, McKee and Atkinson 2000). Consequently, at colder temperatures, this relationship is reversed, resulting in slower development but greater adult body size following emergence (Sweeny and Vannote 1978, Markarian 1980). Slight changes in development rate may then cause aquatic insects to emerge earlier or later and with a smaller or larger body size relative to water temperature and cumulative growing degree day accumulation. Given that fecundity is directly related to adult body size, and that most aquatic insect taxa have brief adult stages, changes in emergence timing and maturation size may have strong consequences for maintaining reproductive success, adequate fitness, and survivorship (Sweeny and Vannote 1978). Sweeny and Vannote (1978) proposed that aquatic insects have a narrow temperature range within which adult body size and fecundity are optimized, and they hypothesized that changes of only 2–3° C could lead to population extirpations at the limits of species' geographic ranges. Aquatic insects that are specialists with narrow thermal niche requirements are particularly prone to extirpation by sudden shifts in temperature.

The homogenous environmental conditions typical of tailwater systems, especially the homogenization of temperature along an latitudinal gradient, can lead to losses in biodiversity and the exclusion of species ill-adapted to these dam-modified thermal regimes. Natural environmental heterogeneity and disturbance in unregulated rivers constitutes a physical habitat template that dictates the types of species adaptations necessary for local persistence (Poff and Ward 1990, Huryn and Wallace 2000). In addition, a lack of exposure to extreme thermal diversity may exclude a large percentage of taxa from colonizing habitats directly downstream of dams because requisite thermal heterogeneity is not met (Lehmkuhl 1972, Lehmkuhl 1974, Kraft and Mundahl 1984, Mundahl and Kraft 1988, Saltveit et al. 1994). Species currently occupying tailwaters are presumably better adapted to low and relatively constant temperatures relative to those species in unregulated sections upstream of the reservoir. However, these tailwater species may also be poor competitors that are only able to compete successfully in depauperate communities.

We sought to elaborate on the current research regarding tailwater effects on macroinvertebrates by asking the following questions: (1) How do the physiochemical, habitat, and environmental conditions of tailwaters influence macroinvertebrate diversity and abundance and (2) How does temperature, specifically, affect macroinvertebrate diversity and abundance? We also note here that we see great potential in using hypolimnetic tailwaters as model systems for furthering our understanding of thermal constraints on diversity in freshwater systems.

Methods

Study area

Our three test sites are located downstream of South Holston Reservoir on the South Fork Holston River, a hypolimnetic tailwater located near Bristol, Tennessee, and a control site is located upstream of the reservoir (Figure 1). The tailwater is managed by the Tennessee Valley Authority (TVA) for hydroelectric power generation, flood control, and recreation, and it is part of the upper Tennessee River valley. Four seasonal collections were made between January 2015 and March 2016. We selected riffle habitats from three different river reaches (upper, middle, and lower), increasing in distance from the dam. All environmental data including flow rate were collected in the absence of hydropeaking. Each collection site was equipped with a temperature data logger (Onset HOBO U20, Bourne, MA, USA) mounted to a small dolomite boulder in order to determine the thermal profile of each river reach.

Field collection and laboratory processing of macroinvertebrates

To estimate macroinvertebrate community diversity and abundance at each river reach, we established longitudinal transects with six equidistant subsamples harvested from Surber samplers (30.5 cm^2, 500-µm-mesh) after disturbing substrate for three minutes (Cummins 1962, Hynes 1971). The contents of each Surber subsample were placed into collection jars with 80% ethanol. Whole jar samples were vortexed and a 200 mL subsample was quantified after being selected using a random number generator. All macroinvertebrates (except Coleoptera, and Chironomidae) were identified to the genus level using taxonomic keys (Merritt et. al 2008) and enumerated using a stereomicroscope (Olympus SZ61 zoom, Center Valley, PA, USA).

Habitat, physiochemical, and nutrient data collection

In order to accurately characterize the effect of habitat and environment on macroinvertebrate communities, we measured flow rate ($m \bullet s^{-1}$), DO, pH, conductivity, temperature, turbidity, orthophosphate, NO_3^-, and daily mean discharge ($m^3 \bullet s^{-1}$). Flow rate was measured using a hand-held flow probe (Global Water Instrumentation, College Station, TX, USA) at the surface and benthic (substrate) level. DO, pH, conductivity and temperature were measured with a multi-parameter water chemistry meter (Thermo Fisher Scientific Orion, Waltham, MA, USA) and turbidity with a portable turbidimeter (HF Scientific DRT-15CE, Fort Myers, FL, USA). Annual and 5-year discharge data were gathered from the USGS Marion, VA field office (control site) (http://waterdata.usgs.gov/nwis/uv?site_no=03473000) and the TVA Chattanooga, TN field office division of ecological stream monitoring (tailwater sites) (personal communication, Tyler Baker, TVA). We used Ion-exchange Chromatography (Dionex ICS-3000, Sunnyvale, CA, USA) to calculate NO_3^- concentrations at each site. Dissolved inorganic phosphorus (orthophosphate) was determined via the molybdate blue colorimetric method (Murphy and Riley 1962, Stainton et al. 1974) by measuring absorbance with a spectrophotometer (Bruker Vector 22 FT-IR, Billerica, MA, USA) at 885 nm using a 5 cm quartz cell. All water samples for N and P analysis were acidified within 12 hours of collection with 4% conc. H_2SO_4 by volume for a ~30 day maximum holding time. A summary of the annual means and standard errors for each water quality and environmental parameter can be found in Table 2.

Statistical analysis

HOBOware software (HOBOware Pro/3.7, Onset Computer Corp., Bourne, MA, USA) was used to collect, log, and calculate cumulative growing degree-days (GDD) for all water temperature data from data loggers mounted to dolomite boulders. We calculated the number of cumulative annual growing degree-days for each river reach by using the equation: Cumulative GDD = $\sum [(\frac{T_{max}+T_{min}}{2}) - T_{base}]$, where T_{base}=10°C. All data were LOG transformed after failing Shapiro-Wilk normality tests. We calculated Shannon (*H'*) and Simpson (D) Index values and richness (S) and evenness (*J'*) using the macroinvertebrate abundances from each Surber subsample. Pearson correlation matrices determined that Simpson Index (0.940, $p<0.001$), species richness (0.832, $p<0.001$) and evenness (0.789, $p<0.001$) values were significantly and positively correlated to Shannon Index values. Therefore, Shannon Index (*H'*) values were used in all analyses as a dependent variable. To determine any statistical differences in Shannon Index between sites, we performed a one-way ANOVA (SPSS/23, IBM Corp., Armonk, NY, USA) with Fisher's LSD post hoc tests.

Multivariate statistical analyses were used to determine the effect of hypothesized environmental parameters on macroinvertebrate community structure, diversity, and abundance. To do this, we conducted a partial least squares regression analysis (PLS) (JMP/10 Pro, SAS Institute, Cary, NC, USA). Shannon Index (*H'*) was selected as a dependent, response variable while surface flow rate, benthic flow rate, dissolved oxygen, pH, conductivity, temperature, turbidity, orthophosphate, and nitrate were selected as independent, predictor variables. In addition, Nonmetric multidimensional scaling (NMDS) analyses were conducted (PC-ORD/6, Gleneden Beach, OR, USA) to visualize differences in macroinvertebrate community structure at the genus level using Bray-Curtis distance measures with site as a grouping variable. Multi-response permutation procedure (MRPP) analysis was then used to determine any significance differences in community structure between sites. The effects of different environmental variables on the ordination of different sites were then quantified via joint plots and Pearson's r and Kendall's tau correlation coefficients.

Results

Thermal effects

A one-way ANOVA found significant differences between macroinvertebrate diversity [Shannon Index (*H'*)] among sites and seasons with significant pairwise site comparisons between all sites following Fisher's LSD post hoc tests. We found a strong correlation between Shannon Index and the number of cumulative growing degree-days, annual thermal profile, and temperature range and variance (Figure 2). Control site *H'* was ~50% higher than at site one, 33.8% greater than site two (Mean *H'*=1.45, GDD=438.06), and 24.2% greater than at site three (Mean *H'*=1.66, GDD=522.42). In addition, *H'* at site three was 34.9% and 12.7% greater than sites one and two, respectively. Species richness at the control site (Mean *S*=20) was also 2–3× that of the tailwater sites, although there was little variation in richness between tailwater sites (Mean *S*=7–11). More explicitly, we collected 17 Ephemeroptera, 9 Plecoptera, 12 Trichoptera, and 6 Diptera genera from the control site, as compared to 3 Ephemeroptera, 1 Plecoptera, 4 Trichoptera, and 4 Diptera genera at the most diverse tailwater site, which had a narrower annual thermal range than the control. Likewise, site one had 80% and 76% less EPT abundance than sites two and three, but it supported greater populations of Diptera and Oligochaeta and an isolated population of Isopoda. Amphipoda were collected only at tailwater sites two and three, which we suspect prefer a slightly wider thermal range than the Isopoda found immediately downstream of the reservoir.

The reduced *H'* observed directly downstream of the dam (site one) and gradual increase in *H'* with increasing distance from the dam is likely attributable to a change in annual thermal range at downstream sites (Figure 3, Table 4). At the control site, we observed the highest annual *H'* values among collection sites and highest annual temperature variance (45.7) and range (25.4°C); remarkably, productivity is much lower at the control site (1,293 individuals per 30.5 cm^2 habitat sampled) relative to the tailwater reaches (Site 1=5,660, Site 2=2,869, Site 3=4,634; Table 7). We observed the lowest temperature variance (7.3) and range (11.8°C) immediately downstream of the dam, and this site (site one) also had the lowest mean *H'* values among collection sites. Sites two and three had greater annual temperature variances (9.7, 10.5, respectively) and annual temperature ranges (19.4°C, 21.5°C, respectively) compared to site one, and Shannon Index (Table 1) also increased with increasing distance downstream from the dam.

Environmental effects on macroinvertebrates

To examine the influence of physicochemical habitat parameters on macroinvertebrate abundance and diversity, we included 9 different environmental variables in a partial least squares regression model of observed variation in diversity between sites. This suite of environmental predictors accounted for 61.2% of the cumulative variation in *H'*, and several variables were important predictors of Shannon Index (Figure 5, Table 5). Surface flow (VIP=1.36), benthic flow (VIP=1.22), temperature (VIP=1.16), conductivity (VIP=1.50) and turbidity (VIP=0.85) were significant contributors to the PLS model of Shannon Index.

NMDS ordination plots revealed that three distinct communities are present within the study area. Joint plots explained the directionality of specific environmental parameters influencing the ordination of the data (Figure 6). Macroinvertebrate communities at site 1 and the control site formed separate groupings within the NMDS plot, whereas sites two and three exhibited considerable overlap in ordination space but grouped separately from site one and the control site. NMDS axes 1 and 2 explained 49% and 28% of the respective variation in the ordination.

MRPP analysis showed highly significant differences ($p<0.001$) for all pairwise comparison except for sites two and three, which were slightly different ($p=0.022$, Table 6). Kendall correlation coefficients quantified the correlation of each environmental variable and the ordination of community data along each NMDS axis. Surface flow ($r^2=0.217$, tau=0.322) and benthic flow (r^2=0.111, tau=0.251) exhibited a positive relationship with the distribution of the control and sites two and three along NMDS axis 1 (Table 6; Figure 5). In addition, conductivity (r^2=0.604, tau=-0.446; r^2=0.115, tau=-0.268) had a highly negative correlation with the ordination of sites one, two, and three along the 1st and 2nd NMDS axis (Table 6; Figure 5). Environmental parameters represented by individual joints did not explain the ordination of the site one community within ordination space. Kendall correlation coefficients yielded somewhat similar results to the PLS regression, but produced a more sensitive analysis. Temperature and turbidity were the only environmental predictors to be significant variables of importance in the PLS model, but not as Kendall correlation coefficients and were the least significant of the five VIPS greater than 0.80.

Discussion

Large hypolimnetic tailwaters are among the most widely-occurring and yet poorly-understood lotic ecosystems in the southeastern USA. Aquatic biota occurring in these systems experience daily flow extremes that may exceed naturally occurring annual extremes as well as greatly altered nutrient and temperature regimes relative to undammed rivers. Here, we highlight the effect of daily flow extremes and the altered daily and annual thermal regime on macroinvertebrate benthic community shifts in diversity and abundance in a dolomitic tailwater river. Specifically, we focus on the reduction of sensitive macroinvertebrate groups (e.g., Ephemeroptera, Plecoptera, and Trichoptera [EPT]) in favor of other taxa that are better adapted to radical variation in temperature and hydrology.

Our work complements work of prior investigators in that we also found that limited thermal diversity excludes a large percentage of taxa from colonizing habitats immediately downstream of hypolimnetic-release dams. We analyzed annual and 5-year daily mean discharge data and found that TVA river operations managers consistently reduce power generation flows during the mid–late summer time period. In 1991, the TVA, in cooperation with Trout Unlimited, completed construction of an aeration weir to increase tailwater DO concentrations and raise the minimum tailwater baseflow from 0 to 2.6 $m^3 \bullet s^{-1}$ (Habera et al. 2005). The latter was achieved by building a weir pool to collect water during power generation that is then continually released downstream to decrease riverbed desiccation when power is not being generated. Currently, the lack of consistent summer hydropeaking in the form of short-term "pulses" results in significant warming to downstream river sections (Figure 3). During full power-generation periods, flows are sufficient to cool the entire 22 km tailwater reach and to produce thermal homogeneity across sites. Summer pulses are designed to conserve thermal habitat for macroinvertebrates, but other aquatic communities are not factored into management decisions. Power generation pulses average 1–2 hours in duration with discharges of approximately 42.9 $m^3 \bullet s^{-1}$. In contrast, full power generation flows typically last 10–12 hours in duration with a discharge of approximately 70.7 $m^3 \bullet s^{-1}$. Pulsed releases do not provide sufficient cold water to mitigate the effects of natural warming on lower river reaches during the day, especially during the spring and summer. Natural warming of the upper river is effectively eliminated in the tailwater reach, and temperatures are influenced by the continual release of cool water from the reservoir's hypolimnion. Reduced daily discharge and increased natural warming result in a 25.5% and 34.9% increase in *H'* at sites two and three relative to site one. Site one is ~13.7 km and 20 km from sites two and three, respectively (personal communication, Tyler Baker, TVA;

Table 1). The increase in annual Shannon diversity at sites two and three compared to site one is notable given the limited distance between sites.

Additionally, it is important to note that PLSR and NDMS ordination analyses utilized temperature data measured only during macroinvertebrate collections and not from temperature data loggers, which were analyzed separately. Significant variation in daily mean temperature cannot be captured by these statistical methods from limitations of each procedure's ability to handle data sets with a significantly different number of observations (e.g. not enough Shannon Index observations for temperature observations). Therefore, we acknowledge that the actual impact of temperature on tailwater macroinvertebrate communities may either be obscured or understated when considering only the macroinvertebrate collection-sensitive temperature data incorporated as a predictor into the PLS model and NMDS ordination.

In 1972, Dennis Lehmkuhl hypothesized that increases to macroinvertebrate diversity directly downstream of a reservoir were impeded by the insects' inability to terminate egg diapause, a period of arrested metabolism in the egg phase until environmental token stimuli trigger physiological processes that resume normal development (Koštál 2006). Lehmkuhl (1972) claimed that water temperatures below the dam were simply too warm during the winter and too cold during the summer and that temperatures required for diapause termination in the late summer–fall (overwintering species) and winter–spring (species with summer and fall generations) were never met. Although the environmental stimuli that trigger termination of diapause of aquatic macroinvertebrates are not yet well known, air temperature, in particular, is known to lead to diapause termination in terrestrial insects (Horie et al. 1999, Denlinger 2002). We reasonably hypothesize, then, that water temperature, in addition to an unknown suite of other environmental conditions, is responsible for diapause termination in aquatic insects. In so doing, we support Lehmkuhl's diapause failure hypothesis as one reason for reduced macroinvertebrate diversity directly below dams.

We establish the term "diapause termination temperature thresholds" to explain the diversity patterns seen in our own data. For example, if water temperatures do not meet the diapause termination temperature threshold of a particular species, the egg stage never breaks diapause, and the species is extirpated from the community. We find that tailwater sections immediately below hypolimnetic-release dams may favor species with ample diapause plasticity to either forego diapause entirely or to shorten their diapause periods to adapt to thermal variation.

Individual flow rates at subsampling locations across river reaches could also have influenced diversity observed among control and tailwater sites. Surface and benthic flow rates at the control site were almost twice as high as each of the tailwater sites (Table 2) and may have favored species that have high velocity microhabitat requirements. Macroinvertebrate richness and EPT richness, in particular, are typically highest in riffle habitats; some EPT taxa,for example, are exclusive to microhabitats with high flow velocities in order to supplement aerobic metabolism or to gain access to specialized food resources trapped in the substrate (Lancaster and Hildrew 1993, Connolly et al. 2004, Lamouroux et al. 2004, Brooks et al. 2005). Although changes to flow regime may explain why some species appear to prefer higher flow rates in these systems, changes to thermal variation may also represent important drivers of decreasing tailwater macroinvertebrate diversity. Few studies have examined the physiological effects of temperature on the survivorship and development of macroinvertebrate early life history stages, and such studies are sorely needed to better elucidate interacting effects of flow and thermal range on benthic macroinvertebrate diversity.

PLS models and NMDS ordinations revealed strong correlations between variation in *H'* and conductivity across sites. The South Fork Holston River Tailwater is an important tributary to the Tennessee River and rises in the lower Great Appalachian Valley, which is underlain by primary dolomite and secondary limestone bedrock layers, specifically the Knox-Dolomite Formation (Oder 1934, Davis 1990). Dolomite is a sedimentary carbonate rock that is composed of 70% magnesium and 30% calcium carbonate ($CaCO_3$). In dolomitic and limestone rich rivers, excess Ca^{2+} becomes bioavailable from the reaction of $CaCO_3$ with H_2CO_3 to yield HCO_3^- and Ca^{2+} (Plummer and Busenberg 1982). Reservoirs in the lower great valley act as stores for bicarbonate and dissolved Ca ions, which are acquired by aquatic insects via chloride cells, chloride epithelia, or anal papillae (Komnick 1977, Poteat and Buchwalter 2014). Recent work has shown that macroinvertebrates absorb and utilize Ca^{2+} at different rates and that these differences are deeply rooted in phylogeny (Poteat and Buchwalter 2014). The average conductivity of tailwater sites ranged from 239.1–263.2 µs cm^{-1} while the control averaged 174.3 µs cm^{-1} (Table 2). It is possible that variation in Ca^{2+}-dependent specific conductivity may explain the decrease in *H'* at tailwater sites relative to the control, as some species may simply outcompete others for available Ca^{2+} creating less diverse macroinvertebrate communities that are able to respond strongly to Ca^{2+} gradients. Additionally, many of the true limestone springs and seeps that enter the tailwater have incredibly high conductivities (~600 µs cm^{-1}) from significant contributions of Ca^{2+} from limestone caves and secondary bedrock layers. These springs have a lower species richness than the South Fork Holston River, but they also support populations of ionically and thermally adaptable benthic macroinvertebrates (e.g., *Ephemerella* spp., *Baetis* spp., *Tricorythodes* spp., *Micrasema* spp., Amphipoda, and Isopoda). Hence, the increased bioavailability of Ca^{2+} in the tailwater and limestone feeder springs likely leads to Ca^{2+}over enrichment producing an invertebrate community dominated by the effects of Ca^{2+} in addition to thermal variation.

In conclusion, the significant loss of macroinvertebrate diversity in the South Fork Holston tailwater is likely the product of reduced thermal range and increased variability in discharge at sites closest to the dam, and thus these effects are mitigated at downstream sites. Numerous other studies in addition to our own also have shown that the limited diel and annual range of tailwater temperatures can have a profound impact on benthic macroinvertebrate communities. Further, our data suggest that temperature or other life-history mis-matches may occur in tailwaters, thereby supporting the ”diapause termination hypothesis” explanation of low macroinvertebrate diversity below hypolimnetic dams on mid-order streams. We hope that further investigations will be undertaken to test our diapause termination hypothesis and, if supported, to determine how the physiology of macroinvertebrate egg diapause termination is interrelated to species presence and absence in thermally variable or invariable habitats.

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Table 1. GPS coordinates, elevation (m), USA topographic map river mile markers, and distance from the dam (km) for four sites in the South Fork of the Holston River in northeastern Tennessee and southwestern Virginia..

Table 1.
Study Site Summary

Site	Coordinates	Elevation	River Mile Marker	Distance From Dam
Control	36°39'10.80", -081°53'13.20"	534.62 m	73.7	0.0 km
Site 1	36°31’33.60”, -082°06’50.40”	450.50 m	48.0	1.2 km
Site 2	36°29'16.80", -082°11'42.00”	436.17 m	39.5	14.9 km
Site 3	36°28'04.00", -082°14'13.20"	427.94 m	36.2	20.2 km

Table 2. Mean (and standard errors) for a range of physiochemical, water quality, and habitat data for each sampling site.

Table 2.
Physiochemical, Water Quality, and Habitat Data

Environmental Variable	Control	Site 1	Site 2	Site 3
Surface Flow Rate (m s^{-1})	0.91 (0.07)	0.34 (0.04)	0.52 (0.06)	0.59 (0.04)
Benthic Flow Rate (m s^{-1})	0.56 (0.06)	0.20 (0.02)	0.36 (0.04)	0.37 (0.04)
Dissolved Oxygen (mg L^{-1})	11.20 (0.32)	11.83 (0.17)	12.45 (0.31)	12.45 (0.23)
Temperature (°C)	12.93 (1.47)	7.96 (0.26)	10.58 (0.70)	9.57 (0.73)
Conductivity (µs cm^{-1})	174.3 (2.84)	239.1 (1.09)	247.5 (3.08)	263.2 (1.97)
pH	8.11 (0.05)	7.82 (0.06)	8.27 (0.05)	8.26 (0.08)
Turbidity (NTU)	2.22 (0.12)	1.25 (0.11)	1.40 (0.13)	2.20 (0.20)
NO_3^- (mg L^{-1})	10.63 (1.55)	13.01 (1.34)	10.59 (2.11)	7.95 (0.50)
PO_4^{3-} (µg L^{-1})	71.42 (8.31)	70.77 (10.51)	80.74 (6.32)	81.82 (6.44)

Note. All data are annual means with standard errors in parenthesis. NTU, nephelometric turbidity unit; NO_3^-, nitrate; PO_4^{3-}, orthophosphate

Table 3. Results of one-way ANOVA of the effect of site on macroinvertebrate Shannon Index (*H'*) score for sites in the South Fork of the Holston River in northeastern Tennessee and southwestern Virginia.

Table 3.
One-way ANOVA Summary

Dependent Variable	Source	df	*F*-value	*P* value
Shannon Index (*H'*)	Site	3	58.827	<0.001
	Error	86		
	Fisher's LSD Post Hoc Tests	*P* value		
	0 vs. 1	<0.001*		
	0 vs. 2	<0.001*		
	0 vs. 3	<0.001*		
	1 vs. 2	<0.001*		
	1 vs. 3	<0.001*		
	2 vs. 3	0.014*		

Note. –Zero is equivalent to the control site. All variables transformed using the following equation: LOG(Data). LSD, least significant difference

*p<0.05

Table 4. Temperature data summary statistics for control and tailwater sampling sites. Note the differences in temperature variance and range between sites.

Table 4.
Temperature Data Summary Statistics

Statistic	Control	Site 1	Site 2	Site 3
Mean	12.52	8.32	9.46	9.80
Standard Error	0.07	0.03	0.03	0.03
Median	12.40	7.68	9.37	9.87
Mode	-0.10	4.93	11.24	11.24
Standard Deviation	6.76	2.70	3.12	9.73
Variance	45.67	7.30	9.73	10.47
Minimum	-0.10	3.26	1.22	0.89
Maximum	25.32	15.09	20.62	22.43
Range	25.42	11.83	19.39	21.54

Table 5. Partial least squares (PLS) regression with Shannon Index (*H'*) as a dependent variable.

Table 5.
Partial Least Squares Regression

Dependent Variable	Independent Variable	VIP	Number of VIP>0.8	Method	Number of Factors	Percent Variation Explained For Cumulative X	Percent Variation Explained For Cumulative Y
Shannon Index (*H'*)	Surface Flow	1.36*	5	NIPALS	4	70.00	61.12
	Benthic Flow	1.22*					
	Temperature	1.16*					
	DO	0.60					
	Conductivity	1.50*					
	pH	0.77					
	Turbidity	0.85*					
	Nitrate	0.37					
	Phosphate	0.50					

Note. –VIFs derived from an initial least squares multiple linear regression. All variables transformed using the following equation: LOG(Data). VIP, variable important to projection; VIF, variance inflation factor; NIPALS, non-linear iterative partial least squares algorithm

*VIP>0.8.

Table 6. Multi-response permutation procedure by site for macroinvertebrate abundance and diversity using Bray-Curtis distance measures. Also shown are Pearson's r and Kendall's tau correlations to determine significant relationships between environmental variables and the ordination of different sites.

Table 6.
Multi-response Permutation Procedure (MRPP)
Pearson's r and Kendall tau Correlation Summary

Multi-response Permutation Procedure (MRPP)			Pearson and Kendall Correlations With Ordination Axes						
Comparison	A value	P-value	Environmental Variable	Axis 1			Axis 2		
				R	r^2	Tau	r	r^2	tau
All Sites	0.223	<0.001$^+$	Surface Flow	0.466	0.217*	0.322$^{\bullet}$	-0.071	0.005	-0.104
0 vs. 1	0.181	<0.001$^+$	Benthic Flow	0.333	0.111**	0.251$^{\bullet}$	-0.072	0.005	-0.124
0 vs. 2	0.203	<0.001$^+$	Temperature	0.235	0.055	0.166	-0.175	0.030	-0.109
0 vs. 3	0.191	<0.001$^+$	DO	-0.274	0.075	-0.126	-0.168	0.028	-0.074
1 vs. 2	0.201	<0.001$^+$	Conductivity	-0.777	0.604*	-0.446$^{\bullet}$	-0.340	0.115**	-0.268$^{\bullet}$
1 vs. 3	0.142	<0.001$^+$	pH	-0.214	0.046	-0.219$^{\bullet}$	-0.267	0.072	-0.233$^{\bullet}$
2 vs. 3	0.021	0.022$^+$	Turbidity	0.242	0.059	0.220$^{\bullet}$	-0.195	0.038	-0.139
			Nitrate	0.128	0.016	0.129	-0.040	0.002	-0.009
			Phosphate	0.000	0.000	-0.063	-0.195	0.038	0.027

Note. –Zero is equivalent to the control site. All variables transformed using the following equation: LOG(Data). r^2, Pearson's r; tau, Kendall rank correlation coefficient

$^+p<0.05$; $^{**}r^2>0.100$; $^{*}r^2>0.200$, $^{\bullet}$tau>0.2

Table 7. Average annual macroinvertebrate abundance, Shannon (*H'*) and Simpson (D) Diversity indices, richness (*S*), and evenness (*J'*) for all sites.

Table 7.
Average Annual Macroinvertebrate Abundance, Shannon and Simpson Index, Richness, and Evenness Values

	Control	Site 1	Site 2	Site 3
Ephemeroptera	542	370	973	1108
Plecoptera	10	0	0	1
Trichoptera	285	6	71	210
Diptera	53	54	34	41
Chironomidae	304	2260	391	699
Annelida	46	2890	382	471
Isopoda	0	54	2	1
Amphipoda	0	14	979	1795
Elmidae	46	2	30	206
Mollusca	7	10	7	102
Total Abundance	1293	5660	2869	4634
H'	2.19	1.08	1.45	1.66
D	0.82	0.53	0.70	0.76
S	20	7	9	11
J'	0.74	0.56	0.67	0.71

Note.—Annelida includes Oligochaeta and Turbellaria; *H'*, Shannon Diversity Index; D, Simpson Diversity Index; *S*, richness; *J'*, evenness

Figure 1. Topographic map showing approximate field sampling locations along the South Fork Holston River with control (non-tailwater) site (red) and tailwater sites (black). Note the location of sampling sites to the city of Bristol, TN.

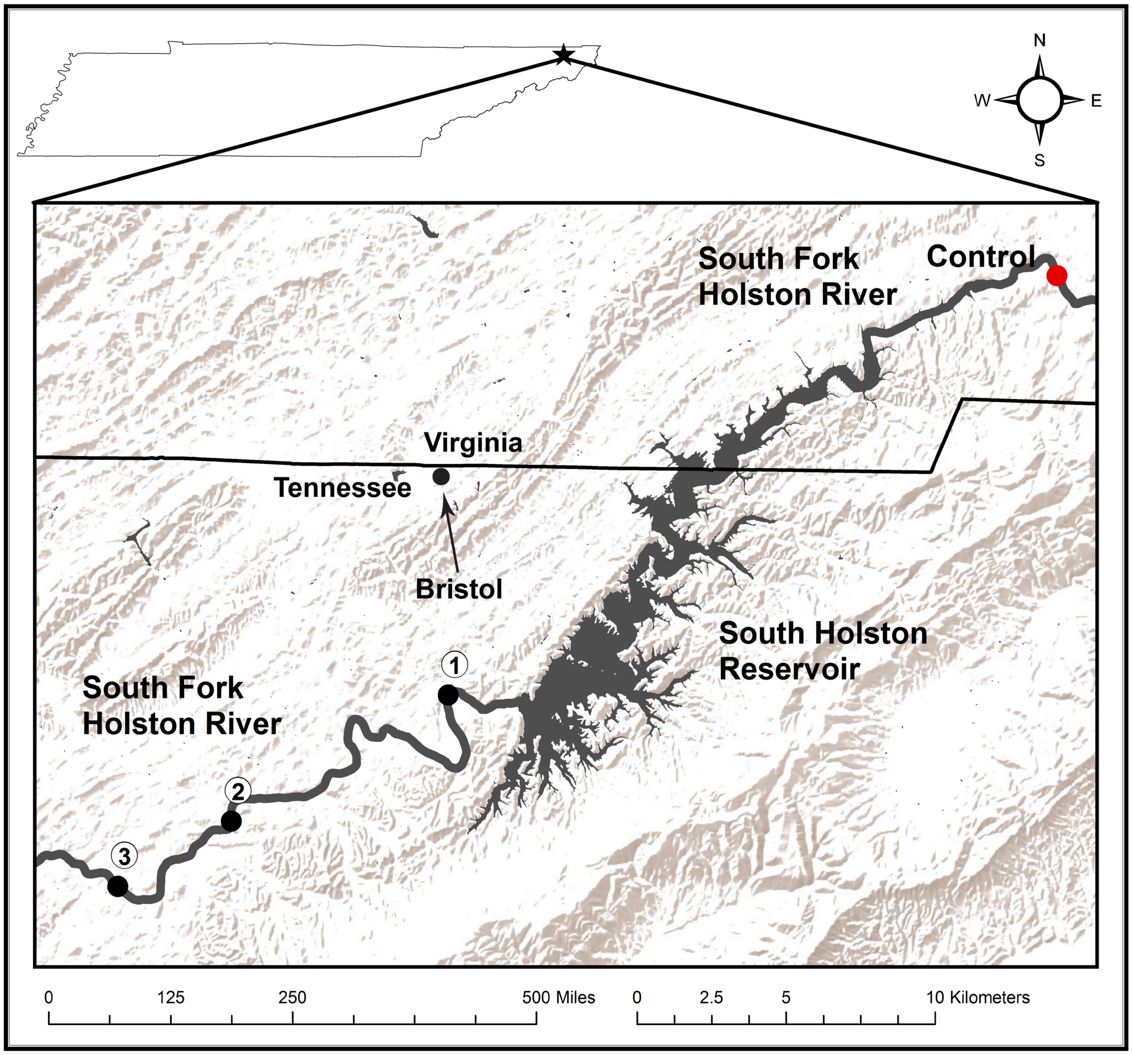


Figure 2. Average Shannon Index (*H'*) (top) and cumulative annual growing degree days (GDD) (bottom) for control (A, E), site 1 (B, F), site 2 (C, G), and site 3 (D, H) between February 2015-2016. Note the relationship between increasing Shannon Index and increasing GDD

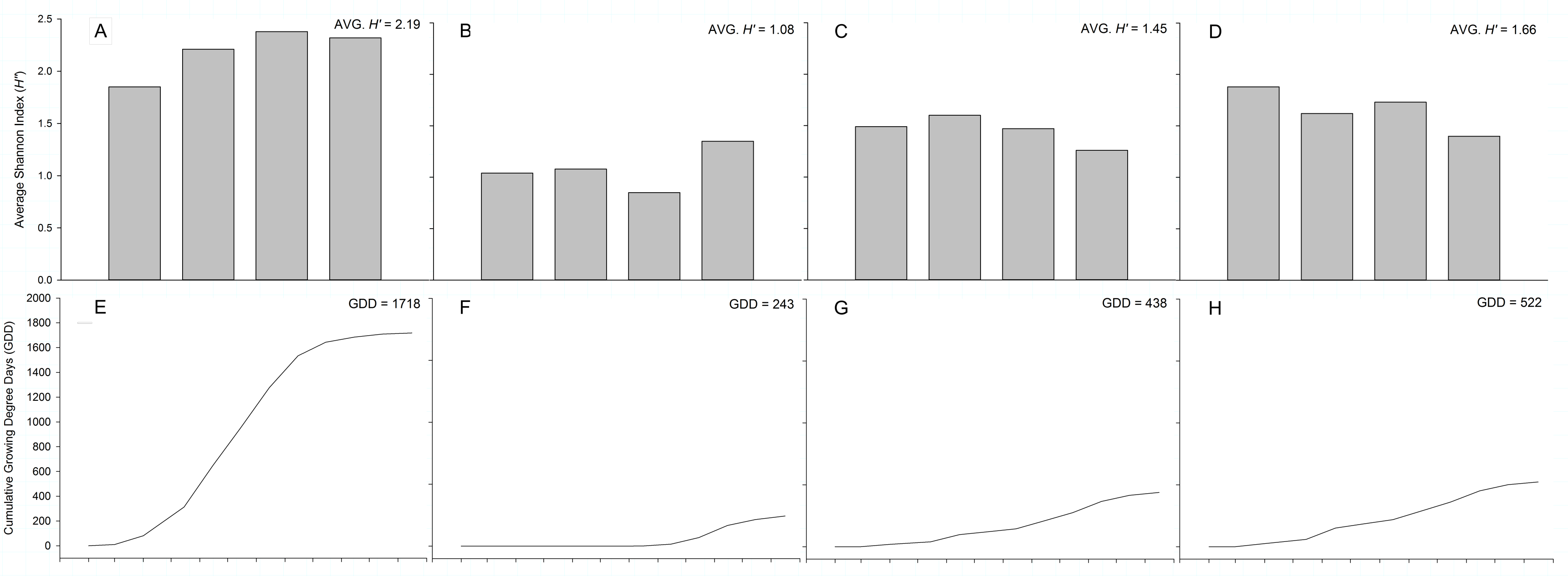


Figure 3. Annual thermal profiles of four sites in the South Fork Holston River in northeastern Tennessee and Southeastern Virginia from February 2015-February 2016. From the top left, upstream control site (A), Site 1- 1.2 km downstream from the dam (B), Site 2- 14.9 km downstream (C) and Site 3- 20.2 km downstream of the dam (D)..

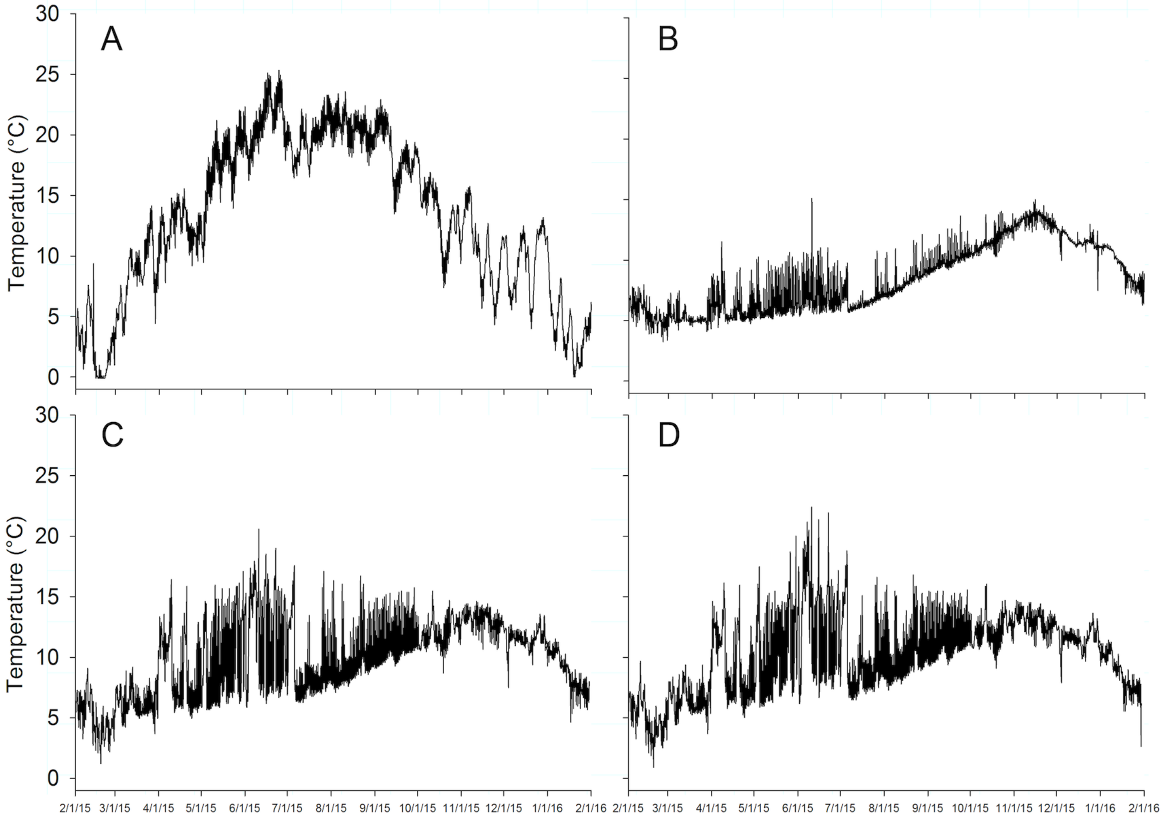


Figure 4. Annual daily mean discharge ($m^3 \bullet s^{-1}$) hydrographs for control (A), site one (B), site two (C), and site three (D) between February 2015-2016 (left) and February 2011-2016 (right). Note the reduction in daily mean discharge during the mid-late summer months for each year.

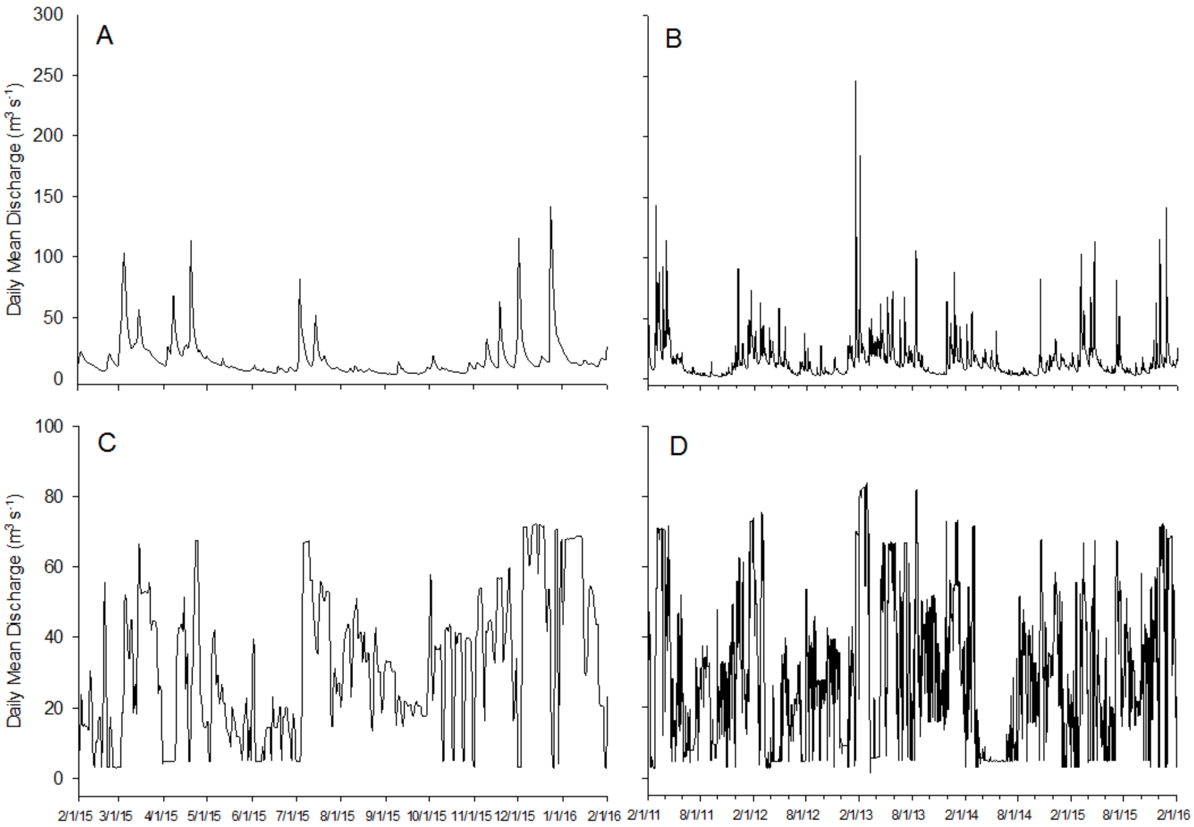


Figure 5. Simple linear regression of predicted LOG transformed Shannon Index (*H'*) by actual LOG transformed Shannon Index (*H'*) following a PLS regression analysis. Hypothesized environmental parameters used to predict differences in Shannon Index (*H'*) between sites explained 61.2% of the total variation *H'* between sites.

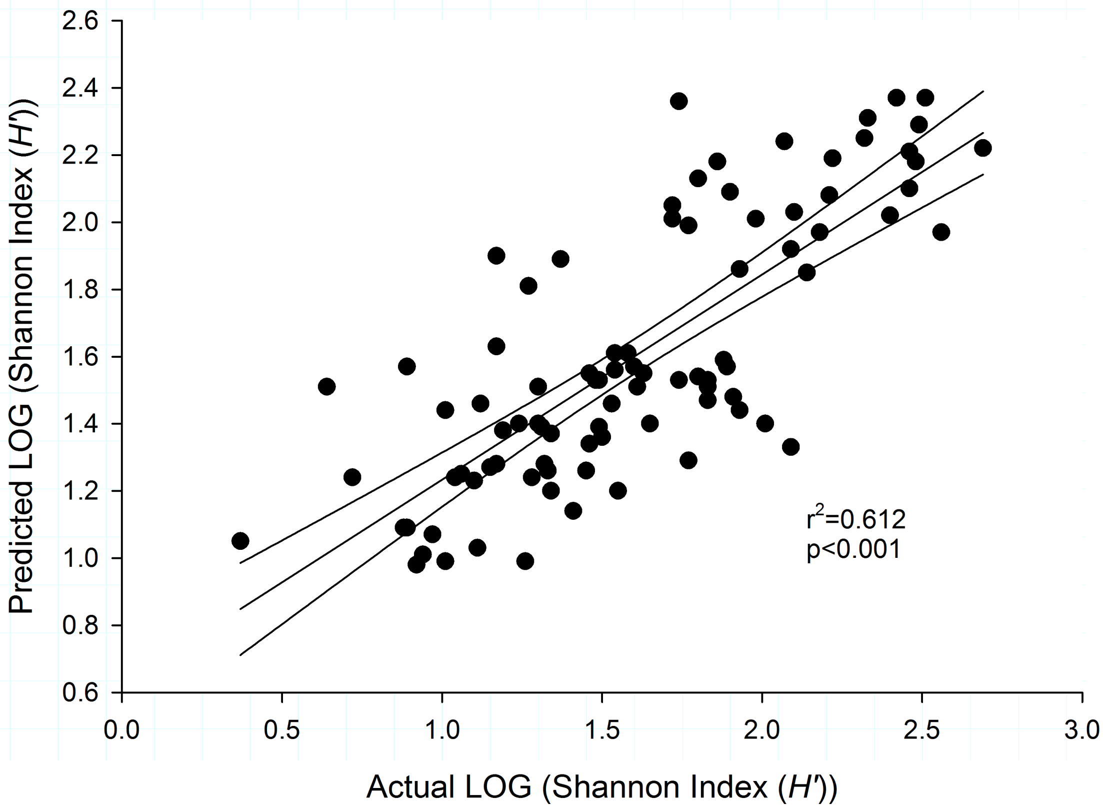
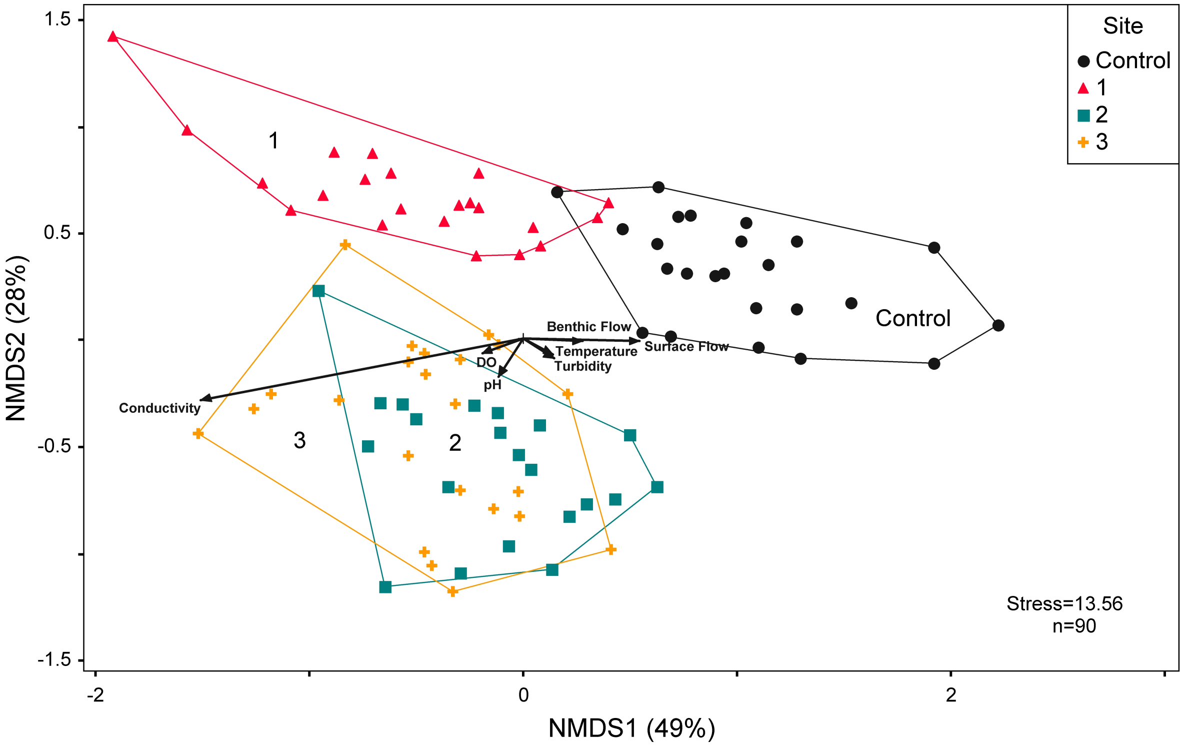


Figure 6. NMDS ordination joint plots showing the influence of environmental variables on the placement of macroinvertebrate communities within ordination space.



Chapter 2

26 July 2016

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Effect of habitat and *Didymosphenia geminata* on aquatic macroinvertebrates in the South Fork Holston River Tailwater, near Bristol, Tennessee

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Abstract

We conducted a large-scale field experiment in the South Fork Holston River near Bristol, Tennessee to understand how *Didymosphenia geminata* affects tailwater macroinvertebrate communities. *Didymosphenia geminata* and macroinvertebrate collections were made seasonally between January 2015 and March 2016 in riffle and run mesohabitats at three different tailwater reaches at increasing distance from the dam and at a single control site upstream of South Holston Reservoir. We found that *D. geminata* ash-free dry mass (AFDM) and dry mass differed significantly between mesohabitats with significantly higher *D. geminata* AFDM and dry mass in run habitats. Partial least squares regression (PLS) revealed that surface flow, benthic flow, dissolved oxygen, conductivity, and turbidity were significant variables of importance for predicting the observed variation in *D. geminata* AFDM and accounted for 45.2% of the total variation in *D. geminata* AFDM between sites. A second PLS regression found that surface flow, benthic flow, temperature, conductivity, turbidity, and *D. geminata* AFDM were significant variables of importance for predicting Shannon diversity between sites and accounted for 51.4% of the variation in *H'*. Nonmetric multidimensional scaling (NMDS) ordination plots indicated three distinct communities present, with joint plots and Pearson and Kendall correlation coefficients confirming PLS results except for the exclusion of temperature as a variable of importance. Relative abundance of specific taxonomic groups differed between mesohabitats, which is attributable to the significant difference in *D. geminata* between riffles and runs. Riffle habitats had increased Ephemeroptera (especially *Ephemerella* and *Baetis* spp.), EPT (Ephemeroptera + Plecoptera + Trichoptera) taxa in general, and Amphipoda. Run habitats saw increases in Oligochaeta, Turbellaria, and Isopoda. Diptera and Chironomidae increased only at runs furthest downstream, where *D. geminata* AFDM and dry mass were statistically different between mesohabitats. Our results indicate that surface and benthic flow rate, specifically low flow rates, along with dissolved oxygen, conductivity, and turbidity influence *D. geminata* AFDM, which in turn affects macroinvertebrate abundance and diversity. Our research suggests that federal and state resource managers should consider increasing minimum tailwater base flows, the frequency of high discharge pulses, and the maximum discharge during power generation, to minimize the effects of low flows on *D. geminata* growth and macroinvertebrates.

Key words: Invasive species, mesohabitat effects, dolomitic rivers, upper Tennessee River valley, partial least squares regression, NMDS

Introduction

The construction of dams to regulate the flow of rivers for drinking water storage, flood control, and power generation has resulted in numerous social and economic benefits (Lessard et al. 2013). However, the altered flow regimes of regulated rivers promote the establishment of invasive species. Invasive species are usually non-native or exotic species that threaten the diversity and abundance of native species and the ecological integrity of infested habitats. Most invasive species become introduced outside of their native range with the help of humans and result in environmental (changes to trophic system energy dynamics, nutrient cycling, extinction of native species, etc.), economic (~\$120 billion a year in damages), and social (negative public perception) consequences that demand management action (Pimental et al. 2005). Few non-native immigrant species actually become truly invasive following their introduction to new habitats. Invasion success is typically demonstrated by the Tens rule of Williamson, which states that for every ~10% of species that become introduced outside of their native range, ~10% of these species become invasive (Williamson 1996). Some have challenged the Williamson rule citing original research that of vertebrate introductions to North America and Europe, approximately 50% of introduced species have become invasive (Jeschke and Strayer 2005). Currently, similar research has not been undertaken to determine the invasive success of non-native aquatic species in North America, specifically periphyton and aquatic emergent and submergent plants.

Invasive species can also become nuisance species or a species that poses an adverse effect on and to humans. These effects can be direct (a species that causes direct physical harm) or indirect (a species that destroys habitats that humans find enjoyable for recreation, vacation, and leisure) (Colautti and MacIsaac 2004). *Didymosphenia geminata* has become affiliated with indirect negative social connotations from recreational fisherman and outdoor enthusiasts, which validate its classification as an invasive, nuisance species. In the southeastern United States, the species is also considered cryptogenic or a species with unknown origins (Carlton 1996). Some investigators contend that *D. geminata* is an invasive species originally native to Vancouver Island, British Columbia and western North America (Bothwell et al. 2009), whereas others suggest that *D. geminata* is a native nuisance species (Taylor and Bothwell 2014). However, regardless of *D. geminata*'s status as a cryptogenic, invasive, or nuisance species, its extensive bloom forming behavior and the resultant effects on aquatic macroinvertebrate communities pose a threat to aquatic life in southeastern USA tailwaters.

Algal blooms of the diatom *D. geminata* were first reported in the South Holston River, Bristol, Tennessee, USA, downstream of South Holston Reservoir in 2005 by Tennessee Valley Authority (TVA) limnologist Tyler Baker. Invasive diatom species like *D. geminata* flourish in tailwater rivers because these regulated rivers typically have homogeneous flow regimes with little or no variation in flow rate, with extended periods of below average discharge relative to pre-dam levels (Segura et al. 2010, Lessard et al. 2013). Kirkwood et al. (2007) found that discharge on the Bow and Red Deer rivers in Manitoba is inversely correlated with *D. geminata* biomass. Additionally, Richardson et al. (2014) found that high mean flow rates result in lower *D. geminata* biomass and that low flows promote *D. geminata* blooms. Substrate mobility has also been shown to affect *D. geminata* biomass (Miller et al. 2009, Cullis et al. 2013). Miller et al. (2009) found that *D. geminata* biomass in regulated streams along the Colorado Front Range was higher in streams with less bed movement compared to less stable channels. Because regulated river tailwaters are typically sediment-starved and do not experience large magnitude, substrate-mobilizing floods, benthic algal assemblages are frequently dominated by mat-forming diatoms like *D. geminata* (Lessard et al. 2013).

Unlike nuisance green algae (e.g., *Cladophora* spp.) that form large mats in eutrophic lakes and rivers, *D. geminata* prefers the oligotrophic conditions of high-elevation and tailwater streams (Ellwood and Whitton 2007, Kirkwood et al. 2007, Kirkwood et al. 2009, Miller et al. 2009, Bothwell and Kilroy 2011, Kilroy and Bothwell 2011, Kilroy and Bothwell 2012, Richardson et al. 2014, Reid and Torres 2014, Taylor and Bothwell 2014). In addition, turbidity is important for *D. geminata* proliferation and stalk establishment because river systems with low turbidity have greater light penetration, and cell division is more rapid (Kirkwood et al. 2007, Kirkwood et. 2009, Bothwell and Kilroy 2011, James et al. 2014).

In the southeastern USA*, D. geminata* only occurs in tailwater rivers with regulated flows and thermal regimes that are artificially cooled by hypolimnetic releases from large upstream reservoirs. *Didymosphenia geminata* populations have been reported from Kentucky, Maryland, North Carolina, Tennessee and Virginia (Spaulding and Elwell 2007). *Didymosphenia geminata* may prefer tailwaters because these habitats have stable, homogeneous flows, low water temperatures, low levels of dissolved inorganic phosphorus and low turbidity (Taylor and Bothwell 2014). These conditions are also associated with significant changes to macroinvertebrate community structure relative to unimpacted sites. Changes include reduced species richness and increased dominance of disturbance-tolerant taxa such as oligochaetes, crustaceans and some Chironomidae (Larson 2007, Kilroy et al. 2009, Gillis and Chalifour 2010, James et al. 2010, Richardson et al. 2014).

No studies have investigated the effects of *D. geminata* stalk and cell biomass on macroinvertebrate diversity in tailwaters of the southeastern USA. However, studies from elsewhere suggest that *D. geminata* blooms may negatively affect benthic community structure due to proliferation of mucopolysaccharide stalk and algal cell biomass (Larson 2007, Kilroy and Bothwell 2009, Gillis and Chalifour 2010, James et al. 2010, Richardson et al. 2014). Specifically, *D. geminata* blooms are associated with reductions in Ephemeroptera, Plecoptera, and Trichoptera (EPT) abundance and diversity and increases in more tolerant species including Chironomidae and oligochaetes (Larson 2007, Kilroy et al. 2009, James et al. 2010, Gillis and Chalifour 2010, Richardson et al. 2014).

Changes to macroinvertebrate community structure likely reflect larger community-wide shifts in riverine trophic interactions and habitat integrity (Gillis and Chalifour 2010). Explanations for observed shifts in community structure following *D. geminata* introductions and nuisance bloom presence include the trapping of fine sediment within interstitial spaces typically inhabited by invertebrates (Larson 2007); the creation of novel, patchy mixed *D. geminata* benthic habitat that supports an abundant and diverse Dipteran fauna (Gillis and Chalifour 2010); and the proliferation of *D. geminata* coverage during base flow conditions (Richardson et al. 2014).

We conducted a pilot study in July 2014 and found that flow rate influences *D. geminata* stalk and cell growth in a southeastern USA tailwater and ultimately results in significant alterations to the macroinvertebrate community there. Low flow rates led to increases in *D. geminata* stalk and cell biomass, which, in turn, resulted in macroinvertebrate communities that are more tolerant of *D. geminata* blooms (e.g., Chironomidae, oligochaetes, and isopods) (M. Green, unpublished data). Thus, we decided to build upon the current literature and our pilot study by investigating the effect of flow rate and *D. geminata* biomass on macroinvertebrate community abundance and diversity in riffle and run mesohabitats found in a southeastern USA tailwater. This work was conducted to inform management of how tailwater environmental conditions affect *D. geminata* and macroinvertebrates with the goal to encourage federal and state agencies to apply our results to current tailwater management plans. Specifically, we sought to answer the following questions:

1) Which environmental and habitat parameters are associated with increased *D. geminata* biomass?
2) How do *D. geminata* and macroinvertebrate abundance vary among and within sites and mesohabitats?

Methods

Study area

Downstream of South Holston Reservoir, the South Fork Holston River is managed by the Tennessee Valley Authority (TVA) as a ~20 km long hydroelectric and recreational cool-water tailwater river with an average base flow of 2.5 $m^3 \bullet s^{-1}$. However, the tailwater picks up considerable flow volume from first- and second-order tributaries that increase base flow by approximately ~2.8 $m^3 \bullet s^{-1}$. During hydropeaking, base flow can increase to 70.8 $m^3 \bullet s^{-1}$and remain so until power generation ceases. In addition to hydropeaking, the TVA often uses an hour long “pulse” of approximately 42.8 $m^3 \bullet s^{-1}$ (Habera et al. 2005) during the summer months to mitigate the negative effects of seasonal warming on the recreational trout fishery. Upstream of the reservoir, the control site is best characterized as an unregulated, fourth-order Blue Ridge Mountain stream with moderate to large cobble and boulder substrate with natural thermal and flow conditions. The bedrock layer for both tailwater and control sites is mostly comprised of dolomite ridges from the Knox Dolomite Formation in the lower Great Appalachian Valley (Oder 1934, Davis 1990), In the tailwater, numerous species of aquatic macrophytes flourish including *Fontinalis antipyretica* (common river moss), Bryaceae, *Chara* spp. (musk grasses), *Elodea canadensis* (water weed), *Potamogeton crispus* (curly leaf pondweed), and *Potamogeton* spp. (pondweeds), *Ludwigia* sp. (water primrose), and *Nasturtium officinale* (watercress). Field surveys also identified dominant populations of *Gomphonema* diatoms at control sites and small, scattered distributions of *Rhizoclonium* green algae at tailwater sites.

It is important to note that *D. geminata* blooms were never confirmed from control sites upstream of South Holston Reservoir. Microscopic evaluation of periphyton collected from the control site yielded spring and summer populations of *Gomphonema* spp., which are similar to *D. geminata* in cell morphology, mat coloration, and stalk structure.

Fieldwork was conducted between January 2015 and March 2016. We established four study sites; three sites were located downstream from the dam and a fourth site was located upstream of the dam, which served as a control site under a natural temperature and flow regime (see discussion above). Field collections were made in winter (January/February), spring (April/May), summer (August/September) 2015 and late winter (late January-early March) of 2016. Mesohabitats with different flow rates were sampled (e.g., riffles and runs) from three different tailwater sites [upper (1), middle (2), and lower (3)], increasing in distance from the dam (Figure 1, Table 1). Riffles (mean depth=0.32 m) had shallow, turbulent fast-flowing water and cobble/gravel substrates, whereas runs (mean depth=0.41 m) had more laminar and slow-moving water and sandy substrates interspersed with occasional cobbles and bedrock outcroppings. Surface and benthic flow rates were measured under baseflow conditions in the absence of scheduled dam water-releases and were significantly different between mesohabitats at all sites (Figure 2).

Macroinvertebrate and* Didymosphenia geminata *collection and processing

For collecting *D. geminata*, we placed 5 cm x 25 cm x 4 cm artificial substrates of Tennessee grey flagstone (n=6) at each sampling site with *D. geminata* harvested approximately every 2-3 months. Three subsamples of *D. geminata* were pooled and randomly selected from artificial substrates using a random number generator and grid and harvested using a 5 cm wide PVC coupling and a scalpel. Harvested algae were transferred into a 150 mL solution of 8% lugols iodine and deionized water (Smayda 1978) for preservation and further processing.

At each artificial substrate, aquatic macroinvertebrates were harvested from Surber samplers (30.5 cm^2; 500-µm-mesh) to characterize benthic community structure (Hynes 1971). Sampling units were modified to accommodate a wooden pole that was fastened to the metal rim of the Surber sampler to serve as a handle. Surber samples were collected adjacent to artificial tiles and substrate was sampled for three minutes. All specimens were preserved in 80% ethanol. Benthic samples were vortexed and a 200 mL subsample was selected using a random number generator. Macroinvertebrates (except Coleoptera and Chironomidae) were identified to the genus level using taxonomic keys (Merritt et al. 2008) and a stereomicroscope (Olympus SZ61 zoom, Center Valley, PA, USA), and the abundance of all taxa within each sample was recorded.

Didymosphenia geminata samples with minimal mat biomass were filtered by vacuum using a Corning PYREX 47 mm microfiltration glass assembly and filter flask onto pre-weighed 55 mm borosilicate glass microfiber filters (Whatman 934-AH, 1 µm nominal pore size). Many collection jars contained suspended algal cells and much greater mat biomass, proving difficult to vacuum filter. Therefore, these samples were sieved using a #230 (63 µm-mesh-size; 20.32 x 5.08 cm diameter) Standard Testing Sieve (Dual Manufacturing Co., Inc., Franklin Park, IL, USA). Algal cells and mat biomass not retrieved by the sieve were collected with a funnel and Erlenmeyer flask and centrifuged in nalgene bottles at 5000 rpm for 15 minutes using a Sorvall RC 6+ centrifuge and FIBERlite F14-6x250y rotor (Thermo Fisher Scientific, Waltham, MA, USA). All filtered, sieved, and centrifuged samples were transferred to pre-weighed aluminum weigh boats and dried in a drying oven (Quincy Lab, Inc. 40GC, Chicago, IL, USA) for 7 days at 60° C to a constant mass. Dried samples were then re-weighed and placed into a muffle furnace (Sybron Thermolyne 1500, Milwaukee, WI, USA) for 1 hr at 550°C using metal racks. Once samples had cooled to room temperature, the mass of the ash product was subtracted from the mass of the dried algae to calculate the ash-free dry mass (AFDM) of algae in the sample and scaled to 0.15 m^2 of total habitat sampled accounting for all three *D geminata* subsamples. It should be noted that due to the thickness and density of *D. geminata* mats, muffled samples were ground with a mortar and pestle every 30 minutes to insure homogeneous Carbon oxidation during ashing.

Habitat, physiochemical, and nutrient data collection

In order to characterize the effect of habitat on *D. geminata* blooms, we measured flow rate, dissolved oxygen (DO), pH, conductivity, temperature, turbidity, orthophosphate (PO_4^{3-}) and nitrate (NO_3^-) concentrations during macroinvertebrate and *D. geminata* collection. Flow rate ($m•s^{-1}$) was measured using a hand-held flow meter (Global Water Instrumentation, College Station, TX, USA) at the surface and substrate level. DO (mg L^{-1}), pH, conductivity (µs cm^{-1}), and temperature were measured with a multi-parameter water chemistry meter (Thermo Fisher Scientific Orion, Waltham, MA, USA) and turbidity (NTU) with a portable turbidimeter (HF Scientific DRT-15CE, Fort Myers, FL, USA)**.** Ion-exchange Chromatography (Dionex ICS-3000, Sunnyvale, CA, USA) was used to calculate the concentration of NO_3^- (mg L^{-1}) at each site. Orthophosphate (µg L^{-1}) concentration was determined via the molybdate blue colorimetric method (Murphy and Riley 1962, Stainton et al. 1974) by measuring absorbance with a spectrophotometer (Bruker Vector 22 FT-IR, Billerica, MA, USA) at 885 nm using a 5 cm quartz cell. All water samples for N and P analysis were acidified within 12 hours of collection with 4% conc. H_2SO_4 by volume for a ~30 day maximum holding time.

Statistical analysis

Shapiro-Wilk and quick frequency distribution tests were conducted to assess normality and detected significant right and left skewed data. In addition, *D. geminata* were absent from some riffle subsamples, so we choose to transform the data using the function LOG (data + 2). We calculated Shannon (*H'*) and Simpson (D) Diversity Index values and richness (S) and evenness (*J'*) using the macroinvertebrate abundances for each Surber subsample. Pearson correlation matrices determined that *D. geminata* dry mass (0.718; p<0.001) and inorganic mass (0.663; p<0.001) were significantly and positively correlated to *D. geminata* AFDM; Simpson Index (0.927; p<0.001), richness (0.787; p<0.001), and evenness (0.762; p<0.001) values were significantly and positively correlated to Shannon Index values. Therefore, *D. geminata* AFDM and Shannon Index values were used in all analyses as dependent variables. To determine whether *D. geminata*, benthic invertebrate communities, and environmental parameters were different between sites and mesohabitats, SPSS software was used to perform 2-way ANOVAs with *D. geminata* AFDM and Shannon Index as mutually exclusive dependent variables and site and mesohabitat as fixed factors (SPSS/23, IBM Corp., Armonk, NY, USA). We used Student's t-tests to determine whether *D. geminata* AFDM and dry mass and surface and benthic flow rates differed between mesohabitats.

Nonmetric multidimensional scaling (NMDS) was conducted (PC-ORD/6, Gleneden Beach, OR, USA) to visualize differences in macroinvertebrate community structure using Sorenson (Bray-Curtis) distance measures and site as a grouping variable. Multi-response permutation procedure (MRPP) analysis was then used to determine whether macroinvertebrate community metrics were significantly different among sites using Sorenson (Bray-Curtis) distances. Joint plots were fitted to the NMDS ordination plot showing the relationship of environmental variables to the NMDS structure and Pearson's r and Kendall's tau correlation coefficients were calculated to quantify the relationship and "fit" of environmental variables to the macroinvertebrate ordination along NMDS axes 1 and 2.

In order to determine the effect of habitat, physiochemical data, and nutrients on *D. geminata* AFDM and *D. geminata* effects on Shannon diversity, We conducted partial least squares regression analyses (PLS, JMP/10 Pro, SAS Institute, Cary, NC, USA) by utilizing the NIPALS method with k-fold cross validation (k=7). *Didymosphenia geminata* AFDM and Shannon Index values were each selected as dependent response variables whereas surface flow rate, benthic flow rate, DO, pH, conductivity, temperature, turbidity, orthophosphate, and nitrate were selected as independent predictor variables. In addition, we included *D. geminata* AFDM as an independent variable in the PLS model to predict Shannon Index to determine if *D. geminata* contributed to the overall variation in *H'* between sites. PLS is based on a projection of the predictor (x) and response (y) variables into a set of latent, uncorrelated predictor variables (or PLS factors) and corresponding scores, which minimizes potential collinearity between individual predictors and maximizes predictor and response covariance (Carrascal et al. 2009). Prior to performing a PLSR with the data, a multiple linear regression found our independent variables to exhibit moderate collinearity (Table 4), and therefore we chose to negate these effects by choosing partial least squares regression (PLS).

Results

Habitat, Environment, and Nutrients

Two-way ANOVAs found that temperature (p<0.001), DO (p<0.001), pH (p<0.001), and orthophosphate (p=0.008) were significantly different between sites across all seasons but not between mesohabitats, with no significant interaction between site and mesohabitat. Nitrate was only found to be significantly different between mesohabitats (p=0.006).

Turbidity was different between sites (p<0.001) but not mesohabitats, with a significant interaction between site and mesohabitat (p=0.043). Surface flow rate (p<0.001) and benthic flow rate (p<0.001) were significantly different between sites and mesohabitats but did not have a significant interaction between site and mesohabitats (Figure 2). Conductivity was significantly different for all three main effects. In addition, significant differences in surface and benthic flow rates between mesohabitats were proportional to the significant difference in *D. geminata* AFDM and dry mass between mesohabitats at each site (Figure 2). A summary of the annual means and standard errors for each water quality and environmental parameter can be found in Table 2.

D. geminata and macroinvertebrate communities

Macroinvertebrate abundance and diversity [Shannon index (*H'*)] differed significantly among sites, and Fisher's LSD post hoc tests found all sites to be significantly different from each other. However, *H'* was slightly insignificant between mesohabitats, and there was not a significant interaction between site and mesohabitat (Table 3).

Didymosphenia geminata AFDM did not differ significantly among sites downstream of South Holston Dam. However, *D. geminata* AFDM was significantly different between mesohabitats, with a significant interaction between site and mesohabitat (Table 3). *T*-tests found that *D. geminata* AFDM was significantly different between riffle and run mesohabitats at sites two (p<0.001) and three (p=0.016) but not at site one (p=0.169). *Didymosphenia geminata* dry mass was also significantly different between run and riffle mesohabitats at sites two (p<0.001) and three (p=0.006) but not at site one (p=0.214) (Figure 2).

Habitat and D. geminata abundance

There was little seasonal variation in *D. geminata* AFDM at site one (Figure 3), but at sites two and three, *D. geminata* AFDM was significantly lower in the summer than in the winter and spring. Of the 10 environmental variables hypothesized to influence variation in *D. geminata* AFDM, 6 were significant predictors (or variables of importance) in the PLS model (Table 4). Surface flow (VIP=1.28), benthic flow (VIP=1.17), DO (VIP=1.30), conductivity (VIP=1.13), and turbidity (VIP=1.09 were all significant predictors of the observed variation in *D. geminata* AFDM. All 9 measured environmental variables explained 45.2% of the total variation in *D. geminata* AFDM (Figure 4).

***D. geminata* and macroinvertebrates**

Environmental predictor variables and *D. geminata* AFDM accounted for 51.4% of the total variation in Shannon *H'*. Six variables (Surface flow [VIP=1.52], benthic flow [VIP=1.42], temperature [VIP=1.11], conductivity [VIP=1.26)], turbidity [VIP=0.86], and *D. geminata* AFDM [VIP=0.91]) were significant predictors of overall variation in Shannon *H'* values (Figure 4, Table 4). NMDS ordination plots revealed three distinct communities present within the study area (Figure 5). The control site and site one had distinct communities whereas sites two and three exhibited a substantial degree of overlap within the NMDS plot. NMDS axes 1 and 2 explained 54% and 23% respectively of the overall variation in macroinvertebrate community structure. MRPP analysis found significant differences for each pairwise comparison but did not find differences at sites two and three (Table 5). Surface flow (r^2=0.242, tau=0.366), benthic flow (r^2=0.162, tau=0.309), and turbidity (r^2=0.187, tau=0.281) all positively influenced the placement of the control site and sites two and three along NMDS axis 1 (Figure 5). In addition, conductivity (r^2=0.164, tau=-0.076; r^2=0.335, tau=0.422) had a positive effect on the placement of sites two and three along the 1st and 2nd NMDS axes. Finally, *D. geminata* AFDM (r^2=0.143, tau=-0.355) negatively influenced the placement of site one along NMDS axis 1 (Figure 5). Pearson and Kendall tau correlation coefficients nearly validated the PLS regression and found that temperature was the only environmental predictor considered significant in the PLS model, but not as a correlation coefficient.

The relative abundance of several macroinvertebrate groups changed between mesohabitats across all tailwater sites (Table 6). For all seasonal collections, riffle habitats had increased abundance of EPT taxa, Ephemeroptera in general, and *Baetis* spp., in particular, at all sites. Also for all seasonal collections, riffle habitats had increased abundance of Amphipoda at sites two and three. Run mesohabitats had higher densities of Oligochaeta, Turbellaria (flat worms), and Isopoda at all sites, whereas Diptera in general and Chironomidae in particular were more abundant in run mesohabitats at sites two and three for all collections. At site one, *D. geminata* AFDM and dry mass were not statistically different between mesohabitats (Figure 2), and this may explain the similar densities of Diptera and Chironomidae observed between riffles and run mesohabitats at this site. Surprisingly, however, the abundance of *Ephemerella* spp. did not differ significantly between mesohabitats at site two but did at sites one and three.

Discussion

The nuisance diatom *D. geminata* has radically transformed lotic ecosystems across North America, but because it is uncommon in southeastern USA tailwaters, populations have not been well studied in this region until now. Our study identified several environmental parameters that appear to explain variation in the abundance of *D. geminata* among sites and mesohabitats in a large southeastern tailwater stream, the South Holston River. Additionally, we were able to establish a relationship between *D. geminata* biomass and sensitive benthic macroinvertebrate groups.

Didymosphenia geminata may prefer tailwaters because these habitats have stable, homogeneous flows, cold-water temperatures, low levels of orthophosphate and low turbidity (Taylor and Bothwell 2014). Although *D. geminata* normally occurs in oligotrophic rivers were P is limited, Bothwell and Kilroy (2011) discovered via P addition experiments that PO_4^{3-} does not limit cell division rates. Rather, phosphate enrichment triggers excessive cell division in combination with high concentrations of NO_3^-. Our study reached similar conclusions regarding N and P, as concentrations in the South Holston River were much higher than those reported by others (e.g. <10 μg/L) and are more in line with those reported by Kawecka and Sanecki (2003) from southern Poland. These differences can be explained through variation in land use and geology. The Upper Tennessee River Basin is composed of approximately 64% forestland, 27% agricultural land (mainly pastureland), 6% urban, 2% open water, and 1% barren land and mines (Hampson et al. 2000). Although we were unable to find land use statistics for all *D. geminata* study areas, we did find that at least some study sites were impacted by semi-agricultural land use (e.g., Kilroy et al. 2009). Other studies for which we could find land use statistics described study areas in well-forested, mountainous regions (Spaulding and Elwell 2007, Larson 2007, Richardson et al. 2014), with some of these freshwater systems being fed by glaciers and alpine snowmelt (Spaulding and Elwell 2007, Kirkwood et al. 2007, Gillis and Chalifour 2010). Our study sites are in well forested, agricultural farmland with significant non-point source runoff of nutrients. In addition, the surrounding karst topography of our study area (Oder 1934; Davis 1990) creates nutrient sinks for agricultural runoff to infiltrate groundwater leading to slow dispersion of nutrients to surface water. While, future experiments with increased temporal sampling frequency of P and N may better elucidate the effects of nutrient dispersion on *D. geminata* growth, bloom formation, and persistence over time, our limited nutrient data suggest that P and N are relatively unimportant drivers of bloom coverage and presence as indicated by PLS regression models and NMDS joint plots.

As noted above, flow rate has a significant effect on *D. geminata* AFDM, with increasing and decreasing flow rates being proportional to significant increases and decreases in algal biomass. While it has been shown that increased discharge, flow rate and shear stress may significantly decrease *D. geminata* benthic cover (Kirkwood et al. 2007, Miller et al. 2009, Segura et al. 2010, Cullis et al. 2013, Lessard et al. 2013, Richardson et al. 2014), the influence of flow rate on *D. geminata* biomass and its subsequent effects on macroinvertebrate communities in different mesohabitats has not been explored unti now.

After running the PLS model, we discovered that the observed or actual variation in *D. geminata* AFDM between sites was best predicted by a group of variables that included surface flow and benthic flow, conductivity, DO, and turbidity. When we used Shannon *H’* as a response variable in PLS models, we found that surface and benthic flow, temperature, conductivity, turbidity, and *D. geminata* all contributed significantly to the observed variation in Shannon *H’* between sites, with *D. geminata* AFDM having the lowest significant VIP other than turbidity. Variables important to projection in PLS are equivalent to calculating the coefficient of partial determination (partial r^2) for predictor (x) variables in a multiple linear regression and explain the contribution of each predictor to the observed variation in the response. Therefore, the higher the VIP value, the greater the predictor's importance to the observed variation in the response variable. Surface flow, benthic flow, conductivity, DO, and temperature all had greater VIPs than *D. geminata* AFDM. Although *D. geminata* AFDM was significant, its contribution to the PLS model to predict Shannon diversity between sites is relatively minor compared to abiotic predictors such as surface flow, benthic flow, conductivity, and temperature. These results are not surprising given the nature of the system—one that is dominated by hypolimnetic water releases from the bottom of a reservoir. However, PLS and NMDS analyses could not account for differences in *D. geminata* AFDM between mesohabitats for all sites. If the latter could be incorporated into PLS and NMDS analyses given the significant difference in *D. geminata* AFDM between riffle and run mesohabitats, *D. geminata*'s VIP value and Pearson and Kendall correlation coefficients might increase, or at least become equal to other environmental parameter VIPs and coefficients in each analysis.

The results of our NMDS ordination and corresponding Pearson and Kendall correlation coefficients confirmed PLS model runs and further strengthened our understanding of the important role that surface flow, benthic flow, conductivity, and *D. geminata* AFDM play in structuring macroinvertebrate communities in the South Fork Holston River Tailwater.

Remarkably, *D. geminata* appears to influence the site one community more than it influences the communities of sites two and three (Figure 5). Site one has significantly more taxa known to be *D. geminata* tolerant (e.g., oligochaetes, flat worms, chironomids, and isopods) (Larson 2007, Kilroy et al. 2009, Gillis and Chalifour 2010, James et al. 2010, Richardson et al. 2014) relative to sites two and three, and it did not have significantly different *D. geminata* AFDM between mesohabitats.

The increased abundance of *Baetis* spp., other Ephemeroptera, all EPT taxa, and amphipods in riffle relative to run habitats may be due to the fact that riffles generally had lower *D. geminata* biomass. *Didymosphenia geminata* mats consist of interlinking stalks with distal cells forming a complex web of detritus, trapped inorganic sediment, and other diatom species. The composition of these mats restrict which benthic macroinvertebrate taxa may exist in them, based on the biological attributes of those taxa. For example, the mats may impair the navigation and dispersal of certain benthic macroinvertebrates (Spaulding et al. 2007), perhaps decreasing their occurrence at such sites. At our study sites, we note that runs had significantly higher *D. geminata* dry mass compared to riffles. The composition of inorganic mat constituents can vary, but generally consists of inorganic frustule mass, sediment, and non-algal plant biomass (McLaughlin 2009). *Didymosphenia geminata* dry mass in run habitats was a mean of 73.6% inorganic material compared to 55% in riffles. Again considering the biological attributes of macroinvertebrate denizens of the benthos, especially mayflies, it is known that inorganic sediments entrained in algal mats may negatively affect respiration (Larson 2007) and thus exclude some individuals or some taxa altogether.

We observed in run mesohabitats that *D. geminata* appears to exclude the usual native algae, bryophytes, and submergent macrophytes found in riffle mesohabitats. In riffles, these native periphyton and bryophytes may provide a physical refuge from hydro-peaking flows and offer benthic macroinvertebrates a stable food resource. We generally collected more *Ephemerella* spp., *Baetis* spp. and to a certain extent, EPT taxa in general, in bryophytes, particularly *Fontinalis antipyretica* and mosses of the family Bryaceae, and near *Rhizoclonium* sp. (a native green alga), which were all dominant in riffles that lacked significant *D. geminata* inundation.. Limited gut dissection of *Ephemerella* spp. and *Baetis* spp. from study sites did not reveal any *D. geminata* frustules, cell fragments, or stalk biomass. However, gut dissection of Chironomidae did reveal significant consumption of *D. geminata*, which provides a possible reason for the increase in chironomids in run habitats that are dominated by *D. geminata* (Green and Blum, unpublished data).

Management Recommendations

Our research on *D. geminata* in the South Holston tailwaters is the first of its kind in the southeastern USA and builds upon previous research, while also adding new information that can be applied to management. Above, we provided statistical evidence that *D. geminata* AFDM increases with decreasing flow rate and that high water temperatures are associated with reduced *D. geminata* AFDM. Specifically, we attributed decreases in *D. geminata* biomass to a cumulative increase in water temperature at sites two and three during the spring and summer (Figure 3). This is consistent with what is known of *D. geminata*'s biology and ecology, as reviewed above. Thus, river operation managers may face an interesting paradox: increased power generation frequency and discharge may mitigate *D. geminata* blooms through increased shear stress, but these increase could also create more favorable thermal conditions for continued bloom survivorship by lowering tailwater water temperatures.

Here exists a no-win situation if management plans to mitigate blooms are not implemented properly. Given that *D. geminata* AFDM is lowest during the summer, a series of “flood pulse discharges” during the spring and summer months may scour algal mats while warm temperatures help to mitigate bloom formation. If initiated in proper time intervals (e.g. early morning and late evening), these flood pulses should flush algal mats downstream in between periods of short growth (during hydropeaking) and widespread die off (during the day) due to natural warming. Consideration in the timing of water releases should reflect the need for mat removal in specific river reaches. For example, water released from the dam takes an average of 4–5 hours to reach lower river reaches. If morning hours are target periods for flushing to allow adequate die off during the day, then water must be released from the dam during the night to insure an early morning water arrival time and fulfillment of desired management objectives. Significant deviations from the latter may result in undesired effects or wasted management effort to mitigate bloom formation. Lastly, modification to existing dam infrastructure to allow surface releases from the epilimnion may prove to be the ultimate mitigation solution for *D. geminata* blooms, but it may not be considered feasible by federal and state agencies due to high cost and potential negative effects to the tailwater’s recreational trout fishery, which has had an historical annual economic value of ~1 million dollars (Williams and Bettoli 2003).

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Table 1. Study site GPS coordinates with elevation (m), river mile marker, and distance between sites (km) for all mesohabitats.

Table 1.
Study Sites

Site	Coordinates	Elevation	River Mile Marker	Distance Between Sites
Control	36°39'10.80", -081°53'13.20"	534.62 m	73.7	0.0 km
Site 1				
Run	36°31’33.60”, -082°06’43.20”	450.50 m	48.2	41.2 km
Riffle	36°31’33.60”, -082°06’50.40”	450.50 m	48.0	
Site 2				
Run	36°29'20.40", -082°11'42.00”	436.78 m	39.6	13.8 km
Riffle	36°29'16.80", -082°11'42.00”	436.17 m	39.5	
Site 3				
Run	36°28'01.20", -082°14'09.60"	426.11 m	36.35	5.3 km
Riffle	36°28'04.00", -082°14'13.20"	427.94 m	36.2	

Table 2. Mean (and standard errors) for a range of physiochemical, water quality, and habitat data for each sampling site.

Table 2.
Physiochemical Data

Environmental Variable	Control	Site 1 Flat	Site 1 Riffle	Site 2 Flat	Site 2 Riffle	Site 3 Flat	Site 3 Riffle
Surface Flow Rate (m s^{-1})	0.91	0.15	0.34	0.28	0.52	0.31	0.59
	(0.07)	(0.02)	(0.04)	(0.02)	(0.06)	(0.02)	(0.04)
Benthic Flow Rate (m s^{-1})	0.56	0.07	0.20	0.13	0.36	0.17	0.37
	(0.06)	(0.01)	(0.02)	(0.02)	(0.04)	(0.02)	(0.04)
Dissolved Oxygen (mg L^{-1})	11.20	11.43	11.83	12.50	12.45	12.66	12.45
	(0.32)	(0.18)	(0.17)	(0.28)	(0.31)	(0.32)	(0.23)
Temperature (°C)	12.93	7.73	7.96	11.09	10.58	10.26	9.57
	(1.47)	(0.24)	(0.26)	(0.67)	(0.70)	(0.72)	(0.73)
Conductivity (µs cm^{-1})	174.3	237.1	239.1	260.5	247.5	264.0	263.2
	(2.84)	(0.97)	(1.09)	(3.51)	(3.08)	(3.00)	(1.97)
pH	8.11	8.09	7.82	8.28	8.27	8.21	8.26
	(0.05)	(0.07)	(0.06)	(0.03)	(0.05)	(0.07)	(0.08)
Turbidity (NTU)	2.22	1.19	1.25	1.59	1.40	1.64	2.20
	(0.12)	(0.08)	(0.11)	(0.17)	(0.13)	(0.19)	(0.20)
NO_3^- (mg L^{-1})	10.63	16.73	13.01	14.71	10.59	18.64	7.95
	(1.55)	(3.19)	(1.34)	(2.71)	(2.11)	(4.43)	(0.50)
PO_4^{3-} (µg L^{-1})	71.42	54.78	70.77	85.66	80.74	91.84	81.82
	(8.31)	(7.07)	(10.51)	(4.70)	(6.32)	(9.20)	(6.44)

Note. All data are annual means with standard errors in parenthesis. NTU, nephelometric turbidity unit; NO_3^-, nitrate; PO_4^{3-}, orthophosphate

Table 3. Two-way ANOVAs with *D. geminata* AFDM and Shannon Index (*H'*) as dependent variables and site and mesohabitat as fixed factors with site X mesohabitat as an interaction effect.

Table 3.
Two-way ANOVA Summary

Dependent Variable	Source	df	*F*-value	*P* value
D. geminata AFDM	Site	3	2.004	0.116
	Mesohabitat	1	6.384	0.012*
	Site x Mesohabitat	3	2.961	0.034*
	Error	161		
	Fisher's LSD Post Hoc Tests	*P* value		
	0 vs. 1	<0.001*		
	0 vs. 2	<0.001*		
	0 vs. 3	<0.001*		
	1 vs. 2	0.762		
	1 vs. 3	0.147		
	2 vs. 3	0.249		
Shannon Index (*H'*)	Site	3	81.511	<0.001*
	Mesohabitat	1	3.185	0.076
	Site x Mesohabitat	2	1.050	0.352
	Error	155		
	Fisher's LSD Post Hoc Tests	*P* value		
	0 vs. 1	<0.001*		
	0 vs. 2	<0.001*		
	0 vs. 3	<0.001*		
	1 vs. 2	<0.001*		
	1 vs. 3	<0.001*		
	2 vs. 3	0.016*		

Note. –Zero is equivalent to the control site. All variables transformed using the following equation: LOG(Data+2). LSD, least significant difference; AFDM, ash-free dry mass

*p<0.05

Table 4. Partial least squares (PLS) regression with *D. geminata* AFDM and Shannon Index (*H'*) as a dependent variable.

Table 4.
Partial Least Squares Regression Summary

Dependent Variable	Independent Variable	VIP	VIF	Number of VIP>0.8	Method	Number of Factors	Percent Variation Explained For Cumulative X	Percent Variation Explained For Cumulative Y
D. geminata AFDM	Surface Flow	1.28*	5.43	5	NIPALS	5	85.81	45.19
	Benthic Flow	1.17*	5.01					
	Temperature	0.78	1.69					
	DO	1.30*	2.31					
	Conductivity	1.13*	2.05					
	pH	0.68	1.74					
	Turbidity	1.09*	1.91					
	Nitrate	0.38	2.07					
	Orthophosphate	0.75	1.74					
Shannon Index (*H'*)	Surface Flow	1.52*	5.42	6	NIPALS	3	58.52	51.40
	Benthic Flow	1.42*	5.03					
	Temperature	1.11*	1.60					
	DO	0.53	2.64					
	Conductivity	1.26*	1.81					
	pH	0.46	1.69					
	Turbidity	0.86*	2.12					
	Nitrate	0.40	2.07					
	Orthophosphate	0.79	1.59					
	D. geminata AFDM	0.91*	1.85					

Note. –VIFs derived from an initial least squares multiple linear regression. All variables transformed using the following equation: LOG(Data+2). VIP, variable important to projection; VIF, variance inflation factor; NIPALS, non-linear iterative partial least squares algorithm; AFDM, ash-free dry mass

*VIP>0.8

Table 5. Multi-response permutation procedure by site for macroinvertebrate abundance and diversity using Bray-Curtis distance measures. Pearson’s r and Kendall tau Correlations determining significant correlations and fit between environmental variables and each NMDS (nonmetric multidimensional scaling) axis via joint plots are also shown.

Table 5.
Multi-response Permutation Procedure (MRPP)
Pearson’s r and Kendall tau Correlation Summary

Multi-response Permutation Procedure (MRPP)			Pearson and Kendall Correlations With Ordination Axes						
Comparison	A-value	P-value	Environmental Variable	Axis 1			Axis 2		
				r	r^2	Tau	r	r^2	tau
All Sites	0.151	$<0.001^{+}$	*D. geminata* AFDM	-0.378	0.143**	$-0.355^{\bullet}$	-0.088	0.008	-0.012
0 vs. 1	0.186	$<0.001^{+}$	Surface Flow	0.492	0.242*	$0.366^{\bullet}$	-0.014	0.000	0.082
0 vs. 2	0.136	$<0.001^{+}$	Benthic Flow	0.403	0.162**	$0.309^{\bullet}$	0.014	0.000	0.045
0 vs. 3	0.126	$<0.001^{+}$	Temperature	0.144	0.021	0.093	-0.005	0.000	-0.022
1 vs. 2	0.121	$<0.001^{+}$	DO	0.066	0.004	0.072	0.309	0.096	$0.221^{\bullet}$
1 vs. 3	0.102	$<0.001^{+}$	Conductivity	-0.405	0.164**	-0.076	0.579	0.335*	$0.422^{\bullet}$
2 vs. 3	0.004	0.147	pH	-0.145	0.021	-0.155	0.209	0.043	0.199
			Turbidity	0.433	0.187**	$0.281^{\bullet}$	0.168	0.028	0.020
			Nitrate	0.248	0.062	0.161	0.048	0.002	-0.016
			Orthophosphate	0.074	0.006	-0.058	0.132	0.017	0.028

Note. –Zero is equivalent to the control site. All variables transformed using the following equation: LOG(Data+2). AFDM, ash-free dry mass; r^2, Pearson’s r; tau, Kendall rank correlation coefficient

$^{+}p<0.05$

$*r^2>0.200$

$**r^2>0.100$

$^{\bullet}tau>0.2$

Table 6. Average annual macroinvertebrate abundance, *D. geminata* AFDM, *D. geminata* Dry Mass, Shannon Diversity Index (*H'*), and Richness (*S*) for all sites.

Table 6.
Average Annual Macroinvertebrate Abundance, *D. geminata* AFDM, *D. geminata* Dry Mass, Shannon, and Richness

Site	*Ephemerella* spp.	*Baetis* spp.	EPT Taxa	Diptera	Chironomidae	Annelida	Isopoda	Amphipoda	Elmidae	Mollusca	*D. geminata* AFDM ($g\ m^{-2}$)	*D. geminata* Dry Mass ($g\ m^{-2}$)	*H'*	*S*
Control	227	42	876	53	304	46	0	0	46	7	Not Present	Not Present	2.19	20
Site 1 Run	44	12	69	44	1839	4648	376	59	0	1	0.363	1.257	1.00	7
Site 1 Riffle	278	92	376	54	2260	2890	54	14	2	10	0.261	0.807	1.08	7
Site 2 Run	258	261	554	14	778	706	14	367	22	21	0.498	3.508	1.42	9
Site 2 Riffle	254	718	1044	34	391	382	2	979	30	7	0.098	0.494	1.45	9
Site 3 Run	98	143	300	11	914	720	0	301	48	17	0.325	4.379	1.48	8
Site 3 Riffle	296	811	1318	41	699	471	1	1795	206	102	0.115	0.500	1.66	11

Note. –Annelida includes Oligochaeta and Turbellaria; AFDM, ash-free dry mass; *H'*, Shannon Diversity Index; *S*, richness

Figure 1. Topographic map showing approximate field sampling locations along the South Fork Holston River with control (non-tailwater) site (red) and tailwater sites (black). Two different mesohabitats, riffles and flats, were sampled at each tailwater site. Note the location of sampling site to the city of Bristol, TN.

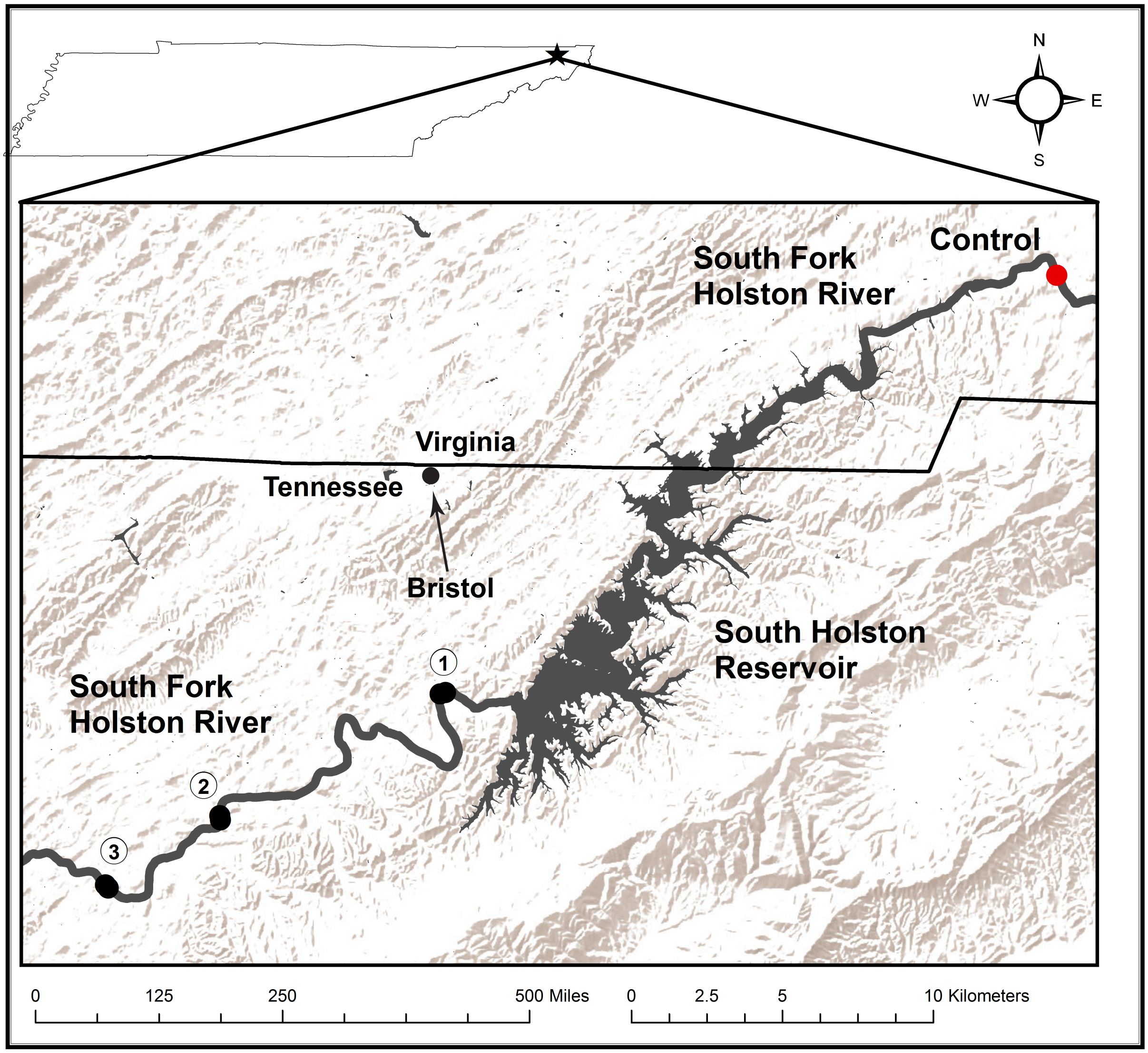


Figure 2. Boxplots of *D. geminata* AFDM (A), *D. geminata* dry mass (B), surface flow rate (C), and benthic flow rate (D) between flats (solid white) and riffle (solid black) mesohabitats for all sites. Note that *D. geminata* was not present at the control site.

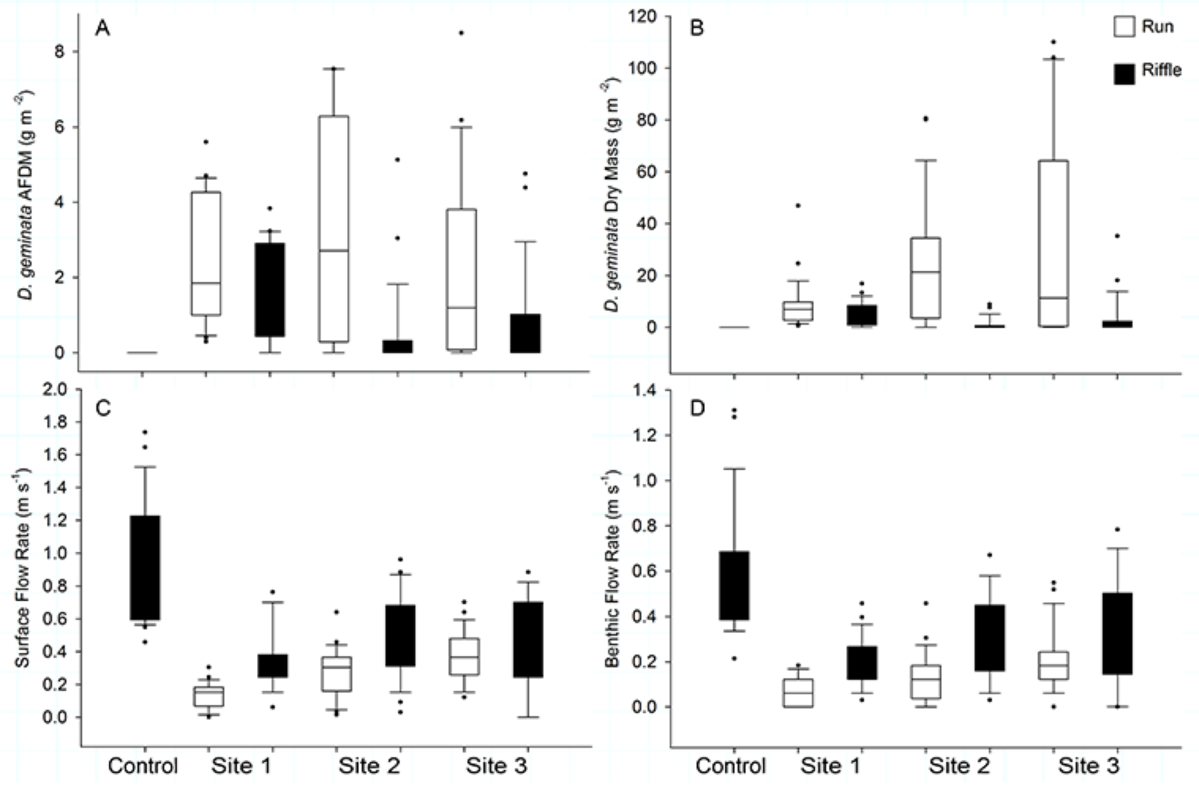


Figure 3. Boxplots of *D. geminata* AFDM (A) and temperature (B) by site and grouped by season. Boxplots in order: winter (solid white), spring (dark striped), and summer (white with dotted lines).

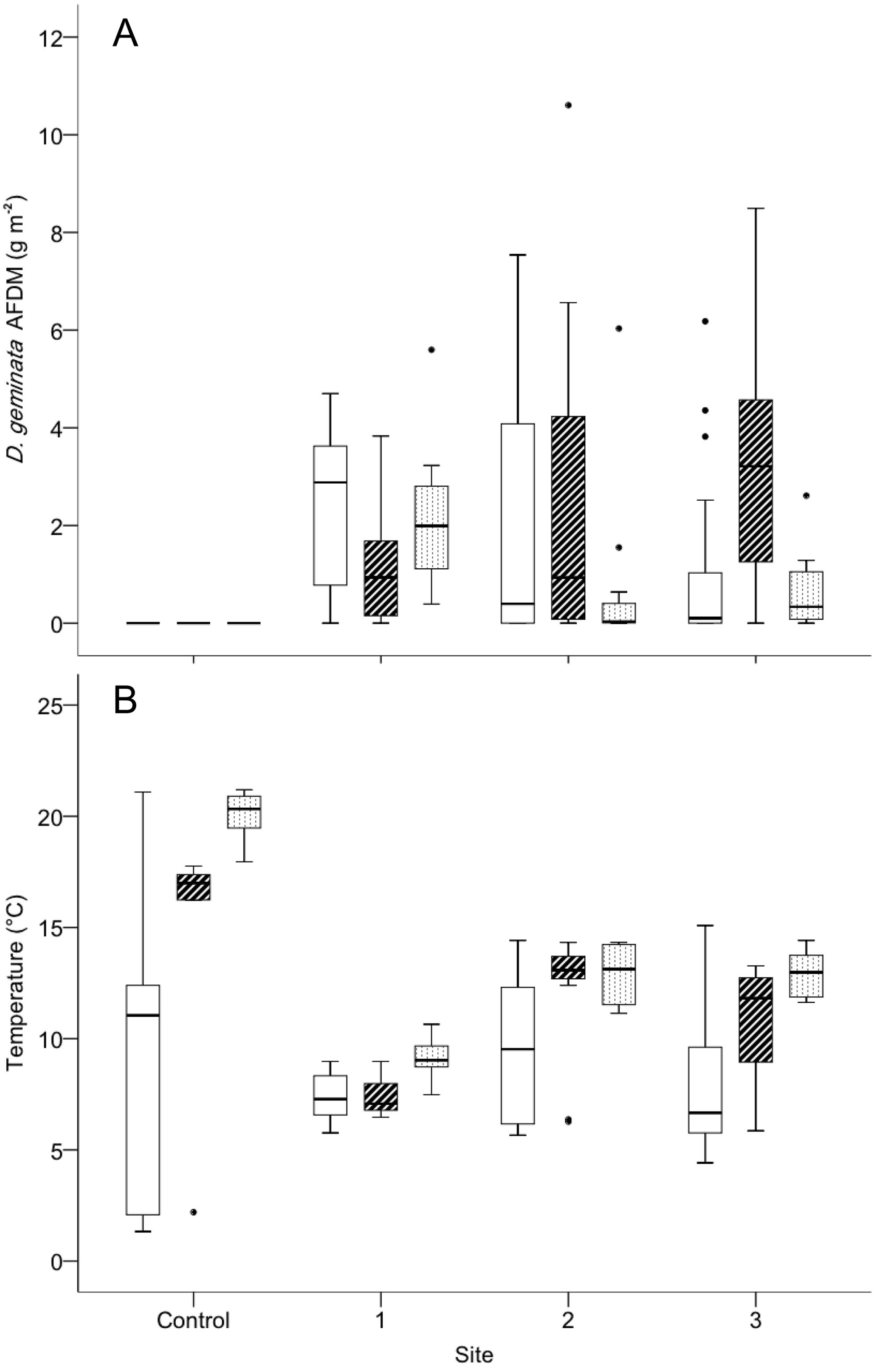


Figure 4. Simple linear regression of predicted LOG transformed *D. geminata* AFDM by actual LOG transformed *D. geminata* AFDM (A) and of predicted LOG transformed Shannon Index (*H'*) by actual LOG transformed Shannon Index (*H'*) (B) following a PLS regression analysis.

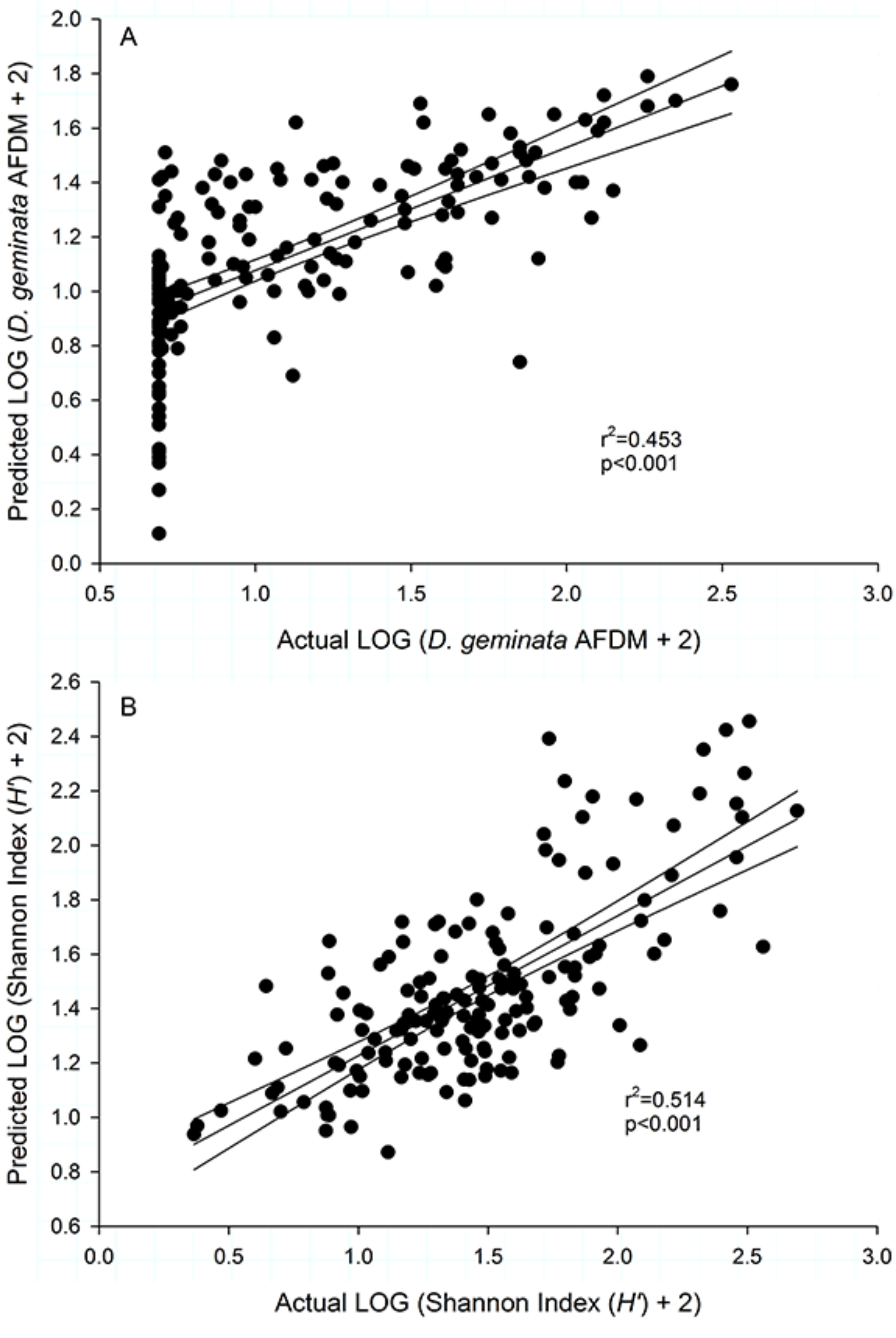
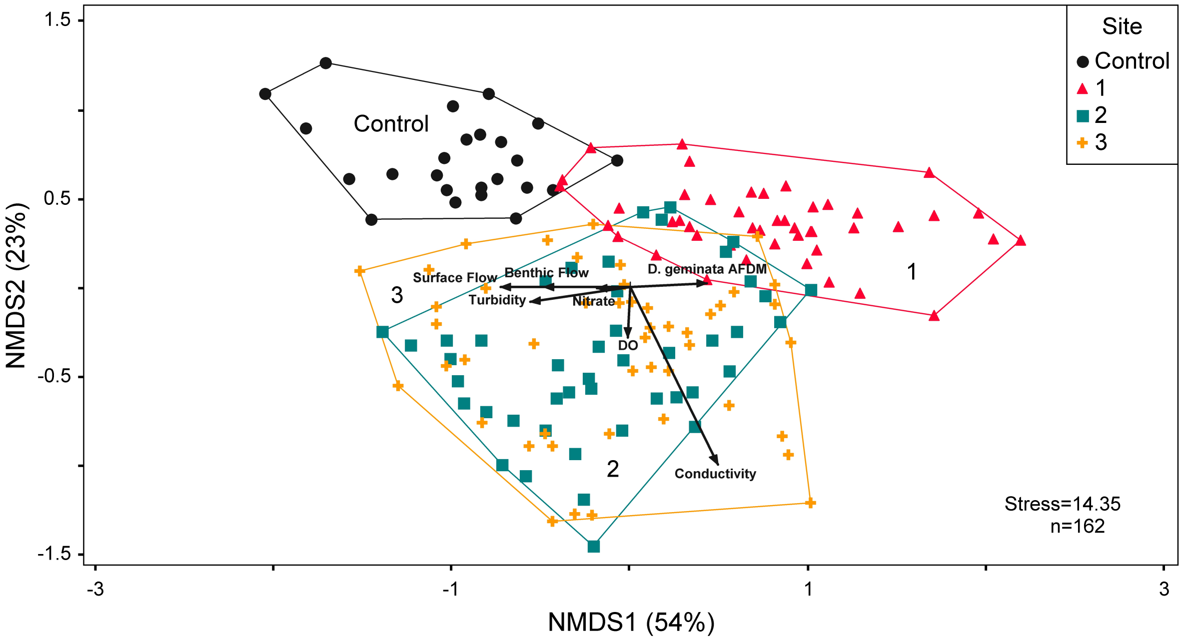


Figure 5. NMDS ordination joint plots showing the influence of environmental variables on the placement of macroinvertebrate communities within ordination space.



Vita

Matthew William Green was born in Clemmons, NC, to Chuck and Claudia Green. He graduated from West Forsyth High School in Clemmons, North Carolina in May 2007 and enrolled in North Carolina State University, Raleigh, North Carolina to study Anthropology the following Fall. In December 2011, he was awarded a Bachelor of Arts degree in General Anthropology. After graduating, he began work as a laboratory assistant in the NCSU Department of Entomology and then accepted a research assistantship in aquatic entomology in pursuit of a Master of Science degree. In October 2013, he left North Carolina State University and transferred to Appalachian State University in Boone, North Carolina to complete the remainder of his prior research and begin new projects starting January 2014. The M.S. from Appalachian State University was awarded in August 2016.

In this free-time, Mr. Green is an ardent fly fisherman who contributes popular science articles to the fly fishing literature on imitating aquatic insects and baitfish to catch fish, especially trout. He resides in the Cumberland Valley of south-central Pennsylvania near his favorite limestone fishing streams.