ASSESSING LONG-TERM ECOLOGICAL CHANGE IN THE OGEECHEE RIVER USING AQUATIC INVERTEBRATE COMMUNITIES

by

KELLY MACKENZIE MURRAY-STOKER

(Under the Direction of Darold Batzer and Joseph McHugh)

ABSTRACT

Anthropogenic impacts are a threat to rivers worldwide, and aquatic insects are important to assessing environmental change. In the 1980s, researchers preformed collections of macroinvertebrates on submerged woody debris in the Ogeechee River (Georgia, USA) for two years. We collected invertebrates from 2015 to 2017 with the same methodology to compare communities. We documented greater taxonomic richness and change in community structure compared to the 1980s. We also documented a decline in total insect biomass; however, overall abundance was similar, indicating a decline in size of individuals. Specifically, larger consumer taxa mostly decreased, while smaller consumer taxa and most predator taxa increased in number. Additionally, functional trait richness of the community increased over three decades, but functional dispersion has not changed. Focusing on caddisflies, we examined ecological basis of change and larval morphology of an understudied genus. These results provide a unique perspective on long-term environmental changes in river systems.

INDEX WORDS: Aquatic ecology, Aquatic insects, Ogeechee River, Community ecology, Biomass, Discharge, Trichoptera, Functional traits, Structural equation modeling

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION

"The river just isn't the same as it used to be." This was a statement often heard by staff of Ogeechee Riverkeeper over the years from citizens concerned about the state of the Ogeechee River. In May of 2011, a major chemical spill, exacerbated by high temperatures and low flows, wreaked havoc on the Ogeechee ecosystem. The associated fish kill was the largest in the state of Georgia's history, and the Ogeechee River's reputation as a near-pristine system was marred. Though these events alone support the idea that the river no longer exists in a reference condition, the question of whether the river is "the same as it used to be" is testable with data.

In the 1980s, the aquatic invertebrate communities of the relatively undisturbed Ogeechee River were categorized and quantified through extensive sampling by Arthur Benke, Bruce Wallace, Keith Parsons, and other researchers. Studies were performed on drifting insects, benthic organisms, growth rates, tributary habitats, and invertebrates living on submerged woody debris. Ultimately, evaluating the snag habitat provided the most information about the river's aquatic invertebrates, and a two year study was carried out consisting of monthly sampling.

Over thirty years later, we replicated the study design of Benke and others to compare invertebrate communities of the early 1980s with those of the mid-2010s. Here we present the results of analyzing the differences between the two time periods in terms of biomass, abundance, taxonomic structure, and functional trait diversity, in an effort to answer the question of whether both acute environmental damage and long term changes in climate and human impact have led to a community of aquatic invertebrates that isn't the same as it once was.

CHAPTER 2

LONG-TERM COMPARISONS OF RIVERINE INVERTEBRATE COMMUNITIES REVEALS BIOMASS DECLINE

Introduction

Invertebrate abundance and diversity has been declining on a global scale (Dirzo et al. 2014). A recent, high-profile study from protected natural areas in Germany documented a more than 75% decline in flying insect biomass over several decades, though the reasons for the decline remain ambiguous (Hallmann et al. 2017). The relationship of organism metabolism to temperature (Gillooly et al. 2001) suggests that a warming environment would select for declines in size, with some taxa already showing these effects in response to modern anthropogenic climate change (Sheridan & Bickford 2011, Baudron et al. 2014). However, recent experiments testing the effects of warming on invertebrate biomass and production in freshwater environments found that responses were taxon-dependent and results did not match predictions of overall decreases due to higher temperatures (Jonsson et al. 2015, Nelson et al. 2017). Potential effects of warming also need to be assessed in the context of other environmental stressors, which may act antagonistically or synergistically with warming effects (Jackson et al. 2016). Long-term comparisons of freshwater invertebrates, crucial organisms in both aquatic and terrestrial food webs (Baxter et al. 2005), can promote understanding of present and future conditions of freshwater habitats.

In the early 1980s, researchers conducted a multi-year study on the Ogeechee River, including two years of monthly sampling of aquatic invertebrates inhabiting submerged woody

debris and attached organic detritus (termed "snags") to determine the annual production of the most common groups (Benke and Wallace 2015). This dataset represents a rare quantitative characterization of invertebrate community from a river in reference condition in North America. The Ogeechee River runs through the Coastal Plain physiographic region of the Southeastern United States and was considered until recently to be a relatively "pristine" system (Benke & Wallace 2015); it provides habitat for rare and endangered fish species such as Robust Redhorse, *Moxostoma robustum*, (Slaughter 2013) and Shortnose Sturgeon, *Acipenser brevirostrum* (Farrae et al. 2014). Benke and Wallace (2015) concluded that aquatic invertebrate secondary production (or, the annual turnover in biomass) was higher than most other rivers of similar size.

Here, we present results from replicating the 1980s study more than 30 years later, from 2015-2017. We compared abundance, biomass, and community structure of aquatic invertebrates of the Ogeechee River, and we found significant changes in biomass across the 35-year time period. We also present evidence that these biomass declines are potentially linked to patterns of river discharge and carbon delivery.

Biomass decline driven by consumers

We documented a significant decline in invertebrate biomass ($p = 0.0223$) between the 1981-1983 time period (hereafter: "1980s") and the 2015-2017 time period (hereafter: "2010s"), with the monthly biomass of the 2010s, on average, only 62% of the 1980s (Fig 2.1). There was no significant difference by season. By contrast, there was no difference in the abundance of individual invertebrates between time periods, though there was a significant difference in abundance among seasons ($p = 0.0396$). Through both sampling periods, autumn contained the highest abundance of invertebrates. Because biomass estimates incorporate measures of both abundance and individual size, a difference between responses of abundance and biomass

indicates changes in size. We therefore conclude the biomass decline is not a result of fewer invertebrates living in the Ogeechee River, but of a decline in average size.

We separated the invertebrate community into two broad feeding groups: consumers (including both herbivores and detritivores) and predators. In the 1980s, consumers comprised 85% of the total snag invertebrate biomass (Fig 2.2). Biomass and abundance changes in this group accentuated results from analyzing total community: there was a large decrease in consumer biomass between time periods ($p = 0.0372$), but not by season. Consumer abundance was significantly different by season ($p = 0.0057$), but not by time period. Consumer biomass in the 2010s sampling period was on average only 47% of monthly values in the 1980s.

Predators exhibited a dissimilar response in comparison to the previous analyses of overall communities or consumers. There was a significant increase in predator abundance from the 1980s to the 2010s ($p = 0.0239$), but no significant differences in biomass by time period or season. Predator abundance increased by 98% from the 1980s to the 2010s. Again, the difference between biomass and abundance suggests an impact of invertebrate size, which in this case was due to an increase in the proportion of smaller-bodied individuals.

The similarity between results of the full community and solely the consumers indicates that the declines in consumer taxa are driving the overall patterns. The increase in predator abundance did not have an impact on total numbers due to their relatively low numbers; the average of monthly consumer abundance across both time periods (94,768 individuals per square meter) was an order of magnitude higher than predator abundance (2,804 individuals per square meter).

Shifts in community structure

Communities of invertebrates were significantly different from each other between the 1980s and the 2010s periods ($p<0.00001$), and among seasons of the year ($p<0.00001$; Fig 2.3). Seasonal differences were expected, as analyses of the 1980s data alone showed consistent patterns in peaks of particular dominant taxa (Benke & Wallace 2015). There was no interaction between season and period, suggesting that phenological cycles for common taxa were similar across time periods. An indicator taxa analysis showed two taxa characteristic of the 1980s period that substantially declined in the 2010s: *Pteronarycs* (Giant Salmonfly; Plecoptera: Pteronarcyidae) and Tipulidae (crane fly; Diptera), two of the largest invertebrate consumers in the river (Table 2.S1). In the 2010s, 20 taxa had notably increased in relative community abundance compared to the 1980s, including small-bodied consumers such as *Microcylloepus* (riffle beetle; Coleoptera: Elmidae) and *Baetsica* (Armored Mayfly; Ephemeroptera: Baetiscidae), and assorted predators such as *Argia* and *Enallagma* (damselflies; Odonata: Coenagrionidae) and *Boyeria* (dragonfly; Odonata: Aeshnidae). These results provide evidence that at least part of the change in biomass is due to increased presence of different taxa, rather than smaller individuals of the same taxa.

In the 1980s, the top three taxa in terms of overall biomass contribution were caddisflies (Trichoptera): *Hydropsyche* (Hydropsychidae), *Chimarra* (Philopotamidae), and *Cheumatopsyche* (Hydropsychidae), comprising 60.8% of the total measured biomass. In the 2010s, these groups were still some of the largest in terms of invertebrate biomass in snag habitats, yet all three genera exhibited declines relative to the 1980s and together made up only 38.9% of biomass. The mean monthly biomass of *Hydropsyche* in the 2010s period was 33.2% of that in 1980s. *Chimarra* experienced the least dramatic decline of the three, with the 2010s biomass being 58.7% of the 1980s mean monthly biomass, but *Cheumatopsyche* biomass in the

2010s was reduced to 15.6% of that in the 1980s, and were surpassed in percent contribution to total community biomass by two predators, *Corydalus* (Neuroptera: Corydalidae) and *Neurocordulia* (Odonata: Corduliidae), and midges (Diptera: Chironomidae).

Annual patterns in invertebrate biomass and river discharge

Gut content analyses conducted during the 1980s study determined that the most productive groups relied primarily upon amorphous detritus (Wallace et al. 1987, Benke & Wallace 2015); Hydropsychid caddisflies, for example, filter detritus from the water column with silken nets. Studies of the river's metabolism showed that most of the carbon originates from the adjacent floodplain (Meyer & Edwards 1990, Meyer et al. 1997). In this subtropical system, the undammed river floods its banks as a result of high precipitation and inundates the forested floodplain (Benke et al. 2000), which consists of blackwater swamps. Once the river level recedes, it carries detritus back into the main channel. In the 1980s, an ENSO event in the second sampling year (1982-1983) was associated with higher winter-spring flows, and secondary production of that year (concentrated in the summer) was 1.5x higher than the first year (1981-1982; Benke & Wallace 2015). The reliance of river metabolism on flood pulses has also been prominently documented by Junk et al. (1989) in Amazon river-floodplain systems.

Examining the relationship between river discharge and changing invertebrate biomass in the Ogeechee River, we found differences in the annual flow patterns between time periods. The highest monthly average discharge of the two sampling periods of the 1980s was in March; the highest monthly average in the 2010s was in January (Fig 2.4). The winter-spring flooding in the Ogeechee is a biologically important and historically consistent characteristic (Benke 2001). We determined that spring discharge levels were significantly lower in the 2010s ($p = 0.0291$), though earlier winter flows were not significantly different between periods. The highest average

discharge in the 2010s was about two-thirds as much as that in the 1980s. In both periods, peaks in invertebrate biomass occurred later in the year than peaks in discharge, with biomass measurements highest on average in November in both time periods, though patterns appear more variable in the 2010s (Fig 2.4).

Declining discharge and consequences for invertebrate biomass

To determine whether the lower discharge in the 2010s was the result of an ongoing trend, we analyzed continuous, long-term discharge data from the United States Geological Survey on the Ogeechee River near the sampling site (USGS 2018a). The average daily discharge across the winter-spring period was calculated for each year from 1969-70 to 2016-17. We found there was a significant trend over time of decreasing average daily discharge ($p = 0.020$, Fig 2.5). This may be a result of decreasing precipitation associated with climate change in the southeast (USGCRP 2017). The large amount of agricultural land usage in Georgia has also resulted in high amounts of surface and groundwater withdrawal, which has been increasing in the Ogeechee basin (Georgia Water Coalition 2017).

We anticipated that the levels of discharge would be associated with the delivery of carbon to the river channel, so we compared average discharge values with measurements of dissolved carbon (USGS 2018b) between the 1980s and 2010s. There was a significant, positive relationship between discharge and carbon ($p < 0.00001$), but no difference in the relationship between time periods (Fig 2.S1). More work is needed to determine whether the floodplain remains the source of the bulk of carbon, but our results provide evidence that decreased delivery of carbon due to decreases in spring flows may be the underlying reason for the observed declines in dominant consumers in our study.

Conclusions

Many studies have explored the potential impacts of climate change on freshwater invertebrate communities. Results have shown differential effects of increases in temperature based on invertebrate size (Merckx et al. 2018, Nelson et al. 2017, Jonsson et al. 2015). The results from our long-term comparisons of aquatic invertebrate communities also show a disparity in responses, but invertebrates are primarily divided along feeding group boundaries.

The most dramatic changes in biomass between sampling periods was concentrated in consumers, specifically large-bodied ones, with various smaller consumer taxa becoming more prominent in the community, while abundances of predators increased. Other work has shown ecosystem change (specifically, nutrient addition) to differentially affect aquatic invertebrate consumers and predators, but in the opposite manner: production of large-bodied consumers increased, while predators did not (Davis et al. 2010). In both cases, however, it seems the large consumer taxa "invulnerable" to predation dictate community-level responses to environmental change. Here, hypothesized changes in carbon delivery due to decreases in seasonal discharge may be responsible for a decline in the large filter-feeding caddisflies (Hydropsychidae and Philopotamidae) that dominated in the 1980s; in turn, smaller consumer taxa have become more abundant, which may be beneficial to predators.

A long-term study of aquatic macroinvertebrate communities in Europe also found that climate impacts alter the composition of feeding groups (Jourdan et al. 2018). Our results similarly suggest that examining responses of individual feeding groups and, ultimately, the energy resources of focal taxa to environmental change may provide insight to the future of river ecosystem functioning. Our study provides an example of potential impacts on size-specific

invertebrate biomass from changes in river flows, which has also been shown to affect invertebrate abundance and diversity (Kakouei et al. 2018, Ruhi et al. 2018, Holt et al. 2015).

The Ogeechee River, once a model of an undisturbed Coastal Plain blackwater system, is under threat from floodplain development and logging, human use of water, and chemical spills (a major spill in the 2011 caused the largest fish kill in the history of Georgia; Barrett & Fleming 2011). A multifaceted approach is necessary to determine what will drive invertebrate biomass in a changing climate. A decline in overall assemblage biomass is emerging as an indicator of pervasive ecosystem change (Hallmann et al. 2017, Lister & Garcia 2018). In aquatic systems, workers have relied heavily on the responses of sensitive indicator species (Bonada et al. 2006); however, in the Ogeechee River, many of the small-bodied taxa that were indicators of the 2010s would still be consider environmentally sensitive, yet they became more, not less prevalent. If biomass change is related to climate change, the largest-bodied organisms should receive special focus. The *Pteronarcys* stonefly nymphs, hydropyschid caddisfly larvae, and tipulid crane fly larvae, which contributed the most to biomass declines and community change, are proportionally larger than the rest of the aquatic invertebrate community in much the same way that the biomass of threatened elephants and baleen whales are to the biomass of co-existing vertebrates.

Supplementary Material

Methods

Study Site

The Ogeechee River is a sixth-order blackwater system in southeastern Georgia, U.S.A. It has a low gradient and is one of the largest free-flowing rivers on the east coast of North America (Benke 1990); as a result, the river is highly connected to the floodplain. The forested floodplain

is characterized by plants such as willow (*Salix* spp.) and bald cypress (*Taxodium distichum*) as well as bottomland hardwoods. The benthic substrate is primarily sand.

For both sampling periods (1980s and 2010s), the same 1 km reach of the river near Eden, GA was used (32°11′29″ N, 81°24′58″ W). Sampling was conducted monthly from December 1981 to November 1983 (comprised of 25 dates) and from July 2015 to August 2017 (24 dates). Logistical barriers precluded sampling for two (non-consecutive) months in the second time period, so the sampling period was extended until 24 sampling events were reached. In each sampling period, there was an ENSO event; the winter of 1982-83 and 2015-16.

Field Sampling & Laboratory Processing

Our sampling scheme in the 2010s was designed to follow that of the 1980s (e.g. Benke & Parsons 1990) as closely as possible; Parsons directed the sampling in the 1980s and participated in most sampling events in the 2010s, guiding that effort. Samples were collected within the main channel from canoe or boat, and followed a haphazard sampling scheme. We collected submerged woody debris (henceforth: "snags") from both banks along the entire sampling reach. In the 1980s sampling period, 20 snags per date were collected in the first year. However, thereafter, it was determined that the processing time for this number of samples was inhibitory, and thus only 10 snags were processed in the second year. In the 2010s period, we collected 10 snags on every sampling date. Snags were classified as collectable if they were physically attached: either living material from submerged roots or branches from trees on the bank or dead material embedded in the benthic substrate. Unattached, free-floating woody debris were not collected. Snags were pulled from the water with a specially-designed longitudinal sieve with 100µm stainless steel mesh (University of Georgia Instrument Shop, Athens, GA, U.S.A.) to contain associated organic material and organisms, and were removed with loppers. The wood,

organic material, and invertebrates from each sample were placed in plastic bags and kept on ice for transport to the lab. Any vertebrates such as fish or salamanders were returned to the water.

Within 24 hours, the samples were processed in the lab. All sample material was rinsed over a round 90µm sieve. Organic debris and invertebrates were separated from the wood and initially preserved in 10% formalin to fix tissues. A toothbrush was used to further remove organisms and debris from the surface of the wood. Wood was placed in plastic bags containing 95% ethanol until further processing. After three days, the formalin was washed from samples by transferring material to DI water for 24 hours, then to 70% ethanol as a long-term preservative.

We approximated the surface area of the wood material by using the equation for surface area of an elliptical cylinder:

$$\pi \frac{ab}{2} + \left[\pi \frac{h}{2} (a+b) \times \left(1 + \frac{q^2}{4} + \frac{q^4}{64} + \frac{q^6}{256}\right)\right]$$

where h = height, a = semi major axis, and b = semi minor axis, and $q = (a\text{-}b)/(a\text{+}b)$. Because the dimensions of each end of the snag were not always similar, we took two measurements at both ends and used an average for each a and b. Measurements were taken in centimeters, then the surface area was converted to square meters. Each whole snag was cut several times and we used the sum of surface areas of the individual pieces to minimize loss of variation in shape that would affect actual the surface area. We also subsampled each snag to search for invertebrates inside or firmly attached to the wood (e.g. insect pupae, wood-mining organisms such as *Stenochironomus* midge larvae) that did not get removed during initial rinsing and brushing.

Invertebrate Sorting

Macroinvertebrates were removed from detritus in preserved samples by first splitting the sample into three size fractions, >1mm, >250µm, >90µm, by rinsing the samples overs stacked sieves. Subsampling was used to reduce processing time to a manageable amount. Unless it contained a very large amount of material, the >1mm size fraction was not subsampled because the large organic material was difficult to homogenize. The two smaller size fractions were typically subsampled within a range of 1/8 to 1/64 of the total fraction (though more or less subsampling was done depending on the sample) in order to keep the total amount of invertebrates removed between 100 and 500 individuals. Subsampling was done by homogenizing the sample in a petri dish marked to have 8 equal sections and pipetting out a section at random.

Invertebrates were separated from the organic material under a stereoscope and placed into vials of 70% ethanol. After removing all invertebrates, organic material was classified by dominant type (leaves, macrophytes, wood, moss, roots, or fine particulate organic matter) and placed in the drying oven at 60° C for at least 24 hours. The organic material was then weighed to get the dry mass associated with each snag in grams.

Identification and measurements

Macroinvertebrates were identified under a dissecting microscope using keys from Merritt et al. (2008), Morse et al. (2017a) and Epler (2010). Individuals were primarily identified to genus. For some taxa however, due to a high level of time and expertise needed for a genus-level identification, family was used instead, e.g. Chironomidae (Diptera), following protocols from the 1980s study (Benke & Wallace 2015). Also, if an individual was too small to be reliably assigned to a genus, lower-resolution designations were given (i.e. at the family or order level). In the second year of the 1980s study, mayflies (Ephemeroptera) were only identified to family,

so while we identified most mayfly individuals to genus in the 2010s, family level designations are used here for comparisons of all datasets.

Taxa were designated as either "consumer" or "predator" according to Merritt et al. (2008). Even though caddisflies in the family Hydropsychidae are known to ingest animal material (Benke et al. 2001), we considered them consumers based on their feeding strategy (i.e., filtering detritus and other material from the water column rather than having to actively capture prey).

Measurements were taken to the nearest 0.1mm of each individual's head capsule width and/or body length, depending on the taxon, using a paper ruler placed under a petri dish. To obtain a calculation of the number of individuals per square meter of wood surface area, we multiplied the number of individuals measured by the subsampled value and divided by the wood surface area, then divided the total of each taxon by 10 to get the average per date.

Biomass values for each individual were calculated using the formula

$$M = aL^b$$

where *a* and *b* are derived from length-mass regressions and are specific to each taxon and body part. We used values of *a* and *b* published by Benke et al. (1999), which included many calculations from individuals specific to the Ogeechee River. We were not able to calculate biomass for all individual macroinvertebrates: some taxa or life stages do not have length-mass regressions yet developed that would allow us to use the above equation, and some individual specimens were not in good enough condition to obtain accurate measurements. However, our dataset represents the vast majority of the biomass within the samples. Additionally, our main goal was to make comparisons between the two time periods, and data from each time period included the same restrictions.

Water Data

We obtained both average daily discharge and water chemistry metrics from the United States Geological Survey. Discharge data was obtained from the USGS online database using gage 02202500 (Ogeechee River near Eden, GA; USGS 2018a). We calculated the average cubic meter per second over a two-week period preceding each sampling date using the *waterData* package in R (Ryberg & Vecchia 2017). We also obtained historical data starting in December 1969 to determine average daily discharge for the winter-spring season of each year until 2017. Dissolved carbon values for each month corresponding to snag sampling in both time periods were obtained from an online database of USGS water quality testing at gage 02202190 (Ogeechee River near Oliver, GA; USGS 2018b), which is upstream of the Eden gage and the closest location to the snag sampling site where water chemistry measurements were collected consistently in the 1980s and the 2010s.

Statistics

We performed an analysis of variance (ANOVA) to test for differences in invertebrate abundance (no. per square meter) and biomass (mg per square meter) by period (1980s vs 2010s) and by season (winter, spring, summer fall). We used a $ln(x+1)$ transformation on both abundance and biomass data, since a few dominant taxa occurred at many orders of magnitude above others. We conducted separate analyses for all macroinvertebrates as a whole, those classified as primary consumers, and those classified as predators (Merritt et al. 2008).

We calculated Bray-Curtis distances from a $ln(x + 1)$-transformed taxon by sample biomass matrix, and conducted a permutational analysis of variance (PERMANOVA) with 10,000 permutations to test for differences in community structure by period and season, using the *vegan* package in R (Oksanen et al. 2018). We also conducted an indicator species analysis

with 10,000 permutations using the *indicspecies* package in R (De Caceres & Lengendre 2016) to determine which taxa characterized any significant differences between period and season groups. Taxa are not considered indicator species in this analysis if they are rare or generalists.

To study characteristics and relationships of macroinvertebrate biomass and discharge, we averaged each of these values by month for each sampling period (typically two values each month) to obtain an average annual pattern. We separated winter months (December, January, and February) and spring months (March, April, May) and performed two-sample unpaired t-tests on discharge each season group, using the two-week mean discharge values prior to each invertebrate sampling date (winter: 1980s $n = 6$; 2010s $n = 5$; spring: 1980s $n = 6$, 2010s $n = 6$). Discharge data was *ln*-transformed to meet assumptions of normality.

We also performed a Mann-Kendall test on the continuous historical data to assess whether seasonal discharge was exhibiting a long-term trend, after testing the data for autocorrelation and partial autocorrelation (using the *Kendall* package in R; McLeod 2011). We then performed a linear regression between the average two-week discharge and dissolved carbon values corresponding to each sampling date in the 1980s and 2010s periods.

Significance was considered $P<0.05$. All analyses were conducted in the R statistical program (version 3.5.0, R Core Team 2018) in R Studio.

Tables

Table 2.S1. Results of ANOVA on abundance and biomass of aquatic invertebrates by time period (1980s vs 2010s) and season. Biomass values were *ln(x+1)*-transformed.

Total density	*Df*	*SS*	*MS*	*F*	*p*
Period	1	0.12	0.1224	0.123	0.7271
Season	3	9.04	3.0143	3.0143	**0.0396**
Period * Season	3	6.31	2.1044	2.123	0.1121
Residuals	41	40.65	0.9915		
Total biomass					
Period	1	6.33	6.331	5.639	**0.0223**
Season	3	6.09	2.029	1.807	0.1610
Period * Season	3	3.24	1.079	0.961	0.4204
Residuals	41	46.03	1.123		
Consumer density					
Period	1	0.17	0.1744	0.176	0.6775
Season	3	9.23	3.0766	3.096	**0.0372**
Period * Season	3	6.58	2.1940	2.208	0.1017
Residuals	41	40.75	0.9938		
Consumer biomass					
Period	1	9.44	9.439	8.513	**0.0057**
Season	3	5.40	1.801	1.624	0.1985
Period * Season	3	4.58	1.528	1.378	0.2630
Residuals	41	45.46	1.109		
Predator density					
Period	1	7.68	7.685	5.506	**0.0239**
Season	3	4.20	1.399	1.003	0.4014
Period * Season	3	0.97	0.323	0.231	0.8742
Residuals	41	57.23	1.396		
Predator biomass					
Period	1	0.25	0.250	0.125	0.7258
Season	3	13.57	4.522	2.253	0.0965
Period * Season	3	1.59	0.530	0.264	0.8510
Residuals	41	82.28	2.007		

Table 2.S2. Results of PERMANOVA on Bray-Curtis distance matrices of aquatic invertebrate communities defined by time period and by season. Biomass values were *ln(x+1)*-transformed.

	df	*SS*	*MS*	*F*	R^2	*p*
Period	1	0.9883	0.98831	14.7591	0.19580	**<0.0001**
Season	3	1.0510	0.35033	5.2317	0.20822	**<0.0001**
Period * Season	3	0.2627	0.08758	1.3079	0.05205	0.1549
Residuals	41	2.7455	0.06696		0.54392	
Total	48	5.0475			1.00000	

Table 2.S3. Results of indicator taxa analyses by time period and season on *ln(x+1)*-transformed community biomass matrices.

Analysis	Group	Taxon	Higher Taxon	stat	p-value
Period	1980s	*Pteronarcys*	Plecoptera	0.937	<0.001
		Tipulidae	Diptera	0.529	0.008
	2010s	Ephemeroptera	Ephemeroptera	0.996	<0.001
		Elmidae	Coleoptera	0.945	<0.001
		Nectopsyche	Trichoptera	0.891	<0.001
		Leptoceridae	Trichoptera	0.888	<0.001
		Amphipoda	Crustacea	0.874	<0.001
		Macrostemum	Trichoptera	0.839	<0.001
		Brachycentrus	Trichoptera	0.764	<0.001
		Argia	Odonata	0.744	<0.001
		Triaenodes	Trichoptera	0.733	<0.001
		Microcylloepus	Coleoptera	0.704	<0.001
		Ceraclea	Trichoptera	0.679	0.002
		Polycentropodidae	Trichoptera	0.660	0.001
		Cyrnellus	Trichoptera	0.645	0.0003
		Leptophlebiidae	Ephemeroptera	0.599	0.002
		Cernotina	Trichoptera	0.577	0.0009
		Lepidoptera	Lepidoptera	0.568	0.0047
		Baetisca	Ephemeroptera	0.551	0.0275
		Enallagma	Odonata	0.537	0.0057
		Trichoptera	Trichoptera	0.534	0.0397
		Boyeria	Odonata	0.507	0.037
Season	Spring	*Ironoquia*	Trichoptera	0.496	0.263
	Winter, Spring	Ephemerellidae	Ephemeroptera	0.911	<0.001
		Isopoda	Crustacea	0.894	<0.001
		Isoperla	Plecoptera	0.552	0.0179
	Fall, Winter	*Taeniopteryx*	Plecoptera	0.788	<0.001
	Spring, Summer	*Tricorythodes*	Ephemeroptera	0.876	<0.001
		Caenidae	Ephemeroptera	0.862	<0.001
	Winter, Spring, Summer	*Perlesta*	Plecoptera	0.814	0.024
	Spring, Summer, Fall	*Ancyronyx*	Coleoptera	0.870	0.0164

Figures

Fig 2.1. Boxplots representing comparisons of abundance and biomass between the 1980s and the 2010s. Significant differences between periods were assessed by a factorial analysis of variance (ANOVA), taking into account period, season, and the interaction between season and period. Values represented graphically and used in analyses were transformed by *ln(x+1)*. ANOVA tables for each analysis are included in Supplementary Materials. 1980s $n = 25$; 2010s $n = 24$.

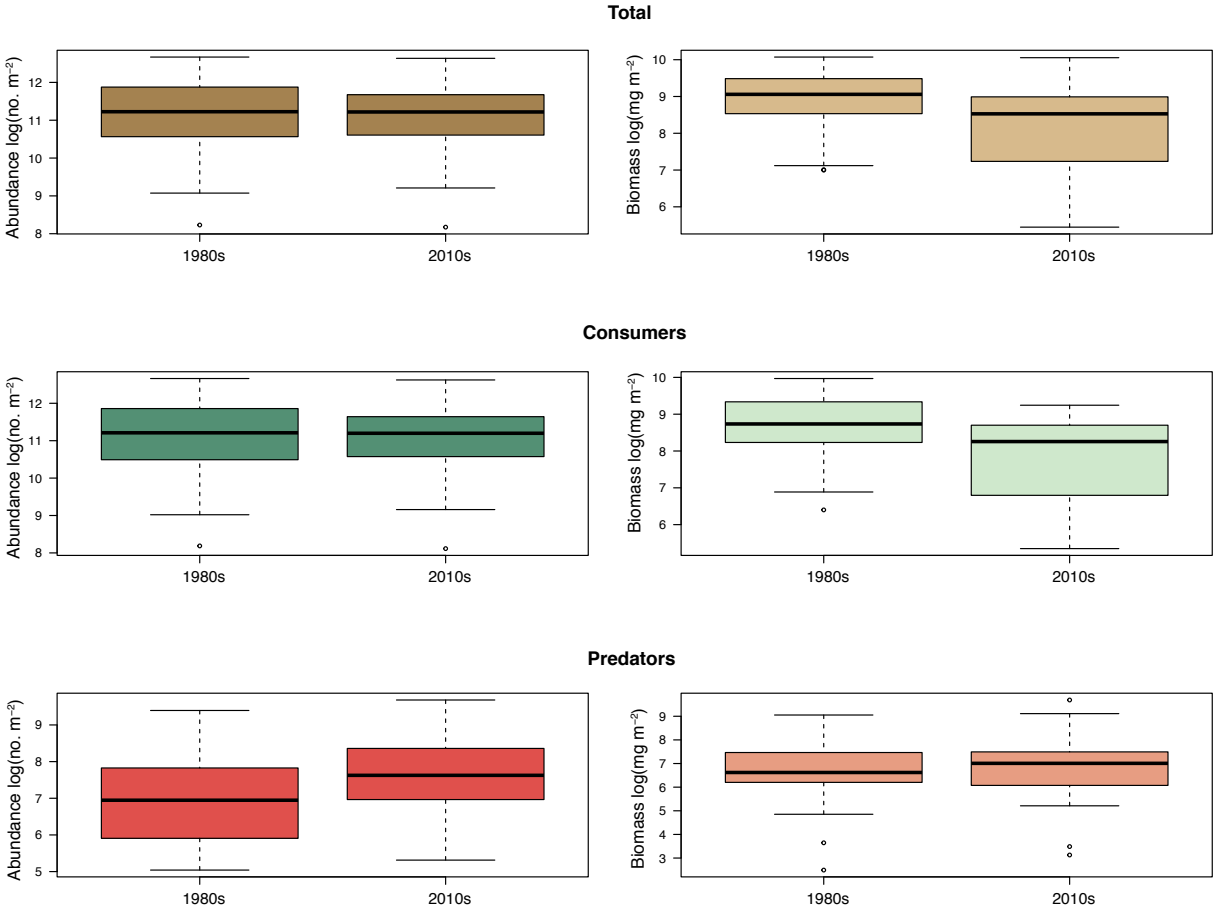


Fig 2.2. Schematic of invertebrate biomass changes between feeding groups. Circle sizes are representative of relative amount of average monthly biomass (mg/m^2). Insect images represent the most dominant families in each group. 1980s consumer taxa: Hydropsychidae (Trichoptera), Philopotamidae (Trichoptera), Chironomidae (Diptera), Pteronarcyidae (Plecoptera). 2010s consumer taxa: Philopotamidae, Hydropsychidae, Chironomidae, Heptageniidae (Ephemeroptera). 1980s predator taxa: Corydalidae (Neuroptera), Perlidae (Plecoptera). 2010s predator taxa: Corydalidae, Corduliidae (Odonata).

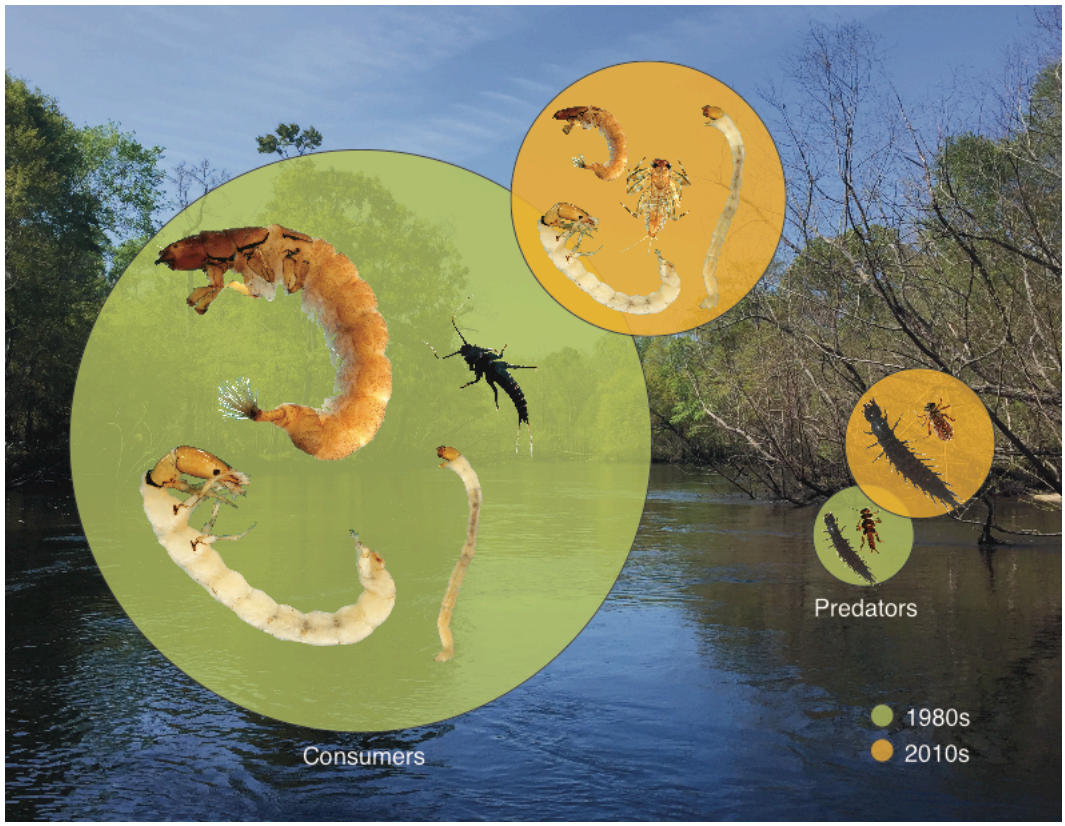


Fig 2.3. Non-metric multidimensional scaling (NMDS) ordination of snag invertebrate communities by season and time period. Bray-Curtis distance was calculated from a *ln(x+1)*-transformed family/genus-level taxon matrix, with each monthly sample defined as a community. Relative distance of points along the NMDS axes represents dissimilarity in taxonomic structure between communities. Significant differences were assessed with a permutational analysis of variance (PERMANOVA), using the *vegan* package in R (Oksanen et al. 2018). Taxa most characteristic of each period and season were assessed with an indicator taxa analysis with the *indicspecies* package in R (De Caceres & Lengendre 2016; Table 2.S1). 1980s $n = 25$; 2010s $n = 24$.

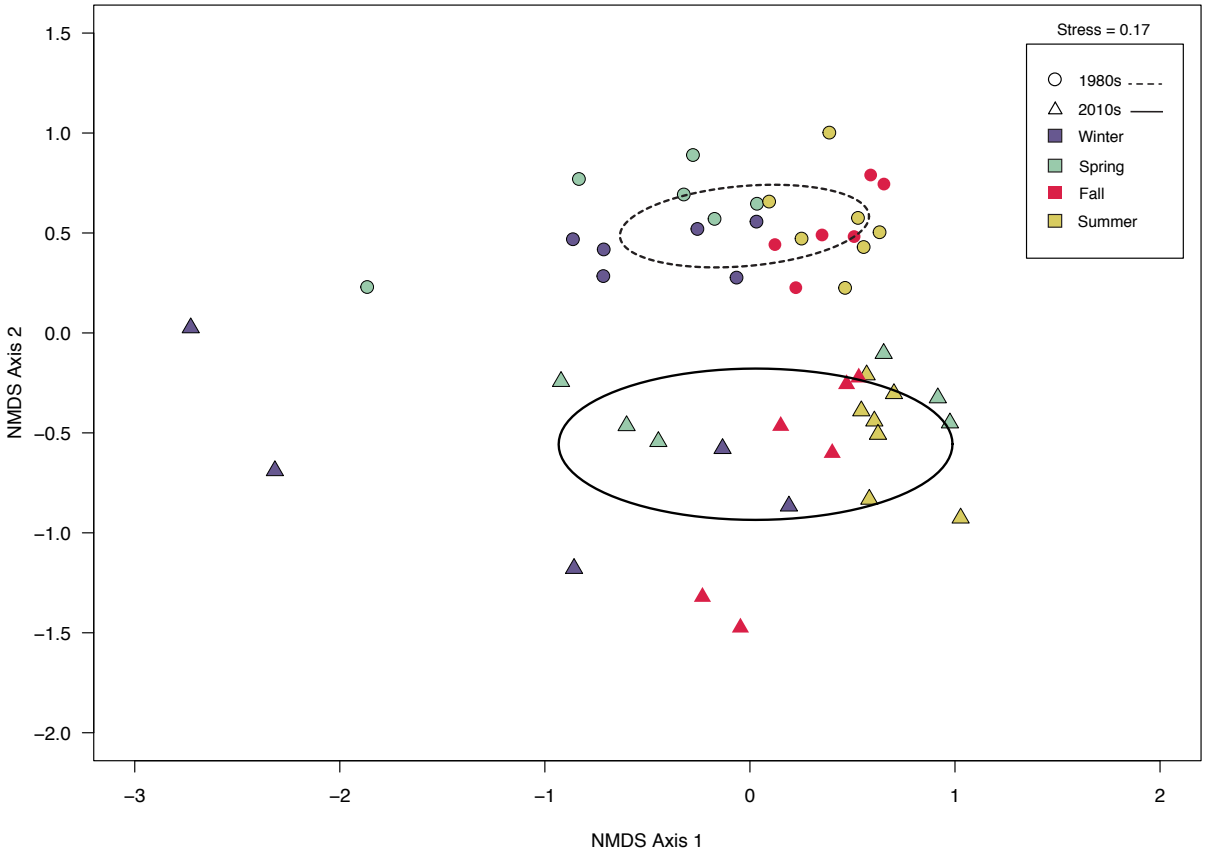


Fig 2.4. Bar plot of monthly averages of biomass on an annual cycle from each time period. Each time period represents two years of data. Error bars represent standard error. Discharge values calculated from average daily discharge for two-week period prior to invertebrate sampling date each month, using data from the United States Geological Survey (Gage 02202500; USGS 2018a).

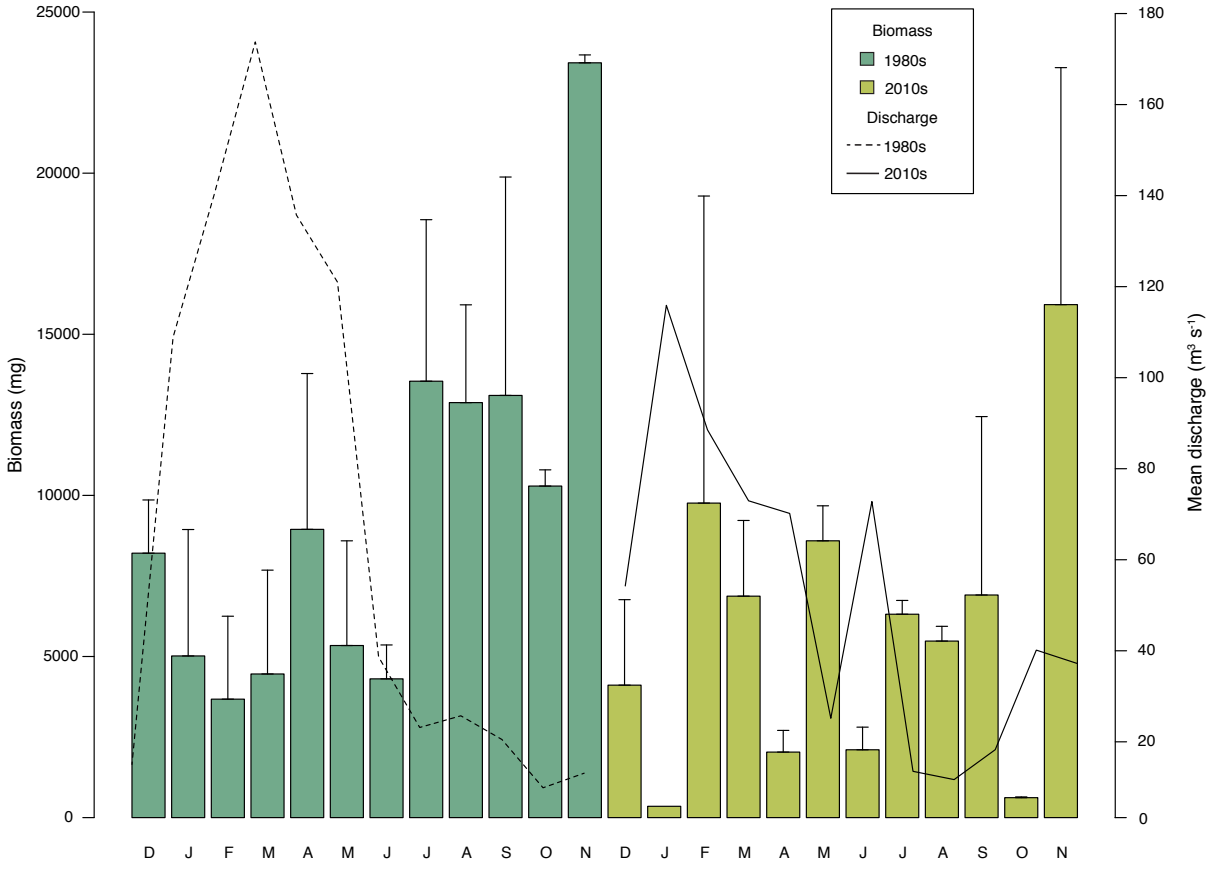


Fig 2.5. Averages of daily discharge from continuous annual winter/spring flows (December–May) from December 1969 to May 2017, fitted with a locally weighted scatterplot smoothing curve. After testing for autocorrelation and partial autocorrelation, a two-sided Mann-Kendall test was performed to test for a significant time-series trend, using the *Kendall* package in R (McLeod 2011) Discharge data was obtained from the United States Geological Survey (Gage 02202500; USGS 2018) and the waterData package in R (Ryberg & Vecchia 2017).

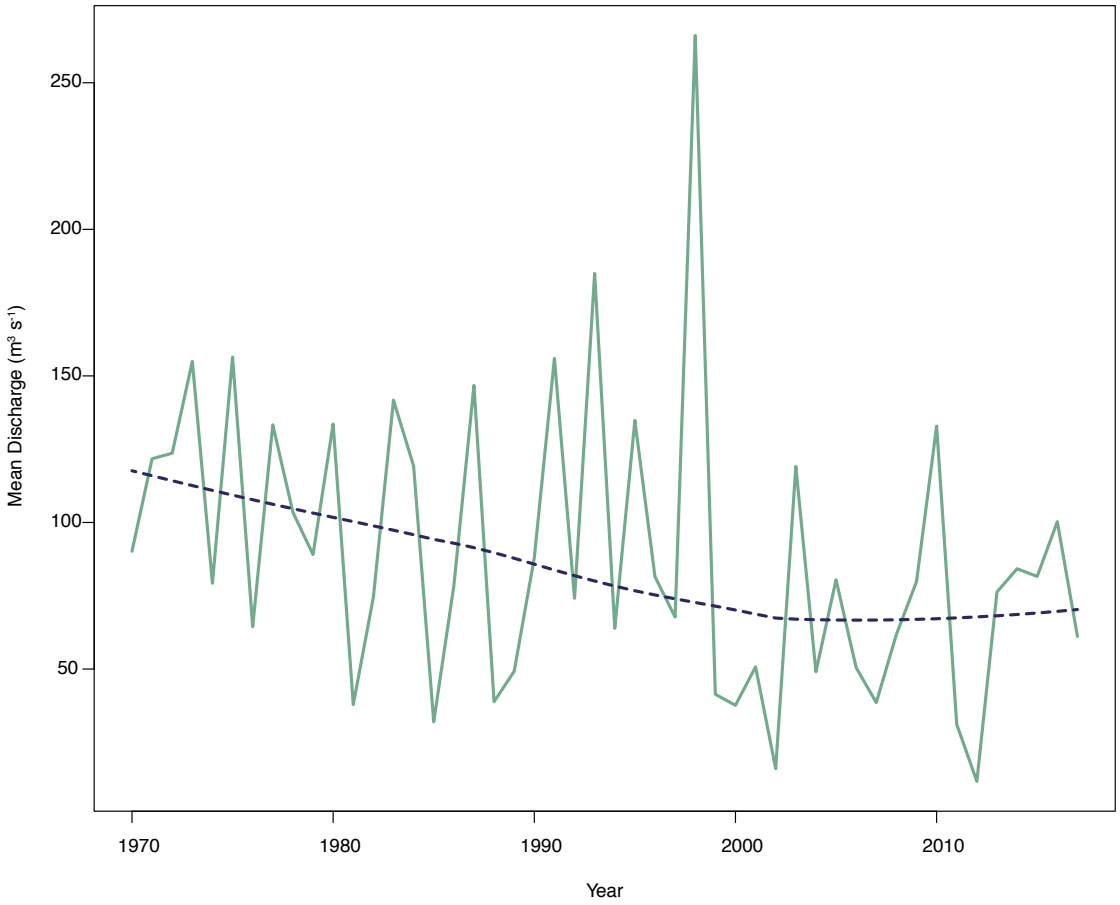
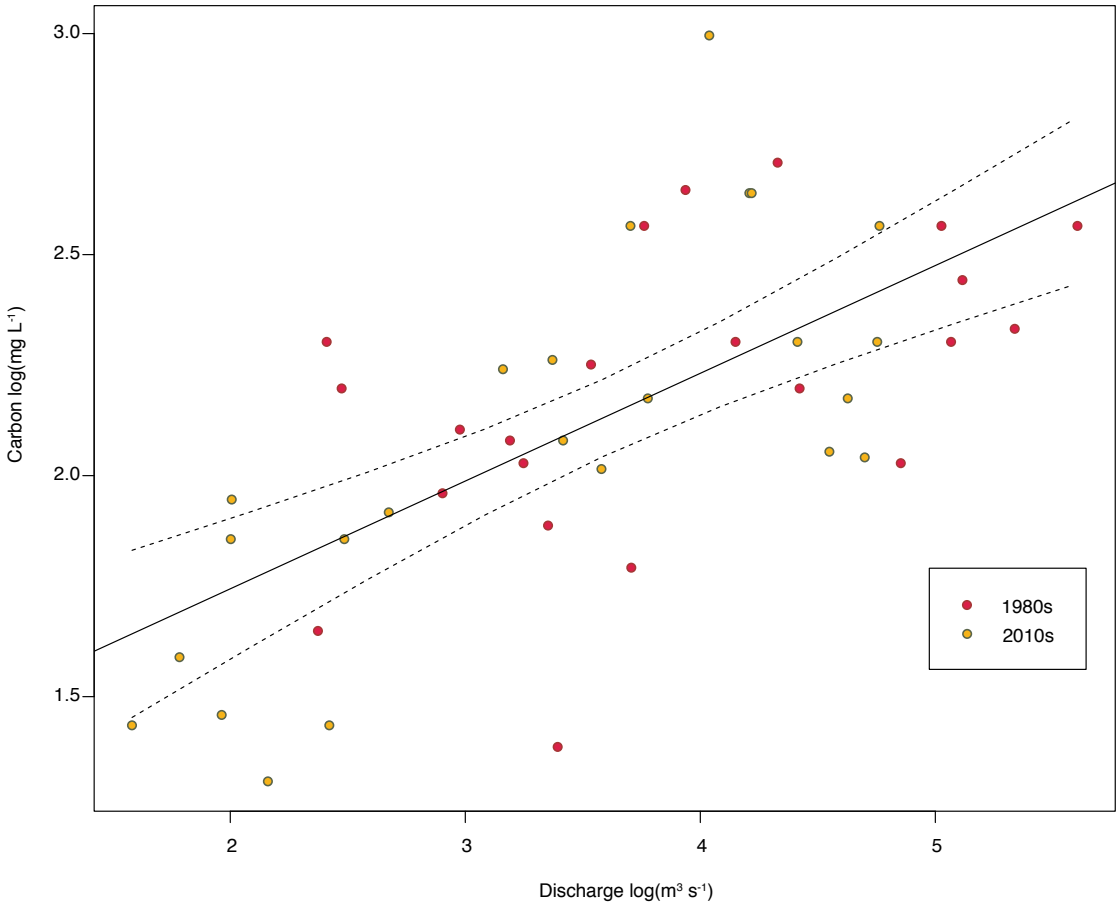


Fig 2.S1. Linear regression of dissolved carbon and two-week averages of daily discharge, each point corresponding to values collected closest to an invertebrate sampling date in each time period. All sampling months from both time periods were used in this analysis. Dotted lines represent 95% confidence interval. Both carbon and discharge values were *ln(x+1)*-transformed. Data was obtained from the United States Geological Survey (discharge: gage 02202500, USGS 2018a; carbon: gage 02202190, USGS 2018b).



CHAPTER 3

COMMUNITY COMPOSITION OF CADDISFLIES (INSECTA: TRICHOPTERA) IN THE OGEECHEE RIVER: SHIFTS OVER THREE DECADES

Introduction

Trichoptera is the most speciose of the fully-aquatic insect orders and is only surpassed by Diptera in the number of described aquatic species (Malm et al. 2013). The order is also ecologically diverse (Mackay & Wiggins 1979), including representatives of virtually all types of aquatic macroinvertebrate functional feeding groups (Wiggins & Currie 2008). Caddisflies are considered to be relatively intolerant of pollution, which makes them a frequent focus of biomonitoring programs, along with Ephemeroptera and Plecoptera (Lenat 1993).

While measures of percent Trichoptera in a collection of aquatic macroinvertebrates can be informative, it is clear that data with finer taxonomic resolution allows for more precise assessment of environmental conditions (Lenat & Resh 2001). Species-level identifications of aquatic larvae are ideal but can be limited by gaps in knowledge of the immature stages. For example, the microcaddisflies (Hydroptilidae) comprise the most species-rich family of Trichoptera in the Nearctic biogeographic region (Rasmussen & Morse 2018), yet a very small percentage of species have been described in the larval form (Wiggins 1977, Morse et al. 2017b). Once a strong taxonomic foundation is established for such poorly known groups, ecological studies could produce evaluations of functional traits (e.g. Poff et al. 2006), which could then be used to assess community responses to disturbance (Mouillot et al. 2013); however, it is essential to understand which traits are relevant to changing environmental conditions.

A study of aquatic invertebrates in the Ogeechee River (Georgia, USA) in the 1980s determined that net-spinning caddisflies were responsible for much of the riverine secondary production (Benke & Wallace 2015). Since then, the river has been altered from its reference condition. For example, a chemical spill in 2011 was associated with significant ecological damage (Barrett & Fleming 2011), and anthropogenic climate change has resulted in increasing droughts across the southeastern United States (USGCRP 2017). We replicated the 1980s aquatic invertebrate study (Benke & Wallace 2015) to understand how long-term ecological change manifested in the riverine insect community. In this paper, we focus specifically on Trichoptera because of the order's diversity and its known importance to ecosystem functioning in the 1980s. Here, we present results from analyses of caddisfly abundance, richness, and community composition, and then attempt to improve the state of taxonomy for the larval forms of the genus *Hydroptila* (Hydroptilidae) by focusing on the species occurring in the Ogeechee River.

Methods

Study site and sample collection

The Ogeechee River is a sixth-order blackwater system that runs through the Coastal Plain physiographic region of Georgia (U.S.A.) to the Atlantic Ocean. The river has sandy benthic substrate and is highly connected to the adjacent forested floodplain, both physically and by energy flows (Meyer et al. 1997, Benke et al. 2000). Sampling for both the 1980s and 2010s took place along a 1-km reach of the river near Eden, GA (32°11′29″ N, 81°24′58″ W), upstream of the confluence with Black Creek, a fourth-order tributary.

Invertebrates were sampled by collecting submerged woody debris ("snags") in the main channel. Ten snags were collected monthly for two years in each time period. The 1980s study included 25 sampling dates from December 1981 to November 1983; the 2010s study included

24 sampling dates from July 2015 to August 2017. Collections in the 2010s were designed to follow the protocols used in the 1980s as closely as possible (described in Benke & Parsons 1990); Parsons participated in sampling during both periods, helping to ensure methodological consistency. Snags were collected from canoe or boat by lifting wood with a longitudinal sieve fitted with 100µm mesh, lopping an approximately 50 cm section into the sieve, and then transferring all contents into plastic bags. Sample bags were placed on ice and transported to the lab to be processed within 24 hours. The samples from the first 20 months of the 2010s effort were initially fixed in 10% formalin for 72 hours, washed by soaking in DI water for 24 hours, then permanently preserved in 70% ethanol. The final 4 monthly samples (May-August 2017) were directly preserved in 95% in ethanol to allow for DNA extraction. Invertebrate densities were calculated per square meter of wood surface area. A full description of field sampling and laboratory sorting is included in Chapter 2.

We also collected adult caddisflies during the evenings prior to two sampling dates, July and August 2017. We used an ultraviolet light placed over a pan of 95% ethanol, placed adjacent to the Ogeechee River sampling reach, to attract and preserve adults. The trapping was conducted from dusk to 1 hour after sunset.

Identification

Macroinvertebrates were identified under a dissecting microscope using keys to Trichoptera of the southeastern United States by Morse et al. (2017b). Larvae were primarily identified to genus, although family and order designations were used for individuals that were insufficiently mature for reliable genus determinations. Pupae were typically identified to family using Wiggins and Currie (2008). Hydroptilidae adults were identified to genus using Blickle (1979).

Voucher specimens from both the 1980s and 2010s are deposited in the University of Georgia Collection of Arthropods (UGCA).

Statistical analysis

Densities from the ten snag samples per sampling date were averaged to compute monthly means. We conducted a two-sample t-test of average monthly caddisfly densities between the 1980s and the 2010s time periods. Abundances were *ln(x+1)*-transformed prior to analysis. We analyzed community composition by applying a Bray Curtis dissimilarity index to an *ln(x+1)*-transformed abundance matrix and performing a permutational analysis of variance (PERMANOVA; 10000 permutations), partitioning the effects of season, time period, and the interaction of season and time period. Analyses were conducted with the *vegan* package in R (Oksanen et al. 2018), with significance considered at $p < 0.05$.

To determine changes for individual genera, we calculated the percent difference in average monthly densities relative to the 2010s time period [(2010s density - 1980s density) / 2010s density * 100]. Therefore, negative values represent a decrease from the 1980s to the 2010s and positive values represent an increase. We then generated a bootstrapped distribution of these percent changes by resampling the data 5000 times with replacement. We calculated 95% confidence intervals (CI) from the bootstrapped distribution, and changes in relative abundances were considered significant if the 95% CI did not overlap 0.

All analyses were conducted using the R statistical program (version 3.5.0, R Core Team 2018) with the R Studio interface.

Hydroptila Morphology and Genetics

We identified larval morphotypes of *Hydroptila* using macrophotography and compound microscopy, initially differentiating specimens based on case construction and thoracic

pigmentation. Macrophotographic images were captured with a Canon EOS-1 digital camera and a Canon Macro Photo MP-E 65mm lens. Lighting consisted of two Yongnuo Digital Speedlite YN560 III speed flashes. Sequential images were made at different focal depths and combined to create a deep focus image, using Helicon Focus 6.4.2 Pro software. The composite images were edited with Adobe Photoshop CS6 (Adobe Systems, Inc., 2003, San Jose, California). We focused on a set of characters that were shown to be diagnostic at the species level for some larvae of *Hydroptila* (Keiper & Foote 1999, 2000). Temporary slide mounts were made of legs dissected from the thorax at the coxa and cleared in warm 10% potassium hydroxide solution, rinsed in DI water, mounted in glycerol, and viewed with a Leica Leitz ® DMRB compound microscope. Line drawings of structures were created using Adobe Illustrator CC.

For high magnification study and imaging of the mandibles of *Hydroptila* larvae, we used an FEI Teneo (FEI, Inc., Hillsboro OR USA) field emission scanning electron microscope at 5.00 kV (Georgia Electron Microscopy, University of Georgia). Mandibles were dissected from head capsules, air dried, and mounted on SEM stubs with adhesive carbon tape. Specimens were sputter coated with gold for 60s before viewing.

To determine if 1) the *Hydroptila* morphotypes were genetically distinct species, and 2) the larvae could be associated with described adults, we extracted and sequenced the mitochondrial cytochrome oxidase *c* subunit I (COI), which is commonly used to identify Trichoptera species (Zhou et al. 2011, Ruiter et al. 2013, Zhou et al. 2016). We subsampled the head and thorax of specimens and used the DNeasy Blood & Tissue Kit from QIAGEN® for extraction. We used two forward (LepF1: ATTCAACCAATCATAAAGATATTGG; LCO1490: GGTCAACAAATCATAAAGATATTGG) and two reverse primers (LepR1: TAAACTTCTGGATGTCCAAAAAATCA; HCO2198: TAAACTTCAGGGTGACCAAAAA

ATCA), following the methods of Ruiter et al. (2013).

We amplified for the COI region using polymerase chain reaction (PCR). We used a total 10µl reaction volume: 7.2µl water; 1µl 10x reaction buffer, 0.2µl dNTP, 0.1µl each forward primer, 0.1 each reverse primer, 0.2µl *Taq* polymerase, and 2µl template DNA. The reaction was thermocycled for 94°C 180s; 5 cycles of 94°C 40s, 45°C 40s, 68C 60s; and 35 cycles of 94°C 40s, 51°C 40s, 68°C 60s. PCR results were checked using agarose gels with ethidium bromide stain under UV light. Successful reactions were cleansed of excess primer with 0.2µl Exonuclease I and 0.2µl Calf Intestinal Phosphatase for each reaction, incubated at 37°C for 30 minutes and at 85°C for 15 minutes, and diluted 1:1 with DI water.

Samples were sequenced by Eurofins Genomics LLC (Louisville, KY, USA) in the forward direction. Sequences were edited and aligned in Geneious® version 11.1 (https://www.geneious.com). The BLAST function in Geneious was used to find the most similar existing COI sequences in the Barcode of Life Database (BOLD; http://www.boldsystems.org) and GenBank (www.ncbi.nlm.nih.gov/genbank/). Neighbor Joining methods were used for tree-building with a Tamura-Nei genetic distance model.

Percent change of caddisfly taxa

There was no significant difference in average monthly density of all Trichoptera between time periods ($p = 0.4031$). Community composition was significantly different between time periods (PERMANOVA, $p < 0.0001$, Table 3.1) and among seasons ($p < 0.0001$); there was also a significant interaction of season and time periods on caddisfly community composition ($p = 0.0309$). Throughout the 1980s sampling period, 13 genera in 6 families were collected; 17 genera in 7 families were collected in the 2010s. Changes for specific genera are detailed below, grouped into families (Fig 3.1).

Polycentropodidae

Two new genera appeared in the 2010s samples that were not found in the 1980s: *Cyrnellus* and *Cernotina*. *Cyrnellus* occurred in 10 out of 24 samples in the 2010s across summer, fall, and winter. It was represented by *Cyrnellus fraternus* (Banks 1905), the only species in the genus occurring outside the Neotropics (Morse 2018). *Cernotina* occurred in 8 out of 24 samples in the 2010s, and was found in all seasons.

Neureclipsis occurred in both time periods; it was present in 7 out of 25 samples in the 1980s representing fall, summer, and winter. In the 2010s, it was found in 10 out of 24 samples, spanning all seasons. There was no significant difference in the percent 1980s abundance relative to the 2010s (95% CI: -31.8, 92.3).

Philopotamidae

One genus in this family, *Chimarra*, was present in both time periods. *Chimarra* was the most abundant Trichoptera genus overall in both time periods; occurring in all months sampled, except one, in each time period. Average monthly abundance decreased in the 2010s, but this decline was not significant (95% CI: -363.8, 5.6).

Hydropsychidae

Hydropsyche was the second most abundant genus overall in the 1980s and the third most abundant genus overall in the 2010s. *Hydropsyche* significantly declined in abundance from the 1980s (95% CI: -840.4, -15.2). *Cheumatopsyche* was the third most abundant genus in the 1980s; it declined significantly (95% CI: -723.7, -81.1) in the 2010s, in which it was the fourth most abundant caddisfly genus. Both *Hydropsyche* and *Cheumatopsyche* were present every month over both time periods.

Macrostemum occurred in both time periods; voucher specimens from both time periods were identified as *M. carolina* (Banks 1909). It occurred in 8 months out of 25 in the 1980s. In the 2010s, *Macrostemum* occurred in 20 months out of the 24 sampled, and significantly increased in abundance from the 1980s (95% CI: 93.5, 99.3).

Hydroptilidae

Hydroptila occurred every month over both time periods. *Hydroptila* was fourth most abundant genus overall in the 1980s; its relative contribution to caddisfly community abundance increased in the 2010s to become the second most abundant genus overall, after *Chimarra*. However, average monthly abundances were similar between time periods (95% CI: -184.6, 63.9).

Neotrichia occurred only in the 2010s. Its appearance in monthly collections was strongly seasonal: *Neotrichia* consistently occurred from May through October, but was never collected from November through April.

Leptoceridae

Four genera of leptocerids occurred, all four in both time periods. *Ceraclea* occurred in 4 out of 25 monthly samples in the 1980s; by contrast, it was present in 14 out of 24 samples in 2010s. It significantly increased from the 1980s period to the 2010s period (95% CI: 81.4, 99.6). The genus *Oecetis* occurred in 18 out of 25 months in the 1980s and 22 out of 24 months in the 2010s. Percent change in abundances between time periods was not significantly different (95% CI: -143.6, 73.7). *Nectopsyche* shifted in prevalence from appearing in 10 out of 25 months in the 1980s to being present in every one of the 24 months sampled in the 2010s, with a significant increase in abundance (95% CI: 29.4, 97.0). *Triaenodes* only occurred in one month in the 1980s collections, but in the 2010s collections, it was present in 13 months and showed a significant increase in abundance (95% CI: 83.8, 97.7).

Brachycentridae

This family was newly documented in the 2010s compared to the 1980s. The genus *Brachycentrus* occurred in 15 out of 24 months in the 2010s. Larval specimens were identified as *B. numerosus* (Say 1823) using a morphological key (Morse et al. 2017b), then confirmed with DNA analysis (our specimen was matched to specimens identified as *B. numerosus* in BOLD with 99.79% similarity). This is a new record for this species for the state of Georgia; it has, however, previously been documented in surrounding southeastern states, including North Carolina, South Carolina, Alabama, Tennessee, and Florida (Rasmussen & Morse 2018).

Limnephilidae

Relative to the other Trichoptera, Limnephilidae was uncommon in both time periods. *Pycnopsyche* occurred in one of the 1980s samples (in February) and in one of the 2010s samples (in May). *Ironoquia* occurred in 3 monthly samples from the 1980s (in March and April), and in 2 monthly samples from the 2010s (in December and March). Neither *Ironoquia* (95% CI: -236.4, 89.5) nor *Pycnopsyche* (95% CI: -32.7, 63.5) exhibited significant change between time periods.

***Hydroptila* larval taxonomy**

Two morphotypes of *Hydroptila* larvae were distinguished using specimens from the 2010s collections. "*Hydroptila* morphotype I" had a case constructed of algae (Fig 3.2); "*Hydroptila* morphotype II" had a case constructed of minerals (Fig 3.3). There were also differences in coloration patterns on the thoracic nota and dorsum of the head capsule whereby pigmentation was more extensive on *Hydroptila* morphotype I. Temporary slide mounts of each morphotype's legs viewed under a compound microscope allowed for digital illustration of structures (Fig 3.4). The overall structure of the legs is similar with a divided trochanter on all

three legs, though *Hydroptila* morphotype II has fewer robust setae on each trochanter than *Hydroptila* morphotype I (Fig 3.4).

The morphotypes were further differentiated with scanning electron microscopy of the mandibles (Fig 3.5). Both morphotypes had asymmetrical mandibles, with the left mandible bearing a large median excavation of the mesal margin containing a brush of setae. The mesal margin of the right mandible of both morphotypes is entire and bears a broad membranous prostheca. The apical tooth of the left mandible of *Hydroptila* morphotype I was more bluntly rounded than that of *Hydroptila* morphotype II. The apex of the right mandible of *Hydroptila* morphotype I was evenly divided into two teeth, while that of *Hydroptila* morphotype II was unevenly divided, with the apical tooth being larger.

DNA analysis provided evidence that these two morphotypes represent distinct species. DNA extractions were successful for one individual of each larval morphotype and one unidentified adult *Hydroptila* female. None of these specimens matched sequences with identified species of *Hydroptila* in BOLD, but a Neighbor-joining tree created with the ten most similar sequences in GenBank for each of the three specimens placed the two morphotypes in different clades (Fig 3.6). The adult *Hydroptila* female and *Hydroptila* morphotype I appear to be the same species.

Discussion

Our study of the caddisflies of the Ogeechee River demonstrated that community compositions have significantly shifted over the past three decades. Some of the dominant genera from the 1980s declined, while other, less dominant, taxa increased in abundance. Trichoptera richness also increased from the 1980s, with four new genera appearing in the 2010s collections. These changes provide context to explore caddisfly ecological responses to environmental change.

Significant declines in overall invertebrate biomass in the Ogeechee River have occurred since the 1980s that may be linked to patterns of discharge and subsequent effects on carbon delivery from the floodplain (see Chapter 2). *Hydropsyche*, *Cheumatopsyche*, and *Chimarra* in particular were the source of much of the 1980s invertebrate community biomass. These three taxa are filtering collectors that rely on amorphous detritus (Benke & Wallace 1997), which primarily originates from the floodplain (Meyer et al. 1997). These declines may have community-level consequences for other caddisflies.

Macrostemum, a net-spinning, filter-feeding caddisfly like *Hydropsyche* and *Cheumatopsyche*, increased in the 2010s relative to the 1980s. Average abundances in the 2010s, however, were still less than those of the two more dominant genera. In the 1980s, *Macrostemum* populations may have been limited by competition with *Hydropsyche* and *Cheumatopsyche* and their subsequent declines then allowed *Macrostemum* to increase; these populations have not yet begun to be limited by carbon delivery. Patterns for leptocerids may also be related to *Hydropsyche* and *Cheumatopsyche* changes. *Oecetis* is predatory, and Benke and Wallace (1997) reported that it is one of the few predators that ingests *Hydropsyche* and *Cheumatopsyche*, among other prey. *Oecetis* was the only genus in Leptoceridae from the Ogeechee not exhibiting a significant increase in the 2010s. The longhorn caddisflies as a family show generalist tendencies in habitat type and trophic relationships (Wiggins 1977), which may explain the apparent success of most other genera (*Triaenodes*, *Nectopsyche*, and *Ceraclea*) in the Ogeechee River with increasing niche space.

Four genera were unique to the 2010s: *Cyrnellus* and *Cernotina* (Polycentropodidae), *Neotrichia* (Hydroptilidae), and *Brachycentrus* (Brachycentridae). Sources for new populations in the Ogeechee River study reach may include mainstem reaches further upstream, lower-order

tributaries, or, for taxa that can tolerate lentic conditions, the floodplain. *Cyrnellus* individuals were documented from the nearby Black Creek in the 1980s (Benke, unpublished data). *Brachycentrus*, *Cernotina*, and *Neotrichia*, however, were not documented in Black Creek in the 1980s, nor have they been documented in other streams in the Ogeechee Basin (unpublished data from a 2001-2007 sampling effort by the Georgia Department of Natural Resources, Environmental Protection Division). However, *Cernotina* is known to also inhabit lentic habitats (Morse & Holzenthal 2008), and *Neotrichia* occurs in Upper Three Runs Creek in the adjacent Savannah River basin (Floyd et al. 1993). Elsewhere, populations of *Brachycentrus* larvae are phosphorus limited (Veldboom & Haro 2011), and densities of this genus increased in response to experimental phosphorus addition in Alaska (Hershey & Hiltner 1988). Recent chemical changes in the Ogeechee River may have made it a more suitable habitat for *Brachycentrus*. However, relaxed competition may also be involved; *Brachycentrus* larvae are filter-feeders, like the hydropsychid caddisflies, and it may be due to the aforementioned declines in this group that a population of *Brachycentrus* larvae was able to establish.

Studying the caddisflies of the Ogeechee River has also helped illuminate aspects of Hydroptilidae taxonomy. Larval microcaddisflies are poorly studied at the species level (Wiggins 1977, Morse et al. 2017b). Ross (1944) associated the larvae and adults of several species, but larval characters described were not consistently reliable for identification (Wiggins 1977). Here, we were able to distinguish two morphotypes of larval *Hydroptila* from our Ogeechee River collections as separate species using DNA techniques to confirm morphological studies. The larval case and mandibles were the most helpful characters in differentiating the two. We were not able to associate the larvae to identified adults due to the lack of adult male *Hydroptila*

specimens collected and the currently incomplete nature of BOLD, but we anticipate that future molecular work will enable species designations.

This study highlights the importance of considering the ecological context of individual taxa when assessing long-term environmental change. Hydropsychid taxa are considered fairly tolerant to environmental change compared to other caddisfly groups (Lenat 1993). However, hydropsychids exhibited the only significant declines in the Trichoptera community, likely due to food web dynamics. Meanwhile, genera considered more sensitive, such as *Brachycentrus*, became established, producing an unexpectedly higher richness in the 2010s. Coarse metrics of order-level abundance were clearly inadequate to judge long-term change in the Ogeechee River. As rivers and streams are continually impacted by human activity, perspectives on how changes will manifest in the aquatic invertebrate assemblage will benefit from detailed documentation of the Trichoptera community.

Table

Table 3.1. Results of PERMANOVA on Bray-Curtis distance matrices of caddisfly communities defined by time period and by season. Abundance values were *ln(x+1)*-transformed.

	df	*SS*	*MS*	*F*	R^2	*p*
Period	1	0.8726	0.87262	21.3666	0.26623	**<0.0001**
Season	3	0.4969	0.16564	4.0557	0.15160	**<0.0001**
Period * Season	3	0.2337	0.07791	1.9078	0.07131	**0.0321**
Residuals	41	1.6744	0.04084		0.51086	
Total	48	3.2777			1.00000	

Figures

Fig 3.1. Average percent difference of Trichoptera genera density per square meter of snag surface area between 1980s and 2010s, relative to 2010s densities. Differences in average density per month were analyzed with bootstrapped 95% confidence intervals. Bars marked with an asterisk (*) are significantly different between time periods, i.e. confidence intervals do not overlap zero. Bars are colored to group genera by family.

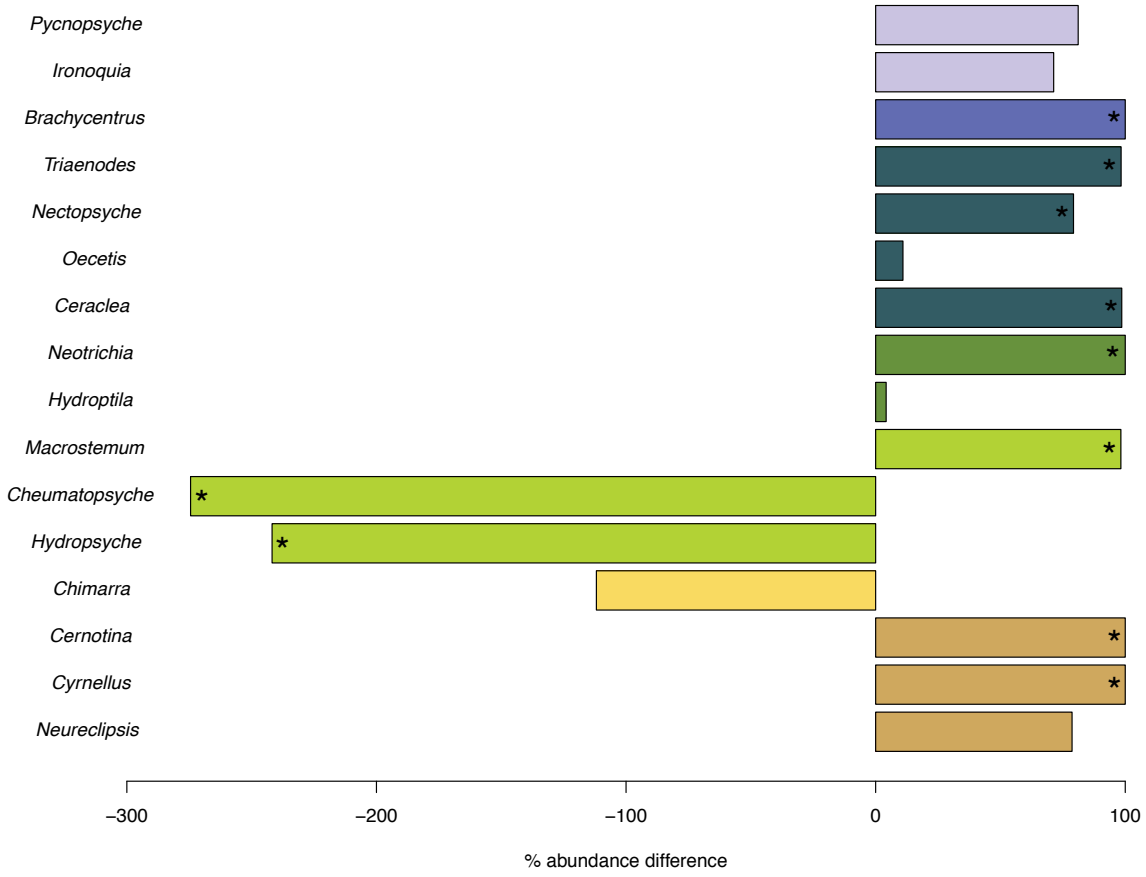


Fig 3.2. *Hydroptila* larval morphotype I, right lateral habitus (A), larval case (B), and dorsal view of head and thorax (C).

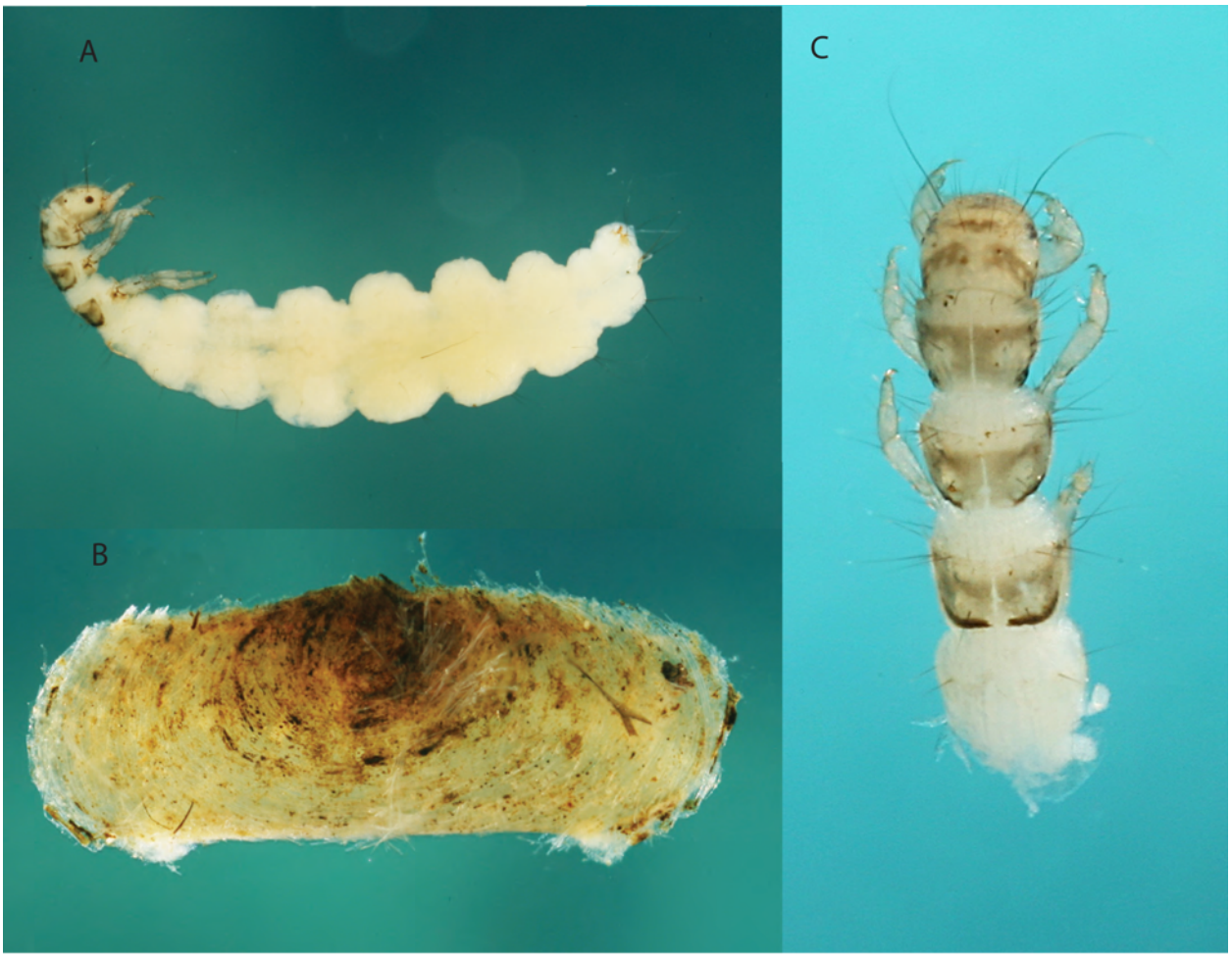


Fig 3.3. *Hydroptila* larval morphotype II, right lateral habitus (A), larval case (B), and dorsal view of head and thorax (C).

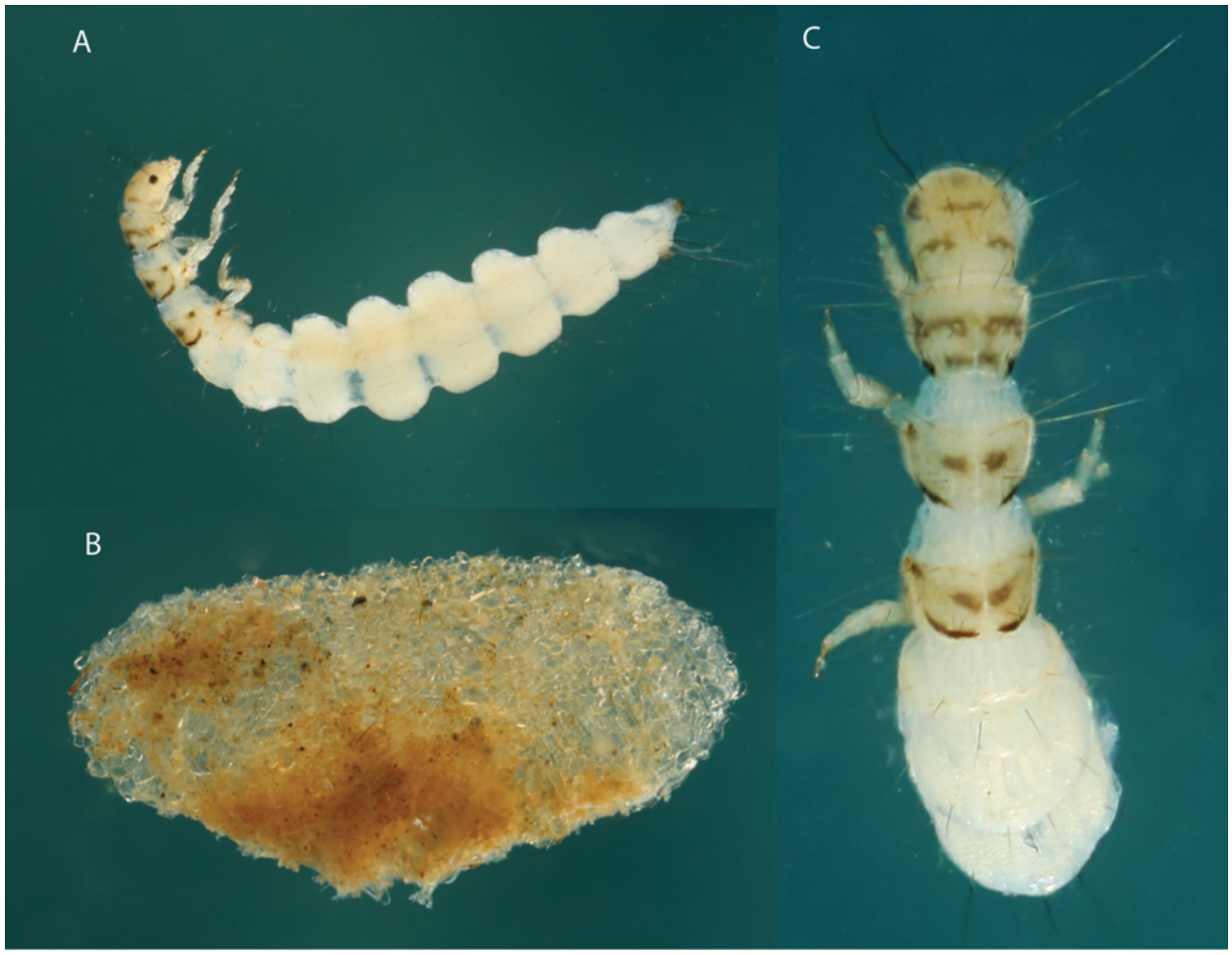


Fig 3.4. Digital illustrations of *Hydroptila* morphotype I prothoracic (A), mesothoracic (B), and metathoracic (C) legs, and *Hydroptila* morphotype II prothoracic (D), mesothoracic (E), and metathoracic (F) legs. All illustrations are right side, posterior view, beginning at trochanter.

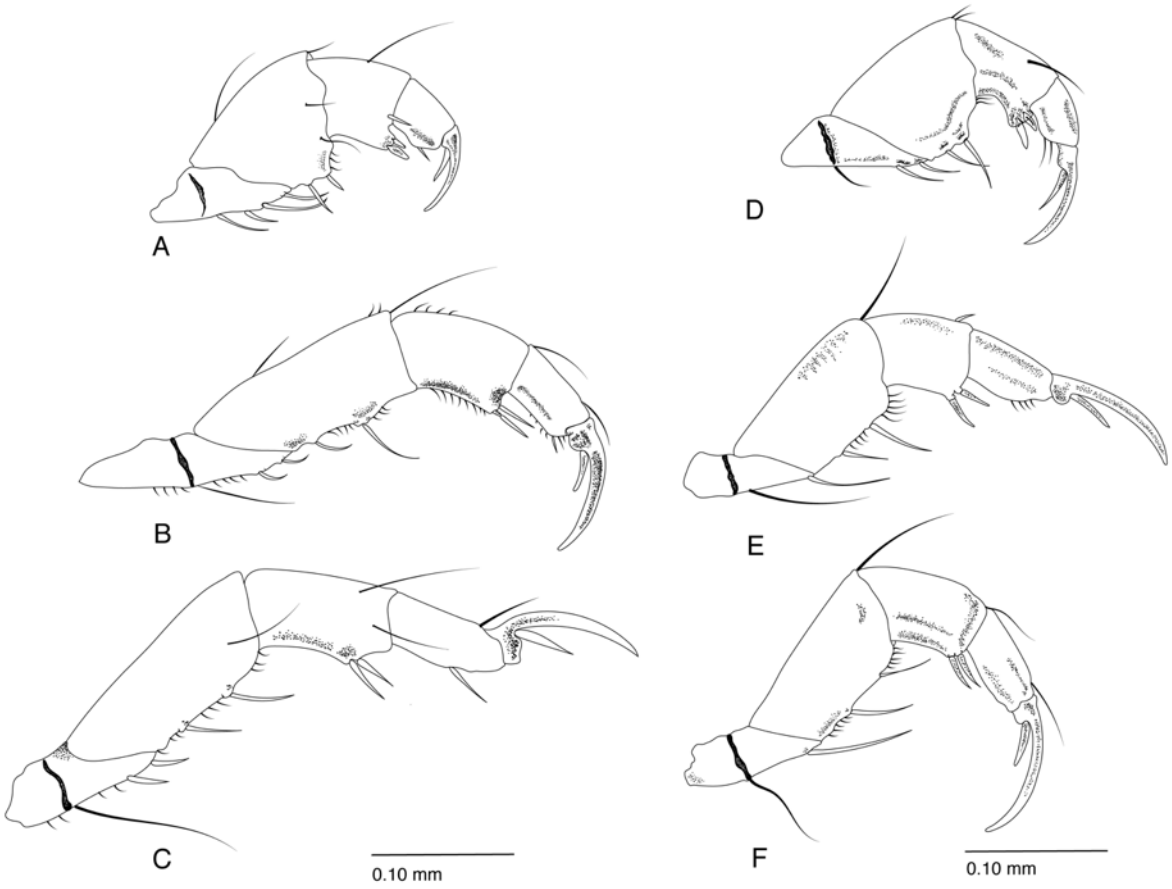


Fig 3.5. Scanning electron micrographs of *Hydroptila* morphotype I mandibles, left (A) and right (B), and *Hydroptila* morphotype II mandibles, left (C) and right (D). All mandibles in dorsal view. Base of right mandible of *Hydroptila* morphotype II obscured by part of the larval head capsule.

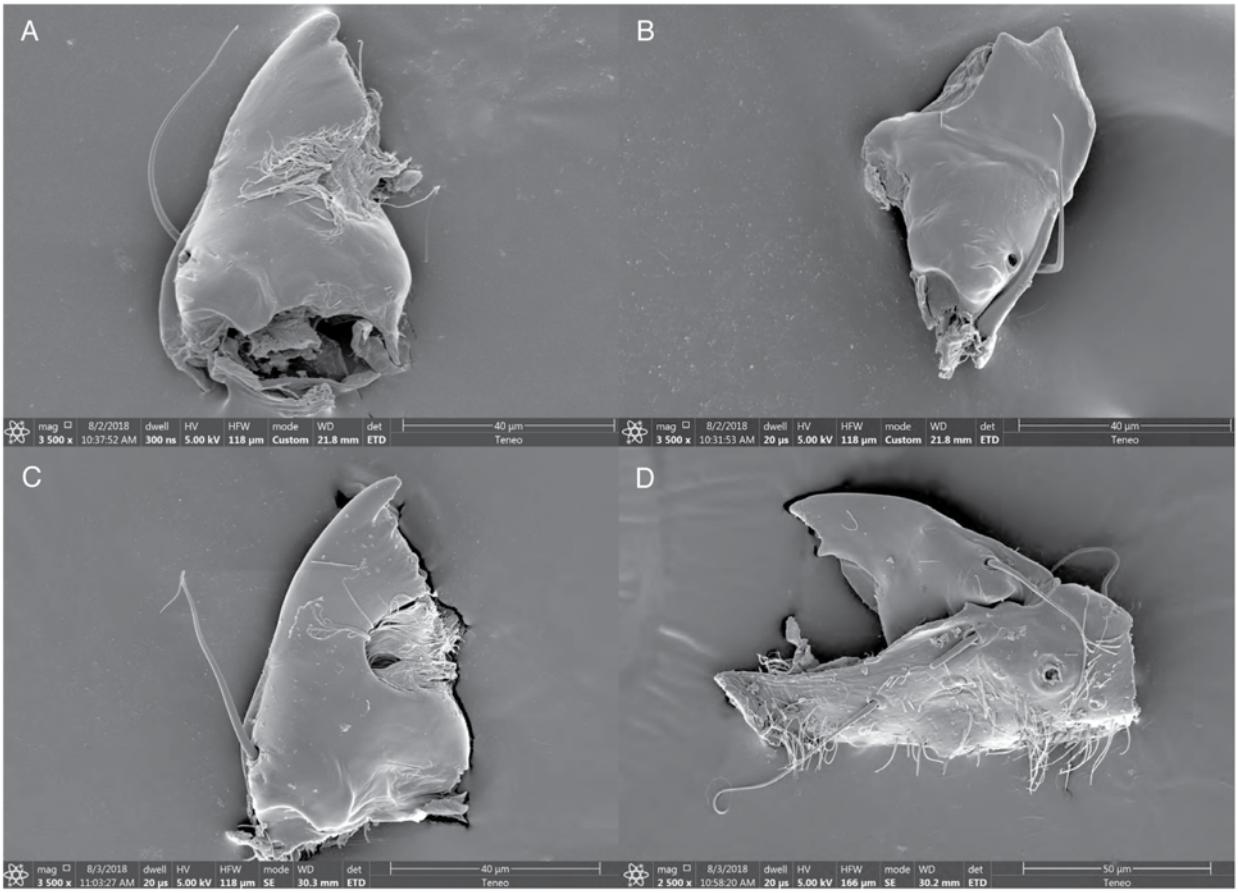
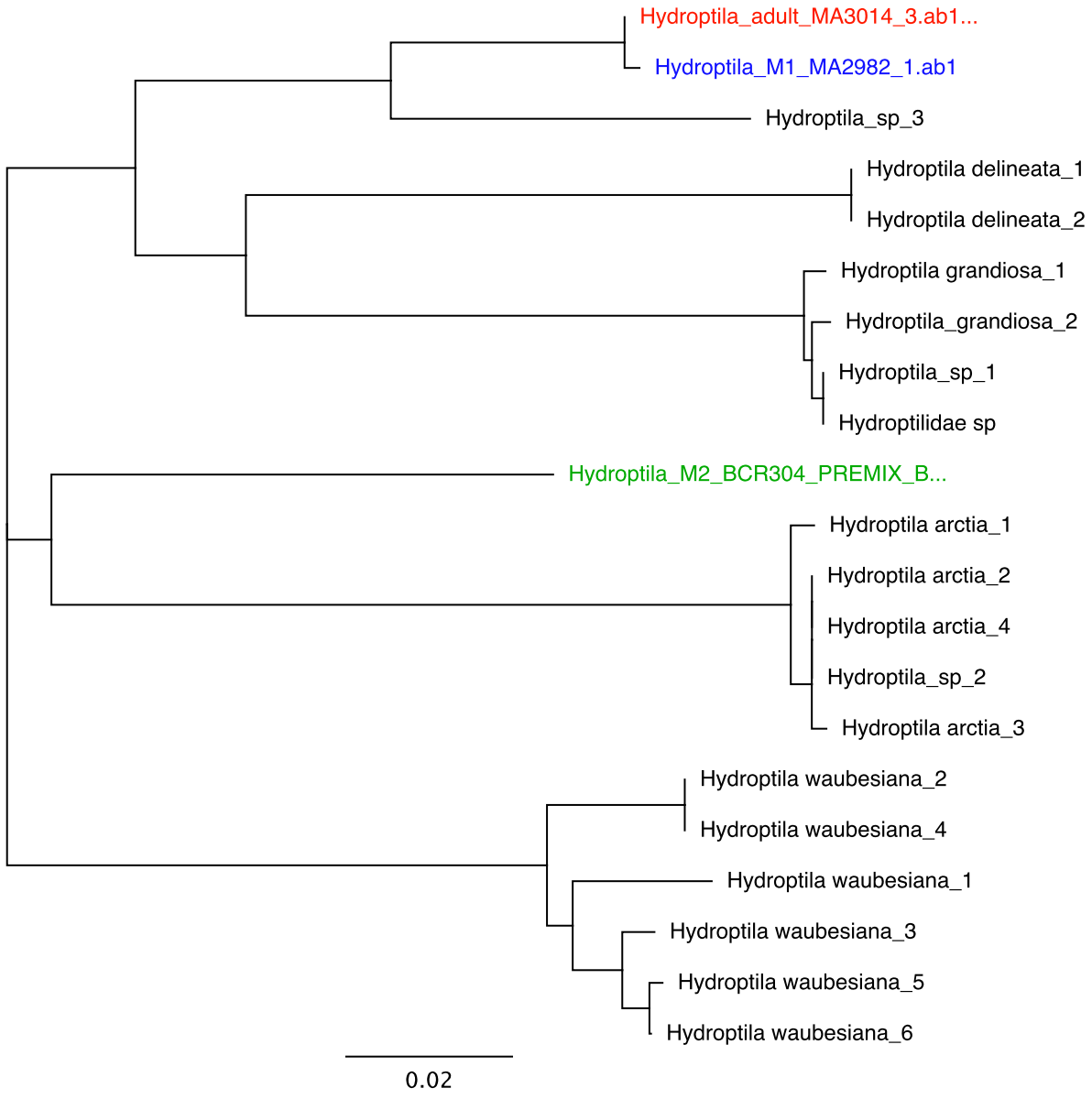


Fig 3.6. Neighbor-joining tree of aligned cytochrome oxidase I sequences of collected specimens (in color) and sequences from GenBank (in black). “M1” and “M2” abbreviations refer to *Hydroptila* morphotype I and *Hydroptila* morphotype II, respectively. Scale represents number of substitutions per site of sequence alignment. Tree created in Geneious version 11.1 (https://www.geneious.com).



CHAPTER 4

LONG-TERM CHANGE IN AQUATIC INSECT COMMUNITIES IS CHARACTERIZED BY FUNCTIONAL REDUNDANCY AND SHIFTING INFLUENCE OF ENVIRONMENTAL PARAMETERS

Introduction

Worldwide, rivers and streams are under threat from anthropomorphic change (Vörösmarty et al. 2010). Evaluating the specific influence of stressors such as increased temperature and drying due to climate change, altered hydrology due to urbanization, and changes in chemical composition due to industrial influences will help predict future community characteristics and cascading ecosystem effects. Focal traits can be selected based on their relationship to known stressors (Poff et al. 2006), and modeling of environmental measurements can help illuminate their influence on trait diversity, which may be altered based on overall community structure. Comte et al. (2016) found that functional diversity of fish communities characterized by nonnative species was less structured by environmental factors.

Functional traits of organisms and their structure within communities provide a link from biodiversity to ecosystem processes. These metrics are useful in understanding the determinants of community composition (Klais et al. 2017, Start et al. 2018), as the prevalence of certain traits over others can indicate the role of environmental filtering. Specific indices of trait diversity measure the utilization of trait space by taxa; the degree of clustering of taxa within trait space indicates niche differentiation (Schleuter et al. 2010). Aspects of functional trait diversity can

also provide a nuanced evaluation of human disturbance and other environmental changes (Mouillot et al. 2013).

Measuring diversity of functional traits is thought to provide a metric of resilience to environmental change that may not be predicted by taxonomic biodiversity alone, since different taxa may overlap in trait space (Schleuter et al. 2010). There is evidence of a positive relationship between functional diversity and ecosystem function (Kirwan et al. 2007, McKie et al. 2008), though depending on the community and ecological process of interest, this is not always the case (Stoker et al. 2017). Ultimately, our understanding of how environmental effects on taxa scale up through the ecosystem can be aided by functional trait analyses.

Here, we employed both taxonomic and trait metrics to assess the characteristics of aquatic insect communities in response to three decades of ecological change. We used datasets from the 1980s and 2010s to compare responses of community structure and functional diversity in the Ogeechee River, a well-studied system characterized by high invertebrate productivity due to inputs of terrestrial detritus from the floodplain (Benke and Wallace 2015). Previous examinations of invertebrate community change within the Ogeechee showed decreases in biomass and abundance of dominant consumer taxa (Chapter 2) and increases in caddisfly generic richness (Chapter 3). Consequently, we expected that functional richness would also increase, due to the potential for new taxa to introduce unique traits to the community. We also expected that current communities would be associated with higher functional dispersion, indicating a change in distribution of traits following the declines of dominant taxa with similar traits. We analyzed the environmental determinants of functional trait indices using structural equation modeling to determine how different environmental stressors structure functional diversity.

Methods

Study site and invertebrate collections

The Ogeechee River is a sixth-order blackwater system located in southeastern Georgia (U.S.A.) that runs through the Coastal Plain and drains into the Atlantic Ocean. The Ogeechee River is unregulated and low-gradient; consequently, it floods its banks during periods of high precipitation, creating a dynamic similar to that described by the Flood Pulse Concept (Junk et al. 1989). The source of most of the seston in the river, an important food resource for macroinvertebrates (Benke & Wallace 2015), is the forested floodplain (Meyer 1990). Sampling in both the 1980s time period and the 2010s time period took place along the same 1-km reach of the Ogeechee River (32°11′29″ N, 81°24′58″ W).

In the 2010s, we conducted two years of approximately monthly sampling designed to follow that in the 1980s (Benke & Parsons 1990); fidelity to the original methods was enabled by the participation of Parsons in both periods of sampling. The 1980s sampling was conducted from December 1981 to November 1983 (25 dates). We conducted the 2010s sampling from July 2015 to August 2017 (24 dates). We collected submerged woody debris ("snags") from the main channel by boat or canoe using a longitudinal sieve with 100μm mesh. We collected ten snags each date. Invertebrates were washed from snags in the lab, fixed in 10% formalin, then preserved in 70% ethanol. Surface area of snags was measured, and invertebrate densities were calculated per square meter of wood, then the average density was calculated from the ten snags collected on each sampling date. A full description of field and lab methods is included in Chapter 2.

Invertebrates were identified using keys by Merritt et al. (2008), Epler (2010), and Morse et al. (2017a) with a dissecting microscope. Taxonomic resolution of identifications primarily

followed that of the 1980s data; insects were identified to genus except Chironomidae and Simuliidae (Diptera). Mayflies (Ephemeroptera) were identified to family in one year of the 1980s sampling, so to maintain consistency, all genera were grouped into family unless the family was represented by a single genus in the Ogeechee collections, e.g. *Tricorythodes* (Leptohypidae). For the analyses presented here, non-insect taxa, individuals in a condition that could only be identified to order, and any taxa that totaled less than 100 individuals m^{-2} across both time periods were removed from the dataset.

Environmental data

We obtained discharge and water chemistry data from the Ogeechee River in both time periods from the United States Geological Survey gage 02202500 (Ogeechee River near Eden, GA; USGS 2018a) using and the *waterData* package in R (Ryberg & Vecchia 2017). We calculated the mean daily discharge for the two weeks prior to each sampling date. We obtained water temperature (°C), pH, dissolved organic carbon (unfiltered, mg L^{-1}), dissolved oxygen (mg L^{-1}), conductivity (µS cm^{-1} at 25°C), and alkalinity (mg L^{-1} $CaCO_3$) values for each month corresponding to snag sampling in both time periods from an online database of USGS water quality testing at gage 02202190 (Ogeechee River near Oliver, GA; USGS 2018b). Each of the environmental metrics were compared by season, period, and the two-way interaction using an analysis of variance (ANOVA). Significance was considered at $P<0.001$ to account for multiple comparisons.

Community analyses

Differences in aquatic insect community abundances were analyzed with a permutational analysis of variance (PERMANOVA) with 10,000 permutations. A Bray-Curtis measure of distance was applied to a *ln(x+1)*-transformed abundance matrix. We compared communities by

season, period, and the two-way interaction using the *vegan* package in R (Oksanen et al. 2018). We conducted an indicator taxa analysis with 10,000 permutations on insect communities by time period and by season within each time period using the *indicspecies* package in R (De Caceres & Legendre 2016).

Functional trait metrics

We assigned trait values to insect taxa using designations following Poff et al. (2006). We selected the following traits: Development, Female dispersal, Rheophily, Thermal preference, Habit, and Trophic habit. We chose these traits to represent a mixture of potential responses based on life history, the environment, and trophic position. Taxa were scored categorically within each trait; the categories included in each trait are shown in Table 4.1.

We calculated functional richness (FRic) and functional dispersion (FDis) using *FD* package in R (Laliberté & Legendre 2010, Laliberté et al. 2014). FRic corresponds to the amount of trait space occupied (Villéger et al. 2008, Schleuter et al. 2010); FDis measures the average distance of each taxon to the centroid of the community, and provides a measure of trait complementarity (Laliberté & Legendre 2010). FDis is weighted by the abundance of taxa, while FRic is independent of abundance. We estimated differences in both indices by period, season, and the two-way interaction using an ANOVA. FDis was *ln(x)*-transformed to meet test assumptions.

Path analysis

We used structural equation models (SEMs) to assess environmental drivers of FRic and FDis in both time periods. Model fit was evaluated using chi-square tests comparing expected and observed covariance of model structures (as in Grace 2006). Structural equation models were considered to fit the data when there was no difference in expected and observed covariance ($p >$

0.05). Causal pathways or correlations between variables were added to the original hypothesized model following single degree of freedom chi-square criteria (Grace et al. 2010).

We constructed two sets of two SEMs: FRic and FDis for both the 1980s and 2010s. All SEMs were estimated by maximum likelihood. The direction and magnitude of the causal relationship between variables are represented by standardized path coefficients; these values allow for the comparison of relationship strengths within the SEM (Grace 2006). All data were scaled (i.e. centered on the mean and then divided by the standard deviation) prior to analysis. Analyses were conducted using the *lavaan* package in R (Rosseel 2012).

Endogenous environmental variables included discharge, temperature, pH, dissolved carbon, dissolved oxygen, conductivity, calcium carbonate. Season was the only exogenous variable (i.e., not affected by other variables). Season was scored 1-4, with winter being 1 and fall being 4.

All above analyses were conducted in the R statistical program (version 3.5.0, R Core Team 2018). Significance was considered at $p < 0.05$ unless otherwise stated.

Results

Environmental data

Most of the environmental characteristics varied by season: temperature ($F_{3,41} = 40.79$, $p < 0.0001$), dissolved oxygen ($F_{3,41} = 18.49$, $p < 0.0001$), calcium carbonate ($F_{3,40} = 7.73$, $p = 0.0003$), and conductivity ($F_{3,40} = 6.98$, $p = 0.0007$). Water temperature ranged from 5.5°C to 29.5°C. Dissolved oxygen was highest in winter and lowest in the summer, with a maximum of 12 mg L^{-1} and a minimum of 4.6 mg L^{-1}. Calcium carbonate was highest in fall and lowest in winter, ranging from 6 mg L^{-1} to 52 mg L^{-1}. Conductivity was also highest in fall and lowest in winter, ranging from 47 µS cm^{-1} to 171 µS cm^{-1}. None of these variables varied significantly by

time period or by the interaction of season and time period. Dissolved carbon and pH did not differ significantly by season, period, or the season-period interaction.

Community analysis

The 1980s community matrix consisted of 54 taxa; that of 2010s consisted of 59 taxa. The 1980s contained 3 unique taxa; the 2010s contained 8 unique taxa. Community structure was significantly different between time periods ($F_{1,41} = 17.37$, $R^2 = 0.213$, $p < 0.0001$) and among seasons ($F_{3,41} = 6.04$, $R^2 = 0.222$, $p < 0.0001$); there was also a significant interaction of season and time periods on community composition ($F_{3,41} = 1.68$, $R^2 = 0.062$, $p = 0.0437$). A nonmetric multidimensional scaling ordination of the distance between communities showed that, in the 1980s, winter and spring communities were mostly similar to each other, while summer and fall communities were relatively clustered; however, communities were less clustered by season in the 2010s (Fig 4.1).

The results of the indicator taxa analyses by period included two taxa that were characteristic of the 1980s relative to the 2010s, while 18 taxa that were characteristic of the 2010s (Table 4.2). We conducted seasonal indicator taxa analyses separately for each time period due to the significant interaction between season and time period indicated by the PERMANOVA. Certain taxa retained significant seasonal characterization across time periods: *Taeniopteryx* (Plecoptera)*, Tricorythodes,* and Ephemerellidae (Ephemeroptera), but for the most part seasonal indicator taxa were different between time periods (Table 4.2). One example, *Nectopsyche* (Trichoptera) was strongly seasonal in the 1980s, but had increased in the 2010s to be a characteristic taxon of the overall time period.

Functional diversity metrics

We observed a significant difference in FRic by period ($F_{3,41} = 8.47$, $p = 0.0058$), but not by season or by the season-period interaction (Fig 4.2A). FRic was 0.6x higher in the 2010s. FDis was significantly different by season ($F_{3,41} = 2.91$, $p = 0.0458$), but not by period or by the season-period interaction. FDis was highest in summer and fall (Fig 4.2B).

Path analysis

FRic and FDis varied between time periods in the extent to which values were explained by environmental parameters. Models for FRic and FDis in the 1980s had identical fit (both: Chi-square = 11.883, DF = 11, $p = 0.372$). However, there were not any significant environmental predictors of FRic in the 1980s (Fig 4.3A), while FDis was significantly predicted by temperature, conductivity, and dissolved oxygen (Fig 4.3B). Both models for the 2010s required more pathways to increase model fit than the 1980s. In the 2010s, model fit for FDis (Chi-square = 7.40, DF = 3, $p = 0.060$) was better relative to that of FRic (Chi-square = 16.21, DF = 5, $p = 0.006$). 2010s FRic was significantly explained by carbon and pH (Fig 4.3C); 2010s FDis was significantly explained by conductivity (Fig 4.3D).

Discharge was consistently negatively affected by advancing season (starting in winter) in all models with similar magnitude; likewise, temperature was consistently positively affected by season, though to a lower magnitude in the 2010s compared to the 1980s. In the 1980s, carbon decreased with seasonal progression (path coefficient of -0.42), but in the 2010s FRic model, carbon was positively linked to season (0.37). Season and carbon were not significantly related in the 2010s FDis model. In both periods, temperature had a consistent negative effect on dissolved oxygen. Discharge was negatively related to conductivity and calcium carbonate, with higher magnitude in the 2010s.

Discussion

The effects of long term ecological change on functional diversity are beginning to be characterized in a variety of environments and taxa (Villéger et al. 2010, Frainer et al. 2017, Jarzyna & Jetz 2018). Here, we showed that the aquatic insect communities of the Ogeechee River have undergone both taxonomic and functional change over the past 30 years. Taxonomic community composition was significantly different between time periods, and the taxa characterizing seasonal communities have also changed. Functional richness increased from the 1980s to the 2010s, while functional dispersion varied by season. We also found differences in environmental determinants of functional trait metrics between time periods.

Similar to our findings, an increase in functional richness and lack of change in functional dispersion between communities separated by more than a decade was reported by Villéger et al. (2010) studying tropical fish communities; Frainer et al. (2017) also found increases in functional richness in arctic fish communities after 8 years of warming, due to range expansions of generalist species. In Chapter 3, we studied caddisfly taxa that had appeared or increased from the 1980s to the 2010s; several of these taxa were either generalists or had a similar trophic habit to the declining Hydropsychidae. Therefore, while species and functional richness increased, the similarity of functional dispersion between the two time periods suggests that newly established taxa are largely functionally redundant.

The results of measuring functional diversity, however, can be relative. For example, we used invertebrate densities to weight estimates of functional dispersion. Density was chosen over biomass to be inclusive of taxa for which biomass calculations were unavailable, but using biomass would also likely return different results. Additionally, these changes are examined on a local scale. Jarzyna and Jetz (2018) showed that taxonomic and functional richness of bird

communities increased over time on small scales, but these changes diminished over global scales. Most importantly, values of functional diversity indices depend on the traits chosen to analyze, so the selection of traits must pertain to focal questions of the study (Saito et al. 2015). For our analysis, we selected traits with the relevance to organisms' ecological niche and seasonal presence in the community (Poff et al. 2006), but an analysis focusing more on life history, morphology, or mobility may return different results.

Using structural equation modeling, we were able to associate the richness and dispersion of functional traits with individual parameters. This approach allowed us to examine functional diversity outside of the categorical groupings of period and season, as some environmental metrics were not associated with seasonal change. We expected discharge to be important to functional diversity metrics, as it appeared to be correlated with trophic habit-specific declines in biomass and abundance in the Ogeechee River (Chapter 2). Also, discharge has been shown to be an important metric structuring both functional (Schriever et al. 2015, Ruhi et al. 2018) and taxonomic (Scholl et al. 2016) aspects of communities in other systems. Discharge was not directly related to functional indices in either time period, but it did indirectly affect significant predictors such as carbon (2010s FRic; though this model had the lowest fit) and conductivity (1980s and 2010s FDis). Long-term changes in discharge may be responsible for differences observed between the 1980s and the 2010s, but monthly flow variation does not have an impact within each time period.

The parameters with direct impacts on functional indices varied between time periods. Functional richness in the 1980s had no significant environmental predictors, while it was positively correlated to carbon and pH in the 2010s. Neither carbon nor pH exhibited significant variation by time period or season, but it appears that variation of these characteristics within the

2010s has become more important to the composition of traits. Functional dispersion was negatively related to conductivity in both time periods. Conductivity, though sometimes an indication of pollution (Walsh et al. 2001, Morgan et al. 2012), was positively related to alkalinity and negatively related to discharge in both time periods. A limestone spring is responsible for high-alkalinity inputs in the Ogeechee River (Meyer et al. 1997).

Using functional diversity metrics in biomonitoring has been proposed as way to assess human impact on rivers and streams (Saito et al. 2015). This approach may enable the identification of specific stressors in a region. Our study shows that the influence of various environmental characteristics on functional diversity is changing; specifically, continuing assessment of functional richness and dispersion may illuminate the relationship of these metrics with conductivity, carbon, and pH in the Ogeechee River. Also, the evaluation of functional diversity may provide context for changes in traditional taxonomic metrics; our studies supported previous findings that that increases in taxonomic richness and changes in community composition do not necessarily correspond to novel niche occupation. Furthermore, potential studies of present ecological metabolism of the Ogeechee compared to that of the 1980s (Meyer and Edwards 1990, Meyer et al. 1997) would provide insight into whether these community-level changes in aquatic insects correspond to a change in carbon processing, with potential impacts on other members of the food web and downstream ecosystems.

Tables

Table 4.1. Traits and trait states used to calculate metrics of functional diversity.

Trait	Trait state
Development	Semivoltine
	Univoltine
	Multivoltine
Female dispersal	High (<1 km)
	Low (>1 km)
Rheophily	Depositional
	Depositional and erosional
	Erosional
Thermal preference	Cold/cool
	Cool/warm
	Warm
Habit	Burrow
	Climb
	Sprawl
	Cling
	Swim
Trophic habit	Collector-gatherer
	Collector-filterer
	Herbivore
	Predator
	Detritivore

Table 4.2. Results of indicator taxa analyses by time period and season on *ln(x+1)*-transformed community density matrices.

Analysis	Group	Taxon	Higher taxon	stat	p-value
Period	1980s	*Pteronarcys*	Plecoptera	0.960	<0.001
		Tipulidae	Diptera	0.529	0.009
	2010s	Elmidae	Coleoptera	0.948	<0.001
		Hydroptilidae	Trichoptera	0.942	<0.001
		Leptoceridae	Trichoptera	0.866	<0.001
		Nectopsyche	Trichoptera	0.861	<0.001
		Macrostemum	Trichoptera	0.846	<0.001
		Brachycentrus	Trichoptera	0.791	<0.001
		Argia	Odonata	0.761	<0.001
		Triaenodes	Trichoptera	0.715	<0.001
		Neotrichia	Trichoptera	0.707	<0.001
		Ceraclea	Trichoptera	0.705	<0.001
		Polycentropodidae	Trichoptera	0.650	0.002
		Cyrnellus	Trichoptera	0.645	<0.001
		Coenagrionidae	Odonata	0.635	<0.001
		Scirtidae	Coleoptera	0.612	<0.001
		Cernotina	Trichoptera	0.577	<0.001
		Paraleptophlebia	Ephemeroptera	0.540	0.004
		Baetisca	Ephemeroptera	0.538	0.015
		Enallagma	Odonata	0.524	0.009
1980s	Spring	*Ironoquia*	Trichoptera	0.707	0.027
	Summer	*Dineutus*	Coleoptera	0.82	0.005
	Fall, Winter	*Taeniopteryx*	Plecoptera	0.763	0.016
	Summer, Fall	*Microcylloepus*	Coleoptera	0.903	0.003
		Caenidae	Ephemeroptera	0.901	<0.001
		Tricorythodes	Ephemeroptera	0.877	<0.001
		Nectopsyche	Trichoptera	0.803	0.007
	Winter, Spring, Summer	*Isonychia*	Ephemeroptera	0.949	0.003
	Fall, Winter, Spring	Ephemerellidae	Ephemeroptera	0.942	0.005
		Neoperla	Plecoptera	0.892	0.010
2010s	Winter	*Taeniopteryx*	Plecoptera	0.805	0.003
		Paraleptophlebia	Ephemeroptera	0.730	0.027
	Summer	Gyrinidae	Coleoptera	0.655	0.043
	Winter, Spring	*Perlesta*	Plecoptera	0.944	<0.001
		Ephemerellidae	Ephemeroptera	0.905	<0.001
	Spring, Summer	*Brachycentrus*	Trichoptera	0.908	0.002
	Summer, Fall	*Corydalus*	Neuroptera	0.896	0.012
		Neotrichia	Trichoptera	0.876	0.001
	Spring, Summer, Fall	*Macrostemum*	Trichoptera	0.917	0.017
		Tricorythodes	Ephemeroptera	0.895	0.012
		Paragnetina	Plecoptera	0.880	0.030

Figures

Fig 4.1. Non-metric multidimensional scaling (NMDS) ordination of Bray-Curtis distance of insect communities by season and period. Relative distance of points along the NMDS axes represents dissimilarity among communities. 1980s n = 25; 2010s n = 24.

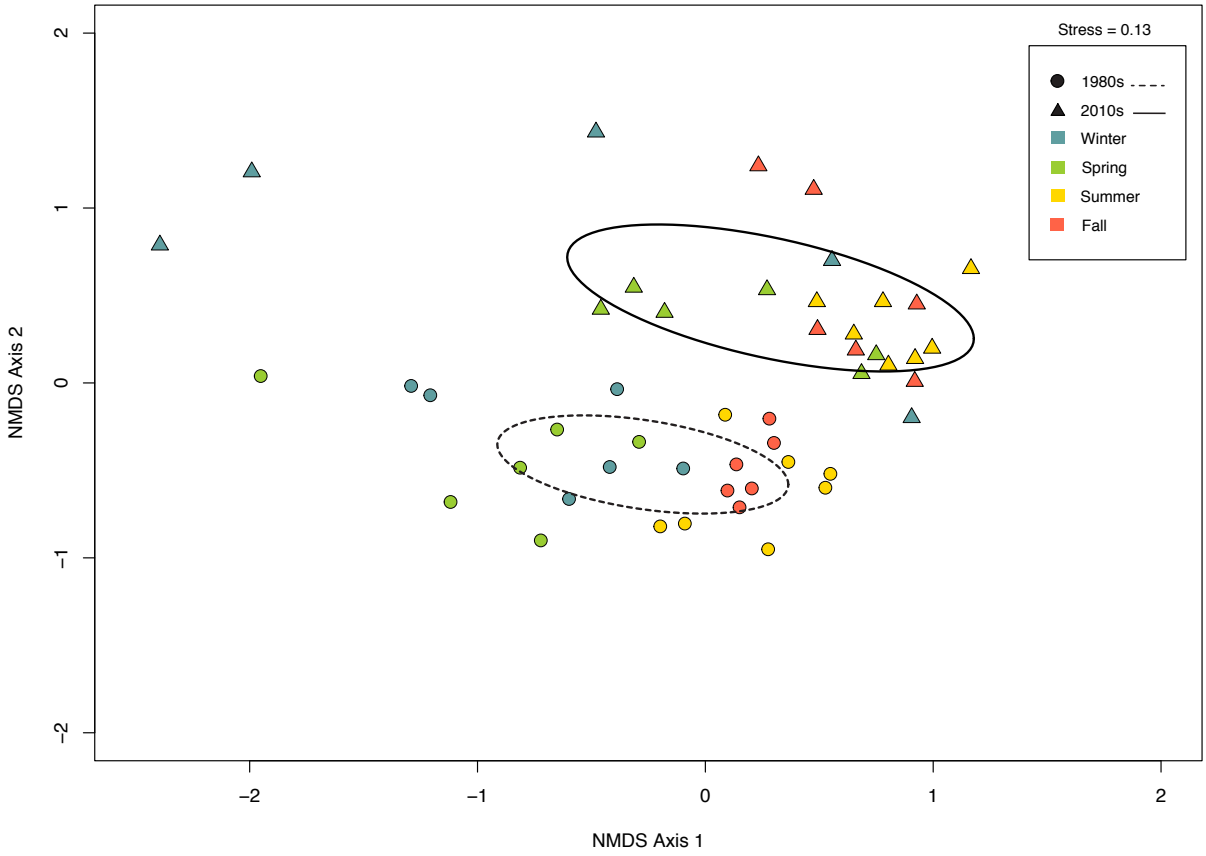


Fig 4.2. Estimates of functional richness (FRic; A) and functional dispersion (FDis; B) between 1980s and 2010s time periods and among seasons. Error bars represent 95% confidence intervals. Dashed gray lines represent 1980s means; solid gray lines represent 2010s means.

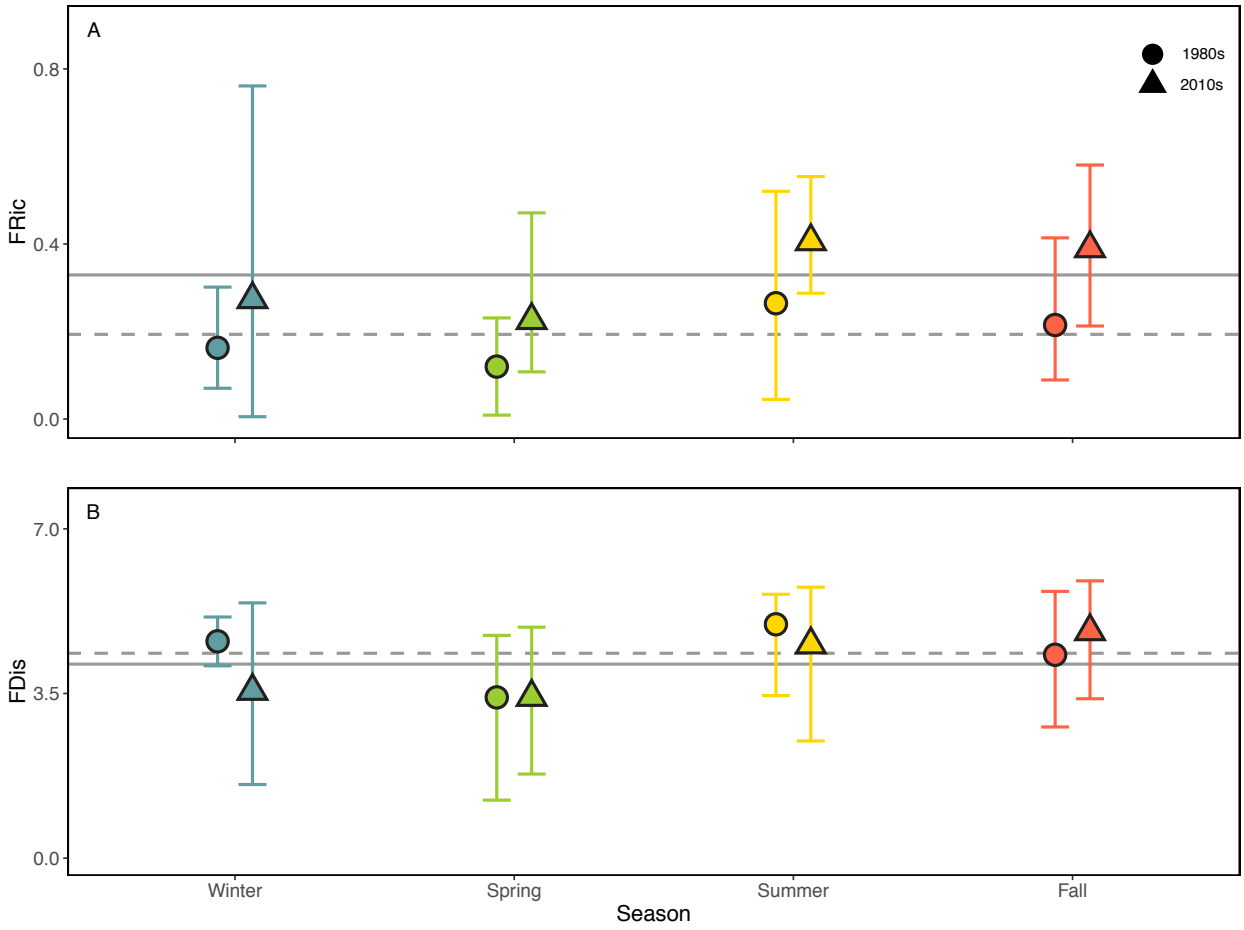
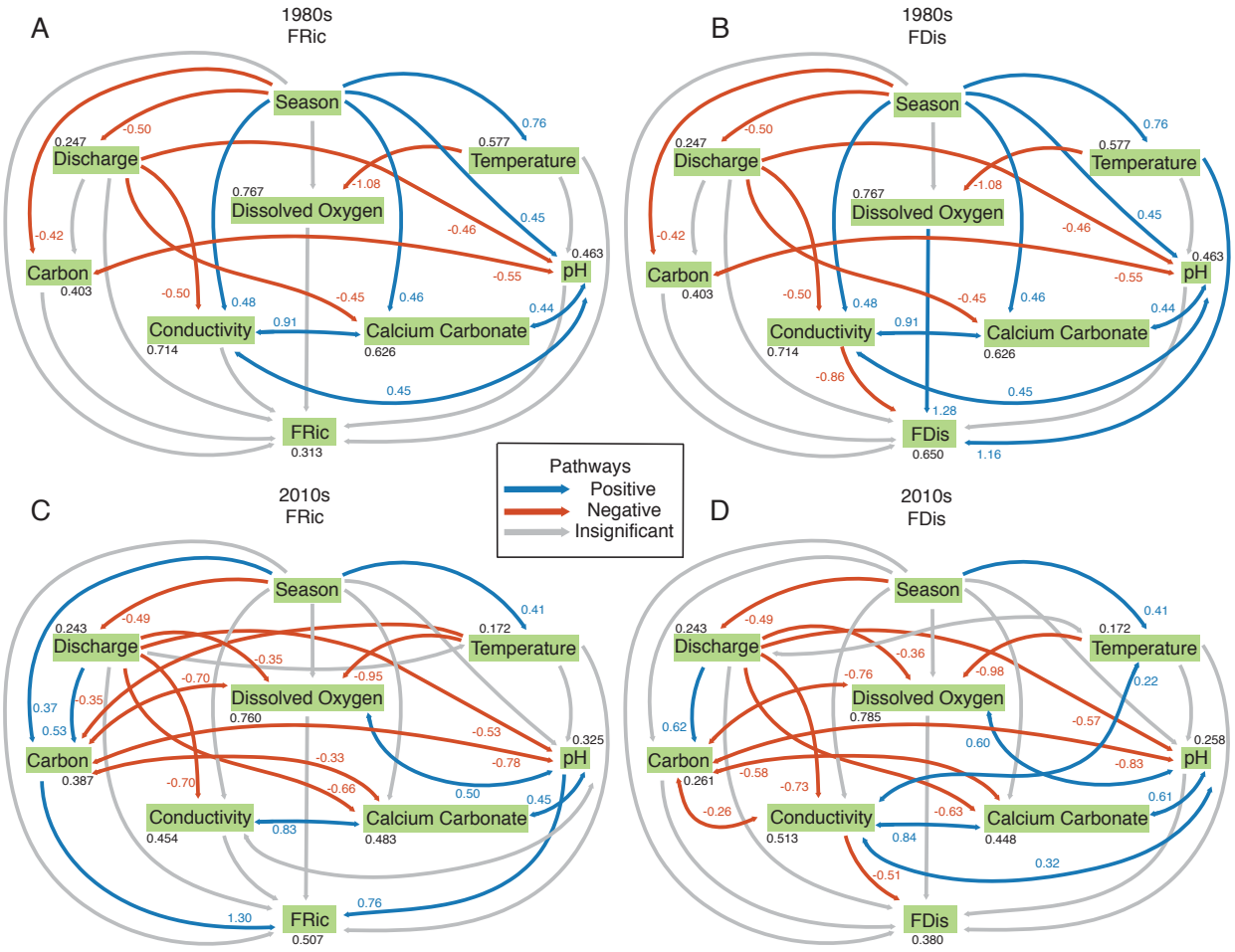


Fig 4.3. Path diagrams representing structural equation models (SEMs) for FRic and FDis individually in the 1980s (A, B respectively) and 2010s (C, D respectively). Unidirectional arrows represent causal pathways; bidirectional arrows represent correlational pathways. Path coefficients representing the magnitude and direction of effect are associated with each pathway. Numbers in black below each response variable are the R-squared values.



CHAPTER 5

CONCLUSION

In this series of studies, we analyzed the changing composition of aquatic invertebrates inhabiting the Ogeechee River. Similar to studies of terrestrial invertebrates populations, we found that insect biomass has declined significantly over the past few decades, potentially associated with changes in the timing and magnitude of discharge and subsequent carbon delivery by the river-floodplain dynamic. Focusing on caddisflies of the Ogeechee River, we showed that Trichoptera generic richness had increased over the past 30 years, as had the abundance of several genera that were relatively scarce in the 1980s. An analysis of functional trait diversity suggested that newly-established taxa such as these were functionally redundant in the system, and that the influences of various environmental parameters on community trait structure have changed. We hope our study will contribute to characterizing the changes that can be expected from river communities in this epoch of worldwide declines in biomass and changes in biodiversity.

REFERENCES

Banks, N. (1905). Descriptions of new Nearctic Neuropteroid insects. *Transactions of the American Entomological Society*, 32, 1–20.

Banks, N. (1909). Two new caddice flies. *Entomological News*, 20, 342.

Barrett, T., & Fleming, J. (2011). *Fish kill investigation report: Ogeechee River; Effingham, Bulloch, Screven, Bryan, and Chatham Counties, Georgia*. Georgia Department of Natural Resources, Wildlife Resources Division, Fisheries Section.

Baudron, A. R., Needle, C. L., Rijnsdorp, A. D., & Tara Marshall, C. (2014). Warming temperatures and smaller body sizes: Synchronous changes in growth of North Sea fishes. *Global Change Biology*, 20, 1023–1031. https://doi.org/10.1111/gcb.12514

Baxter, C. V, Fausch, K. D., & Saunders, W. C. (2005). Tangled webs: Reciprocal flows of invertebrate prey link streams and riparian zones. *Freshwater Biology*, 50, 201–220.

Benke, A. C. (1990). A perspective on America's vanishing streams. *Journal of the North American Benthological Society*, 9, 77–88.

Benke, A. C. (2001). Importance of flood regime to invertebrate habitat in an unregulated river – floodplain ecosystem. *Journal of the North American Benthological Society*, 20, 225–240.

Benke, A. C. (2018). River food webs: an integrative approach to bottom-up flow webs, top-down impact webs, and trophic position. *Ecology*, 99, 1370–1381.

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

Benke, A. C., Chaubey, I., Ward, G. M., Dunn, E. L., Oct, N., Ward, M., & Dunn, E. L. (2000). Flood pulse dynamics of an unregulated river floodplain in the Southeastern U.S. Coastal Plain, *Ecology*, 81, 2730–2741.

Benke, A. C., & Parsons, K. A. (1990). Modelling black fly production dynamics in blackwater streams. *Freshwater Biology*, 24, 167–180.

Benke, A. C., & Wallace, J. B. (1997). Trophic basis of production among riverine caddisflies: implications for food web analysis. *Ecology*, 78, 1132–1145.

Benke, A. C., & Wallace, J. B. (2015). High secondary production in a Coastal Plain river is dominated by snag invertebrates and fuelled mainly by amorphous detritus. *Freshwater Biology*, 60, 236–255.

Benke, A. C., Wallace, J. B., Harrison, J. W., & Koebel, J. W. (2001). Food web quantification using secondary production analysis: predaceous invertebrates of the snag habitat in a subtropical river. *Freshwater Biology*, 46, 329–346.

Blickle, R. (1979). Hydroptilidae (Trichoptera) of America north of Mexico. *New Hampshire Agricultural Experiment Station Bulletin*, 509, 1–97.

Bonada, N., Prat, N., Resh, V. H., & Statzner, B. (2006). Developments in aquatic insects biomonitoring: A comparative analysis of recent approaches. *Annual Review of Entomology*, 51, 495–523.

Comte, L., Cucherousset, J., Boulêtreau, S., & Olden, J. D. (2016). Resource partitioning and functional diversity of worldwide freshwater fish communities. *Ecosphere*, 7, e01356.

Davis, J. M., Rosemond, A. D., Eggert, S. L., Cross, W. F., & Wallace, J. B. (2010). Long-term nutrient enrichment decouples predator and prey production. *Proceedings of the National Academy of Sciences*, 107, 121–126

De Caceres, M., & Legendre, P. (2016). indicspecies: Relationship between species and groups of sites. R package version 1.7.6.

Dirzo, R., Young, H. S., Galetti, M., Ceballos, G., Isaac, N. J. B., & Collen, B. (2014). Defaunation in the Anthropocene. *Science*, 345, 401–406.

Epler, J. (2010). *The water beetles of Florida: an identification manual for the families Chrysomelidae, Curculionidae, Dryopidae, Dytiscidae, Elmidae, Gyrinidae, Haliplidae, Helophoridae, Hydraenidae, Hydrochidae, Hydrophilidae, Noteridae, Psephenidae, Ptilodactylidae and Scirtidae*. State of Florida Department of Environmental Protection. Tallahassee, Florida.

Farrae, D. J., Albeke, S. E., Pacifici, K., Nibbelink, N. P., & Peterson, D. L. (2014). Assessing the influence of habitat quality on movements of the endangered shortnose sturgeon. *Environmental Biology of Fishes*, 97: 691–699.

Floyd, M., Morse, J., & McArthur, J. (1993). Aquatic insects of Upper Three Runs Creek, Savannah River Site, South Carolina. Part IV: Caddisflies (Trichoptera) of the Lower Reaches. *Journal of Entomological Science*, 28, 85–95.

Frainer, A., Primicerio, R., Kortsch, S., Aune, M., Dolgov, A. V., Fossheim, M., & Aschan, M. M. (2017). Climate-driven changes in functional biogeography of Arctic marine fish communities. *Proceedings of the National Academy of Sciences*, 114, 12202–12207.

Georgia Water Coalition. (2017). *Watering Georgia: The state of water and agriculture in Georgia*. Athens, GA.

Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science*, 293, 2248–2251.

Grace, J. B. (2006). *Structural Equation Modeling and Natural Systems*. Cambridge University Press, Cambridge, UK.

Grace, J. B., Anderson, T. M., Olff, H., & Scheiner, S. M. (2010). On the specification of structural equation models for ecological systems. *Ecological Monographs*, 80, 67–87.

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., … De Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. *PLoS ONE*, 12, e0185809.

Hershey, A., & Hiltner, A. (1988). Effect of a caddisfly on black fly density: Interspecific interactions limit black flies in an arctic river. *Journal of the North American Benthological Society*, 7, 188–196.

Holt, C., Pfitzer, D., Scalley, C., Caldwell, B., & Batzer, D. (2015). Macroinvertebrate community responses to annual flow variation from river regulation: An 11-year study. *River Research and Applications*, 31, 798–807.

Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. *Global Change Biology*, 22, 180–189.

Jarzyna, M. A., & Jetz, W. (2018). Taxonomic and functional diversity change is scale dependent. *Nature Communications*, 9, 2565.

Jonsson, M., Hedström, P., Stenroth, K., Hotchkiss, E. R., Vasconcelos, F. R., Karlsson, J., & Byström, P. (2015). Climate change modifies the size structure of assemblages of emerging aquatic insects. *Freshwater Biology*, 60, 78–88.

Jourdan, J., O'hara, R. B., Bottarin, R., Huttunen, K.-L., Kuemmerlen, M., Monteith, D., … Haase, P. (2018). Effects of changing climate on European stream invertebrate communities: A long-term data analysis. *Science of the Total Environment*, 621, 588–599.

Junk, W., Bayley, P. B., & Sparks, R. E. (1989). The Flood Pulse Concept in River-Floodplain Systems. In D. P. Dodge (Ed.), *Proceedings of the International Large River Symposium* (pp. 110–127). Canadian Special Publication of Fisheries and Aquatic Sciences 106.

Kakouei, K., Kiesel, J., Domisch, S., Irving, K. S., Jähnig, S. C., & Kail, J. (2018). Projected effects of climate-change-induced flow alterations on stream macroinvertebrate abundances. *Ecology and Evolution*, 8, 3393.

Keiper JB, & Foote BA. (1999). Biology and immature stages of two species of Hydroptila Dalman (Trichoptera: Hydroptilidae) which consume *Cladophora* (Chlorophyta). *Proceedings of the Entomological Society of Washington*, 101, 514–521.

Keiper, J. B., & Foote, B. A. (2000). Biology and larval feeding habits of coexisting Hydroptilidae (Trichoptera) from a small woodland stream in northeastern Ohio. *Annals of the Entomological Society of America*, 93, 225–234.

Kirwan, L., Lüscher, A., Sebastià, M. T., Finn, J. A., Collins, R. P., Porqueddu, C., … Connolly, J. (2007). Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *Journal of Ecology*, 95, 530–539.

Klais, R., Norros, V., Lehtinen, S., Tamminen, T., & Olli, K. (2017). Community assembly and drivers of phytoplankton functional structure. *Functional Ecology*, 31, 760–767.

Laliberté, E., & Legendre, P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology*, 91, 299–305.

Laliberté, E., Legendre, P., & Shipley, B. (2014). FD: measuring functional diversity from multiple traits, and other tools for functional ecology. R package version 1.0–12.

Lenat, D. R. (1993). A biotic index for the Southeastern United States: Derivation and list of tolerance values, with criteria for assigning water-quality ratings. *Journal of the North American Benthological Society*, 12, 279–290.

Lenat, D., & Resh, V. (2001). Taxonomy and stream ecology: The benefits of genus- and species-level identifications. *Journal of the North American Benthological Society*, 20, 287–298.

Lister, B. C., & Garcia, A. (2018). Climate-driven declines in arthropod abundance restructure a rainforest food web. *Proceedings of the National Academy of Sciences*, 1–10.

Mackay, R. J., & Wiggins, G. B. (1979). Ecological diversity in Trichoptera. *Annual Review of Entomology*, 24, 185–208.

Malm, T., Johanson, K. A., & Wahlberg, N. (2013). The evolutionary history of Trichoptera (Insecta): A case of successful adaptation to life in freshwater. *Systematic Entomology*, 38, 459–473.

McLeod, A. (2011). Kendall: Kendall rank correlation and Mann-Kendall trend test. R package version 2.2.

McKie, B. G., Woodward, G., Hladyz, S., Nistorescu, M., Preda, E., Popescu, C., … Malmqvist, B. (2008). Ecosystem functioning in stream assemblages from different regions: Contrasting responses to variation in detritivore richness, evenness and density. *Journal of Animal Ecology*, 77, 495–504.

Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L. F., Backeljau, T., Bonte, D., … Van Dyke, H. (2018). Body-size shifts in aquatic and terrestrial urban communities. *Nature*, 558, 113–116.

Merritt, R. W., Cummins, K. W., & Berg, M. B. (2008). *An Introduction to the Aquatic Insects of North America* (4th ed.). Dubuque, Iowa: Kendall/Hunt Publishing.

Meyer, J. L. (1990). A blackwater perspective on riverine ecosystems. *BioScience*, 40, 643–651.

Meyer, J. L., Benke, A. C., Edwards, R. T., & Wallace, J. B. (1997). Organic matter dynamics in the Ogeechee River, a blackwater river in Georgia, USA. *Journal of the North American Benthological Society*, 16, 82–87.

Meyer, J. L., & Edwards, R. T. (1990). Ecosystem metabolism and turnover of organic carbon along a blackwater river continuum. *Ecology*, 71, 668–677.

Morgan, R. P., Kline, K. M., Kline, M. J., Cushman, S. F., Sell, M. T., Weitzell, R. E., & Churchill, J. B. (2012). Stream conductivity: Relationships to land use, chloride, and fishes in Maryland streams. *North American Journal of Fisheries Management*, 32, 941–952.

Morse, J.C. (2018). Trichoptera World Checklist. http://entweb.clemson.edu/database/trichopt/index.htm [Accessed 9 October 2018.]

Morse, J. C., McCafferty, W. P., Stark, B. P., & Jacobus, L. M. (2017a). *Larvae of the Southeastern USA Mayfly, Stonefly, and Caddisfly Species (Ephemeroptera, Plecoptera, and Trichoptera)*. Clemson, South Carolina: Clemson University Public Service Publishing.

Morse, J., & Holzenthal, R. (2008). Trichoptera genera. In R. Merritt, K. Cummins, & M. Berg (Eds.), *An Introduction to the Aquatic Insects of North America* (4th ed., pp. 481–552). Dubuque, IA: Kendall Hunt Publishing.

Morse, J., Holzenthal, R., & Yadamsuren, O. (2017b). Trichoptera. In J. Morse, W. McCafferty, B. Stark, & L. Jacobus (Eds.), *Larvae of the Southeastern USA Mayfly, Stonefly, and Caddisfly Species* (pp. 248–442). Clemson, SC: Clemson University Public Service Publishing.

Mouillot, D., Graham, N. A. J., Villéger, S., Mason, N. W. H., & Bellwood, D. R. (2013). A functional approach reveals community responses to disturbances. *Trends in Ecology and Evolution*, 28, 167–177.

Nelson, D., Benstead, J. P., Huryn, A. D., Cross, W. F., Hood, J. M., Johnson, P. W., … Ólafsson, J. S. (2017). Shifts in community size structure drive temperature invariance of secondary production in a stream-warming experiment. *Ecology*, 98, 1797–1806.

Oksanen, J., Blanchet, F., Friendly, M., Roeland, K., Legendre, P., McGlinn, D., … Wagner, H. (2018). vegan: Community ecology package. R package version 2.5–2.

Poff, N. L., Olden, J. D., Vieira, N. K. M., Finn, D. S., Simmons, M. P., & Kondratieff, B. C. (2006). Functional trait niches of North American lotic insects: Traits-based ecological applications in light of phylogenetic relationships. *Journal of the North American Benthological Society*, 25, 730–755.

R Core Team. (2018). R: A language and environment for statistical computing.

Rasmussen, A. K., & Morse, J. C. (2018). Distributional Checklist of Nearctic Trichoptera (August 2018 Revision). Unpublished, Florida A&M University, Tallahassee. 506 pp. [Available at http://www.Trichoptera.org]

Ratnasingham, S., & Hebert, P. (2007). BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364.

Ross, H. H. (1944). The Caddis Flies, or Trichoptera, of Illinois. *Bulletin of the Illinois Natural History Survey*, 23, 1–326.

Rosseel, Y. (2012). lavaan: An R package for structural equation modeling. *Journal of Statistical Software*, 48, 1–36.

Ruhi, A., Dong, X., McDaniel, C. H., Batzer, D. P., & Sabo, J. L. (2018). Detrimental effects of a novel flow regime on the functional trajectory of an aquatic invertebrate metacommunity. *Global Change Biology*, 24, 3749–3765.

Ruiter, D. E., Boyle, E. E., & Zhou, X. (2013). DNA barcoding facilitates associations and diagnoses for Trichoptera larvae of the Churchill (Manitoba, Canada) area. *BMC Ecology*, 13, 1–39.

Ryberg, K., & Vecchia, A. (2017). waterData: Retrieval, analysis, and anomaly calculation of daily hydrologic time series data. R package version 1.0.8.

Saito, V., Siqueira, T., & Fonseca-Gessner, A. (2015). Should phylogenetic and functional diversity metrics compose macroinvertebrate multimetric indices for stream biomonitoring? *Hydrobiologia*, 745, 167–179.

Saito, V. S., Cianciaruso, M. V., Siqueira, T., Fonseca-Gessner, A. A., & Pavoine, S. (2016). Phylogenies and traits provide distinct insights about the historical and contemporary assembly of aquatic insect communities. *Ecology and Evolution*, 6, 2965–2937.

Say, T. (1823). Descriptions of insects belonging to the order Neuroptera Linné and Latreille, collected by the expedition under the command of Major Long. *Western Quarterly Reporter*, Cincinnati, 2, 160–165.

Schleuter, D., Daufresne, M., Massol, F., & Argillier, C. (2010). A user's guide to functional diversity indices. *Ecological Monographs*, 80, 469–484.

Scholl, E. A., Rantala, H. M., Whiles, M. R., & Wilkerson, G. V. (2016). Influence of flow on community structure and production of snag-dwelling macroinvertebrates in an impaired low-gradient river. *River Research and Applications*, 32, 677–688.

Schriever, T. A., Bogan, M. T., Boersma, K. S. Cañedo-Argüelles, M., Jaeger, K. L., Olden, J. D., & Lytle, D. A. (2015). Hydrology shapes taxonomic and functional structure of desert stream invertebrate communities. *Freshwater Science*, 34, 399–409.

Sheridan, J. A., & Bickford, D. (2011). Shrinking body size as an ecological response to climate change. *Nature Climate Change*, 1, 401–406.

Slaughter, J. E. (2013). *Conservation and Restoration of the Robust Redhorse Moxostoma robustum in the Oconee River, Georgia Volume 8*. Retrieved from http://www.robustredhorse.com/f/2013 FERC Volume 8 Report.pdf

Start, D., McCauley, S., & Gilbert, B. (2018). Physiology underlies the assembly of ecological communities. *Proceedings of National Academy of Sciences*, 115, 23, 6016–6021.

Stoker, D., Falkner, A. J., Murray, K. M., Lang, A. K., Barnum, T. R., Hepinstall-Cymerman, J., … Pringle, C. M. (2017). Decomposition of terrestrial resource subsidies in headwater streams: Does consumer diversity matter? *Ecosphere*, 8, e01868.

United States Geological Survey. (2018a). Surface Water data for USA: USGS Surface-Water daily statistics. https://waterdata.usgs.gov/nwis/dvstat?

United States Geological Survey. (2018b). Water quality samples. https://nwis.waterdata.usgs.gov/ga/nwis/qwdata?

USGCRP. (2017). *Climate science special report: A sustained assessment of the U.S. Global Change Research Program*. Washington DC.

Veldboom, J. A., & Haro, R. J. (2011). Stoichiometric relationship between suspension-feeding caddisfly (Trichoptera: Brachycentridae) and seston. *Hydrobiologia*, 675, 129–141.

Villéger, S., Mason, N. W. H., & Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, 89, 2290–2301.

Villéger, S., Ramos Miranda, J., & Flores Hernández, D. (2010). Contrasting changes in taxonomic vs. functional diversity of tropical fish communities after habitat degradation. *Ecological Applications*, 20, 1512–1522.

Vörösmarty, C. J., McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., … Davies, P. M. (2010). Global threats to human water security and river biodiversity. *Nature*, 467, 555–561.

Wallace, J. B., Benke, A. C., Lingle, A. H., & Parsons, K. (1987). Trophic pathways of macroinvertebrate primary consumers in sub-tropical blackwater streams. *Archiv Für Hydrobiologie*, 4, 423–451.

Walsh, C. J., Sharpe, A. K., Breen, P. F., & Neman, J. A. S. (2001). Effects of urbanization on streams of the Melbourne region, Victoria, Australia. I. Benthic macroinvertebrate communities. *Freshwater Biology*, 46, 535-551.

Wiggins, G. (1977). *Larvae of the North American caddisfly genera (Trichoptera)*. Toronto, ON: University of Toronto Press.

Wiggins, G., & Currie, D. (2008). Trichoptera families. In R. Merritt, K. Cummins, & M. Berg (Eds.), *An Introduction to the Aquatic Insects of North America* (4th ed., pp. 439–480). Dubuque, IA: Kendall Hunt Publishing.

Zhou, X., Robinson, J. L., Geraci, C. J., Parker, C. R., Flint, O. S., Etnier, D. A., … Hebert, P. D. N. (2011). Accelerated construction of a regional DNA-barcode reference library: caddisflies (Trichoptera) in the Great Smoky Mountains National Park. *Journal of the North American Benthological Society*, 30, 131–162.

Zhou, X., Kjer, K. M., Frandsen, P. B., Holzenthal, R. W., Beet, C. R., Bennett, K. R., … Koenig, A. (2016). The Trichoptera barcode initiative: A strategy for generating a species-level Tree of Life. *Philosophical Transaction of the Royal Society B*, 371, 20160025.