ZOOPLANKTON SPECIES DIVERSITY IN THE TEMPORARY WETLAND SYSTEM OF THE SAVANNAH RIVER SITE, SOUTH CAROLINA, USA

by

MARCUS ALEXANDER ZOKAN

(Under the Direction of JOHN M. DRAKE)

ABSTRACT

Understanding how diverse species communities develop and how the species within them coexist is one of the central questions in community ecology. The temporary wetland system occurring on the Savannah River Site near Aiken, South Carolina is home to the most species rich temporary wetland zooplankton assemblage known in the world. While previous research has documented this remarkable diversity, there has been little study directed at understanding how diversity is distributed at the landscape and local scales or on investigating potential mechanisms of what has led to the high richness of this system. The collection of studies presented here examine diversity patterns in the zooplankton community, links these patterns to spatial and temporal variation, experimentally tests the effects of two important environmental factors on diversity, and describes two new species. Results indicate that long hydroperiod lengths were associated with high species richness. Wetlands with similar species assemblages were generally closer together, suggesting the importance of dispersal. Over the course of a year, diversity increased during the spring and summer months and declined toward the fall, these changes were associated with low pH, low conductivity, and high water temperature. Vegetated areas within wetlands had greater diversity than did unvegetated areas, and diversity was particularly low in areas of decaying vegetation. Temporal comparisons provide evidence for distinct seasonal communities that arise every year. Experimental tests of the impact of hydroperiod length on diversity found that shorter hydroperiods resulted in reduced species richness, and communities dominated by just a few

species. Predation was found to have no effect on diversity or community composition. During investigation of the diversity of these wetlands, two new species of the genus *Chydorus* were discovered and described. These two species differ from congeners both in morphology and phylogenetically. Together these studies describe how environmental variation can impact the diversity of the zooplankton communities within temporary wetlands and show how hydroperiod limits the richness of these systems. The results presented here provide insight into the forces that may lead to diverse communities in temporary wetlands, providing direction for future research into these dynamic ecosystems.

INDEX WORDS: diversity, zooplankton, wetland, hydroperiod, mesocosm, *Chydorus*

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2015

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ACKNOWLEDGEMENTS

I wish to thank my advisor John Drake for his enthusiasm, guidance, support, and patience throughout my graduate school career. I also thank my advisory committee of Alan Covich, Amy Rosemond, Rebecca Sharitz, and John Wares for their guidance, advice, and questions, which greatly improved the quality of my work. Special thanks goes to Rebecca Sharitz, Linda Lee, Stacey Lance, and David Scott of SREL for use of their labs, equipment, logistical support, knowledge, and providing salamanders for the mesocosm experiment. I thank the Wares lab, particularly John Wares and John Robinson, for allowing and assisting me with genetic techniques and analyses. A special thanks goes to all Drake Lab members both past and present who all contributed to my graduate career in some way, but particularly Andrea Silletti for logistical assistance, Drew Kramer for his zooplankton knowledge and help with analyses, and Reni Kaul for completing the zooplankton genetic work. I also thank the Odum School of Ecology for monetary support, and its staff for all their assistance throughout my time here.

I greatly want to thank my family, particularly my parents, Mark and Gloria Zokan, for their love and support, and for fostering and encouraging my interests throughout my life. I also thank my grandmother Lorraine Zokan and my uncle Alberto Cambra for their roles in shaping my love of nature. A special thanks to Eileen, my partner in all aspects of my life, her love, support, and patience have been unwavering; her assistance, physical, emotional, and intellectual, I could not have been without.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

General context - The wetlands of the Savannah River Site (SRS) near Aiken, South Carolina has one of the most species-rich zooplankton assemblages yet known from any temporary wetland system in the world (Mahoney et al. 1990; DeBiase & Taylor 2005). How and why should these wetlands be more species-rich than seemingly comparable systems around the world? This diverse set of wetlands provides an ideal study system to investigate questions that are broadly applicable to other temporary wetland systems. For example, are there particular environmental conditions that lead to greater diversity? Do the effects of these environmental conditions change at different scales? How does variable hydroperiod affect diversity? What effect does predation have? How do spatial and temporal heterogeneity affect diversity? While I ask these questions specifically about temporary wetlands, they arise from and feed into the large body of research on species diversity patterns and coexistence.

The problem of how species coexist in diverse communities has long been a central question of community ecology. In the mid-20th century, the question of why so many species exist was first discussed by Hutchinson (1959), shortly followed by the question of how species coexist in diverse communities (Hutchinson 1961). Research at the time focused largely on deterministic processes associated with the niche concept, namely abiotic factors, competition and predation (MacArthur 1958; MacArthur 1964; Paine 1966). However, the conceptual seeds were present for the growth of theory that included the role of non-equilibrium and stochastic processes (MacArthur & Wilson 1967; Connell 1978). The field of community ecology expanded greatly from the 1990s to the present, which brought about more extensive theoretical work attempting to explain the existence of diverse communities and the processes that lead to them (Hubbell 2001; Leibold et al. 2004; Clark 2010). Although numerous mechanisms are potentially involved in the creation and maintenance of the diversity we observe, a

conceptual synthesis of community ecology by Vellend (2010) was able to reduce all of these processes into four: selection, drift, speciation and dispersal.

In Vellend's framework, selection is the realm of deterministic processes such as response to the abiotic environment and interspecific interactions. Drift refers to the random changes in species abundances due to stochastic demographic processes. Speciation is of course the evolution of new species, whereas dispersal is the movement of organisms across the landscape. Current theories for the maintenance of diversity and coexistence often include aspects of several of these process categories.

In my second chapter I present the results of an intensive two-year survey of the zooplankton communities of a set of 14 temporary wetlands at the SRS that were selected to represent a range in environmental conditions. While I primarily focus on selection processes, these wetlands are part of a larger metacommunity through which dispersal and drift occur. Previous work has brought attention to the high diversity of the system and related it on the landscape level to hydroperiod length and wetland area (Mahoney et al. 1990). I expand on this work with greater taxonomic resolution and higher sampling frequency to more fully describe the diversity of the system. The primary goal of this chapter was to investigate patterns of α- and β-diversity on both the landscape and local scales, and ultimately link these patterns with a set of environmental factors. In addition, I specifically examine how α- and β-diversity change over time, which has been largely neglected in wetland studies despite the important seasonal dynamics of these ecosystems. The potential effects of dispersal are examined by comparing similarity and distance among these wetlands.

In the third chapter I experimentally test the effects of two selection processes, the constraint of hydroperiod length on life history and the effects of predation, on diversity and community composition. The constraint of hydroperiod length has been established as an important factor limiting the occurrence of species in temporary wetland habitats (Mahoney et al. 1990; Wellborn et al. 1996). In addition, predation by salamander larvae has been noted to have top-down effects on zooplankton communities (Holomuzki et al. 1994; Blaustein et al. 1996). These two factors were brought together in a conceptual model by Wellborn et al. (1996) termed the "predation-permanence gradient". This model states that the

constraints of hydroperiod length are strongest in wetlands with short hydroperiods, whereas the effects of predation are most important in long hydroperiod wetlands, and that both of these forces interact to produce observed zooplankton communities. In this chapter I conducted a mesocosm experiment to test the effects of these two forces on the assembly of wetland zooplankton communities.

In the fourth chapter I touch on speciation (but not the actual process) by describing two new species of the genus *Chydorus*. These two organisms were among the most abundant and widespread cladocerans in my samples; however, neither matched descriptions of known species. Here I describe these two new species morphologically and compare them to other currently known *Chydorus*. I also conducted a phylogenetic analysis using the CO1 and 16S to support the morphological data and to place these two species in a broader phylogenetic context.

Review of temporary wetland species richness - The zooplankton community of the SRS has exceptionally high species richness, with 60 cladoceran species, 25 cyclopoid copepods, 11 calanoid copepods, 2 fairy shrimp and 2 clam shrimp recorded from the site (DeBiase & Taylor 2005). In addition to the high richness on the landscape level, up to 25 cladoceran species have been collected from a single wetland (Mahoney et al. 1990). To compare how impressive this species richness is, Mahoney et al. (1990) conducted a literature review on the subject, and determined that the wetlands of SRS held the most species rich zooplankton fauna known from any temporary wetland system in the world. Because the biodiversity of temporary wetlands has become more recognized, there have been many studies published since then that have described the diversity of these unique systems around the world. Therefore, I conducted a literature review as an update to Mahoney et al. (1990).

In my review, I focused only on cladoceran species richness because they are the most species rich taxonomic group that I focus on for my own research and they are also the only group that is consistently reported in most studies. I also took care to only use data from hydrologically isolated temporary wetland systems so that studies were comparable to the wetlands of SRS. Hydrologically isolated wetlands are defined as wetlands that have no natural surface water connections to permanent aquatic habitats. This eliminates floodplain wetlands and lake-associated wetlands that periodically

receive direct inputs from a potentially larger species pool, which may include species not specifically adapted to the conditions of temporary wetland environments. Since I am most interested in the upper range of cladoceran richness in temporary wetlands, there are several studies that had few species that I chose not to include. I did not include these studies, as well as some others, because I did not believe the taxonomic resolution and sampling intensity was good enough to make for a fair comparison.

The updated literature review found that the wetland system of SRS was the most species rich temporary wetland system yet studied. The nearest in richness was a set of 200 temporary wetlands in Sicily that held 55 cladoceran species (Marrone et al. 2006) and a set of 21 wetlands in Macaé, Brazil that had 47 (Lopes et al. 2014). Of the 43 other wetland systems included, 35 of them had fewer than half as many species as SRS. In my own survey of 14 wetlands on SRS I identified 52 cladoceran species, which would place my study behind only the Sicilian wetlands. Even more amazing was the 43 cladoceran species I collected from a single wetland in SRS. Very few of the included studies stated the richness of any single wetland; however, this wetland alone exceeds the richness of all but two of the included temporary wetland systems. This particular wetland, known as Sarracenia Bay, had only 21 cladoceran species recorded from it in the earlier survey of Mahoney et al. (1990). The most species-rich wetland from Mahoney et al. (1990) was the adjacent Craig's Pond, with 25 recorded cladoceran species. This leaves open the possibility that Craig's Pond or other wetlands in SRS have even greater species richness.

It should be noted in this literature review that vast regions of the world were hardly represented. The areas with the best coverage were North America, South America, the Mediterranean region, and northern Europe. Sub-Saharan Africa was represented by just three studies, Australia by two, and Asia represented by just a single study. These regions are known to have high species richness (Forró et al. 2008), which leaves the possibility open that there may be additional temporary wetland systems of comparable or greater diversity yet to be described. Regardless, the SRS represents a true outlier in temporary wetland zooplankton species richness.

Review of Carolina bays and the processes that effect diversity in temporary wetlands - The temporary wetlands of the SRS largely belong to a class of wetlands known as Carolina Bays. They are

distinguished from similar appearing wetlands due to their unique geologic formation. They are characterized by their shallow, elliptical basins with the longest dimension oriented on a northwest to southeast axis and by the presence of a distinct sand rim (Prouty 1952; Sharitz 2003). Their origin has been the subject of some debate, but the currently accepted explanation is the modification by prevailing winds of shallow ponds that developed in landscape depressions during the mid Holocene (Grant et al. 1998; Gaiser et al. 2001). While widely distributed on the southeastern Atlantic Coastal Plain from New Jersey to northern Florida, they reach their greatest abundance in North Carolina and South Carolina (Sharitz 2003).

Carolina bays are a type of depression wetland and are functionally similar to other types of depression wetlands found throughout the southeastern USA including cypress domes and limesinks (Battle and Golladay 2001; Tiner 2003). Carolina bays usually have no natural surface drainage and receive most of their water through direct precipitation and lose it through evapotranspiration; however, at least some bays have lateral exchanges with groundwater when water levels of both are high (Schalles and Shure 1989; Lide et al. 1995; Pyzoha et al. 2008). Water conditions within Carolina bays are typically of low pH and dilute, with low concentrations of most ions (Schalles and Shure 1989; Newman & Schalles 1990). Dissolved oxygen levels vary considerably over the course of a year, becoming especially low during the summer months (Schalles and Shure 1989). These wetlands are considered polymictic, with stratification occurring diurnally and destratification occurring over night (Schalles and Shure 1989). Stratification occurs primarily in the warmer months of the year and may be more stable in forested wetlands (Moore 1970). Primary productivity is low and is dominated by macrophytes, diatoms, desmids and photosynthetic bacteria (Schalles and Shure 1989).

There are at least 300 known Carolina bays on the SRS in addition to other temporary depression wetlands that are functionally similar (Schalles et al. 1989; Kirkman et al. 1996). They are widely distributed around the SRS and primarily clustered on interfluvial ridges (Schalles et al. 1989). They typically fill over the winter and maintain water levels into spring before drying down in summer (Sharitz 2003). Some of these wetlands dry completely on an annual basis, whereas others dry fully only in

droughts (Sharitz 2003). Soils in bays on the SRS are typically sandy to sandy-loam over soil layers with greater clay content (Newman & Schalles 1990). This clay layer is less permeable than the overlying sediment and slows water loss into the ground (Lide et al. 1995). The vegetation of bays on the SRS is quite variable and is linked to hydroperiod (Sharitz 2003). Forested bays typically have shorter hydroperiods than those dominated by herbaceous vegetation; however, fire also plays a role in vegetation type (De Steven & Toner 2004). The type of vegetation in a single bay varies spatially and bays often show distinct vegetation zones (Schalles et al. 1989). The vegetation type of a bay is not a static feature and varies with drought and flood cycles in addition to fire (Kirkman & Sharitz 1994; Mulhouse et al. 2005).

Processes that impact diversity on the SRS operate on both the local scale and the landscape scale. Within a wetland, selection processes such as niche-based adaptation to microhabitats may occur. The most readily observable heterogeneity at this scale is differences in vegetation and substrate, and littoral zooplankton are known to segregate according to their apparent preferences in these factors (Tremel et al 2000; Nevalainen 2012). This niche adaptation may allow more species to occur in wetlands that have a greater number or complexity of habitat types. Another potential scenario is that at least some zooplankton species have temporal niches (Schoener 1974), where species avoid interspecific interaction by utilizing resources at different times and thus allow more species to exist in a particular wetland. Seasonal succession in zooplankton is a well-known phenomenon and there is evidence for distinct warm and cool season fauna in wetlands (Frey 1982c). Of course, the likelihood for this to occur increases with hydroperiod, as short hydroperiod sites may lack the adequate time to be partitioned.

On the other hand, species may not show affiliation to any particular habitat. This could be due to the effects of unmeasured factors or to the effects of drift and dispersal. The fluctuating water levels of temporary wetlands affect different parts of the wetland at different times, thus there are likely to be differences in location and density of resting stages for different organisms. These differences may persist in a refilled wetland and result in asynchrony in zooplankton populations and species distributions (Takahashi et al. 2008) unless evened out through dispersal to preferred habitats. Asynchrony in

environmental factors has been shown to increase local diversity in zooplankton (Steiner et al. 2011), but it is unclear if this idea can be extended to asynchrony in demography.

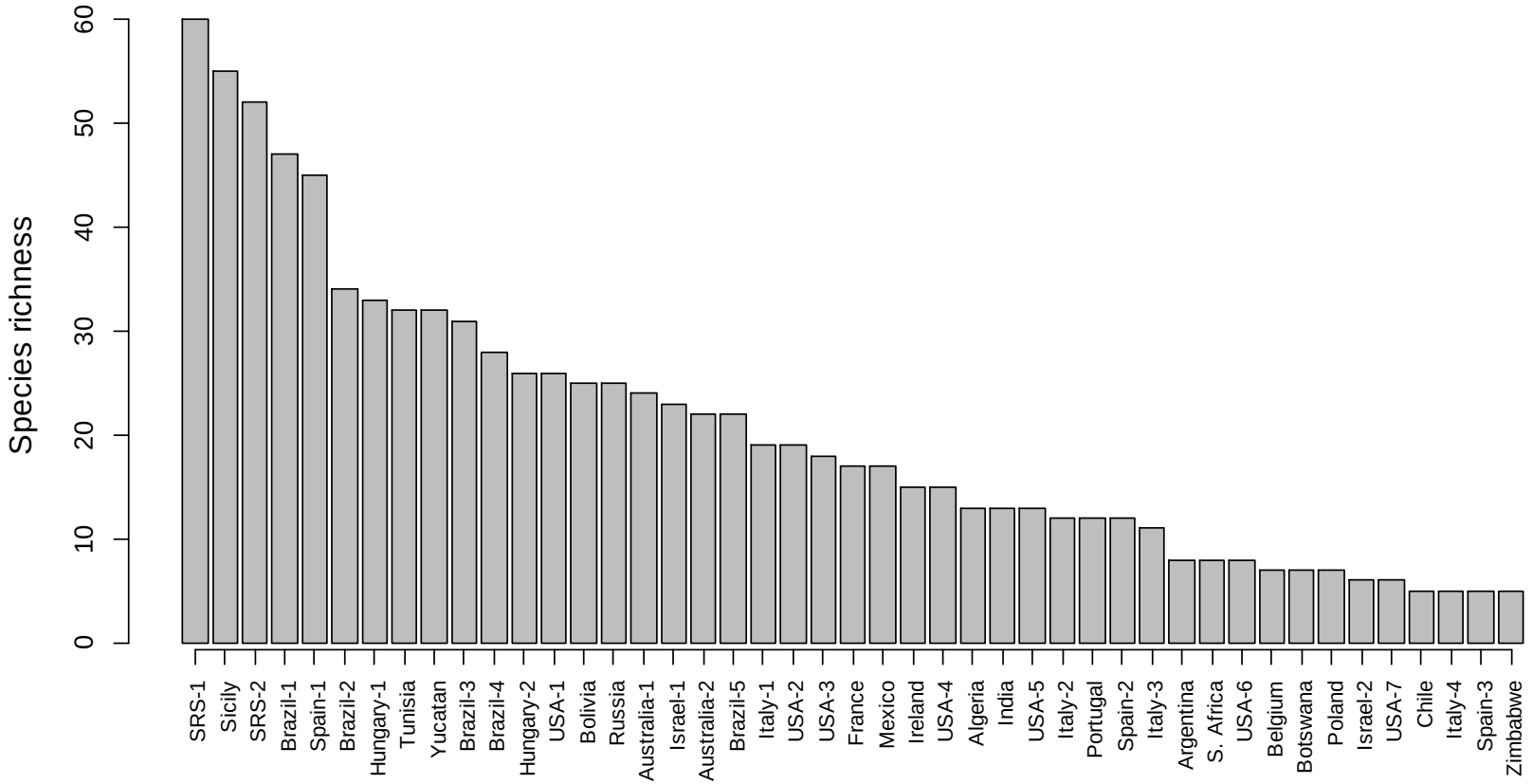
On the landscape scale, heterogeneity between wetlands is a probable cause of high beta diversity. At this scale the most obvious selection process is again niche-based adaptation to variation in the environment, as Carolina bays exhibit variation in water chemistry, vegetation and hydroperiod among others. Water chemistry in bays varies between wetlands, and differences in water chemistry, particularly pH and conductivity, can alter zooplankton species assemblages (Nevalainen et al. 2011; Korosi & Smol 2012). DeBiase & Taylor (2005) indicated that vegetation type may impact diversity, finding that forested bays had lower zooplankton species richness than open, herbaceous bays. Hydroperiod is perhaps the most notable environmental factor in temporary wetlands, since wetland drying acts as a major constraint on the life histories of aquatic organisms (Wellborn et al. 1996). Results from Carolina bays indicate that longer hydroperiod bays generally have greater zooplankton species richness than those with shorter hydroperiods (Mahoney et al. 1990; DeBiase & Taylor 2005). However, it is unclear if species within short hydroperiod wetlands are merely a subset of the community found in long hydroperiod sites or if there is in fact species turnover along a hydroperiod gradient as suggested by Wellborn et al. (1996). Another selection process, predation, could also impact diversity between wetlands, since predation pressure increases with hydroperiod and could lead to the elimination of susceptible species (Wellborn et al. 1996).

Wetland size may also have an effect on diversity. Mahoney et al. (1990) found a strong positive relationship between cladoceran richness and wetland area. However, it is unclear what wetland size represents in this context. It could be that it simply allows a greater amount of space for local scale processes, it could correlate with a greater number or diversity of microhabitats, or it could be confounded with wetland hydroperiod. Another possibility is that larger wetlands provide a larger target for potential dispersers whether passive or active. In this view, species communities are not saturated and organisms dispersing in may increase richness (Leibold et al. 2004), thus larger wetlands should accumulate greater diversity than smaller wetlands. However, genetic evidence from zooplankton in Carolina bays shows

high differentiation between sites indicating either low dispersal rates or persistent founder effects (Boileau & Taylor 1991). Distance between wetlands may also play a role in the importance of dispersal within this system, as zooplankton assemblage similarity was greater over short distances (Mahoney et al. 1990). Whether this is due to dispersal or that nearby wetlands are more similar in habitat is unclear.

Figure 1.1. A comparison of cladoceran species richness from temporary wetland systems around the world, values were taken from published studies. The studies included are as follows from left to right: SRS-1 = Savannah River Site, USA – Debiase and Taylor (2005), Sicily = Marrone et al. (2006), SRS-2 = the present study, Brazil-1 = Lopes et al. (2014), Spain-1 = Fahd et al. (2009), Brazil-2 = Van Damme and Dumont (2010), Hungary-1 = Boven (2009), Tunisia = Turki and Turki (2010), Yucatan = Elías-Gutiérrez et al. (2006), Brazil-3 = Lopes et al. (2014), Brazil-4 = Hollwedel et al. (2003), Hungary-2 = Toth et al. (2014), USA-1 = Gaiser and Lang (1998), Bolivia = Declerck et al. (2010), Russia = Yevdokimov and Yermokhin (2009), Australia-1 = Bayly (1997), Israel-1 = Bromley (1993), Australia-2 = Morton and Bayly (1977), Brazil-5 = Dinizl (2013), Italy-1 = Crosetti and Margaritora (1987), USA-2 = Mackin (1931), USA-3 = King et al. (1996), France = Waterkeyn et al. (2008), Mexico = Rodriguez-Almaraz and Leija-Tristan (1995), Ireland = Reynolds et al. (2004), USA-4 = Jenkins et al. (2003), Algeria = Samraoui (2002), India = Karuthapandi et al. (2012), USA-5 = Prophet et al. (1959), Italy-2 = Vagaggini et al. (2002), Portugal = Caramujo and Boavida (2010), Spain-2 = Sahuquillo and Miracle (2010), Italy-3 = Mura and Brecciaroli (2003), Argentina = Echaniz and Vignatti (2010), South Africa = Coetzer (1981), USA-6 = Modlin (1982), Belgium = Forro et al. (2003), Botswana = Lindholm et al. (2009), Poland = Kuczynska-Kippen et al. (2013), Israel-2 = Eitam et al. (2004), USA-7 = Ripley and Simovich (2009), Chile = De Los Ríos-Escalante (2012), Italy-4 = Tavernini et al. (2005), Spain-3 = Boix et al. (2001), Zimbabwe = Anusa et al. (2012)

Figure 1.1.


Species richness
0
10
20
30
40
50
60
SRS-1
Sicily
SRS-2
Brazil-1
Spain-1
Brazil-2
Hungary-1
Tunisia
Yucatan
Brazil-3
Brazil-4
Hungary-2
USA-1
Bolivia
Russia
Australia-1
Israel-1
Australia-2
Brazil-5
Italy-1
USA-2
USA-3
France
Mexico
Ireland
USA-4
Algeria
India
USA-5
Italy-2
Portugal
Spain-2
Italy-3
Argentina
S. Africa
USA-6
Belgium
Botswana
Poland
Israel-2
USA-7
Chile
Italy-4
Spain-3
Zimbabwe

CHAPTER 2

ZOOPLANKTON SPECIES DIVERSITY IN THE TEMPORARY WETLAND SYSTEM OF THE SAVANNAH RIVER SITE, SOUTH CAROLINA, USA

Abstract

The Savannah River Site (SRS) near Aiken, South Carolina, USA, is home to the most diverse assemblage of zooplankton found in any temporary wetland system in the world. However, the diversity of this system has not been fully characterized. Here we present the results of a two-year biweekly survey of the zooplankton communities of 14 wetlands on SRS. Our goal was to examine patterns in α- and β-diversity on both the landscape and local scales and link these patterns to environmental variation among and within these wetlands. We also examined how α- and β-diversity change in these dynamic systems over time. Hydroperiod length proved to be an important factor that led to high species richness on the landscape scale, whereas distance, hydroperiod, and pH were related to community differences between wetlands. Both α- and β-diversity varied considerably over time. High α-diversity was associated with the warmer months of the year when pH and conductivity were lower. Temporal changes in β-diversity suggest that similar species assemblages arise under similar environmental conditions annually. Wetlands that had a forested canopy generally had lower α-diversity than open, herbaceous wetlands; vegetated areas within a wetland had greater α-diversity, regardless of the vegetation type. These results build on previous work in this system to provide a better understanding of zooplankton diversity in the temporary wetlands of SRS, and should be broadly applicable to temporary wetland systems in the surrounding region.

Introduction

Temporary wetlands are unique habitats because they function as both terrestrial and aquatic systems at different points in time; they may hold water for just a few days, or for years at a time before they dry down. They form important habitats for many types of plants and animals and are particularly notable for their diverse assemblages of amphibians and aquatic invertebrates (Mahoney et al. 1990; Leeper and Taylor 1998; Semlitsch and Bodie 1998). Not generally recognized are the extremely rich zooplankton communities that inhabit some temporary wetland systems (DeBiase & Taylor 2005; Fahd et al. 2009). Lake zooplankton communities are relatively well studied, but wetland zooplankton communities are not. Since zooplankton form a critical link in aquatic food webs, understanding their diversity patterns and especially changes in diversity over time are essential to understanding these dynamic ecosystems. A particularly notable temporary wetland system is found on the Savannah River Site (SRS) near Aiken, South Carolina, where over 100 species of zooplankton have been recorded (DeBiase & Taylor 2005). The sheer richness of the zooplankton community in this wetland system provokes questions on how this diversity is distributed both on the landscape and local scales and begs investigation of the environmental factors associated with these diverse wetland communities.

Zooplankton species richness in temporary wetland systems varies considerably across the landscape, and individual wetlands can contain a substantial proportion of the regional species richness (Mahoney et al. 1990). High species richness has been associated with both long hydroperiod lengths and larger wetland area (Mahoney et al. 1990), but other factors have not been investigated. High variability in richness between wetlands suggests that β-diversity among temporary wetlands may be significant. Mahoney et al. (1990) found that β-diversity was indeed high between wetlands and that similarity had no relationship with distance. Aside from hydroperiod, wetland area, and distance, no other relationships with richness have been investigated in SRS or in other comparable wetlands with respect to the zooplankton community. Previous work has focused entirely on the landscape scale, leaving the effects of within-wetland variation on diversity unclear. In addition, temporal changes in α- and β-diversity are likely, but remain unstudied.

To build on previous work and to more fully examine the relationships between diversity and spatial and temporal environmental variation we investigated the following questions. 1) Why does α-diversity vary between wetlands? Here we examined whether certain environmental factors explained variation in diversity among wetlands. Previous work established that species richness was positively correlated with hydroperiod length and wetland area (Mahoney et al 1990); we add water temperature, pH, conductivity, soil type and canopy cover. This comparison was made among the cumulative α-diversities of 14 wetlands and the mean environmental conditions of each wetland. 2) Why does β-diversity vary between wetlands? Beta diversity has been measured in this system, but its relationship with distance was the only comparison tested (Mahoney et al 1990). We compared between-wetland β-diversity with the environmental parameters listed in question one to investigate whether zooplankton communities aligned with certain environmental conditions, or whether they were spatially clustered, suggesting dispersal may be important. 3) Does α-diversity vary within a wetland and why? Here we examined whether α-diversity changes over time, and whether these changes were associated with changes in environmental parameters. In addition, we compared α-diversity between different vegetation types to test if certain microhabitats have greater diversity. 4) Does beta diversity vary within a wetland and why? Here we investigated whether there is evidence for temporal communities within a wetland, and whether changes in community similarity are explicable due to changes in environment. To explore these questions we surveyed 14 wetlands biweekly over a two-year study period. We chose each wetland to emphasize the inherent variability in their characteristics; environmental parameters used in our diversity comparisons included water temperature, temperature variation, pH, specific conductivity, day of the year, soil type, canopy cover, and vegetation type.

Overall, we found that species richness on the landscape-level was strongly correlated with hydroperiod length, but that forested wetlands had lower α-diversity than their open-canopied counterparts. Similar zooplankton communities among wetlands were associated with short distances, similar hydroperiod, and similar pH. In general, high α-diversity within a wetland was associated with the spring and summer months, when water pH and conductivity were low and water temperatures were high.

In addition, vegetated microhabitats had greater α-diversity than unvegetated areas. Lastly, there was strong evidence for seasonal zooplankton communities. The results of this study add considerably to the understanding of zooplankton diversity patterns within the temporary wetland system of SRS. However, it is likely these results will also be applicable to similar wetlands present throughout the southeastern USA.

Methods

Study Location

The location of this study was the Savannah River Site (SRS), a nuclear reserve owned by the United States Department of Energy. The SRS was aquired in 1950, began to be developed in 1951 and was closed to the public the following year (Debiase and Taylor 2005). The site is located in the upper coastal plain of South Carolina 32 km south of Aiken and includes portions of Aiken, Barnwell and Allendale counties. Most of the SRS is within a region known as the Sand Hills, which consists of rolling, hilly terrain with deep, well-drained soils (Griffith et al. 2002). The portion of the Sand Hills on which the SRS is located is referred to as the Aiken Plateau and includes areas of loamy soils in addition to the more typical deep sands (Griffith et al. 2002). Portions adjacent to the Savannah River consist of the modern river floodplain and Pleistocene river terraces with soils of alluvium, sand and clay (Griffith et al. 2002). When SRS was acquired, it consisted of approximately 52% agricultural land and open land, with the remaining 48% forested (White and Gaines 2000). Since then, much of the site has been reforested and managed for timber or left as natural land with only 19% of the 893 km^2 site currently developed (Debiase and Taylor 2005).

Site Selection

The wetlands sampled were selected in an effort to maximize the variation within a set of environmental variables that include water temperature, conductivity, pH, hydroperiod, vegetation type, and wetland area. The first criteria used was soil type, as minerals in the soil presumably have effects on water chemistry, soil characteristics on hydrology, and a combination of both on vegetation (Moore 1970; De Steven and Toner 2004). Soil maps provided in Rogers (1990) were used to determine the major soil groupings on which bays at SRS occur. The first is the Rembert-Hornsville association, which is poorly

drained to moderately well drained and predominantly sandy loam with some clay. The second is the Fuquay-Blanton-Dothan association, which consists of well-drained sandy soils with loamy iron-rich sub-soils. The third group is the Blanton-Lakeland association, which are very well drained with a thick sandy surface layer. To simplify, I call these soil associations terrace, upland, and sandhill respectively, based upon their characteristics and position on the landscape. Five wetlands were chosen to represent each of the three soil groups.

Data presented in Schalles et al. (1989) were used to select wetlands within each soil group according to canopy type (forested versus open) and by wetland area. Effort was made to keep the proportion of wetlands of each canopy type equal across the three soil groups, but this was not possible due to unequal distribution of forested wetlands among them. Canopy type at each of the selected wetlands was confirmed by visits during January 2009. Vegetation was further characterized through plant surveys during August and September 2009. Open herbaceous wetlands were surveyed via a transect method where three linear transects were measured from the deepest point of each wetland (or another set reference point) to the transition to upland vegetation. Transect direction was determined via randomly generated numbers representing compass degree headings. Vegetation was quantified as percentage cover per species in each square meter centered on the transect tape. Forested wetlands were surveyed with five 10 X 10 m vegetation plots per site, with their location determined through randomly generated GPS points. All tree species that were greater than 10 cm diameter at breast height (dbh) were identified, enumerated, and their dbh measured. Hydrographs produced by R. Lide (unpublished data) were used to select wetlands by hydroperiod; and of those eventually chosen, hydrographs were available for all but one. In addition, wetlands were selected to consist of two separate clusters within each soil grouping for comparison purposes in distance-similarity analyses.

Sampling Methods

In total, 15 wetlands were selected for the field survey; however, one wetland from the sandhill soil group did not hold water during the sampling period and was eliminated from the study. The 14 remaining wetlands (Figure 2.1.) were sampled biweekly beginning in January 2009 and continuing

through December 2010 for two years of data. Samples continued to be collected beyond this date, but were not included in this study. Wetlands were split into two groups, which were sampled in alternating weeks for reasons of sampling logistics. Due to schedule conflicts, there were occasionally weeks that could not be sampled. In anticipation of these events, both groups were sampled over consecutive days and again two weeks later before reverting to the regular alternating schedule. In these events, one group of wetlands was sampled two weeks in a row before the biweekly schedule resumed. In only two cases did I fail to sample on at least a biweekly basis; these occurred in early February and early March 2009 when one group of wetlands were sampled on a three week interval. When most wetlands were dry, the remainder could all be sampled in one day and groups were not alternated.

Zooplankton were sampled quantitatively using a device called a tube trap sampler that was constructed using the design of Paggi et al. (2001) by the University of Georgia instrument shop. This device effectively samples the entire water column from the surface to just above the sediment layer, which is then filtered through an 80-micron plankton net and into a sample bottle. The volume of water sampled could be calculated by using the depth of water that was sampled. The first sample at each wetland was taken at a set reference point, which was usually a depth gauge installed for earlier hydrological studies. As these gauges were usually located in the lowest point within a wetland, they were ideal for monitoring the overall depth of water; however, sometimes they were absent or not located in the lowest point. In these cases a length of 1-inch diameter PVC pipe was driven into the ground to serve as the reference point instead. Effort was made to select the lowest point to better monitor the overall depth of water in each wetland; however, this was not always possible. For example, a few wetlands had ditches running through their centers that varied only slightly in depth throughout their length, thus choosing a reference point was not straightforward and the points ultimately chosen for sampling were a few centimeters shallower than some other points along the ditch. In addition, wild pigs created wallows within some sites that were deeper than the reference depth points, but these were ignored as they were not present at the beginning of the study period.

Five samples were taken in each wetland when sufficient water was present. As mentioned above, the first was taken at the reference point, with the others taken at random GPS points that were generated prior to each sampling day. In most cases, the potential area from which random points were drawn covered the extent of the wetland, but for a few wetlands they were restricted to avoid the danger of falling into deep submerged holes or to avoid densely vegetated areas that could not be sampled. When the inundated area of a wetland was reduced in extent to where randomly located sample points were no longer feasible, then samples were collected in a non-random transect formation. Each transect was selected so that it crossed the widest portion of remaining water while passing through the reference point. A few sites dried in a manner that produced scattered pools of water that would be missed if the transect method were used; therefore, haphazard sampling of these individual pools was used instead. Samples were spaced as evenly as possible and were no closer than one meter apart to avoid disturbing the water column in the next sample. When taking five samples within a wetland would fail to meet the above spacing criteria, then the number of samples was reduced to three and would subsequently be reduced to one when a wetland was nearly dry. In some cases, even when the inundated area was wide enough for five samples, fewer were taken because heavy vegetation (such as *Cephalanthus* thickets) prevented sampling much of that area. In practice, the tube trap sampler does not work as effectively at depths of less than 10 cm; therefore, when wetlands were shallower than this depth, they were sampled qualitatively by submerging a 125 ml sample bottle and allowing it to fill completely with water. Samples were preserved in the field by pouring 95% ethanol into sample bottles that contained the sample and some residual water, leading to a final ethanol concentration of 70-80%.

Location, depth, and habitat type were recorded for each sample point. The two broadest categories for habitat type were vegetated and open water. The vegetated category referred to samples in which living or dead plants occupied approximately 25% or more of the water column, or any portion of the water surface. Open water was used to refer to samples in which vegetation was absent, contributed little three dimensional structure, or could not be determined due to a lack of water clarity. Sub-categories included floating-leaved plants (*Nymphaea odorata*, *Lemna* spp.), submersed plants (*Callitriche*

heterophylla, Juncus repens, Polygonum hydropiperoides, Potemogeton spp., *Sphagnum* spp., *Utricularia* spp.), emergent (*Carex* spp., *Eleocharis* spp. *Juncus* spp. *Leersia hexandra, Panicum hemitomon, Panicum virgatum, Rhynchospora macrostachya, Rhynchospora tracyi, Saccarhum giganteum, Scirpus cyperinus*), thatch (dead emergent vegetation no longer in an upright position), leaf litter (fallen pine needles and leaves of hardwood trees) and algae (clumps of filamentous algae unattached to vegetation). The vegetated category included the entirety of the subcategories of floating vegetation, emergent vegetation and the majority of submerged vegetation; it also included some thatch. The open water category contained the algae and leaf litter subcategories, and most of thatch; bare substrate was also included here.

Several water quality parameters were measured in the field, whereas others were measured in the lab. In both cases, these measurements were made using a YSI Professional Plus (Yellow Springs Instruments Inc.). Field measured parameters included water temperature (0.1 °C) and specific conductivity (0.1 mS/m). Field measurements were taken immediately adjacent to the reference point approximately 5 cm below the surface; water samples taken for lab measurements were collected in the same location by submerging a 125 ml sample bottle. Initially pH was measured in the field, but a probe failure and calibration issues resulted in pH being measured in the lab instead (0.01 pH units). After the study period, ammonium (0.1 mg/l) and nitrate (0.1 mg/l) were also measured; however, readings were inconsistent and did not appear trustworthy, thus they have not been included here. Beginning in November 2009, temperature loggers were deployed in each wetland to monitor temperature (0.5 °C) every four hours. Each temperature logger was moored to a reference point and rested on the substrate. Data were periodically downloaded and loggers were redeployed. In some cases loggers failed for unknown reasons and resulted in data gaps.

Sample Processing

Field samples were filtered in the lab through 63 micron mesh and stored in 95% ethanol in 20 ml scintillation vials. Organisms within samples were counted and identified under a Leica MZ75 dissection scope using a plankton wheel. Specimens in which identification required greater magnification were

separated from the sample and identified in a drop of glycerin under a compound microscope. Specimens were returned to the sample after identification if dissection was not required; those dissected were discarded. Whenever possible, all zooplankton within a sample were identified and counted; however, if densities exceeded 50 individuals per ml, processing time became excessively long and a sub-sampling method was sometimes employed instead. The sub-sampling methodology used here was modified from a protocol developed by the EPA for sampling the Great Lakes (Great Lakes National Program Office 2010). First, the volume of the sample was measured and its contents suspended. A portion of known volume was removed, and all taxa were enumerated and identified. The abundance of taxa that exceeded a density of 20 individuals per ml in the subsample was then estimated in the remainder of the sample based on the volume. Taxa that were lower in abundance were completely counted and identified in the remaining sample.

Environmental Variables

Mean water temperature, mean temperature variation, mean specific conductivity, mean pH, hydroperiod, and wetland area were the wetland-level environmental variables. Mean water temperature and mean temperature variation were calculated from the measurements of the temperature loggers. The temperature measurements used were from the period January 1st 2010 through May 5th 2010; this period was chosen because there were no gaps in the data and all wetlands were inundated. Mean specific conductivity and mean pH were both calculated from the measurements taken while sampling. Hydroperiod was measured as the estimated total number of days over the two-year study period that a wetland was inundated. Because wetlands were sampled biweekly, the actual date of drying and refilling was not observed. Therefore, we designated these dates as the day halfway between the day drydown or refill was observed, and the previous sampling date. Wetland area was taken from Schalles et al. (1989).

Date level environmental variables included temperature, specific conductivity and pH and were the measurements taken during sampling trips. Because temperature could vary due to time of day, this measurement was somewhat dependent on when the wetland was sampled. For examining β-diversity relationships, the absolute value of each pairwise difference was calculated for both wetland and date-

level environmental parameters. In most cases, wetland-level environmental variables were used when comparing between wetlands and date-level environmental variables were used when investigating within-wetland diversity. However, in a few analyses we were able to use date-level environmental variables in wetland-level comparisons. Using date-level variables was more favorable than wetland-level variables due to the much greater degrees of freedom.

Richness and diversity calculations

Recent scholarship on diversity measurement regards Hill numbers as the most appropriate diversity metrics (Jost 2007; Chao et al. 2012). There are three orders of Hill numbers designated q_0, q_1, and q_2, and are separated based on their sensitivities to common and rare species. All three are related through a single equation, can be derived from diversity metrics that have long been in common use in community ecology (Shannon index, Simpson Index), and have the benefit of being represented as numbers of species (Jost 2007). Since they describe different aspects of a community we examine all three in our analyses. Order q_0 is equivalent to species richness and gives greater weight to rare species than the other two orders because frequencies are not taken into account (Chao et al. 2012). Order q_1 weights species according to their frequencies and can be viewed as the number of species of average abundance in the community (Chao et al. 2012). Order q_2 favors the abundant species and can be interpreted as the number of dominant species in the community (Chao et al. 2012).

Alpha diversity represents the total diversity of a single sampling unit and was calculated at several levels: for each sample (sample-level), pooled samples by date within a wetland (date-level), and cumulative per wetland (wetland-level). True species richness was estimated in the program Spade (Chao & Shen 2010) using both the bias-corrected Chao1 estimator (Chao 2005) and ACE estimator (Chao and Lee 1992). For wetland-level q_0, the ACE estimator was chosen as it is considered more accurate; however, it sometimes could not be calculated at date and sample levels due to properties of the species abundance distributions. In addition, both the ACE and Chao1 estimators were occasionally overinflated at the date and sample levels due to low sample sizes. To reduce both of these problems we took a conservative approach and took the lower of the two estimates as q_0 for date and sample levels. Alpha

diversity of q_1 was calculated as the exponential of the Shannon index estimator of Chao and Shen (2003); q_2 was calculated as the inverse of the minimum variance unbiased estimator of the Simpson index (Magurran 1988). Both q_1 and q_2 were calculated using Spade. Gamma diversity, or the total diversity of all sampling units combined, was calculated for all 14 ponds as species richness and was estimated using the ACE estimator (Chao and Lee 1992)

Beta diversity represents the degree of overlap between the species communities of two sampling units and was calculated from species abundance matrices both between wetlands (wetland-level), and within wetlands between sampling dates (date-level); all were pairwise measures. As with α-diversity, β-diversity was calculated at the q_0, q_1, and q_2 diversity orders. The Sørensen index (βq_0) represents the overlap between two communities in species presence/absence. The Horn index (βq_1) could be interpreted as the community overlap of average species. The Morisita-Horn index (βq_2) represents the overlap in dominant species between two communities. All β-diversity measures were calculated in R (R core team 2013) using the 'vegetarian' package (Charney and Record 2012).

Data analysis

Type II ANOVA was used to examine differences between soil groups and canopy type in date-level temperature, conductivity, and pH data, and wetland-level hydroperiod and area data. The relationships between wetland-level α-diversity and wetland-level environmental parameters (mean temperature, mean temperature variation, mean conductivity, mean pH, hydroperiod, and area) were investigated with Pearson correlation. Differences in α-diversity between soil groups and canopy type were examined with type II ANOVA using date-level q_0, q_1 and q_2. Beta diversity differences among wetlands were examined using linear regression. The relationship between wetland-level β-diversity, soil type, and canopy type were tested using type II ANOVA.

Within-wetland environmental parameters were first examined in pairs using linear regression to determine the shape of their relationships. These were then combined in linear mixed-effects regression to investigate the relationships between date-level environmental variables, with wetland identity the random effect. The effect of environmental variables on within-wetland α-diversity was also investigated

with linear mixed-effects regression, again with wetland identity as the random effect. Mixed effect ANOVA was used to test for differences in α-diversity between vegetation types. The relationships between within-wetland β-diversity and environmental parameters were examined with linear regression, testing each variable separately. The cumulative effects of these environmental parameters on β-diversity were then tested using linear mixed-effects regression.

Results

A total of 485,047 organisms were identified in this study; of these, 308,481 were microcrustacean zooplankton (Table 2.1.). The ten most abundant zooplankton taxa identified to species level were *Bosmina tubicen* (59,275), *Diaphanosoma cf. brachyurum* (35,520), *Daphnia laevis* (19,662), *Chydorus freyi* (15,654), *Chydorus carolinensis* (14,250), *Ceriodaphnia laticaudata* (10,203), *Aglaodiaptomus atomicus* (10,181), *Ceriodaphnia megops* (9,441), *Ceriodaphnia cf. dubia* (8,502), and *Pseudosida bidentata* (5,144); all other taxa were represented by fewer than 5,000 individuals each (Table 2.1.). Harpacticoida and Ostracoda made up a substantial portion of the total abundance (10,738 and 37,760 respectively), but were not identified to a lower taxonomic level.

The total richness of the pooled zooplankton community (γ-diversity) was 86 taxa; we believe we captured most of the species present on the dates sampled, as the estimated species richness was 87 species. The sampled richness by higher taxonomic group was two Anostraca, one Laevicaudata, seven Calanoida, 22 Cyclopoida, and 52 Cladocera (Table 2.1.). Within Cladocera, the most diverse family was Chydoridae with 26 species, followed by the Daphniidae with eight species and Macrothricidae with seven.

Landscape level environmental variables

The waters of all sampled wetlands were acidic with mean pH per wetland ranging from 4.47 to 5.65 ($\bar{x}$ = 5.18, SD = 0.38) and soft, with mean specific conductivities ranging from 27.49 to 60.39 mS/m ($\bar{x}$ = 39.23, SD = 9.61; Table 2.2.). Mean temperature did not vary much between wetlands (range: 8.6-12.6, $\bar{x}$ = 39.23, SD = 0.92), although differences in temperature variance were greater (range: 5.7-20.5, $\bar{x}$ = 16.19, SD = 4.82).

Temperature (F = 5.093, p =0.006), pH (F = 127.286, p < 0.001), and specific conductivity (F = 14.474, p < 0.001) all differed by soil type. Both pH and specific conductivity were significantly greater in wetlands on upland soil types versus terrace and sandhill soils. Temperature was lower in wetlands on sandhill soils than the other two soil types.

There were differences in water temperature (F = 11.009, p< 0.001) and specific conductivity (F = 4.147, p=0.042) between wetlands that had a forested canopy versus those that had an open canopy, although there were no differences in pH (F = 0.452, p = 0.502). Specific conductivities were higher in forested wetlands and water temperatures were lower.

The estimated hydroperiod over two years of sampling ranged from 302 to 730 days ($\bar{x}$ = 486.36, SD = 133.02, Table 2.2.). Hydroperiod length did not differ between wetlands on different soil types (F=0.867, p=0.456). Forested wetlands had a lower mean hydroperiod ($\bar{x}$ = 420.167, SD = 96.874) than did open wetlands ($\bar{x}$ = 536.000, SD = 139.944), but this was not statistically significant (F = 3.529, p = 0.097).

The smallest wetland was 0.12 hectares and the largest was 11.29 ($\bar{x}$ = 2.76, SD = 3.01; Table 2.2.). Wetland size did not differ between wetlands of different soil types (F = 1.208, p = 0.348) or between canopy types (F = 1.760, p = 0.220)

Landscape level α-diversity

The species richness of the sampled wetlands ranged from 19 to 60 species ($\bar{x}$ = 36.79, SD = 10.50; Table 2.1.). Anostraca were present in only four wetlands and Laevicaudata in only three wetlands during the study period. Calanoida ranged in richness from 0-3 species per wetland ($\bar{x}$ = 2.14, SD = 0.95), whereas Cyclopoida ranged from 5-14 ($\bar{x}$ = 10.00, SD = 2.60). Cladocera were the most species rich group, with a range in richness of 10 to 43 species ($\bar{x}$ = 22.21, SD = 8.29). The average cladoceran community consisted of 10 chydorids, six daphnids, two macrothricids, two sidids, one ilyocryptid, and one bosminid.

Mean water parameters (pH, specific conductivity, temperature, temperature variation) were uncorrelated with total α-diversity per bay or with mean α-diversity per sample per wetland (Table 2.3.; Table 2.4.). Wetland area also had no relationship with any diversity metric. However, hydroperiod was correlated with total q_0 (t = 3.581, p = 0.004, correlation = 0.719; Figure 2.1.) and mean q_0 (t = 3.214, p = 0.007, correlation = 0.680; Figure 2.2.), but not with total or mean q_1 and q_2. Wetlands that held water longer had greater total and average sample richness than those that had shorter hydroperiods. There were significant differences in α-diversity between wetlands on different soil types and with different canopy cover (Table 2.5.; Figures 2.3.-2.5.). Wetlands on sandhill soils had lower q_0, q_1, and q_2 than those on terrace soils; there were no differences between the other soil type comparisons.

Landscape level β-diversity

Wetlands that were proximal to each other were more similar in βq_0 (t = -4.321, p < 0.001), βq_1 (t = -2.390, p = 0.019), and βq_2 (t = -2.396, p = 0.019) than those more distal (Figures 2.6.-2.8.). Hydroperiod also had a strong effect on βq_0 (t = -3.623, p < 0.001), with wetlands of similar hydroperiod having comparable species assemblages (Figure 2.9.). Low difference in pH (t = -3.137, p = 0.936; Figure 2.10.) and temperature variation (t = -2.163, p = 0.033) were related to similar βq_1 between wetlands; pH was similarly related to βq_2 (t = -2.617, p = 0.011).

Wetlands on the same soil type tended to be more similar in species community at all diversity levels (βq_0: F= 18.069, p < 0.001; βq_1: F = 33.623, p < 0.001; βq_2: F = 29.886, p < 0.001; Figure 2.11.). Wetlands with the same canopy cover were no more similar than those with a different canopy cover (βq_0: F= 0.779, p < 0.001; βq_1: F = 3.109, p = 0.081; βq_2: F = 0.882, p = 0.350). This indicates that there was not a notable assemblage difference between open and forested wetlands.

Wetland level environmental variables

Temperature oscillated seasonally over the sampling period (Figure 2.12.). The fewer data points on the decreasing portions of the cycle were because many wetlands were in their dry phase. Conductivity (Figure 2.13.) and pH (Figure 2.14.) did not display any clear trends, but like with temperature, there were periods of sparse data due to wetland drying. It is notable that the highest conductivities generally

occurred as wetlands refilled. Wetland depth oscillated seasonally, with two drying and refill cycles over the sampling period (Figure 2.15.). The refilling in 2010 had deeper inundation than in 2009.

Water temperature had a strong relationship with day of the year ($r^2 = 0.840$, $p < 0.001$), with temperatures peaking in midsummer (Figure 2.16). Day of year explained little variation in either conductivity ($r^2 = 0.037$, $p<0.001$; Figure 2.17.) or pH ($r^2 = -0.002$, $p = 0.345$; Figure 2.18.) although there were slight decreases in both parameters during the first half of the year. This time period was also when wetlands were at their deepest (Figure 2.19.). Wetland depth had a moderate relationship with day of the year ($r^2 = 0.231$, $p < 0.001$).

Temperature had a strong relationship to day of the year ($t = 21.58$, $p < 0.001$; Figure 2.16) and a weaker relationship with wetland depth ($t = -2.339$, $p = 0.020$; $r^2 = 0.845$; Table 2.6.; Figure 2.20.). Conductivity had a positive relationship with pH ($t = 2.687$, $p = 0.008$; Figure 2.21), but was unrelated to water depth or day of the year (Table 2.6.). Water pH had a positive relationship to specific conductivity ($t = 3.200$, $p < 0.001$; Figure 2.21.) and its interaction with day of the year ($t = -3.316$, $p < 0.001$; Table 2.6.).

Wetland level α-diversity

Species richness generally increased as a hydroperiod proceeded, with a slight decrease as drydown approached (Figure 2.22.); q_1 and q_2 displayed a similar pattern (Figures 2.23-2.24). Day of year ($t = -5.008$, $p < 0.001$), pH ($t = -4.027$, $p < 0.001$) and conductivity ($t = -4.171$, $p < 0.001$) all had negative relationships with q_0 (Table 2.7.). Both q_1 and q_2 were related largely to temperature (q_1: $t = 3.476$, $p = 0.002$; q_2: $t = 3.995$, $p < 0.001$) and secondarily to conductivity (q_1: $t = -2.255$, $p = 0.025$; q_2: $t = -2.010$, $p = 0.045$). This indicates that q_0 was generally greatest within a wetland relatively early in the year and when pH and conductivity were low; q_1 and q_2 were highest when temperatures were high and conductivity low.

Species richness was greater in samples from vegetated areas ($\bar{x} = 13.75$, SD = 7.27) than from samples that lacked vegetation ($\bar{x} = 9.49$, SD = 5.90; $F = 47.075$, $p < 0.001$; Figure 2.25.). Similarly, both q_1 ($F = 43.005$, $p < 0.001$) and q_2 ($F = 24.647$, $p < 0.001$) were greater in vegetated samples (q_1: $\bar{x} = 5.73$,

SD = 3.39; q_2: $\bar{x}$ = 4.06, SD = 2.83) than in unvegetated ones (q_1: $\bar{x}$ = 4.17, SD = 2.37; q_2: $\bar{x}$ = 3.09, SD = 1.91). Diversity of all levels differed by vegetation type ($p < 0.001$; Table 2.8.). At all levels of diversity, samples from floating, emergent, and submerged vegetation did not differ from each other (Table 2.8.; Figures 2.26.-2.28.). Thatch had the lowest diversity and differed from all habitat types except leaf litter; leaf litter differed from all other habitat types in q_2 of open water. Open water and floating vegetation were different from one another only in q_1.

Wetland level β-diversity

The highest βq_0 occurred when samples were near to each other in time or when they were approximately one year apart, and were most different when they were separated in time by around 180 days and 540 days (Figure 2.29.); there were similar, but less distinct patterns for βq_1 and βq_2 (Figures 2.30.-2.31.). The relationship between β-diversity and day of year difference was best described by a quadratic relationship (βq_0: $p < 0.001$, $r^2 = 0.302$; βq_1: $p < 0.001$, $r^2 = 0.298$; βq_2: $p < 0.001$, $r^2 = 0.209$; Figures 2.32.-2.34.). Temperature difference had a negative linear relationship with β-diversity (βq_0: $t = -42.44$, $p < 0.001$, $r^2 = 0.1614$; βq_1: $t = -39.70$, $p < 0.001$, $r^2 = 0.150$; βq_2: $t = -30.55$, $p < 0.001$, $r^2 = 0.095$). Both conductivity difference and pH difference also had negative linear relationships to β-diversity, but explained little of the variation (conductivity: βq_0: $t = -28.38$, $p < 0.001$, $r^2 = 0.096$; βq_1: $t = -17.25$, $p < 0.001$, $r^2 = 0.040$; βq_2: $t = -12.97$, $p < 0.001$, $r^2 = 0.022$; pH: βq_0: $t = -19.03$, $p < 0.001$, $r^2 = 0.036$; βq_1: $t = -14.37$, $p < 0.001$, $r^2 = 0.025$; βq_2: $t = -12.22$, $p < 0.001$, $r^2 = 0.019$). Linear mixed-effect models were used to determine which pairwise variables were related to β-diversity within a wetland. However, no single factor could be pinpointed and instead similarity between samples was related to a combination of day of year differences, temperature difference, conductivity difference, pH difference, and their interactions (Table 2.9.).

Discussion

The wetlands studied here were relatively similar in most of the environmental parameters examined, although they differed considerably in α- and β-diversity. Alpha diversity, particularly q_0, was quite high, but was mostly uncorrelated with environmental variation. The exception was hydroperiod length, which had a strongly positive relationship with species richness. Wetlands that had a forested canopy had lower α-diversity than those with an open canopy. Beta diversity also varied considerably among wetlands. Distance between wetlands was an important correlate with all levels of β-diversity; similar hydroperiod length was important for βq_0, whereas pH similarity was important for βq_1 and βq_2. Wetlands on the same soil type had greater overlap in zooplankton communities; canopy type was not an important factor. Environmental parameters within a wetland varied seasonally, but relationships were only notable for water temperature and wetland depth. High species richness within a wetland was associated with time of year, low pH, and conductivity; high q_1 and q_2 were most related to low conductivity and high water temperature. Samples from vegetated sites had greater α-diversity than those from open water samples, and diversity was particularly low in samples from substrates of decaying plant material. Samples that were nearest temporally tended to have highly overlapping zooplankton communities; however, this was also true of samples collected approximately a year apart, indicating that there are similar temporal communities that arise every year. Multiple environmental factors were associated with within-wetland temporal community overlap.

Our study reaffirmed that the wetlands of SRS are home to the most species rich temporary wetland zooplankton community reported in the world. The known cladoceran community of SRS consists of at least 60 species (Debiase and Taylor 2005), of which we sampled 52 species in the present study. The nearest comparable community in richness were the 55 cladoceran species that were sampled by Marrone et al. (2006) from 200 ponds throughout the island of Sicily. Other notable assemblages were 47 cladoceran species from Macaé, Brazil (Lopes et al. 2014) and 45 cladocerans from Doñana, Spain (Fahd et al. 2009). Even more notable was the high species richness within individual wetlands. A single wetland sampled during our study was home to 43 cladoderan species, which is by itself greater than all

other temporary wetland systems known, other than the three listed above. There is some indication that there are wetlands containing even greater richness at SRS than the ones we sampled (Mahoney et al. 1990).

The significance of hydroperiod on species richness in temporary wetland systems has been well established (Mahoney et al. 1990; Wellborn et al. 1996; Fahd et al. 2009) and is further supported by our results. The importance of this single factor indicates that shorter hydroperiods do in fact limit the presence of species; the most likely route of this limitation is through demographic impacts on life history and a reduction in potential niche space temporally. The effect of hydroperiod on higher orders of diversity had not been investigated previously. The lack of relationship with q_1 and q_2 indicates that hydroperiod has no effect on species frequencies. The differences between forested and open canopy wetlands; however, involved all three orders of α-diversity. The reasons why forested wetlands had lower α-diversity cannot be fully explained by our data, but they may be related to shorter hydroperiods and lower habitat complexity. Although not significant, forested wetlands did have shorter hydroperiods on average than did open canopy wetlands. Forested wetlands generally have shorter hydroperiods because long inundations tend to limit the establishment of trees (Kirkman et al. 1996; Mulhouse et al. 2005). In addition, the shading of the water surface limited macrophyte growth, thus aquatic vegetation was minimal; as our within-wetland analyses indicated, α-diversity was greater in vegetated areas than in the open water, leaf-litter substrates that predominate in forested wetlands.

The distance between wetlands was the only factor impacting β-diversity that was common to all three orders of diversity. Wetlands that were near spatially tended to have similar species communities in presence/absence, average species, and dominant species. This is not simply explicable by nearby wetlands having similar environmental conditions, because distance did not correlate with similarity in environment. Instead, it could be related to the dispersal of species between wetlands. Zooplankton species in general are capable of dispersing effectively over short distances (Cohen and Shurin 2003) although species vary in both dispersal and colonization ability (Shurin 2000; Cáceres and Soluk 2002). Since dispersal should be greater between two wetlands that are closer together, the likelihood of shared

species is greater. The similarity in community composition between wetlands on the same soil group is likely related to distance and dispersal, as wetlands in the same soil group were usually nearer to each other than to those in other soil groups. In addition to distance, both hydroperiod and pH were important factors associated with differences between wetlands. Wetlands that had similar hydroperiod lengths had more shared species, again indicating the importance of hydroperiod as a driver of species presence/absence. Interestingly, pH similarities led to similar community composition in both average and dominant species, but not in presence/absence. Shifts in zooplankton communities are well-documented in aquatic systems that have experienced anthropogenic acidification (Nevalainen et al. 2011; Korosi and Small 2012; Labaj et al. 2015); these and the present study indicate that pH is an important factor in structuring zooplankton assemblages.

Alpha diversity varied over the course of a year due to season and changing environmental conditions. In general species richness was greater in the spring through summer and declined in the fall. High species richness was also associated with low pH and low conductivity, although pH and conductivity did not vary in any consistent pattern. The number of common and abundant species increased with low conductivity and with high temperature. These patterns indicate that not only did richness increase during warmer months of the year, but that the zooplankton community also became more diverse, with greater equitability. Other diversity patterns within wetlands relate to vegetation. Communities within vegetated areas had greater α-diversity than those in areas that were unvegetated; however, there were no detectable differences between floating, emergent, and submerged vegetation. Interestingly, α-diversity was lower in thatch and leaf litter than in areas of bare substrate. It is unclear why substrates of seeming greater structural complexity and food resources would have lower diversity, but it may be related to low dissolved oxygen. Similar wetlands with substrates covered with leaf litter often have depleted oxygen levels due to low primary production and high bacterial decomposition (Moore 1970); therefore, it is likely that areas of thatch and leaf litter within the study wetlands experience similar conditions periodically.

Zooplankton communities varied considerably over the course of a year, but these changes were cyclical. Samples taken in close proximity temporally tended to be similar to each other in species presence/absence, common species, and dominant species. Interestingly, there were comparable levels of community overlap in samples taken approximately one year apart. This clearly indicates that very similar communities arose around the same time each year. In addition the communities approximately six months and 18 months apart were highly dissimilar, showing that communities changed completely in the intervening periods. The occurrences of similar communities were related to most of the measured environmental parameters and are not likely to be the result of any single environmental change. It is well known that some zooplankton species appear under similar conditions annually (Hammer and Sawchyn 1968; Taylor et al. 1990; Medland and Taylor 2001); however, many are known to be more haphazard (Taylor and Mahoney 1990; Medland and Taylor 2001). Thus this annual turnover in zooplankton communities remains an interesting result.

On two points, the present study disagreed with the previous research of Mahoney et al. (1990). First was the lack of a relationship between area and α-diversity, which was somewhat surprising given that species-area relationships are well established in community ecology (Schoener 1976; Fryer 1985). This is likely an issue of lower sample size in the present study, as Mahoney et al. (1990) examined nine more wetlands. The correlation between area and q_0 was reasonably high in our study, but the test lacked the power to detect a significant result. The other disagreement was regarding the β-diversity distance relationship. Mahoney et al. (1990) did not detect a relationship between community similarity and distance between wetlands; in the present study, there was a significant relationship. Again, Mahoney et al. (1990) brought more data to bear against this question. It is unclear why there was a disparity in this result; one possibility is that the greater taxonomic resolution of our study and the inclusion of cyclopoid copepods led to greater separation of otherwise similar wetland communities.

In addition to the low sample size issues for some of our wetland-level comparisons, our study was also limited by the low number of potential explanatory variables. We did not collect data on dissolved oxygen, nutrient levels, primary production or phytoplankton composition, which could all have

had important effects on diversity and community composition. Similar wetlands are documented to undergo important changes in dissolved oxygen levels, which potentially effect aquatic biota (Moore 1970). Several study wetlands were suspected to have limiting dissolved oxygen levels during certain times of the year due to observation of very low zooplankton densities. In addition, shifts in zooplankton communities in wetlands are known to occur due to fluctuations in nutrient dynamics and food resources (Schoenburg 1988) and the inclusion of these interactions are likely an important missing piece in our understanding of these wetland systems.

Despite these limitations, our study provides important conclusions regarding the temporary wetlands of SRS. Both α- and β -diversity are high on the landscape scale driven largely by hydroperiod length, differences in pH, and dispersal between wetlands. On the local scale, α- and β -diversity can vary considerably temporally and spatially. Spatial differences arise from habitat heterogeneity, whereas temporal differences are linked to seasonal changes in water chemistry and temperature. Temporary wetland systems similar to those of SRS are widespread in the southeastern USA and form an important component of the regional biodiversity. Due to their similarities in environmental characteristics, our results should be broadly applicable to other wetland systems of the southeast. In addition, some of our conclusions may be true of temporary wetlands in general. The value of temporary wetlands is now being recognized and there are pushes to preserve temporary wetlands from development and degradation. Results from our study may prove useful in deciding how these dynamic ecosystems should be conserved and managed.

Table 2.1. Total number of individuals of each taxon collected between January 2009 and December 2010 from the 14 study wetlands. Wetlands are labeled by the identity number given in Schalles et al (1989) at the top of each column.

	3	**4**	**7**	**9**	**11**	**25**	**26**	**40**	**41**	**44**	**66**	**78**	**79**	**80**
Anostraca														
Eubranchipus stegosus	14	6								16	2			
Streptocephalus sealii											1			
Laevicaudata														
Lynceus gracilicornis								3			11		329	
Calanoida														
Aglaodiaptomus atomicus	3717	456			2394		634	957				2023		
Aglaodiaptomus clavipoides										18	403			
Aglaodiaptomus stagnalis		25	90						14	8	394	8		
Hesperodiaptomus augustaensis			469	1	399	4								
Leptodiaptomus moorei		343						6	3	1591	3035			1
Onychodiaptomus birgei						1523								
Onychodiaptomus sanguineus	178		1351	24	728	78								
Cyclopoida														
Acanthocyclops robustus	202	332	534	253	409	184	236	92	52	179	123	104	91	207
Acanthocyclops venustoides			6	55	29	11								
Diacyclops cf. languidus				397										
Diacyclops crassicaudis		4	1				4		2	15	1		192	17
Diacyclops haueri		119								264				
Diacyclops navus			89	21	19	187								
Diacyclops nearcticus	3	7	12	23	1	9	10	2	35	25	13	25	10	50
Diacyclops thomasi	241		112	133	250	65	44	162	531			502		1
Ectocyclops phaleratus					1			2				1		
Eucyclops elegans			2		8	4	6	19			1	51	2	
Eucyclops pectinifer	1		16	328	19	6	13	25	24	1	34	110		
Homocyclops ater							2							

Macrocyclops albidus	1					1	7	51	19	5	1	6		
Macrocyclops fuscus	3	11	1	55	44		12	5	14	5		3		
Megacyclops cf. viridis				6	10							8		12
Mesocyclops americanus								37						
Microcyclops sp.	112	26	36	124	344		197	41		64	53	243	4	
Orthocyclops modestus	1	56	1				4	79	17	46		1	1	
Paracyclops cf. smileyi								2						
Paracyclops chiltoni					3							2		
Thermocyclops parvus	18							19						
Tropocyclops sp.		49	33	1889	156	718	67	120	221	7	7	950	35	
Harpacticoida	1287	1372	1906	854	194	41	136	310	2052	2533		50		3
Cladocera														
Acroperus sp.										1	1	757	1	
Alona cf. quadrangularis												4		
Alona costata	13				14		72	5			19	41	1	1
Alona guttata			5	8	21						11	140		2
Alona manueli	1305									1	62	1		
Alona ossiani	28						696	47				116		
Alona rustica americana							10				2	51		
Alonella excisa	24	100	141	394	738	12	753		8	169	224	634	1	1
Alonella exigua				139	422							18		
Camptocercus sp.	887		1631	403	862	297	875					33		
Karualona pennuelasi							324							
Kurzia cf. media	702	181	1030	1	21	128	506	43	752	85	40	570		
Oxyurella brevicaudis		1	1				2	9				5		
Bosmina tubicen	287	1	2768	5900	2026	4022	2671	613		8	9153	31107	478	241
Chydorus carolinensis	530	2672	1248	687	910	1519	2205	265	2219	1412		437	6	140
Chydorus eurynotus	2864	195								438				
Chydorus freyi	582		860	1948	1045	24	7697	896	251	1	705	1605		40
Chydorus linguilabrus									1		1	73		
Disparalona acutirostris												2		

Dunhevedia cf. crassa	1													
Ephemeroporus hybridus							1					30		
Paralona cf. pigra									2			18		
Picripleuroxus denticulatus	239	799	468	1091	180	24	774	371	152	646		1	14	
Picripleuroxus stramineus	23				409	5	1					573		2
Pseudochydorus cf. globosus	1	9	10	3	7		31	12	3	1		17		
Ceriodaphnia laticaudata	1778	117	652	729	494	219	2746	1118	278	215	4	1853		
Ceriodaphnia megops			497	863	597	2902	409	108	1208	7	1311	54	1358	127
Ceriodaphnia sp. A	202	2649		1801	462		167	510	1			2710		
Daphnia laevis	4390	2337	3	5	306	59		1639	8075	2745		92	11	
Scapholebris armata	14	19	11	146	40	240	37	198	636	44		114		
Scapholebris freyi	99	110	179	605	148	132	458	105	253	122	559	645	628	21
Simocephalus cf. exspinosus	764	522	72	1	119	20	159	81	110	112	34			
Simocephalus serrulatus	82	145		245	177	3	79	82	99		38	145		
Eurycercus longirostris	668	1						376	7	3				
Eurycercus microdontis								8				180		
Ilyocryptus bernerae												14		
Ilyocryptus gouldeni					162									
Ilyocryptus silvaeducensis												160		
Ilyocryptus spinifer	15		1	50			261	117	2		51	290		
Acantholebris curvirostris	8						1370	4	4		49	560	1	
Grimaldina brazzai	7						1409	21	1		91	418		
Lathonura cf. rectirostris			49	109	677									
Macrothrix cf. spinosa								3	1			284		
Macrothrix elegans							1232	7	9		1067	665		
Macrothrix sp. A												33		
Streblocercus pygmaeus									18			5		
Streblocercus serrulatus											9	572		4
Moina micrura	1207													
Moinodaphnia macleayii					203									
Polyphemus cf. pediculus												425		

Diaphanosoma cf. brachyurum	2026		6510	3764	1838	4659	6227	841	2		1544	8109		
Pseudosida bidentata	767		587	509	591	327	685	388	94	169	615	411		1
Ostracoda	5686	1011	1480	2526	6152	17	4145	4218	9267	2208	63	74	36	877

Table 2.2. Environmental parameters for the 14 study wetlands; values for temperature, temperature variation, specific conductivity, and pH represent mean values from measurements taken on sampling trips between January 2009 and December 2010. Wetlands are labeled by the identity number given in Schalles et al (1989). Temp. = water temperature, Temp. var = temperature variation, Cond = specific conductivity, Hydro = hydroperiod length, Soil = soil type (ter = terrace, up = upland, sh = sandhill), Canopy = canopy type (f = forested, o = open canopy)

Wetland	Temp. (°C)	Temp. var	Cond (mS/m)	pH	Hydro (days)	Soil	Canopy	Area (Ha)
3	9.9 (±0.22)	11.7	46.15 (±3.94)	5.65 (±0.08)	485	up	o	5.67
4	10.0 (±0.24)	14.5	40.76 (±1.00)	5.58 (±0.05)	422	up	f	1.29
7	10.2 (±0.28)	20.0	31.08 (±1.25)	5.21 (±0.04)	392	ter	f	2.51
9	9.5 (±0.22)	11.6	53.04 (±4.64)	4.47 (±0.06)	491	ter	o	1.74
11	10.0 (±0.24)	14.7	33.69 (±3.49)	5.23 (±0.05)	562	ter	o	0.77
25	10.6 (±0.29)	21.5	43.16 (±1.47)	4.78 (±0.07)	449	ter	f	1.01
26	11.1 (±0.26)	17.2	27.49 (±1.83)	5.05 (±0.06)	548	ter	o	0.49
40	10.3 (±0.29)	20.5	41.58 (±1.82)	5.55 (±0.04)	730	up	o	5.26
41	8.6 (±0.15)	5.7	60.39 (±3.36)	5.53 (±0.04)	589	up	f	0.12
44	9.5 (±0.21)	11.3	40.22 (±1.43)	5.65 (±0.04)	367	up	f	2.02
66	10.8 (±0.29)	21.2	34.40 (±6.11)	5.26 (±0.06)	402	sh	o	11.29
78	12.6 (±0.29)	20.5	29.17 (±1.87)	4.91 (±0.06)	730	sh	o	4.01
79	10.3 (±0.28)	20.1	27.81 (±1.57)	4.93 (±0.10)	340	sh	o	0.49
80	9.9 (±0.25)	16.1	40.33 (±1.73)	4.79 (±0.04)	302	sh	f	2.02

Table 2.3. Pairwise Pearson correlations for total q_0, q_1, and q_2 compared with six environmental parameters. Hydroperiod length was positively correlated with total q_0; all other tests were not significant. The three orders of α-diversity were calculated from the total pooled species frequencies for each wetland. A Bonferroni corrected p-value of 0.008 was used for significance tests.

pH	**t**	**p**	**correlation**
total q_0	0.695	0.500	0.197
total q_1	0.696	0.500	0.197
total q_2	0.854	0.410	0.239
specific conductivity			
total q_0	-0.597	0.561	-0.170
total q_1	0.012	0.991	0.003
total q_2	0.342	0.739	0.098
temperature			
total q_0	2.115	0.056	0.521
total q_1	-0.300	0.770	-0.086
total q_2	-0.839	0.418	-0.235
temperature variation			
total q_0	0.300	0.769	0.086
total q_1	-0.578	0.574	-0.164
total q_2	-1.007	0.334	-0.279
hydroperiod length			
total q_0	**3.581**	**0.004**	**0.719**
total q_1	1.262	0.231	0.342
total q_2	0.461	0.653	0.132
wetland area			
total q_0	1.770	0.102	0.455
total q_1	-0.218	0.831	-0.063
total q_2	-0.516	0.615	-0.147

Table 2.4. Pearson correlations for mean q_0, q_1, and q_2 compared with six environmental parameters. Hydroperiod length was positively correlated with mean q_0; all other tests were not significant. The three orders of α-diversity were calculated as mean diversity per sample for each wetland. A Bonferroni corrected p-value of 0.008 was used for significance tests.

pH	**t**	**p**	**correlation**
mean q_0	0.537	0.601	0.153
mean q_1	0.417	0.684	0.120
mean q_2	0.354	0.730	0.102
specific conductivity			
mean q_0	-0.489	0.634	-0.140
mean q_1	-0.749	0.469	-0.211
mean q_2	-0.728	0.481	-0.206
temperature			
mean q_0	1.597	0.136	0.419
mean q_1	1.260	0.232	0.342
mean q_2	1.037	0.320	0.287
temperature variation			
mean q_0	0.229	0.823	0.066
mean q_1	0.253	0.805	0.073
mean q_2	0.202	0.844	0.058
hydroperiod length			
mean q_0	**3.214**	**0.007**	**0.680**
mean q_1	2.699	0.019	0.615
mean q_2	2.377	0.035	0.566
wetland area			
mean q_0	0.688	0.504	0.195
mean q_1	-0.0538,	0.958	-0.016
mean q_2	-0.219	0.831	-0.063

Table 2.5. Type II ANOVA comparing total q_0, q_1, and q_2 with canopy type (forested vs. open canopy) and soil type (terrace, upland, sandhill). Below the ANOVA table are the results of a Tukey's test, which gives p-values from comparisons between soil types; TR-UP = terrace vs. upland, TR-SH = terrace vs. sandhill, UP-SH = upland vs. sandhill. There were significant differences in q_0, q_1, and q_2 among both canopy types and soil types.

Type II ANOVA

		F	p
q_0	canopy type	**70.431**	**< 0.001**
	soil type	**10.032**	**< 0.001**
	interaction	**3.207**	**0.041**
q_1	canopy type	**36.819**	**< 0.001**
	soil type	**12.011**	**< 0.001**
	interaction	1.161	0.314
q_2	canopy type	**18.932**	**< 0.001**
	soil type	**7.583**	**< 0.001**
	interaction	0.803	0.449

Tukey's test of differences between soil types

	TR-UP	TR-SH	UP-SH
q_0	0.169	**0.008**	0.363
q_1	0.168	**0.000**	0.069
q_2	0.337	**0.004**	0.136

Table 2.6. Linear mixed-effects regression of within wetland changes in environmental parameters; the fixed effect was wetland identity and the random effects were pH, specific conductivity, water temperature, and day of the year. Water temperature had a significant relationship with day of the year and water depth; conductivity had a significant relationship with pH.

Regression of day of year & depth on temperature

	t	p
day of the year	**21.580**	**< 0.001**
water depth	**-2.339**	**0.020**
interaction	0.620	0.536

Regression of day of year, depth &pH on conductivity

	t	p
day of the year	1.328	0.185
water depth	1.039	0.299
pH	**2.687**	**0.007**
pH:depth	-0.578	0.564
pH:day	-0.929	0.353
day:depth	-1.101	0.271
day:depth:ph	0.279	0.780

Regression of day of year & conductivity on pH

	t	p
day of the year	1.877	0.061
conductivity	**3.200**	**< 0.001**
interaction	**-3.316**	**< 0.001**

Table 2.7. Linear mixed-effects regression of within-wetland α-diversity; the fixed effect was wetland identity and the random effects were pH, specific conductivity, water temperature, and day of the year. Day of year is represented by a cubic relationship with each diversity metric. Day of year, pH, and conductivity had significant negative relationships with q_0, whereas q_1 and q_1 had significant negative relationships with conductivity and significant positive relationships with water temperature.

q_0	t	p
pH	**-4.027**	**< 0.001**
conductivity	**-4.171**	**< 0.001**
temperature	0.521	0.603
day of year	**-5.008**	**< 0.001**

q_1	t	p
pH	-0.124	0.901
conductivity	**-2.255**	**0.025**
temperature	**3.476**	**0.002**
day of year	-1.287	0.199

q_2	t	p
pH	0.686	0.503
conductivity	**-2.010**	**0.045**
temperature	**3.995**	**< 0.001**
day of year	-0.811	0.418

Table 2.8. Type II ANOVA of the effect of vegetation type on three diversity metrics (upper table) and the results of a Tukey's test of the pairwise differences in diversity metrics by vegetation type (lower table). The columns in the Tukey's test represent p-values for q_0, q_1, and q_2. FL = floating vegetation, EM = emergent vegetation, SB = submerged vegetation, O = open water, LL = leaf litter, TH = thatch

Type II ANOVA

	F	p
q_0	**14.555**	**< 0.001**
q_1	**11.470**	**< 0.001**
q_2	**7.100**	**< 0.001**

Tukey's test

	p (q_0)	p (q_1)	p (q_2)
FL-EM	0.729	0.998	1.000
LL-EM	**< 0.001**	**< 0.001**	**< 0.001**
O-EM	**< 0.001**	**< 0.001**	**0.002**
SB-EM	0.775	0.994	0.920
TH-EM	**< 0.001**	**< 0.001**	**< 0.001**
LL-FL	**< 0.001**	**< 0.001**	**0.003**
O-FL	0.096	**0.007**	0.108
SB-FL	0.143	0.951	0.926
TH-FL	**< 0.001**	**< 0.001**	**< 0.001**
O-LL	**< 0.001**	**0.036**	0.351
SB-LL	**< 0.001**	**< 0.001**	**< 0.001**
TH-LL	0.208	0.053	0.117
SB-O	**< 0.001**	**< 0.001**	**< 0.001**
TH-O	**< 0.001**	**< 0.001**	**0.001**
TH-SB	**< 0.001**	**< 0.001**	**< 0.001**

Table 2.9. Linear mixed-effects regression of within-wetland β-diversity; the fixed effect was wetland identity and the random effects were day of year, water temperature, specific conductivity, pH, and their paired interactions. Day of year is represented by a quadratic relationship with each β-diversity metric.

q_0		t	p
	day of year	**38.451**	< **0.001**
	temperature	-1.542	0.123
	conductivity	**-15.082**	< **0.001**
	pH	**-5.959**	< **0.001**
	day:temp	**-6.113**	< **0.001**
	day:cond	**9.576**	< **0.001**
	day:pH	-0.224	0.823
	temp:cond	-1.344	0.179
	temp:pH	0.612	0.541
	cond:pH	**3.498**	< **0.001**
q_1		t	p
	day of year	**41.732**	< **0.001**
	temperature	**-2.099**	**0.036**
	conductivity	**-13.819**	< **0.001**
	pH	**-8.270**	< **0.001**
	day:temp	**-2.284**	< **0.001**
	day:cond	**13.863**	< **0.001**
	day:pH	**6.157**	< **0.001**
	temp:cond	**-2.126**	**0.034**
	temp:pH	0.118	0.907
	cond:pH	**5.395**	< **0.001**
q_2		t	p
	day of year	**39.673**	< **0.001**
	temperature	**-3.393**	**0.015**
	conductivity	**-12.594**	< **0.001**
	pH	**-8.826**	< **0.001**
	day:temp	-1.498	0.134
	day:cond	**12.527**	< **0.001**
	day:pH	**5.805**	< **0.001**
	temp:cond	**-2.190**	**0.029**
	temp:pH	1.302	0.193
	cond:pH	**3.662**	< **0.001**

Figure 2.1. Map of Savannah River Site showing the locations of the 14 wetlands sampled in this study (red dots). The map also displays the major soil types. Light green = terrace, brown = upland, yellow = sandhill.

Figure 2.2. (A) Correlation between wetland-level species richness (q_0) and hydroperiod length; (B) correlation between mean sample species richness and hydroperiod length

Figure 2.3. Boxplots of q_0 by canopy and soil type groups. The dark bar within each box represents the median. The lower and upper margins of each box represent the first and third quartiles respectively. The whiskers extend to the most extreme point that is no greater than 1.5 times the interquartile range from the box. Notches on the vertical margins of the boxes are calculated as +/- 1.58 x the interquartile range/ square root (n), and represent approximately the 95% confidence interval surrounding the median. Canopy types are denoted by F (forested) and O (open canopy). Soil groups are denoted by TR (terrace), UP (upland), and SH (sandhill).

Figure 2.4. Boxplots of q_1 by canopy and soil type groups. The dark bar within each box represents the median. The lower and upper margins of each box represent the first and third quartiles respectively. The whiskers extend to the most extreme point that is no greater than 1.5 times the interquartile range from the box. Notches on the vertical margins of the boxes are calculated as +/- 1.58 x the interquartile range/ square root (n), and represent approximately the 95% confidence interval surrounding the median. Canopy types are denoted by F (forested) and O (open canopy). Soil groups are denoted by TR (terrace), UP (upland), and SH (sandhill).

Figure 2.5. Boxplots of q_2 by canopy and soil type groups. The dark bar within each box represents the median. The lower and upper margins of each box represent the first and third quartiles respectively. The whiskers extend to the most extreme point that is no greater than 1.5 times the interquartile range from the box. Notches on the vertical margins of the boxes are calculated as +/- 1.58 x the interquartile range/ square root (n), and represent approximately the 95% confidence interval surrounding the median. Canopy types are denoted by F (forested) and O (open canopy). Soil groups are denoted by TR (terrace), UP (upland), and SH (sandhill).

Figure 2.20. The relationship between water temperature and water depth for all 14 wetlands from January 2009 through December 2010

Figure 2.21. The relationship between specific conductivity and pH for all 14 wetlands from January 2009 through December 2010

Figure 2.22. The relationship between within-wetland q_0 and day of the year for all 14 wetlands from January 2009 through December 2010

Figure 2.23. The relationship between within-wetland q_1 and day of the year for all 14 wetlands from January 2009 through December 2010

Figure 2.24. The relationship between within-wetland q_2 and day of the year for all 14 wetlands from January 2009 through December 2010

Figure 2.25. Boxplots of pairwise differences in within-wetland α-diversity in samples from vegetated areas and from samples in unvegetated areas. The dark bar within each box represents the median. The lower and upper margins of each box represent the first and third quartiles respectively. The whiskers extend to the most extreme point that is no greater than 1.5 times the interquartile range from the box. Notches on the vertical margins of the boxes are calculated as +/- 1.58 x the interquartile range/ square root (n), and represent approximately the 95% confidence interval surrounding the median.

Figure 2.26. Boxplots of within-wetland q_0 for each vegetation type. The dark bar within each box represents the median. The lower and upper margins of each box represent the first and third quartiles respectively. The whiskers extend to the most extreme point that is no greater than 1.5 times the interquartile range from the box. Notches on the vertical margins of the boxes are calculated as +/- 1.58 x the interquartile range/ square root (n), and represent approximately the 95% confidence interval surrounding the median. FL = floating vegetation, EM = emergent vegetation, SB = submerged vegetation, O = open water, LL = leaf litter, TH = thatch

Figure 2.27. Boxplots of within-wetland q_1 for each vegetation type. The dark bar within each box represents the median. The lower and upper margins of each box represent the first and third quartiles respectively. The whiskers extend to the most extreme point that is no greater than 1.5 times the

interquartile range from the box. Notches on the vertical margins of the boxes are calculated as +/- 1.58 x the interquartile range/ square root (n), and represent approximately the 95% confidence interval surrounding the median. FL = floating vegetation, EM = emergent vegetation, SB = submerged vegetation, O = open water, LL = leaf litter, TH = thatch

Figure 2.28. Boxplots of within-wetland q_2 for each vegetation type. The dark bar within each box represents the median. The lower and upper margins of each box represent the first and third quartiles respectively. The whiskers extend to the most extreme point that is no greater than 1.5 times the interquartile range from the box. Notches on the vertical margins of the boxes are calculated as +/- 1.58 x the interquartile range/ square root (n), and represent approximately the 95% confidence interval surrounding the median. FL = floating vegetation, EM = emergent vegetation, SB = submerged vegetation, O = open water, LL = leaf litter, TH = thatch

Figure 2.29. Plot of pairwise within-wetland q_0 by difference in sampling date

Figure 2.30. Plot of pairwise within-wetland q_1 by difference in sampling date

Figure 2.31. Plot of pairwise within-wetland q_2 by difference in sampling date

Figure 2.32. Plot of pairwise within-wetland q_0 by difference in day of year

Figure 2.33. Plot of pairwise within-wetland q_1 by difference in day of year

Figure 2.34. Plot of pairwise within-wetland q_2 by difference in day of year

Figure 2.1.

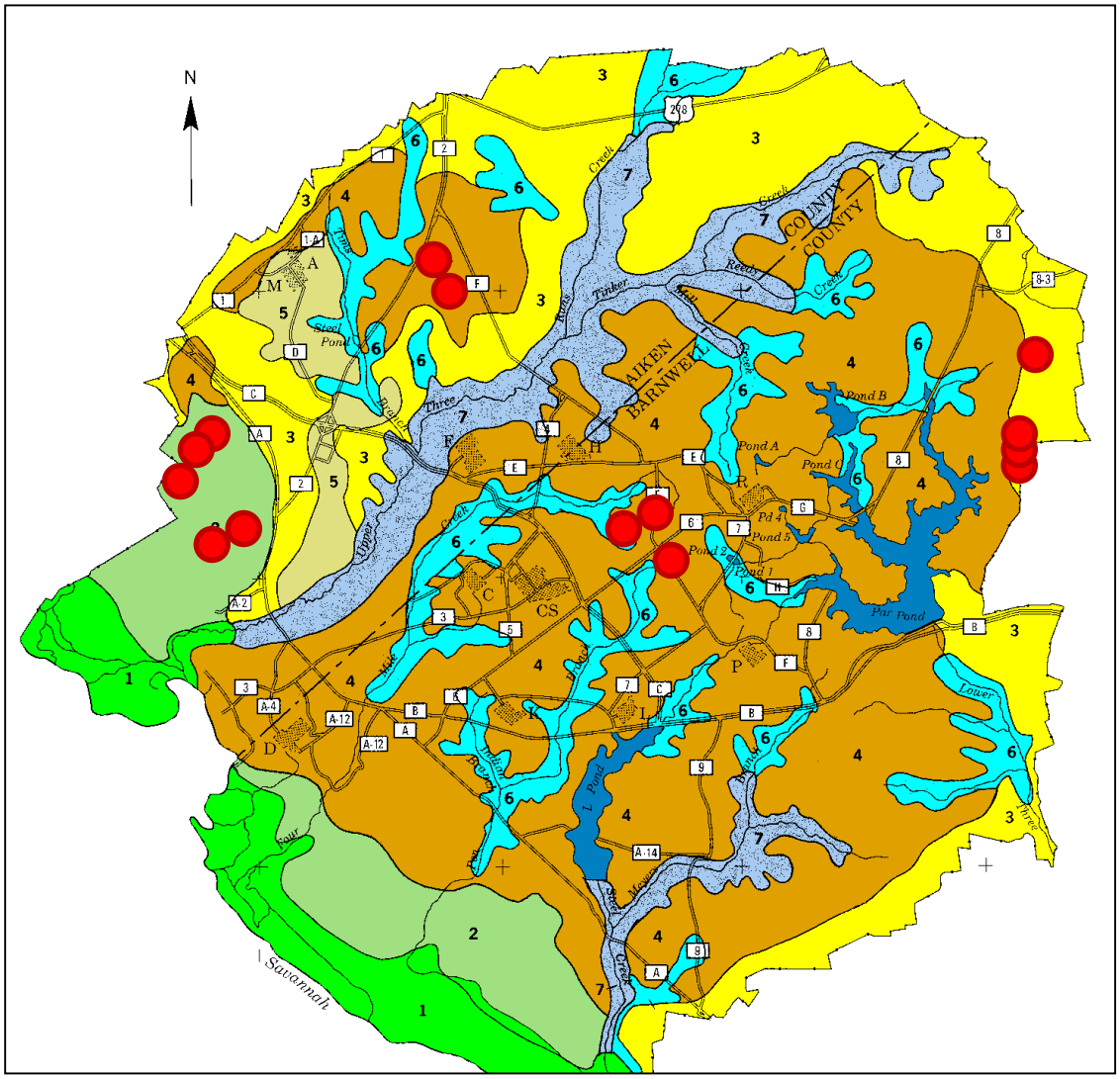

N
AIKEN
BARNWELL
COUNTY
COUNTY
Pond A
Pond B
Par Pond
Pond 1
Pond 5
Upper Three Runs
Lower Three Runs
Steel Pond
Tinker Creek
Reedy Creek
Beaver Dam Creek
Indian Grave Branch
Meyers Creek
Four Hole Swamp
Savannah

Figure 2.2.

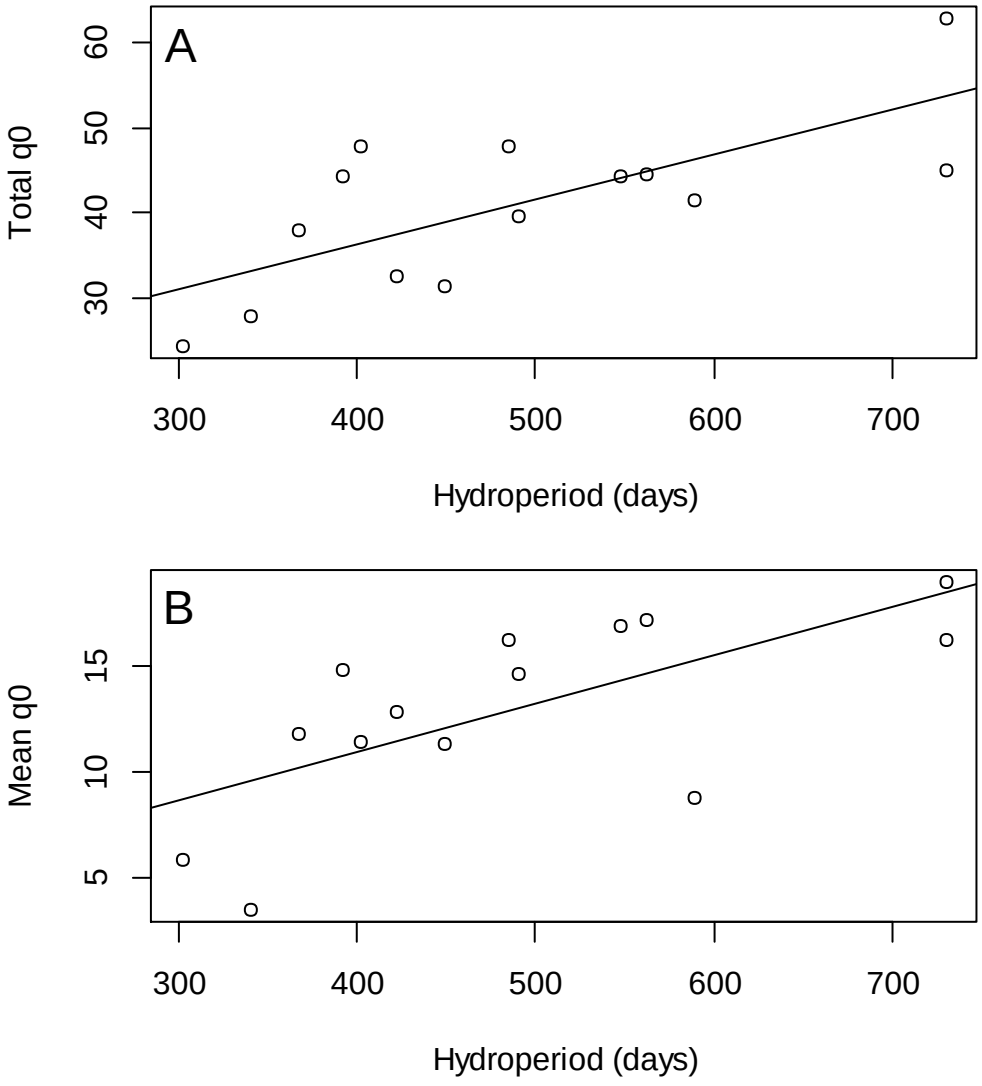
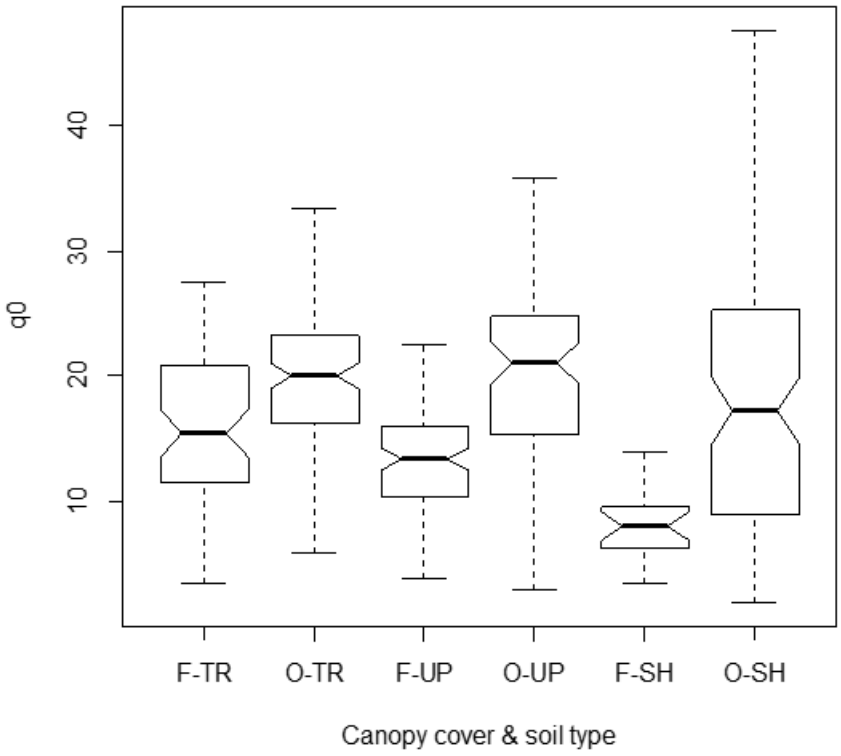

A
Total q0
30
40
50
60
300
400
500
600
700
Hydroperiod (days)
B
Mean q0
5
10
15
300
400
500
600
700
Hydroperiod (days)

Figure 2.3.



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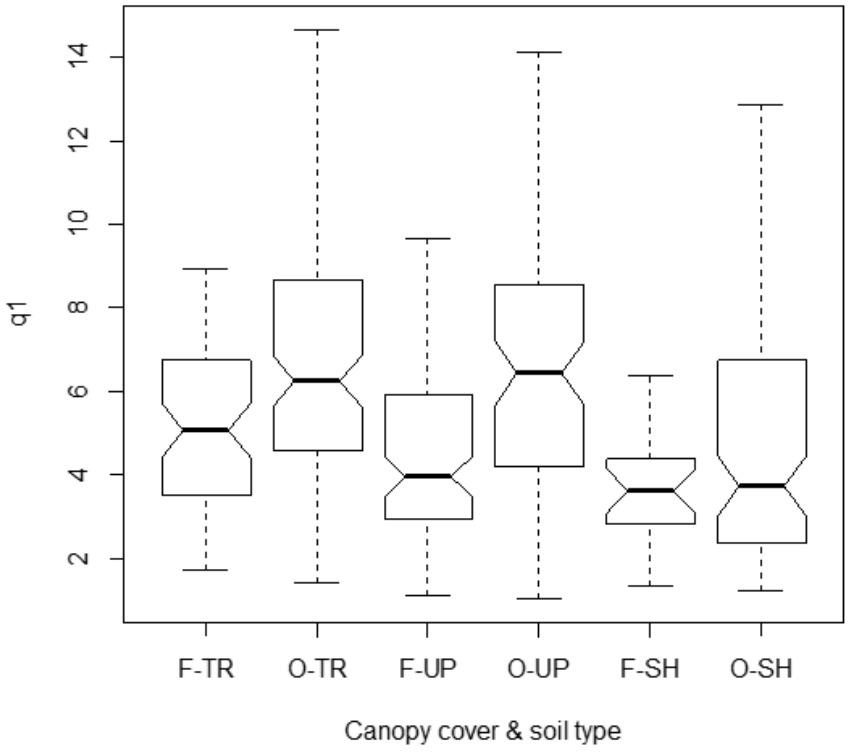

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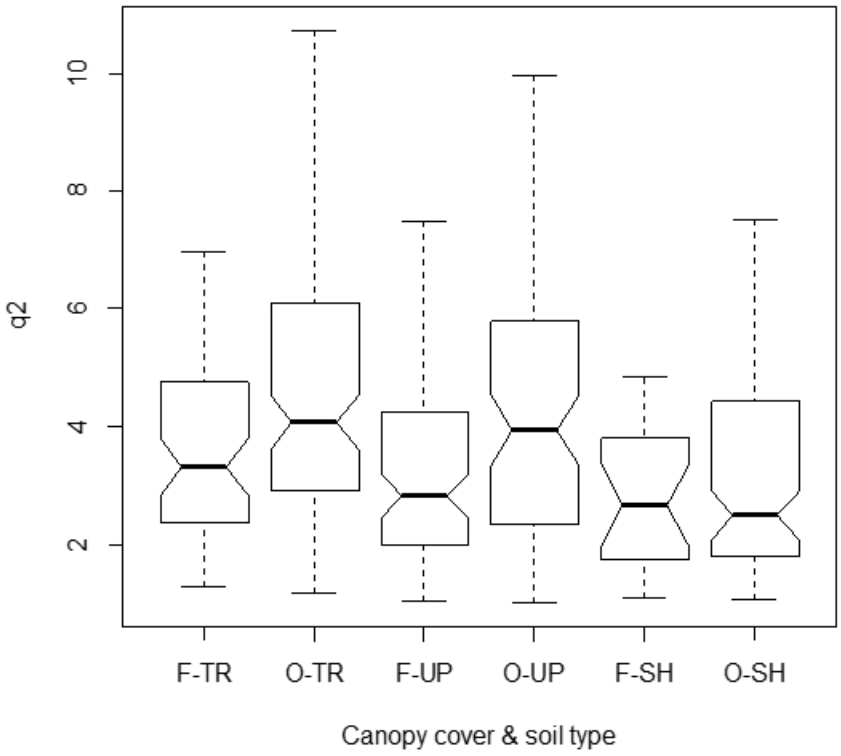


Figure 2.6.

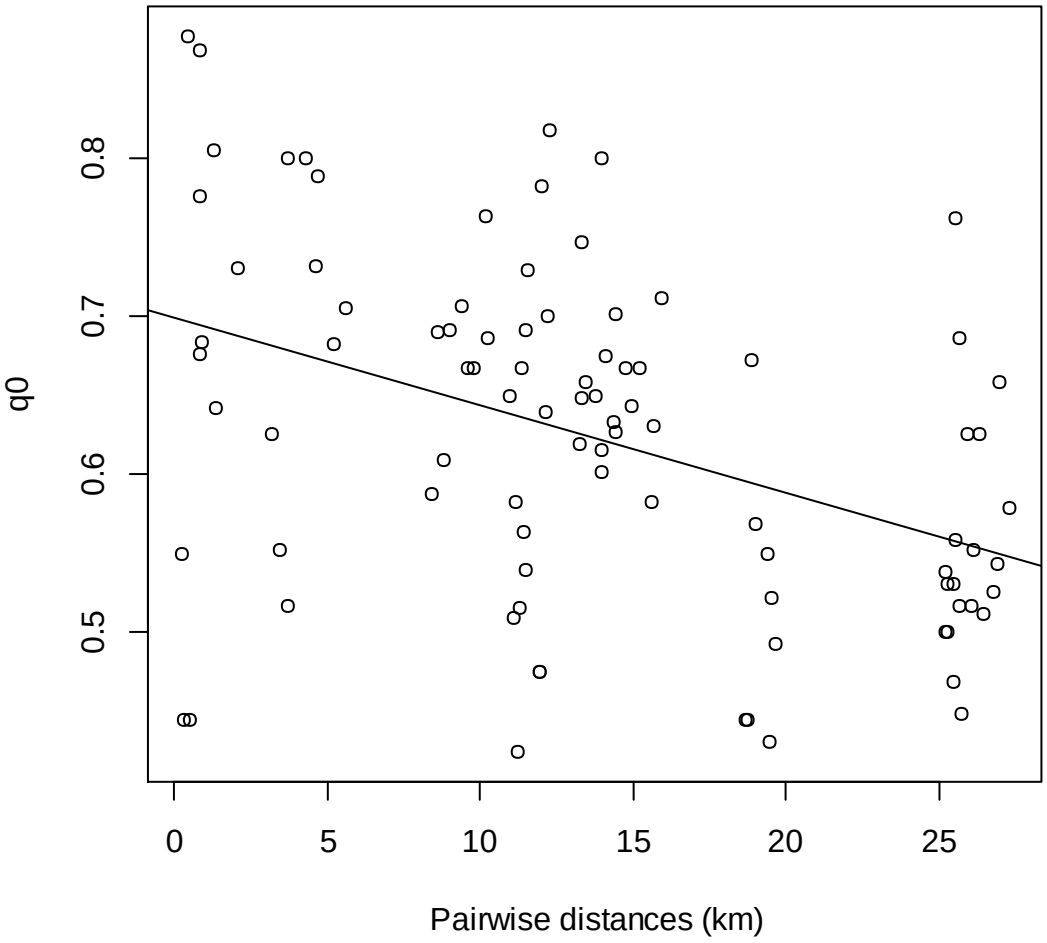


Figure 2.7.

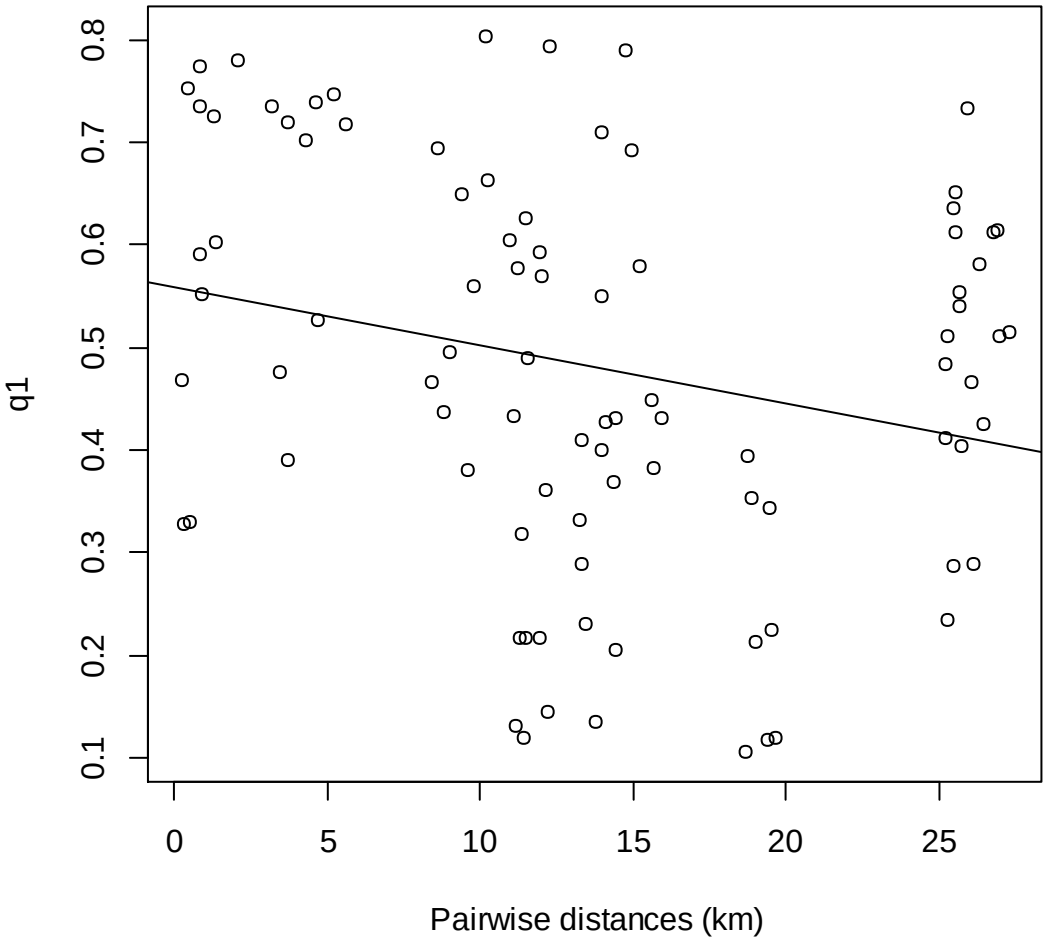


Figure 2.8.

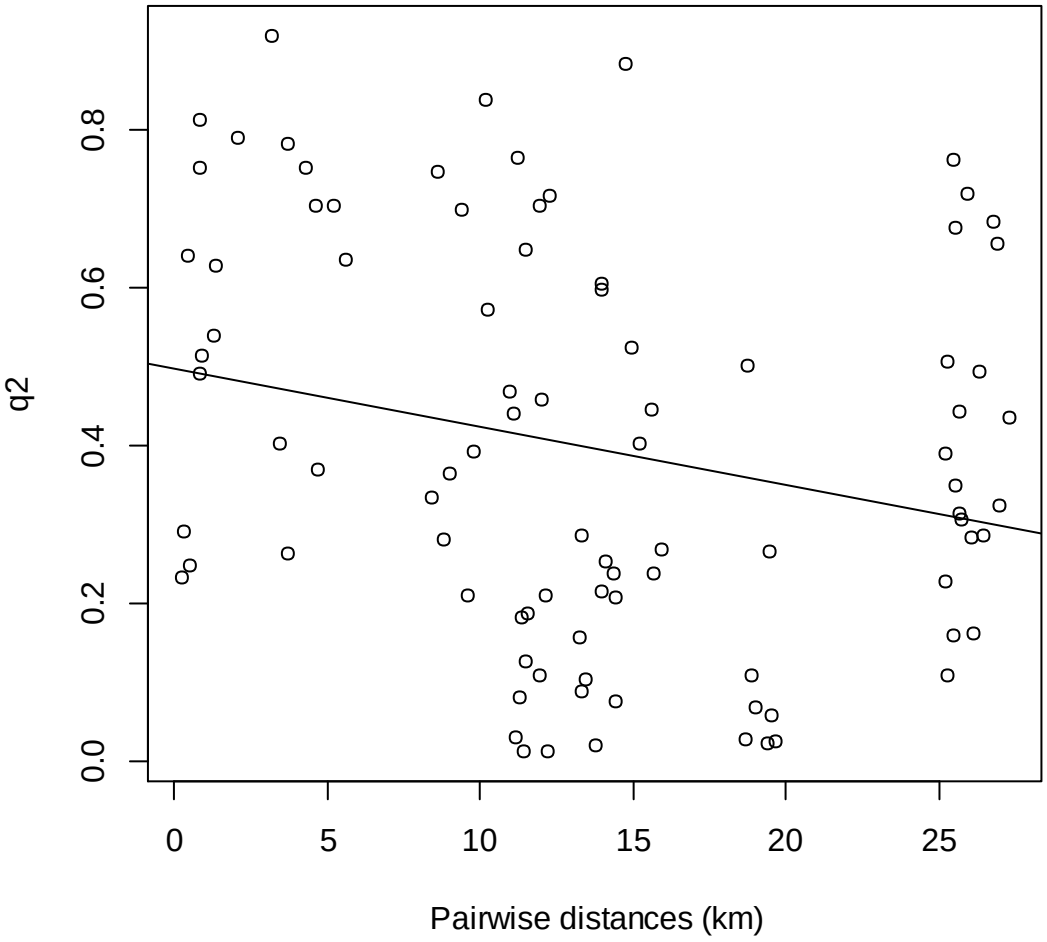


Figure 2.9.

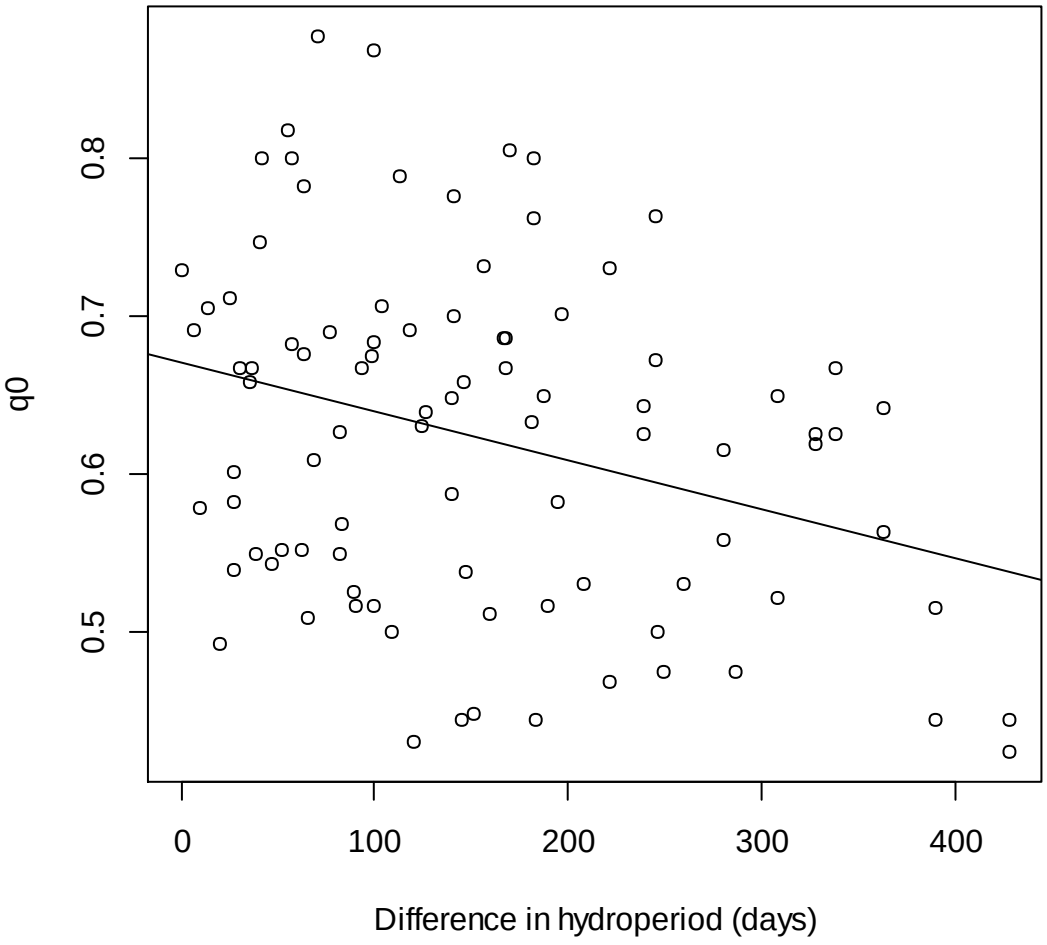

q0
0.5
0.6
0.7
0.8
0
100
200
300
400
Difference in hydroperiod (days)

Figure 2.10.

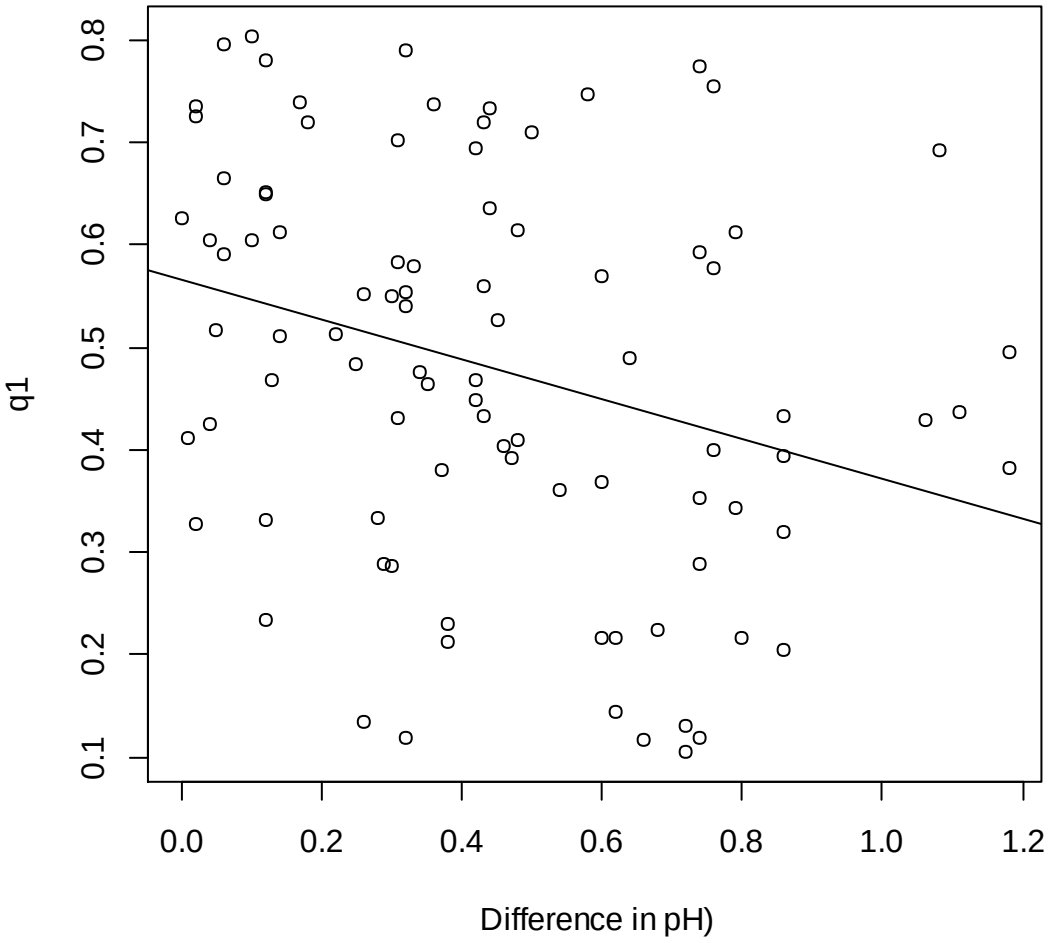


Figure 2.11.

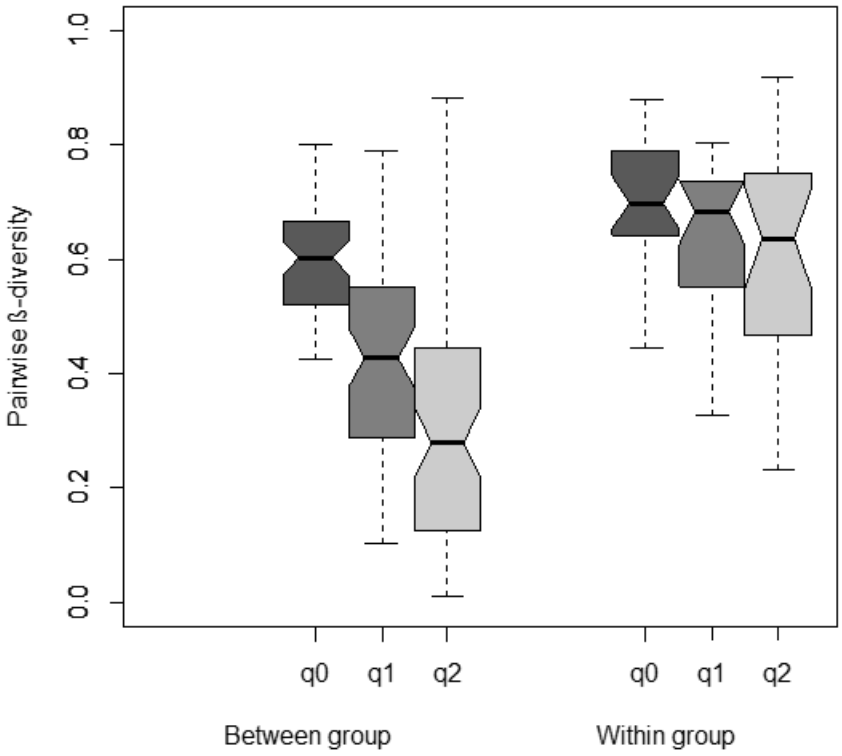

Pairwise ß-diversity
0.0
0.2
0.4
0.6
0.8
1.0
q0
q1
q2
q0
q1
q2
Between group
Within group

Figure 2.12.

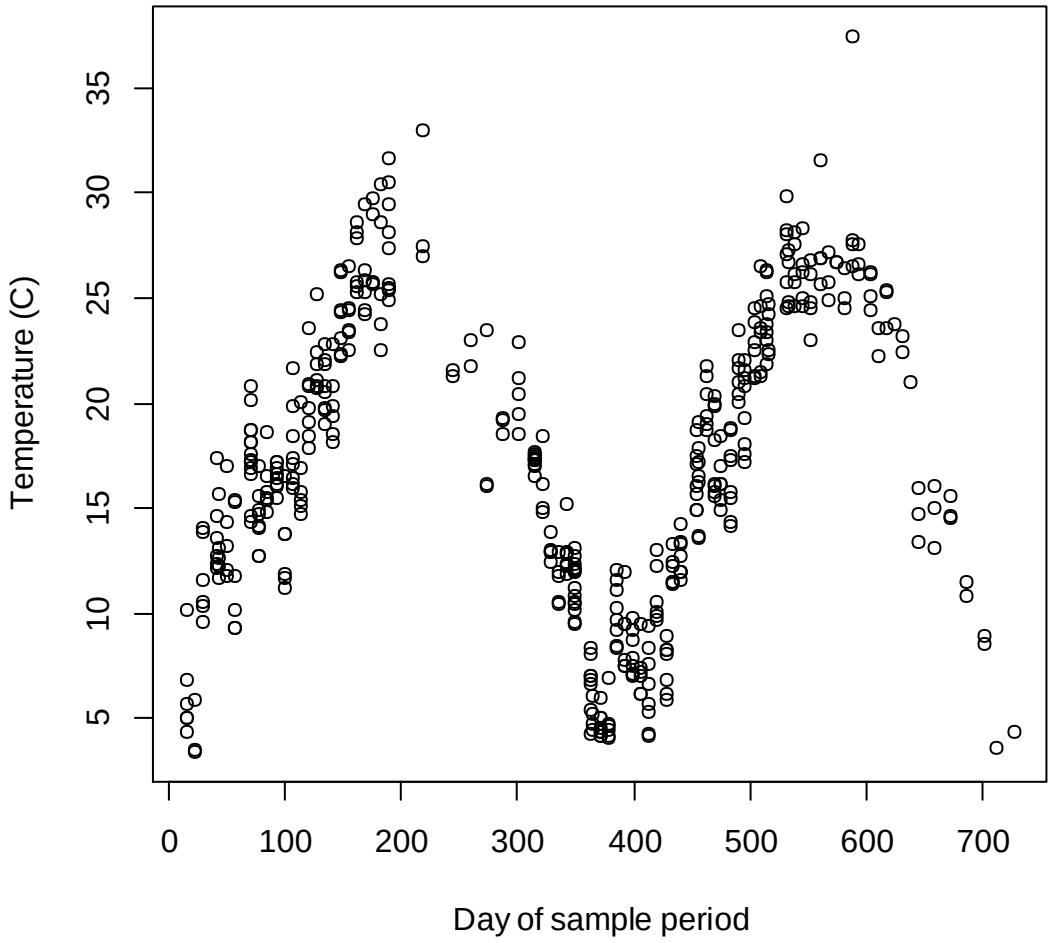

Temperature (C)
5
10
15
20
25
30
35
0
100
200
300
400
500
600
700
Day of sample period

Figure 2.13.

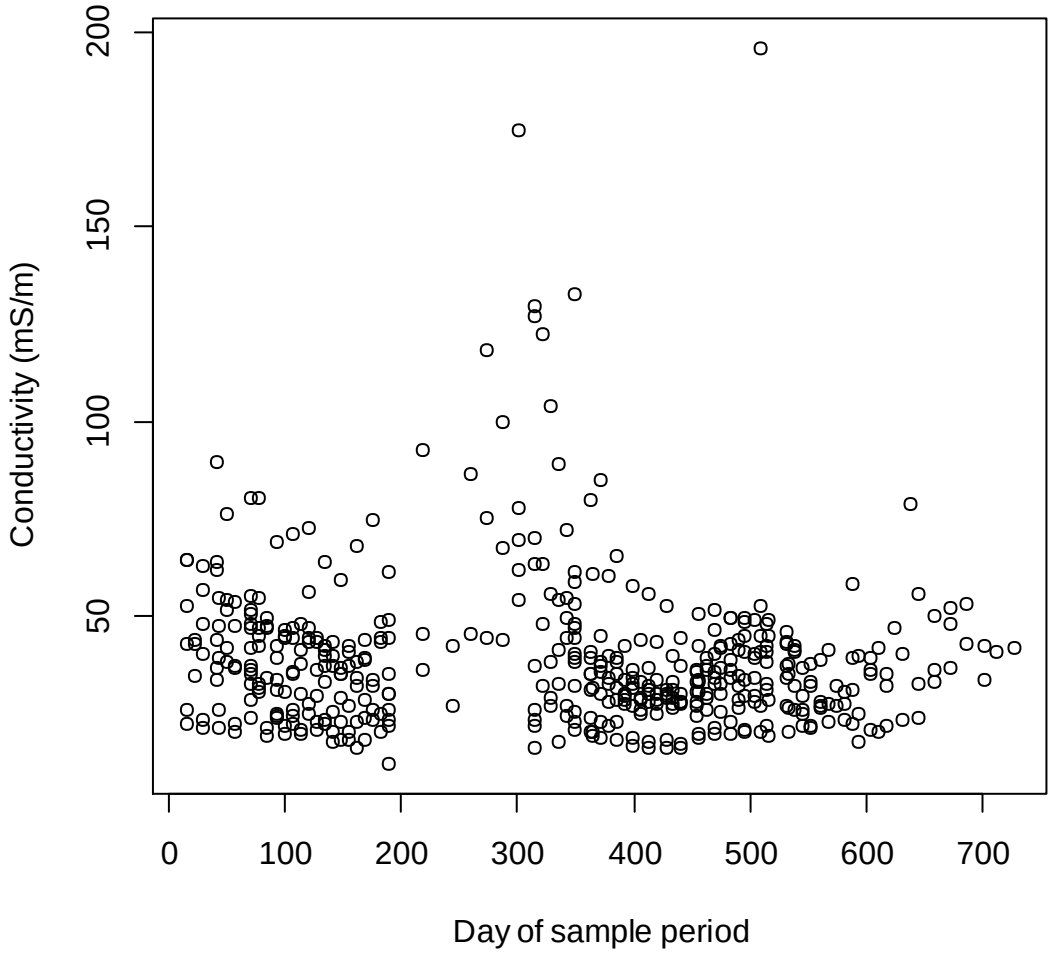

Conductivity (mS/m)
50
100
150
200
0
100
200
300
400
500
600
700
Day of sample period

Figure 2.14.

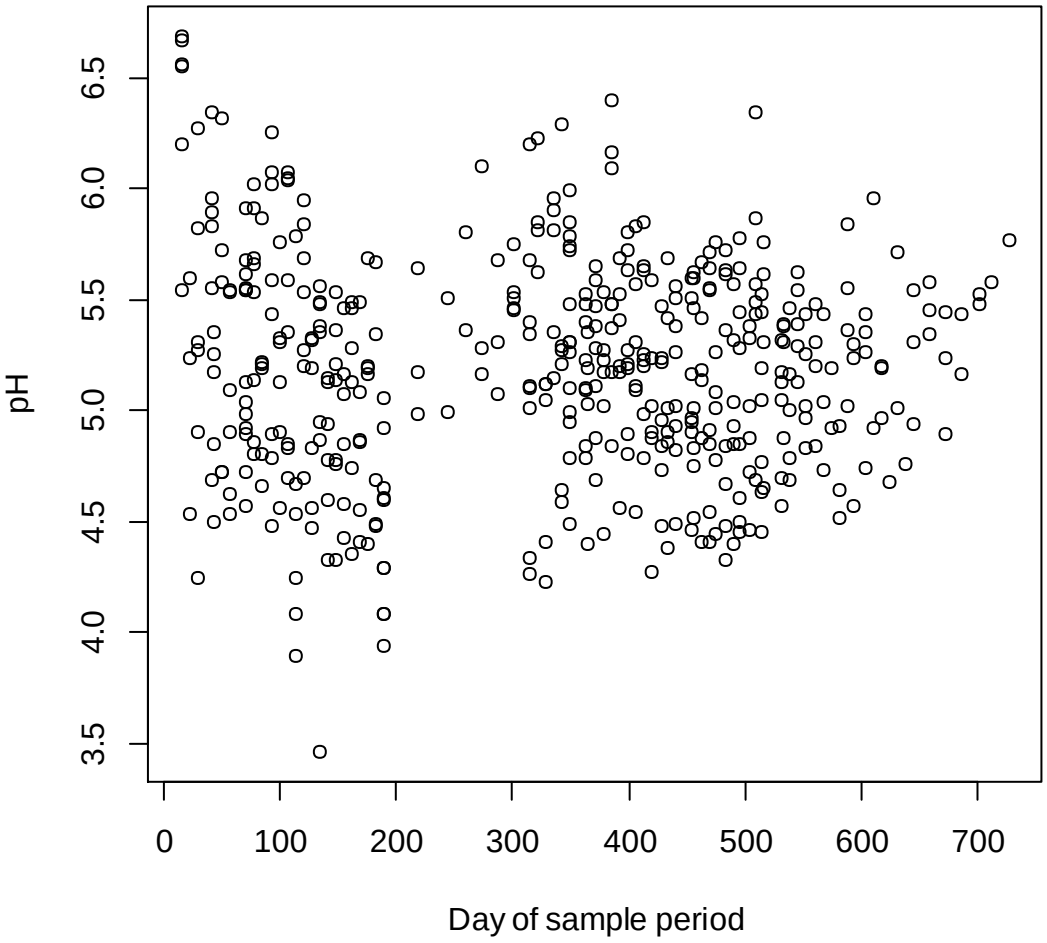

pH
3.5
4.0
4.5
5.0
5.5
6.0
6.5
0
100
200
300
400
500
600
700
Day of sample period

Figure 2.15.

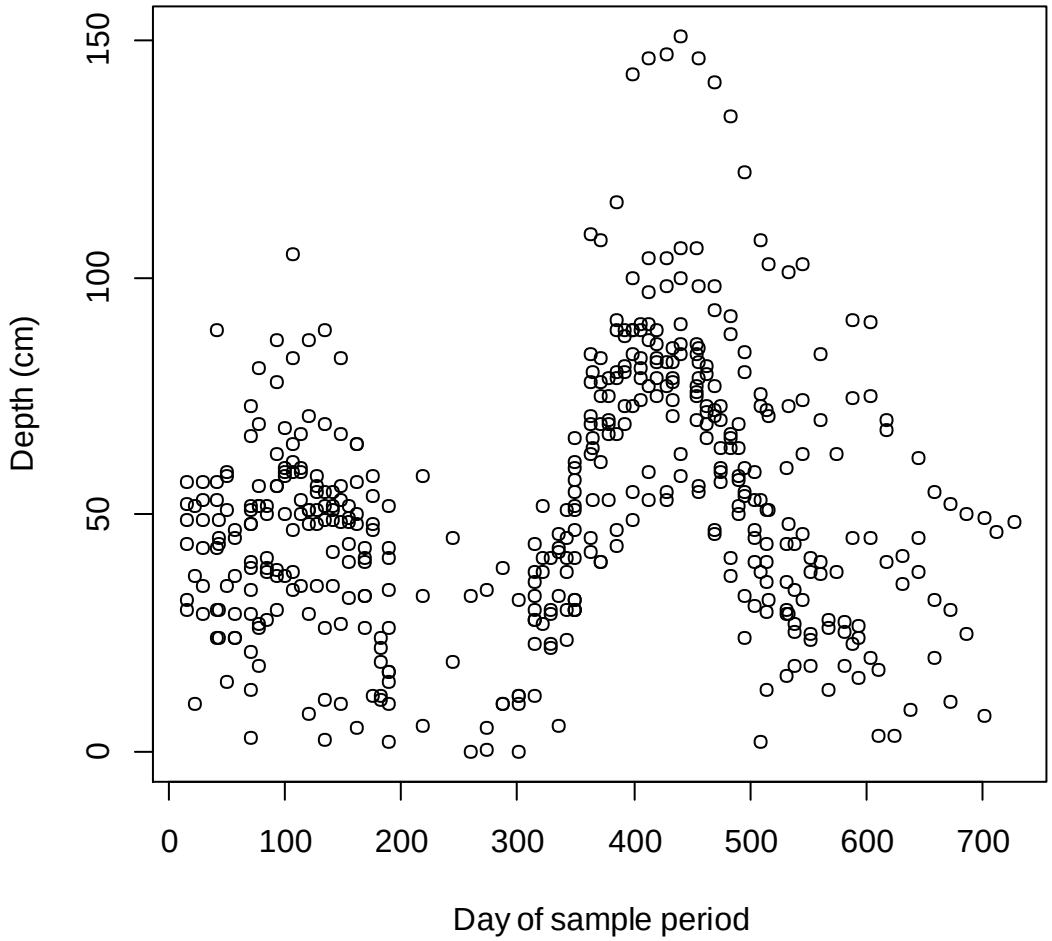

Depth (cm)
0
50
100
150
0
100
200
300
400
500
600
700
Day of sample period

Figure 2.16.

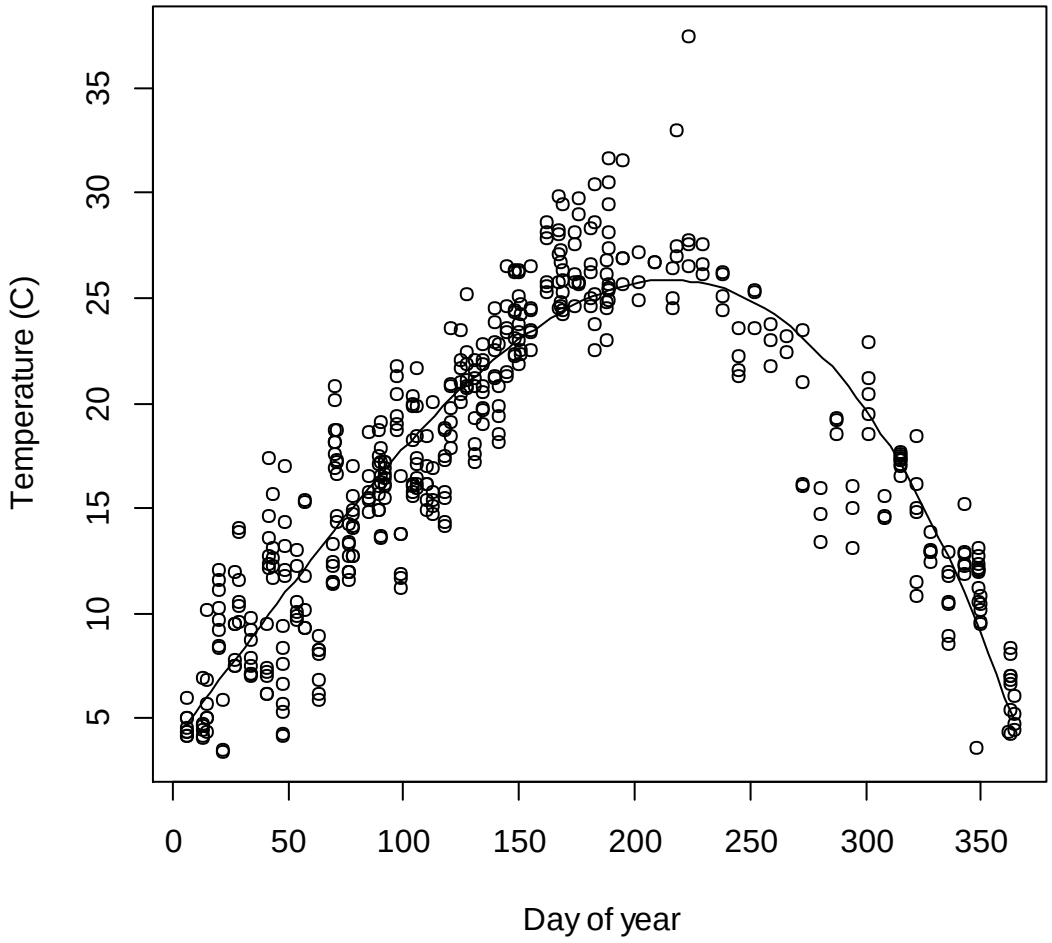

Temperature (C)
5
10
15
20
25
30
35
0
50
100
150
200
250
300
350
Day of year

Figure 2.17.

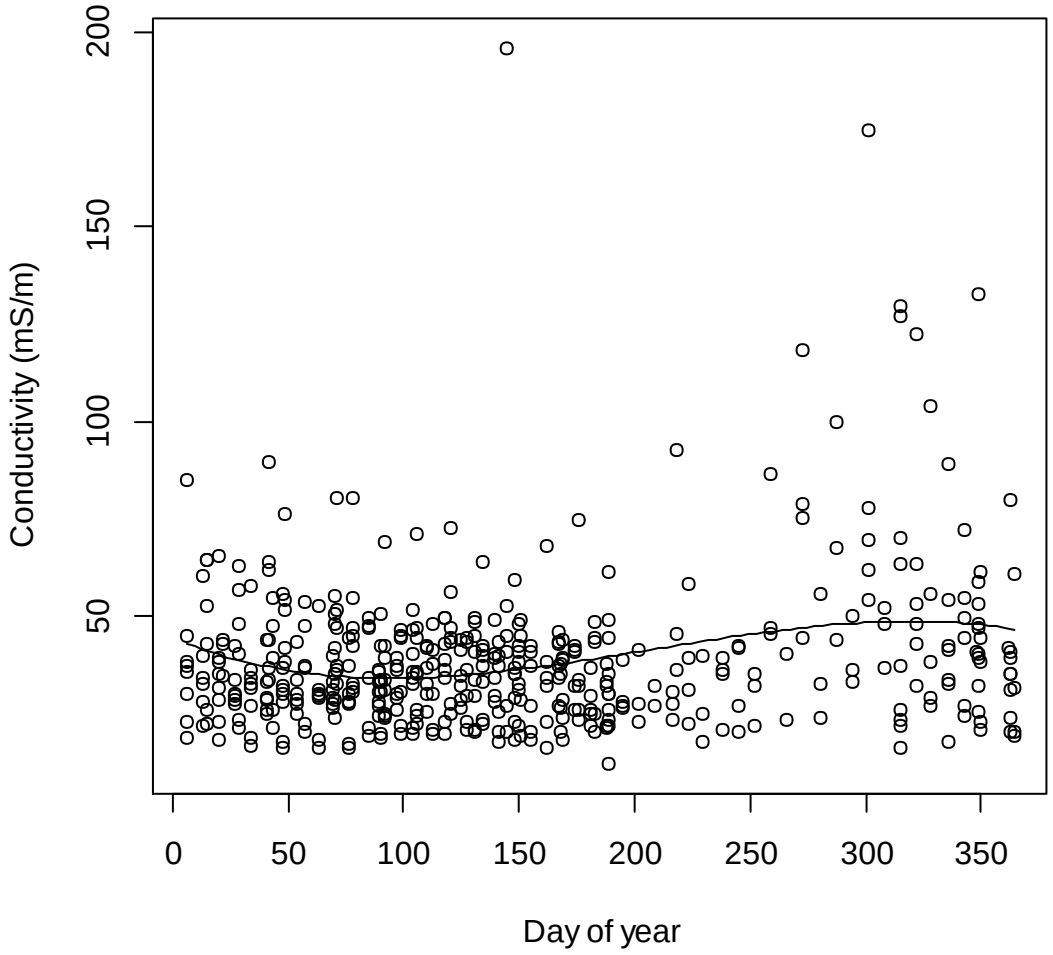

Conductivity (mS/m)
50
100
150
200
0
50
100
150
200
250
300
350
Day of year

Figure 2.18.

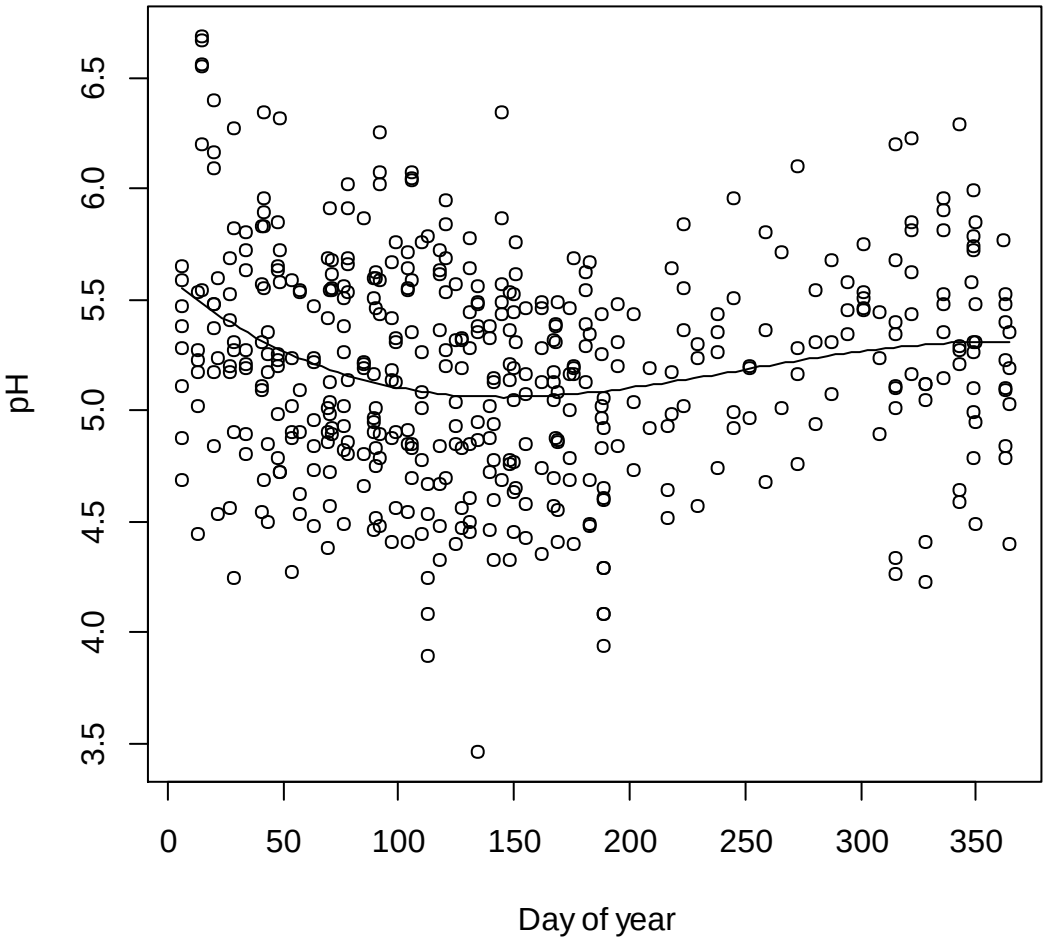


Figure 2.19.

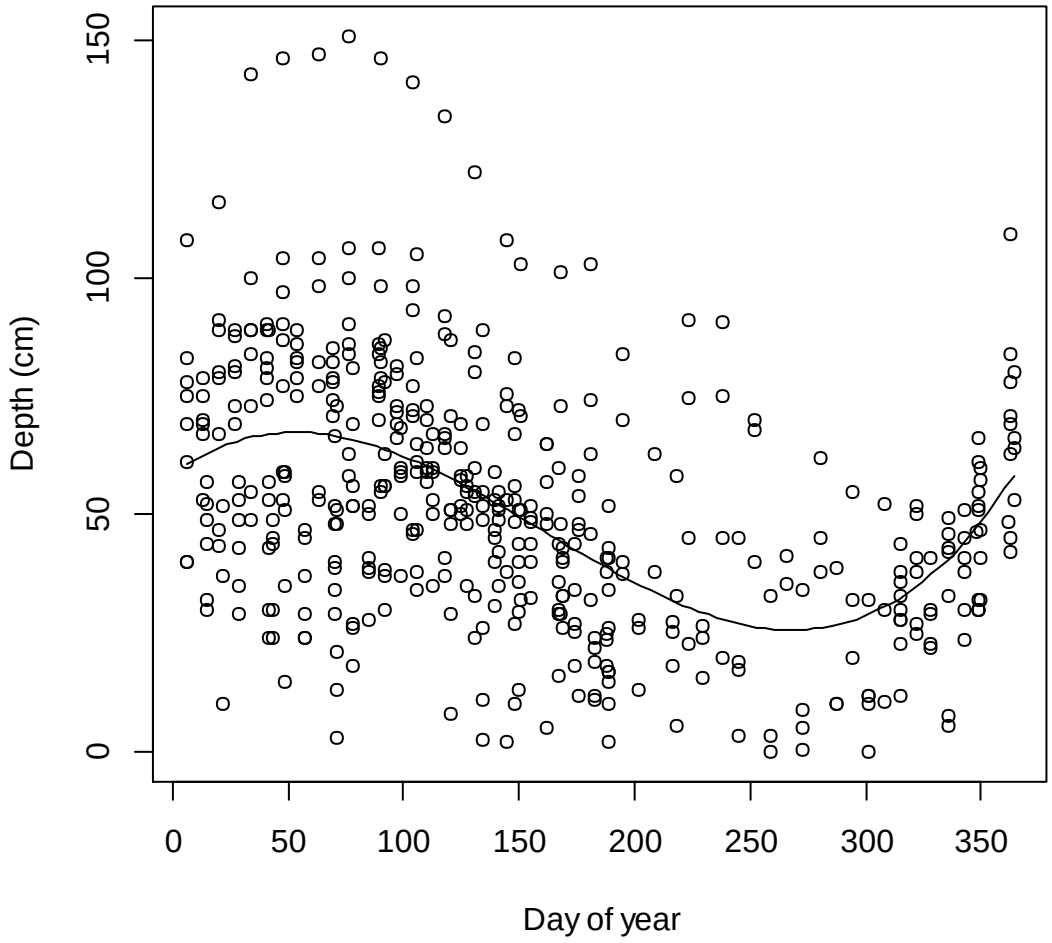


Figure 2.20.

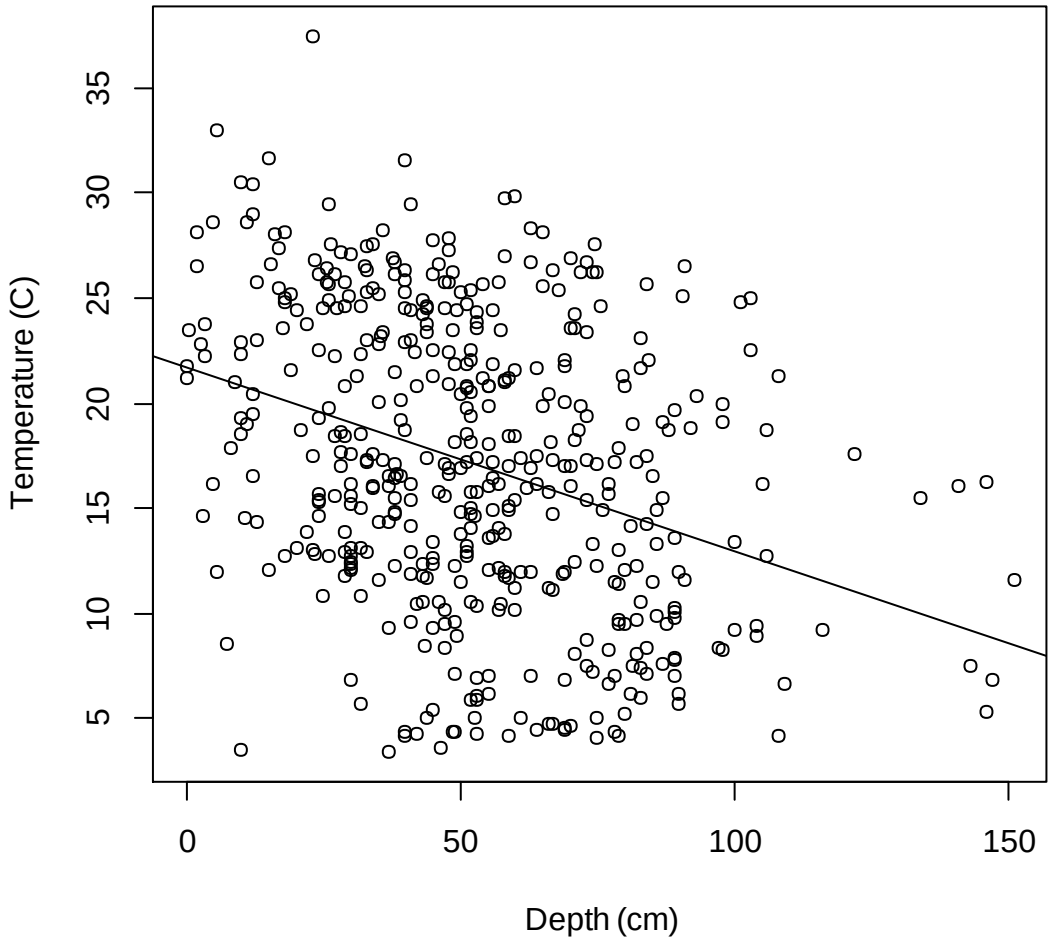


Figure 2.21.

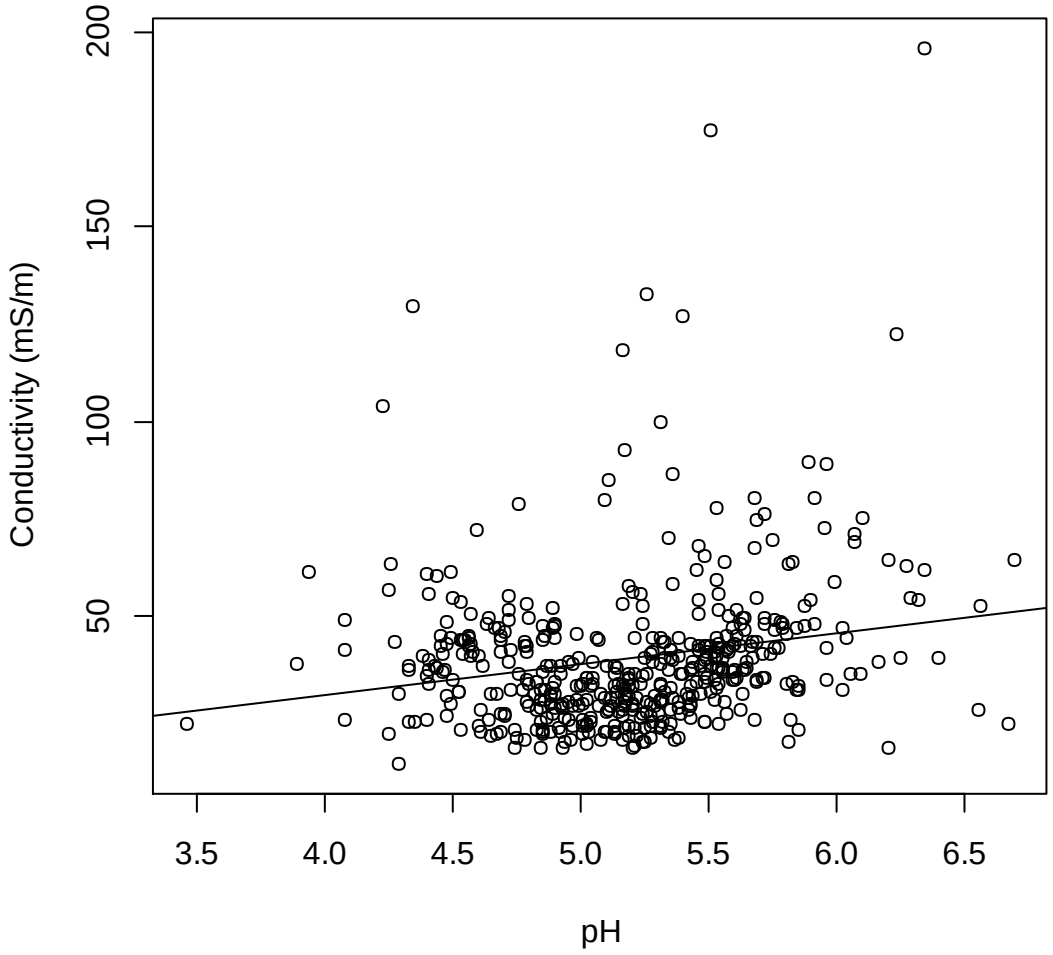


Figure 2.22.

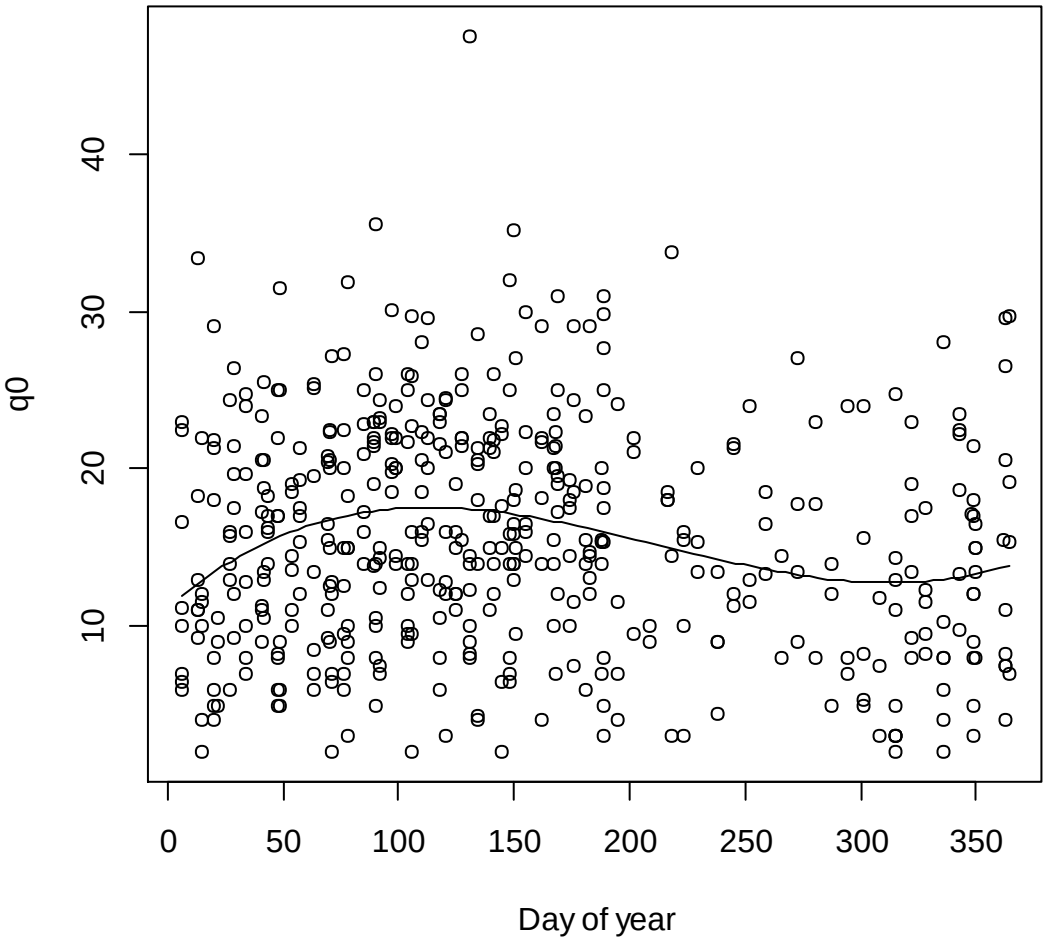
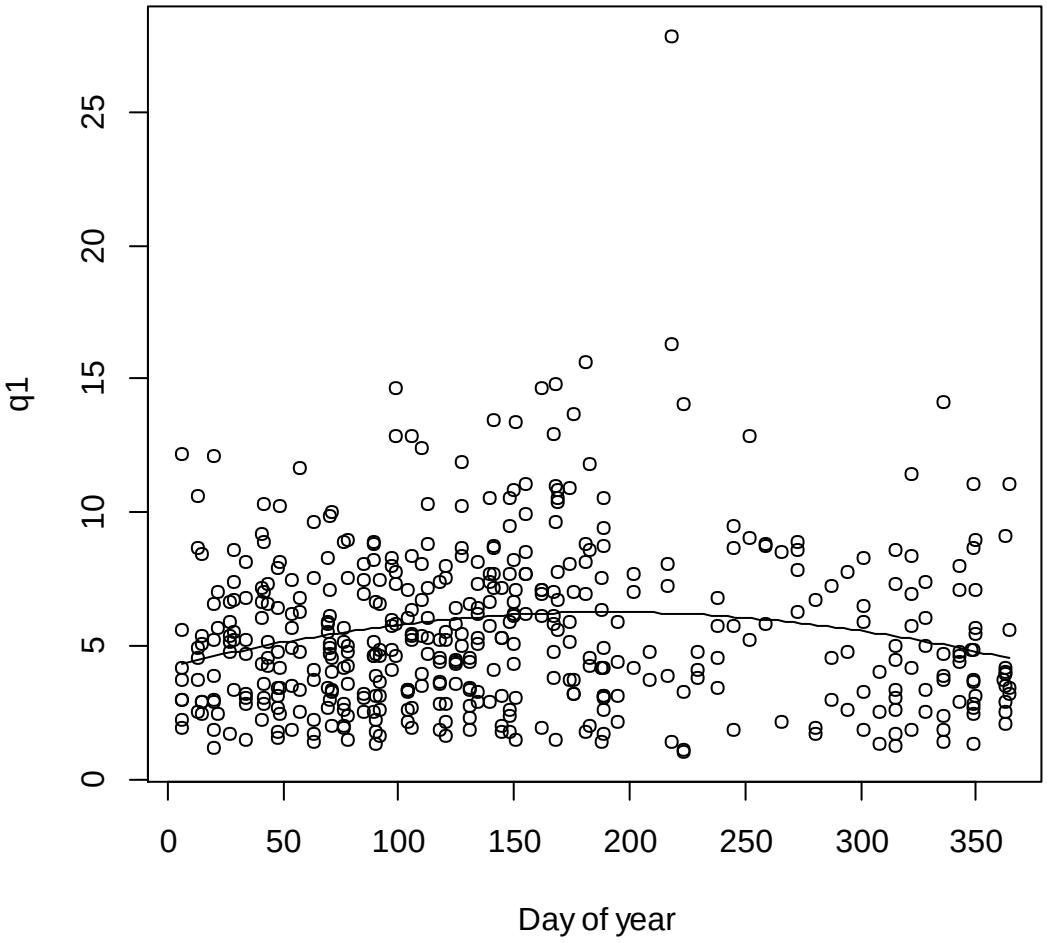


Figure 2.23.



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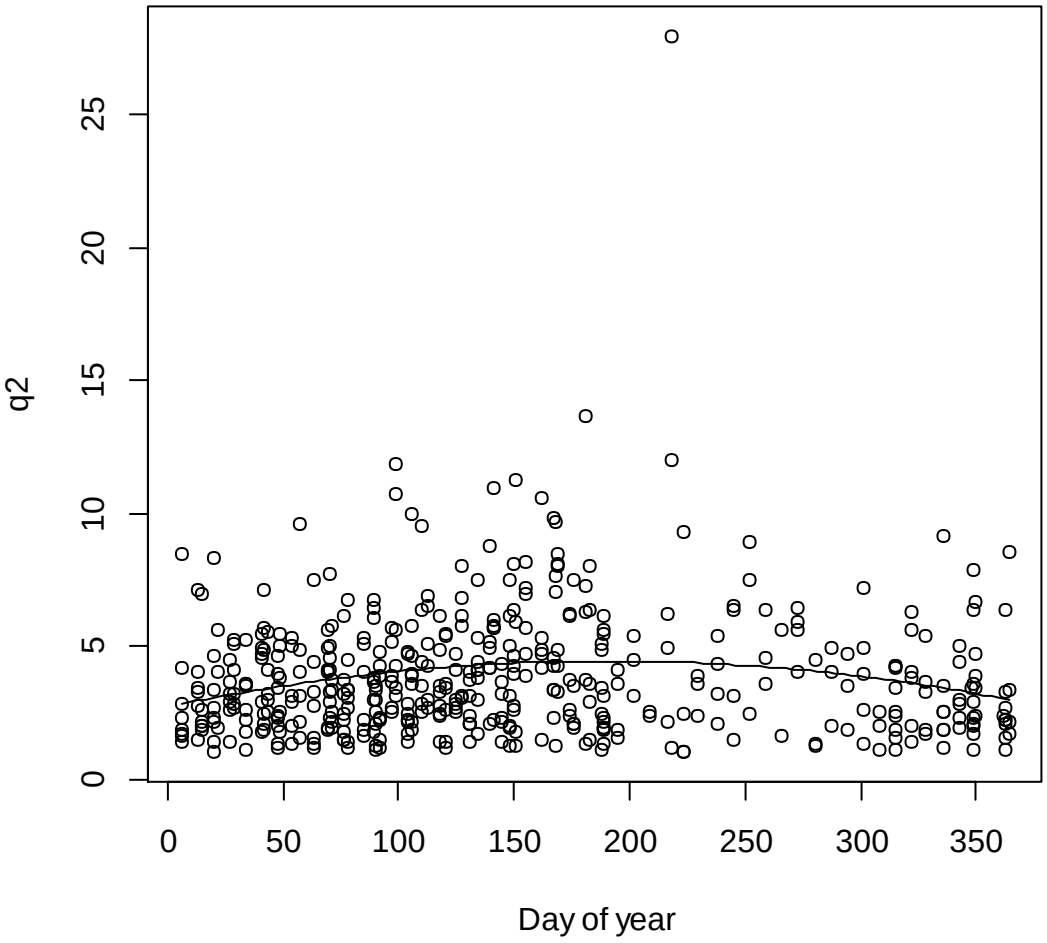


Figure 2.25.

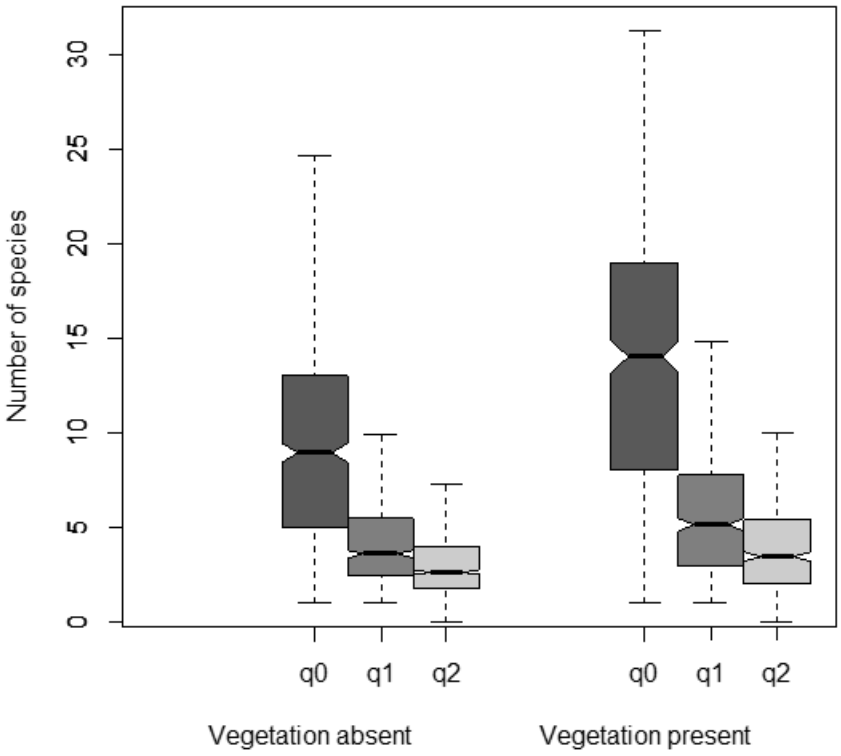

Number of species
0
5
10
15
20
25
30
q0
q1
q2
q0
q1
q2
Vegetation absent
Vegetation present

Figure 2.26.

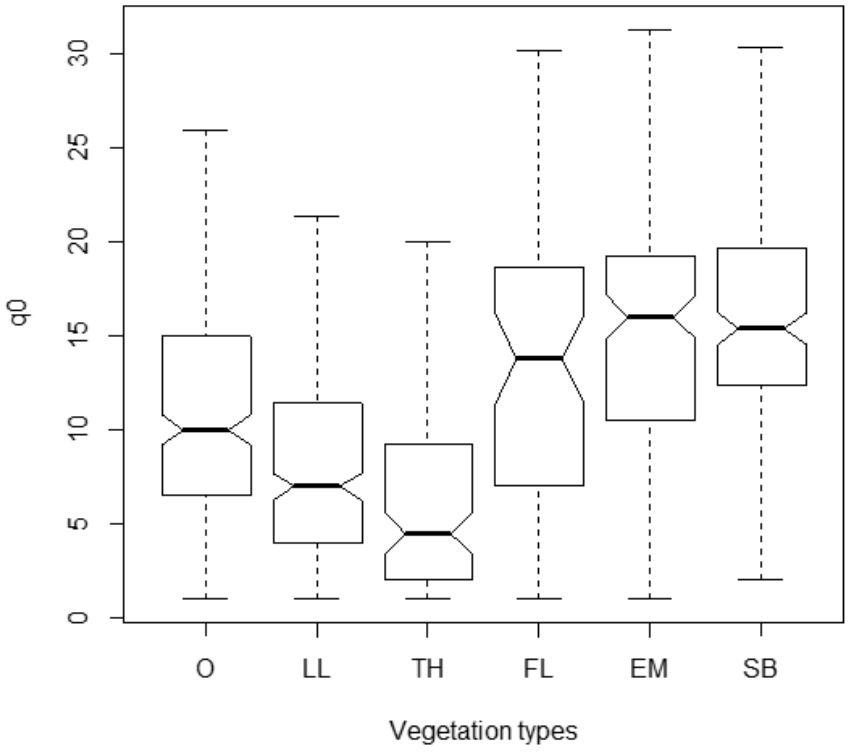


Figure 2.27.

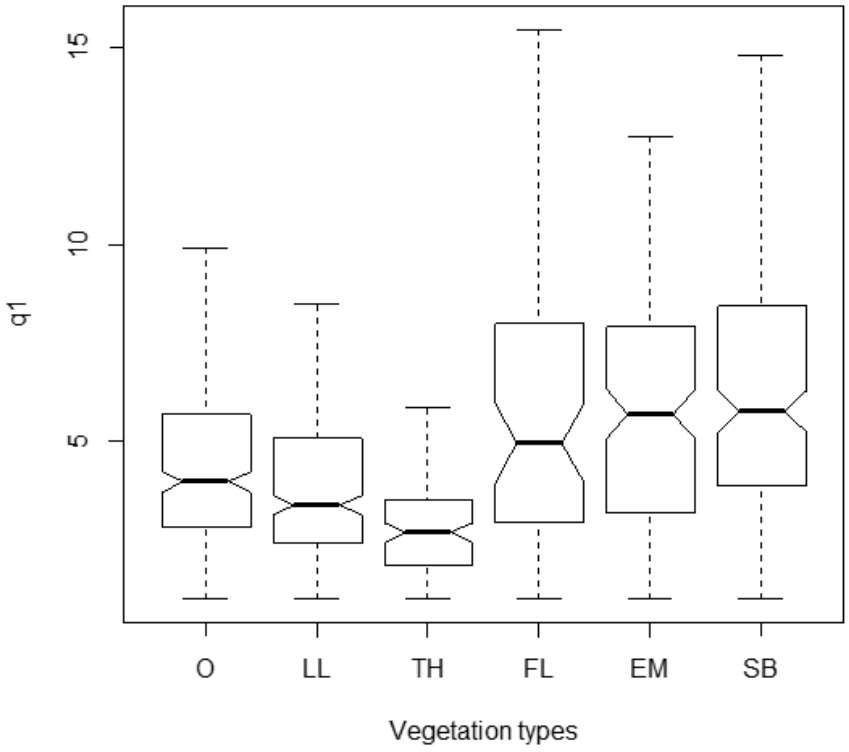


Figure 2.28.

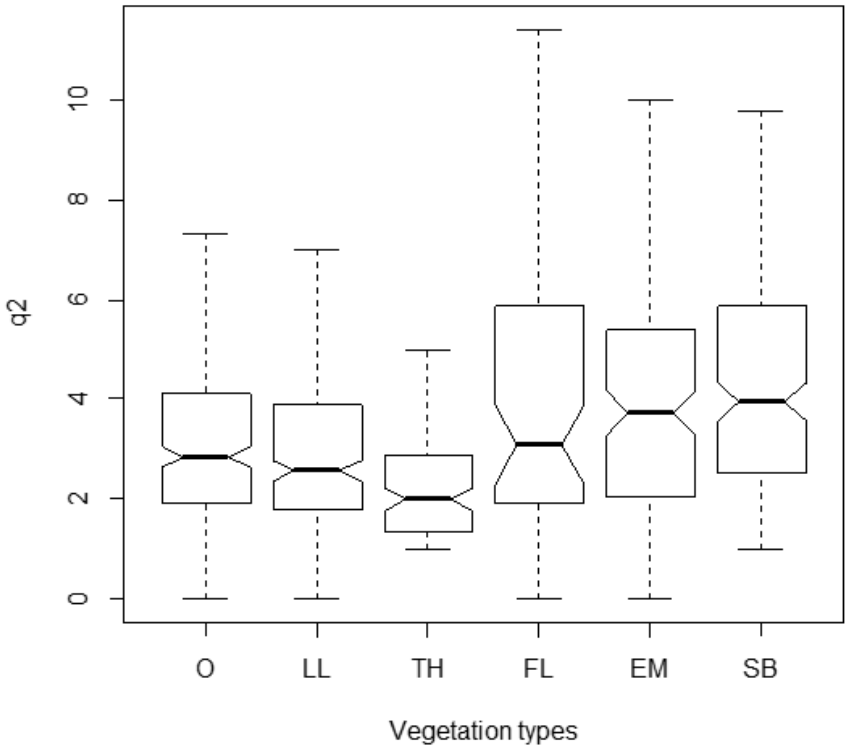


Figure 2.29.

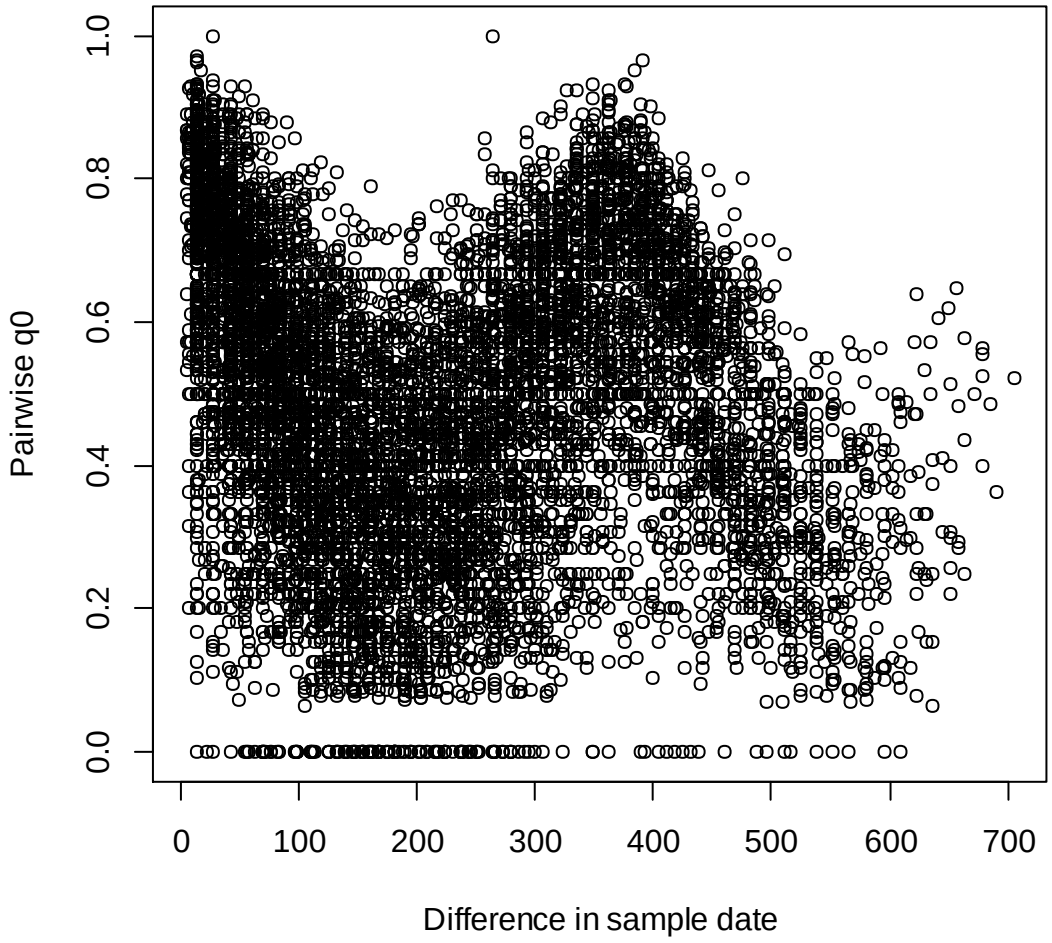

Pairwise q0
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
700
Difference in sample date

Figure 2.30.

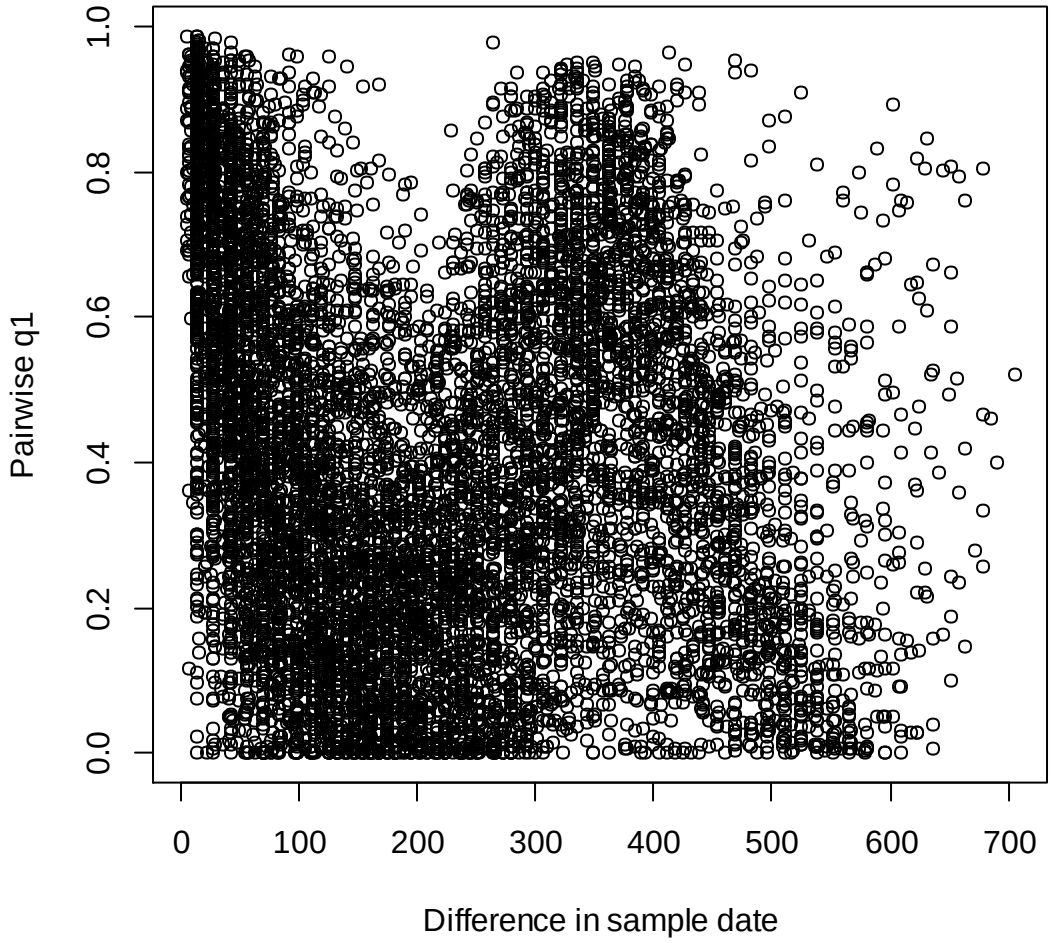

Pairwise q1
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
700
Difference in sample date

Figure 2.31.

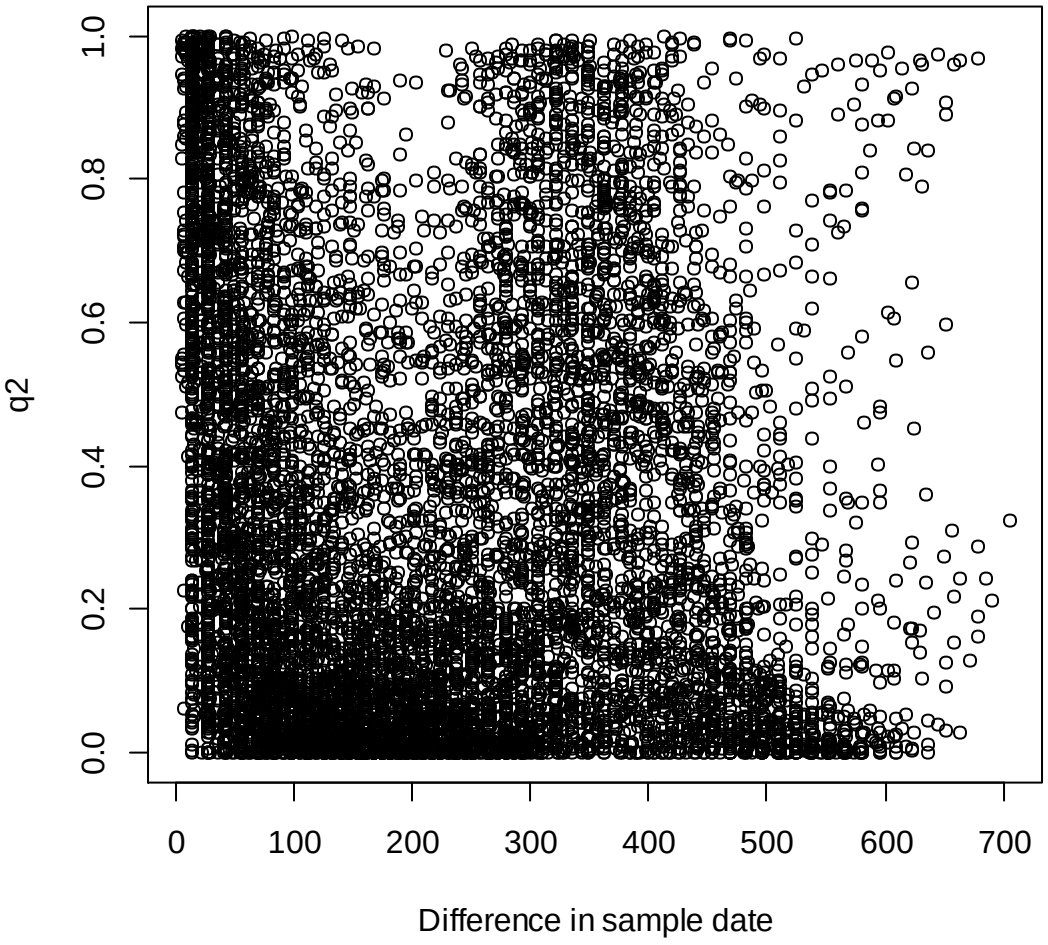

q2
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
700
Difference in sample date

Figure 2.32.

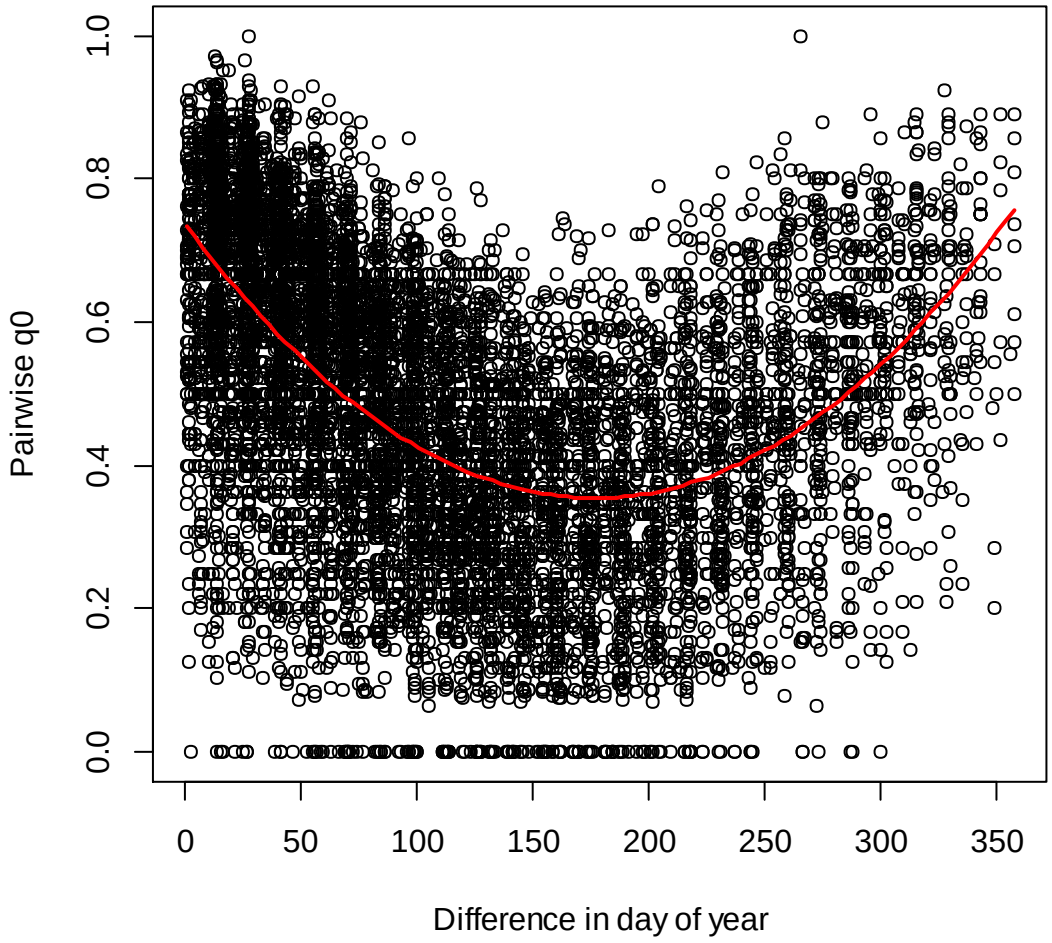

Pairwise q0
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
Difference in day of year

Figure 2.33.

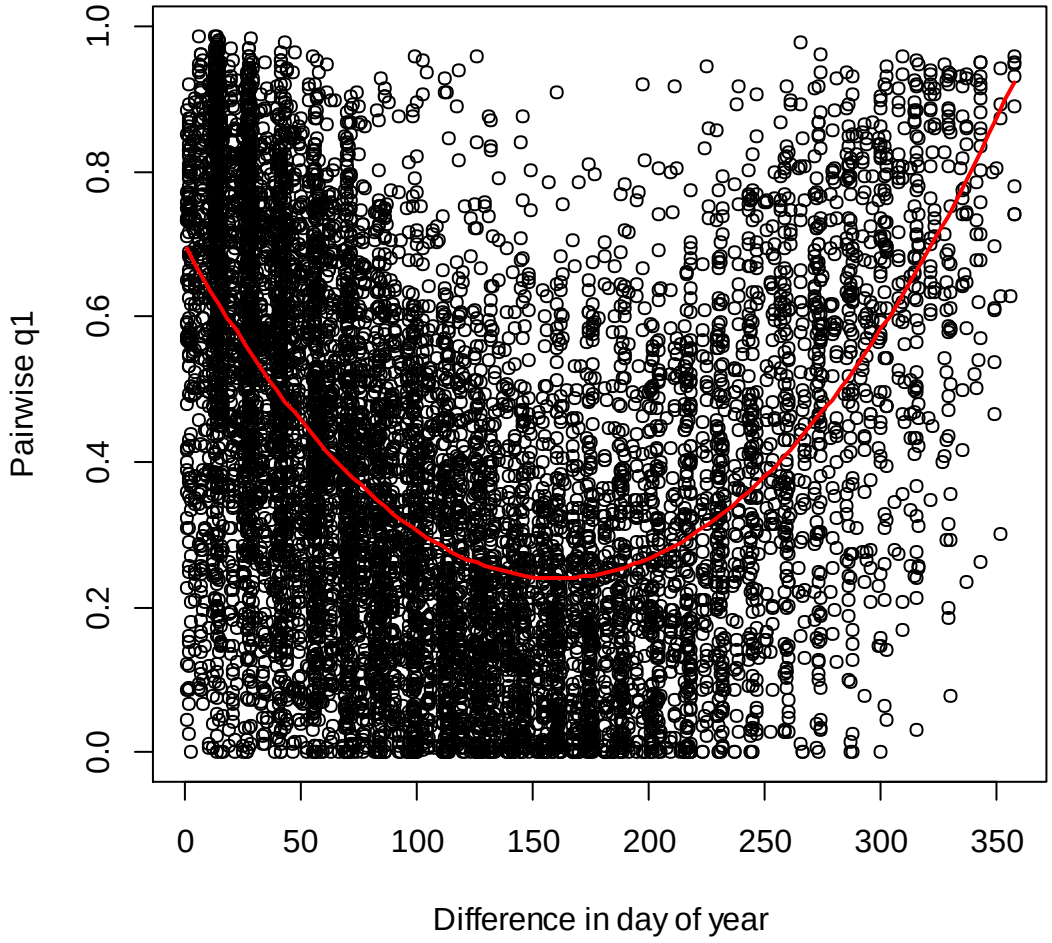
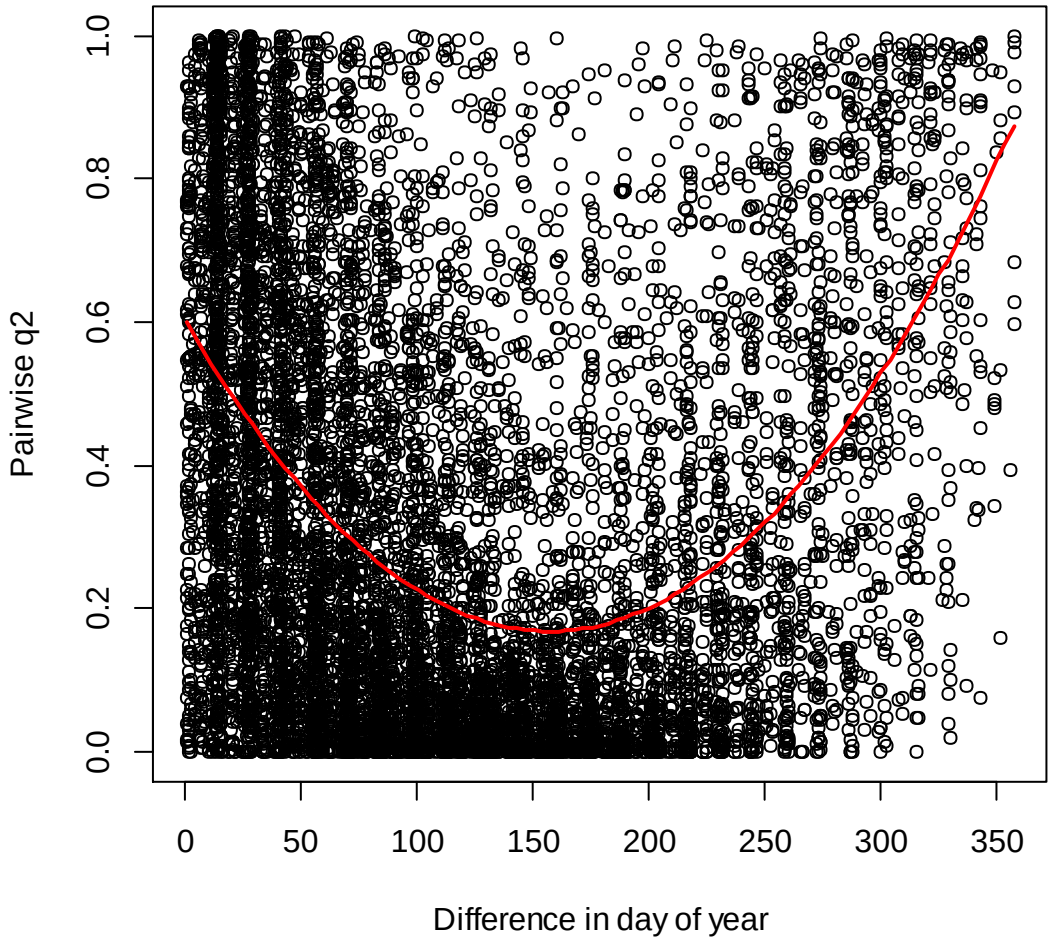

Pairwise q1
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
Difference in day of year

Figure 2.34.


Pairwise q2
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250
300
350
Difference in day of year

CHAPTER 3

THE EFFECT OF HYDROPERIOD AND PREDATION ON THE DIVERSITY OF TEMPORARY POND ZOOPLANKTON COMMUNITIES

Abstract

The communities observed in nature are the result of multiple low variance (niche) and high variance (neutral) processes interacting on a potential species pool. In temporary pond ecosystems it is hypothesized that the two dominant structuring forces on zooplankton communities are predation and demographic constraints due to wetland drying. Both of these forces are low variance processes, but act most strongly at opposing ends of a hydroperiod gradient. Our objective was to test how these two processes affect α- and β-diversity of zooplankton communities derived from a diverse temporary pond system. We hypothesized that decreased hydroperiod length and the presence of salamander larvae as predators would decrease β-diversity and that intermediate hydroperiod communities would have the greatest species richness. Our 1-year mesocosm experiment (n=36) consisted of two predation treatments (present/absent) and three hydroperiod treatments (short/medium/long) fully crossed, seeded from the resting egg bank of multiple temporary ponds. In total, we collected a total of 37 species of microcrustacean zooplankton from our mesocosms. A reduction in hydroperiod length resulted in lower α-diversity, with short hydroperiod treatments affected most strongly. However, salamander predation had little observable effect on zooplankton community diversity or size structure. Endpoint community dissimilarity (β-diversity) was greatest in the medium hydroperiod treatment with regards to species presence/absence, but was greatest in the long hydroperiod treatment when abundances were included. Our results indicate that hydroperiod length is an important force impacting the diversity of zooplankton communities in temporary wetland habitats, suggesting that environmental changes that reduce hydroperiod length would result in diversity loss.

Introduction

Understanding the factors that govern the development and distribution of species diversity within a landscape is an area of wide interest in ecology and conservation biology. Community assembly theory provides a process-based explanation for observed patterns of co-occurrence among species with particular reference to the sequence of species introductions (Diamond 1975; Chase 2003; Weiher et al. 2011; Vellend et al. 2014). Processes that govern community assembly can be classified as *low variance* or *high variance*, depending on how dissimilar the resulting species assemblage will be among communities undergoing assembly forces. Communities dominated by low variance processes exhibit high similarity in species composition under similar environmental conditions, whereas communities dominated by high variance processes can have dissimilar species assemblages although the environments themselves may be very similar (Chase 2003). Low variance interactions include competition, predation and niche adaptation that can have predictable outcomes and are often referred to as deterministic or niche processes. High variance examples include dispersal and drift (Vellend 2010), and are often referred to as neutral processes (Hubbell 2001). How these different processes interact to structure communities remains an interesting avenue of research.

Study System

Carolina bays are home to one of the most species-dense zooplankton communities within temporary ponds on Earth (Mahoney et al. 1990). These wetland ecosystems are unique to the Atlantic coastal plain of the United States and display a wide range of hydrologic conditions from ephemeral pools inundated only a few months per year to semi-permanent ponds that dry completely during droughts (Sharitz 2003). Additionally, there is great inter-annual variation in hydroperiod within a single wetland (Wyngaard et al. 1991; Sharitz 2003). Species that coexist within these ecosystems are well adapted to the variable hydrologic conditions, having various forms of resting stages or the ability to recolonize when conditions are favorable (Wellborn et al. 1996). In general, wetland hydrology appears to be an important driver of community diversity among these wetlands (DeBiase & Taylor 2005).

The Carolina bays on the Savannah River Site (SRS), South Carolina, USA, have exceptionally high species richness, with over 96 species of cladocerans and copepods (DeBiase & Taylor 2005). In addition to the high richness on the landscape level, up to 43 cladoceran species have been collected from a single wetland (personal observations, MZ). This degree of zooplankton species concentration is much greater than in any temporary wetland system yet studied (Mahoney et al. 1990).

Predation on zooplankton in this ecosystem comes largely from a diverse assemblage of aquatic insects and from salamander larvae (Taylor & Mahoney 1990). While the effects of fish predation on zooplankton and their cascading effects on the aquatic ecosystem are well studied (Brooks & Dodson 1965; Carpenter et al. 2011), salamander predation has received comparatively little research. Some mesocosm studies of salamander predation indicate that they can have important top-down effects on the community, such as decreased zooplankton density and biomass, and increased periphyton, bacteria and chlorophyll *a* (Holomuzki et al. 1994; Blaustein et al. 1996). In addition, Blaustein et al. (1996) found that salamander predation can reduce species richness. In the Carolina bays of SRS the primary salamander species are *Ambystoma opacum, A. talpoideum, A. tigrinum, Eurycea quadridigitata* and *Notophthalmus viridescens*, all of which include cladocerans as a substantial proportion of their diet

(Taylor et al. 1988). These predators substantially reduce zooplankton densities in field experiments (Scott 1990; Holomuzki et al. 1994; Blaustein et al. 1996).

The predation-permanence gradient

Current theory holds that communities are the product of four classes of processes: selection, drift, speciation, and dispersal (Vellend 2010). Low variance processes can be classified within selection, whereas high variance processes are represented by drift and dispersal. In this study we examine how two low variance selection processes – predation and demographic constraints imposed by temporal environmental variation (hydrological dry down) – affect diversity in wetlands of the SRS. This focus is generated from a conceptual model developed by Wellborn et al. (1996) that hypothesized the existence of a "predation-permanence gradient" (Figure 3.1.). The model of the predation-permanence gradient predicts that predation is the most important community-structuring force in long-hydroperiod wetlands (wetlands that typically contain standing water and where dry down is infrequent); whereas wetland drying is the most important force in short-hydroperiod wetlands (wetlands that frequently dry down). According to the model, these two factors determine the presence or absence of species, according to whether species are sensitive to predation or adapted to drying. By definition, predation-sensitive species will not survive in permanent wetlands where vertebrate predators such as fish can persist, but will survive in the latter, where predators are reduced. Species that inhabit temporary ponds are adapted to wetland drying. For those that produce resting stages, they must produce them prior to a dry-down and in sufficient quantities for their population to survive the dry phase and truncated wet phases. Because wetland drying and predation are both low variance processes and act in opposing directions, community similarity should be greatest at either end of the predation-permanence gradient. Alternatively, Chase (2003; 2007) suggests that community dissimilarity, or β-diversity, increases with increasing hydroperiod, but that predation increases similarity (decreases β-diversity) in permanent wetlands (Chase et al. 2009). It remains unclear what the net effect of these processes will be when both are acting together. However, because predation and wetland drying act most strongly at opposing ends of the hydroperiod gradient and are reduced in intermediate hydroperiods, dissimilarity could be increased at intermediate levels of both.

This greater dissimilarity could result from greater species richness or α-diversity at some level of intermediate hydroperiod. Preliminary observational data supports this prediction (Chase 2003; Debiase & Taylor 2005). High dissimilarity among their respective communities is needed to sustain high diversity within a group of wetlands (Chase 2003). This dissimilarity could occur either through differences in environmental conditions among wetlands or through high variance processes.

Experiment

Because these predictions have not been tested empirically, we conducted a mesocosm experiment in which predation and hydroperiod were manipulated to test the following hypotheses:

- **H1 – zooplankton community dissimilarity (β-diversity) increases with hydroperiod length.** More specifically, we predict that endpoint communities in long hydroperiod treatments will have greater β-diversity among replicates than those in short hydroperiod treatments. This is because the constraint on life history due to wetland drying is a low variance process, which is lessened as hydroperiod length increases;
- **H2 – zooplankton community similarity increases in the presence of predation.** We predict endpoint communities in the predation treatment will have lower β-diversity among replicates than those in which predation is absent. Since predation acts as a low variance process, the presence of a predator should lead to greater community similarity; and
- **H3 – intermediate hydroperiods will have the greatest zooplankton species richness (α-diversity).** We predict endpoint communities in medium hydroperiod treatments will have the greatest species richness. The low variance pressures of predation and wetland drying are reduced in intermediate hydroperiods, leading to the possibility of greater species richness when both of these pressures are lessened.

Treatments consisted of three hydroperiod manipulations of different duration (short, medium, and long) and two predation treatments (salamander larvae present versus absent) in a fully crossed, balanced design with six replicates per treatment. Mesocosms were seeded with soil from several natural wetlands

to provide a large representative species pool from which communities could assemble in response to the imposed treatments. This design addresses if and how the impacts of salamander predation and the demographic constraints imposed by wetland drying reduce a large species pool to the smaller communities observed in natural wetlands.

Methods

The experiment was conducted at the SRS, South Carolina near the Savannah River Ecology Laboratory (SREL). Experimental mesocosms consisted of 189 L plastic containers with overall dimensions of 108 cm X 55 cm X 45 cm (Figure 3.2.). Each mesocosm was seeded with ~ 200g of sediment from each of five nearby wetlands that span a hydrologic gradient from ephemeral pools to semi-permanent ponds. The sediments contained the resting stages of zooplankton and other organisms that exist at each of these sites, and were mixed and spread among all 36 mesocosms. By using this range of sediment samples, the intention was that each mesocosm would be inoculated with a good representation of the regional species pool. Well water was used to fill the mesocosms initially, and was used in refilling mesocosms after drydown. Rainfall was sufficient to keep the mesocosms filled throughout the remainder of the experiment. In addition, a well-mixed 18 L water sample was taken from one wetland, and 0.5 L of it were added to each mesocosm to provide a base level of primary and secondary production in anticipation of the addition of salamander larvae. The tops of all mesocosms were screened to prevent colonization by vertebrate-transported plankton species. Treatments implemented on each mesocosm were assigned randomly.

Hydrology treatments.

To simulate environmental drydown, water was released from a drainage valve on each mesocosm and run through a 183μm mesh plankton net to collect any zooplankton resting stages that were washed out, which were then returned to the mesocosm. The short-hydroperiod treatment was inundated for spans of 132 days, 96 days, and 64 days (the experimental endpoint). The medium-hydroperiod treatment was inundated for 218 days and for 92 days. The permanent treatment remained wet the entire 350 day duration of the experiment. In simulated drydowns, mesocosms were left dry for at

least 28 days before refilling. During the first drydown of the short-hydroperiod treatment, repeated rainfall kept shallow puddles (2-3 cm) within those mesocosms; however, in subsequent drydowns, mesocosms were tipped on their sides to prevent water from entering.

Predation treatments.

Predator treatments involved the addition of (n=2) marbled salamander (*Ambystoma opacum*) or mole salamander (*Ambystoma talpoideum*) larvae. First, larvae of *A. opacum* were added at day 132. These were replaced by *A. talpoideum* beginning on day 258 to reflect characteristic seasonal periodicities in their life histories. Specifically, *Ambystoma opacum* in SRS migrate to breeding ponds in the fall and larvae metamorphose between April and June (Pechmann 1995). In contrast, *Ambystoma talpoideum* larvae hatch in winter and metamorphose over the summer (Scott 1993). However, many become paedomorphic in wetlands that maintain constant water levels and are present throughout the year (Semlitsch 1987; Pechmann 1995). Thus, the change in species at day 258 (June 4th) mimics the seasonal replacement of species seen in these types of wetlands. The 18 mesocosms in the predation treatment were stocked with two salamander larvae per mesocosm (3.37/m^2), which is at the low end of natural hatching densities, but is within range of densities present as larvae approach metamorphosis (Scott 1990). When mesocosms were dry, salamanders were removed to a holding tank and fed a diet of zooplankton and insect larvae; they were returned to the mesocosms once the containers were refilled. Salamander density was monitored periodically by sweeping a dip net through each mesocosm until all were accounted for or three consecutive sweeps failed to produce another individual. Additional larvae were added as needed to maintain the treatment density. Our experimental protocol was in accordance with the procedures of and approved by The University of Georgia Institutional Animal Care and Use Committee.

Sample collection.

One zooplankton sample per mesocosm was taken monthly using a tube trap sampler (Paggi et al. 2001). One mesocosm in the short-hydroperiod/no-predator treatment group was damaged and drained out between the penultimate and final sampling day and was removed from all analyses for that date.

Cladocerans, calanoid copepods and female cyclopoid copepods were identified to the species level where possible. Immature Calanoida, immature and male Cyclopoida, Harpacticoida, Ostracoda, and Anostraca were identified to class level and were counted and designated as pseudo-species. Water conditions (pH, conductivity, temperature) were monitored in conjunction with each sampling using a YSI Professional Plus.

Statistical analysis.

To examine the effect of experimental treatments on community similarity, the abundances of each species present on the final sampling date were converted to a community matrix. Following Chao et al. (2012), two pairwise measures of β-diversity (Sørensen–Dice index and Morisita's overlap index) were calculated from each matrix, reflecting dissimilarity in presence/absence and relative abundances, respectively. These measures can be derived from the classical definition of β-diversity and are themselves transformations of a single diversity metric, but with different weights given to species frequencies (Jost 2011; Chao et al. 2012). The Sørensen–Dice index represents differences in species presence (q=0) and the Morisita's overlap index represents differences in dominant species (q=2) in the Hill number diversity framework (Jost 2011). The Sørensen–Dice index and Morisita's overlap index were calculated using the 'vegan' package in R. Within treatment β-diversity was compared using ANOVA and Tukey's HSD.

Sample species richness was calculated for each sample as total number of species and pseudo-species, omitting immature and male cyclopoids, since females were identified to species. The lower bound of true species richness was estimated using the bias-corrected Chao1 estimator (Chao 2005). Two additional measures of α diversity, estimators of Shannon's index (Chao and Shen 2003) and the Simpson index (minimum variance unbiased estimator) (Magurran 1988) that account for unseen species, were calculated for each sample using abundances of the same taxonomic units used for species richness calculation. Shannon's index may be interpreted as species evenness and the Simpson Index as species dominance (Whitaker 1972). Species richness, Shannon's Index and the Simpson Index represent the three levels of α diversity (q) recommended by Chao et al. (2012) to characterize a community of species.

These metrics can be converted to Hill numbers, with the interpretation of species richness (q=0) as the number of species, exponential of Shannon's Index (q=1) as the number of average species, and inverse Simpson Index (q=2) as the number of dominant species (Chao et al. 2012). The levels of diversity (q) in measures of both α and β diversity are equivalent regarding the weights given to species frequencies. Richness and diversity estimates were calculated using the program SPADE (Chao and Shen 2010). Effects of experimental treatments were analyzed using repeated measures ANOVA and pairwise t-tests. Differences between treatments for each date were analyzed with ANOVA and Tukey's HSD.

To determine if salamander predation had a size-selective effect on the zooplankton community, we compared the mean length of zooplankton between treatments. Length data for each species of zooplankton collected were obtained from the literature. Maximum length was chosen for the analyses because it was available for all species. The maximum length of a species was multiplied by the number of individuals collected and summed with that of all other species within a sample; the mean was then taken to produce the average maximum zooplankton length for that sample. Effects of experimental treatments on mean length were analyzed using repeated measures ANOVA. Differences between treatments for each date were analyzed with ANOVA and Tukey's HSD.

Result

Over 40,000 individuals of at least 37 species were collected during this study (Table 3.1.); representing 46% of taxa known from the wetlands from which the mesocosm communities were derived. Total mesocosm sample species richness ranged from 12 to 23 species (mean=16.19, SD = 2.86). The endpoint communities held 19 total species and ranged from 1 to 8 species per mesocosm (mean=3.37, SD = 1.97).

Beta diversity within hydroperiod treatments measured using the Sørensen–Dice index differed between treatments ($F=24.46$, $p<0.001$) and was greatest within the medium-hydroperiod treatment (Figure 3.3.), which differed from both the short ($p<0.001$) and long-hydroperiod treatments ($p<0.001$). The long-hydroperiod treatment was also more dissimilar than the short-hydroperiod treatment ($p=0.03$). The same analysis performed on Morisita's overlap index also found differences between treatments

(F=29.78, p<0.001). However, in this analysis the short-hydroperiod treatment had greater similarity within treatment than both the medium (p<0.001) and long-hydroperiod treatments (p<0.001). β-diversity within predation treatments differed in Sørensen–Dice index calculations (F=10.06, p=0.002), with dissimilarity greater within the no predator treatment than the predator treatment (Figure 3.4.). The Morisita overlap index did not differ between predation treatments (F=0.68, p=0.41).

Neither hydroperiod (F=1.88, p=0.17), or predation (F=0.01, p=0.91), or their interaction (F=1.33, p=0.28) had a statistically significant effect on estimated species richness. However, species richness did vary over the duration of the experiment (Figure 3.5.) and differed significantly between hydroperiod treatments on day 321 (F=5.37, p=0.01) and day 350 (F=4.41, p=0.02). On day 321, species richness of the short-hydroperiod treatment was significantly lower than in both the medium (p=0.01) and long-hydroperiod treatments (p=0.002); on day 350, only the long and short-hydroperiod treatments differed (p=0.02; Figure 3.6.).

Similar to species richness, there were no differences between hydroperiod treatments (F=2.68, p=0.09), predation treatments (F=0.002, p=0.96) nor the interaction (F=0.003, p=0.99). However, Shannon's index did differ between hydroperiod treatments on day 321 (F=8.12, p=0.002) and day 350 (F=19.62, p<0.001) (Figure 3.7.). On day 321, the short-hydroperiod treatment was significantly lower than both medium (p=0.003) and long-hydroperiod treatments (p=0.006), whereas on day 350, the long-hydroperiod treatment had a greater Shannon index than both short (p<0.001) and medium-hydroperiod treatments (p<0.001; Figure 3.6.).

Hydroperiod (F=4.28, p=0.02; Figure 3.8.), but not predation (F=0.04, p=0.84) or the interaction (F=0.15, p=0.86) had a statistically significant effect on the estimated Simpson index. The Simpson index according to hydroperiod treatment differed significantly on day 321 (F=6.52, p=0.005) and day 350 (F=22.25, p<0.001) of the experiment. The short-hydroperiod treatment differed from both the medium (p=0.01) and long-hydroperiod (p=0.01) treatments on day 321, whereas the long-hydroperiod treatment differed from both the short (p<0.001) and medium-hydroperiod (p<0.001) treatments on day 350 (Figure 3.6.).

Predation did not have an effect on the size structure of the zooplankton community either overall ($F=0.002$, $p=0.97$; Figure 3.9.), or on any sampling date. Mean maximum length varied little over the course of the experiment (mean=1.09, SD = 0.06), with a range of 0.48-1.34 among samples.

Discussion

β-diversity of the endpoint communities in this experiment were strongly affected by hydroperiod. The short-hydroperiod treatment exhibited moderate community dissimilarity when rare species were considered, but these communities were nearly identical with respect to the dominant species. In contrast, long-hydroperiod communities showed moderate community dissimilarity when both rare and dominant species were considered. The medium-hydroperiod treatment had much greater dissimilarity with respect to rare species than the other two treatments, but communities were much more similar in dominant species. The directionality and relative magnitude of the shift in dissimilarity from $q=0$ to $q=2$ were similar in both drydown treatments. This similarity suggests that some species respond more favorably to wetland drying and come to dominate the community once wetlands are re-flooded. At both levels of β-diversity, the long-hydroperiod treatment had significantly greater dissimilarity than did the short-hydroperiod treatment, which supports the prediction that wetland drying promotes community similarity. This corroborates the study of Chase (2007), which found greater community similarity among ponds that experience drought than those that do not.

The effect of hydroperiod on α-diversity was significant in a way similar to its effects on β-diversity. The endpoint communities within the short-hydroperiod treatment were low in richness, low in equitability, and low in dominant species relative to the other two treatments. The medium and long-hydroperiod treatments were not significantly different from each other in species richness, but differed at higher orders of diversity. Compared to the long-hydroperiod treatment, the medium-hydroperiod treatment had lower species evenness and a low number of dominant species. Wetland drying appears to impact all levels of α-diversity. A moderate frequency of drying led to communities dominated by just a few abundant and common species with a relatively high number of rare species. More frequent drydown led to the loss of the rarer species and a relatively low-diversity community.

Predation appeared to have a smaller impact on β-diversity. While it had no effect on dominant species, it had some effect on the presence of rare species, leading to slightly more similar communities when salamander larvae were present. This result partially supports the prediction that predation should increase community similarity. However, there were no differences between predation treatments in analyses of α-diversity. This lack of difference suggests that salamander predation, at least at the densities used in this experiment had little effect on diversity. These effects might have been observed had we used higher predator densities. Studies that have noted effects of salamander larvae predation on zooplankton densities had predator densities that were two to eight times greater than the density we used (Scott 1990; Blaustein et al. 1996).

Predation appeared to have no effect on the size distribution of the zooplankton community, although the preference for larger zooplankton as prey has been observed in multiple salamander predation studies (Taylor et al. 1988; Holomuzki et al. 1994; Blaustein et al. 1996). This could be due to the overall paucity of larger zooplankton species (e.g. *Daphnia*, *Simocephalus*, Calanoid copepods) in our mesocosms. Since large zooplankton species were few, the communities in the predator and no-predator treatments were both dominated by smaller species. The lack of large zooplankton species was puzzling, but was clearly not a predation effect as they were scarce in both treatment groups.

The third prediction that species richness would be greater in intermediate hydroperiod treatments was not supported by the results. Mean species richness per mesocosm was lower in the medium-hydroperiod treatment than the long-hydroperiod treatment, though this difference was small and not statistically significant. The model that led to this prediction suggested that dissimilarity may be greater in intermediate hydroperiods. Our interpretation was that greater species richness would be the cause. Dissimilarity was indeed greater within the medium-hydroperiod treatment, but species richness was not. Instead, it appears that the greater dissimilarity was the result of differences in species presence/absence between mesocosms.

Following the changes in diversity throughout the experiment, it becomes notable that the hydroperiod treatments largely tracked each other's changes over time and only diverged during the last two sampling

dates. These coincide with the re-inundation of the medium and short-hydroperiod mesocosms following drydown; in the case of the latter, it was the second of two drydowns. The medium-hydroperiod treatment did not diverge from the long hydroperiod treatment in species richness, but did so in both Shannon's and Simpson indices indicating that community structure changed from one more even in species abundance to one that became dominated by a few species. The short-hydroperiod treatment showed a similar pattern of community structure, but with lower diversity at all levels. Interestingly, there was not a perceptible difference in any diversity measure between hydroperiod treatments after the first drydown of the short-hydroperiod treatment. This result may be partially explained by the mesocosms on this occasion retaining a small amount of water through the drydown due to frequent rains. However, there must have been some effect of this drydown to result in the drastic change observed after the second drydown. Studies in temporary wetlands have found that frequent drydowns can deplete the resting egg bank (Taylor et al. 1990), which is a plausible explanation for the pattern observed here.

The predation-permanence gradient model predicts that the low variance processes of predation and demographic constraint due to wetland drying are greatest at opposite ends of the hydrologic gradient (Wellborn et al. 1996). Our data support the latter, but provide little support for the former. A reduced set of species was able to persist in the short hydroperiod treatments, whereas a richer assemblage was found in the other two hydroperiod treatments. In contrast, predation had little impact on the experimental communities. However, our experiment was not designed to test increasing intensity of predation as the predation-permanence gradient hypothesizes, but simply whether or not predation could influence diversity. An extension of the predation-permanence gradient model is that low variance processes are lessened in intermediate portions of the hydrologic gradient, so that high variance stochastic processes take on greater importance. This was manifested as greater dissimilarity within the medium hydroperiod treatment instead of differences in species richness as had been anticipated.

A key prediction of the predation-permanence gradient model, the increase in community similarity as hydroperiod is shortened, was supported by this experiment. In addition, shorter hydroperiod communities had lower richness than longer hydroperiod communities. One implication is that a

reduction in hydroperiod length among temporary wetlands could lead to a loss of diversity. However, another interesting finding is the increase in dissimilarity among intermediate hydroperiod wetlands. This increase indicates that at some level of drying frequency, diversity could remain high at least among a group of wetlands. Within our study system it appears that high dissimilarity among wetlands may be an important factor in maintaining high diversity on the landscape level.

Acknowledgements

We wish to thank Rebecca Sharitz (SREL/UGA) and Linda Lee (SREL) for the use of lab space, equipment and logistical support. We also wish to thank David Scott (SREL) and Stacy Lance (SREL/UGA) for collecting the salamander larvae used in this experiment, advising us in salamander care and logistical support in mesocosm setup. We thank the Odum School of Ecology for funding this research through two OSE Small Grants. Our experimental protocol was approved by The University of Georgia Institutional Animal Care and Use Committee under Animal Use Protocol # A2012 04-024-A2 and UGA Animal Welfare Assurance # A3437-01.

Table 3.1: Table of zooplankton taxa collected from the wetlands from which the communities in the mesocosms were derived. Taxa that have a frequency listed were also collected within mesocosms. Frequency refers to the number of mesocosms a species was collected in during the experiment.

Species/pseudospecies	Freq.	Species/pseudospecies	Freq.
Anostraca	2	*Bosmina tubicen*	34
Streptocephalus seali		*Camptocercus cf. rectirostris*	31
Eubranchipus stegosus		*Ceriodaphnia laticaudata*	1
Laevicaudata		*Ceriodaphnia megops*	
Lynceus gracilicornis		*Ceriodaphnia cf. dubia*	
Calanoida	1	*Chydorus eurynotus*	
Agalaodiaptomus atomicus		*Chydorus linguilabrus*	
Agalaodiaptomus clavipoides		*Chydorus sp. A*	22
Agalaodiaptomus stagnalis	1	*Chydorus sp. B*	36
Hesperodiaptomus augustaensis		*Daphnia laevis*	6
Leptodiaptomus moorei		*Diaphanosoma cf. brachyurum*	36
Onychodiaptomus sanguineus		*Disparalona acutirostris*	
Cyclopoida		*Dunhevedia cf. crassa*	
Acanthocyclops robustus	15	*Ephemeroporus hybridus*	3
Acanthocyclops venustoides		*Eurycercus longirostris*	
Diacyclops crassicaudis		*Eurycercus microdontus*	
Diacyclops navus		*Grimaldina brazzai*	1
Diacyclops nearcticus		*Ilyocryptus bernerae*	1
Diacyclops thomasi		*Ilyocryptus gouldeni*	6
Ectocyclops phaleratus		*Ilyocryptus silvaeducensis*	24
Eucyclops elegans		*Ilyocryptus spinifer*	7
Eucyclops pectinifer	25	*Kurzia cf. media*	2
Macrocyclops albidus		*Lathonura cf. rectirostris*	
Macrocyclops fuscus		*Macrothrix elegans*	31
Megacyclops cf. viridis		*Macrothrix cf. spinosa*	11
Microcyclops sp.		*Macrothrix sp. B*	
Orthocyclops modestus		*Moina micrura*	15
Paracyclops chiltoni		*Moinodaphnia macleayii*	
Thermocyclops parvus		*Oxyurella brevicaudis*	
Tropocyclops sp.	25	*Paralona cf. pigra*	4
Harpacticoida	2	*Picripleuroxus denticulatus*	
Cladocera		*Picripleuroxus stramineus*	
Acantholebris curvirostris		*Polyphemus cf. pediculus*	
Acroperus sp.		*Pseudochydorus cf. globosus*	
Alona costata	31	*Pseudosida bidentata*	27
Alona guttata	4	*Scapholebris armata*	12
Alona manueli	1	*Scapholebris freyi*	33
Alona ossiani	6	*Simocephalus cf. exspinosus*	
Alona quadrangularis		*Simocephalus serrulatus*	2
Alona rustica americana	1	*Streblocercus pygmaeus*	1

Alonella excisa	33	*Streblocercus serrulatus*	
Alonella exigua		**Ostracoda**	22

Figure 3.1. A conceptual diagram of the predation-permanence gradient in temporary wetlands, developed from Wellborn et al. (1996). The strength of the environmental constraint on life history due to wetland drying is greatest when hydroperiod is short and alleviates as hydroperiod increases. On the other hand, the strength of predation is greatest in long hydroperiod ponds and diminishes in shorter hydroperiod wetlands due to fewer predators capable of sustaining populations in these habitats. The intersection of these two relationships suggests that the pressure exerted by these two processes may be lessened in moderate hydroperiods, although the true shape of these relationships is unknown.

Figure 3.2. Photograph of the experimental setup. Mesocosms consisted of thirty-six 189 L plastic containers with overall dimensions of 108 cm X 55 cm X 45 cm arranged in two rows of eighteen. Mesocosms were covered with screen and had drainage valves installed near the bottom to manipulate water levels.

Figure 3.3. Mean community dissimilarity calculated within each hydroperiod treatment for endpoint communities at two diversity levels, Sørensen–Dice index (q=0) and Morisita's overlap index (q=2). The colored lines indicate +/- 1 S.E.

Figure 3.4. Boxplot of within treatment β-diversity measured as Sørensen–Dice index dissimilarity for the three hydroperiod treatments calculated from samples collected on day 350, the experimental endpoint. The dark bar within each box represents the median. The lower and upper margins of each box represent the first and third quartiles respectively. The whiskers extend to the most extreme point that is no greater than 1.5 times the interquartile range from the box. Notches on the vertical margins of the boxes are calculated as +/- 1.58 x the interquartile range/ square root (n), and represent approximately the 95% confidence interval surrounding the median.

Figure 3.5. Mean species richness estimated using the Chao1 estimator by hydroperiod treatment over the 350 day experiment. The colored polygons enclose regions bounded by +/- 1 standard error. Breaks in the polygons represent periods when the mesocosms within that treatment were dry.

Figure 3.6. Number of species represented as Hill numbers. Mean values were calculated within each hydroperiod treatment for endpoint communities at three diversity levels, Chao 1 species richness (q=0), estimated exponential Shannon's index (q=1), and estimated inverse Simpson index (q=2). The colored polygons enclose regions bounded by +/- 1 standard error.

Figure 3.7. Mean estimated Shannon's index by hydroperiod treatment over the 350 day experiment. The colored polygons enclose regions bounded by +/- 1 standard error. Breaks in the polygons represent periods when the mesocosms within that treatment were dry.

Figure 3.8. Mean estimated Simpson index by hydroperiod treatment over the 350 day experiment. The colored polygons enclose regions bounded by +/- 1 standard error. Breaks in the polygons represent periods when the mesocosms within that treatment were dry.

Figure 3.9. Mean maximum length of the zooplankton community by predation treatment over the 350 day experiment. The colored polygons enclose regions bounded by +/- 1 standard error. Note that the scale of the y-axis does not begin at zero

Figure 3.1.

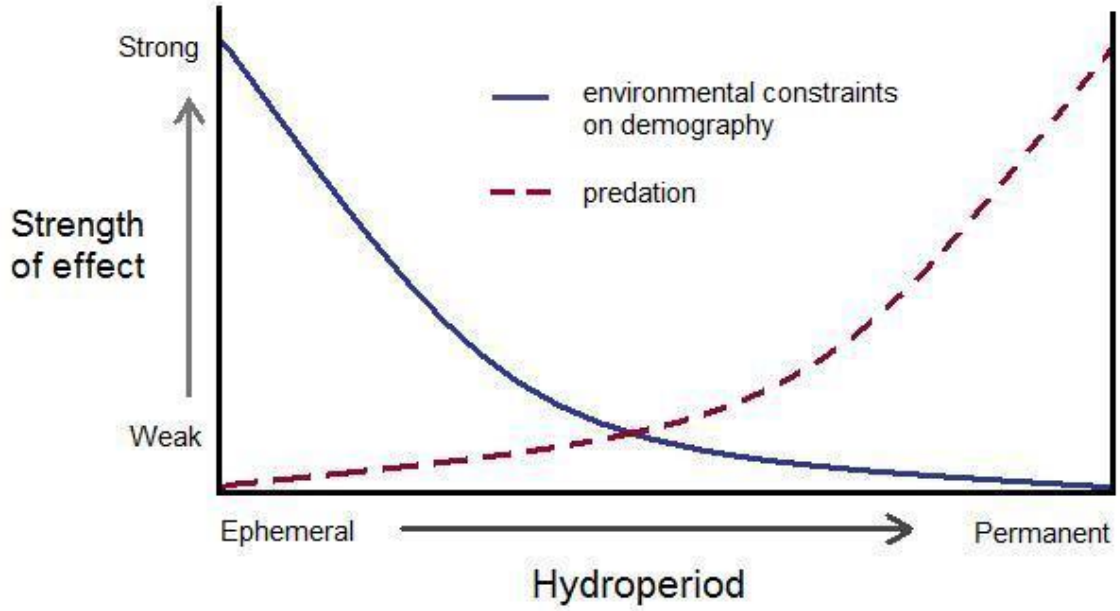

Strong
Weak
Strength of effect
environmental constraints on demography
predation
Ephemeral
Permanent
Hydroperiod

Figure 3.2.

Figure 3.3.

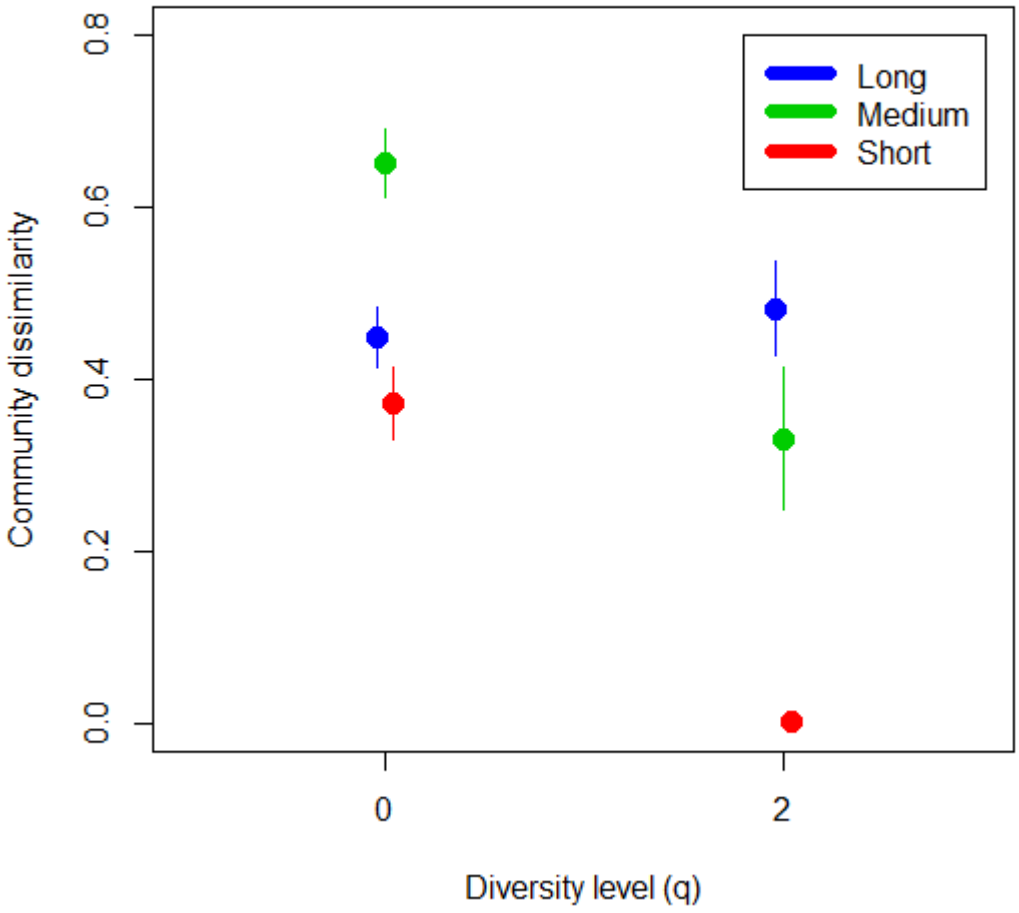

Community dissimilarity
0.0
0.2
0.4
0.6
0.8
Long
Medium
Short
0
2
Diversity level (q)

Figure 3.4.

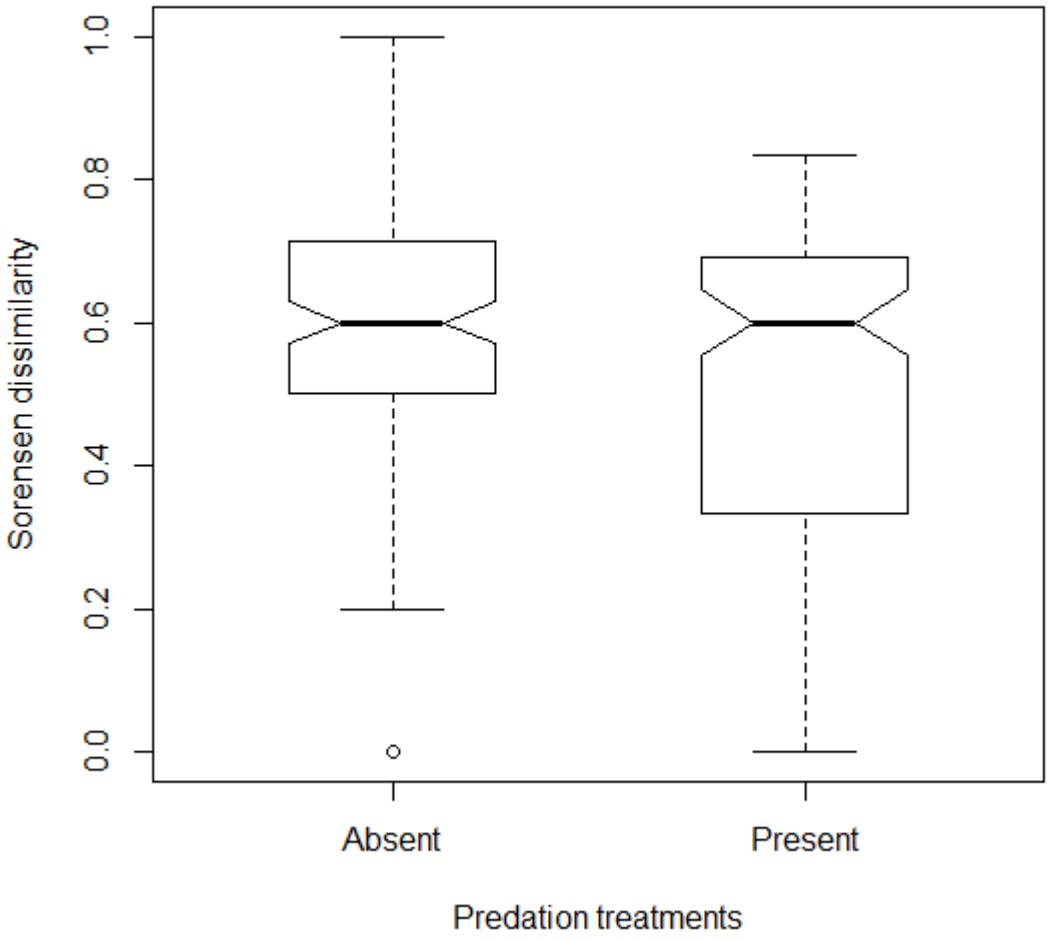

Sorensen dissimilarity
0.0
0.2
0.4
0.6
0.8
1.0
Absent
Present
Predation treatments

Figure 3.5.

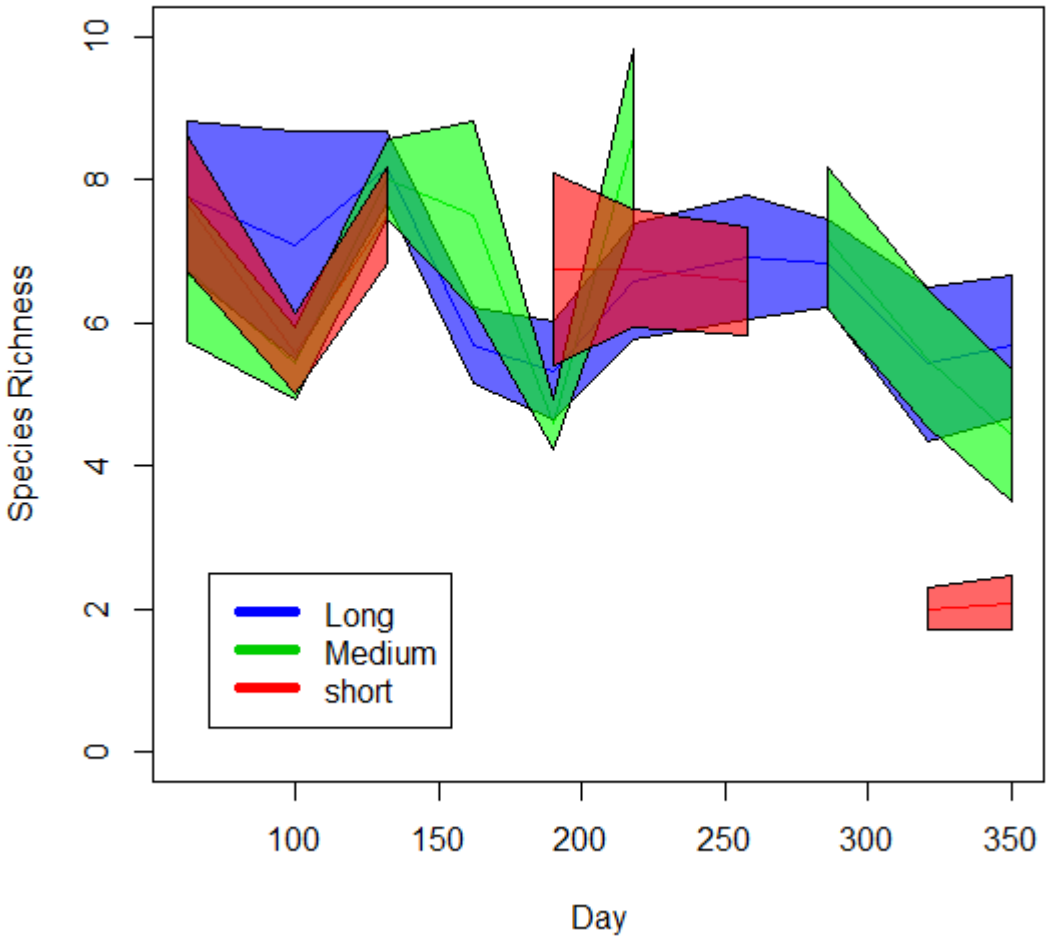

Species Richness
0
2
4
6
8
10
100
150
200
250
300
350
Day
Long
Medium
short

Figure 3.6.

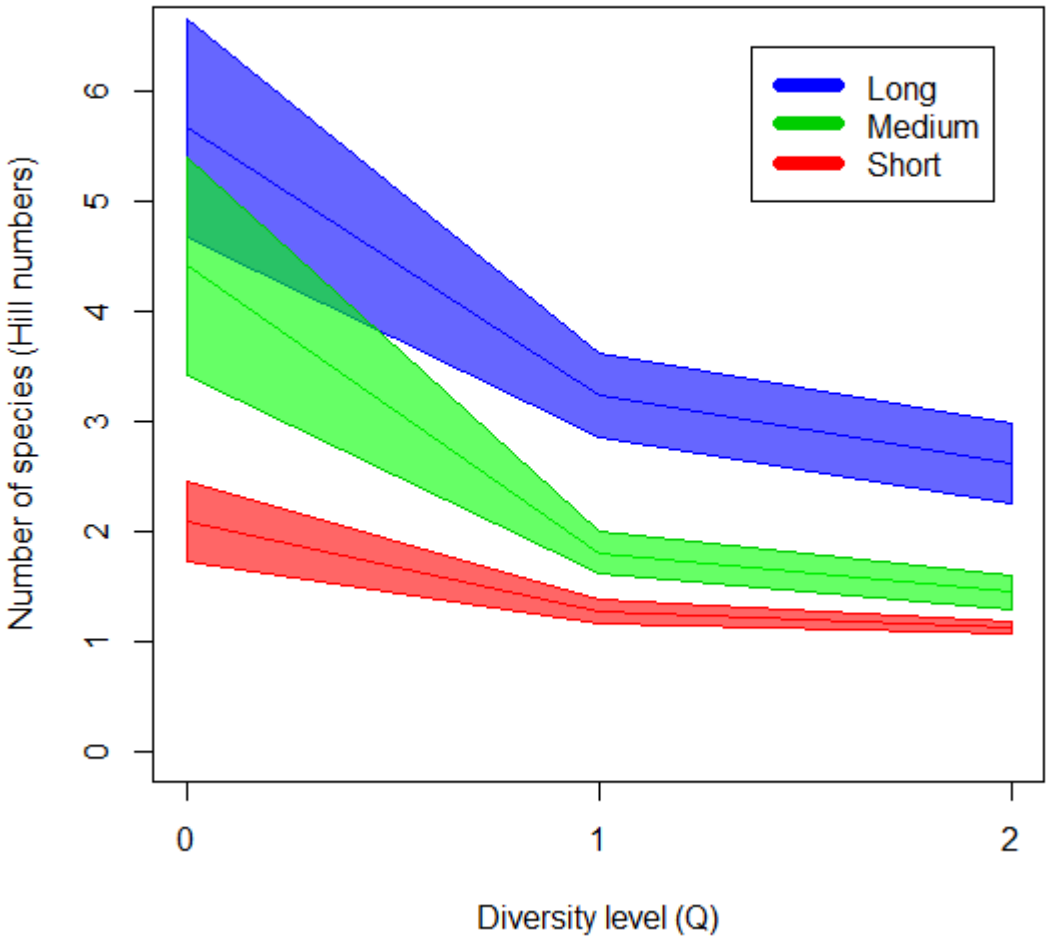

Long
Medium
Short
Number of species (Hill numbers)
0
1
2
3
4
5
6
0
1
2
Diversity level (Q)

Figure 3.7.

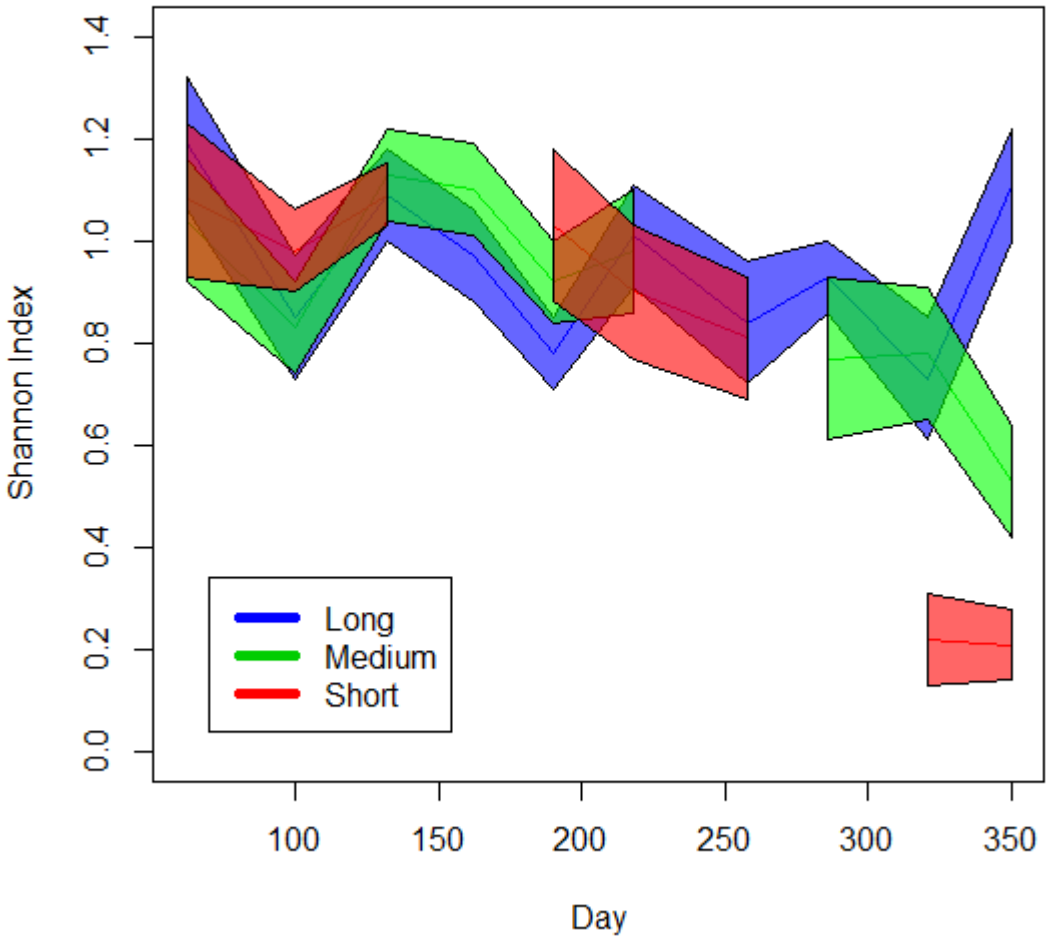

Shannon Index
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
100
150
200
250
300
350
Day
Long
Medium
Short

Figure 3.8.

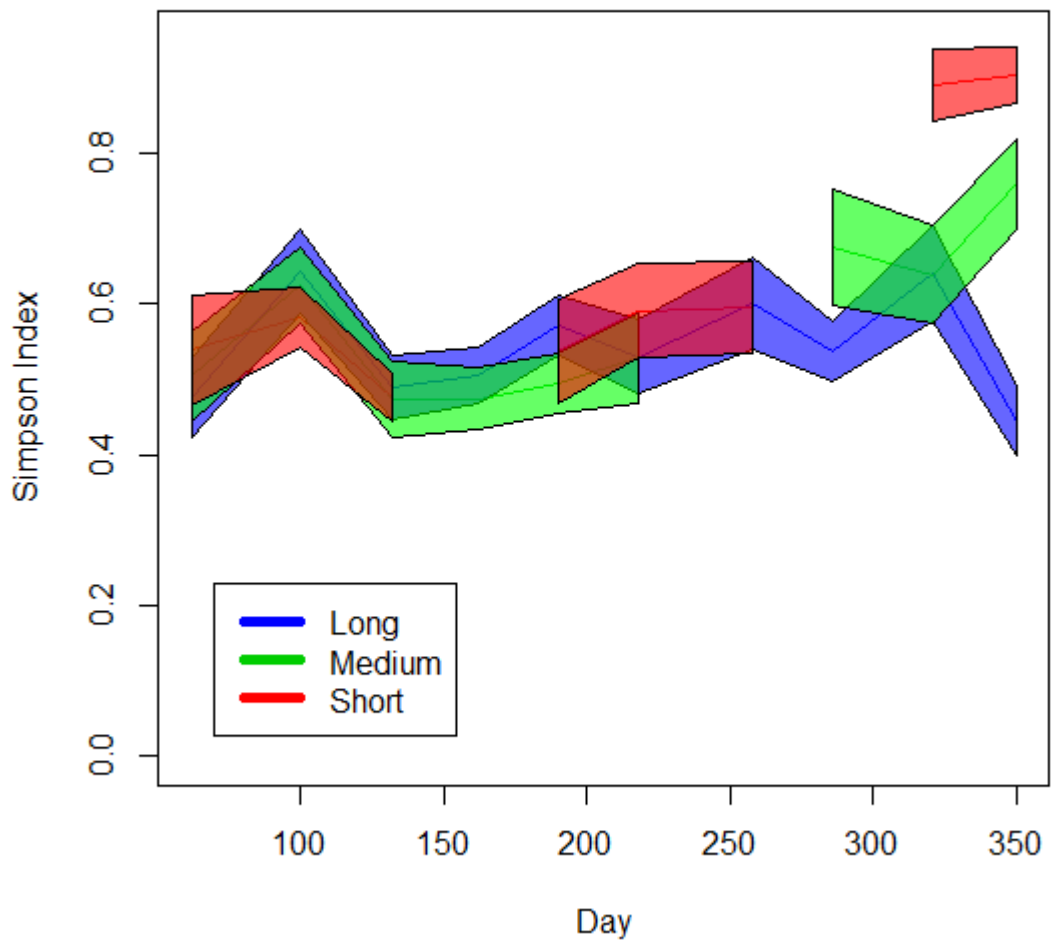
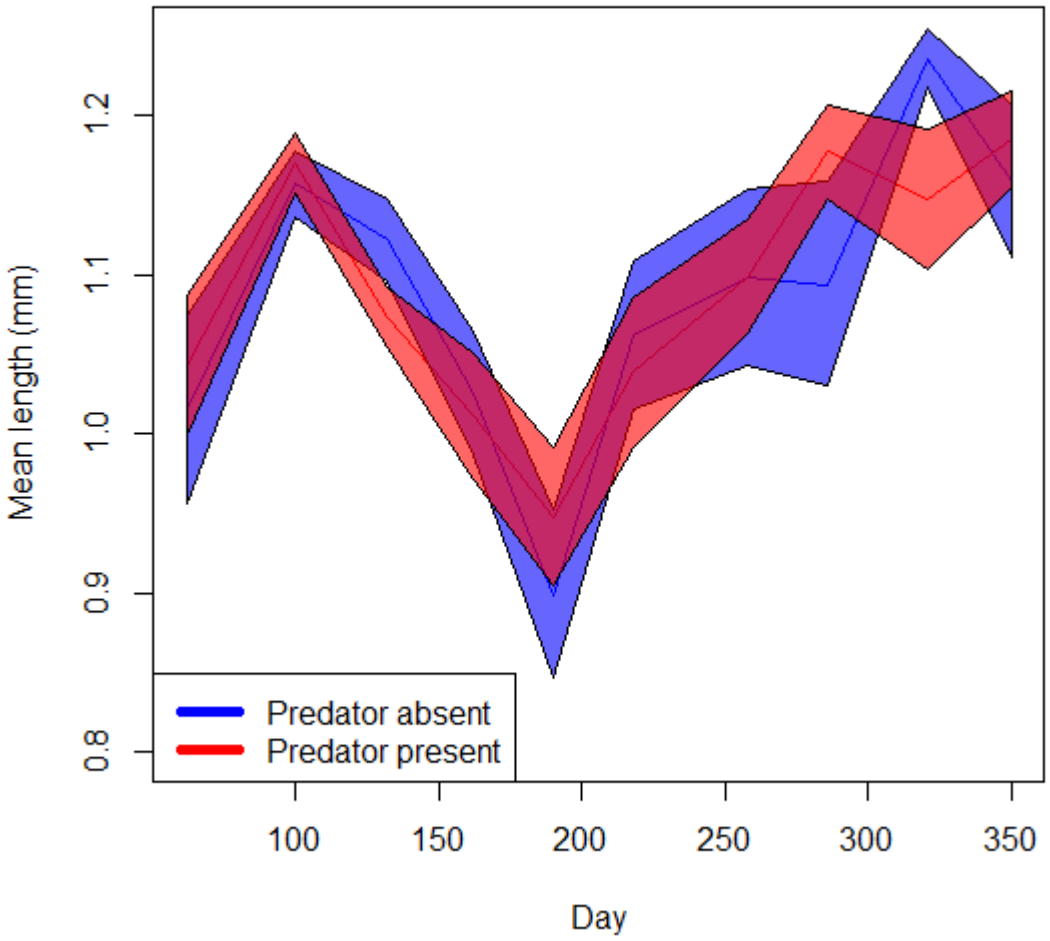

Simpson Index
0.0
0.2
0.4
0.6
0.8
100
150
200
250
300
350
Day
Long
Medium
Short

Figure 3.9.


Mean length (mm)
0.8
0.9
1.0
1.1
1.2
100
150
200
250
300
350
Day
Predator absent
Predator present

CHAPTER 4

TWO NEW SPECIES OF *CHYDORUS* (BRANCHIOPODA: ANOMOPODA: CHYDROIDAE) FROM THE SOUTHEASTERN USA

Abstract

Examination of *Chydorus* specimens from South Carolina, USA has revealed the presence of two previously unknown taxa. *Chydorus freyi* **sp. nov.** is a member of the *C. sphaericus* group and can be distinguished by its distinctive labral keel, which has a notably concave margin near the apex. *Chydorus carolinensis* **sp. nov.** is a member of the *C. eurynotus* group and differs from the above species by the distinct postanal angle on the postabdomen, and from all other *Chydorus* by its relatively long postanal margin and the unique morphology of the male postabdomen. A combined 16S and CO1 phylogeny was constructed including the two new species and six other congeners present in the eastern USA. Species groups within the genus are discussed.

Introduction

One of the largest genera in the family Chydoridae is *Chydorus*. This genus is diverse in species, but not in form, its 32 valid taxa (Smirnov 1996; Smirnov and Sheveleva 2010; Sinev 2014) are largely defined by their distinctive globular body shape (Smirnov 1996). Due to their morphological similarities, many were lumped into worldwide species that have since been found to have more restricted distributions (Frey 1982b; Frey 1987; Frey 1995). In North America, *C. sphaericus* was long considered the most common species; however, detailed study revealed that it consisted of at least two taxa, *C. biovatus* and *C. brevilabris* (Frey 1980; 1985). In fact, *C. sphaericus* as currently defined may not occur in North America at all (Frey 1995). Instead, *C. brevilabris* is considered the most common and widespread species on the continent (Chengalath 1982; Frey 1985). Five additional endemic North American *Chydorus* have been described: *C. bicornutus*, *C. faviformis*, *C. bicollaris*, *C. canadensis*, and *C. linguilabris* (Chengalath & Hann 1981; Frey 1982a; 1982b, Frey 1987). Also recorded from the

United States and Canada are the apparently holarctic *C. gibbus* and *C. ovalis* (Chengalath & Hann 1981; Chengalath 1982; Smirnov 1996). The tropical *C. pubescens*, *C. nitidulus*, *C. eurynotus* and *C.*cf. *invaginatus* have been recorded from the southern USA, Mexico and northern Central America (Frey 1982c; Elías-Gutiérrez et al. 1999; Elías-Gutiérrez et al. 2006). However, there are likely more undescribed species occurring in North America, particularly among *sphaericus*-like populations in the Arctic region (Belyaeva & Taylor 2009) and the extensive wetlands of the southern USA (Frey 1985).

One such area of the southern USA, the Savannah River Site (SRS), South Carolina, contains a large system of temporary wetlands notable for having extremely high species richness, surpassing that of any temporary wetland system yet studied in the world (Mahoney et al. 1990; DeBiase & Taylor 2005). These wetlands have been well sampled in the last 25 years, with at least six *Chydorus* species previously reported; however, some could not be assigned to known species (DeBiase & Taylor 2005). In a recent survey at SRS, we collected samples of *Chydorus* that were initially identified as *C. brevilabris*, but closer inspection revealed two morphologically distinct forms. Neither matched the description of *C. brevilabris* and instead represent two new species. Here we describe these two morphologically and place them in a combined CO1 and 16S phylogeny containing six of the other *Chydorus* species present in the USA to further support their distinct identities.

Material and Methods

Morphology: The samples examined in this study were collected between January 2009 and December 2010 as part of a larger survey of the zooplankton fauna of 14 temporary wetlands. They were collected using a tube trap sampler (Paggi et al. 2001) and were preserved with 100% ethanol. The samples selected for study were chosen based on the presence of parthenogenetic females, ephippial females, and males all in a single sample. Samples were initially examined under a binocular stereoscopic microscope. Specimens were selected and placed on glass slides in a drop of glycerol and ethanol, and were then studied and dissected under a compound microscope. Drawings were made in GIMP 2.8.10 from micrographs taken with a camera attached to the compound microscope. Specimens studied with SEM were critical point dried, sputter coated with gold and mounted on aluminum stubs and then

examined with a Zeiss 1450EP. Our morphological descriptions closely follow the format used in Sinev (2014), as it is the most complete taxonomic treatment of *Chydorus* species. Holotypes and paratypes of both species will be sent to the National Museum of Natural History, Washington D.C. upon acceptance of this manuscript for publication.

Phylogenetics: Specimens of the new species used in genetic analyses were collected from several localities in SRS (Table 1). Samples of additional species were collected from Athens, Georgia (*C. brevilabris*), Laurinburg, North Carolina (*C. bicollaris, C. bicornutus*) and SRS (*C.* cf. *eurynotus, C. linguilabris, Picripleuroxus denticulatus*). Specimens were placed live into individually marked tubes of 25 µl of Quickextract (Epicentre®), which were then incubated for 2 hours at 65°C followed by 10 minutes at 95°C following the methods of Belyaeva and Taylor (2009). The mitochondrial cytochrome *c* oxidase subunit I (COI) gene and the ribosomal large subunit rDNA (16S) were amplified using polymerase chain reaction (PCR).

PCR protocols were modified from the methods of Sacherová and Hebert (2003). The COI primers used (Chy-f, Chy-r) were designed by Belyaeva and Taylor (2009); the 16S primers were 16Sch-a (Sacherová and Hebert 2003) and 16Sbr (Palumbi et al. 1991). COI PCR was performed in 25uL 1x buffer containing, 1.5mM MgCl2, 0.2mM of each dATP,dTTP, dGTP, and dCTP, 0.5uM forward and reverse primers, 2.5 uL of 10X BSA, and 1.25U GoTaq® DNA Polymerase (Promega, Madison, WI USA). The 16S rDNA region was amplified using the same chemistry with BSA omitted and only 1U Taq in a total of 50uL volume. The PCR protocol consisted of 1 cycle of 1.5 minutes at 94°C, 35 cycles of 45 seconds at 93°C, 1 minute at 50°C, 1 minute at 72°C, followed by 1 cycle of 5 minutes at 72°C. PCR product was visualized on an agarose gel to determine if the extracted samples amplified. Amplified products were sequenced by the Georgia Genomics Facility using an Applied Biosystems 3730xl 96-capillary DNA Analyzer (Thermo Fisher Scientific Inc.,Waltham, MA USA).

Sequences were edited using Sequencher 5.0.1 and aligned in MEGA 6.0. Additional sequences provided by V. Sacherová (Sacherová and Hebert 2003) were added to the alignment. Phylogenetic model testing was done using jModelTest 2.1.5 (Posada 2008) to select the appropriate nucleotide substitution

model. The GTR+G was selected for the 16S and HKY+I+G was selected for CO1. Phylogenies were reconstructed using Bayesian phylogenetic analyses in MrBayes 3.2 (Holsenbeck & Ronquist 2001). The analysis ran for 10,000,000 generations, sampling every 100 generations; the first 25% of trees were discarded and posterior probabilities were calculated. The concensus tree was viewed in MEGA 6.0 and rooted with *Picripleuroxus denticulatus* as the outgroup.

Abbreviations used in illustrations and text: cbs – copulatory brush seta of limb I; ep – epipodite; ex – exopodite, gfp – gnathobasic filter plate of limbs II-V; il – inner lobe of limb V; IDL – inner distal lobe of limb I; ms – male seta of limb I; ODL – outer distal lobe of limb I; pep – pre-epipodite; s – sensillum.

Taxonomic descriptions

***Chydorus freyi* sp. nov.**

(Figs. 4.1.-4.4.)

Etymology: This species name is in honor of cladoceran taxonomist David G. Frey for his extensive contributions to *Chydorus* taxonomy. He may have been aware of this species through his collecting expeditions in the southeastern USA based on statements mentioning undescribed *brevilabris*-like species in the region (Frey 1980; 1985).

Type locality:

Mona Bay, a temporary wetland in Savannah River Site, South Carolina, USA (33° 19' 04.03" N, 81° 28' 35.88" W)

Holotype:

A parthenogenetic female from the type locality collected on May 11th, 2010

Paratypes:

Over 100 individuals collected with the holotype including multiple males and ephippial females

Description: Parthenogenetic female: *General:* in lateral view (Fig. 4.1.A), individuals are relatively rounded in profile with a height/length ratio approximately 0.97 (SE = 0.009). In rear view (Fig. 4.1.B) the body is slightly compressed and somewhat oval in shape.

Valves: The anterior portion of the ventral margin has 7-10 setae and the posterior portion has 33-35 setae attached on the inner side of the valve. The anterior ventral setae are long and without setules. The posterior ventral setae are setulated. Setae shorten abruptly at the postero-ventral corner; there are no denticles. The valves are decorated with polygons having straight margins; they become less distinct away from the ventral margins of the valves. Some individuals have dimples on the valve and head shield.

Head: (Fig. 4.1.A) eye is about 1.5 times larger than the ocellus. In lateral view the rostrum is oriented downward and posteriorly. In frontal view (Fig. 4.1.C) the rostrum is triangular and the apex has a very small notch at the tip. There are two major head pores (Fig. 4.1.D); post pore distance is 1.6 times the inter pore distance. Lateral head pores are small and are around equidistant between the major head pores; they are near the midline and are not situated symmetrically. The rear portion of the head shield is rounded.

Labrum: (Fig. 4.2.A) anterior margin of labral keel is convex with a distinctly concave area near the apex; posterior margin convex; apex rounded and pointing slightly outward.

Postabdomen: (Fig. 4.2.B, 4.3.A) short, gradually narrowing distally; ventral margin straight. There is a distinct incision on the distal margin near the claw base. Distal angle rounded. The dorsal margin is slightly concave; preanal angle prominent, postanal angle absent. The postanal portion is slightly longer than anal portion. The dorsal margin has 7-10 pairs of narrow, sharp denticles. The length of the longest denticles is slightly longer than the width of the postabdominal claw base.

Postabdominal claw: (Fig. 4.2.B) slender and slightly curved, equal in length to the postanal portion of the postabdomen. The claw has two basal spines; distal spine approximately 4.5 times shorter than claw; proximal spine 3 times shorter than the distal spine. There is a pecten of spinules on the dorsal margin of the claw; the spinules become larger in the distal portion.

Antennule: (Fig. 4.4.A) short, half the length of rostrum. Length is approximately 1.5 times the width. The seta originates around 1/2 of the distance from the base of the antennule and is about 0.4 times the length of the antennule. Aesthetascs are terminal and the longest are approximately equal in length to the antennules.

Antenna: (Fig. 4.2.C) antennal arrangement for the setae is 0-0-3/0-1-3; for spines it is 0-0-1/0-0-1.

Limb 1: (Fig. 4.4.B-C) has 6 rows of long setules on the ventral side. The epipodite has a long projection 2 times longer than the epipodite itself. The ODL has two setae, one very long and the other very short. IDL with 3 setae; seta 1 2/3 the length of the longest ODL seta; seta 2 slender and 4/5 the length of the longest ODL seta; seta 3 robust and curved, similar to the length of the longest ODL seta; seta 3 has prominent setules on the distal portion. The base of IDL seta 3 is three times greater than the width of the base of seta 1. Endite 1 has 3 setae (g-i) that are approximately equal in length and have setules on their distal portions, a plumose seta (j), and a naked seta (3). Endite 2 has two long setae (e-f) that have setules on their distal portions, a shorter seta (d) also with setules on the distal portion, and a naked seta (2). Endite 3 has four setae (a-c, 1) that are similar in length; all have setules on their distal portions.

Limb 2: (Fig. 4.4.D) is somewhat triangular in shape. The exopodite has a long seta approximately 2 times longer than the length of the exopodite. There are eight scraping setae with denticles on their distal portions; the three smallest (6-8) are approximately equal in size, while the remaining setae (5-1) are of increasing length. The gnathobasic filter plate consists of eight setulated setae.

Limb 3: (Fig. 4.4.E-G) the exopodite is somewhat rectangular in shape with three lateral setae (1-3) and four terminal setae (4-7). Setae 1-3 are roughly equal in length and are 1/3 the length of seta 4; seta 5 is about 2/5 the length of seta 4; seta 6 is approximately 3/4 the length of seta 4, and seta 7 is around 1/2 the length of seta 4. Setae 1-6 are plumose, seta 7 is naked. The distal part of endite has two large denticulate setae (1-2); the basal portion of endite has six plumose setae. There are four inner setae and the filter plate has eight plumose setae.

Limb 4: (Fig. 4.4.H-I) the pre-epipodite has fine setules, while the epipodite has a projection equal in length to the epipodite itself. The exopodite is relatively round in shape with seven setae; setae 1-5 are plumose, and setae 6-7 have setules on their basal portions. The inner portion of the limb has four setae. The gnathobase has a long projection, a sensillum and one long seta. There are four inner setae on the limb and filter plate has six setae.

Limb 5: (Fig. 4.4.J) the pre-epipodite is rounded with many setules; the epipodite is irregular in shape with a long projection. The exopodite is oval in shape with four plumose setae. On the basal side of the exopodite, adjacent to seta 4, there two clusters of setules. The exopodite margin between seta 4 and 3 is long and setulated. The inner lobe of the limb is finger-like and setulated along the inner margin. There are two setae on the inner face of the limb, both with setules on their distal portions; the innermost seta has particularly long setules. The gnathobase filter plate has four setae.

Ephippial female: The body is similar to the parthenogenetic female in lateral view (Fig. 4.1.E), but has a straight margin above the postero-dorsal angle. It is also more compressed laterally (Fig. 4.1.F). The ephippium has 1 egg and is dark brown in color.

Male: *General:* The shape is more oval than the female in lateral view (Fig. 4.1.G; 4.3.C), with a height/length ratio of 0.85 (SE = 0.008). It is moderately compressed laterally (Fig. 4.1.H).

Head: (Fig. 4.1.G) in lateral view the rostrum projects downward and posteriorly. In frontal view (Fig. 4.1.I) the rostrum is triangular with convex margins and a rounded apex with two small spines. The labrum (Fig. 4.2.D) is similar to the parthenogenetic female, but the margin is less concave near the apex.

Postabdomen: short, finger-like and expanded distally (Fig. 4.2.E; 4.3.D). The preanal angle is prominent, the anal margin is deeply concave, and the distal angle not incised. There are 3-5 pairs of long, thin denticles on the postanal margin.

Postabdominal claw: is slender and slightly curved and setulated (Fig. 4.2.E). There are two basal spines present, the distal spine is about 1/4 the length of the claw, and the proximal spine is 0.4 times shorter than the distal spine.

Antennule: (Fig. 4.4.K) is 3/4 the length of the rostrum and has six terminal, three sub terminal and one lateral aesthetascs. The longest aesthetascs are slightly longer than the antennule. There is a large seta originating at the middle of the antennule that is approximately equal to the antennule in length. The length of the antennule is approximately 1.5 times the width.

Limb 1: (Fig. 4.4.L) has a U-shaped copulatory hook with two ridges on the distal end. The IDL has four setae; setae 1 and 3 are similar in length, with seta 2 about 2/3 shorter than setae 1; the male seta is curved and is about the same length as seta 1.

Size: The length of ovigerous females was 0.29-0.38 mm and a height of 0.29-0.34 mm. Adult males had a length of 0.22-0.24 mm and a height of 0.19-0.21 mm.

Differential diagnosis: The shape of the labrum is distinctive and distinguishes it from all other described *Chydorus*. Among species known from the North America, it can be easily distinguished from the honeycombed *C. bicollaris*, *C. bicornutus*, *C. faviformis* and the reticulated *C. linguilabris* and *C. cf. invaginatus* by lacking these valve features. It is readily separated from *C. canadensis*, *C. carolinensis* **sp. nov.,** *C. eurynotus, C. pubescens* and *C. nitidulus* by the relatively short postabdomen lacking a postanal angle. It is most similar to *C. biovatus, C. brevilabris* and undescribed species of the *C. sphaericus* group, but can be identified by the labrum shape, the presence of basal spines on the postabdominal claw of the male, and the long thin denticles on the male postabdomen.

Distribution and ecology: This species was collected from 12 of 14 temporary wetlands in the SRS (Aiken and Barnwell Counties) that were sampled regularly between January 2009 and December 2010, and was also collected from a roadside ditch in Colleton County, South Carolina (33° 6' 22.25" N, 80° 43' 43.61" W) on April 3, 2012. Specimens were collected throughout the year and parthenogenetic reproduction occurred in all months. Ephippial females were most often present in April and May, but were collected from March through July. Similarly, males were most frequent in April and May, but were present from February through June. The proportion of males and ephippial females was greatest in May, indicating this was the peak timing of sexual reproduction in these populations. This species was most abundant in samples from vegetated habitats versus unvegetated habitats (Kruskal-Wallis test, chi-squared = 127.03, $p < 0.001$). It was present in multiple vegetation types, but was most abundant among submerged plants (*Polygonum hydropiperoides, Callitriche heterophylla, Juncus repens, Luziola fluitans, Utricularia* spp., *Sphagnum* spp.) and secondarily among emergent grasses and sedges (*Panicum* spp.,

Leersia hexandra, Rhynchospora spp.). Congenerics that occurred with it include *C. carolinensis* **sp. nov.,** *C. linguilabris* and *C. cf. eurynotus*.

***Chydorus carolinensis* sp. nov.**

(Figs. 4.3.; 4.5.-4.7.)

Etymology: The species name refers to the region it is known to inhabit, the coastal plain of South Carolina. It also refers to a type of temporary wetland unique to this region known as a Carolina Bay, in which this species is found.

Type locality:

Flamingo Bay, a temporary wetland in Savannah River Site, South Carolina, United States (33° 20' 15.99" N, 81° 40' 43.92" W)

Holotype:

A parthenogenetic female from the type locality collected on April 14th, 2010

Paratypes:

Over 50 individuals collected with the holotype including multiple males and ephippial females

Description: Parthenogenetic female: *General:* in lateral view (Fig. 4.5.A), individuals are relatively rounded in profile with a height/length ratio approximately 0.90 (SE = 0.011). In rear view (Fig. 4.4.B) the body is slightly compressed and somewhat obovate in shape.

Valves: the anterior portion of the ventral margin has about 21-23 setae and the posterior portion has around 40-42 setae attached on the inner side of the valve. The anterior ventral setae are long and without setules. The posterior ventral setae are setulated. Setae shorten abruptly at the postero-ventral corner; there are no denticles. The valves lack obvious polygons or other decoration.

Head: (Fig. 4.5.A) eye is about 1.6 times larger than the ocellus. The rostrum is oriented downward and posteriorly. In frontal view (Fig. 4.5.C), the rostrum is sharply triangular with slightly concave margins; the apex has a very small notch. There are two major head pores (Fig. 4.5.D); post pore distance is 3/4 the inter pore distance. Lateral head pores are small and are slightly closer to the anterior major

head pore; they are near the midline and are not situated symmetrically. The rear portion of the head shield is rounded.

Labrum: (Fig. 4.6.A) anterior margin of labral keel is convex; the apex is short and rounded.

Postabdomen: (Fig. 4.6.B; 4.3.B) narrowing distally; ventral margin very slightly concave. There is a distinct incision on the distal margin near the claw base. Distal angle rounded. The preanal angle is prominent and the postanal angle is distinct. The postanal portion is straight to convex and is equal in length to the anal portion, which is concave. The dorsal margin has 10-14 sharp denticles, several of which may be doubled. There are clusters of setules positioned laterally and there are groups of long setules along the anal margin. The length of the longest denticles is slightly shorter than the width of the postabdominal claw base.

Postabdominal claw: (Fig. 4.6.B) slender and slightly curved, equal in length to the postanal portion of the postabdomen. The claw has two basal spines; distal spine approximately 4.5 times shorter than claw; proximal spine 3 times shorter than the distal spine. There is a pecten of spinules on the dorsal margin of the claw.

Antennule: (Fig. 4.6.C) short, half the length of rostrum. Length is approximately 2.3 times the width. The seta originates around 2/5 of the distance from the base of the antennule and is about 0.3 times the length of the antennule. Aesthetascs are terminal and the longest are approximately half the length of the antennules.

Antenna: (Fig. 4.6.D) antennal arrangement for the setae is 0-0-3/0-1-3; for spines it is 0-0-1/0-0-1.

Limb 1: (Fig. 4.7.A-B) has 7-8 rows of long setules on the ventral side. The epipodite is without a projection. The ODL has 2 setae, one very long and the other very short. IDL with 3 setae; seta 1 is 0.45 times the length of the longest ODL seta; seta 2 slender and 3/4 the length of the longest ODL seta; seta 3 robust and curved, about 3/4 the length of the longest ODL seta; seta 3 has prominent setules on the distal portion. The base of IDL seta 3 is about 2 times greater than the width of the base of seta 1. Endite 1 has 3 setae (g-i) that are approximately equal in length and have setules on their distal portions and spines on their basal portions, a plumose seta (j), and a naked seta (3). Endite 2 has two long setae (e-f) that have

setules on their distal portions, a shorter seta (d) also with setules on the distal portion, and a naked seta (2). Endite 3 has four setae (a-c, 1) that are similar in length; all have setules on their distal portions.

Limb 2: (Fig. 4.7.C) is somewhat triangular in shape. The exopodite has a long seta approximately 1.3 times longer than the length of the exopodite. There are eight scraping setae with denticles on their distal portions; the three smallest (6-8) are approximately equal in size, while the remaining setae (5-1) are of increasing length. The gnathobasic filter plate consists of eight setulated setae.

Limb 3: (Fig. 4.7.D-F) the exopodite is somewhat rectangular in shape with three lateral setae (1-3) and four terminal setae (4-7). Setae 1 is approximately 1/3 the length of seta 4; setae 2-3 are roughly equal in length and are 1/5 the length of seta 4; seta 5 is about 2/5 the length of seta 4; seta 6 is approximately 3/4 the length of seta 4, and seta 7 is around 2/3 the length of seta 4. Setae 1-6 are plumose, seta 7 is naked. The distal part of endite has two large denticulate setae (1-2); the basal portion of endite has six plumose setae. There are four inner setae, four elements on the distal gnathobase, and the filter plate has eight plumose setae.

Limb 4: (Fig. 4.7.G-H) the pre-epipodite has fine setules, while the epipodite has a very short projection. The exopodite is relatively round in shape with seven setae; setae 1-5 are plumose, and setae 6-7 have setules on their basal portions. The inner portion of the limb has four setae. The gnathobase has a long projection, a sensillum and one long seta. There are four inner setae on the limb, and filter plate has six setae.

Limb 5: (Fig. 4.7.I) the pre-epipodite is rounded with many setules; the epipodite is oval in shape with a very short projection. The exopodite is oval in shape with four plumose setae. On the basal side of the exopodite, adjacent to seta 4, there two clusters of setules. The exopodite margin between seta 4 and 3 is long and setulated. The inner lobe of the limb is finger-like and setulated along the inner margin. There are two setae on the inner face of the limb, both with setules on their distal portions; the innermost seta has particularly long setules. The gnathobase filter plate has four setae.

Ephippial female: The body is similar to the parthenogenetic female in lateral view (Fig. 4.5.E). In frontal view, it is more compressed laterally (Fig. 4.5.F). The ephippium has 1 egg and is only slightly darker in color.

Male: *General:* The shape is more oval than the female in lateral view (Fig. 4.5.G; 4.3.E), with a height/length ratio of 0.83 (SE = 0.011). It is moderately compressed laterally (Fig. 4.5.H).

Head: (Fig. 4.5.G) in lateral view the rostrum projects downward and posteriorly. In frontal view (Fig. 4.5.I) the apex of the rostrum is rounded with a spine at the apex.

Postabdomen: long, narrow and finger-like (Fig. 4.6.E; 4.3.F). The preanal angle is indistinct; the anal margin is straight, and the postanal margin is slightly concave. There are 12-14 clusters of small, sharp denticles on the anal and postanal margins, usually in groups of three; there are approximately equal numbers of spinule clusters laterally.

Postabdominal claw: is small, with a sinuate curve (Fig. 4.6.E). There is one basal spine present that is about 1/5 the length of the claw.

Antennule: (Fig. 4.6.F) is 3/4 the length of the rostrum and has six terminal and three sub terminal aesthetascs. The longest aesthetascs are slightly shorter than the antennule. There is a large seta originating at the middle of the antennule that is nearly equal to the antennule in length. The length of the antennule is approximately 2 times the width.

Limb 1: (Fig. 4.67.J) has a U-shaped copulatory hook with one ridge on the distal end. The IDL has four setae; setae 1-3 are similar in length; the male seta is curved and is about the same length as seta 1.

Size: The length of ovigerous females was 0.35-0.45 mm and a height of 0.31-0.40 mm. Adult males had a length of 0.30-0.32 mm and a height of 0.24-0.27 mm.

Differential diagnosis: This species can be easily distinguished from honeycombed and reticulated *Chydorus* by the lack of these features on the valves. It can be separated from *C. brevilabris*, *C. freyi* **sp. nov.** and *sphaericus*-group species by the longer postabdomen with a prominent postanal angle. It differs from *C. nitidulus* by the absence of a spine at the postero-ventral corner, from *C. pubescens* by the lack of setules on the valves, and from *C. canadensis* by the rounded labrum shape. It appears most similar to *C.*

eurynotus, but it can be distinguished by the ornamentation of the antenna, the longer postanal margin of the postabdomen and by the unique postabdomen morphology of the male, which differs substantially from all described species in the genus.

Distribution and ecology: This species was collected from 13 of 14 temporary wetlands in the SRS (Aiken and Barnwell Counties) that were sampled regularly between January 2009 and December 2010. Specimens were collected throughout the year and parthenogenetic reproduction occurred in all months; however, they were most abundant from December through June. Ephippial females were most often present in March through May, but were also collected in June. Similarly, males were most frequent March through May, but were present from February through June. The proportion of males and ephippial females was greatest in April, indicating this was the peak timing of sexual reproduction in these populations. Peak parthenogenetic reproduction occurred from December through February. This species was most abundant in samples from vegetated habitats versus unvegetated habitats (Kruskal-Wallis test, chi-squared = 8.75, p = 0.003). This species was most abundant among dead, recumbent stems of emergent vegetation (*Panicum spp.*, *Leersia hexandra*, *Rhynchospora* spp.), but was also common in submerged vegetation (*Polygonum hydropiperoides, Callitriche heterophylla*, *Juncus repens, Utricularia* spp., *Sphagnum* spp.) and among the fallen leaves of hardwood and pine trees. Congenerics that occurred with it include *C. freyi* **sp. nov.,** *C. linguilabris* and *C. cf. eurynotus*.

Phylogeny: The combined 16S and CO1 dataset had 397 variable sites out of 1081; 289 sites were parsimony informative. Based on our phylogeny (Fig. 4.8), the nearest congener to *C. freyi* **sp. nov.** is *C. brevilabris* (pairwise distance, mean= 0.060, SD = 0.004). These two species are part of a larger grouping that included *C. linguilabris* (pairwise distance, mean = 0.122, SD = 0.004) and *C. bicornutus* (pairwise distance, mean = 0.132, SD = 0.004). The closest relations to *C. carolinensis* **sp. nov.** are *C. canadensis* (pairwise distance, mean = 0.133, SD = 0.000) and *C.* cf. *eurynotus* (pairwise distance, mean = 0.111, SD = 0.000); the only other species on this branch of the phylogeny is *C. bicollaris* (pairwise distance, mean = 0.316, SD = 0.000). Both new species are well differentiated phylogenetically from other congeners that were included. Genetic structure within both species was relatively shallow. Node

support between species all had posterior probabilities exceeding 85 with the exception of the basal node separating the two major groupings of *Chydorus*. The two species with honeycombed valves, *C. bicornutus* and *C. bicollaris* (pairwise distance, mean = 0.277, SD = 0.004), did not appear closely related to each other.

Discussion

The relationships among species within the genus *Chydorus* are unclear and the genus is in need of greater taxonomic study. Recent taxonomic work among other members of the family Chydoridae has redefined genera, identified many species groups, and described new genera (Dumont & Silva-Briano 2000; Van Damme & Dumont 2008; Van Damme et al. 2011). This work in *Chydorus* has been hampered by the lack of variability in the morphological features that have proved useful in studies of other genera to define lineages within *Chydorus*. Head pore number and arrangement is relatively consistent across the group, limb morphology is largely conserved, and valve sculpture and labrum shape may be homoplasious (Sinev 2014). Features which may hold some promise are postabdomen morphology, particularly that of males; however, a combined genetic and morphologic approach may be the most effective way to address this problem (Belyaeva and Taylor 2009).

The most well-defined species group within *Chydorus* is the *C. sphaericus* group. Since *C. sphaericus* is the type species of the genus, members of this group are *Chydorus* sensu stricto (Frey 1980). This group may be best defined by the shapes of both the female and male postabdomen. Females have a relatively short postabdomen with a prominent preanal angle and lack a postanal angle; denticles are long and spine-like. Males have a finger-like postabdomen with a notably concave anal margin; it is expanded distally, and the preanal angle may or may not be prominent. Described species in this group include *C. biovatus*, *C. brevilabris* and *C. sphaericus* (Frey 1980; Frey 1985); despite its honeycombed valve sculpture, *C. faviformis* also appears to be a member (Frey 1982a, Frey 1987). This species group is global in distribution (Smirnov 1996); however, its diversity is underrepresented as shown by Belyaeva and Taylor (2009). Study of male morphology, including postabdomen denticulation, the presence or

absence of basal spines on the postabdominal claws, and the shape of the preanal angle, should prove useful in distinguishing new species (Belyaeva and Taylor 2009).

Chydorus freyi **sp. nov.** belongs to the *C. sphaericus* group based on the shape of the female and male postabdomen. It appears most similar to *C. brevilabris* due to the short and rounded labrum; however, the distinctly concave margin on the labrum readily separates them. The prominent preanal angle on the male postabdomen distinguishes it from *C. biovatus*, and the long thin denticles on the postanal margin and the presence of a basal spine on the postabdominal claw separates it from all described members of the *sphaericus* group. There is an undescribed form that has a basal spine on the male postabdominal claw, but it lacks a prominent preanal angle (Belyaeva and Taylor 2009). Phylogenetic analysis supports the close relationship between *C. freyi* **sp. nov.** and *C. brevilabris*; the relationship between *C. brevilabris*, *C. sphaericus*, and similar forms was previously established (Frey 1980; Belyaeva and Taylor 2009).

Another species group, labeled by Sinev (2014) as the *C. eurynotus* group, is less well defined. According to Sinev (2014), it includes *C. eurynotus*, *C. idrisi*, *C. pubescens*, *C. ventricosus*, *C. parvus,* and *C. brevilabris*; the defining features given were a small rounded labral plate and the absence of honeycomb sculpture on the valves. We believe *C. brevilabris* is not part of this group for the reasons given above. Features shared among the remaining species include a prominent postanal angle on the female postabdomen and a sharply triangular rostrum. The morphology of the male postabdomen among these species varies considerably, which suggests this may not be a natural group. Species in this group are largely tropical to subtropical in distribution (Smirnov 1996; Sinev 2014); however, we believe *C. canadensis* and *C. irinae* may also be members, both of which are from temperate regions (Chengalath & Hann 1981; Smirnov and Sheveleva 2010).

Chydorus carolinensis **sp. nov.** appears to be a member of the *C. eurynotus* species group based on the prominent postanal angle, the sharply triangular rostrum and the small rounded labrum. Of species known from North America, *C. eurynotus, C. pubescens*, and *C. canadensis* are the most similar. It differs from all three in having a longer postanal margin. The male postabdomen in particular is unique; no other

Chydorus has a postabdomen so long and narrow, or with so many denticle clusters. It should be noted that the male of *C. canadensis* has not been described, so its appearance relative to C. *carolinensis* **sp. nov.** is unknown. Phylogenetic analysis revealed that *C. canadensis* and *C. cf. eurynotus* were both more closely related to C. *carolinensis* **sp. nov.** than any of these were to the *sphaericus* group species. The phylogenetic relationship among these species indicates that the *eurynotus* group may be a valid species group.

Interestingly, the two honeycombed species, *C. bicornutus* and *C. bicollaris*, were not close phylogenetically, indicating that honeycomb valve sculpture evolved independently in multiple lineages. However, this is not too surprising, since Frey (1982a) concluded that the honeycombed valves of these two taxa and of *C. faviformis* masked their considerable morphological differences and suggested that they were not closely related. Frey (1982a) also noted that the male postabdomen of *C. bicollaris* and *C. linguilabris* bore some resemblance, though he did not suggest they were closely related. Our analysis did not recover these two as close relatives, indicating that the physical resemblance is coincidental. Incidentally, our collection of *C. bicornutus* near Laurinberg, North Carolina represents a substantial range extension from the nearest known population in Bamber Lake, New Jersey, a distance of over 700 km; most records of this species have been from eastern Canada and the northeastern USA (Chengalath 1982; Frey 1982a)

The two new species have so far been found in a relatively small region, but their ranges are likely more extensive. Frey (1980; 1985) mentioned undescribed *brevilabris*-like forms found throughout the southeastern USA; it is probable that at least some of the specimens he collected were *C. freyi* **sp. nov.** His collections spanned from North Carolina to Louisiana, largely in the coastal plain; however, specimens were reported as *C. cf. sphaericus*, so it is unclear what taxa were actually collected (Frey 1982c). Nevertheless, it seems likely that *C. freyi* **sp. nov.** occurs in a broad swath of coastal plain in the southeast USA. It is less clear how widespread C. *carolinensis* **sp. nov.** may be, as records of *eurynotus* group species in the USA are few. Frey (1982c) reported *C. cf. eurynotus* from Louisiana and *C. pubescens* from North Carolina and Florida. It is unclear which, if any, of these records could represent C.

carolinensis **sp. nov.** However, it is clear that there are multiple species of *Chydorus* occurring in the southeastern USA which are undescribed or poorly documented. The two species descriptions presented here add considerably to the taxonomic knowledge of North American *Chydorus*, and should prove useful in clarifying which species are present within the region.

Acknowledgements

We would like to thank Rebecca Sharitz and Linda Lee of the Savannah River Ecology Lab for logistical assistance during sampling. We thank John Robinson for assistance with PCR and Veronika Sacherová for sharing *Chydorus* sequences with us. We also thank John Shields for aiding us with SEM images.

Table 4.1. Sample codes and collection locations for the specimens used in phylogenetic analyses; SRS = Savannah River Site.

Sample code	Species	Latitude	Longitude	Sample Location
Chy bicoll GS 1	*Chydorus bicollaris*	34°54'35.09"N	79°33'50.78"W	Gum Swamp Lake, Laurinburg, North Carolina, USA
Chy bicorn GS 1	*Chydorus bicornutus*	34°54'35.09"N	79°33'50.78"W	Gum Swamp Lake, Laurinburg, North Carolina, USA
Chy bicorn GS 2	*Chydorus bicornutus*	34°54'35.09"N	79°33'50.78"W	Gum Swamp Lake, Laurinburg, North Carolina, USA
Chy bicorn ONT	*Chydorus bicornutus*	45°34'N	78°30'W	Starling Lake, Ontario, Canada (Sacherová and Taylor 2003)
Chy brev Ath 1	*Chydorus brevilabris*	34°01'19.44"N	83°22'47.39"W	temporary pond, near Athens, Georgia, USA
Chy brev Ath 2	*Chydorus brevilabris*	34°01'19.44"N	83°22'47.39"W	temporary pond, near Athens, Georgia, USA
Chy brev Ath 3	*Chydorus brevilabris*	34°01'19.44"N	83°22'47.39"W	temporary pond, near Athens, Georgia, USA
Chy brev Ath 5	*Chydorus brevilabris*	34°01'19.44"N	83°22'47.39"W	temporary pond, near Athens, Georgia, USA
Chy brev Ath 6	*Chydorus brevilabris*	34°01'19.44"N	83°22'47.39"W	utemporary pond, near Athens, Georgia, USA
Chy canad ONT	*Chydorus canadensis*	45°22'N	75°52'W	Ottawa, Ontario, Canada (Sacherová and Taylor 2003)
ChyA 176 1	*Chydorus carolinensis*	33°13'18.89"N	81°44'49.46"W	Ellenton Bay (176), SRS, South Carolina, USA
ChyA 176 2	*Chydorus carolinensis*	33°13'18.89"N	81°44'49.46"W	Ellenton Bay (176), SRS, South Carolina, USA
ChyA 176 4	*Chydorus carolinensis*	33°13'18.89"N	81°44'49.46"W	Ellenton Bay (176), SRS, South Carolina, USA
ChyA 176 6	*Chydorus carolinensis*	33°13'18.89"N	81°44'49.46"W	Ellenton Bay (176), SRS, South Carolina, USA
ChyA 78 14	*Chydorus carolinensis*	33°13'18.89"N	81°44'49.46"W	Saracennia Bay (78), SRS, South Carolina, USA
ChyA 78 15	*Chydorus carolinensis*	33°13'18.89"N	81°44'49.46"W	Saracennia Bay (78), SRS, South Carolina, USA
Chy eury 4 1	*Chydorus cf. eurynotus*	33°20'34.50"N	81°41'06.33"W	bay 4, SRS, South Carolina, USA
ChyB 7 1	*Chydorus freyi*	33°17'26.15"N	81°46'02.52"W	bay 7, SRS, South Carolina, USA
ChyB 7 2	*Chydorus freyi*	33°17'26.15"N	81°46'02.52"W	bay 7, SRS, South Carolina, USA
ChyB 7 3	*Chydorus freyi*	33°17'26.15"N	81°46'02.52"W	bay 7, SRS, South Carolina, USA
ChyB 7 4	*Chydorus freyi*	33°17'26.15"N	81°46'02.52"W	bay 7, SRS, South Carolina, USA
ChyB 11 1	*Chydorus freyi*	33°18'03.15"N	81°45'37.12"W	bay 11, SRS, South Carolina, USA
ChyB 11 2	*Chydorus freyi*	33°18'03.15"N	81°45'37.12"W	bay 11, SRS, South Carolina, USA
ChyB 11 3	*Chydorus freyi*	33°18'03.15"N	81°45'37.12"W	bay 11, SRS, South Carolina, USA
ChyB 11 4	*Chydorus freyi*	33°18'03.15"N	81°45'37.12"W	bay 11, SRS, South Carolina, USA
ChyB 66 1	*Chydorus freyi*	33°19'04.03"N	81°28'35.88"W	Mona Bay (66), SRS, South Carolina, USA
ChyB 78 3	*Chydorus freyi*	33°17'23.47"N	81°29'06.32"W	Saracennia Bay (78), SRS, South Carolina, USA
ChyB 78 5	*Chydorus freyi*	33°17'23.47"N	81°29'06.32"W	Saracennia Bay (78), SRS, South Carolina, USA

ChyB 78 11	*Chydorus freyi*	33°17'23.47"N	81°29'06.32"W	Saracennia Bay (78), SRS, South Carolina, USA
Chy ling 78 3	*Chydorus linguilabris*	33°17'23.47"N	81°29'06.32"W	Saracennia Bay (78), SRS, South Carolina, USA
Chy ling 78 4	*Chydorus linguilabris*	33°17'23.47"N	81°29'06.32"W	Saracennia Bay (78), SRS, South Carolina, USA

Figure 4.1. *Chydorus freyi* **sp. nov.** from the type locality, a temporary pond in Savannah River Site, South Carolina, USA. A-D: parthenogenetic female; A, lateral view; B, rear view; C, apex of the rostrum; D, head shield. E-F: ephippial female in lateral and rear views. G-I: mature male; G, lateral view; H, rear view; I apex of rostrum.

Figure 4.2. *Chydorus freyi* **sp. nov.** from the type locality, a temporary pond in Savannah River Site, South Carolina, USA. A-C: parthenogenetic female; A, labrum; B, postabdomen; C, antenna. D-E: mature male; D, labrum; E, postabdomen.

Figure 4.3. Scanning electron micrographs of *Chydorus freyi* **sp. nov.** (A, C-D) and *Chydorus carolinensis* **sp. nov.** (B, E-F). A-B: postabdomen of a parthenogenetic female; C, E: mature male in lateral view; D, F: postabdomen of mature male.

Figure 4.4. *Chydorus freyi* **sp. nov.** from the type locality, a temporary pond in Savannah River Site, South Carolina, USA. A-J: parthenogenetic female; A, antennule; B, limb I; C; IDL and ODL; D, limb II; E, exopodite of limb III; F, limb III; G, inner portion of limb III; H, limb IV; I, inner portion of limb IV; J, limb V. K-L: mature male; K, antennule; L, limb I.

Figure 4.5. *Chydorus carolinensis* **sp. nov.** from the type locality, a temporary pond in Savannah River Site, South Carolina, USA. A-D: parthenogenetic female; A, lateral view; B, rear view; C, apex of the rostrum; D, head shield. E-F: ephippial female in lateral and rear views. G-I: mature male; G, lateral view; H, rear view; I apex of rostrum.

Figure 4.6. *Chydorus carolinensis* **sp. nov.** from the type locality, a temporary pond in Savannah River Site, South Carolina, USA. A-C: parthenogenetic female; A, labrum; B, postabdomen; C, antennule; D, antenna. E-F: mature male; E, postabdomen; F, antennule.

Figure 4.7. *Chydorus carolinensis* **sp. nov.** from the type locality, a temporary pond in Savannah River Site, South Carolina, USA. A-I: parthenogenetic female; A, limb I; B; IDL and ODL; C, limb II; D, exopodite of limb III; E, limb III; F, inner portion of limb III; G, limb IV; H, inner portion of limb IV; I, limb V. J, limb I of mature male.

Figure 4.8. A combined 16S and CO1 Bayesian phylogeny of *Chydorus* species from the eastern USA, rooted with *Picripleuroxus denticulatus* as an outgroup. Samples of *Chydorus carolinensis* **sp. nov.** are indicated by the black bar and samples of *C. freyi* **sp. nov.** are indicated with the grey bar. Posterior probabilities are given for nodes having > 50% support. Sample codes are given in Table 1.

Figure 4.1.

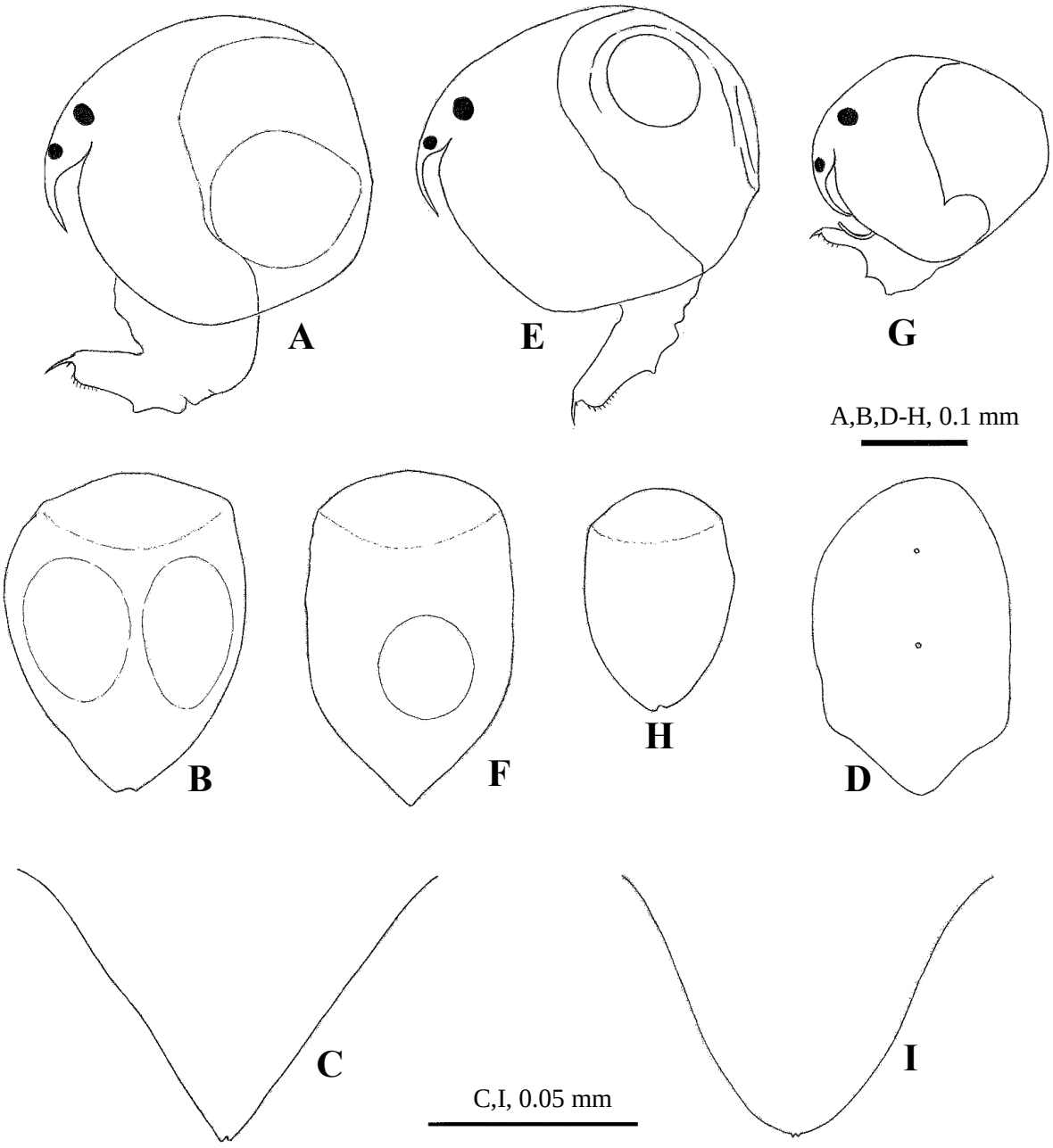


Figure 4.2.

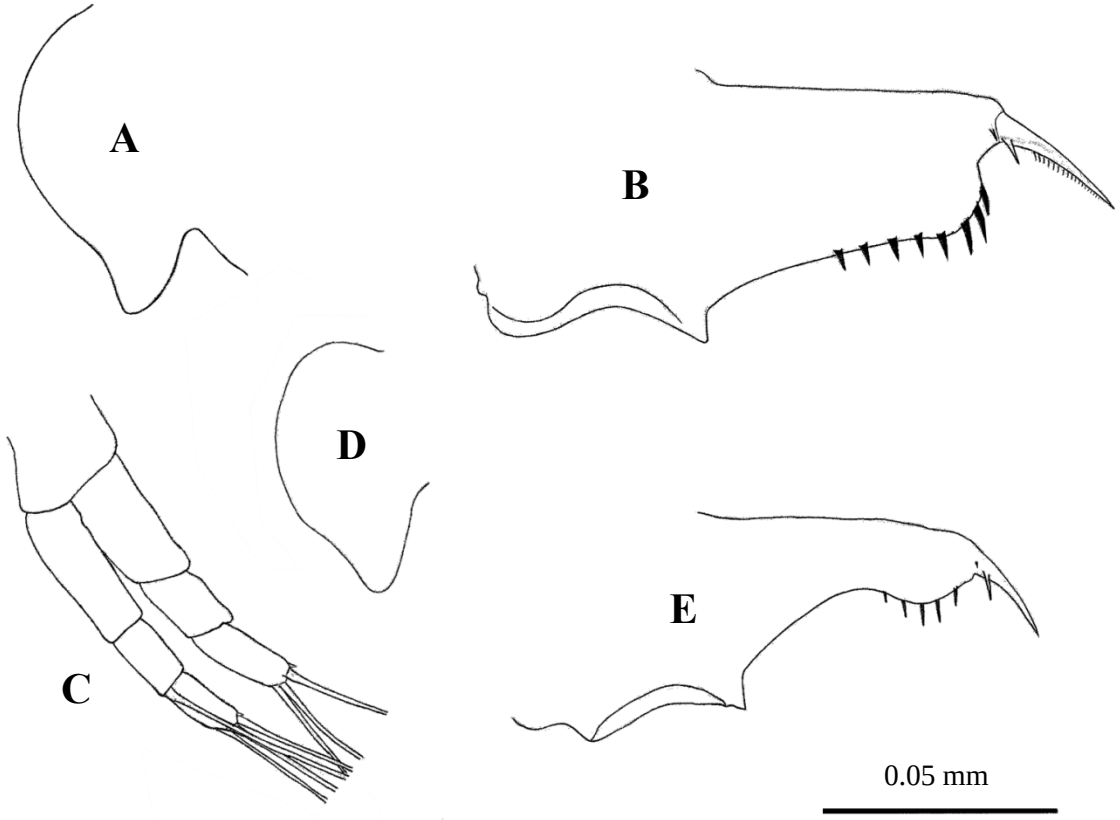


Figure 4.3.

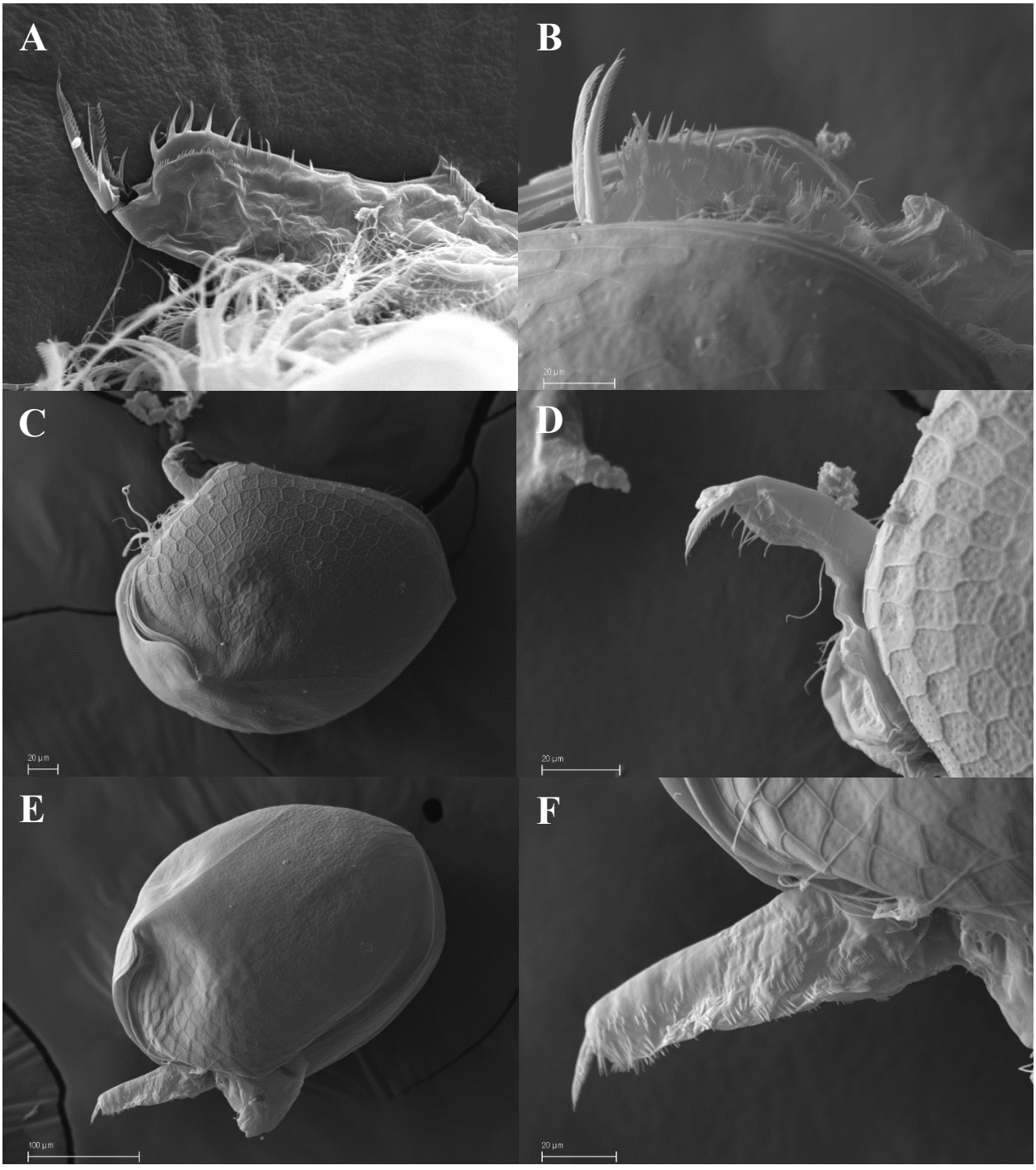


Figure 4.4.

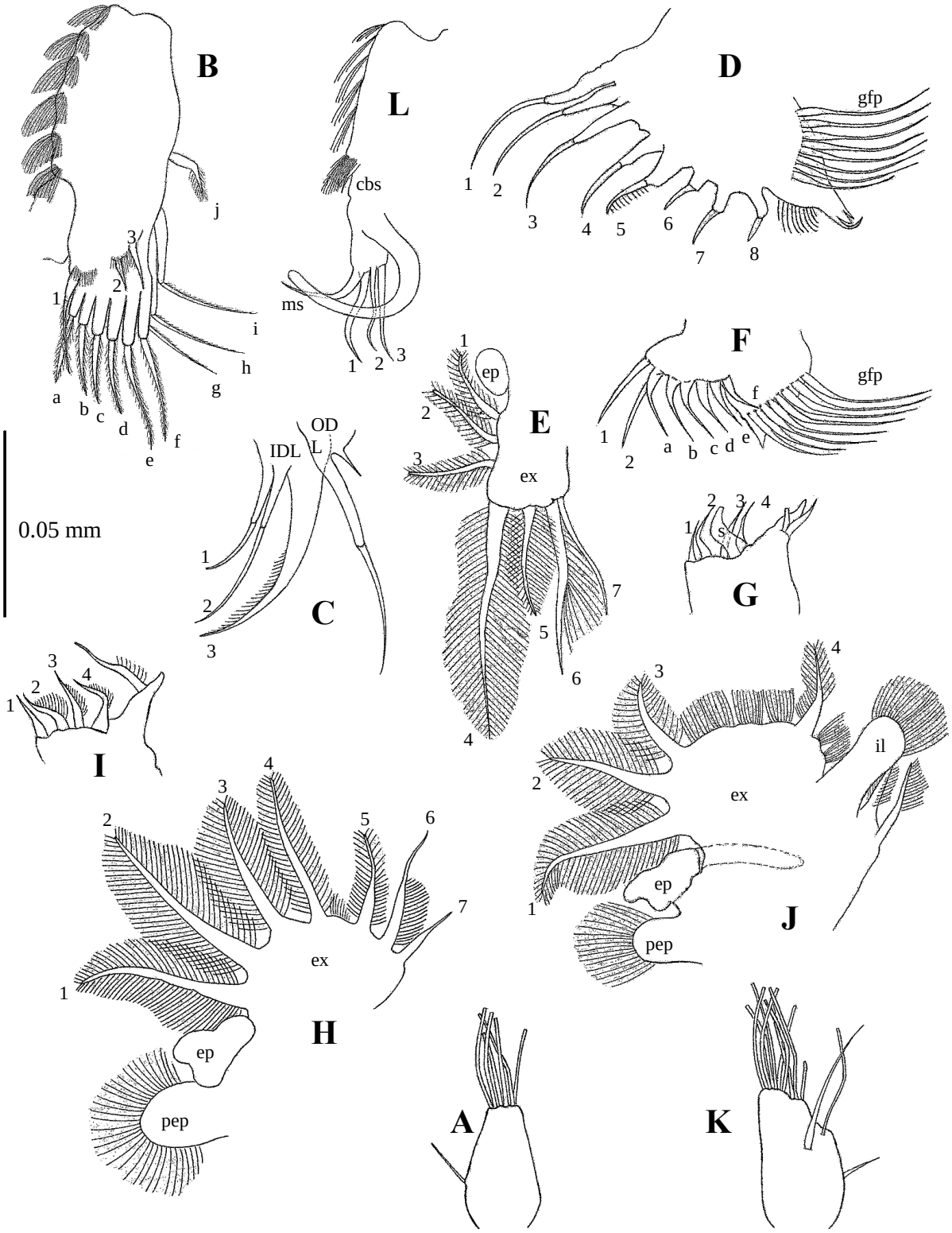

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Figure 4.5.

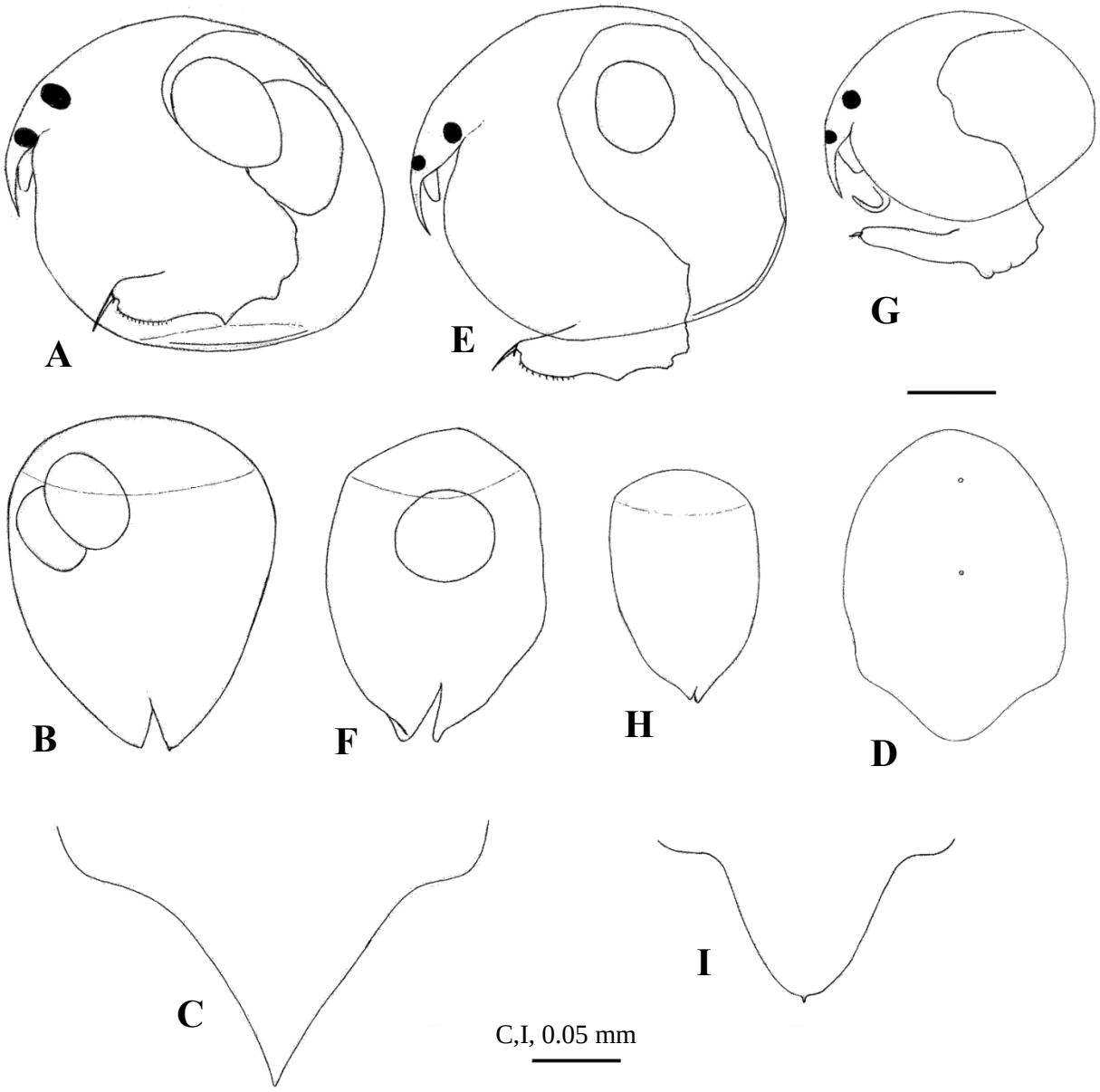

Figure 4.6.

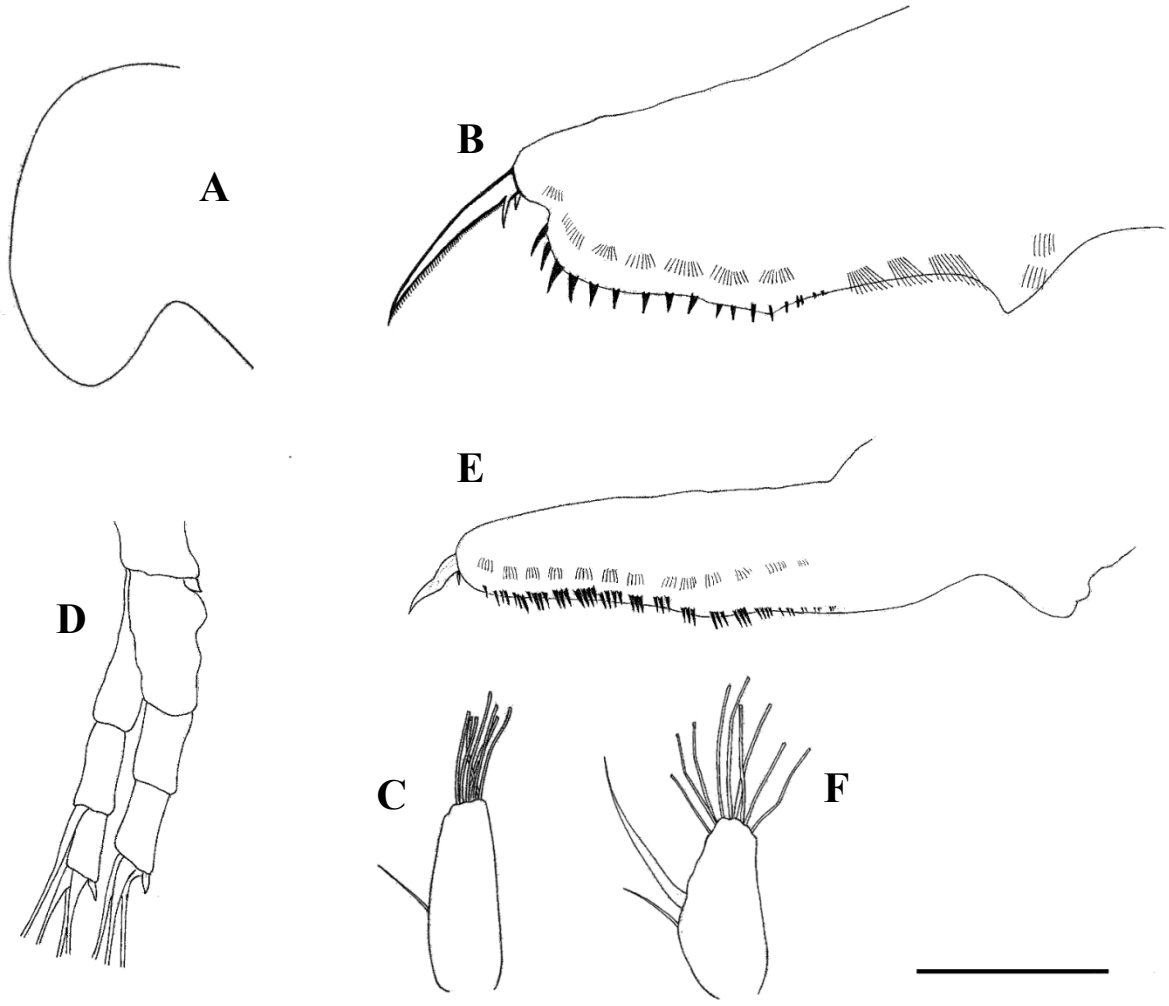

Figure 4.7.

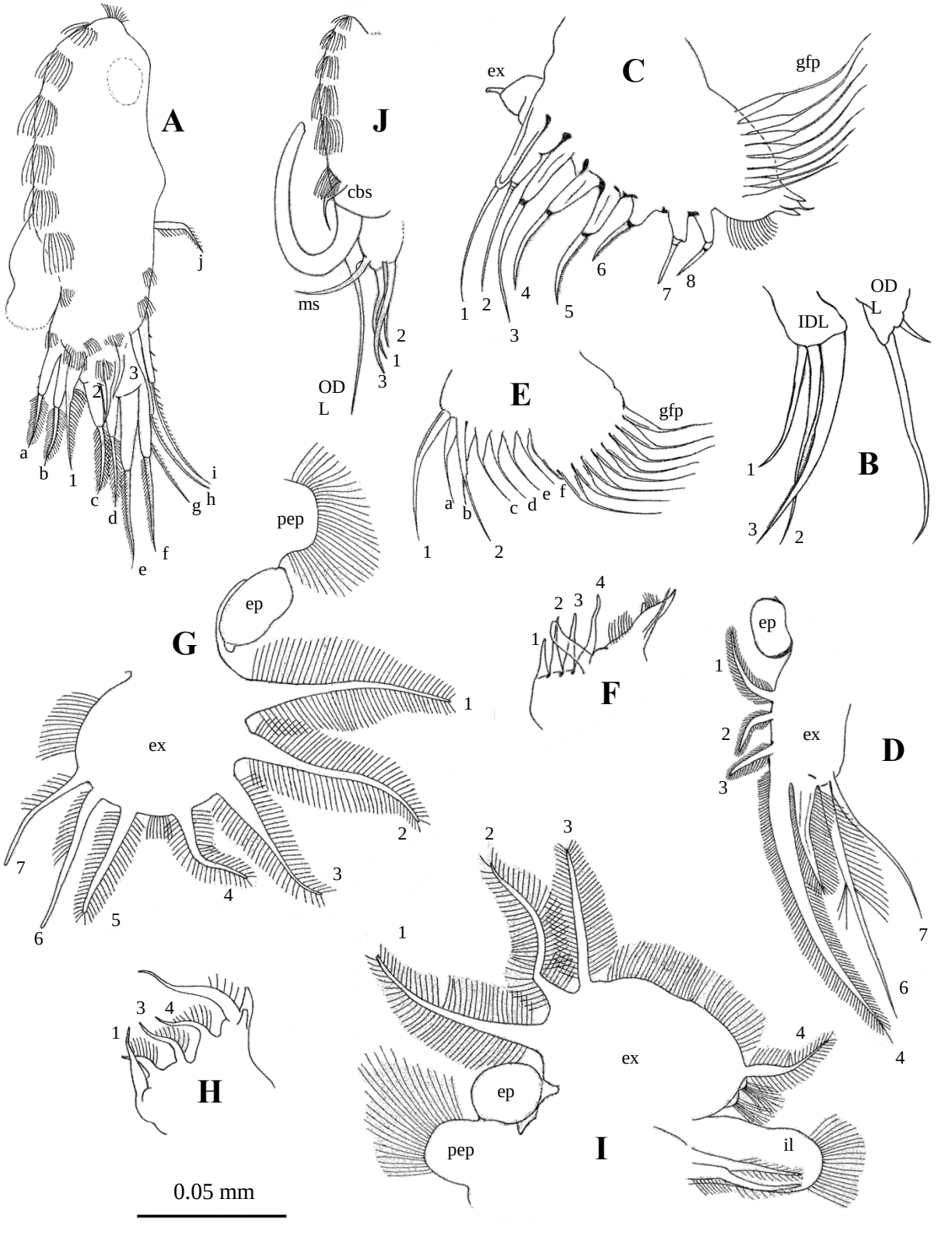

Figure 4.8.


Chy bicoll GS 1
Chy eury 4 1
Chy canad ONT
ChyA 176 1
ChyA 176 2
ChyA 176 4
ChyA 176 6
ChyA 78 14
ChyA 78 15
Chy bicorn GS 1
Chy bicorn GS 2
Chy bicorn ONT
Chy brev Ath 1
Chy brev Ath 5
Chy brev Ath 6
Chy brev Ath 2
Chy brev Ath 3
ChyB 11 1
ChyB 11 3
ChyB 78 11
ChyB 66 1
ChyB 78 3
ChyB 78 5
ChyB 11 2
ChyB 7 1
ChyB 7 2
ChyB 11 4
ChyB 7 4
ChyB 7 3
Chy ling 78 3
Chy ling 78 4
Pic dent 176 2
85
98
91
99
100
70
61
100
60
100
67
98
100
86
90
97
100
95
94
50
100
0.05
Chydorus carolinensis **sp. nov.**
Chydorus freyi **sp. nov.**

CHAPTER 5

CONCLUSIONS

The two year survey of 14 wetlands in SRS answered many questions regarding the distribution of diversity within this system. High diversity on the landscape scale was associated only with hydroperiod length, and only with species richness and not the numbers of common or dominant species. Overall, wetlands with a longer hydroperiod length had greater species richness. The vegetation type of the wetland was also of importance, as forested wetlands had lower α-diversity than those with an open canopy. Beta diversity also varied considerably among wetlands, with distance between wetlands a significant correlate, suggesting dispersal between wetlands was of some importance. Wetlands that had similar hydroperiod length were home to a similar group of species, whereas wetlands with similar pH had like groups of common and dominant species. Wetlands on the same soil type had greater overlap in zooplankton communities, but canopy type was not an important factor, suggesting that forested wetlands did not have a unique species community. On the local scale, high α-diversity was associated with the warmer months of the year, when pH and conductivity were low. Spatial heterogeneity was also of importance at the local scale, as vegetated sites had greater α-diversity than those from open water samples; diversity was particularly low in samples from substrates of decaying plant material. Beta-diversity varied considerably over time, with similar communities occurring both near in time and approximately one year apart. This indicates that similar temporal communities arise every year; these temporal changes were the result of multiple factors.

While the wetland survey found that hydroperiod length was correlated with high species richness and was associated with community differences between wetlands, the mesocosm experiment in chapter 3 was able to test whether hydroperiod was indeed a causal agent. In addition, the effect of salamander predation was examined to determine if there were important top-down effects on zooplankton diversity and community composition. Hydroperiod was found to have a strong impact on α-diversity; species richness

was reduced considerably in the short hydroperiod treatment by the experimental endpoint. Shortened hydroperiods also resulted in the domination of the zooplankton community by only a few species. Differences in hydroperiod length led to differences in community composition as well. Communities of intermediate hydroperiod length had the greatest within-treatment differences in species presence/absence, whereas short hydroperiod treatments came to be dominated by a nearly identical community among replicates. Interestingly, these community patterns were not observed in the field survey data; the shortest hydroperiod wetlands were not similar to each other, indicating that in absence of identical conditions and founding communities, their communities can diverge considerably. Salamander predation had little impact on zooplankton diversity, community composition, or size structure.

Two of the most important members of the zooplankton community numerically were *Chydorus carolinensis* and *C. freyi*, which is interesting because neither had species identities prior to this study. These two species were both widespread among wetlands and usually abundant where they were found, contributing considerably to the overall diversity of the wetlands of this system. Given their distribution in SRS, both species are likely to be widespread in similar wetlands of the southern USA. The descriptions of these two species add to an increasing list of endemic North American cladoceran species.

The studies presented in this dissertation used both descriptive and experimental approaches to address diversity in temporary wetlands systems. The field survey provides a broad view of spatial and temporal patterns in diversity of a set of highly species rich temporary wetlands. The mesocosm study experimentally tests the impact of a factor that was identified in the field survey as an important correlate of species richness. The final chapter describes two new species, which add to the overall richness of the SRS wetlands. This combination of studies provides an important contribution to our understanding of the temporary wetlands of SRS. However, since similar stressors impact temporary wetlands of all types, the conclusions presented here should be broadly applicable beyond the borders of SRS. In addition, they provide a solid base which future studies can build upon to increase knowledge of how diverse communities develop and how species coexist within them.

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