Abstract

THE EFFECTS OF ZOOPLANKTON DISPERSAL ON COMMUNITY ASSEMBLY OF TEMPORARY PONDS

by

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Interdisciplinary Doctoral Program in Biological Sciences

The dispersal of individuals among habitat patches is thought to have an important effect on ecological communities as it can influence both population dynamics and community assembly. Though much work on dispersal has been completed, zooplankton offer an interesting opportunity to study dispersal. They can disperse not only among ponds, but they can also disperse within and among ponds through time as their eggs can remain dormant for long periods until environmental conditions initiate their hatching. The dissertation has focused on the role of temporal and spatial dispersal on the assembly of zooplankton communities, an assessment of whether predators weaken the effect of early dispersing zooplankton species on late dispersing zooplankton species through differences in zooplankton hatching phenology, and the effects of environment and space on temporary pond zooplankton communities in the Croatan National Forest, NC. Differences in spatial and temporal dispersal had a weak effect on the number of zooplankton species present. Nonetheless, both spatial and temporal dispersal strongly affected the total abundance and species composition of zooplankton present, but their effects were interdependent. When predation and the effects of zooplankton hatching phenology were considered predators and one zooplankton species that arrived early slowed the growth of the

later arriving zooplankton species. Algal resources were not affected by predators, but were affected by the order of arrival of the different zooplankton species. Lastly, the role of environment and space on zooplankton temporary pond communities showed that spatial and environmental factors explained similar amounts of the variation in zooplankton composition in the Croatan National Forest, with environmental factors explaining more of the variation in the spring and summer than in the fall and winter. Together these results indicate that zooplankton composition in pond communities can be affected by interactions between spatial and temporal dispersal, the presence of predators and differences in hatching phenology, as well as by environmental factors such as temperature and spatial factors such as pond size and pond density. Thus, highlighting the importance of dispersal but also its interaction with other abiotic and biotic factors to form zooplankton communities.

THE EFFECTS OF ZOOPLANKTON DISPERSAL ON COMMUNITY ASSEMBLY OF TEMPORARY PONDS

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Introduction

The movement of individuals from one population to another through dispersal is a fundamental ecological process that can prevent local extinction (Matthysen 2012), help maintain species diversity at local and regional scales (Cadotte 2006), provide novel genetic variation to a population (Baguette et al. 2012, Edelaar & Bolnick 2012), and reduce kin competition and inbreeding depression (Ronce 2007). Though a great deal of work has focused on the active or passive dispersal (via wind, flowing water, or animal vectors) of individuals among populations separated by space (Levin et al. 2003, Benton & Bowler 2012), many plants and invertebrates possess life history stages where the individuals have the capacity to disperse among populations separated by space or time. Temporal dispersal in these organisms is facilitated by the production of dormant life history stages (i.e., seeds or eggs) that allow individuals to disperse and become incorporated into a future (sometimes by many generations) population that might be quite different from that in which the dormant individual was produced (De Stasio 1989, Evans & Dennehy 2005). A number of studies of organisms that disperse both spatially and temporally have found that both spatial (Shurin et al. 2000, Cáceres & Soluk 2002, Levine & Murrell 2003) and temporal (Hairston & Kearns 2002, Jones et al. 2010) dispersal can have profound impacts on ecological communities by altering the composition, diversity, and relative abundances of species present. Nonetheless, few studies have directly compared how spatial and temporal dispersal contribute to the development of ecological communities (but see Nathan & Muller-Landau 2000, Gray & Arnott 2011), nor have they evaluated the extent to

which one form of dispersal (i.e., temporal or spatial) alters the influence of the other form of dispersal on community assembly.

Spatial and temporal dispersal may affect ecological communities differently for a variety of reasons. Temporal dispersal may have a different effect on the development of ecological communities than spatial dispersal because 1) the in situ production of dormant individuals provides a large pool of potential colonists for a patch (Brock et al. 2003, De Stasio 2007) and the number of spatially dispersing colonists from other patches declines very rapidly with distance from their source patch (Allen 2007, Frisch et al. 2012), and 2) dormant individuals are already adapted to the timing and release from dormancy specific to the habitat (Gyllström & Hansson 2004, Evans & Dennehy 2005), and dormant individuals may be better adapted to living in the habitat patch than individuals dispersing from elsewhere (Bohonak & Jenkins 2003, Brendonck & De Meester 2003). Temporal dispersal is also more likely to drive the development of an ecological community in a consistent direction because the dispersers produced within the community are more likely to be similar to each other and those released from dormancy; whereas, spatial dispersal is more likely to drive the development of ecological communities into different directions, because it can be rather idiosyncratic from which patch a particular disperser arrives (Forbes & Chase 2002, Shurin et al. 2009, Simonis & Ellis 2014). Patches will also likely vary in the kind of dispersers (i.e., different species or different phenotypes/genotypes of a given species) they produce (McPeek & Holt 1992, Benard & McCauley 2008).

In addition to having different effects, one form of dispersal (i.e., temporal versus spatial) may alter the influence that the other form of dispersal has on the development of ecological communities. Such an influence could be produced if individuals arriving via one form of

dispersal are able to augment or reduce the likelihood that individuals arriving via the other mode of dispersal would persist in the community. Two possible ways in which this could happen include: 1) phenotypes/species that disperse via one mode of dispersal are inherently superior competitors on a per capita basis and/or arrive in such great numbers they reduce the likelihood individuals dispersed via the other dispersal method can persist in the habitat and 2) phenotypes/species that disperse via one mode of dispersal arrive to a site earlier and this earlier arrival influences the performance of phenotypes/species that arrive later. Indeed, a number of studies have found that the outcome of competitive interactions can depend on which species achieves a particular threshold population size first (Park 1954, Matveev 1985, Walters & Mackay 2005) and the order in which species arrive to a patch (Alford & Wilbur 1985, Drake 1990, Chase 2003, Fukami 2004, Hernandez & Chalcraft 2012, Amoroso & Chalcraft 2015) can have significant consequences on the development of ecological communities and the performance of species. Consequently, temporal dispersal could weaken the effect of spatial dispersal on community development if temporal dispersers emerge earlier than spatial dispersers (Jenkins & Buikema 1998) or if temporal dispersers emerge within a patch faster than the rate at which spatial dispersers arrive (De Meester et al. 2002). Spatial dispersers could weaken the effect of temporal dispersers on community development if the occurrence of cues that trigger emergence of individuals from dormant stages is delayed or other factors (i.e., deep burial, infection, etc.) reduce the number of dormant individuals that emerge from dormancy (Bohonak & Jenkins 2003, Allen 2007, De Stasio 2007).

We investigated the independent and interactive effects of spatial and temporal dispersal on zooplankton communities by conducting an experiment in mesocosms designed to mimic features of natural ponds. Zooplankton have the ability to produce dormant eggs that can survive

conditions unfavorable for non-dormant life history stages (Brendonck & De Meester 2003, De Stasio 2007). The active zooplankton community ("zooplankton community" hereafter) in a water body is generally described on the basis of the non-dormant individuals present in a water column, where the impacts that dormant individuals may have on the active zooplankton community is often neglected (Gyllström & Hansson 2004). Dormant zooplankton eggs can persist for hundreds of years (Hairston et al. 1995, Mergeay et al. 2007) and can accumulate in high densities on pond bottoms ($10^3 – 10^5$ eggs per m^2 eggs depending on the species; Brendonck & De Meester 2003) in what is referred to as an egg bank. Dormant individuals may disperse through time by delaying hatching (Bilton 2001, Havel & Shurin 2004) and/or passively disperse through space via biotic (Figuerola et al. 2005, van de Meutter et al. 2008) or abiotic vectors (Brendonck & Riddoch 1999, Cáceres & Soluk 2002). Though rates of emergence from dormant eggs is largely dependent on environmental cues and varies considerably among water bodies and across years within a water body, the proportion of individuals exiting dormancy among lakes ranged from 6% to 50% for *Daphnia pulicaria* (Cáceres & Tessier 2003). Non-dormant stages of zooplankton cannot disperse through time, but can disperse across space via multiple mechanisms (Michels et al 2001, Lopez et al. 2005, Kelly et al. 2013). Though great leaps across space are possible (Green & Figuerola 2005, Vanshoenwinkel et al. 2011), the number of spatially dispersing zooplankton generally declines very rapidly as distance increases from a source pond (ponds located 60 meters away from a source pond receive only 10% of the dispersers that ponds 10 meters from a source pond receive; Allen 2007).

We hypothesized that 1) increasing spatial dispersal would alter the development of the active zooplankton community when temporal dispersal was limited and 2) increasing temporal dispersal would alter the development of the active zooplankton community when spatial

dispersal was limited. We also hypothesized that increasing rates of emergence of individuals from dormant egg banks would weaken the effect of spatial dispersal on community development. We expected higher rates of temporal dispersal would produce an active zooplankton community that already had many individuals present by the time spatial dispersers arrived to a pond, which would reduce the likelihood of spatially dispersing zooplankton establishing in a pond.

Methods

We independently manipulated rates of spatial dispersal (i.e., high versus low rate of spatial dispersal) and temporal dispersal (i.e., natural versus reduced rate of temporal dispersal) into the active zooplankton community found within newly filled artificial ponds or mesocosms (38 L plastic containers) containing a dormant egg bank. These manipulations produced four experimental treatments that were each replicated once within each of five transects stemming from a borrow pit pond (area: 2,733 m^2, max. depth on 21 September 2011: 1.01 m) located in a forested area in Greenville, NC (Fig. 1). The borrow pit pond contained fish and served as a source of spatially dispersing zooplankton. A list of the active zooplankton community found in this and other nearby ponds can be found in Supplement 1A.

Mesocosms remained uncovered throughout the experiment and each was filled with approximately 30 L of well water, received 25 g of turkey oak leaves to provide a natural source of nutrients, a 900 gram sediment sample containing a dormant egg bank, and had treatment manipulations applied to them on a transect by transect basis on 23 June 2011. Sediment samples were derived from a homogenized mixture of thirty squares (25 cm^2) of sediment cut from the periphery (n=15) and interior portion (n=15) of a dried pond basin in the Croatan

National Forest on 21 June 2011. The depth of each square was 3 cm, as this represents the portion of sediment in a pond containing the active egg bank (Brendonck & De Meester 2003). Zooplankton that were present in the pond from which the sediment samples were obtained were identified from resting eggs present in the sediment and active members of the zooplankton community that were collected after the pond refilled (samples collected bimonthly for another project from September 2012-September 2013). A list of the zooplankton assemblage present in the pond from which the egg bank was derived can be found in Supplement 1A.

We manipulated spatial dispersal into mesocosms by placing mesocosms either 3 m (near treatment) or 50 m (far treatment) away from the borrow pit pond. The number of dispersing zooplankton declines exponentially with distance from a source pond such that ponds located 60 meters away from a source pond receives 10% of the number of dispersing zooplankton that ponds located 10 meters away from a source pond receives (Allen 2007). Consequently, near mesocosms will experience a higher rate of spatial dispersal than far mesocosms. Recruitment from the dormant egg bank in each mesocosm was manipulated by placing the dormant egg bank into a closed 1 gallon Ziploc® bag that had a 16 cm^2 hole cut on one side and either leaving this opening uncovered or by gluing fine mesh (30 μm) over the opening. Uncovered bags allowed dormant individuals to enter the active zooplankton community and allowed nutrients from the sediment to diffuse into the mesocosm (egg bank treatment). Bags covered with a fine mesh, however, hindered the ability of dormant individuals to enter the active zooplankton community but would still allow nutrients from the sediment to diffuse into the mesocosm (no egg bank treatment).

The active zooplankton community in each mesocosm was sampled on 3 August and 7 September 2011 by filtering (mesh size=64 μm) a 1 L sample of water collected from the

mesocosm using a PVC tube sample. The contents retained in the filter were preserved in >70% ethanol for later identification in the lab. For identification purposes, we focused on cladoceran zooplankton specifically (excluding other zooplankton groups such as copepods, rotifers, and protozoa) and cladocerans were identified to the species level. All sampling materials were thoroughly rinsed after each sample was collected, and all water samples were collected from mesocosms in the same transect before collecting additional samples from other transects. Standing stock of phytoplankton present in each mesocosm at the end of the experiment was estimated on the basis of chlorophyll a extraction (APHA 1995) from a 550 mL sample of water.

Statistical Analyses

We utilized PROC MIXED in SAS version 9.4 (SAS Institute 2013) to evaluate how treatments differed in the total abundance of cladocerans and the number of cladoceran species present in a mesocosm. Transects were treated as a random factor, treatments were treated as a fixed factor, and time (August or September) was treated as a repeated factor. Mesocosm was identified as the subject of repeated measurements. Planned contrasts were performed to evaluate 1) the effect of spatial dispersal (i.e., distance) when there was little temporal dispersal, 2) the effect of temporal dispersal (i.e., recruitment from the egg bank) when spatial dispersal was low 3), differences in the effect of temporal and spatial dispersal, and 4) the extent to which the combined effect of high spatial and temporal dispersal differed from that expected if the effects of each mode of dispersal were independent of each other (Supplement 1B). A difference between the observed and expected effect when both spatial and temporal dispersal was high would indicate that one or both modes of dispersal affected the influence of the other mode of dispersal. In such an event that observed and expected responses differed, we used the

LSMESTIMATE option when implementing PROC MIXED to estimate the least square or marginal effect of 1) temporal dispersal when spatial dispersal was low (far egg bank vs. far no egg bank), 2) temporal dispersal when spatial dispersal was high (near egg bank vs. near no egg bank), 3) spatial dispersal when temporal dispersal was low (near no egg bank vs. far no egg bank) and 4) spatial dispersal when temporal dispersal was high (near egg bank vs. far egg bank). P-values for each contrast were adjusted to control the False Discovery Rate (FDR; Verhoeven et. al 2005) for each response variable by using PROC MULTTEST in SAS v.9.4 (SAS Institute 2013). Both unadjusted and FDR adjusted p-values (p_{FDR}) are reported.

We described how mesocosms differed in the species composition of the active cladoceran community with three dissimilarity matrices. One matrix described Bray-Curtis differences in the abundances of all taxa (BCA), the second matrix described Bray-Curtis differences in the presence/absence of all taxa (BCP), and the third matrix described Raup-Crick differences in the presence/absence of all taxa (RC). Each matrix provides a different descriptor of differences in species composition. BCA emphasizes differences that are due to taxa that may be extremely common in one community but less common in another community, while BCP emphasizes differences that are due to some taxa being present in one community but absent in another. Differences in species composition as described by BCA or BCP could also derive from one community having more species present within it than the other (Raup & Crick 1979, Chase et al. 2011). RC emphasizes differences in which species are present/absent in communities independent of differences in the number of species present (Raup & Crick 1979, Chase et al. 2011). A dummy species with the value 1 was added when calculating the abundance matrix due to an excess of 0 values in the abundance data (Clarke et al. 2006). Bray-Curtis matrices were

generated in PRIMER-E version 6 (Clarke & Gorley 2006) while the Raup-Crick matrix was generated using the Raup-Crick function in the R Vegan package (Chase et al. 2011).

We evaluated how species composition varied among treatments and through time by conducting a PERMANOVA using PRIMER-E v.6 (Clarke & Gorley 2006) on each of the three dissimilarity matrices. Our PERMANOVA model included the fixed effects of treatment, time, and their interaction, as well as the random effect of transect. The same planned contrasts that were performed for total abundance of cladocerans and the number of cladoceran species were also performed on species composition (Supplement 1B). We also evaluated whether certain treatments contained mesocosms that were more heterogeneous in their species composition than other treatments and whether the extent of heterogeneity in species composition among mesocosms changed through time by conducting a PERMDISP using PRIMER-E v.6 (Clarke & Gorley 2006) on each of the three dissimilarity matrices. The PERMDISP model included fixed effects of treatment and time but PERMDISP does not allow for the specification of interactive or random effects. Treatments with greater heterogeneity in species composition would indicate the species composition of mesocosms in that treatment are less deterministic than in other treatments. Similarly, increasing heterogeneity in species composition though time would indicate that mesocosms are diverging in their species composition. Pairwise comparisons were performed among different levels of fixed factors for PERMDISP if the test of the fixed factor suggested that species composition varied significantly, as planned contrasts cannot be performed for PERMDISP. We adjusted p-values from pairwise comparisons to control the FDR across comparisons, but we report both unadjusted and FDR (p_{FDR}) adjusted p-values.

To describe which species contributed the most to differences in species composition among treatments for each sampling period, we performed a SIMPER analysis in PRIMER-E v.6

(Clarke & Gorley 2006) on BCA and BCP data matrices. A SIMPER analysis performed on abundance data (BCA) identifies which taxa contribute the most to differences in species composition as the result of differences in the abundance of those taxa regardless of whether those taxa are present in both treatments or not. In contrast, a SIMPER analysis performed on presence/absence data (BCP) identifies which taxa contribute the most to differences in species composition between treatments as the result of taxa having a higher likelihood of being present (i.e., proportion of mesocosms in a treatment in which the taxa is present) in one treatment than in another treatment.

Results

A total of 12,268 cladocerans comprising 12 species were sampled in mesocosms throughout the experiment. The effects of treatment on the number of cladoceran species present did not vary with time ($F_{3, 28} = 0.88$, $p \geq 0.463$). The mean number of cladoceran species present in a mesocosm declined, however, between August (Least square [LS] mean ± 1SE = 2.5 ± 0.26 species) and September (LS mean ± 1SE = 1.7 ± 0.26 species)($F_{1,28} = 8.39$, $p = 0.007$). Increasing spatial dispersal enhanced the mean number of cladoceran species present by 0.5 species (± 1SE = 0.415) when temporal dispersal was limited but the effect was not statistically different than 0 ($F_{1,28} = 1.45$, $p = 0.238$, $p_{FDR} = 0.687$; Fig. 2). Increasing temporal dispersal had a very weak effect (LS ± 1SE = 0.1 ± 0.415 species) on the mean number of cladoceran species present when spatial dispersal was limited ($F_{1,28}=0.06$, $p=0.811$, $p_{FDR} = 0.866$; Fig. 2). Consequently, we did not find a statistically meaningful difference in how spatial and temporal dispersal affected the mean number of cladoceran species present ($F_{1,28} = 0.93$, $p = 0.343$, $p_{FDR} = 0.687$; Fig. 2). Furthermore, the two modes of dispersal did not alter the influence of the other

mode of dispersal on the number of cladoceran species as the observed number of cladoceran species present when rates of both temporal and spatial dispersal were high did not differ substantially from that expected if the effects of spatial and temporal dispersal were independent of each other ($F_{1,28} = 0.03$, $p = 0.866$, $p_{FDR} = 0.866$; Fig. 2).

The effect of treatments on the abundance of cladocerans present did not vary with time ($F_{1,28} = 0.54$, $p = 0.57$). We observed very little change in the mean abundance of cladocerans present in mesocosms between August (LS mean ± 1SE = 336.10 ± 81.22 individuals) and September (LS mean ± 1SE = 277.30 ± 81.22 individuals)($F_{1,28} = 0.46$, $p = 0.501$). The mean number of cladocerans present in mesocosms increased when spatial dispersal increased ($F_{1,28} = 8.88$, $p = 0.006$, $p_{FDR} = 0.024$) and when temporal dispersal increased ($F_{1,28} = 6.01$, $p = 0.021$, $p_{FDR} = 0.028$; Fig. 3). The effects of spatial dispersal and temporal dispersal on mean cladoceran abundance were similar ($F_{1,28} = 0.28$, $p = 0.601$, $p_{FDR} = 0.601$; Fig. 3). The observed mean abundance of cladocerans present when the rates of both spatial and temporal dispersal were high was different from that expected if the effects of each mode of dispersal were independent of each other ($F_{1,28} = 6.33$, $p = 0.018$, $p_{FDR} = 0.028$; Fig. 3). Increasing spatial dispersal had a much weaker effect on mean cladoceran abundance when temporal dispersal was high (LS mean effect [±1 SE] of spatial dispersal = -70.60 ± 121.99 individuals) than when temporal dispersal was low (LS mean effect [±1 SE] of spatial dispersal = 363.60 ± 121.99 individuals). Similarly, increasing temporal dispersal had a much weaker effect on mean cladoceran abundance when spatial dispersal was high (LS mean effect [±1 SE] of temporal dispersal = -135.1 ± 121.99 individuals) than when spatial dispersal was low (LS mean effect [±1 SE] of temporal dispersal = 299.10 ± 121.99 individuals; Fig. 3).

PERMANOVA revealed that the species composition of mesocosms varied among

treatments but the effects of treatment did not vary through time (Fig. 4, Table 1). There was a strong trend for increased spatial dispersal to alter species composition when temporal dispersal was limited, regardless of how differences in species composition was assessed (BCA $p = 0.056$, $p_{FDR} = 0.074$; BCP $p = 0.059$, $p_{FDR} = 0.118$; RC $p = 0.069$, $p_{FDR} = 0.091$). SIMPER analyses indicated the majority of differences in species composition stemming from increasing spatial dispersal (92% and 83% of the differences in species composition as assessed by BCA and BCP, respectively), was due to spatial dispersal enhancing the abundances and likelihood of occurrence of *D. brachyurum*, *K. latissima* and *B. longirostris* but reducing the abundances and likelihood of occurrence of *S. crystallina* and *S. mucronata* (Table 2). Increasing temporal dispersal altered species composition when differences in species composition were based on BCA ($p = 0.047$, $p_{FDR} = 0.074$), but not when differences were based on BCP ($p = 0.234$) or RC ($p = 0.319$; Table 1). The majority of differences in species composition (91%) stemming from increasing temporal dispersal, was due to temporal dispersal enhancing the abundances of *D. brachyurum* and *S. crystallina* (Table 2). Spatial and temporal dispersal did not differ in their effects on species composition when differences in species composition were described by BCA ($p = 0.171$), but they did differ in their effect on species composition when differences in species composition was described by either BCP ($p = 0.044$, $p_{FDR} = 0.118$) or RC ($p = 0.043$, $p_{FDR} = 0.091$; Table 1). Mesocosms with high temporal dispersal were less likely to have *K. latissima*, *B. longirostris*, and *D. brachyurum* and were more likely to have *S. crystallina* than mesocosms with high spatial dispersal, where the presence of *S. mucronata* was similar in both (Table 2).

We also found strong evidence to indicate that the effects of spatial and temporal dispersal were different when both spatial and temporal dispersal were high than when one of the modes of dispersal was low (i.e., there is a significant interaction between the effects of spatial

and temporal dispersal; Table 1). The interaction was mainly driven by differences in species composition from increasing temporal dispersal when spatial dispersal was high, as increasing spatial dispersal when temporal dispersal was high had less of an effect on differences in species composition. The majority of differences in species composition (96% and 78% of the differences in species composition as assessed by BCA and BCP, respectively) due to increased temporal dispersal when spatial dispersal was high was largely due to temporal dispersal increasing the abundance and likelihood of occurrence of *S. crystallina* and reducing the abundances and likelihood of occurrence of *D. brachyurum*, *K. latissima*, and *B. longirostris* (Table 2). There were not large differences in species composition based on increased spatial dispersal when temporal dispersal was high (Table 2).

We found that species composition of mesocosms changed through time when differences in species composition were assessed by BCA (Fig. 4a), with a trend for differences based on BCP (Fig. 4b), and no differences based on RC (Fig. 4c, Table 1). Simper analysis revealed that the majority of differences in species composition (93% and 74% of the differences in species composition as assessed by BCA and BCP, respectively) through time was due to *D. brachyurum*, *K. latissima*, and *S. mucronata* being less abundant and less likely to occupy mesocosms in September, and *S. crystallina* being slightly more abundant but less likely to occupy mesocosms in September. Species that were sampled in August but absent in September, although in low abundances, were *B. longirostris*, *C. laticaudata*, *S. vetulus*, *C. dubia*, *and D. americana*. Conversely, *S. serrulatus* and *A. excisa* were sampled only in September (Table 2).

PERMDISP and inspection of NMDS plots revealed that mesocosms were more dissimilar in their species composition during September than August but only when differences in species composition were described by BCA and not by BCP or RC (Fig. 4, Table 3). The

extent of heterogeneity in species composition was significantly different among treatments regardless of how differences in species composition were described (BCA $p = 0.010$, BCP $p = <.001$, RC $p = 0.004$; Fig. 4; Table 3). Pairwise comparisons among treatments revealed more variability in species composition among the mesocosms with low rates of both spatial and temporal dispersal than among mesososms within treatments that had high temporal dispersal (Table 3). In addition, when differences in species composition were described by BCP, there was a trend for mesocosms with high spatial dispersal and low temporal dispersal to be more variable than mesocosms in other treatments (Fig. 4b, Table 3).

Phytoplankton abundance, as indicated by chlorophyll-a concentrations, did not significantly differ among treatments ($F_{3,16} = 0.11$, $p = 0.952$).

Discussion

Spatial and temporal dispersal had a weak effect on cladoceran species richness, but had an important effect on cladoceran abundance and composition. The effects of spatial and temporal dispersal on cladoceran abundance and composition, however, were not equivalent and were interdependent. The effect of increasing spatial dispersal on cladoceran abundance and composition was stronger when temporal dispersal was low than when it was high. Similarly, the effect of increasing temporal dispersal on cladoceran abundance was stronger when spatial dispersal was low than when it was high. However, there were differences in species composition with increased temporal dispersal when spatial dispersal was either low or high. Thus, it is also important to consider the interactive effects of temporal and spatial dispersal, as well as their individual impacts on cladoceran abundance and composition.

Effect of spatial dispersal

Spatial dispersal had an important impact on cladoceran abundance and composition when temporal dispersal was low. Our results on the effects of spatial dispersal are in accordance with other studies that evaluated the importance of spatial dispersal of zooplankton on zooplankton community development at small spatial scales (Havel & Shurin 2004, Louette & De Meester 2005, Allen 2007). In agreement with Allen (2007), we also found a similar decline in cladoceran abundances as spatial distance from the source pond increased. Cohen & Shurin (2003) found a decline in species richness with increased distance from one of their source ponds, but also found no effect of increasing distance on species richness from a second source pond they also examined. We also found there was no difference in species richness with increasing spatial distance from the source pond. Similarly, we also found relatively low average species richness of < 3 species as found by other studies investigating colonization of mesocosm and newly dug ponds (Cohen & Shurin 2003, Louette & De Meester 2005, Frisch et al. 2012).

We did find an important effect of distance from the source pond on species composition. Schamp et al. (2015) manually manipulated zooplankton dispersal between mesocosms but also found there were differences in species composition between their lowest dispersal and highest dispersal treatments. We also found greater variability in species composition in mesocosms far from the source pond than near. The greater variability in species composition could have resulted from less spatial dispersal of cladocerans that was more idiosyncratic in species arrival due to differences in cladoceran dispersal abilities (Louette & De Meester 2008, Shurin et al. 2009). Near and far mesocosms shared 4 species, whereas 3 additional species were only found in near mesocosms (*K. latissima*, *B. longirostris*, and *S. vetulus*) and 3 additional species were only found in far mesocsms with low temporal dispersal (*C. dubia*, *D. americana*, and *A. excisa*).

While zooplankton dispersal abilities are known to differ based on experiments looking at colonization, not much is known about the dispersal capacities of different zooplankton species (Jenkins & Buikema 1998, Allen 2007, Louette & De Meester 2008). However, Louette & De Meester (2008) classified *S. vetulus* as being a good short-distance dispersing species based on its ability to colonize a majority of the study ponds and persist throughout their study period. Since *S. vetulus* was only found in mesocosms near our source pond it may have dispersed the short distance from the source pond to our mesocosms, although it was not found in our one time sample of the source pond. Interestingly, *S. crystallina* was found in much higher abundance in the far mesocosms than those near the source pond. Stasko et al. (2012) found *S. crystallina* and *B. longirostris* were relatively rare from their lake samples, but were dominant in their mesocosm experiment and *S. crystallina* was the most abundant zooplankton found attached to canoe hulls. *S. crystallina* was also found to readily attach to Plexiglas blocks after disturbance (Fairchild 1981). These studies indicate that *S. crystallina* may have an excellent ability to attach to dispersal vectors and could explain how it was found in all mesocosms despite distance from the source pond.

Effect of temporal dispersal

Temporal dispersal altered cladoceran abundance and species composition when spatial dispersal was low, but also had a minimal impact on species richness. The importance of an egg bank in providing colonists has been recognized from emergence traps placed in water bodies, from hatching experiments in the laboratory, and from genetic studies (Cáceres 1998, Arnott & Yan 2002, Cáceres & Tessier 2003, De Stasio 2007, Mergeay et al. 2007). One laboratory hatching study found 19-38 cladoceran and copepod species hatched out of the egg bank from

older sites (100+ years) after seven weeks with a range of 56-1246 hatchlings, compared to 1-26 species and 3-167 hatchlings from younger sites (2 years) (Havel et al. 2000). Another study compared zooplankton overland dispersal, dispersal from connected streams, and emergence from the egg bank, which found most dispersal occurred from the egg bank and connected streams with much less dispersal occurring overland, with the egg bank contributing higher proportions of individuals and a higher number of individuals per colonist species (Gray & Arnott 2011). Similarly, a genetic study showed re-colonization of *Daphnia barbata*, after an approximate 50-year absence, was from the dormant egg bank and not the result of passive dispersal from elsewhere (Mergeay et al. 2007). Thus, our results pertaining to the effects of temporal dispersal add to this literature by showing the importance of the egg bank in providing colonists when spatial dispersal is low.

Less is known about the importance of the egg bank in ecological dynamics such as community composition (Brendonck & De Meester 2003). We found there were differences in species composition based on abundance and differences based on heterogeneity in species composition. The low spatial, low temporal mesocosms had much larger variability in their species composition based on abundance, occurrence, and turnover. Four species (*C. laticaudata*, *C. dubia*, *D. americana*, *A. excisa*) were found in very low abundances in the mesocosms that had low temporal dispersal but not in those with high temporal dispersal, such that these species may be driving the variability found. Louette et al. (2008) found some species, including *Ceriodaphnia pulchella*, were unable to persist despite being part of the regional pool and attributed this to competition with larger species or predation. In our mesocosms we did not monitor competition or predation, however, we did not see the presence of predatory aquatic insects when sampling our experiment (L. McCarthy, pers. obs.). Competition could have

occurred and limited the presence of the four species in the mesocosms with an egg bank, such that they could only persist in mesocosms with low temporal dispersal where there might have been less competition. Evidence for competitive exclusion is supported by the presence of 2 large cladocerans, *D. brachyurum and S. crystallina*, which were found in much higher abundances and higher occurrences in mesocosms with an egg bank compared to mesocosms that had low temporal dispersal.

It is counterintuitive that four additional species were found in mesocosms far from the source pond with low temporal dispersal compared to those with high temporal dispersal, as one would expect more species to hatch out of the egg bank than to arrive through space. The arrival of these individuals may have been very idiosyncratic, since they were found in such low abundances and occurrences and were far from the source pond. While we took care not to move individuals between mesocosms, other vectors could have moved cladocerans from our high temporal dispersal mesocosms to our low temporal dispersal mesocosms. Once they arrived in the low temporal mesocosm with potentially less competitors present, they may have been better able to persist. We do not believe dispersal between mesocosms happened with high frequency or we would see similar communities in all mesocosms regardless of spatial or temporal dispersal, which was not the case.

Equivalence of the effects

Spatial and temporal dispersal had a similar effect on cladoceran abundance and a similar effect on both species composition and heterogeneity in species composition based on abundance. However, significant differences in both species composition and heterogeneity in species composition based on occurrence and turnover were found. Interestingly, these results

indicate that spatial and temporal dispersal had similar effects on cladoceran abundance but had different effects on the identity of the species present. Thus, the routes of dispersal are providing different species that are able to colonize the mesocosms, through either passive spatial dispersal that most likely occurred in the high spatial, low temporal mesocosms or through emergence from the egg bank that most likely occurred in the low spatial, high temporal mesocosms.

As mentioned previously, while there is little work on differences in dispersal abilities or dispersal rates of zooplankton, some species might be better at dispersing through vectors across space (Simonis & Ellis 2014). Zooplankton can disperse readily by using certain insect and animal vectors (Allen 2007, van de Meutter et al. 2008) and one potential dispersal vector we saw on our tanks throughout the experiment was 2 different frog species (southern leopard frogs (*Rana sphenocephala*) and squirrel tree frogs (*Hyla squirella*), L. McCarthy per. obs.). Few studies have looked at the potential for frog mediated dispersal but those that have found annelids, ostracods, and ciliates could readily attach to frogs and be moved between bromeliads (Lopez et al. 2005, Sabagh et al. 2011). Thus, if cladoceran species were able to attach to frog vectors moving from the source pond to our experimental mesocosms, they might have affected the composition of the cladoceran community.

The high spatial, low temporal mesocosms were more variable in their species composition based on occurrence and turnover than the low spatial, high temporal mesocosms. More variability in the mesocosms close to the source pond and with low temporal dispersal could happen based on differences in dispersal abilities of cladocerans or rates of dispersal. Dispersing through space is a more random process than hatching from the egg bank. Similarly, it might take more time to "hitch a ride" than to hatch out of the egg bank.

Interaction between spatial and temporal dispersal

The influence of one mode of dispersal on cladoceran abundance and species composition depended on how rapidly the other mode of dispersal was occurring. The interactive effect of spatial and temporal dispersal on cladoceran abundance was lower than expected if the effects were independent. Increasing spatial dispersal had less of an impact on cladoceran abundance and community composition when temporal dispersal was high compared to when temporal dispersal was low and this can be attributed to the presence of an egg bank. Based on our findings when an egg bank is present communities will develop similarly regardless of space, since mesocosms did not differ largely in abundances, species composition, or variability in species composition. Mesocosms with high temporal dispersal also had the lowest mean Bray-Curtis dissimilarity scores based on both abundance (~ 40%) and occurrence (~ 30%). Thus, spatial dispersal has an important effect on abundance and composition when temporal dispersal is low but becomes a weaker force when an egg bank is present (Mergeay et al. 2007, Gray & Arnott 2011). However, there could be instances when an egg bank would not be present, such as newly formed lakes or ponds, where spatial dispersal from nearby water-bodies would be essential to provide colonists. Similarly, an egg bank might not be able to provide colonists that can persist if the environment has changed in some drastic way, like with lake acidification. In this scenario, depletion of the egg bank could also occur, if species were hatching out and dying because of higher pH levels (Gray & Arnott 2011). Yet, if these eggs can persist and hatch at a later time when the pH levels have recovered, the importance of the egg bank in providing colonists returns. Consequently, the particular study system needs to be taken into consideration when analyzing the importance of one form of dispersal versus the other, but

in general if a healthy egg bank is present it will provide colonists and influence the strength of overland spatial dispersal.

Increasing temporal dispersal had more of an impact on cladoceran abundance when spatial dispersal was low, than when spatial dispersal was high. This seems to indicate that when spatial dispersal is high it weakens the effect of temporal dispersal. However, mesocosms close to the source pond with an egg bank were more similar in their species composition to mesocosms far from the source pond with an egg bank than they were to mesocosms close to the source pond with low temporal dispersal. These comparisons indicate that species are able to hatch out of the egg bank and affect community composition even when they are close to a source pond. Furthermore, if high spatial dispersal was affecting mesocosms similarly and temporal dispersal was not having an impact we would expect to see homogenization of the communities based on the high amount of spatial dispersal from the source pond (Schamp et al. 2015). We did not see homogenization of communities near the source pond; instead we saw differences in species composition and variability in composition based on occurrence.

While spatial dispersal did affect mesocosms that had low spatial dispersal and were close to the source pond, spatial dispersal had a weaker effect when there was high temporal dispersal present. As mentioned previously, more variability in the mesocosms close to the source pond and with low temporal dispersal could happen based on differences in dispersal abilities of cladocerans or rates of dispersal, since it might take more time to "hitch a ride" and arrive than to hatch out of the egg bank. If it takes more time to disperse through space than to hatch out of the egg bank, a community that hatched out of the egg bank could have already been present by the time spatially dispersing cladocerans attempted to colonize. Direct comparisons of the different types of dispersal are difficult as researchers usually only consider one type of

dispersal and many do not monitor the exact arrival time of a species due to logistical constraints, rather colonization is inferred from the first sample date of the study. Gray and Arnott (2011) found millions of individuals had hatched out of the egg bank within their first sample date of emergence traps placed in lakes (10 days), compared to much lower spatial dispersal found in overland traps placed 10 meters from lakes (a total of 33 individuals found over the entire 110-day sample period). Thus, in our study the presence of an egg bank when close to the source pond likely impeded the establishment of spatially dispersing individuals in those mesocosms, as more time was needed for spatial dispersers to arrive. Evidence that the egg bank provided a resident community that likely inhibited spatially dispersing cladocerans can be found from a prior zooplankton invasibility study. Shurin (2000) found invasibility of zooplankton communities was higher when the resident zooplankton community was reduced. Thus in our experiment, when the resident community was reduced (low temporal dispersal) spatially dispersing cladocerans could have been invaded more easily than when a resident community was likely present (high temporal dispersal) leading to the differences we saw in composition.

Implications

Our results indicate that temporal and spatial dispersal and their interaction affect the development of pond communities in different ways. Therefore, changes in the rates of either form of dispersal could have important consequences for the zooplankton assemblage present in the pond. Freshwater habitats are becoming rapidly altered and are increasingly affected by habitat degradation, fragmentation, pollution, changes to flow, increases in water extraction, fisheries overexploitation, non-native species introductions, and climate change (Jenkins et al.

2003, Strayer & Dudgeon 2010, Carpenter et al. 2011, Pimm et al. 2014). Anthropogenic destruction of freshwater habitats increases isolation of ponds; cutting off a crucial supply of colonists from nearby ponds and forcing increased reliability on the egg bank. Recent evidence has shown that organic contaminants can accumulate in *Daphnia* resting eggs, which could affect fitness and sexual reproduction (Chiaia-Hernandez et al. 2013). Thus, along with infection, deep burial, and lack of hatching cues, contamination of the egg bank could negatively affect how well an isolated pond can recover from less spatial dispersal.

Increased drought due to climate change could not only adversely affect duration of standing water but also the egg bank as well (Dai 2013). If waterfowl or other dispersers visit an area less due to drought there could be less spatial dispersal of cladocerans, especially long distance dispersal of dormant eggs (Figuerola et al. 2005). The egg bank will be more exposed with drought and if it is not covered with vegetation there could be more dispersal away from the egg bank, which depending on the amount of eggs dispersed and the duration of the drought could negatively impact recruitment when water is restored (Vanschoenwinkel et al. 2008, Tuytens et al. 2014). Ryan et al. (2014) found climate risk is especially high for short interval hydroperiod ponds that contain many unique species not found in other longer hydroperiod ponds or lakes. Thus, conservation efforts should consider how changes to ponds could affect both temporal and spatial dispersal and how those alterations will impact local and regional diversity, as well as restoration and management decisions (Abell et al. 2007, Lemmens et al. 2013).

Conclusions

While there is much interest in zooplankton dispersal, controversies persist surrounding the rates and distances of dispersal and the effect of dispersal on zooplankton community

structure (Havel & Shurin 2004). Our study demonstrates the importance of the individual effects of temporal and spatial dispersal and the interaction between temporal and spatial dispersal on cladoceran communities. Specifically, spatial and temporal dispersal had a strong effect when the other form of dispersal was low. Temporal dispersal also homogenized communities, even with increased spatial dispersal. When an egg bank was present communities were also less variable, compared to more variability in communities when no egg bank was present. Based on our findings on the effects of temporal dispersal, aquatic communities may rely heavily on the egg bank for recruitment of individuals. Future research should investigate potential negative impacts on the egg bank and how they affect community structure, such as infection, contamination, predation, wind dispersal depletions based on climate change, and redistribution of sediments that affect burial as these may drastically affect whether isolated systems can successfully recover when less organisms are temporally and spatially dispersing into the system. These implications do not only apply to pond systems, as plants that produce dormant seed banks may also find a strong effect of temporal dispersal and reliance upon the seed bank when spatial dispersal is low, especially with increased habitat fragmentation in terrestrial habitats. The seed bank may also be similarly affected by infection, contamination, and predation that can affect community structure, and indeed some studies already suggest these findings (Maron & Simms 1997, Dalling et al. 1998, Dalling et al. 2011). Thus, a better understanding is needed of how temporal dispersal can influence community persistence in the face of increased isolation and lower spatial dispersal, while also investigating aspects that could be simultaneously affecting recruitment from dormant reserves.

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Table 1. PERMANOVA results and planned contrasts based on three different dissimilarity indices Bray-Curtis Abundance (BCA), Bray-Curtis presence-absence (BCP), and Raup-Crick (RC). RC results for time were reported as negative value for the Pseudo-F and p-value in PRIMER-E, and are considered non-significant per communication with MJ Anderson, denoted as n.s. Unadjusted p-values were generated and then adjusted for using the FDR (p_{FDR}) for planned contrasts. Planned contrasts show the effect of spatial dispersal on cladoceran composition when temporal dispersal is low, the effect of temporal dispersal when spatial dispersal is low, the equivalence of spatial and temporal dispersal, and the expected cladoceran community composition if the effects of spatial and temporal dispersal were independent. Planned contrast abbreviations: HSLT = High Spatial, Low Temporal; HSHT = High Spatial, High Temporal; LSLT = Low Spatial, Low Temporal; LSHT = Low Spatial, High Temporal.

	BCA			BCP			RC		
Parameter	*df*	*F*	*P (perm)*	*df*	*F*	*P (perm)*	*df*	*F*	*P (perm)*
Treatment	3,39	3.83	0.001	3,39	4.04	0.005	3,39	4.39	0.005
Time	1,39	6.21	0.039	1,39	2.89	0.072	1,39	n.s.	n.s.
Treatment x Time	3,39	1.03	0.427	3,39	0.96	0.468	3,39	1.24	0.364
Planned Contrasts		*p*	p_{FDR}		*p*	p_{FDR}		*p*	p_{FDR}
Effect of spatial dispersal when temporal dispersal low HSLT vs LSLT		0.056	0.074		0.059	0.118		0.069	0.091
Effect of temporal dispersal when spatial dispersal low LSLT vs LSHT		0.047	0.074		0.234	0.234		0.319	0.319
Equivalence of spatial and temporal dispersal HSLT vs LSHT		0.171	0.171		0.044	0.118		0.043	0.091
Interdependence of spatial and temporal dispersal HSLT, HSHT vs LSLT, LSHT		0.032	0.074		0.088	0.118		0.053	0.091

Table 2. SIMPER results pertaining to the cladoceran species contributing the most to the compositional dissimilarity between pairs of treatments and through time based on Bray-Curtis Abundance (BCA), and Bray-Curtis presence-absence (BCP). Numbers in parentheses next to the names of the treatments or times being compared indicate the mean Bray-Curtis dissimilarity between the treatment or time pair. Mean occurrence and mean abundance columns indicate the proportion of mesocosms in a given treatment or time that contain the species and what species abundance levels are like if a species is present, respectively. Contribution indicates the percentage of dissimilarity index that is attributable to differences in the abundance / occurrence of the species. Total contribution of species is 100%, thus all species present in the treatments or during the time period being compared are listed even if they have a small contribution. Treatment abbreviations: HSLT = High Spatial, Low Temporal; HSHT = High Spatial, High Temporal; LSLT = Low Spatial, Low Temporal; LSHT = Low Spatial, High Temporal. Time abbreviations Aug. = August, Sept. = September.

BCA

HSLT vs. LSLT (76.57)

Species	Mean abundance HSLT	Mean abundance LSLT	Contribution
D. brachyurum	16.78	5.18	63.87
S. crystallina	0.67	2.43	13.47
K. latissima	2.56	0.00	9.88
S. mucronata	0.10	0.31	3.36
D. americana	0.00	0.90	2.53
C. laticaudata	0.22	0.28	2.35
B. longirostris	0.44	0.00	1.64
A. excisa	0.00	0.35	1.55
S. vetulus	0.22	0.00	0.86
C. dubia	0.00	0.10	0.48

LSLT vs. LSHT (66.70)

Species	Mean abundance LSLT	Mean abundance LSHT	Contribution
D. brachyurum	5.18	17.09	68.26
S. crystallina	2.43	4.66	22.22
S. mucronata	0.31	0.10	2.76
D. americana	0.90	0.00	2.73
A. excisa	0.35	0.00	1.56
C. laticaudata	0.28	0.00	1.35
S. exspinosus	0.00	0.20	0.63
C. dubia	0.10	0.00	0.48

BCP

HSLT vs LSLT (68.88)

Species	Mean occurrence HSLT	Mean occurrence LSLT	Contribution
D. brachyurum	1.00	0.60	26.81
S. crystallina	0.20	0.60	20.28
K. latissima	0.50	0.00	16.76
S. mucronata	0.10	0.20	9.96
B. longirostris	0.30	0.00	9.17
C. laticaudata	0.10	0.10	6.24
D. americana	0.00	0.10	2.88
A. excisa	0.00	0.10	2.88
S. vetulus	0.10	0.00	2.63
C. dubia	0.00	0.10	2.39

LSLT vs. LSHT (52.59)

Species	Mean occurrence LSLT	Mean occurrence LSHT	Contribution
D. brachyurum	0.60	0.90	34.92
S. crystallina	0.60	0.80	31.79
S. mucronata	0.20	0.10	14.56
S. exspinosus	0.00	0.10	4.26
D. americana	0.10	0.00	3.96
A. excisa	0.10	0.00	3.96
C. dubia	0.10	0.00	3.27
C. laticaudata	0.10	0.00	3.27

Table 2 continued

BCA

HSLT vs. LSHT (52.57)

Species	Mean abundance HSLT	Mean abundance LSHT	Contribution
D. brachyurum	16.78	17.09	62.68
S. crystallina	0.67	4.66	21.79
K. latissima	2.56	0.00	10.15
B. longirostris	0.44	0.00	1.76
C. laticaudata	0.22	0.00	1.15
S. vetulus	0.22	0.00	0.92
S. mucronata	0.10	0.10	0.88
S. exspinosus	0.00	0.20	0.66

HSHT vs. LSHT (38.29)

Species	Mean abundance HSHT	Mean abundance LSHT	Contribution
D. brachyurum	13.58	17.09	64.52
S. crystallina	6.11	4.66	22.43
K. latissima	1.85	0.00	9.14
S. mucronata	0.20	0.10	1.89
S. exspinosus	0.14	0.20	1.54
S. serrulatus	0.10	0.00	0.47

HSHT vs. HSLT (58.03)

Species	Mean abundance HSHT	Mean abundance HSLT	Contribution
D. brachyurum	13.58	16.78	53.91
S. crystallina	6.11	0.67	27.79
K. latissima	1.85	2.56	12.62
B. longirostris	0.00	0.44	1.60
S. mucronata	0.20	0.10	1.31
C. laticaudata	0.00	0.22	1.04
S. vetulus	0.00	0.22	0.84
S. exspinosus	0.14	0.00	0.55
S. serrulatus	0.10	0.00	0.33

BCP

HSLT vs. LSHT (47.49)

Species	Mean occurrence HSLT	Mean occurrence LSHT	Contribution
S. crystallina	0.20	0.80	38.08
K. latissima	0.50	0.00	22.25
B. longirostris	0.30	0.00	12.28
D. brachyurum	1.00	0.90	7.26
S. mucronata	0.10	0.10	6.74
C. laticaudata	0.10	0.00	5.58
S. exspinosus	0.00	0.10	4.18
S. vetulus	0.10	0.00	3.62

HSHT vs. LSHT (27.20)

Species	Mean occurrence HSHT	Mean occurrence LSHT	Contribution
K. latissima	0.30	0.00	22.98
S. crystallina	1.00	0.80	22.06
D. brachyurum	0.90	0.90	20.83
S. mucronata	0.10	0.10	13.24
S. exspinosus	0.10	0.10	13.24
S. serrulatus	0.10	0.00	7.66

HSHT vs. HSLT (49.48)

Species	Mean occurrence HSHT	Mean occurrence HSLT	Contribution
S. crystallina	1.00	0.20	39.79
K. latissima	0.30	0.50	20.75
B. longirostris	0.00	0.30	10.48
D. brachyurum	0.90	1.00	6.97
S. mucronata	0.10	0.10	6.22
C. laticaudata	0.00	0.10	4.61
S. serrulatus	0.10	0.00	4.01
S. exspinosus	0.10	0.00	4.01
S. vetulus	0.00	0.10	3.15

Table 2 continued

	BCA			BCP			
	Aug. vs. Sept. (61.08)				Aug. vs. Sept. (47.65)		
Species	Mean abundance Aug	Mean abundance Sept	Contribution	Species	Mean occurrence Aug	Mean occurrence Sept	Contribution
D. brachyurum	15.73	10.58	63.58	*S. crystallina*	0.70	0.60	27.04
S. crystallina	3.05	3.88	18.56	*D. brachyurum*	0.95	0.75	20.12
K. latissima	1.90	0.31	7.77	*K. latissima*	0.25	0.15	14.84
S. mucronata	0.27	0.09	3.27	*S. mucronata*	0.20	0.05	12.35
D. americana	0.45	0.00	1.80	*B. longirostris*	0.15	0.00	6.56
C. laticaudata	0.25	0.00	1.69	*C. laticaudata*	0.10	0.00	4.96
B. longirostris	0.22	0.00	0.92	*S. exspinosus*	0.05	0.05	4.11
A. excisa	0.00	0.17	0.85	*D. americana*	0.05	0.00	2.33
S. exspinosus	0.07	0.10	0.55	*S. serrulatus*	0.00	0.05	1.95
S. vetulus	0.11	0.00	0.49	*A. excisa*	0.00	0.05	1.95
C. dubia	0.05	0.00	0.37	*C. dubia*	0.05	0.00	1.90
S. serrulatus	0.00	0.05	0.15	*S. vetulus*	0.05	0.00	1.90

Table 3. PERMDISP results and pairwise comparisons based on three different dissimilarity indices Bray-Curtis Abundance (BCA), Bray-Curtis presence-absence (BCP), and Raup-Crick (RC). Unadjusted p-values were generated and then adjusted for using the FDR (p_{FDR}) for pairwise comparisons. Pairwise comparisons abbreviations: HSLT = High Spatial, Low Temporal; HSHT = High Spatial, High Temporal; LSLT = Low Spatial, Low Temporal; LSHT = Low Spatial, High Temporal.

	BCA			BCP			RC		
Parameter	*df*	*F*	*P (perm)*	*df*	*F*	*P (perm)*	*df*	*F*	*P (perm)*
Treatment	3,36	7.2	0.010	3,36	8.23	<.001	3,36	6.15	0.004
Time	1,38	17.2	0.002	1,38	0.005	0.961	1,38	0.22	0.715
Pairwise Comparison		*p*	p_{FDR}		*p*	p_{FDR}		*p*	p_{FDR}
HSLT vs LSLT		0.206	0.247		0.049	0.075		0.076	0.115
LSLT vs LSHT		0.011	0.033		0.006	0.018		0.005	0.019
HSLT vs LSHT		0.104	0.185		0.063	0.075		0.076	0.115
HSHT vs HSLT		0.123	0.185		0.061	0.075		0.120	0.144
HSHT vs LSHT		0.629	0.629		0.820	0.820		0.541	0.541
HSHT vs LSLT		0.004	0.023		0.004	0.018		0.006	0.019

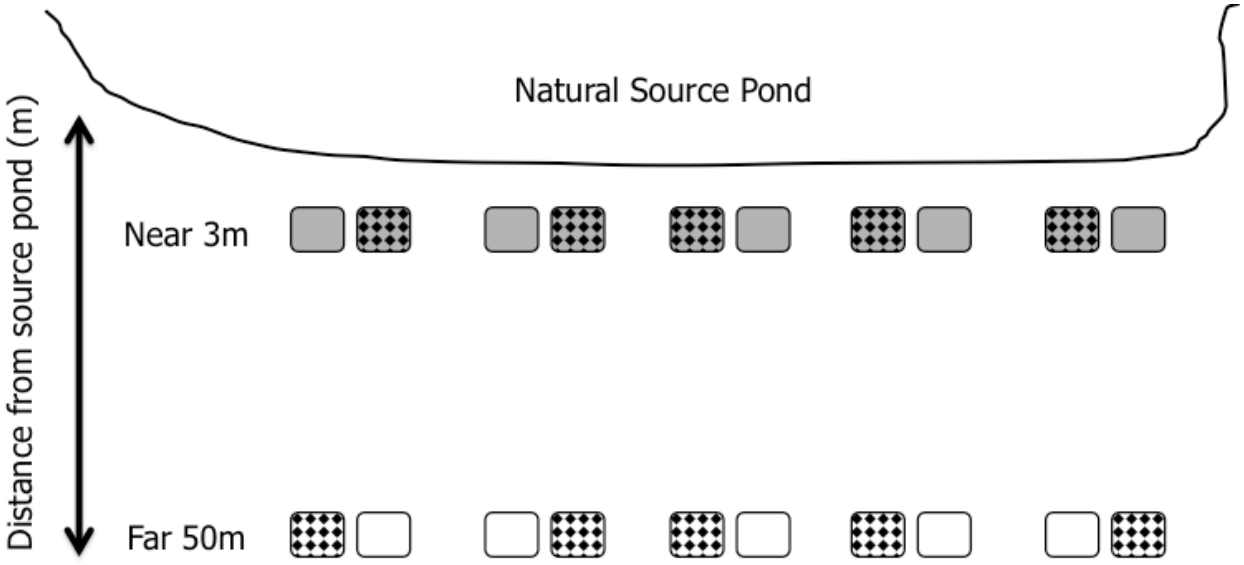

Figure 1. Experimental design setup. Mesocosms near a source pond (gray rectangles) experienced higher rates of spatial dispersal (3m) than mesocosms far from the pond (white rectangles – 50m). Temporal dispersal from an egg bank was either prevented (open rectangle) or allowed (dotted rectangle) with the use of sediment bags that differed in their permeability. Each of these treatments was replicated once within each of five spatial blocks.

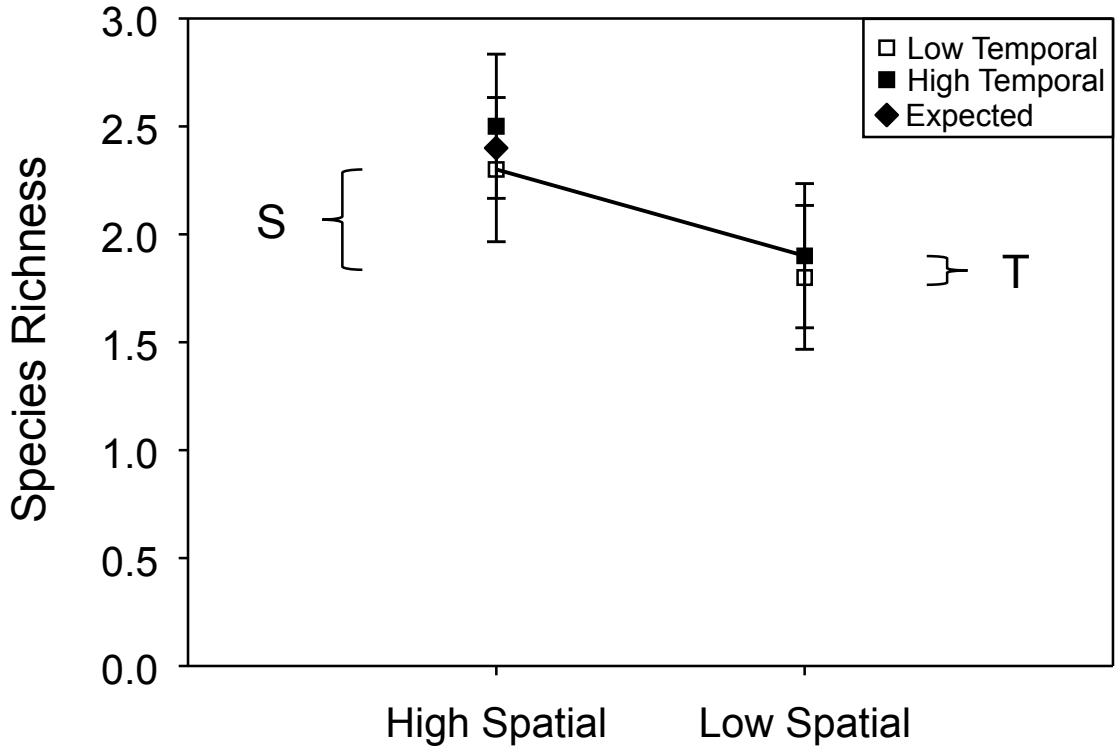

Figure 2. The effect of spatial dispersal on cladoceran species richness (High Spatial, Low Temporal vs. Low Spatial, Low Temporal; depicted by the S), the effect of temporal dispersal (Low Spatial, Low Temporal vs. Low Spatial, High Temporal, depicted by the T), the equivalence of spatial and temporal dispersal (High spatial, Low Temporal vs. Low Spatial, High Temporal, depicted with the line connecting the treatments), and the expected abundance if the effects of spatial and temporal dispersal were independent (depicted with the diamond) (LS-mean ± SE).

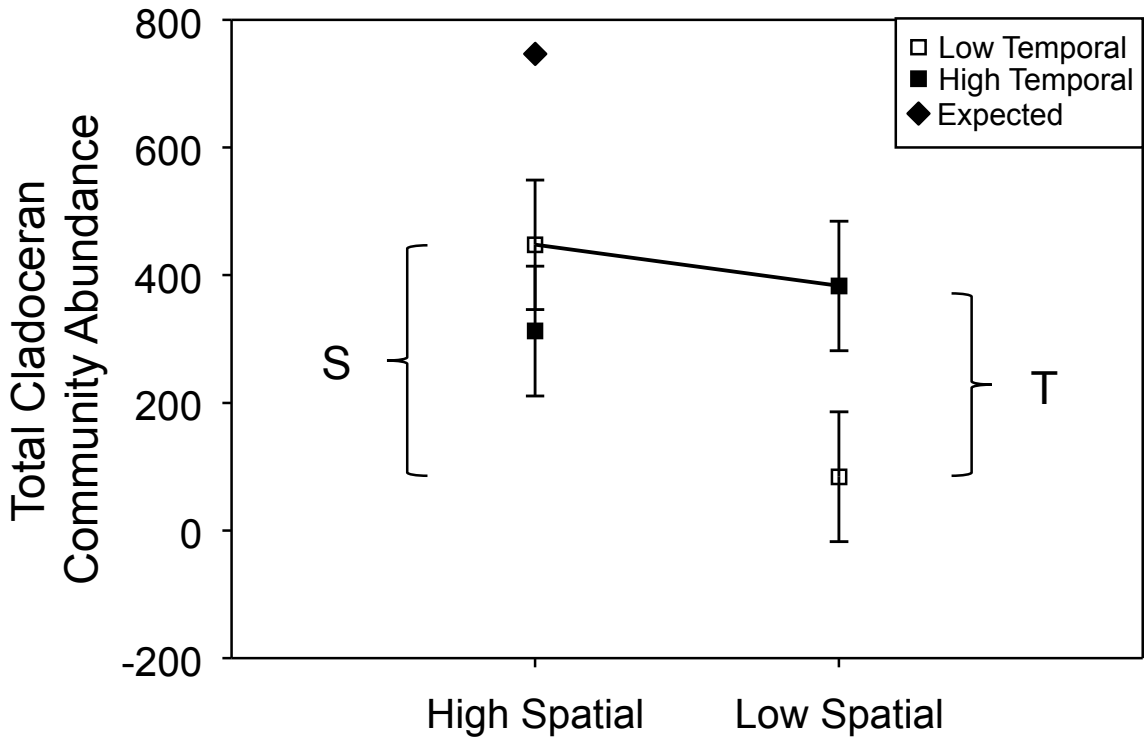

Figure 3. The effect of spatial dispersal on total cladoceran community abundance (High Spatial, Low Temporal vs. Low Spatial, Low Temporal; depicted by the S), the effect of temporal dispersal (Low Spatial, Low Temporal vs. Low Spatial, High Temporal, depicted by the T), the equivalence of spatial and temporal dispersal (High spatial, Low Temporal vs. Low Spatial, High Temporal, depicted with the line connecting the treatments), and the expected abundance if the effects of spatial and temporal dispersal were independent (depicted with the diamond) (LS-mean ± SE).

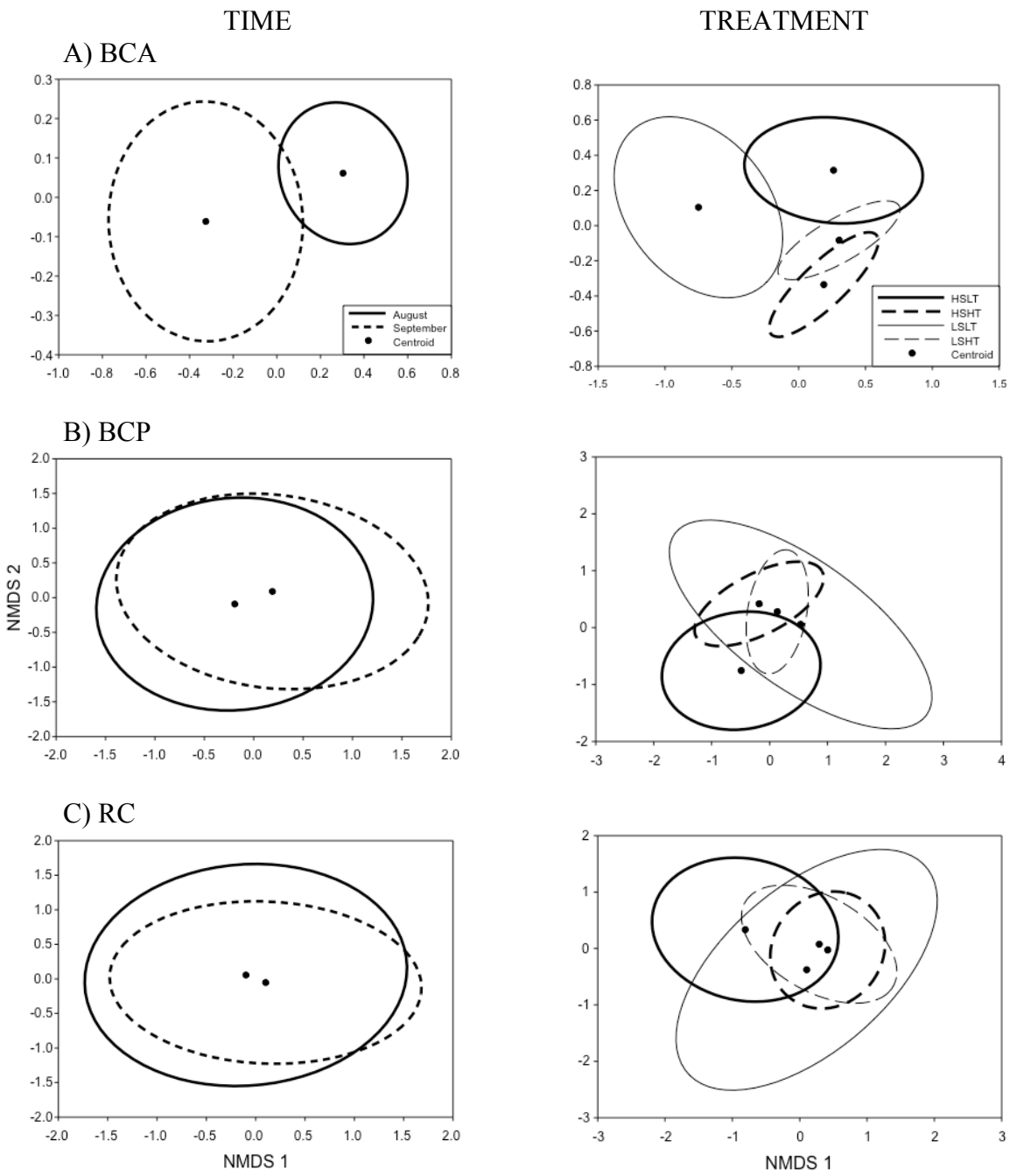

Figure 4. Nonmetric multidimensional scaling (NMDS) ordination plots of community composition based on three different dissimilarity indices A) Bray-Curtis abundance (BCA), B) Bray-Curtis presence-absence (BCP), and C) Raup-Crick (RC). Ellipses represent 95% confidence intervals surrounding the data by month (left) and treatment (right) with filled circles representing group centroids. Months denoted as solid circle = August and dashed circle = September. Treatments denoted as solid bold circle: HSLT = High Spatial, Low Temporal, dashed bold circle: HSHT = High Spatial, High Temporal, solid circle: LSLT = Low Spatial, Low Temporal, dashed circle: LSHT = Low Spatial, High Temporal.

Supplement 1A. Cladoceran species sampled from mesocosms throughout the experiment (August & September 2011), from the borrow pit source pond and other ponds in the study area (September 2011), from the pond where the egg bank was collected in the Croatan National Forest (WT=Watertower pond samples), and from the egg bank placed in mesocosms. The eggs found in the egg bank from WT could only be identified to genus. The WT sample was from multiple samples (samples collected bimonthly from September 2012 - September 2013) taken for another study that was conducted after this experiment, but we have included this information as a reference as to the potential species that could have been in the WT egg bank at the time of this experiment.

		Spatial		Temporal	
Zooplankton Species	Mesocosm samples	Borrow Pit	Other ponds	WT sample	WT egg bank
Acantholeberis curvirostris				x	
Acroperus harpae		x		x	
Alona intermedia				x	
Alona rustica				x	
Alona setulosa				x	
Alonella excisa	x			x	
Alonella exigua				x	
Camptocercus sp.				x	
Bosmina longirostris	x	x	x		
Ceriodaphnia dubia	x			x	*
Ceriodaphnia laticaudata	x		x		
Ceriodaphnia reticulata		x			
Chydorus sphaericus			x	x	
Daphnia laevis		x		x	
Diaphanosoma brachyurum	x	x	x	x	
Disparalona hamata				x	
Dunhevidia americana	x			x	
Ephemeroporus barroisi				x	
Ephemeroporus hybridus				x	
Ephemeroporus tridentatus				x	
Ilyocryptus spinifer				x	
Kurzia latissima	x	x	x	x	
Latonopsis occidentalis				x	
Macrothrix elegans			x		
Moina macrocopa				x	
Moinodaphnia macleayii			x		
Pseudochydorus globosus				x	
Pseudosida bidentata				x	
Scapholeberis mucronata	x	x	x	x	
Sida crystallina	x		x		
Simocephalus expinosus	x	x		x	*
Simocephalus serrulatus	x			x	*
Simocephalus vetulus	x			x	*
Streblocercus serricaudatus				x	

Supplement 1B. Planned contrasts used to compare the individual and interactive effects of spatial and temporal dispersal on cladoceran communities for both the mixed model (PROC MIXED) and multivariate (PERMANOVA) tests.

Contrast	High Spatial (HS) Low Temporal (LT)	High Spatial (HS) High Temporal (HT)	Low Spatial (LS) Low Temporal (LT)	Low Spatial (LS) High Temporal (HT)
1. What is the effect of spatial dispersal when temporal dispersal is low?	1	0	-1	0
2. What is the effect of the temporal dispersal when spatial dispersal is low?	0	0	1	-1
3. Does increasing spatial and temporal dispersal have the same effect?	1	0	0	-1
4. Does the effect of spatial dispersal depend on temporal dispersal?	1	-1	-1	1

CHAPTER 2: How do differences in species hatching phenology and the presence of predators affect temporary pond systems?

Introduction

A growing amount of evidence indicates that both the order in which species enter into a community and the process of predation can play an important role in controlling community dynamics (Louette & De Meester 2007, Chase et al. 2009). One way many organisms differ in their order of arrival into a community is through intra- and inter-specific differences in hatching phenology (Touchon et al. 2006, Altiero et al. 2010, Vanschoenwinkel et al. 2010, Warkentin 2011). If a species hatches out early in the season and grows quickly to reach a larger size, reaches higher population densities, or alters the environment in some way then it might have an advantage over later hatching individuals (Hernandez & Chalcraft 2012, Amoroso & Chalcraft 2015, Rasmussen et al. 2014, Anderson et al. 2015, Rasmussen & Ruldolf 2015). If a species possessing certain key traits is absent from the community because it arrives late and cannot persist based on the reasons described above, these traits would be missing from the community and could change the trajectory of how the community develops. Thus, differences in species hatching phenologies can have important effects on the outcome of community assembly. Furthermore, differences in the order of arrival of species might also affect ecosystem function of those communities (Zhang & Zhang 2007, Kröner et. al 2008, Fukami et al. 2010). This can happen, if for example, abiotic factors are significantly changed by the activity of species as they arrive, where early arriving species might impact not only the establishment and growth of late arriving species but also higher ecosystem properties, such as primary production (Chase 2003, Dickie et al. 2012).

Predation can also have a strong impact on communities (Morin 1984, Price & Morin 2004, Shurin 2001). There is evidence that the presence of predators can change the impact of colonization order and priority effects (Louette & De Meester 2007, Hoverman & Relyea 2008, Jiang et al. 2011) as well as where prey decide to colonize (Resetarits 2001, Forsman et al. 2001, Resetarits & Binckley 2009). If predators themselves vary in their arrival time or the balance between predators and prey shift through ontogeny, predators can also impact prey colonization and long-term community dynamics (Price & Morin 2004, Olito & Fukami 2009, Stier et al. 2013, Rasmussen et al. 2014, Amoroso & Chalcraft 2015, Nosaka et al. 2015).

Although there is some evidence of the effects of hatching phenology and predation on communities, more studies are needed across a variety of study systems and study organisms that investigate the impact of how differences in hatching phenology, the presence of predation, and their interaction affect resources and growth rates of later arriving species (Rasmussen et al. 2014). Accordingly, temporary pond systems provide an excellent opportunity to test these impacts due to the inhabitants exhibiting differences in hatching phenology as well as predation being an important determinant of species composition in temporary ponds (Welborn et al. 1996, Stoks & McPeek 2003). While most work on hatching phenology and predation in temporary pond systems has focused on amphibians (Morin et al. 1990, Sredl & Collins 1991, Boone et al. 2002, Urban 2007, Nosaka et al. 2015) and odonates (Morin 1984, Padeffke & Suhling 2003, Rasmussen et al. 2014), there is little work that investigates the effects of hatching phenology and predation on zooplankton (but see Louette & De Meester 2007).

Zooplankton from temporary ponds, such as large branchiopods (fairy shrimp and clam shrimp) and cladocerans, produce dormant eggs and differ in hatching phenology (Jocque et al. 2010, Vanschoenwinkel et al. 2010, McCarthy pers. obs.). In general, fairy shrimp eggs hatch

quickly after a pond fills and may monopolize resources immediately, thus negatively affecting clam shrimp and cladocerans that take more time to arrive (Jocque et al. 2010, Waterkeyn et al. 2011). Variation in hatching order has been documented for various species, where they differ in their sensitivity to cues, such as temperature or photoperiod, that initiate hatching (Cáceres 1998, Cáceres & Tessier 2003, Gyllström & Hansson 2004). However, further changes to hatching order could occur if dormant eggs miss a hatching cue during a particular season, causing them to fail to terminate dormancy. Dormant eggs can also fail to hatch because of burial depth, infection, contamination, or by predation of the dormant egg (Cáceres & Hairston 1998, De Stasio 2007).

The impact of hatching order by temporally dispersed taxa may be reduced in the presence of predation if predators preferentially consume large branchiopods that enter ponds early (Kneitel & Chase 2004). Flying insect predators, particularly backswimmers, increase in abundance after pond filling, and can readily consume large branchiopods and cladocerans (Murdoch & Scott 1984, Woodward & Kiesecker 1994, Welborn et al. 1996, Brendonck et al. 2002). We hypothesized the larger and more efficient filter feeding branchiopods would have negative impacts on cladoceran growth because they would limit access of the smaller less efficient cladocerans to resources. When predators are present, however, we predict that the better competitors (fairy shrimp and clam shrimp) would be negatively affected by predation, whereas the poor competitors (cladocerans) would be less vulnerable to predation. The cladocerans would be freed from competition as the more vulnerable branchiopods would be preferentially consumed by the backswimmers. We also hypothesized that algal resources would decline in the presence of large branchiopods and cladocerans and that predation would cause an increase in algal resources due to lower consumption by large branchiopods and cladocerans.

Methods

We examined how predators affected the influence of zooplankton hatching phenology in mesocosms designed to simulate freshwater temporary ponds, by manipulating the sequence in which fairy shrimp (*Streptocephalus seali*), clam shrimp (*Eulimnadia astraova*), and a cladoceran (*Daphnia laevis*) arrived to mesocosms that varied in the occurrence of the predatory backswimmer (*Notonecta irrorata*). Treatments consisted of the following hatching phenologies, with zooplankton species listed in their order of arrival: A) fairy shrimp + clam shrimp + cladocerans, B) clam shrimp + cladocerans, C) fairy shrimp + cladocerans, D) fairy shrimp + clam shrimp, E) fairy shrimp only, F) clam shrimp only, and G) cladocerans only (Fig. 5). Each of the hatching phenology treatments (A-G) was also repeated with predators. The inoculation orders were chosen based on hatching phenologies that are most likely to occur in nature. The rationale for the hatching phenologies include: A) is the hatching order that occurs when all species manage to hatch, B-C) fairy shrimp and clam shrimp fail to hatch potentially due to missed hatching cue, D) cladocerans fail to hatch potentially due to pond drying, E-F) fairy shrimp alone and clam shrimp alone, as are the only species that manage to hatch due to infection or contamination of the others species dormant eggs G) both large branchiopods fail to hatch due to predation of dormant eggs. While absolute failure of a species to hatch is unlikely, low levels of hatching ability have been found (6 – 50% hatching fraction of *Daphnia pulicaria* among lakes, Cáceres & Tessier 2003), and the scenarios for failure to hatch (missed hatching cue, pond drying, predation/infection of dormant eggs) have been found to occur in natural systems (Cáceres & Hairston Jr. 1998, Gyllström & Hansson 2004, De Stasio 2007, Mergeay et al. 2007), such that we believe our treatments are realistic of what could occur in natural systems. The scenarios for failure to hatch are proposed to affect specific species in the different

treatments for this experiment, but all of the species could be affected by missed hatching cue, pond drying, and predation/infection of dormant eggs in theory.

Mesocosms consisted of 150-L cattle watering tanks and were filled with approximately 130-L of well water. Mesocosms also had a tight fitting mesh lid to keep unwanted colonizers from entering, while retaining the predatory backswimmers capable of flight. All mesocosms were inoculated with 500 mL of phytoplankton from Croatan National Forest ponds to serve as a resource base for the experimental organisms. The phytoplankton inoculum was filtered with fine mesh before it was added to each mesocosm to ensure zooplankton were not added. Leaf litter (300 g of pine straw and 25 g of turkey oak leaves) was added to each mesocosm on 5 September to provide a source of nutrients for the pond food web and to provide a more natural benthic habitat for prey or predators to use. Experimental organisms were collected from temporary ponds in the Croatan National Forest. Ten fairy shrimp, five clam shrimp, 40 cladocerans, and three total backswimmers were added to the assigned treatments that were slated to contain these organisms. The addition of organisms started on day 1 (8 September) with fairy shrimp (*S. seali*) and continued on day 5 with clam shrimp (*E. astraova*) and day 15 with cladocerans (*D. laevis*). One predator was added on days 22, 29, and 36 for a total of three predators, which simulated an increase in predator abundance found in natural temporary pond systems. Mesocosms were arranged in four spatial blocks with 14 mesocosms, where each treatment was randomly assigned to one mesocosm within each block. Logistical limitations prevented us from replicating each treatment the same number of times. The following treatments were replicated three times: clam shrimp with predators, cladocerans with predators, clam shrimp + cladocerans with predators, and an algae only control. All other treatments were

replicated four times. Blocking allowed us to minimize unexplained environmental differences among treatments within a block (Wilbur 1997).

To assess algal resource levels, periphyton and phytoplankton samples were taken at three times throughout the experiment. The samples were taken before cladocerans were added to see how large branchiopods may have affected resource levels (Day 15), at the middle of the experiment after all study organisms and all predators were added to see how hatching phenology affected resource levels and if predators might have indirectly influenced resource levels (Day 42), and on the last day of the experiment to see if any effects hatching phenology and predators had on algal resources persisted through time (Day 63). Periphytometers were added to each mesocosm and consisted of PVC pipe tied with approximately 30 cm of flagging tape, to allow for periphyton growth on the tape. For periphyton analyses, a measured amount of flagging tape was carefully cut from each mesocosm (6 cm per sample) and placed in deionized water on ice. For phytoplankton, a 550 mL water sample was removed from each mesocosm and placed on ice. Samples were brought back to the lab and prepped for chlorophyll a (chl a) analyses. Periphyton was scraped from the 6 cm portion of flagging tape. Periphyton and phytoplankton samples were then filtered separately through Whatman GF/C filters, the filters were frozen, homogenized in 90% acetone, and chl a concentrations were determined using the phaeophytin-corrected spectrophotometric analysis (APHA 1995).

To assess cladoceran growth through time, cladoceran samples were taken every 7 days from mesocosms containing cladocerans and started on day 21 continuing through day 63. Samples were taken every 7 days, since the age at first reproduction occurs between 4 - 7 days depending on temperature and food conditions (Foran 1986). A PVC tube sampler (.8 m long, 3.81 cm diameter) was used to take samples at multiple, randomly selected locations within each

mesocosm. These sub-samples were combined into a single sample totaling 5 L and filtered with fine mesh (64 um). The samples were filtered over the mesocosms to retain water and were preserved in >70% ethanol for later enumeration. Only cladocerans were preserved, if fairy shrimp or clam shrimp were collected in the sample they were returned back to the mesocosm. The PVC tube sampler, graduated cylinder, and filter were thoroughly rinsed with well water after each sample to avoid moving cladocerans between mesocosms. The experiment ended on day 63, where all surviving fairy shrimp, clam shrimp, and predators were collected.

All cladocerans were counted from each sample for population growth measurements based on abundance (see statistical analyses). A subset of cladocerans collected on 19 October (after all species had been added to mesocosms) and 9 November (the last sample) were photographed for length measurements. When possible 50 randomly chosen cladoceran individuals from each mesocosm were photographed and lengths (mm) were obtained using ImageJ. We used our length estimates along with length-weight regressions from McCauley (1984) to calculate estimates of dry weight for an averaged sized individual in each mesocosm. Average dry weight was calculated for each mesocosm and then multiplied by density of individuals (obtained from abundance samples from 19 October and 9 November) to convert to total biomass of cladocerans (μg/L).

Statistical analyses

We calculated the per capita growth rate (r) of cladocerans from each of the mesocosms that had cladocerans present and calculations of r were based on our weekly samples of cladoceran abundance. We used the following equation to solve for the per capita growth rate, r = ln (N_{t+x} / N_t) / x, where x = the amount of time between population size estimates, N_t =

population size at the start of the time interval, and N_{t+x} = population size at the end of the time interval. Regression analyses were performed in Excel for each mesocosm to evaluate how r changed with population size, where the y-intercept from the regression represented the maximum intrinsic rate of growth (r_{max}), the x-intercept represented the carrying capacity, and the slope represented the strength of density dependence.

All statistical analyses were performed in SAS version 9.4 (SAS Institute 2013). We performed a factorial ANOVA, using PROC MIXED, to evaluate how hatching phenology, predation, and the interaction between hatching phenology and predation affected the following response variables: r_{max}, K, and the slope. Neither hatching phenology, predation, nor the interaction between hatching phenology and predation appeared to produce significant variation in either slope or K. Consequently, we focus our efforts here on the analysis of r_{max} for simplicity. Blocks were treated as a random factor; hatching phenology and predation were treated as fixed factors. Pairwise differences in r_{max} among treatments was assessed via Fisher's LSD. We report both unadjusted p-values from Fisher's LSD and p-values that were adjusted (p_{FDR}) using PROC MULTEST to control the False Discovery Rate (FDR; Verhoeven et. al 2005).

We used a repeated-measures factorial ANOVA to assess how hatching phenology, predation, and the interaction between hatching phenology and predation through time affected cladoceran length and total biomass estimates. Blocks were treated as a random factor, hatching phenology and predation were treated as fixed factor, and time as a repeated factor. Pairwise differences in cladoceran length among treatments as well as pairwise differences in total biomass among treatments was assessed via Fisher's LSD. We report both unadjusted p-values

from Fisher's LSD and p-values that were adjusted (p_{FDR}) using PROC MULTEST to control the FDR (Verhoeven et. al 2005).

The analysis of algal resources required consideration of the fact that treatment identities for some mesocosms changed through time based on when organisms were added. For example, mesocosms designated to only contain cladocerans did not have any zooplankton present on the first algal sampling date and were effectively an "algae" treatment on the first algal sampling date but a "cladoceran" treatment on the second algal sampling date. Consequently, we considered each algal sample date separately: 22 September- before cladocerans were added (Day 15), 19 October - after all study organisms and all predators were added (Day 42), and 9 November - on the last day of the experiment (Day 63).

We used information on the amount of algae present in each mesocosm to quantify how much zooplankton grazing affected algal abundance. We did this for mesocosms containing zooplankton by subtracting the chl a abundance in the mesocosm from the average chl a estimate for all mesocosms lacking zooplankton (algae only controls) during that sample period. PROC MIXED was used to evaluate how hatching phenology and predation affected chl a abundance of both phytoplankton and periphyton. Hatching phenology and predation were treated as fixed factors. Planned contrasts were performed separately for each sample date and therefore change with time based on which organisms were present. Our contrasts evaluated two broad categories, whether zooplankton species affected algal resources differently and whether single species affected algal resources differently than multiple species (see Supplement 2A for a list of specific contrasts under each broad category). P-values for each contrast that pertained to each question were adjusted to control the FDR (Verhoeven et. al 2005) by using PROC MULTTEST. Both unadjusted and p_{FDR} adjusted p-values are reported.

Results

Neither hatching phenology ($F_{3, 18} = 0.55$, $p = 0.652$) nor predation ($F_{1, 18} = 0.25$, $p = 0.620$) affected cladoceran population growth (r_{max}), but there was a trend for the effect of hatching phenology to depend on whether predators were present ($F_{3, 18} = 2.63$, $p = 0.081$; Fig. 6). This trend was mainly driven by the presence of clam shrimp slowing cladoceran population growth when predators were present compared to when no predators were present. The presence of clam shrimp reduced cladoceran population growth by 0.128 (±1 SE = 0.053) when predators were present, although this effect was not statistically different from zero after controlling the FDR ($p = 0.028$, $p_{FDR} = 0.385$; Fig. 6). In the absence of predators, the presence of clam shrimp had little effect on cladoceran population growth.

Cladoceran body length increased by 0.211 mm (±1 SE = 0.034) between October and November ($F_{1, 39} = 38.04$, $p = <.0001$; Fig. 7A). Hatching phenology did not affect cladoceran body length ($F_{3, 39} = 0.29$, $p = 0.831$) but cladocerans became smaller by 0.09 mm (±1 SE = 0.034) when predators were present ($F_{1, 39} = 6.48$, $p = 0.015$; Fig. 7B). There was a trend for an interaction between hatching phenology and predation ($F_{3, 39} = 2.35$, $p=0.087$), where predators seemed to have a stronger effect on cladoceran length when cladocerans were alone ($p=0.004$, $p_{FDR}=0.088$) than when cladocerans were with large branchiopods (Fig. 7C).

Although total cladoceran biomass increased with time between October and November by 31.98 µg/L (± 1SE = 56.64) the effect was not statistically different from zero ($F_{1, 39} = 0.32$, $p = 0.576$). Similarly to cladoceran body length, hatching phenology did not affect total cladoceran biomass ($F_{3, 39} = 1.66$, $p = 0.192$). The presence of predators had an effect on total cladoceran biomass ($F_{1, 39} = 5.88$, $p = 0.020$), with the total cladoceran biomass becoming smaller when predators were present by 137.43 µg/L (±1 SE = 56.70; Fig. 8). There was no

significant interaction between hatching phenology and predation on total cladoceran biomass ($F_{3, 39} = 0.65$, $p = 0.587$).

Predators did not affect the extent of grazing on phytoplankton by zooplankton (October – $F_{1, 39} = 0.92$, $p = 0.343$, November – $F_{1, 39} = 0.01$, $p = 0.907$) and the effect of hatching phenology on zooplankton grazing did not depend on the presence of predators (October – $F_{6, 39} = 1.07$, $p = 0.395$, November – $F_{6, 39} = 0.21$, $p = 0.971$). The extent of grazing by zooplankton on phytoplankton depended on the hatching phenology of zooplankton for each sampling date (September – $F_{2, 43} = 4.68$, $p = 0.015$, October – $F_{6, 39} = 3.04$, $p = 0.015$, November – $F_{6, 39} = 3.19$, $p = 0.012$; Fig. 9). Initially, in September all large branchiopods reduced phytoplankton abundance, both when separate and when together. Clam shrimp reduced phytoplankton abundance less than fairy shrimp ($p=0.013$, $p_{FDR}=0.013$) and less than fairy shrimp + clam shrimp ($p=0.009$, $p_{FDR}=0.017$; Fig. 9A). Fairy shrimp did not differ from fairy shrimp + clam shrimp in their reduction of phytoplankton abundance ($p=0.870$, $p_{FDR}=0.870$; Table 4). Clam shrimp and fairy shrimp + clam shrimp shifted from reducing phytoplankton in September to greatly enhancing phytoplankton abundances in October. The effects of hatching phenology were mainly driven by clam shrimp and fairy shrimp + clam shrimp in October (Fig. 9B). Both enhanced phytoplankton abundance ($p < 0.005$) while other assemblages of zooplankton had no effect ($p > 0.156$; Table 4). Clam shrimp and fairy shrimp + clam shrimp did not differ in their effect of enhancing phytoplankton abundances ($p=0.126$, $p_{FDR}=0.320$; Fig. 9B). Clam shrimp and fairy shrimp + clam shrimp continued to enhance phytoplankton abundances and drive differences until the end of the experiment in November (Table 4, Fig. 9C). These two treatments also continued to have similar effects of enhancement in November ($p=0.385$, $p_{FDR}=0.770$). Clam shrimp enhancement declined slightly from October to November, whereas

fairy shrimp + clam shrimp enhancement of phytoplankton abundance increased slightly from October to November. Hatching phenology (September – $F_{2, 41} = 0.35$, $p = 0.707$, October – $F_{6, 38} = 0.65$, $p = 0.689$, November – $F_{6, 38} = 0.84$, $p = 0.548$), the presence of predators (October – $F_{1, 38} = 0.05$, $p = 0.830$, November – $F_{1, 38} = 1.04$, $p = 0.315$), and the interaction between hatching phenology and predation (October – $F_{6, 38} = 0.96$, $p = 0.467$, November – $F_{6, 38} = 0.97$, $p = 0.458$) did not affect the extent to which zooplankton altered periphyton abundance during any of the sample dates.

Discussion

Differences in hatching phenology affected phytoplankton abundance. Large branchiopods affected phytoplankton abundance by initially reducing phytoplankton but later in the experiment the presence of clam shrimp and fairy shrimp + clam shrimp greatly enhanced phytoplankton abundance. Predation separately affected the impact of the different hatching phenologies on cladoceran population growth and cladoceran body size. We found that clam shrimp and predators reduced cladoceran population growth compared to when no predators were present. Predators were also better able to affect cladoceran length when cladocerans were alone versus when they were present with large branchiopods. Predators also had effects that were independent of hatching phenology, where predators affected cladoceran body size by making them smaller in length, therefore, also causing a similar decline in average total biomass of cladocerans.

Our prediction that large branchiopods would reduce algal resources was true initially, where we did see that large branchiopods were able to reduce phytoplankton abundance, although this reduction did not persist during the later sample dates. The effects of large

branchiopods on phytoplankton abundance are not well known, besides evidence for consumption on a variety of prey items, including phytoplankton, as well as high filtration rates. Fairy shrimp are omnivorous, filter feed by moving their thoracic legs as they swim, and can consume phytoplankton, protozoans, nematodes, rotifers, and even small nauplii of cladocerans (Dodson et al. 2010, Jocque et al. 2010). Spinicaudatan clam shrimp are filter feeders, spending most of their time skimming along the pond bottom in aquatic vegetation or above algal mats, and can consume detritus, algae, small crustaceans, and rotifers (Dodson et al. 2010). Fairy shrimp have also been found to filter water at high rates, between 0.41 – 2.41 L per hour depending on size of the individual (Dumont & Ali 2004). Since fairy shrimp were inoculated first they could have depleted resources based on their high filtration rates. One study did investigate the effects of large branchiopods on primary producers and found no effect of fairy, tadpole, or clam shrimp on primary producers despite being found in high abundances (Brostoff et al. 2010). Since we found effects of large branchiopods on algal resources, the conflicting results could be due to differences between our studies, where we used different genera of large branchiopods or potentially due to differences in phytoplankton found in the clay pans investigated by Brostoff et al. (2010) versus temporary pond habitats we study.

We were surprised to find that in the later sample dates (October and November) clam shrimp and fairy shrimp + clam shrimp greatly enhanced phytoplankton abundance. We are not sure of the exact mechanism behind this as there is little known about large branchiopod ecology and there are few studies using large branchiopods. However, there is some anecdotal evidence that clam shrimp might modify pond ecology as they feed near pond sediments (Frank 1988). Luzier and Summerfelt (1997) observed that clam shrimp (*Caenestheriella belfragei*) fan as they feed which re-suspends lighter pond sediments. Tadpole shrimp (another branchiopod group)

have also been found to disrupt sediment due to burrowing and foraging (Croel & Kneitel 2011). Therefore, large branchiopods might be modifying pond ecology by feeding behaviors that enhance phytoplankton growth. There is also potential for waste excretion of clam shrimp to differ from other zooplankton such that it is beneficial to phytoplankton growth, although this is largely speculation as there is no information that we are aware of on stoichiometry of large branchiopods. If large branchiopods are missing from a community when a pond refills their absence could have negative consequences on other zooplankton if the other zooplankton rely on the enhancement of phytoplankton abundance that large branchiopods provide. However, we do not know for sure if clam shrimp and fairy shrimp + clam shrimp are enhancing edible algae species as we only quantified abundance, but this highlights an important area for further investigation.

When cladocerans were present by themselves or with other large branchiopods in the later sample dates (October and November) they generally reduced phytoplankton although reductions were not significantly different from zero. Some have found that cladocerans, especially Daphniids, are able to reduce phytoplankton (Sarnelle 1993, Schoenberg & Carlson 1984, Arnér et al. 1998, Tessier et al. 2001), while others have found little or no effect of Daphniids on phytoplankton (Turner & Mittelbach 1992, Brett et al. 1994). Why there are such differences in Daphniid grazing is poorly understood, although evidence suggests *Daphnia* are better able to reduce phytoplankton under nutrient enriched conditions (Steiner 2002).

We predicted that large filter feeding branchiopods would negatively affect smaller filter feeding cladocerans when no predators were present. We did not see an impact of large branchiopods on cladoceran population growth, which was surprising since Jocque et al. (2010) found fairy shrimp (*Branchinella longirostris*) reduced densities of a cladoceran (*Macrothrix*

hardingii) and attributed the decline of cladocerans to competition with fairy shrimp. Our grazing evidence indicated that large branchiopods were not competing with cladocerans over resources, as there were no differences between treatments when cladocerans were by themselves compared to when they were with other large branchiopods. Large branchiopods can also negatively affect cladoceran growth by predation on cladoceran nauplii. Yet, zooplankton feeding trials have found *Daphnia* sp. were absent from the gut of fairy shrimp (*Streptocephalus proboscideus*), which the authors attributed to the efficient escape response of *Daphnia* (Mertens et al. 1990, Ali et al. 1996). If competition with cladocerans or predation of cladoceran nauplii by large branchiopods occurred in our study, it was not evident based on our findings, which showed no effect of hatching phenology on cladoceran growth, size, or biomass suggesting competition between the species of large branchiopods and cladocerans we studied is weak. Although all filter feeders, the lack of competition observed might be due to differences in diet of large branchiopods and cladocerans as suggested by others (Thiéry 1991).

We did find that the effect of the different hatching phenologies on cladoceran population growth depended on whether or not predators were present. Predation has been found to affect the outcome of the order of arrival of different cladoceran species, where early arrival allowed *Daphnia magna* to dominate over *Simocephulus vetulus*, but when predation occurred *S. vetulus* became the dominate species (Louette & De Meester 2007). We expected a similar outcome, where cladoceran growth would be reduced when large branchiopods were present, and that cladoceran growth would be enhanced when predators were present due to size selective predation of large branchiopods by predatory backswimmers, since backswimmers have been found to readily consume fairy shrimp, with estimates of about 2 fairy shrimp eaten per hour per backswimmer (Woodward & Kiesecker 1994; Brendonck et al. 2002). Instead we found that

cladoceran population growth was reduced when clam shrimp and backswimmers were present. As stated earlier competition between clam shrimp and cladocerans is unlikely, which is further supported by increased cladoceran population growth when with clam shrimp and no predators. If it is not competition with clam shrimp that is reducing cladoceran population growth, this seems to point to predation of cladocerans by backswimmers that reduces their growth, however, when cladocerans were alone with predators their population growth actually increased slightly compared to when no predators were present. This increase was probably due to predators removing larger cladocerans, which reduced competition between cladocerans allowing for increased growth (Hu & Tessier 1995). Our finding of cladoceran body length becoming smaller when predators were present supports this idea. Thus, when clam shrimp and backswimmers were present an interaction between them occurred, which potentially caused more competition between the cladocerans and slowed their population growth.

Predators had effects that were independent of hatching phenology, where predatory backswimmers reduced cladoceran size and biomass, which is in accordance with other studies (Murdoch & Scott 1984, Arnér et al. 1998, Shurin 2001, Howeth & Leibold 2010). In a laboratory experiment, Murdoch and Scott (1984) found that predation by *Notonecta hoffmani* reduced the average size of individuals of *Daphnia pulex* populations, which also led to reduced average biomass. In a mesocosm experiment, a *Notonecta* sp. selectively preyed on large-bodied *Daphnia magna* over other small-bodied zooplankton that were also present (Arnér et al. 1998). In 2 additional mesocosm experiments, size selective predation by *Notonecta ungulata* (Howeth & Leibold) and *Notonecta undulata* (Shurin 2001) for larger individuals reduced *Daphnia pulex* densities. Although we used a different species of backswimmer (*Notonecta irrorata*) and cladoceran (*Daphnia laevis*) we found similar results of the effect of backswimmers on

cladocerans as these studies, such that size selective predation by backswimmers on large *Daphnia* is likely common across aquatic systems.

We have found evidence that hatching phenology, predation, and the interaction between hatching phenology and predation can have important impacts on temporary pond inhabitants and algal resources. Specifically, algal resources were affected by differences in species hatching phenology, predation impacted cladoceran size and average total biomass, and the extent to which hatching phenology affected cladoceran population growth and cladoceran body size depended on whether predators were present. Understanding how hatching phenology, predation, and their interaction affect aquatic systems is especially important due to climate change, where many organisms might be forced to shift their phenological responses (Winder & Schindler 2004, Yang & Rudolf 2010). Based on our findings, if there are shifts in hatching phenology due to climate change, this could have important impacts on the algal resources, which could affect ecosystem function. If predators also shift their arrival time there could also be important impacts of predation or lack of predation (because of a different arrival time) on the growth rate of later arriving individuals. We have also highlighted the need for more studies on large branchiopods. Not much is known about their impacts on aquatic systems, but as we have shown they are clearly having important effects on algal resources, and in concert with predation can slow the growth of later arriving species.

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Table 4. Results of planned contrasts performed on the extent of zooplankton grazing on phytoplankton (μg/L) during each of the sample dates, 22 September, 19 October, and 9 November. Both unadjusted p-values and p-values adjusted per question to control for the FDR (p_{FDR}) are shown.

	SEPTEMBER	
1. Do large branchipods affect algal resources differently?	p	p_{FDR}
CS vs FS	0.013	0.013
2. Do single species affect algal resources differently than multiple species?		
CS vs FS CS	0.009	0.017
FS vs FS CS	0.870	0.870

	OCTOBER		**NOVEMBER**	
1. Do zooplankton species affect algal resources differently?	p	p_{FDR}	p	p_{FDR}
CS vs FS	0.005	0.008	0.057	0.086
CS vs CLAD	0.003	0.008	0.039	0.086
CLAD vs FS	0.747	0.747	0.803	0.803
2. Do single species affect algal resources differently than multiple species?				
CLAD vs CS CLAD	0.937	0.945	0.948	0.948
CLAD vs FS CLAD	0.911	0.945	0.871	0.948
CLAD vs FS CS	0.095	0.284	0.004	0.032
CLAD vs FS CS CLAD	0.797	0.945	0.727	0.948
CS vs CS CLAD	0.004	0.018	0.046	0.142
CS vs FS CLAD	0.002	0.018	0.047	0.142
CS vs FS CS	0.126	0.302	0.385	0.770
CS vs FS CS CLAD	0.005	0.018	0.070	0.169
FS vs CS CLAD	0.809	0.945	0.856	0.948
FS vs FS CLAD	0.651	0.945	0.928	0.948
FS vs FS CS	0.156	0.313	0.005	0.032
FS vs FS CS CLAD	0.945	0.945	0.917	0.948

Zooplankton abbreviations: FS = Fairy shrimp, CS = clam shrimp, CLAD = cladocerans, CS CLAD = clam shrimp + cladocerans, FS CLAD = fairy shrimp + cladocerans, FS + CS = fairy shrimp + clam shrimp, FS CS CLAD = fairy shrimp + clam shrimp + cladocerans.

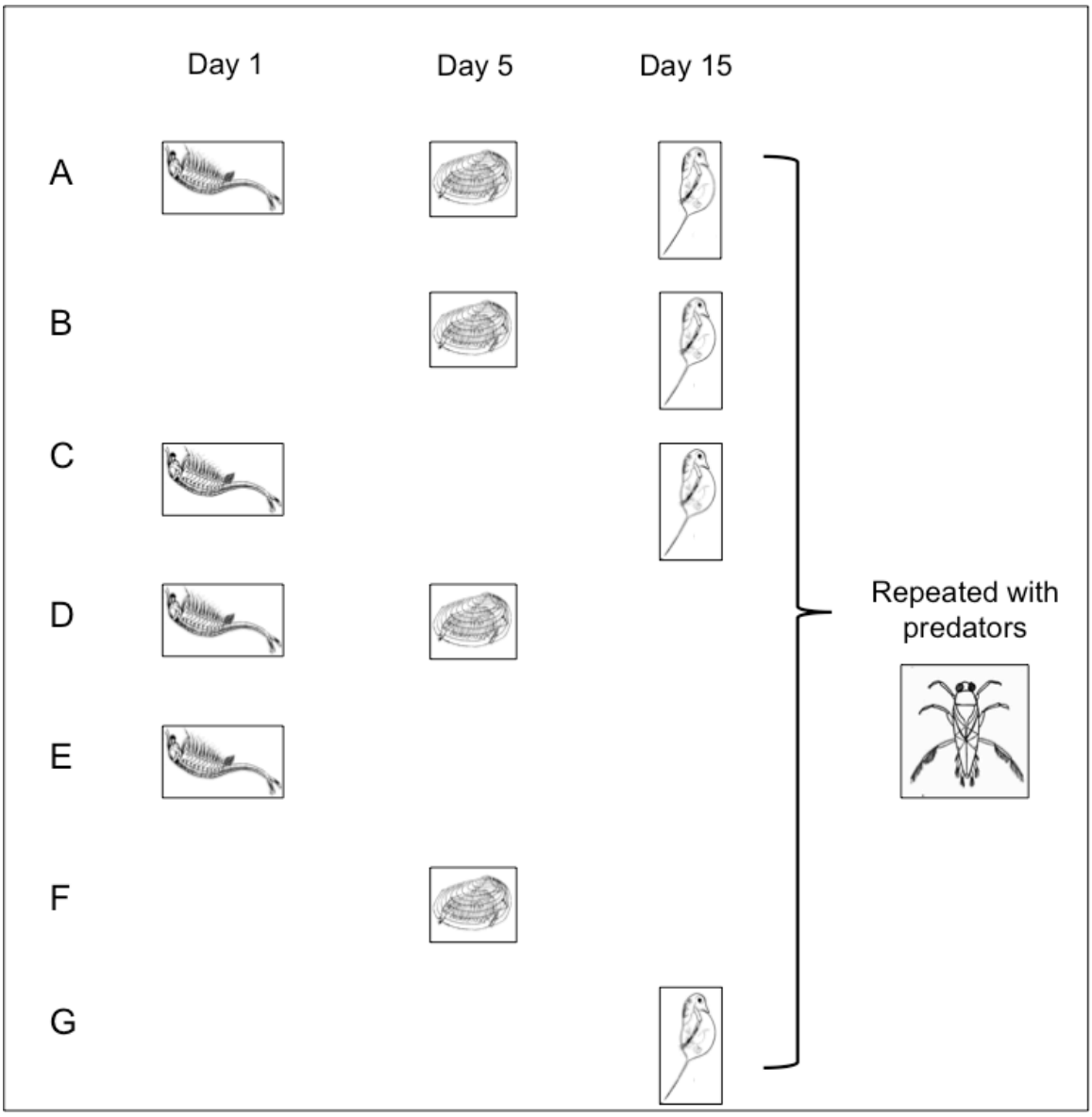

Figure 5. Hatching phenology per treatment (A-G). Day 1 fairy shrimp added, day 5 clam shrimp added, day 15 cladocerans added to specific treatments: A) predicted natural hatching order, B-C) fairy shrimp and clam shrimp fail to hatch due to missed hatching cue, D) cladocerans fail to hatch potentially due to pond drying, E-G) controls and G) both large branchiopods fail to hatch. All treatments were repeated with predatory backswimmers.

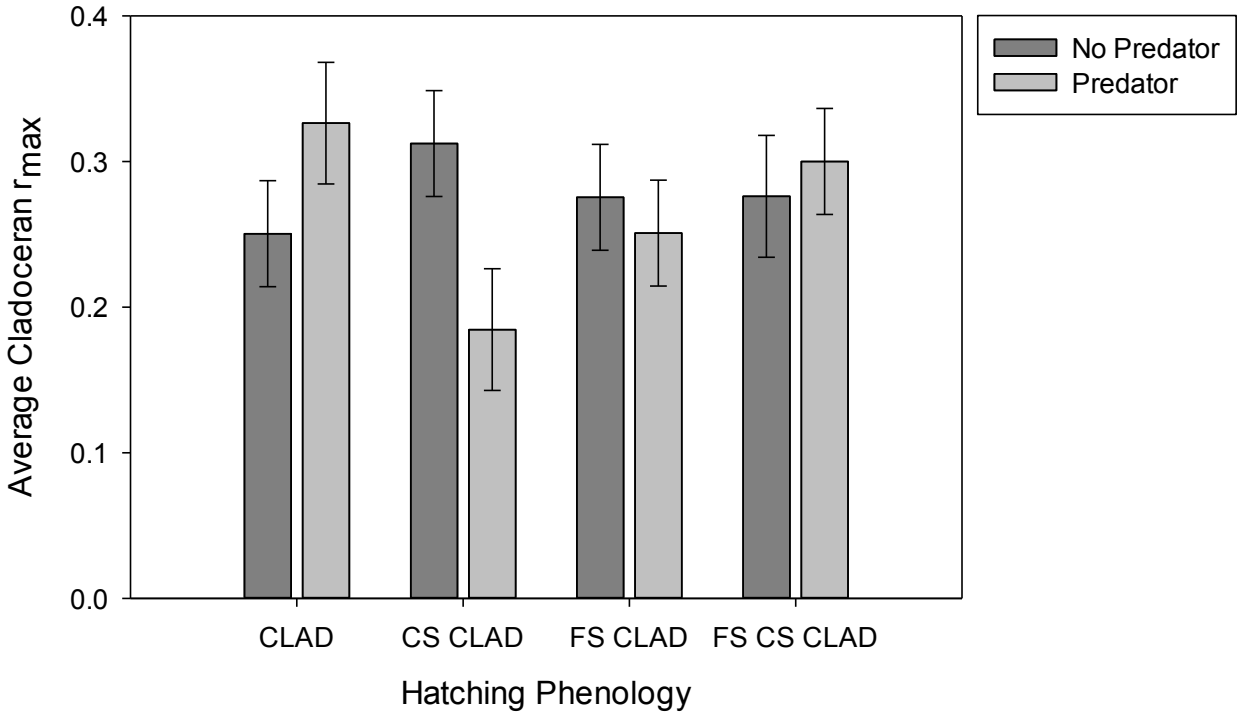

Figure 6. Average maximum intrinsic rate of growth (r_{max}) of cladocerans in the different hatching phenology treatments that contained cladocerans, with and without predatory backswimmers present. Least Square means ± 1 Standard Error are shown. Zooplankton abbreviations: CLAD = cladocerans, CS CLAD = clam shrimp + cladocerans, FS CLAD = fairy shrimp + cladocerans, FS CS CLAD = fairy shrimp + clam shrimp + cladocerans.

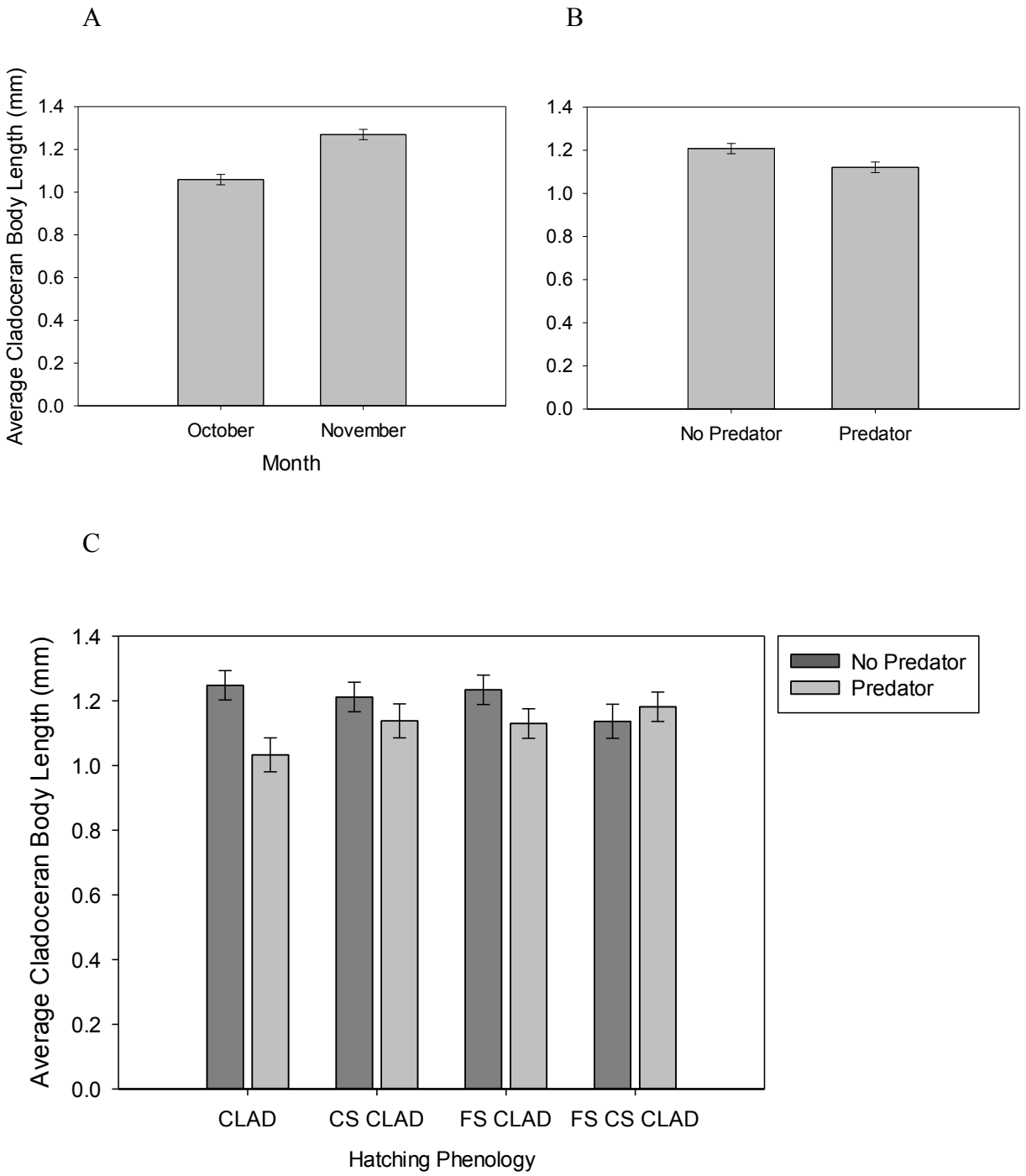

Figure 7. Average length (mm) of cladocerans when A) measured across all mesocosms during October and November, B) measured across mesocosms that either contained or did not contain predators, and C) measured in mesocosms for each of the experimental treatments. Least Square means ± 1 Standard Error are shown. Zooplankton abbreviations: CLAD = cladocerans, CS CLAD = clam shrimp + cladocerans, FS CLAD = fairy shrimp + cladocerans, FS CS CLAD = fairy shrimp + clam shrimp + cladocerans.

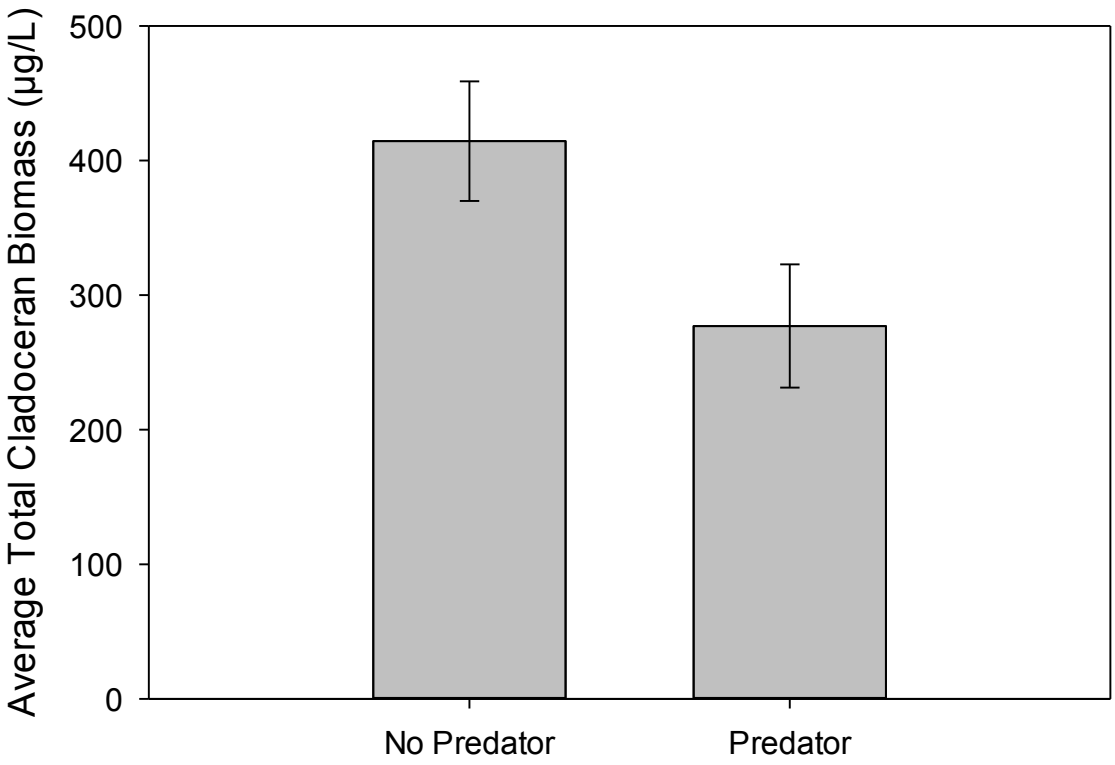

Figure 8. Average total cladoceran biomass (µg/L) in treatments containing or not containing predators. Least Square means ± 1 Standard Error are shown.

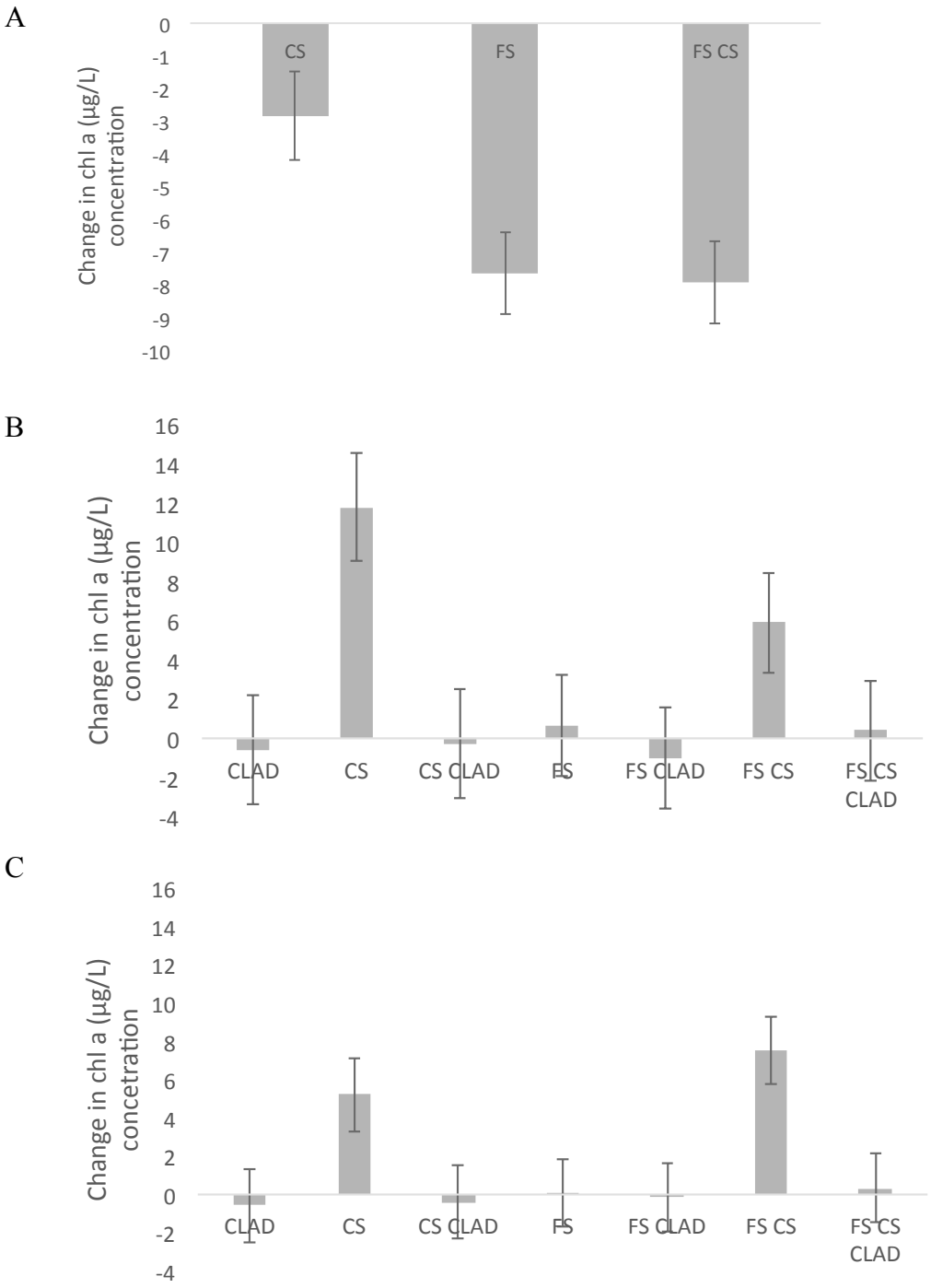

Figure 9. Zooplankton grazing on phytoplankton (chl a (µg/L) concentrations) across the different hatching phenologies during A) 22 September (before cladocerans were added), B) 19 October (after cladocerans and predators were added) and C) 9 November (last sample date of the experiment). Least Square means ± 1 Standard Error are shown. Zooplankton abbreviations: FS = Fairy shrimp, CS = clam shrimp, CLAD = cladocerans, CS CLAD = clam shrimp + cladocerans, FS CLAD = fairy shrimp + cladocerans, FS + CS = fairy shrimp + clam shrimp, FS CS CLAD = fairy shrimp + clam shrimp + cladocerans.

Supplement 2A. Planned contrasts performed on the extent of zooplankton grazing on phytoplankton (µg/L) during each of the sample dates 22 September, 19 October, and 9 November. All species were inoculated by October sample.

	CS	FS	FS CS
SEPTEMBER - Planned contrasts pertainting to each question			
1. Do clam shrimp and fairy shrimp differ in their effect on algal resources?	1	-1	0
2. Do clam shrimp affect algal resources differently than multiple species, (FS + CS) ?	1	0	-1
3. Do fairy shrimp affect algal resources differently than multiple species, (FS + CS)?	0	1	-1

	CLAD	CS	CS CLAD	FS	FS CLAD	FS CS	FS CS CLAD
OCTOBER and NOVEMBER - Planned contrasts pertainting to each question							
1. Do clam shrimp and fairy shrimp differ in their effect on algal resources?	0	1	0	-1	0	0	0
2. Do clam shrimp and cladocerans differ in their effect on algal resources?	1	-1	0	0	0	0	0
3. Do fairy shrimp and cladocerans differ in their effect on algal resources?	1	0	0	-1	0	0	0
4. Do cladocerans affect algal resources differently than multiple species, (CS + CLAD)?	1	0	-1	0	0	0	0
5. Do cladocerans affect algal resources differently than multiple species, (FS + CLAD)?	1	0	0	0	-1	0	0
6. Do cladocerans affect algal resources differently than multiple species, (FS + CS)?	1	0	0	0	0	-1	0
7. Do cladocerans affect algal resources differently than multiple species, (FS + CS + CLAD)?	1	0	0	0	0	0	-1
8. Do clam shrimp affect algal resources differently than multiple species, (CS + CLAD)?	0	1	-1	0	0	0	0
9. Do clam shrimp affect algal resources differently than multiple species, (FS + CLAD)?	0	1	0	0	-1	0	0
10. Do clam shrimp affect algal resources differently than multiple species, (FS + CS)?	0	1	0	0	0	-1	0
11. Do clam shrimp affect algal resources differently than multiple species, (FS + CS + CLAD)?	0	1	0	0	0	0	-1
12. Do fairy shrimp affect algal resources differently than multiple species, (FS + CLAD)?	0	0	0	1	-1	0	0
13. Do fairy shrimp affect algal resources differently than multiple species, (FS + CS)?	0	0	0	1	0	-1	0
14. Do fairy shrimp affect algal resources differently than multiple species, (FS + CS + CLAD)?	0	0	0	1	0	0	-1
15. Do fairy shrimp affect algal resources differently than multiple species, (CS + CLAD)?	0	0	1	-1	0	0	0

CHAPTER 3: How does space and environment affect zooplankton community composition of ponds in the Croatan National Forest, NC?

Introduction

Community ecologists strive to explain which processes are important in regulating communities at local and regional scales (Vellend 2010). At the local level, factors such as abiotic conditions, and competition and predation are important in determining whether species can survive upon arrival into a new community (Hutchinson 1957, Chase & Leibold 2003, Kneitel & Miller 2003). Community composition at the local scale can also be affected by regional factors, such as dispersal (Ricklefs 1987, Leibold et al. 2004). Thus, the composition of a community is a result of a combination of local and regional factors.

One way to investigate local and regional processes is to determine how much of the variation in the species composition of local communities is explained by spatial and environmental variables. Studies have shown that strength of spatial versus environmental factors varies in explaining species composition. For example, Beisner et al. (2006) found that the role of environmental and spatial processes varied depending on the organism investigated and their dispersal ability through space. A meta-analysis that included a broad range of taxa, habitats, spatial scales, body sizes, and dispersal abilities found that 48% of the total variation in community structure was explained by environmental and spatial variables, where 22% was explained by environmental variables independent of space, 16% was explained by spatial variables independent of environment, and the remaining 10% was explained by shared environmental and spatial variables (Cottenie 2005). Thus, both spatial and environmental factors should be considered when investigating drivers of community composition.

Pond systems are an excellent system to study the effects of spatial and environmental factors, as they are discrete aquatic "islands" spread throughout a terrestrial matrix (De Meester et al. 2005). Species composition in ponds can be affected by dispersal between ponds, species interactions, resource levels, and hydroperiod (Welborn et. al 1996, Shurin 2001, Brendonck et al. 2002, Kneitel & Miller 2003, Van Buskirk 2005). Zooplankton are an important component of aquatic systems as they are used to evaluate the ecological health of pond communities, such that understanding the mechanisms behind their distribution is of interest (DeBaise & Taylor 2005).

Our study system, the Croatan National Forest (CNF), located along the SE coastal plain of North Carolina offers a model study system with a high density of ponds that range from temporary to permanent (Chalcraft, unpublished data). Some of the ponds are Carolina bays, which are found throughout the coastal plain, from New Jersey – northern Florida, and are most abundant in North Carolina and South Carolina (Sharitz 2003). Many Carolina bays have been drained for agriculture purposes and few have been cataloged for floral and faunal inhabitants, but those that have been sampled support a diverse assemblage of species (Sharitz 2003). Consequently, the ponds located in the CNF are an excellent system to study the importance of space and the environment on zooplankton composition as they range in size, distance between ponds, and density of nearby ponds. We are not aware of any studies on the zooplankton composition of ponds in the CNF. Other studies have investigated zooplankton composition in ponds and Carolina bays in similar coastal plain habitat at the Savannah River Site in nearby SC, but they have not examined how space and environment affect species composition (Mahoney et al. 1990, Dietz-Brantley et al. 2002, DeBaise & Taylor 2003, DeBaise & Taylor 2005).

Many of the ponds in the CNF have fluctuating hydroperiods, such that organisms that inhabit these ponds have adaptations that allow them to persist under such conditions. Zooplankton have developed unique strategies to overcome desiccation of ponds. Some strategies include reaching reproductive maturity quickly and producing dormant eggs that can survive unpredictable drying events (Brendonck & Riddoch 1999). Another important life history strategy is bet hedging (Stearns 1976). This spreads reproduction (via dormant eggs) over many seasons to avoid the risk of reproductive failure from a drying event or increases in predation (Simovich & Hathaway 1997, Welborn et al. 1996, Brendonck et al. 2002). Delayed hatching also allows for a mixed egg bank with the potential for multiple generations to hatch in a given season (Brendonck & De Meester 2003).

Large branchiopods are mainly found in temporary pond systems that do not contain fish, as they are highly susceptible to fish predators, although invertebrate predators capable of flight are usually present in temporary ponds (Colburn 2004). Fairy and clam shrimp can hatch from dry ponds when the ponds refill in fall, winter, or early spring. Branchiopod species may require certain environmental cues (e.g., exposure to a particular oxygen concentration, temperature, and photoperiod) to hatch (Dodson et al. 2010). With each filling different communities may assemble depending on which species receive the proper cue to hatch out of the dormant egg bank.

Cladocerans and copepods are usually not limited to temporary pond systems and can occur across the majority of the hydroperiod gradient. Cladocerans reproduce through cyclic parthenogenesis, meaning they alternate between sexual and parthenogenic reproduction. Dormant eggs are produced by sexual reproduction when conditions are unfavorable such as increased predation pressure, high conspecific densities, poor food availability or other

environmental factors (Johnson 1973). Copepods reproduce only through sexual reproduction and mostly produce subitaneous eggs that begin to develop immediately. Some calanoid species can also make resting eggs, while cyclopoids can only produce subitaneous eggs (Reid & Williamson 2010). During unfavorable conditions, some late copepodid stages can undergo encystment and diapause (DeBaise & Taylor 2005, Reid & Williamson 2010). Some cladoceran and large branchiopod dormant eggs have spines and/or barbs helping them stick to feathers and fur, but adults could also potentially stick to animal fur and be transferred over short distances (Bilton et al. 2001). Other vectors of transport of zooplankton include wind, rain, flowing water, aquatic insects, consumption then transport of eggs via invertebrates and vertebrates, and human-mediated transport (Havel & Shurin 2004, van de Meutter et al. 2008, Waterkeyn et al. 2010).

It is unlikely that only space or the environment will affect zooplankton composition as most communities are formed by a combination of environmental and spatial factors. Therefore, we hypothesized species would be responsive to environmental characteristics associated with sites, but that high levels of dispersal would allow species to persist in less suitable patches via source-sink dynamics. We hypothesized high levels of dispersal would be important in structuring pond zooplankton communities, due to close proximity of some temporary ponds in our study system (see methods) and an abundance of potential dispersal vectors (wind, rain, invertebrate and vertebrate vectors). If a temporary pond is nearby that dries down the dormant egg bank could be exposed and these ponds could act as potential sources of dormant eggs, as high levels of wind driven dispersal or vector dispersal could move eggs to a nearby pond that holds water (Vanschoenwinkel 2008a). Zooplankton are also known to be affected by environmental characteristics of their habitat (Dodson et al. 2010); therefore, both environmental characteristics and spatial characteristics should act in concert to affect zooplankton composition.

We also hypothesized certain environmental variables might be important in structuring zooplankton communities, such as temperature and pH.

Methods

Field Surveys

A total of 19 ponds were surveyed from September 2012 – September 2013. Ponds were sampled once every 2 months (Sept, Nov, Jan, March, May, July, Sept) and were categorized by approximate size prior to survey work; 7 ponds were large (5,000 – 20,000 m^2), 6 were mid-sized (500 – 2000 m^2) and 6 were small (100 – 350 m^2). Most ponds (13) held water through July but were dry by September 2013. The ponds sampled were from 3 general regions in the Southern CNF separated by approximately 1 km and were referred to as the Pringle road area, Patsy pond area, and Boat Ramp pond area (see Supplements 3A and 3B). Zooplankton samples were performed along a transect, originating from the edge of the pond to the center, and were collected from both vegetated and non-vegetated areas. Since ponds were shallow and highly vegetated we could not use conical plankton nets. Therefore, a PVC tube sampler was used to collect zooplankton from the entire water column and when ponds were very shallow and less than 0.5 m in depth a hand pitcher was used (Steiner 2004). Based on pond size, sample volumes differed, as Steiner (2004) found an amount of 10 – 24 L adequately represented the majority of zooplankton taxa in ponds (Steiner 2004). Therefore, 10 L total was sampled from ponds categorized as small, 20 L total from ponds categorized as mid, and 30 L total from ponds categorized as large. The multiple water column samples totaling the amount specified were emptied and filtered through fine mesh (64 um) and preserved in >70% ethanol. Zooplankton

samples were subsampled in the lab for abundance estimates, where a minimum of 300 individuals were counted and the entire sample was processed when there were less than 300 individuals (Verreydt et al. 2012). We calculated species density as the number of individuals of each species / per L. Large branchiopods and cladocerans were identified to genus or species level. Copepods were categorized as cyclopoid, calanoid, or copepod nauplii.

Environmental variables were measured during each sample date in each pond. Dissolved oxygen (mg/L) was measured using a WTW Oxi 340i dissolved oxygen meter, temperature (°C) and conductivity (µS/cm) were measured using a YSI CastAway CTD and pH was measured using an Oaktron pHTestr 3+. Spatial variables were measured using Google Earth Pro version 7.1, where size of pond was measured to the nearest (m^2). Minimum distance to nearest permanent and minimum distance to nearest temporary pond (m) were also measured even if the nearest pond was not one of the 19 ponds sampled, as the nearest pond might provide colonists to the ponds that were sampled. Nearest temporary and permanent ponds were distinguished since they might be providing different types of colonists (dormant eggs when dry versus live zooplankton when filled). Pond density was measured as the number of ponds within a 200 m radius originating from the center of the study pond. Most dispersal occurs within 60 m of a pond, but there is some evidence for long distance dispersal occurring from over 400 m, such that 200 m should capture most of the dispersal occurring at shorter distances (Allen 2007).

Statistical analyses

We performed analyses for each month separately. Since only 4 ponds held water in September 2013, the second September sample date was dropped from analyses. Analyses were

performed on zooplankton abundance data (species densities per L). All analyses were performed using PRIMER-E version 6 (Clarke & Gorley 2006).

Distance-based redundancy analyses (dbRDA) were used for two purposes in our data analyses. dbRDA is a constrained ordination method, which is an extension of multiple regression analysis to a situation with multiple response variables (Legendre & Legendre 2012). We used dbRDA on Hellinger transformed zooplankton density data as this transformation makes complex data with numerous zero values more suitable for analysis with linear methods (Legendre & Gallagher 2001, Peres-Neto et al. 2006). dbRDA was used first used to partition the total variation in the community matrix into unique environmental and spatial components, as well as to visualize the relationships between communities and explanatory variables (described below). The amount of variation in zooplankton species composition that can be attributed to the different explanatory variables include: the total variation in zooplankton species composition accounted for by both the spatial and environmental variables [E + S], the proportion of variation in zooplankton species composition accounted for by the environmental variables after accounting for the spatial variables [E|S] (pure environmental variation), the proportion of variation in zooplankton species composition accounted for by the spatial variables after accounting for the environmental variables [S|E] (pure spatial variation), and the proportion of variation in zooplankton species composition shared by both environmental and spatial variables. The amount of overlap between spatial and environmental components was computed by subtracting the pure spatial variation [S|E] and the pure environmental [E|S] variation from the total explained variation [E + S], as the pure spatial variation, pure environmental variation, and the variation accounted for by both spatial and environmental variables sum to form the total variation accounted for by the model [E + S] (Stevens et al. 2007). [E] and [S] are also produced

by dbRDA and refer to the amount of variation accounted for by environmental and spatial variables, respectively, without taking into account their correlated effects. Spatial variables included pond size (m^2), minimum distance to temporary pond (m), minimum distance to permanent pond (m) and density of ponds within 200 m of the study pond. Pond size was included as a spatial variable as colonists would have more of a chance of colonizing larger ponds than smaller ponds. Environmental variables included temperature (°C), dissolved oxygen (mg/L), pH, and conductivity (µS/cm).

Relationships between zooplankton composition and potential explanatory variables were then analyzed using dbRDA for each month. To determine which explanatory variables to include we used adjusted R^2 and the forward selection procedure with the correction described by Blanchet et al. (2008) and Borcard et al. (2011) to reduce type I errors. The correction procedure is as follows: the overall adjusted R^2 is generated from the global model with all response variables. Forward selection is then performed and stopped when a variable is either not significant ($p>0.05$) or the adjusted R^2 value of the reduced model is higher than the global model adjusted R^2.

To further account for spatial autocorrelation, relationships between species composition and spatial variables and between environmental and spatial variables were investigated using Mantel tests, using Spearman rank rather than Pearson correlation coefficients (the RELATE procedure) with 4999 permutations. The RELATE procedure tests for the degree of agreement between two datasets. It calculates Spearman rank correlations between the elements of two matrices and produces a matching coefficient (ρ), which is then used in a permutation test. If ρ = 1 or -1 there is a perfect match between the two matrices, if $\rho = 0$ there is no relation between the two matrices (Clark & Gorley 2006). A Hellinger distance matrix was used for zooplankton

species composition data and Euclidean distance matrices were used for spatial and environmental data.

Results

Over 33,000 individuals were counted and identified from ponds in the CNF. We identified 2 large branchiopod species, 39 cladoceran species, and members from the cyclopoid, calanoid, and copepod nauplii groups (see Supplement 3C for a species list).

Variance partitioning based on dbRDA showed that for most of the months, spatial and environmental variables both explained a significant amount of the variation in zooplankton composition, exceptions include January where neither spatial nor environmental variables explained variation in zooplankton composition and March, where environmental variation but not spatial variation explained differences in zooplankton composition (Table 5). Pure spatial and pure environmental variation explained similar amounts of variation for September – January, but pure environmental variation explained more of the variation in zooplankton composition from March – July (Fig. 10). The spatial structuring in the species data that is shared by the environmental data was higher for the months of November, May, and July (Fig. 10).

Neither species composition nor environmental variables were correlated with spatial variables. There were weak correlations between zooplankton composition and all of the spatial variables during each of the study periods ($\rho \leq 0.243$, Table 6). Pond size had the strongest correlation with zooplankton composition in September ($\rho = 0.207$) and November ($\rho = 0.243$), but the correlations were still weak. Similarly, there were weak correlations between environmental variables and spatial variables for all months ($\rho \leq 0.370$, Table 6). Minimum

distance to a permanent pond had the strongest correlation with environmental variables in November ($\rho = 0.235$) and May ($\rho = 0.370$).

dbRDA were also used to visualize the relationships between communities and explanatory variables. The specific spatial and environmental variables that explained significant variation in zooplankton composition varied by month. To interpret dbRDA plots, vectors are shown for the spatial or environmental variables that were significantly correlated ($p \leq 0.05$) with variation in zooplankton composition. Increasing positive values occur along the vectors, such that species that have positive position on the vector axis were more strongly associated with the presence of the variable, while species with negative positions were less strongly associated with the variable. The negative direction of vectors are not shown but can be visualized as the opposite direction of the vectors shown in the plots. Species that corresponded the most to the spatial or environmental variables are shown in a second dbRDA plot. Species that are farther from the origin also responded more strongly to the variable being considered than those closer to the origin, such that species with longer distances from the origin were considered in more detail. For easier visualization all 44 species/groups were not included in the plot, only species whose density represented at least 5% of all the zooplankton sampled per month were considered.

In September, dbRDA showed pond size (Pseudo-F = 2.31, p = 0.011), conductivity (Pseudo-F = 2.30, p = 0.015), and distance to a permanent pond (distance P; Pseudo-F = 1.98, p = 0.042) were significantly correlated with variation in zooplankton composition (Fig 11A). Copepod nauplii were positively associated with larger ponds and *Alonella exigua* was negatively associated with conductivity, meaning it was found in higher densities in ponds with lower conductivity. *Diaphanosoma brachyurum* was positively associated with distance P,

meaning it was found in ponds in higher densities that were far from a permanent pond, whereas *Kurzia latissima* was negatively associated with distance P, meaning it was found in ponds in higher densities that were in closer proximity to a permanent pond (Fig. 11B).

In November, dbRDA showed temperature (Pseudo-F = 3.74, p = 0.002), pond size (Pseudo-F = 2.11, p = 0.016), and conductivity (Pseudo-F = 2.00, p = 0.031) were significantly correlated with variation in zooplankton composition (Fig. 12A). *Pleuroxus straminius* and *Disparalona hamata* were positively associated with temperature meaning they were found in ponds in higher densities that had warmer temperatures, whereas cyclopoid copepods and *Simocephalus exspinosus* were negatively associated with temperature and found in ponds in higher densities that had lower temperatures. *Ephemeroporus tridentatus* was positively associated with conductivity and negatively associated with size, meaning it was found in higher densities in smaller ponds that had higher conductivity (Fig. 12B).

In January, dbRDA found that temperature was the only variable significantly correlated with variation in zooplankton composition (Pseudo-F = 2.41, p = 0.019). Pond temperature only accounted for approximately 13% of the total variation in zooplankton composition (Fig. 13). In March, dbRDA showed temperature (Pseudo-F = 3.27, p = 0.005), pond density (Pseudo-F = 1.86, p = 0.053), and dissolved oxygen (Pseudo-F = 1.87, p = 0.048) were significantly correlated with variation in zooplankton composition (Fig. 14A). *Chydorus linguilabris* was positively correlated with higher pond temperatures, whereas copepod nauplii and *E. tridentatus* were negatively correlated with temperature and found in ponds in higher densities with lower pond temps. *Alona rustica* was positively correlated with higher pond density, meaning it was found in ponds in higher densities that had many ponds in the 200 m surrounding area. *A. exigua* was positively correlated with higher dissolved oxygen levels, whereas calanoid copeopods were

found to be negatively associated with dissolved oxygen and were collected from ponds in higher densities that had low dissolved oxygen levels (Fig. 14B).

In May, dbRDA found dissolved oxygen (Pseudo-F = 5.01, p = 0.002) and pond density (Pseudo-F = 1.66, p = 0.08) were significantly correlated with variation in zooplankton composition (Fig. 15A). *D. hamata* was positively associated with higher pond density, meaning it was found in ponds in higher densities that had many other ponds within 200 m, whereas *Simocephalus serrulatus* and *Simocephalus vetulus* were negatively associated with pond density. *Pseudosida bidentata* and *E. tridentatus* were negatively associated with dissolved oxygen, such that they were found in ponds in higher densities with lower dissolved oxygen (Fig. 15B).

Lastly, in July dbRDA showed all spatial and environmental variables were correlated with variation in zooplankton composition (temperature Pseudo-F = 2.39, p = 0.002; pond density Pseudo-F = 2.04, p = 0.01; conductivity Pseudo-F = 1.81, p = 0.045; pond size Pseudo-F = 1.58, p = 0.101; dissolved oxygen Pseudo-F = 1.35, p = 0.222; distance T Pseudo-F = 1.20, p = 0.294; pH Pseudo-F = 1.06, p = 0.435; and distance P Pseudo-F = 1.15, p = 0.352; Fig. 16A). Although pond size, dissolved oxygen, distance to a temporary pond (distance T), pH, and distance P were not statistically significant, they were included in the model since each of their adjusted R^2 values was lower than the global model's R^2 value of 0.365. *Ephemeroporus hybridus* was positively correlated with higher pond temperatures. *D. hamata* again was positively associated with higher pond density as well as *C. linguilabris.* Cyclopoid copepods were positively associated with higher pond conductivity. *E. tridentatus* and *P. bidentata* were negatively associated with pond size, meaning they were collected from ponds in higher densities

that were smaller in size. *D. brachyurum* was positively associated with distance T, where it was found in ponds in higher densities that were farther from temporary ponds (Fig. 16B).

Discussion

We found that spatial and environmental factors explained similar amounts of the variation in zooplankton composition in the CNF, with environmental factors explaining more of the variation from March – July. Shared variation of environment and space also increased from March – July. The specific environmental and spatial variables that explained variation in zooplankton composition varied by month, with pond temperature being important in four of the six months. Other environmental variables that explained variation across months included conductivity and dissolved oxygen. Spatial variables that explained variation in zooplankton composition across months included pond density and pond size.

Our findings are congruent with a study of lake systems in which zooplankton composition was explained by similar amounts of environmental and spatial variation (Beisner et. al 2006). However, other studies that have investigated spatial and environmental effects on zooplankton composition have found environmental variables explain more of the variation in zooplankton composition than spatial variables (Cottenie et al. 2003, Leibold et al. 2010, Vanschoenwinkel et al. 2007, Declerck et al. 2011, Dallas & Drake 2014). For example, Cottenie et al. (2003) found important effects of environment despite high dispersal abilities of zooplankton that occurred between connected ponds. We did find that environmental variables explained more of the variation in the spring – summer months. Although shared variation also increased during this time, it is smaller than the variation explained by pure spatial or pure environmental variation. We also did not find strong correlations between environmental

variables and spatial variables based on the RELATE analyses, which lessens the probability of abiotic spatial gradients in our study system but is based on the environmental variables we measured.

We predicted that spatial variables would explain variation in zooplankton composition due to potential dispersal from nearby ponds (Havel & Shurin 2004, Louette & De Meester 2005, Allen 2007, Vanschoenwinkel et al. 2008b). While we did not find this effect we did see that certain spatial variables explained variation in zooplankton composition. We would have expected that distances to nearby ponds would have explained the variation in zooplankton composition if dispersal were important, but we only found distance to a permanent pond to be important in September. Frisch et al. (2012) found spatial variables were more important than environmental variables in determining zooplankton composition with distance between ponds and pond surface area being key determinants of colonization rates for copeopods and cladocerans. Although distance to neighboring ponds was generally not important in our study, we did see that the density of ponds was important in March, May, and July. Vanschoenwinkel et al. (2008a) found that the number of dry pools in the study area explained 87% of the total number of dispersing propagules, consisting of mostly crustacean dormant eggs. Thus, it might not be distance to a nearby pond but instead how many ponds are in the area that matters in providing spatially dispersing individuals.

We hypothesized temperature would be an important environmental variable that explained variation in zooplankton composition, and we found it was important across many of the months, which is in accordance with prior studies that have found temperature is an important regulator of zooplankton (Moore & Folt 2003, Steiner 2004, Berger et al. 2007). We also hypothesized that pH might also explain a large portion of the variation in zooplankton

composition as others have found (Holt et al. 2003, Steiner 2004, Dallas & Drake 2014) but this was not the case in our study. pH did not vary greatly across ponds or across months and ranged from 4 – 6.39, with an average of 5.013 for all ponds across all months. Such little variation in pH potentially explains why it did not account for much of the variation in zooplankton composition as all zooplankton in the CNF experienced similar pH conditions.

In addition to temperature, conductivity and dissolved oxygen were important variables the explained significant amounts of variation in zooplankton composition but it depended on the month considered. Specific environmental factors considered by others that explained significant portions of zooplankton composition varied and depend on the study system. In interconnected ponds, turbidity, submerged macrophytes, diversity of macroinvertebrates, and nitrate concentrations significantly explained variation in zooplankton composition (Cottenie et al. 2003). Whereas, in 18 Canadian lakes, total phosphorus explained 5% of the variation in zooplankton composition and total nitrogen, dissolved organic carbon (DOC) and dissolved inorganic carbon each explained about 4% of the variation in lake zooplankton community structure when considering different types of dispersal (Beisner et al. 2006). In another study looking at 139 northeastern US lakes, Dallas & Drake (2014) found a measure of spatial distance, pH, and chlorophyll a (chl a) explained total zooplankton community structure. They considered copepods, cladocerans, and rotifers together and separately and when considered separately cladocerans were structured more by chl a, followed by DOC and spatial distance among sites. This highlights the differences in environmental variables that could potentially explain variation in zooplankton composition, such that some of the variables considered by others might not be important in our study system in explaining more of the environmental variation.

As with any observational multivariate community analysis, there are limitations since we did not measure all possible environmental or spatial variables (Cottenie et al. 2003). Due to study limitations we did not collect chl a data or nutrient data, which has been found to be important in determining zooplankton compositions in lake systems (Beisner et al. 2006, Dallas & Drake 2014). We also did not collect other trophic levels (phytoplankton, bacterioplankton), where considering species composition at other trophic levels has been found to be important to take into account when determining responses of a group of organisms (such as zooplankton) to environmental and spatial factors (Verreydt et al. 2012). Consequently, chl a, biotic variables, trophic interactions, or other variables not considered could account for unexplained variation. However, Steiner (2004) found that *Chaoborus* (predatory midge larvae) abundance, and algal resources surprisingly showed no relationship with *Daphnia pulex* biomass, but instead pond pH and temperature showed the strongest relationship with *D. pulex* biomass.

We did not see general patterns across months when we considered zooplankton species, as individual species were affected by different environmental and spatial variables across months. For example, we did not see the same species affected by temperature across the different months where temperature was found to be an important explanatory variable. However, various species were correlated with temperature such that it does have an important effect on zooplankton composition (Steiner 2004). We also found some species were strongly correlated with the different spatial variables. While there is little known about species specific dispersal rates in zooplankton (Bohonak & Jenkins 2003, Havel & Shurin 2004), we can draw some inferences from prior studies. Louette & De Meester (2008) classified *S. vetulus* as being a good short-distance dispersing species. We found *S. vetulus* and *S. serrulatus* were associated with lower pond densities, such that high pond density might not be necessary for a species that

has high dispersal ability. Conversely, *D. hamata* was associated with higher pond densities in both May and July, where *D. hamata* might need ponds in high densities in order to effectively disperse. *D. brachyurum* was associated with increased distance to temporary (July) and permanent (Sept) ponds, such that dispersal limitation might not be important for this species. However, we could not find any dispersal information for *D. hamata* or *D. brachyurum* from other studies.

Our work highlights the importance of spatial and environmental factors in structuring community composition. We found spatial and environmental variables explained similar amounts of variation in zooplankton composition, with environmental variables becoming stronger in the spring and summer. Specific environmental and spatial variables that explained zooplankton composition varied by month, but temperature was important across many of the months. Temperature is important in determining zooplankton composition, which has important implications for climate change. Indeed, recent studies on warming aquatic systems have found zooplankton will be negatively affected by increasing temperatures (Winder & Schindler 2004, Strecker et al. 2004, Wojtal-Frankiewicz 2012, Thompson & Shurin 2011).

Our work also adds to better understanding of biodiversity in coast plain systems, as this is the first study to our knowledge that investigates zooplankton composition in the CNF. As these systems become increasingly threatened by anthropogenic impacts (Sharitz 2003), a better understanding of what species are present is needed to catalog any changes that might occur to species composition due to future impacts. Future studies could investigate if zooplankton assemblages in ponds are similarly affected by spatial and environmental factors across the coastal plain system, as it ranges from NJ to northern FL. It would be interesting to see if spatial versus environmental factors would differ in importance as spatial scale increased, since

Declerck et al. (2011) found environmental variables were important at the local scale but as spatial scale increased dispersal limitation and neutral processes became more important for cladocerans in High-Andes wetlands. Thus, more work is needed to discern the importance of spatial and environmental variables across different study organisms, study systems, and scales.

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Table 5. Variance partitioning (%) and the associated p-values of the zooplankton data matrix for the different sample months. The different components are: the amount of total variation explained by environment and space [E + S], pure environmental variation [E|S], pure spatial variation [S|E], the spatial structuring in the species data that is shared by the environmental data (Shared), and the unexplained variation. Spatial and environmental refer to the amount of variation accounted for by spatial and environmental variables without taking into account their correlated effects.

Month	Variation	Variance explained (%)	p
SEPTEMBER	S + E	55.39	
	Spatial	31.43	0.013
	Environmental	28.25	0.081
	E | S	23.96	0.131
	S | E	27.14	0.053
	Shared	4.29	
	Unexplained	44.61	
NOVEMBER	S + E	62.06	
	Spatial	38.05	0.002
	Environmental	38.59	0.002
	E | S	24.01	0.067
	S | E	23.47	0.096
	Shared	14.59	
	Unexplained	37.94	
JANUARY	S + E	50.00	
	Spatial	27.97	0.167
	Environmental	27.60	0.199
	E | S	22.07	0.478
	S | E	22.44	0.464
	Shared	5.49	
	Unexplained	50.00	
MARCH	S + E	57.07	
	Spatial	27.93	0.126
	Environmental	33.44	0.014
	E | S	29.14	0.032
	S | E	23.63	0.112
	Shared	4.30	
	Unexplained	42.93	
MAY	S + E	63.51	
	Spatial	32.33	0.09
	Environmental	40.86	0.008
	E | S	31.18	0.038
	S | E	22.65	0.212
	Shared	9.68	
	Unexplained	36.49	
JULY	S + E	78.84	
	Spatial	42.46	0.04
	Environmental	49.59	0.001
	E | S	36.38	0.054
	S | E	29.25	0.169
	Shared	13.21	
	Unexplained	21.16	

Table 6. RELATE analyses showing the correlations (ρ) between individual spatial variables and (i) zooplankton species composition or (ii) environmental variables.

Month	RELATE comparison	
	Zooplankton community matrix (Hellinger)	Environmental matrix (Euclidean)
Variables	Correlation (ρ)	Correlation (ρ)
SEPTEMBER		
SIZE	0.207	0.018
DISTANCE (T)	0	-0.027
DISTANCE (P)	0.003	0.037
DENSITY	-0.092	0.003
NOVEMBER		
SIZE	0.243	-0.060
DISTANCE (T)	0.026	-0.043
DISTANCE (P)	-0.077	0.235
DENSITY	0.140	-0.065
JANUARY		
SIZE	0.081	-0.101
DISTANCE (T)	-0.002	0.175
DISTANCE (P)	-0.049	0.051
DENSITY	0.150	-0.035
MARCH		
SIZE	0.022	-0.103
DISTANCE (T)	-0.028	0.115
DISTANCE (P)	0	0.109
DENSITY	0.162	-0.119
MAY		
SIZE	-0.021	0.020
DISTANCE (T)	0.015	-0.084
DISTANCE (P)	0.030	0.370
DENSITY	-0.034	-0.096
JULY		
SIZE	0.133	-0.110
DISTANCE (T)	0.016	0.009
DISTANCE (P)	0.065	0.129
DENSITY	0.035	-0.094

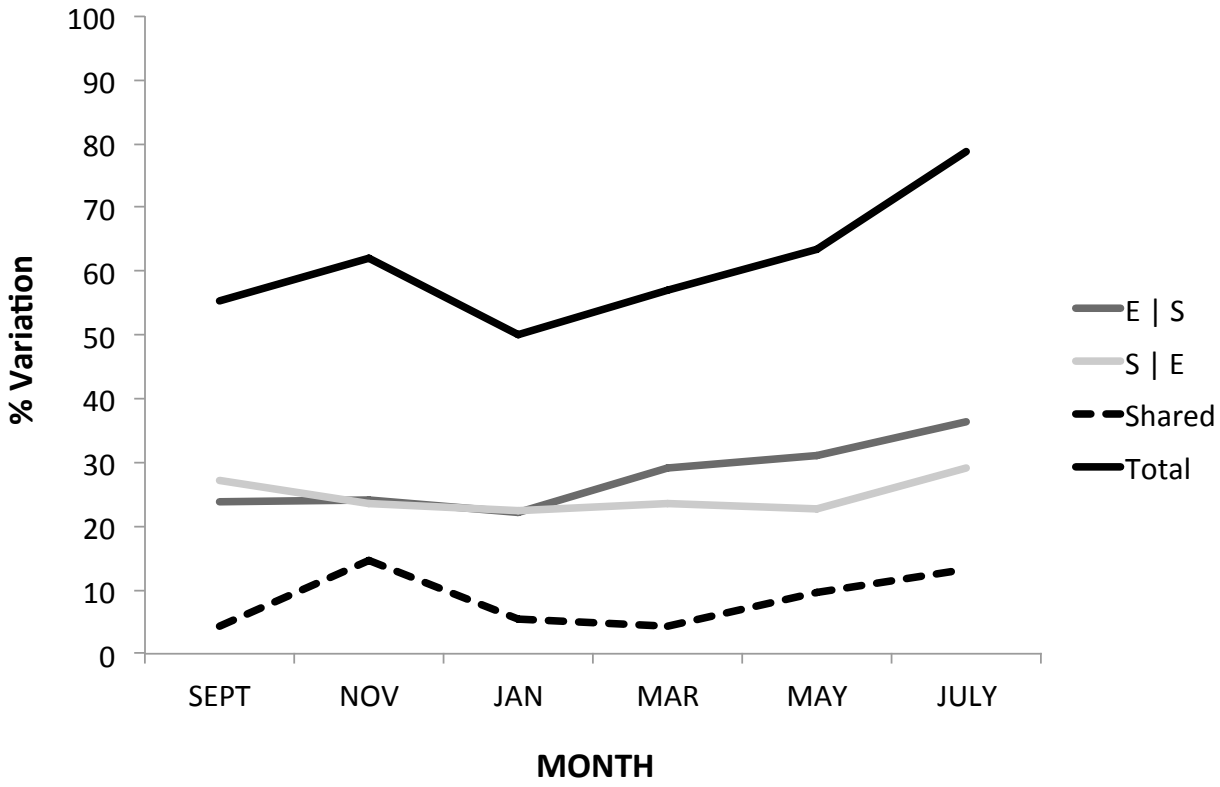


Figure 10. Percent variation explained by the different variance partitioning components across sampling months. Four different components are shown: pure environmental variation, pure spatial variation, the spatial structuring in the species data that is shared by the environmental data, and the total explained variation.

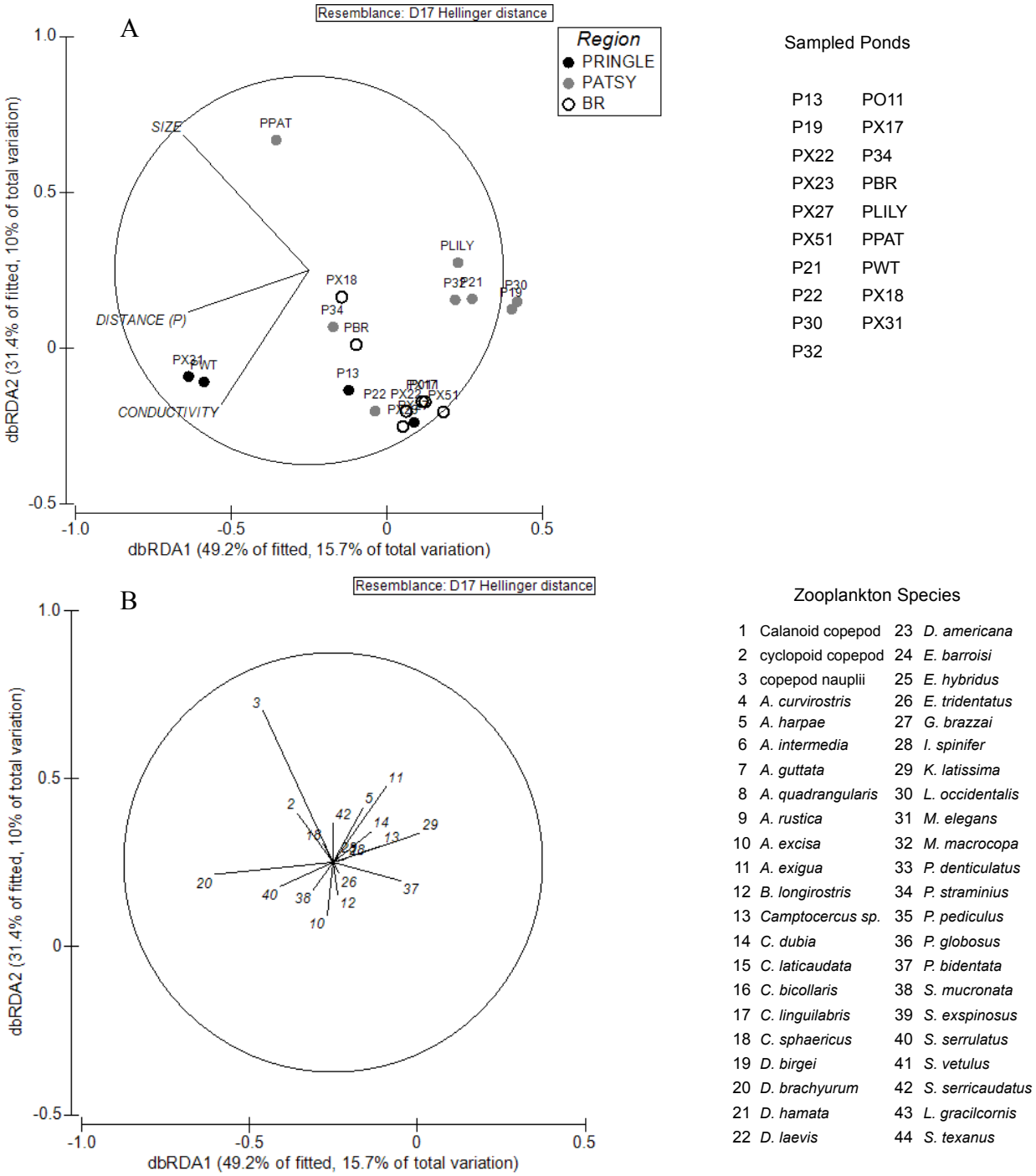


Figure 11. A) dbRDA ordination plots of the community-environment relationship of zooplankton from sampled ponds in the three regions of the CNF during September (see Supplements 3A & 3B for pond information). The different regions are shown by empty, shaded, and filled circles, pond names are listed. B) dbRDA ordination plots of the zooplankton species-environment relationship. Species that contributed at least 5% during September are shown. Each species has a different number, and species names are listed (for full species names see Supplement 3C).

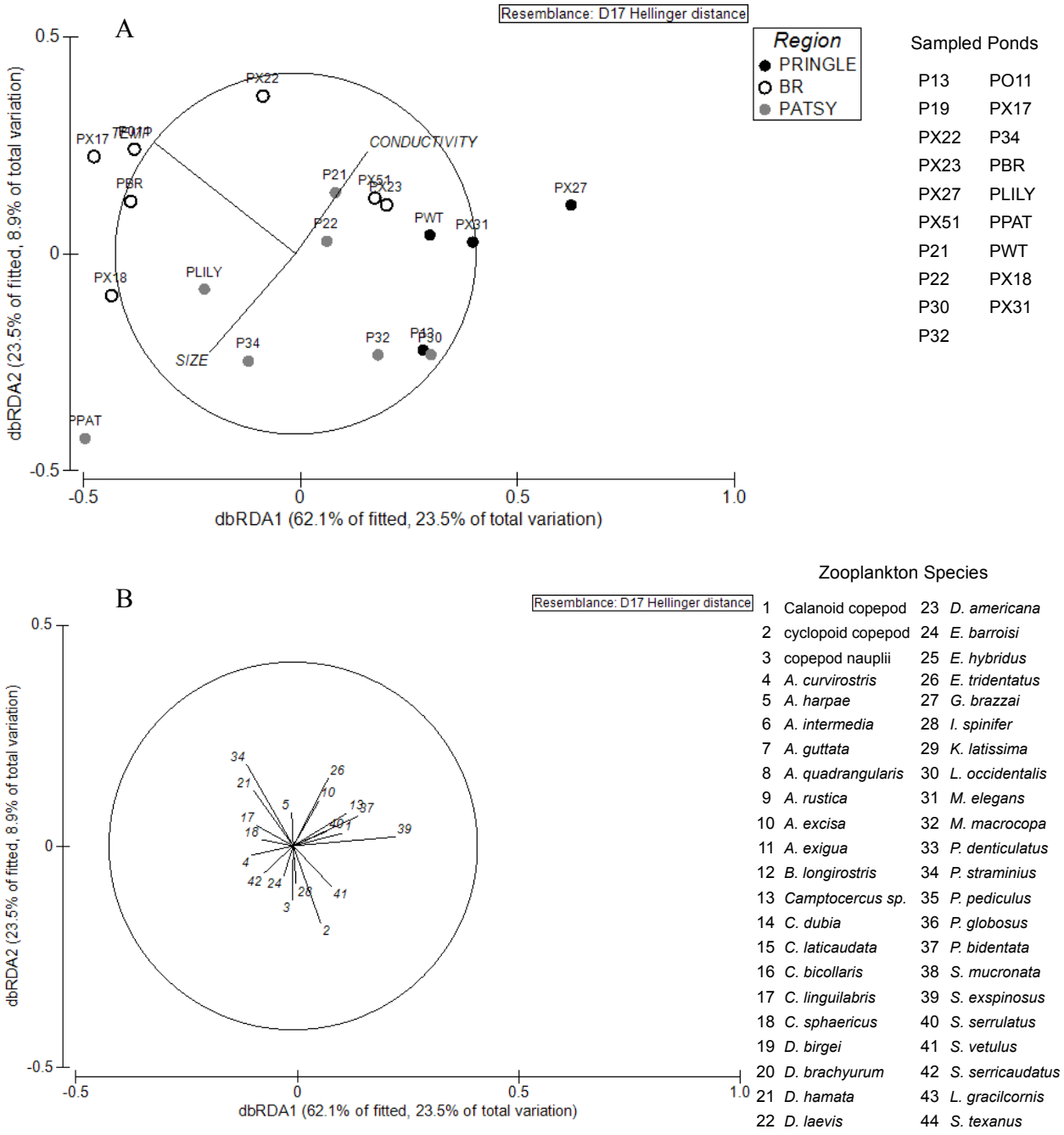


Figure 12. A) dbRDA ordination plots of the community-environment relationship of zooplankton from sampled ponds in the three regions of the CNF during November (see Supplements 3A & 3B for pond information). The different regions are shown by empty, shaded, and filled circles, pond names are listed. B) dbRDA ordination plots of the zooplankton species-environment relationship. Species that contributed at least 5% during November are shown. Each species has a different number, and species names are listed (for full species names see Supplement 3C).

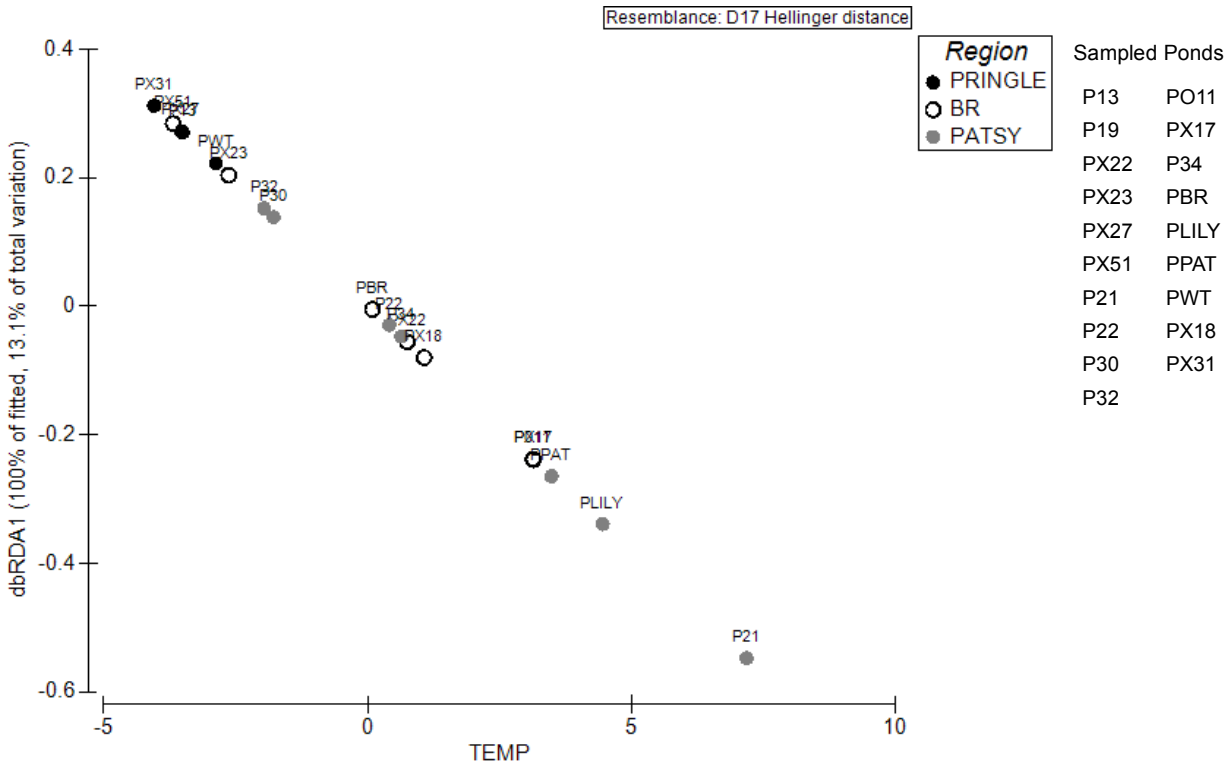


Figure 13. dbRDA ordination plots of the community-environment relationship of zooplankton from sampled ponds in the three regions of the CNF during January (see Supplements 3A & 3B for pond information). The different regions are shown by empty, shaded, and filled circles, pond names are listed.

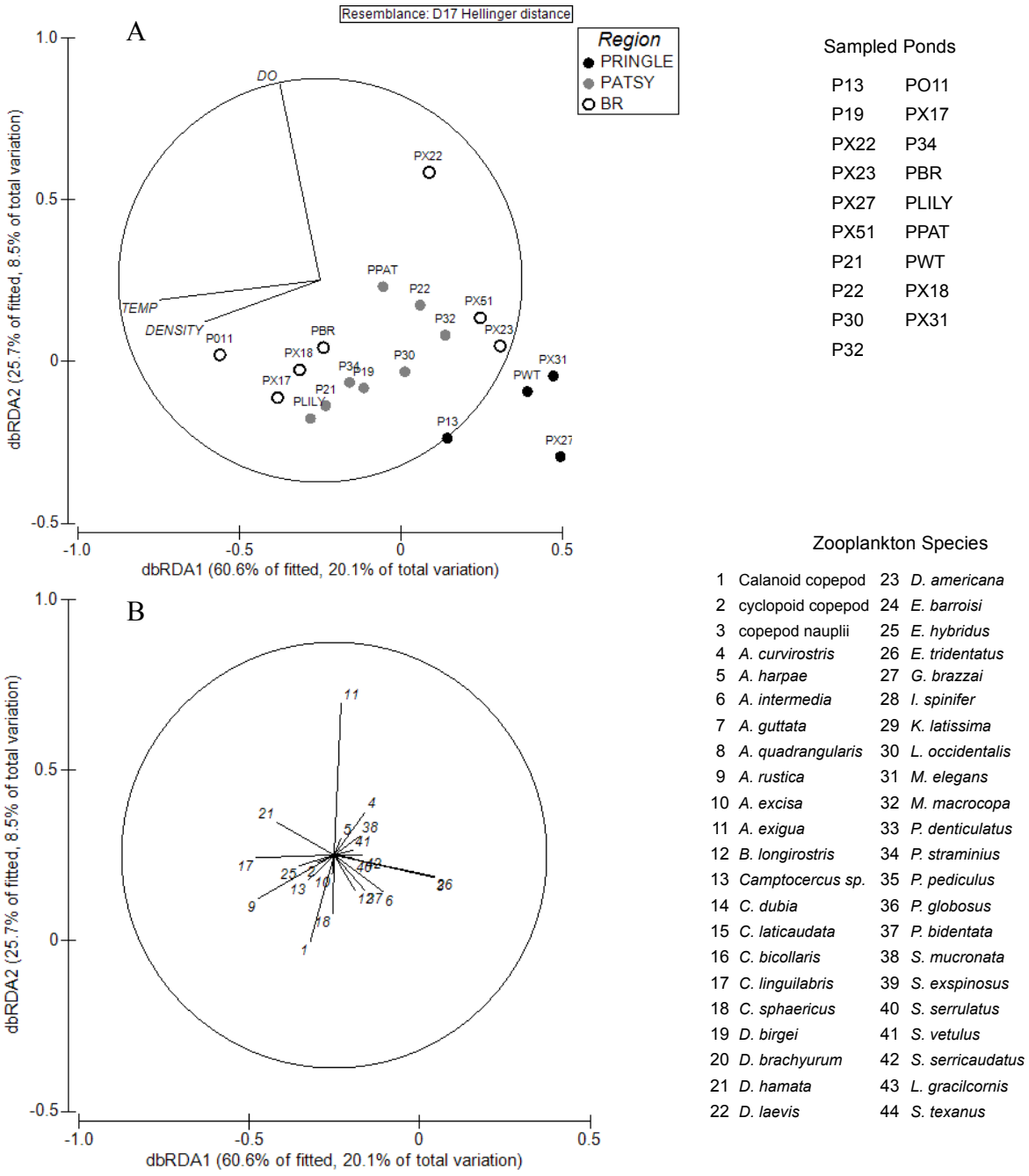


Figure 14. A) dbRDA ordination plots of the community-environment relationship of zooplankton from sampled ponds in the three regions of the CNF during March (see Supplements 3A & 3B for pond information). The different regions are shown by empty, shaded, and filled circles, pond names are listed. B) dbRDA ordination plots of the zooplankton species-environment relationship. Species that contributed at least 5% during March are shown. Each species has a different number, and species names are listed (for full species names see Supplement 3C).

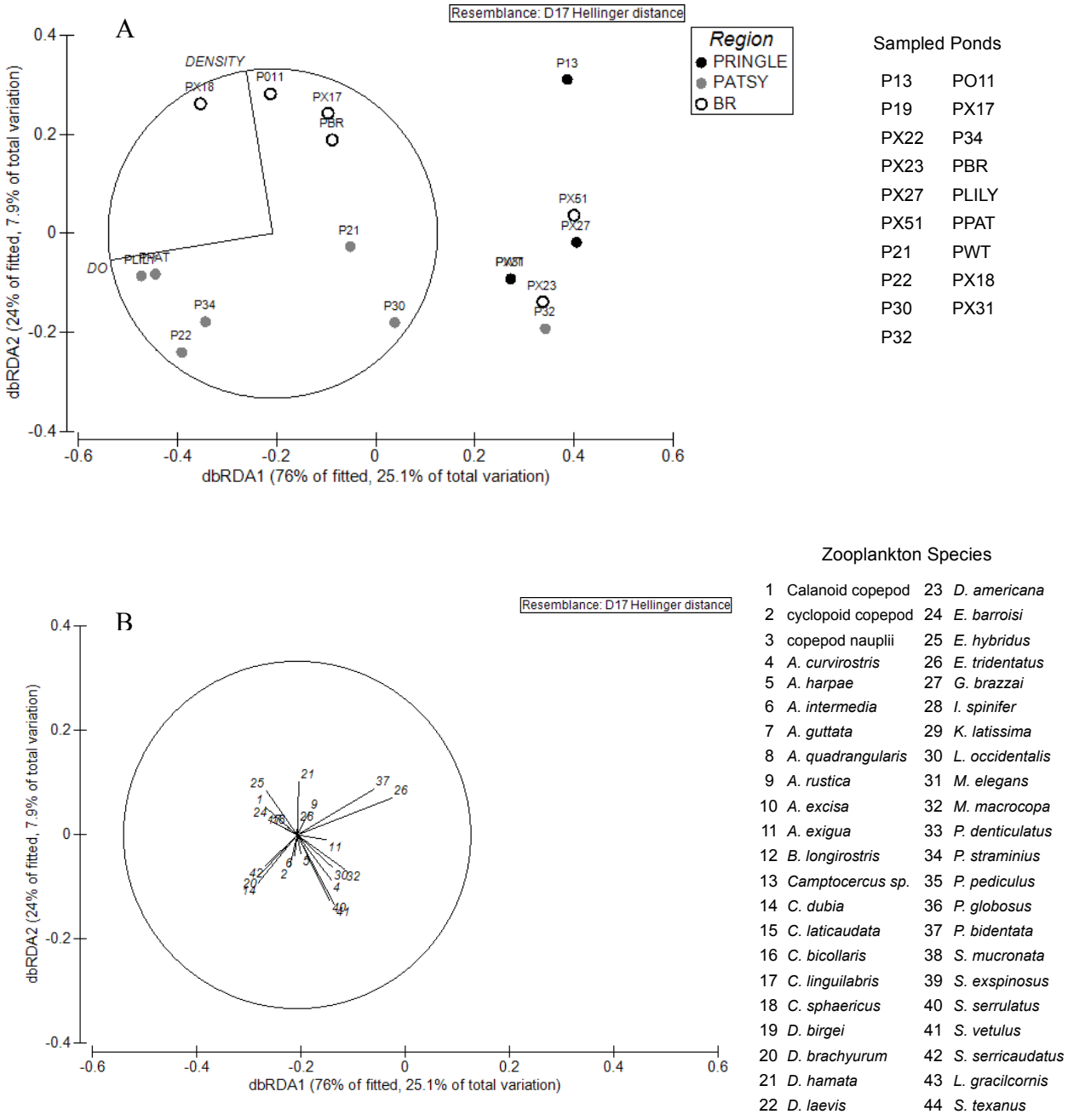


Figure 15. A) dbRDA ordination plots of the community-environment relationship of zooplankton from sampled ponds in the three regions of the CNF during May (see Supplements 3A & 3B for pond information). The different regions are shown by empty, shaded, and filled circles, pond names are listed. B) dbRDA ordination plots of the zooplankton species-environment relationship. Species that contributed at least 5% during May are shown. Each species has a different number, and species names are listed (for full species names see Supplement 3C).

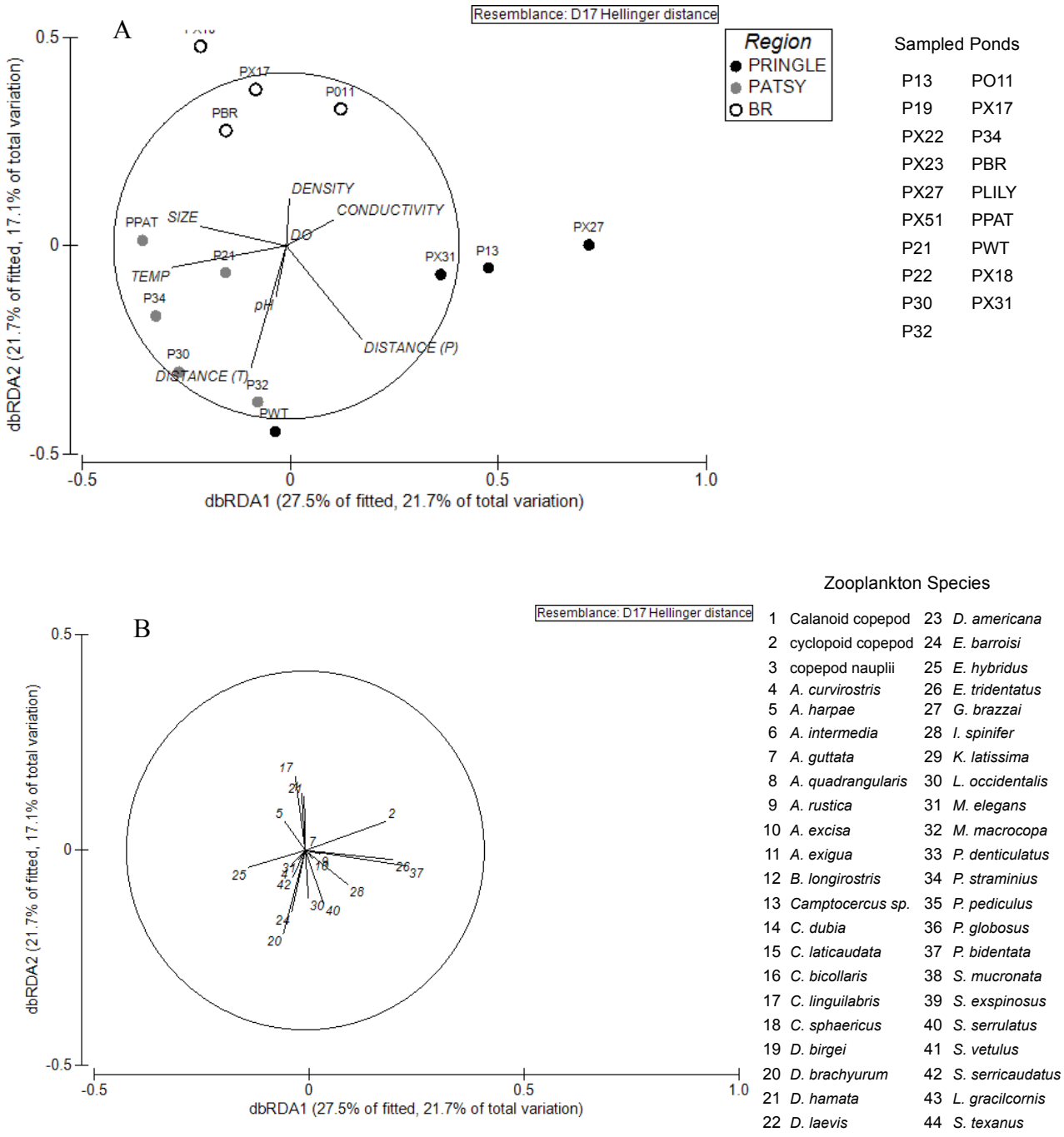
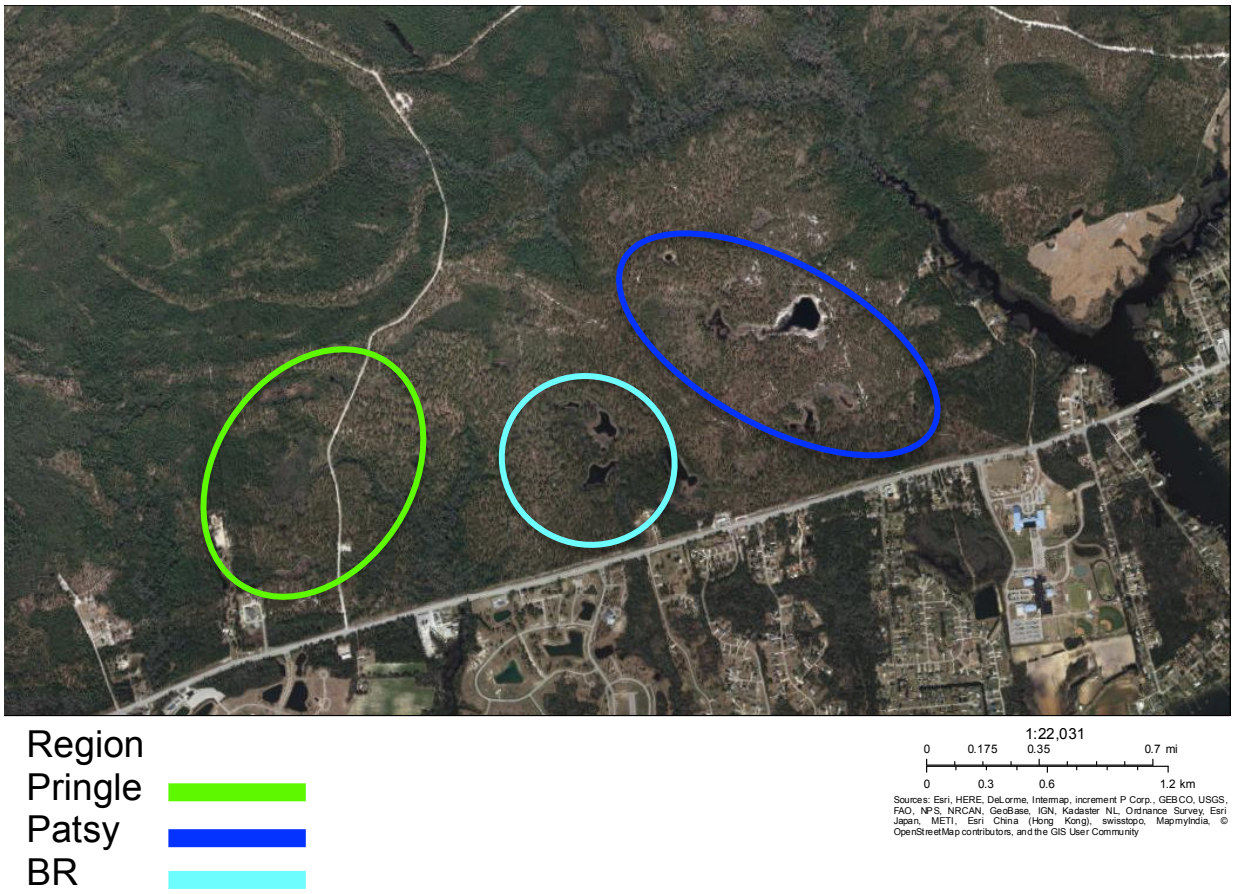


Figure 16. A) dbRDA ordination plots of the community-environment relationship of zooplankton from sampled ponds in the three regions of the CNF during July (see Supplements 3A & 3B for pond information). The different regions are shown by empty, shaded, and filled circles, pond names are listed. B) dbRDA ordination plots of the zooplankton species-environment relationship. Species that contributed at least 5% during July are shown. Each species has a different number, and species names are listed (for full species names see Supplement 3C).



Supplement 3A. The southern end of the Croatan National Park that runs along Highway 24. Circles encompass ponds sampled in the following regions: Pringle road area (green), Patsy pond area (dark blue) and Boat Ramp (BR) area (light blue). Each region is separated by approximately 1 km. See Supplement 2 for more information on ponds sampled in each region.

Supplement 3B – Spatial data for ponds sampled. Ponds belonged to 1 of 3 regions (see supplement 1). Ponds are in order of increasing size for each region.

REGION	Pond Name	Location	SIZE (m^2)	DISTANCE T (m)	DISTANCE P (m)	DENSITY
PRINGLE	PX27	34°43'19.4"N 76°58'55.8"W	112.45	119.11	166.05	3
PRINGLE	P13	34°43'17.5"N 76°58'39.4"W	280.82	25.05	536.2	9
PRINGLE	PWT	34°43'06.3"N 76°58'12.2"W	6831.86	83.6	635.02	2
PRINGLE	PX31	34°43'24.9"N 76°59'02.0"W	7845.83	81.13	635.02	2
PATSY	P19	34°43'21.4"N 76°57'24.8"W	149.42	32.25	128.36	5
PATSY	P30	34°43'38.6"N 76°57'51.4"W	732.51	220.92	82.32	1
PATSY	P22	34°43'27.2"N 76°57'18.7"W	829.74	95.77	308.35	1
PATSY	P21	34°43'23.1"N 76°57'21.3"W	1581.39	69.51	212.75	4
PATSY	P32	34°43'42.4"N 76°58'00.8"W	1708.71	243.39	269.89	0
PATSY	PLILY	34°43'23.5"N 76°57'32.5"W	4986.67	77.75	112.21	4
PATSY	P34	34°43'20.6"N 76°57'39.0"W	8794.45	115.43	112.21	2
PATSY	PPAT	34°43'35.0"N 76°57'38.7"W	18265.35	90.84	197.24	4
BR	PX22	34°43'07.0"N 76°58'21.6"W	195.6	38.6	228.68	3
BR	PX51	34°43'12.7"N 76°58'07.8"W	232.81	193.05	67.33	4
BR	PX23	34°43'06.3"N 76°58'12.2"W	319.66	206.63	181.9	1
BR	PO11	34°43'22.9"N 76°58'19.1"W	588.04	16.37	148.3	10
BR	PX17	34°43'23.1"N 76°58'16.4"W	1640.62	16.37	75.18	9
BR	PBR	34°43'13.9"N 76°58'12.1"W	7784.02	38.44	38.05	8
BR	PX18	34°43'20.3"N 76°58'11.0"W	10459.14	40.52	38.05	10

Supplement 3C. Zooplankton species collected from ponds in the CNF. Numbers correspond to RDA figures.

Copepoda

#	
	Calanoida
1	Calanoid copepod
	Cyclopoida
2	Cyclopoid copepod
3	Copepod nauplii

Branchiopoda

	Cladocerans
	Bosminidae
12	*Bosmina longirostris*
	Chydoridae
5	*Acroperus harpae*
6	*Alona intermedia*
7	*Alona guttata*
8	*Alona quadrangularis*
9	*Alona rustica*
10	*Alonella excisa*
11	*Alonella exigua*
13	*Camptocercus sp.*
16	*Chydorus bicollaris*
17	*Chydorus linguilabris*
18	*Chydorus sphaericus*
21	*Disparalona hamata*
23	*Dunhevidia americana*
24	*Ephemeroporus barroisi*
25	*Ephemeroporus hybridus*
26	*Ephemeroporus tridentatus*
29	*Kurzia latissima*
33	*Pleuroxus denticulatus*
34	*Pleuroxus straminius*
36	*Pseudochydorus globosus*
	Daphniidae
14	*Ceriodaphnia dubia*
15	*Ceriodaphnia laticaudata*
22	*Daphnia laevis*
38	*Scapholeberis mucronata*
39	*Simocephalus exspinosus*
40	*Simocephalus serrulatus*
41	*Simocephalus vetulus*
	Ilyocryptidae
28	*Ilyocryptus spinifer*
	Macrothricidae
4	*Acantholeberis curvirostris*
27	*Grimaldina brazzai*
31	*Macrothrix elegans*
42	*Streblocercus serricaudatus*
	Moinidae
32	*Moina macrocopa*
	Polyphemidae
35	*Polyphemus pediculus*
	Sididae
19	*Diaphanosoma birgei*
20	*Diaphanosoma brachyurum*
30	*Latonopsis occidentalis*
37	*Pseudosida bidentata*
	Laevicaudata
	Lynceidae
43	*Lycneus gracilcornis*
	Anostraca
	Streptocephalidae
44	*Streptocephalus texanus*