ZOOPLANKTON COMMUNITY STRUCTURE AND ECOSYSTEM FUNCTION ACROSS A SALINITY GRADIENT

By Jo Werba

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Pre and post-colonization factors differentially affect community structure. Sea level rise is mixing formerly isoloted freshwater communities with salt water communities. These ponds have a large range of salinity, which is a strong post-colonization factor due to the difficulty of osmoregulation. In this study I seek to understand how changes in pre-colonization factor, dispersal, will affect this system that has a strong post-colonization filter in both community structure and ecosystem function. I manipulated dispersal between mesocosms at four distinct salinities (0ppt, 5ppt, 9ppt, 13ppt). I found that the post-colonization biological filter of salinity is the main driver of both structure and function,with reductions in diversity, decomposition and net primary productivity as salinity increased. This could have dire consequences for our coastal plains where salinity is increasing due to sea level rise.

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Introduction

The theory of island biogeography proposed by MacArthur and Wilson (1967) opened the door for detailed examinations of community organization over space and time. This theory solidified the importance of dispersal, both for establishing new communities, and for regulating the dynamics of existing communities. However, the relative importance of pre-colonization effects, dispersal, versus post-colonization factors (e.g. disturbance regimes, temperature, salinity, priority effects) for regulating community structure is still not well understood. Metacommunity theory is a contemporary extension of island biogeography that is focused on explaining the relative importance of pre and post colonization factors for predicting the structure and dynamics of spatially patchy ecological communities (for a full review see Leibold et al., 2004).

Empirical studies examining the effects of dispersal on community structure (e.g. diversity) have found that the effects of dispersal on community structure depend on spatial scale (Shurin et al., 2000; Amarasekare and Nisbet, 2001; Cadotte, 2006), the collection of species already present in a patch (priority effects) (e.g. Chesson, 2000; Geange and Steir, 2009) and the colonization history of the site (Cadotte, 2006; Drake, 1991; Law and Morton, 1993; Price and Morin, 2004). Dispersal rate can directly affect how important dispersal is to overall diversity (Jones et al., 2015; Logue, 2011). For example, Cadotte (2006) found intermediate dispersal rates increase local (alpha) richness but low rates of dispersal increase regional [gamma] richness. Similarly, fragmented (e.g. reduced dispersal) habitats have been shown to affect species richness and increase extinction in some communities (Staddon et al., 2010; Gilbert et al., 1998).

Besides maintaining richness, dispersal has also been suggested to be an important process in maintaining ecosystems by providing insurance of ecosystem function and stability via introduction of functionally redundant species (Yachi and Loreau, 1999). The insurance hypothesis assumes that more diverse ecosystems are more likely to have species that are ecologically redundant and therefore these ecosystems will be less responsive to environmental perturbations (Hollings et al., 1995; Folke et al., 1996; Yachi and Loreau, 1999; Gonzalez and Loreau, 2009). This can occur if species with similar ecological roles respond differently to the same disturbance; the more tolerant species can functionally compensate for the less resilient species, which makes aggregate community or ecosystem measures more predictable (i.e. stable) (May, 1972). In metacommunities, this phenomenon has been called the *spatial* insurance hypothesis (Loreau et al., 2003), whereby dispersal from a diverse regional species pool compensates for low local [alpha] diversity, which buffers both the local and regional communities against perturbations.

The spatial insurance hypothesis assumes that dispersal is a particularly important force affecting community structure and function following disturbance. For example, Symons and Arnott (2013) found that while dispersal rates did not change diversity in undisturbed systems, recovery of diversity in perturbed systems was more likely in when dispersal rates were higher. Similarly, highly connected communities of rotifers and protozoans with high dispersal rates have been shown to recover faster after disturbance (Altermatt et al, 2011) than communities with low or no dispersal. However, the spatial insurance hypothesis was developed for ecosystems characterized by relatively high diversity, and consequently most empirical tests of the hypothesis have also focused on diverse communities. Less attention has been paid to systems that are characterized by low diversity and high productivity, such as estuaries and salt marsh systems. Understanding how characteristically low diversity meta-communities respond to perturbations or how mixing of low and high diversity communities via the effects of climate change or land use changes will affect patterns of diversity and ecosystem function remains an open question.

In general, it has been suggested that ecosystems can maintain function (are more resilient) better than individual populations (Schindler, 1990; Vitousek, 1990), which is logically consistent with the predictions of the spatial insurance hypothesis. However, empirical support for the spatial insurance hypothesis has been mixed and shown to vary according to the rate of dispersal (Loreau et al., 2003), the specific ecological system under study (Altermatt et al., 2011; Staddon et al., 2010; France and Duffy, 2006; Symons, 2013), and the occupants of the resident community (priority effects) (Jenkins and Buikiema, 1998; Körner et al., 2007; Geange and Stier, 2009). In some cases these patterns in richness also resulted in differences in ecosystem measures (e.g. CO2, nitrogen (Staddon et al., 2010); primary productivity (Symons and Arnott, 2013; Leibold 1999; Dodson, 1992). In the face of impending environmental change, it is imperative to improve our understanding of how diversity and connectivity function to buffer ecosystem functions.

In this study I will investigate how secondary salinization from sea level rise, coastal storm events, and land use practices are affecting coastal wetlands. These processes are both mixing formerly allopatric salt and fresh water communities as well as causing disturbances through flooding and by creating an extreme salinity gradient in coastal wetlands (Doney et al., 2012) via inundation, erosion, and saltwater incursion (Scavia et al., 2002). The effects of these changes in coastal wetland communites are hard to predict because highly productive but low diversity brackish water communities are mixing with more diverse freshwater communities. Indeed, it is unclear whether current meta-community theory and the spatial insurance hypothesis apply to these systems. The effects of a strong ecological gradient and high cost of osmoregulation in different salinity waters poses a physiological barrier for organisms (Kefford et al. 2007; Holst and Peck 2005; Kirst, 1989; Roddie, B.D. et al. 1984) Thus, most species are specialists and only able to tolerate a narrow range of salinities (e.g. Sarma et al. 2005; Bate et al. 2002; Kirst, 1989; Griffith, 1974). In general, there are higher numbers of species that are able to live either in freshwater or high salinity water (e.g. 35ppt- ocean water) than in mid salinities brackish water found in many estuarine systems (Telesh and Khlebovich, 2010). This scenario likely contradicts expectations of the spatial insurance hypothesis because increased mixing of fresh and salt water communities will also increase regional diversity but species many species may not be able to persist in the new osmotic environment. Alternatively, species that are able to survive but not reproduce in sub-optimal salinity ranges may be able to be maintained via dispersal.

In this study, I investigate the composition of pond zooplankton communities across a large salinity gradient, that is characteristic of many coastal isolated wetland pond systems along the eastern coast of the United States where sea level rise is expected to have large impacts (Sallenger et al, 2012). Indeed, many coastal wetlands are already experiencing increased salt water incursions (e.g. Bezirci et al, 2012). For example, natural inland pond communities in North Carolina are experiencing an emerging salinity gradient (Albecker and McCoy, personal communication) (>18-0 ppt) over short (<1mile) distances. While there is an increase in disturbance from saltwater intrusion and an increase in connectivity through flooding, it is unknown if the spatial insurance hypothesis can buffer ecosystem function in this system where there are such strong abiotic pressures and shifts in community structure.

Study system

Zooplankton are useful models for studying the spatial insurance hypothesis because in freshwater wetlands they have been shown to have strong top-down effects on primary producers (Arner et al., 1998). For example, copepods eat microzooplankton which eat phytoplankton. This is an important trophic cascade, that may affect total phytoplankton abundance and affect availability of nitrogen and phosphorus for photosynthesis (Kimmel, 2011; Roddie et al.,1984). And while salinity is hypothesized to be a primary determiniate zooplankton community structure (Breckenridge et al., 2014;Kimmel, 2011;Bate et al., 2002) some estuarine species such as *Eurytemora affins* have been shown to tolerate salinities from 0-15ppt (Appletans et al., 2003; Devreker, 2009) and *Acartia tonsa* to tolerate from 5-30ppt.

Zooplankton have also been the predominant models for research aimed at understanding metacommunity ecology and for testing the spatial insurance hypothesis in aquatic metacommunities (May, 1972). Most research on zooplankton metacommunity dynamics has been focused on isolated interior freshwater pond systems (Heino, 2011; Leibold, 2004). Estuarine habitats, such as the one our study models, tend to be dominated by *Acartia* and *Eurytemora* (Roddie, B.D. et al. 1984). Even in these relatively salinity tolerant species salinity can affect hatching (Holst and Peck 2005) and therefore dispersal and colonization success. Colonization success also depends on the ability to quickly adapt to a new salinity. Therefore, it is unclear if our current understanding of zooplankton metacommunity dynamics can be extrapolated to understand the processes determining the structure of zooplankton communities along an extreme environmental gradient where formerly isolated communities are now mixing.

Freshwater systems are expected to be greatly affected by global climate change (Millennium Ecosystem Assessment, 2005). Changes in salinity due to salt water intrusion and drought could cause shifts in community structure because salt tolerance is a specialized adaptation. Therefore, as seawater invades freshwater systems, environmental filtering (e.g. salt and fresh water tolerance) is expected to be a major driver of community composition. Similarly, because maintaining osmotic balance requires high energy output few species are competitively able to survive therefore species sorting (priority effects and species interactions) is also expected to affect the development of communities in new brackish conditions created by saltwater intrusion. Whether these new communities maintain ecosystem function is not known.

In this study I seek to understand how these novel relationships may be affecting community structure and function. I hypothesize that community structure in fresh and highly saline pools will be driven by priority effects exerted by existing communities because the species that are able to tolerate extreme salinities will be much more competitive than any invader, whereas intermediate saltwater intruded pools will have communities driven by dispersal because few or no species will be highly competitive so species will be maintained predominantly through dispersal (mass effects); and in contrast to predictions from the spatial insurance hypothesis, I expect pools with intermediate salinities to have high diversity via dispersal and high species turnover due to the presence of a strong biological filter but reduced ecosystem functions relative to pools with high or low salinity because of the instability of the communities.

Methods

On May 5, 2015 I collected zooplankton from five ponds near the inner and outer banks of North Carolina. Twenty 1-liter samples of water were collected and strained through 62.5ul mesh filter across a single 100 m transect at each pond. A few ponds were too small to complete a full 100 meter transect and so a second transect was used. These zooplankton were added to our experimental tanks with salinities that matched the zooplankton source ponds. These zooplankton samples served as the starting communities for the experiment. Mesocosms were 567 liter stock watering tanks (filled to 378 liters) with the same salinity as the source ponds. Tanks had salinities of 0, 5, 9 and 13ppt. Each tank was randomly assigned a salinity and dispersal treatment, which consisted of either zooplankton from 13ppt ponds or 50% (by water volume) from freshwater and 50% from 13 ppt ponds (Figure 1). The experiment was replicated four times. Tanks were initially seeded with peat moss to provide a nutrient pulse and the bottoms were covered in sand as a benthic substrate. Mesocosms were covered with 60% shade cloth to prevent macroinvertebrates from and other organisms from colonizing. On June 1, 2015 due to low abundance all tanks at 13ppt salinity were reseeded with zooplankton. To allow populations to stabilize dispersal treatments began 6 weeks after initial seeding on June 11 and 12th, 2015. The dispersal treatment consisted of 2 L of water from the dispersal source tanks. Therefore, actual abundances at each dispersal event varied as the experiment progressed (see table 1 for exact abundance). The treatments will be referred to as “salinity-mixed” for the dispersal treatment consisting of both freshwater and 13ppt, or “salinity-single” for the dispersal treatment just from 13ppt (e.g. 5-mixed is a 5 salinity community receiving both freshwater and salt water communities in the dispersal).

Tanks were sampled every 9 days because for many species this is long enough to complete one-generation cycle (Thompson and Shurin, 2012). Prior to sampling, each tank was well mixed by stirring them in a circular motion around the perimeter five times. Twenty Liters (approximately 5% of total volume) of water was sampled from 20 random locations using an integrated tube sampler. The samples were condensed into 25mL through a 62.5um filter. Zooplankton was preserved in 10% Formalin. For each tank at the time of sampling I measurd DO, NH4, temperature and pH were also measured with YSI pro.

Zooplankton were counted in three 5 mL subsamples and identified to order or genus when feasible using Johnson et al., 2012 and Pennak, 1989. Some studies have suggested that functional groups rather than richness are more appropriate to consider when addressing the spatial insurance hypothesis (e.g. Symons and Arnott, 2013; Barnett et al., 2007); thus only identifying to order should generally be able to address our questions about function.

Testing for effects on community assembly and diversity

To examine alpha diversity I compared “effective” diversity calculated by the exponentiating estimates of Shannon diversity (Jost, 2006) because effective diversity weighs each species precisely by relative abundance (Jost, 2006). I also measured species richness as functions of dispersal treatment and salinity using general linear models with Gaussian errors. Total zooplankton abundance was evaluated using a generalized linear model with the negative binomial distribution using the Mass package in R (Venables and Ripley, 2002). I use negative binomial errors because my abundance data were highly over-dispersed. All general linear models were evaluated using standard F-statistics.

To examine turn-over (beta-diversity), I compared the Whittaker index of beta diversity across salinities for the dispersal treatments. I used this index because it allowed for comparisons that are not strictly pairwise (e.g. jaccard's similarity only allows for comparisons between two locations). Whittaker beta diversity is calculated as gamma (regional diversity)/ alpha (local diversity). Gamma diversity was assumed to be all species found across treatments, while alpha diversity was quantified as richness in individual tanks. I used all of species present across treatments as gamma in order to examine how the potential disperser pool changed community structure and function. Also, since all the natural ponds are in close proximately there should be no barrier for dispersal in the natural system. I used a linear model with a quadratic term for salinity to evaluate the relationship between beta diversity and salinity. Indeed, Beta diversity changed nonlinearly across salinities at the beginning of the experiment, but by the end of the experiment the quadratic term no longer explained the pattern so a simple linear model was used. Inferences for beta diversity are based on tests of parameters using standard F-statistics.

In addition to the Whittaker index, turnover between communities (beta diversity) was also evaluated using nonmetric multidimensional scaling (NMDS) plots based on Bray-Curtis distance matrices. For these analyses one replicate of a 5-mixed treatment was removed because abundances were below our detection limit in our original sample. NMDS were checked for goodness of fit using a stress plot and r-squared values. All fits had r-squared > 0.9. NMDS outputs were compared using analysis of similarity ANOSIM. For any communities that were considered different based on ANOSIM a follow up SIMPER analysis was run in order to determine the species having the largest influence on the difference between communities. NMDS, ANOSIM, and SIMPER analyses were run in R using the Vegan 2.3.3 package (Oksanen et al, 2015).

Ecosystem Function

I assess effects of salinity and zooplankton, and water transfers (presumably also containing phytoplankton, and microbial communities from source ponds) on ecosystem functions using two different proxies for ecosystem function: net ecosystem productivity (NEP), and decomposition rates. NEP was assessed via the continuous diel oxygen method (Sala et al., 2000) using Onset DO data loggers that logged DO at 15 min intervals over 9 day intervals. To examine the effect of treatment on NEP I ran linear mixed effects models with time block, the 9 day intervals, treated as a random effect using the lme4 package in R (Bates et al., 2015). Likelihood ratio tests were used to test for significant treatment effects and inferences among treatments are based on comparisons of model predictions and confidence intervals (Bates et al., 2015, Bolker 2015).

Decomposition was quantified by the difference in dry weight of leaves in decomposition bags from the beginning to end of the experiment. Three different species of plant litter were used in each tank to represent different habitat types, *Spartina sp* found in salt marshes, *Acer rubrum* found in freshwater wetlands; *Phragmites australis* found in both fresh and salt-water wetlands as well as being a successful invasive. Leaves were harvested and air-dried in late May. Leaves were then weighed (maples (4.00g stdev +/- 0.01), *Spartina sp.* (6.99g stdev +/-0.03*),* *Phragmites australis.* (10.01g stdev +/-0.03). *Phragmites australis* and *Acer rubrum* were put into 24” mesh mariculture bags into each tank. *Spartina sp.* was put in metal mesh that had smaller holes so that it would not fall out. The leaf litter remained in the tanks for the duration of the experiment. On day 45 the bags were removed and air-dried for and then oven dried for 48 hours and weighed. Separate generalized linear models were run for each litter type to compare total decomposition between treatments. We ran a linear model with salinity and dispersal as fixed effects. Inferences are based on standard F-statistics. Data were transformed using a log-difference to account for non-normality.

Results

Alpha Diversity

True diversity was used for estimating alpha community diversity in individual pools. There was a significant interaction between salinity and time (F = 5.4143, df = 6,185, p = 0.05). There was also a significant effect of the quadratic salinity term (F=5.0761, df = 6,185, p=0.007). At intermediate salinities (5 and 9 ppt), alpha diversity increased over time while in the freshwater and 13ppt treatments alpha diversity declined (figure 2).

There was a different response when using species richness as an index for alpha diversity. Richness declined on average over time (z= -1.65, p = 0.03, df = 257) (figure 3). There was also a significant effect of salinity (z=-4.492, df = 257, p <0.01) with freshwater having almost double the initial richness compared to the 13ppt pools (figure 3).

I also tested for differences in the total abundance of zooplankton independent of species and found that while diversity decreased, total abundance of zooplankton increased in all treatments over time. Both salinity (z = 2.878, df= 257, p < 0.01) and time (z = -4.218, df = 257, p < 0.01) were significant predictors of abundance. Abundance decreased with increases in salinity (figure 4).

Community Structure (NMDS analysis)

Initial community structure differed between salinities as expected (R=0.377, p=0.001) (figure 5). [Note: Negative R-values suggests that values within group are more variable than between groups, R ~ 1 suggests groups are different and R-values close to 0 suggests that groups are similar.] At the start of the experiment the freshwater communities were clustered and separated from the 5-13 ppt communities. The community structure in freshwater was heavily influenced by the presence of Daphniidae and Cyclopoidia, while higher salinities were dominated by *Acartia*, other members of Calanoida and Harpactoida. After 6 weeks the freshwater community structures still clustered together and were separated from all of the other salinity treatments (figure 6). This difference was mainly driven by the presence of Daphniidae in the freshwater treatments. There was additional separation between the 5 ppt communities (receiving both dispersal treatments) and the 9 and 13 ppt treatments.

The ways in which community compositions changed over time varied among the different salinity treatments. All of the freshwater treatments were similar as expected at the start of the experiment (R value -0.0625, p = 0.694), and their compositions changed significantly over time (R value 0.5972, p=0.001). However, while they diverged from their original starting compositions, they did not become different from each other (R value 0.07639, p=0.262), but, became more variable in composition (see figure 7). Cyclopoida and Chydoridae were important drivers of the observed changes in composition over time.

All mesocosms at 5 ppt salinity also had similar communities at the start of the experiment (R= -0.2037, p= 0.813) and changed significantly over time (R=0.1739, p=0.067). At this salinity, however, dispersal treatment had a significant effect on community development over time, with the mixed salinity dispersal treatments becoming less like the original communities (figure 8). This change over time was associated with large increases in the numbers of Ostracoda and *Acartia* in these ponds. Cyclopoida and Ostracoda accounted for most of the difference between dispersal groups at the end of the experiment.

The 9 ppt communities also changed significantly over time (R =0.3163, p=0.02), but in contrasts to the 5 ppt treatments, these communities became more homogenous over time (figure 9) and the type of dispersal did not cause divergence among the communities (day 1 R= -0.1296, p=0.744; final day R=-0.07407, p=0.581). However, these communities were highly variable (negative R values) which may suggest that they had either high turnover or synchronous dynamics. While differences in the initial communities were associated with Cyclopoida and Calanoida. The difference between the initial and final communities were driven primarily by Harpactoida and Calanoida.

The 13 ppt treatment also had similar initial communities (R= -0.1875, p=0.975), that changed over time (R= 0.2277, p= 0.021) (figure 11). However the communities remained similar to each other regardless of dispersal type (R=0.25, p=0.091). (Figure 10). Differences over time were predominately accounted for by Harpactoida and Calanoida.

Beta Diversity

At the beginning of the experiment there was a nonlinear effect of salinity on beta diversity with higher beta diversity among pools with intermediate salinity compared to either freshwater or 13 ppt salinity (F=14.98, df=2,4, p=0.013) (figure 11). On day 45 this relationship was no longer maintained. Instead I saw a linear relationship. I found a marginally significant relationship between turnover and salinity (t=2.02, p=.078) but no significance based on dispersal treatment (t=-0.241,p=0.81) (figure 12).

Ecosystem Measures

Primary Production

I found a significant effect of salinity and dispersal treatment (chi-squared = 21.002, df = 1, p<0.01) on net ecosystem production, with net production declining with increasing salinity. The freshwater control treatment had the lowest net productivity; whereas salt-water only and mixed salinity dispersal had 38% and 80% increase in productivity respectively (figure 13).

Decomposition

I found a significant inverse relationship between salinity and the amount of decomposition for three leaf types; *Acer rubrum* leaves (F=15.87, df=1,32, p<0.01) (figure 14), *Spartina sp* (F=34.47, df=1,32, p<0.01) (figure 15), and *Phragmites australis* (F=7.04, df=1,32, p<0.01) (figure 16). There was also no significant effect of dispersal type on decomposition rate for any of the leaf types (*Acer rubrum* t=0.548,p=0.548; *Spartina sp* t=1.056,p=0.3), however decomposition of *Phragmites australis* was ~13% greater in the communities saltwater dispersal (t=1.82,p=0.07) (figure 16).

Discussion:

One of the predicted effects of global climate change is that ecological communities that were historically distinct will become merged creating new interaction networks (Parmesan, 2006). However, the effects of such changes on species diversity or on natural ecosystem functions have not been well explored. The spatial insurance hypothesis predicts that increasing regional diversity should increase the potential for functionally redundant species to be present and thus help to maintain ecosystem stability (Loreau et al., 2003). Thus, while the mixing of previously distinct communities from environmental change may have dire consequences for some species, in general increased capacity to maintain ecosystem functions in the face of those same environmental perturbations might be expected. However, this study simulated the effects of increasing salinities and mixing of salt water zooplankton communities into freshwater ponds that are being affected by saltwater incursions, which creates a large abiotic gradient that regardless of proximity and dispersal can limit the ability for species to colonize. This scenario highlights a gap in our understanding of mechansims that maintain biodiversity and ecosystem function in estuarine systems that are characterized by high productivity and also low diversity (Telesh and Khlebovich, 2010). As expected, salinity created a strong biological filter that influenced both structure and function of ecological communities (e.g. Sarma et al., 2005; Bate et al., 2002). The effects of salinity on species richness and diversity were larger than any post colonization effects. This is particularly evident in the freshwater and 13ppt communities where we see only a small change over time and no changes within treatment based on dispersal (figures 8 & 11). However the direction and magnitude of the salinity and dispersal effects varied depending on which measures of structure and function I examined.

The amount of dispersal among habitat patches strongly influences how communities develop and change over time. In general, it is expected that intermediate levels of dispersal should create the most diverse communities (Mouquet and Loreau, 2014; Cadotte, 2006; Mouquet and Loreau, 2002; France and Duffy, 2006), but high levels of dispersal will result in decreases in local richness (Mouquet and Loreau, 2014; Forbes and Chase, 2002; Mouquet and Loreau, 2002) since high levels of dispersal favors the most abundant species. However, in heterogeneous landscapes dispersal affects communities differently (Matthiessen et al., 2014) since species compete differently in different local patches. Specifically, along environmental gradients that are increasing in severity due to climate change, intermediate dispersal increases local richness. In contrasts, in a homogenous landscape species diversity is expected to be highest when dispersal is low (Matthiessen et al., 2014).

In this study, the strong biological filter imposed by our salinity gradient likely swamped out the effects of dispersal. The small effects of dispersal may have stemmed from low ambient abundances in our dispersal source populations (relative to high dispersal rate studies e.g.Thompson and Shurin, 2012; Vanschoenwinkel et al., 2008). However, our two dispersal treatments had very different abundances, with the saltwater only dispersal having much lower abundances than the mixed salinity dispersal, thus I would have expected to see differences among these two dispersal types. Even though the mixed salinity dispersal treatment had greater abundances overall, fewer individuals could survive in the higher salinities. This hypothesis is further supported by the observed patterns in alpha diversity. For example, true diversity increased over time in the intermediate salinity ponds regardless of dispersal type suggesting that local dynamics were more important than dispersal for driving community structure in this connected system (Cottenie et al., 2003). Moreover, salinity of individual pools did not vary much over time (that is little disturbance) and this may have also reduced the influence of dispersal. Indeed, the spatial insurance hypothesis is not well supported in systems that have low levels of disturbance (Symons and Arnott, 2013), because priority effects and competitive dominance hierarchies determine which species persist (e.g. Geange and Stier, 2009).

While alpha diversity was predominately affected by salinity as seen by the decline in richness across salinities, beta diversity was affected by both treatments. Beta diversity was highest in intermediate salinities at the beginning of the experiment, likely because these treatments could accommodate more tolerant fresh and saltwater species. Interestingly, by the end of the experiment there was no longer a significant difference in beta diversity across salinities, which is consistent with the model by Filotas et al. (2010) suggesting the system is functioning as a single community. These results are expected when there is high dispersal, which suggests that despite very low abundances in our manipulated dispersal treatment, the manipulated dispersal rates may still be high relative to ambient rates of dispersal assuming the initial communities were in an equilibrium state. The weak relationship between salinity and beta diversity may suggest that while salinity is still influential dispersal dynamics are creating more homogenous communities.

Interestingly, the 5 ppt salinity treatments increased in beta diversity over time. This makes sense given that 5 ppt is considered a biological boundary for many species (Brand, 1984 (salt tolerant); Sorma et al., 2005 (freshwater species)), so very minor changes in salinity due to rain or minor variations in individuals affecting their ability to survive could more greatly influence community structure at this salinity. Still the five communities end more similar to higher salinity communities rather than fresh. Based on the NMDS plots (figure 10) the 9 ppt communities' turnover decreased over time also supporting the idea of a biological filter. Landscape heterogeneity should be most important factor in beta diversity and across extreme environemtnal gradients rescue effects of dispersal are not expected (Limberger et al., 2014); our study supports this finding.

Based on the *spatial* insurance hypothesis high rates of dispersal were predicted to rescue ecosystem functions (Loreau et al., 2003). However, in this study net primary production decreased across the salinity gradient challenging the spatial insurance hypothesis. In contrast, net ecosystem production increased with dispersal lending support to the spatial insurance hypothesis. While some studies have suggested that changes in zooplankton community structure can influence primary production (e.g. with decreases in Daphnia there are increases small bodied zooplankton (cyclopoids) and higher primary production (Arnѐr et al., 1998). In our study we observed very few daphnids and very high abundances of cyclopoids in the freshwater treatments, which may lend support to this earlier finding that fewer daphnids are correlated with lower rates of primary production. While other studies suggest that using indirect measures of primary production are less likely to show clear patterns (Groner and Novoplansky, 2003) it appears even with small differences in dispersal I were able to detect changes in primary production in this system.

In contrasts to the findings for NEP, in this study there were no differences in decomposition based on dispersal treatment. This may be a result of the strong effects of salinity on the microbial communities, which ultimately control decomposition in this system. As expected maple decomposed most in freshwater, but so did *Spartina sp.*, which was unexpected given that *Spartina sp*. is not found in freshwater systems. *Phragmites australis* decomposed fairly similarly across all salinity treatments and as it is ubiquitous in wetlands of all types this is unsurprising. These plants have different rates of decomposition (e.g *Phragmites australis* decomposes more slowly than *Spartina sp.*(Windham, 2001)) and mixtures of leaves decompose differently than species alone (Gartner and Cardon, 2004), so future work should include mixtures not only of microbial communities but also plant communities since these systems are likely to become more connected.

Species diversity, primary production, and decomposition all decreased as salinity increased in this study, but the effects of dispersal were less clear. While the more abundant and more diverse dispersal treatment (mixed salinities) is predicted to provide a more resilient rescue for both diversity and function, the strong biological filter of salinity overwhelemed these effects in this study. As increases in dispersal and habitat perturbations become more common as a result of sea level rise and other habitat modifications, understanding how extreme gradients and changing patterns of connectivity could impact community structure and ecosystem functions will become increasingly important (Root et al., 2003). While this study is an important first step toward understanding how mixing of communities along an extreme gradient will affect local and regional patterns of diversity and ecosystem function, future research should include perturbations such as variability in salinity within a single season to increase our ability to predict what we should observe in the field. Our study also suggests that metacommunity theory needs to be expanded to more adequately describe expectations in highly heterogeneous landscapes.

Tables and Figures

	1st Dispersal	SD	2nd Dispersal	SD	3rd Dispersal	SD	4th Dispersal	SD	5th Dispersal	SD
13ppt source	1.2	1.7	2.35	2.5	1.8	3.3	1.1	1.5	1.6	2.2
0ppt source	3.4	7.1	7.24	9.9	4.1	6.1	11	18.8	4.6	6.9

Table 1 shows zooplankton abundance per L+/-standard deviation for each dispersal source.

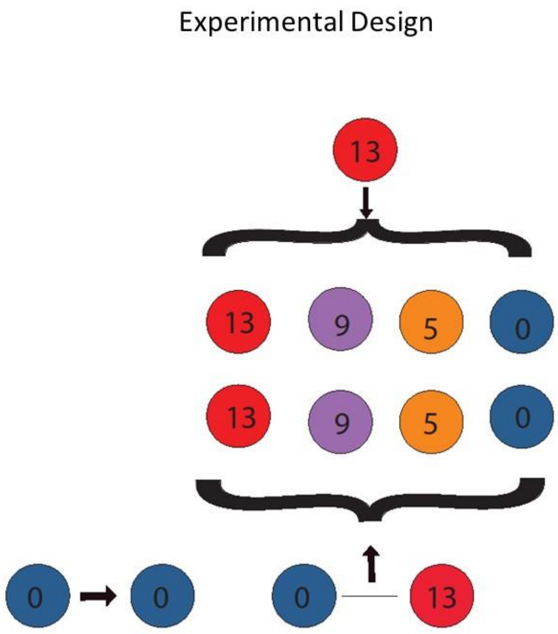


Figure 1 shows the experimental design. Arrows indicate dispersal. This set up was replicated 4 times.

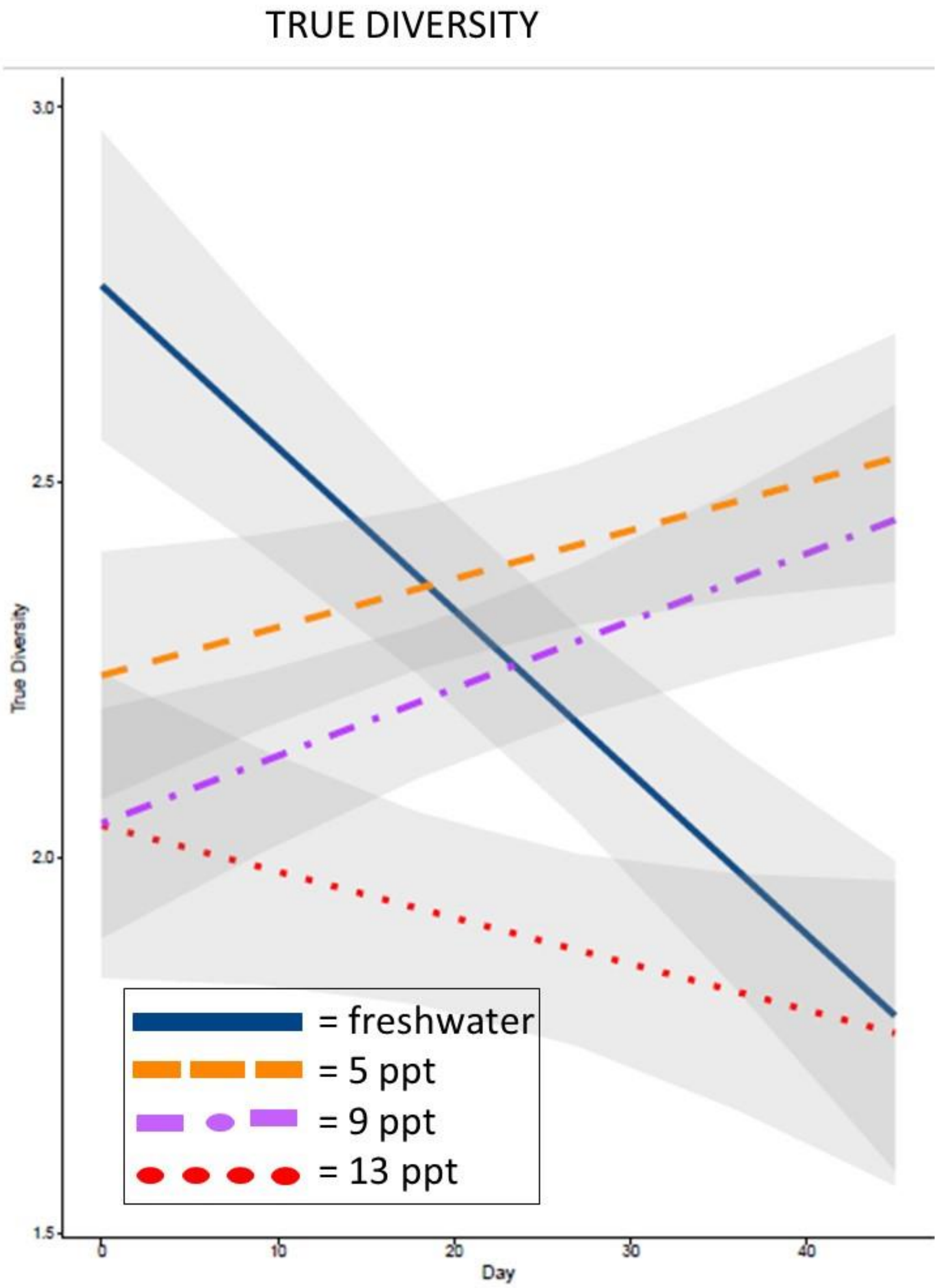
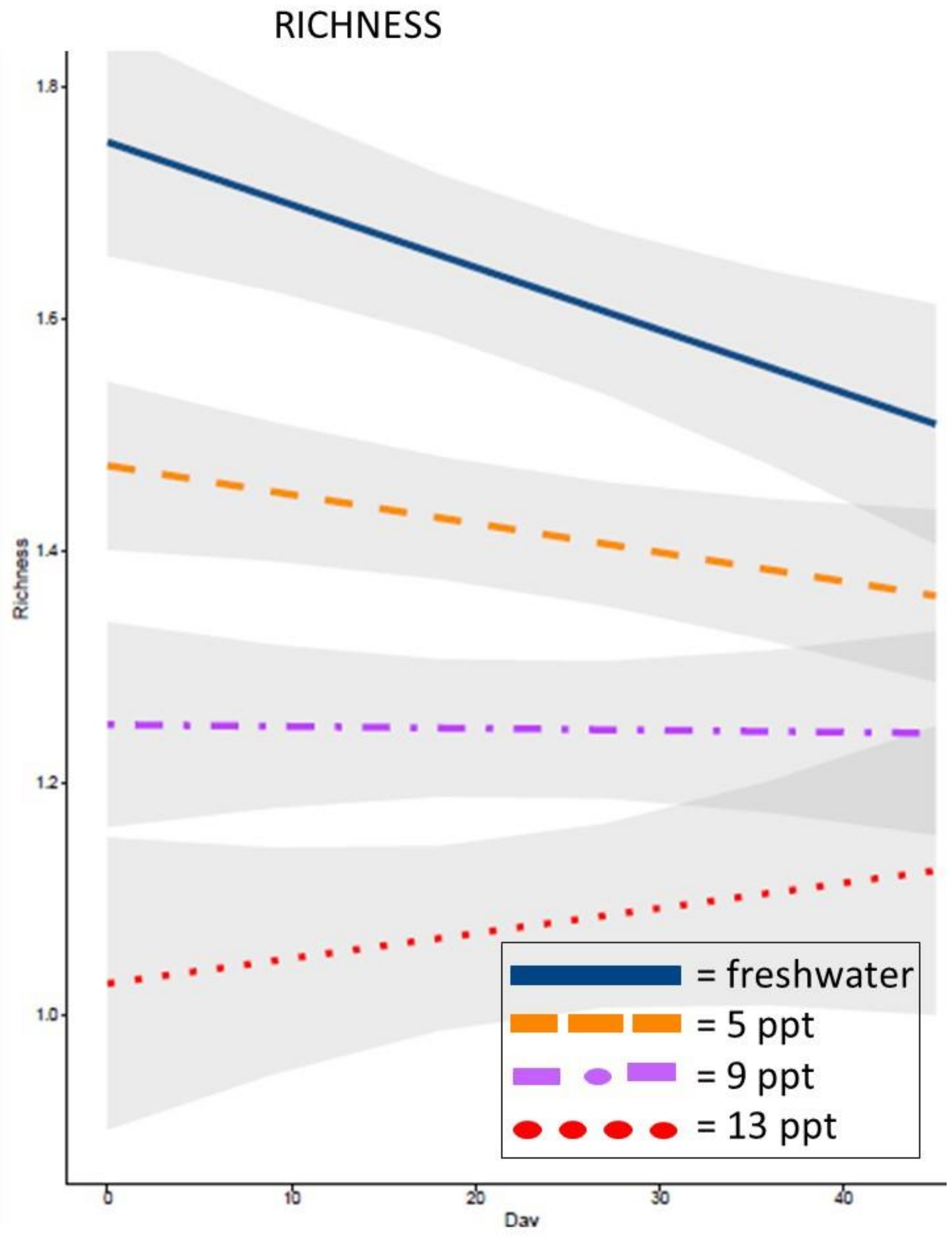


Figure 2 shows zooplankton predicted true diversity, calculated from the exponentiated Shannon Diversity over time . Envelopes represent 95% confidence intervals.



RICHNESS

Figure 3 shows predicted zooplankton richness over times for each salinity . Envelopes represent 95% confidence intervals.

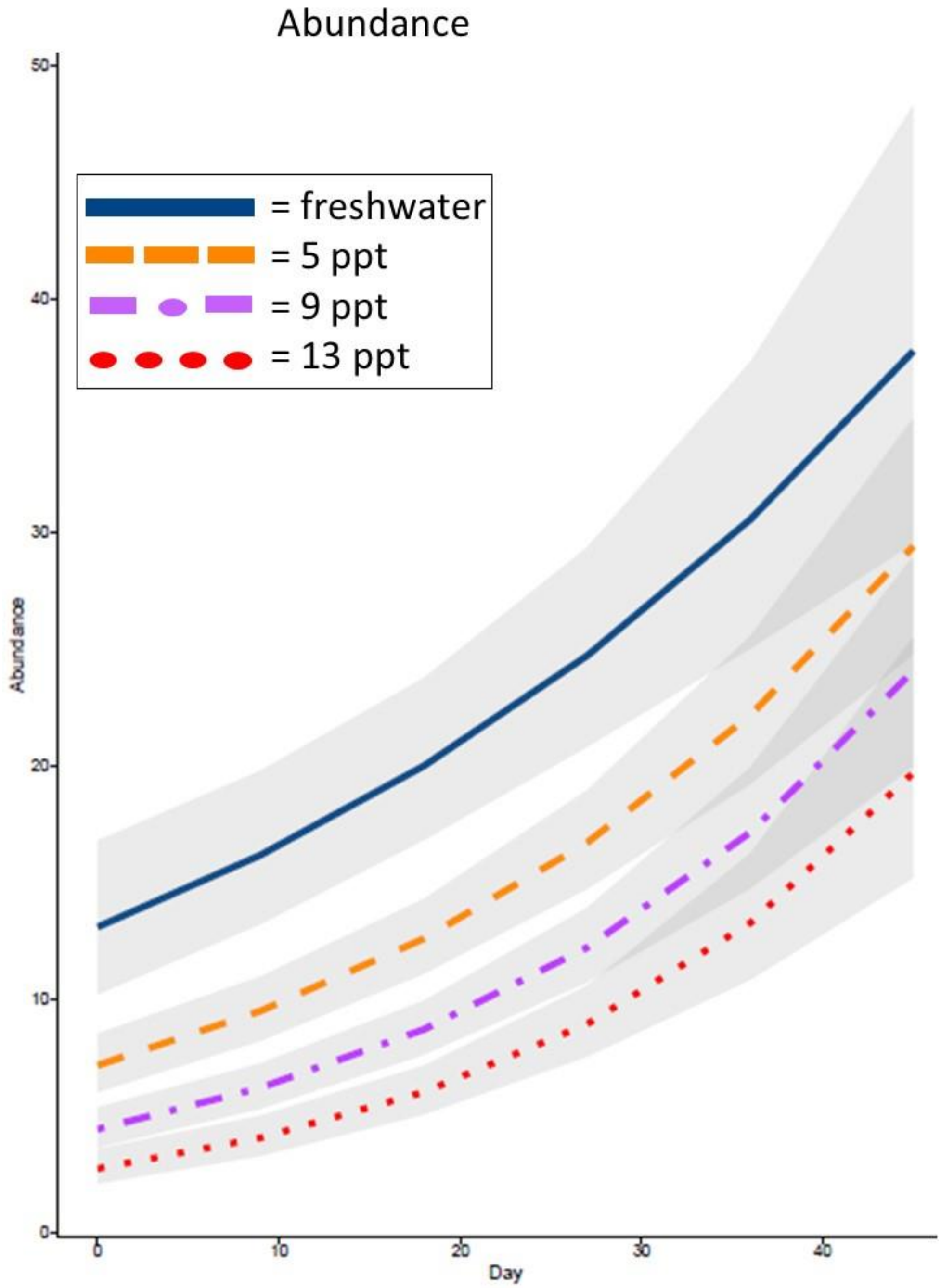


Figure 4 shows predicted zooplankton abundance over time for each salinity. Envelopes represent 95% confidence intervals.

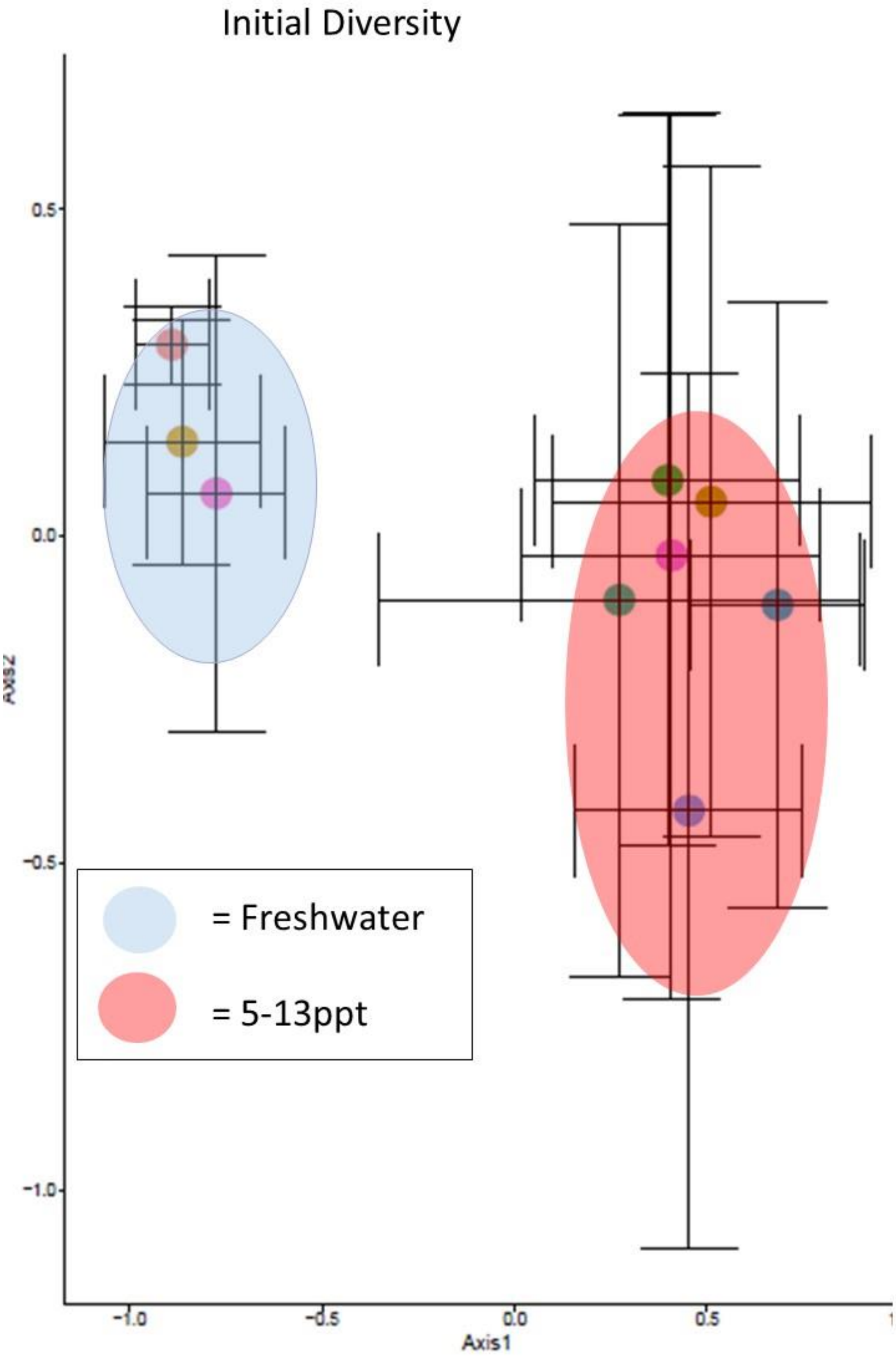


Figure 5 shows initial community structure, all error bars represent standard deviations

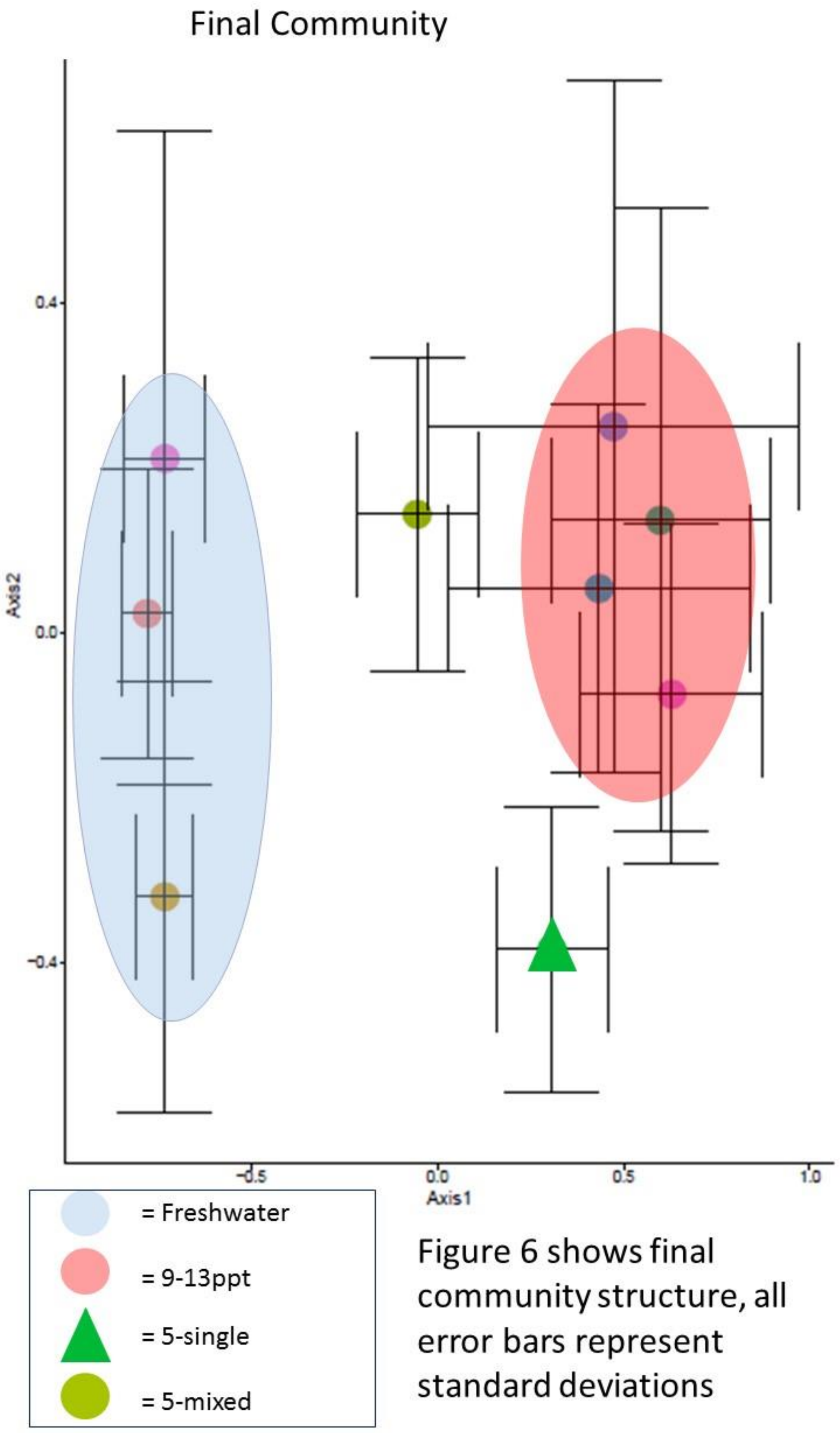


Figure 6 shows final community structure, all error bars represent standard deviations

Freshwater Community

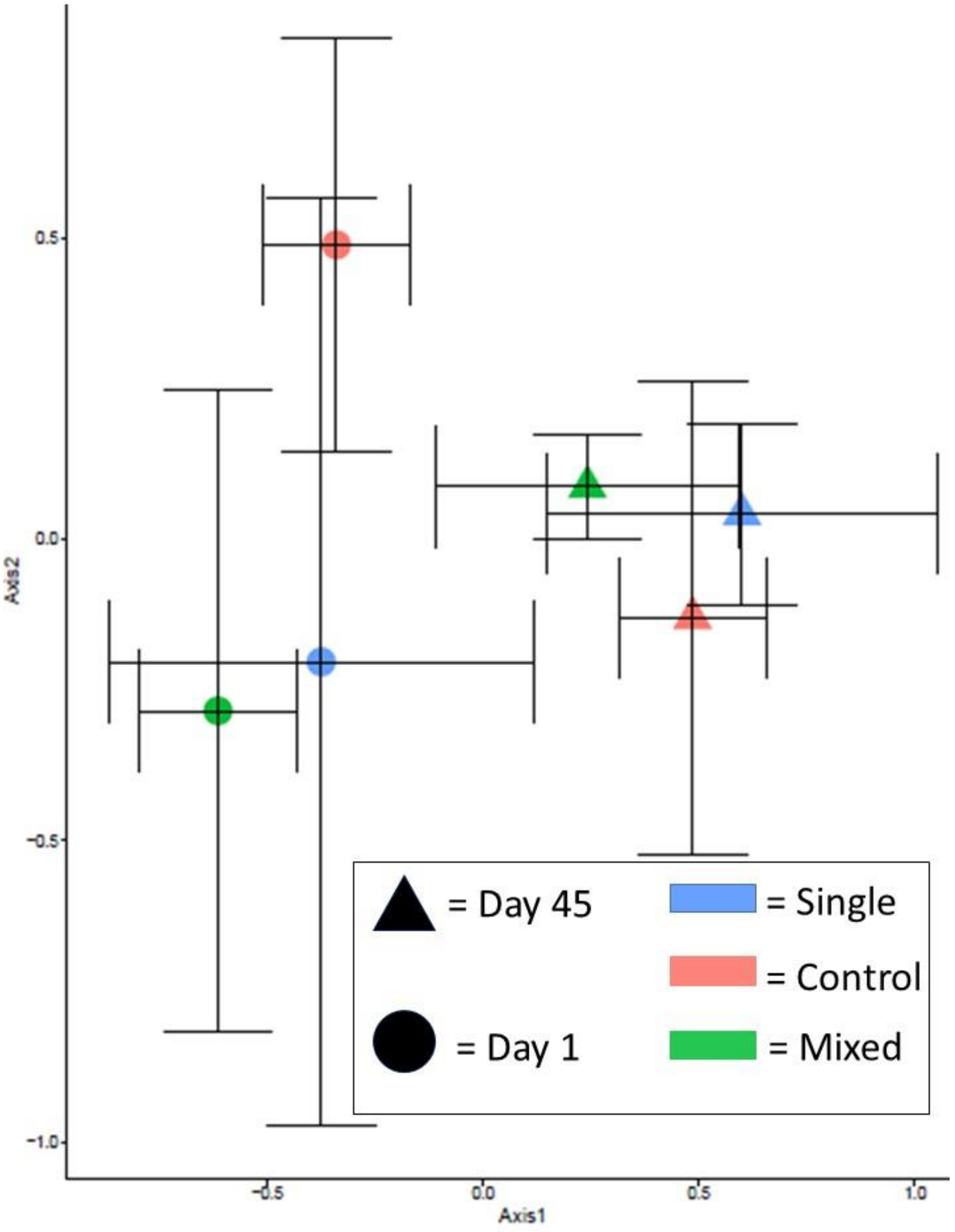
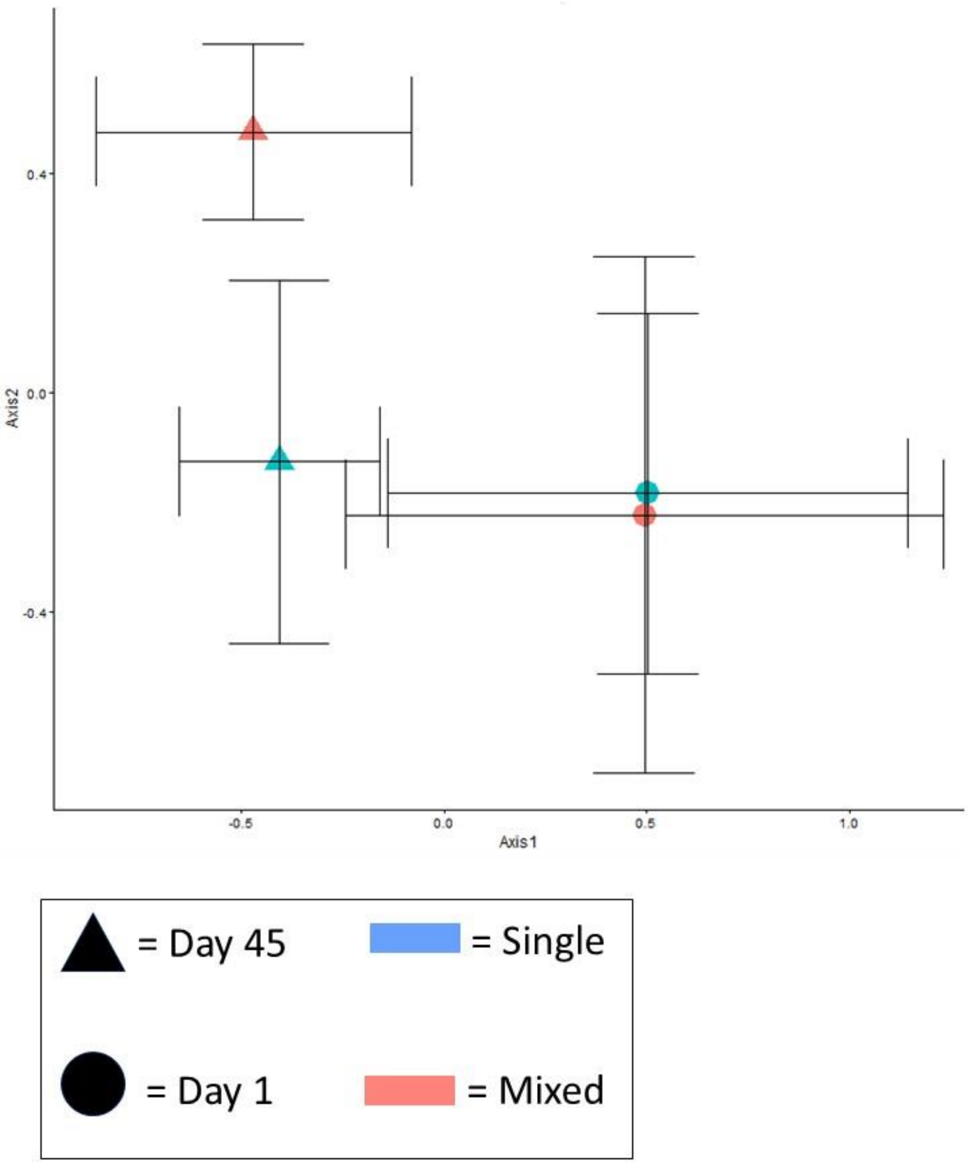


Figure 7 shows the first and final community structures for the freshwater treatments. All bars represent standard deviation



Five Salinity Community

Figure 8 shows the first and final community structures for the five ppt treatments. All bars represent standard deviation

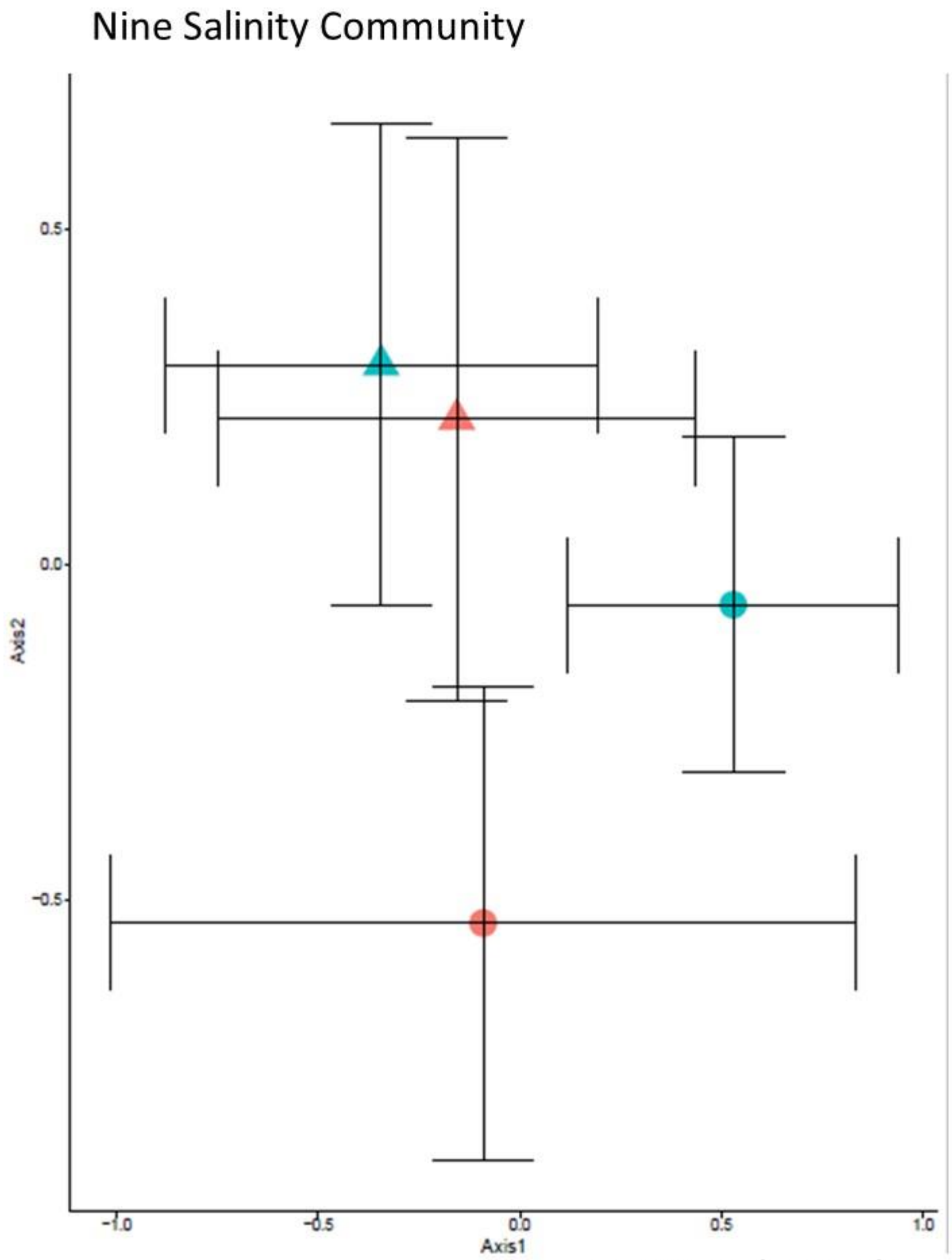


Figure 9 shows the first and final communities for the nine ppt treatments. All bars represent standard deviation

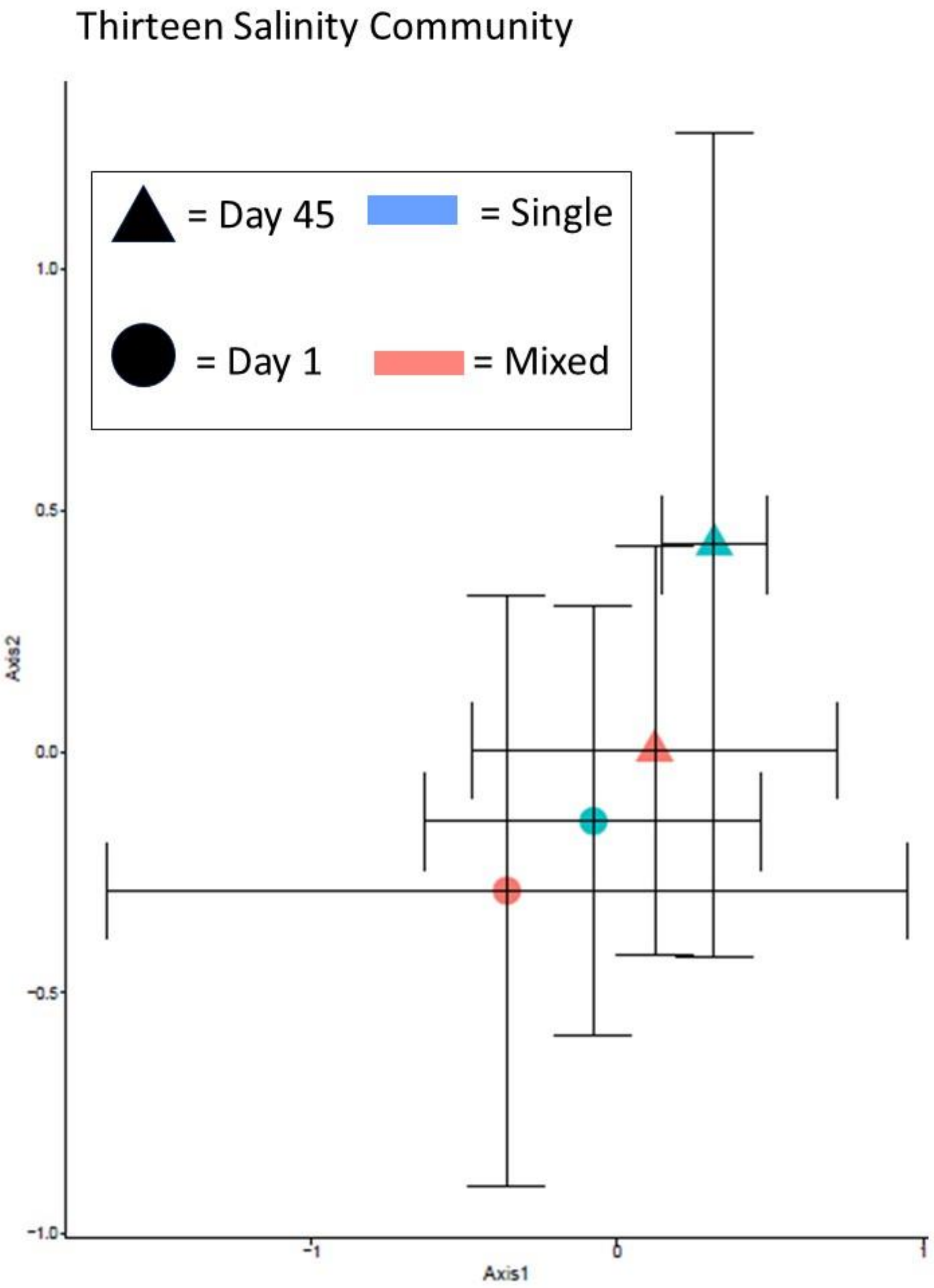


Figure 10 shows the first and final communities for the 13 ppt treatments. All bars represent standard deviation

INITIAL BETA DIVERSITY

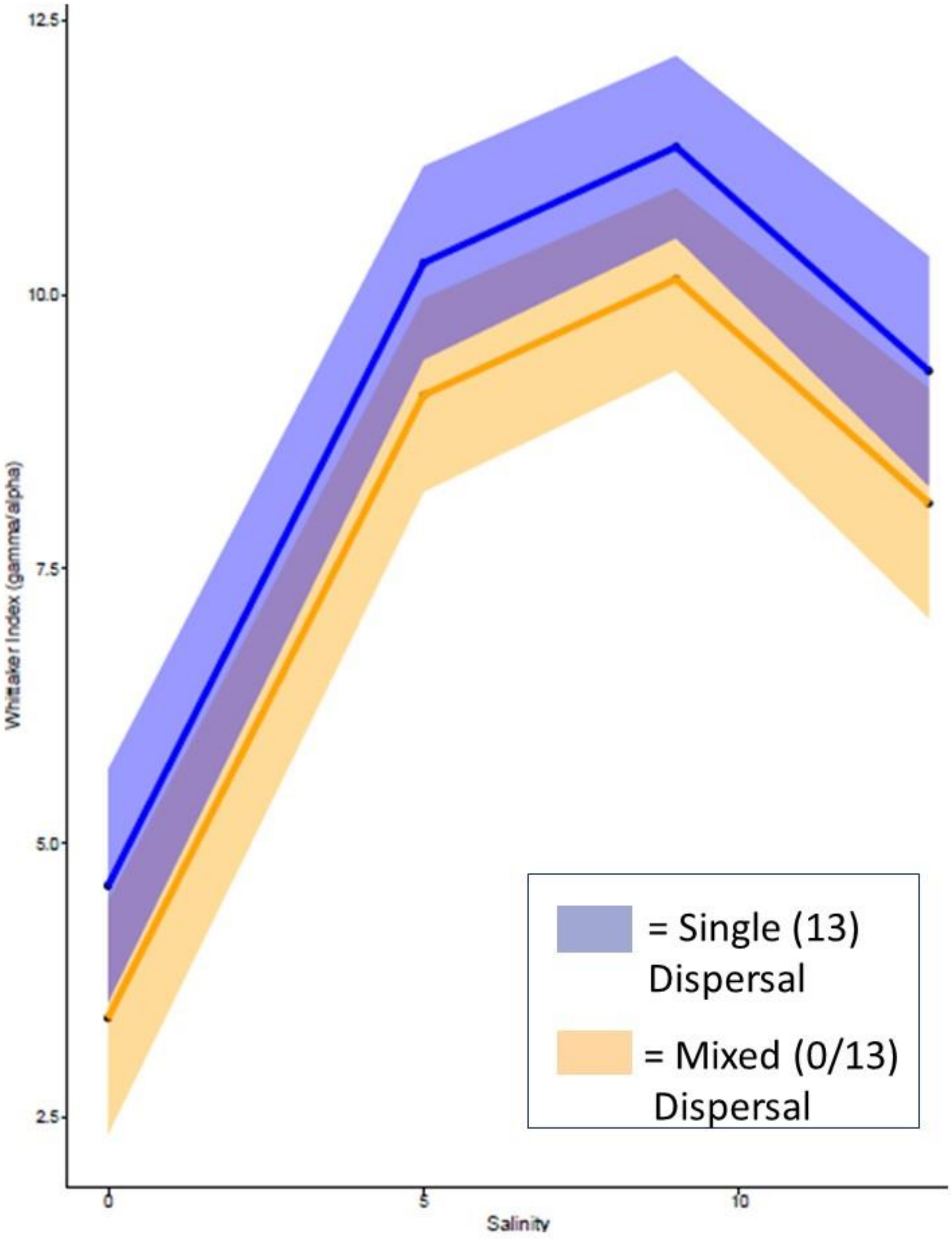


Figure 11 shows predicted initial beta diversity calculated as gamma/alpha across salinity for each dispersal treatment. Error envelope represents 95% confidence intervals.

FINAL BETA DIVERSITY

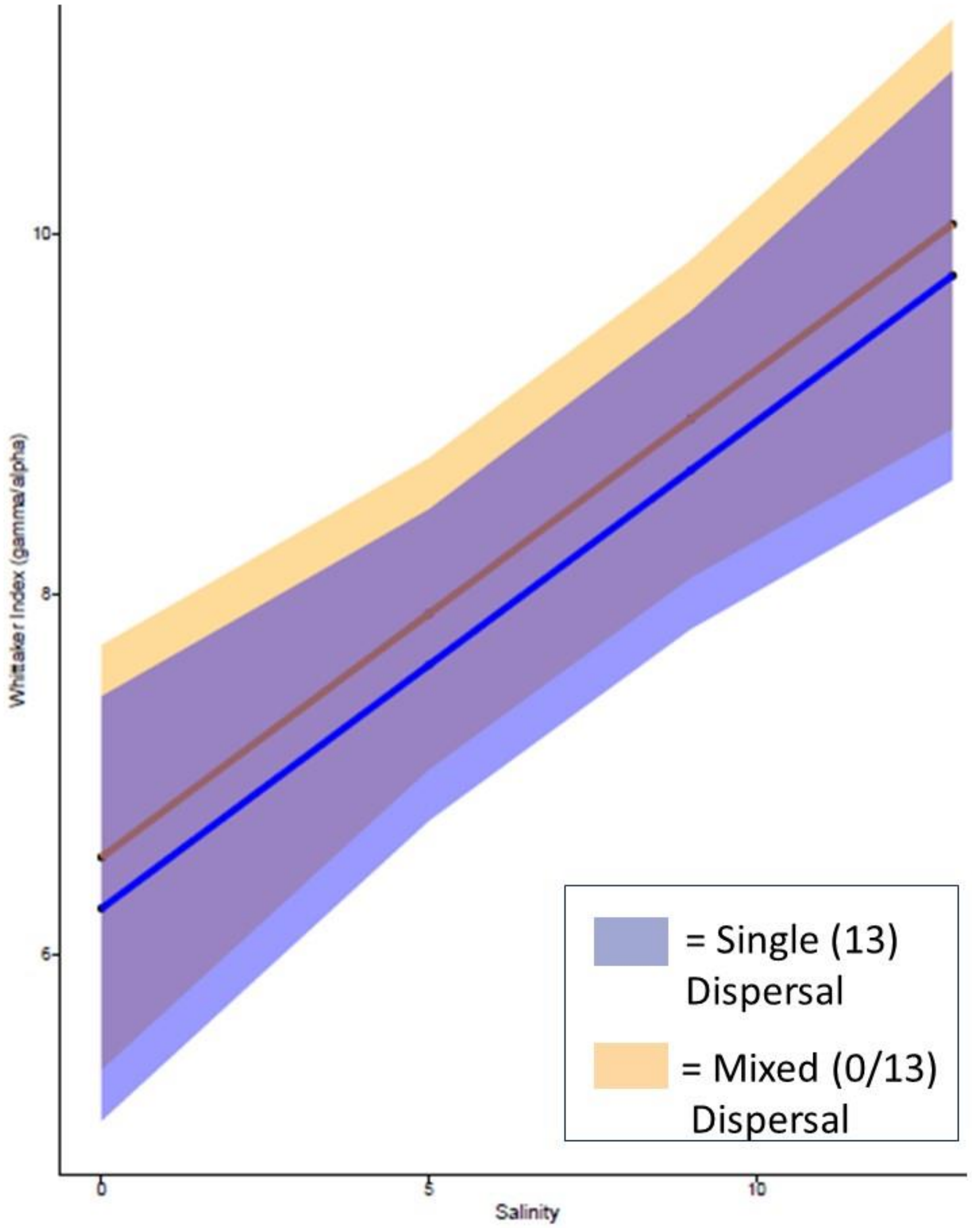


Figure 12 shows predicted final beta diversity calculated as gamma/alpha across salinity for each dispersal treatment. Error envelope represents 95% confidence intervals.

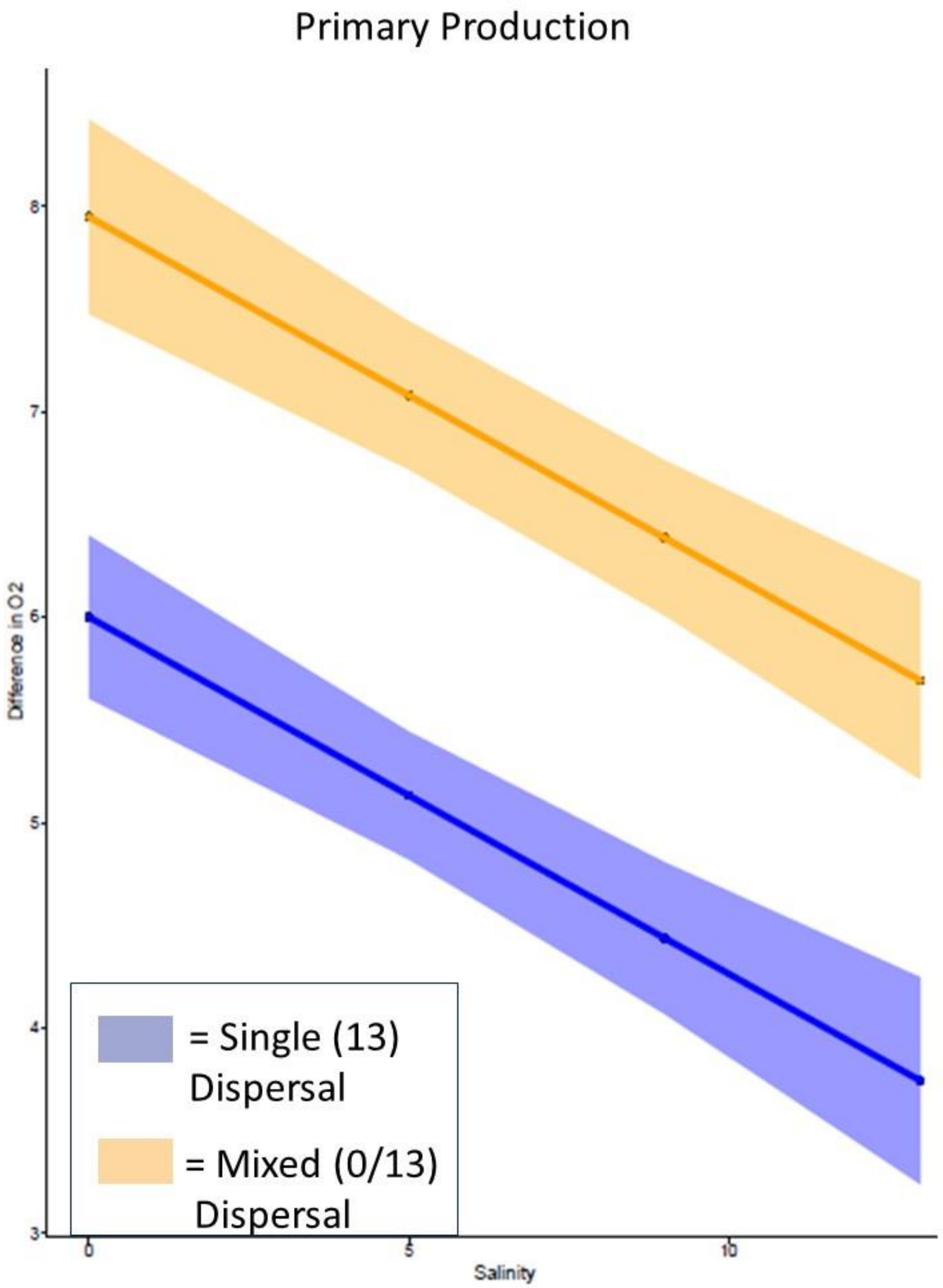


Figure 13 shows the difference in oxygen between the highest and lowest points in the day, across salinity for each dispersal treatment. Lines and error are based on predicted values from model. Error envelope represents 95% confidence intervals.

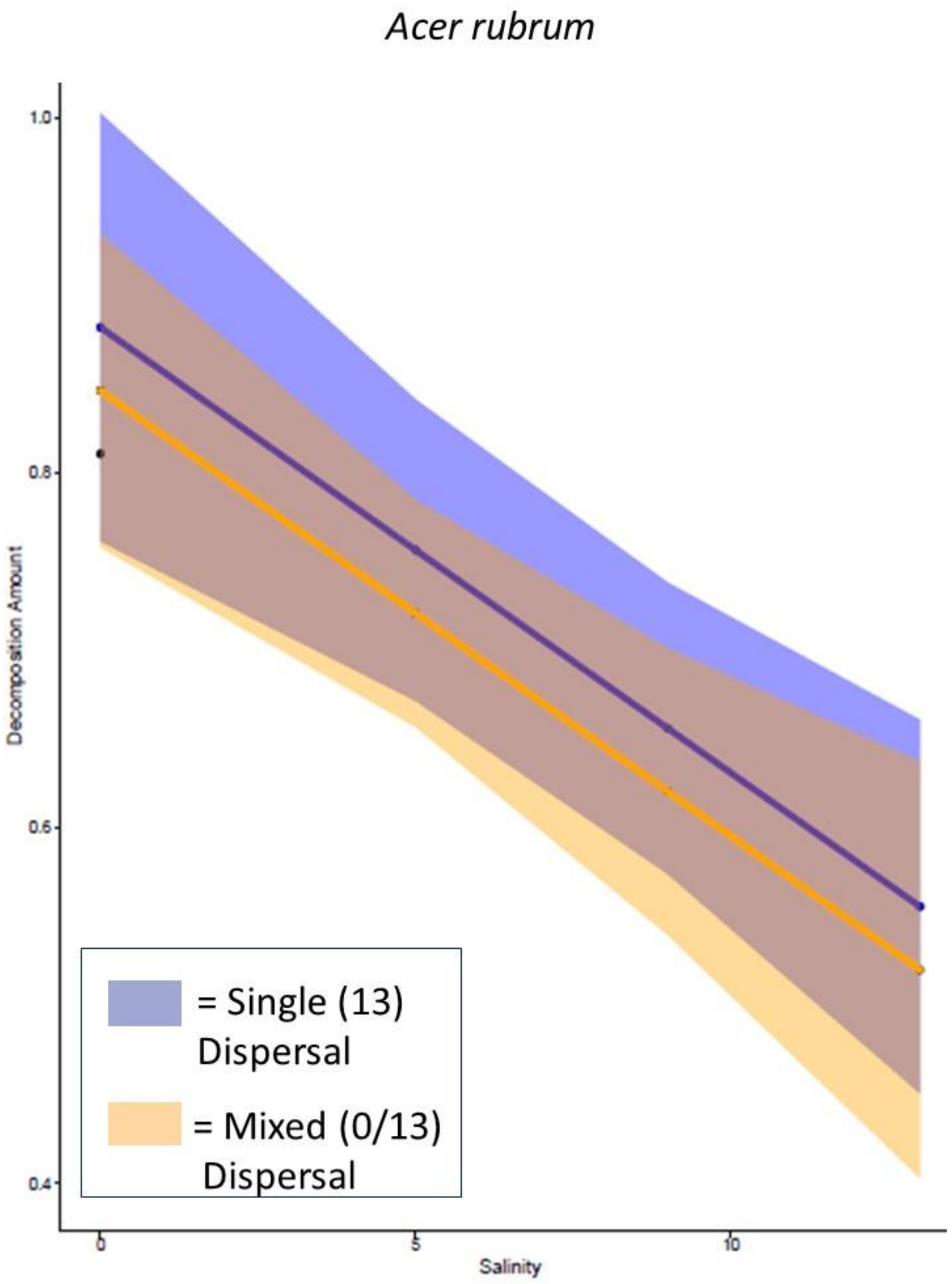


Figure 13 shows decomposition of *Acer rubrum* across salinity for each dispersal treatment. Lines based on predicted values from model. Error envelope represents 95% confidence intervals.

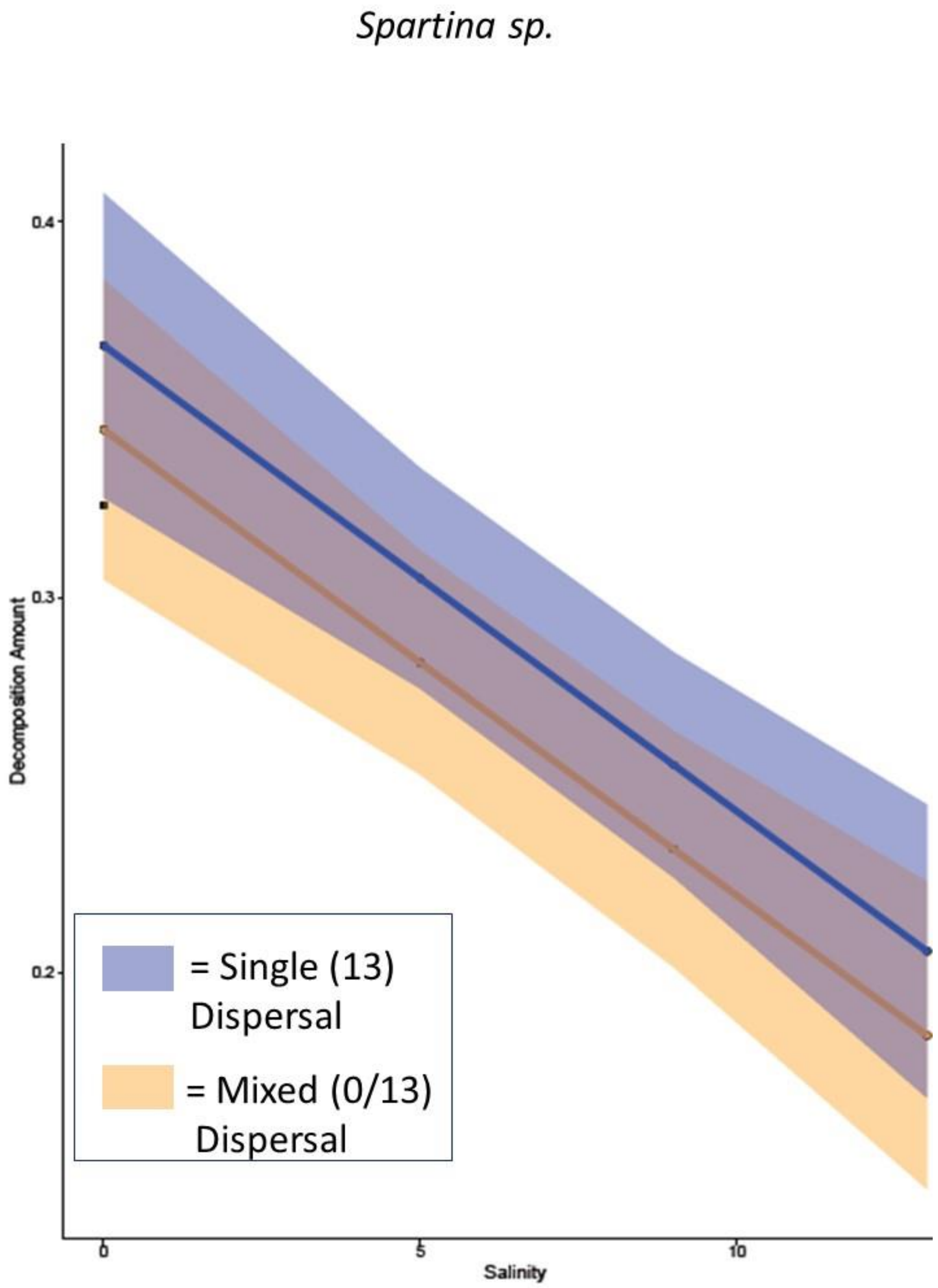
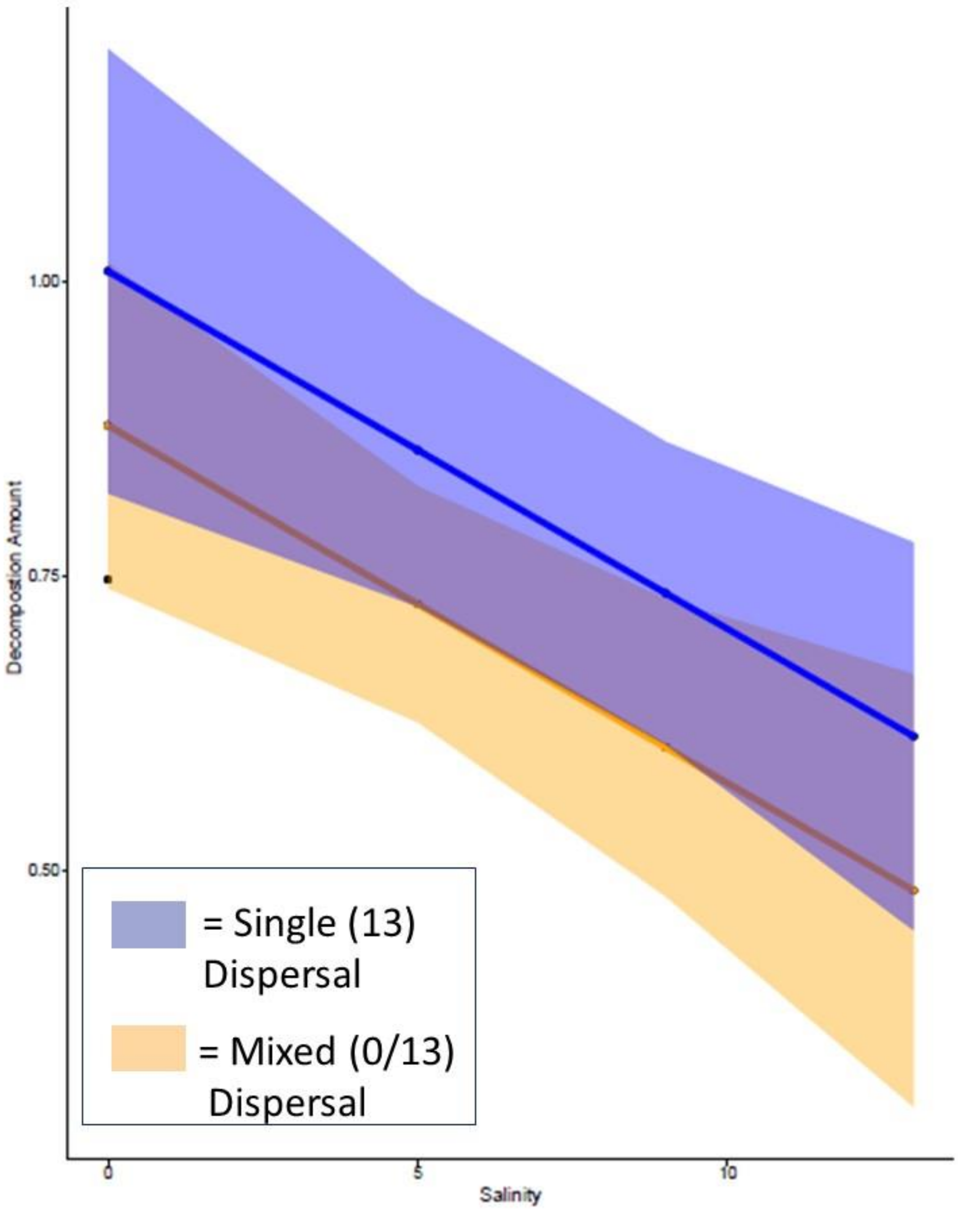

Spartina sp.

Figure 15 shows decomposition of *Spartina sp.* across salinity for each dispersal treatment. Lines based on predicted values from model Error envelope represents 95% confidence intervals.



Phragmites Australis

Figure 16 shows decomposition of *Phragmites australis* across salinity for each dispersal treatment. Lines based on predicted values from model. Error envelope represents 95% confidence intervals.

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