

ENVIRONMENTAL CONTROLS ON AQUATIC INVERTEBRATES

by

SOPHIE I. REINDL

(Under the Direction of Darold Batzer)

ABSTRACT

Gaining a greater understanding of how aquatic invertebrates are environmentally controlled has proven challenging. Batzer ([2013](#)) examined invertebrate ecology in freshwater wetlands across North America and concluded that assemblage controls are commonly weak or operate inconsistently. It is vital that we better understand those factors affecting aquatic invertebrates so that we can anticipate how these organisms are likely to respond to climate change. Wetland researchers frequently attribute invertebrate assemblage variation to spatial or temporal factors (location, hydrology, water quality, plant communities, and predation). When I partitioned the variation of three large wetland datasets (North Dakota prairie potholes, California rock pools, and Georgia Carolina bay wetlands) into spatial, temporal, and unexplained components, variation that was explained by spatial and temporal factors and unexplained variation were of comparable magnitudes (i.e., similar R^2 values of $\sim 50\%$).

To understand what might contribute to the currently unexplained variation, that is unrelated to spatial and temporal controls, I focused on the Georgia Carolina bay wetland data and assessed patterns of secondary succession. I found that non-successional wetlands tended to be smaller and drier, while successional wetlands were larger and wetter. I found that in those wetlands where annual variation was significant, assemblages changed individualistically

following unique trajectories rather than changing in similar ways. However, assemblages in all successional wetlands appeared to reach a compositional threshold that, once reached, resulted in a return towards a regional central tendency in assemblage composition. This range standard may serve as a useful guide when using invertebrate assemblages to assess wetland condition and potential non-natural changes, i.e., assemblages developing outside this natural range of variation are likely aberrant.

INDEX WORDS: Carolina bay, climate change, invertebrate, macroinvertebrate, secondary succession, spatial, temporal, variation, wetland

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DEDICATION

I dedicate this work to my family. Thank you all for your love and support. To my grandparents, Patrick Racey and Sheila Murphy, whose hard work and generosity allowed all their grandchildren to attend college for free. I cannot adequately express how grateful I am for the education you made possible. Thank you for gleefully asking me about the flies. To my grandparents, Fritz and Ellie Schmidbauer, whose lives remind me that hard work for those you love is hardly work at all.

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CHAPTER 1

INTRODUCTION

Invertebrates are a crucial part of aquatic ecosystems. They serve as the primary trophic link between primary production (macrophytes, algae, detritus) and higher order animals (fish, amphibians, birds). They play important roles in nutrient cycling by breaking down organic matter. Their presence in aquatic ecosystems can be important signals of water quality, given their varied tolerance to environmental changes (Batzner and Wissinger 1996; Batzner and Boix 2016). Understanding how aquatic invertebrates are environmentally controlled has proven challenging given the complexity of these ecosystems (Batzner 2013). It is important to understand how environmental controls are acting on aquatic invertebrates so that we can anticipate how these organisms might respond to environmental changes and act in ways consistent with their conservation.

Wetland researchers frequently attribute invertebrate assemblage variation to spatial or temporal factors (location, hydrology, water quality, plant communities, and predation). I wanted to assess how much variation these factors actually explain in these systems. If these factors explain as much variation as is attributed, then researchers are on the right track, but if these factors explain little of the variation seen in wetland invertebrate communities, then research ought to be directed to determining the sources of the unexplained variation. I examined three large wetland datasets and partitioned variation to determine how much could be attributed to spatial and temporal factors, and how much remained unexplained.

Sources of unexplained variation might be more difficult to measure or require new metrics that are not currently in use. I examined the Georgia Carolina bay dataset and tried to look beyond traditional metrics to assess what might be driving unexplained variation in this invertebrate community. I found that wetlands in this complex differentiated themselves as successional and non-successional, and that successional wetlands seemed to reach a kind of compositional threshold. Once reached, this threshold the wetland returned to a kind of regional central tendency of composition. This regional central tendency, if identified in other wetlands, might serve as a new measure against non-natural changes, i.e., invertebrate communities developing outside of the natural range of variation would be considered aberrant.

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CHAPTER 2

DOING THE SAME THING OVER AND OVER AGAIN AND GETTING THE SAME RESULT: ASSESSING VARIANCE IN WETLAND INVERTEBRATE ASSEMBLAGES¹

¹ Reindl, S., *et al.* Doing the Same Thing Over and Over Again and Getting the Same Result: Assessing Variance in Wetland Invertebrate Assemblages. *Wetlands* **43**, 84 (2023).
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Abstract

Past efforts to explain variation of invertebrate assemblages in freshwater wetlands have been less productive than anticipated. To explore why efforts are disappointing, we assembled large invertebrate data sets from North Dakota prairie potholes, California rock pools, and Georgia Carolina bay wetlands that addressed spatial (among wetlands) and temporal (among seasons and years) variation. We anticipated that these large data-set sizes would enable robust conclusions to be drawn, and each place had unique environmental conditions that might contribute to greater explanatory power. We used statistical techniques that partitioned variation in invertebrate assemblages into spatial and/or temporal components, and that also yielded a measure of the amount of unexplained variation; Permutational Multivariate Analysis of Variation and Principal Coordinates Analysis assessed whole assemblage variation, and Analysis of Variance or Analysis of Covariance assessed variation in taxon richness, total abundances, and abundances of wide-spread individual taxa. Across all locations, variation explained by spatial and temporal factors, and unexplained variation were of comparable magnitudes (i.e., similar R^2 values of $\sim 50\%$). Review of other published studies indicate that this pattern is widespread. The 50% or more unexplained variation is typically ignored by researchers, who instead focus on explained fractions. We argue that, besides addressing explained spatial and temporal variation in invertebrate assemblages (e.g., control by hydrology, resources, predation), efforts to understand what contributes to currently unexplained variation, that is unrelated to local spatial or temporal controls (e.g., broad climatic and biogeographic patterns, organism physiology and behavior), will lead to a fuller comprehension of how invertebrates in freshwater wetlands are controlled.

Introduction

Invertebrates are crucial components of wetlands because they are the primary trophic link between primary production (macrophytes, algae, detritus) and higher order animals (fish, amphibians, birds) (Batzner and Wissinger 1996; Batzner and Boix 2016). However, gaining a greater understanding of how invertebrate assemblages in wetlands are ecologically controlled has proved challenging. Batzner (2013) examined invertebrate ecology in freshwater wetlands across North America and concluded that assemblage controls are commonly weak or operate inconsistently.

Studies of the ecology of invertebrates in freshwater wetlands tend to focus on habitat factors such as variation in hydrology (including hydroperiod), water quality, plant communities, and predation (e.g., Batzner and Wissinger 1996; Euliss et al. 2004; Gascón et al. 2016). These factors vary among individual wetland habitats or across different time frames, and thus can be lumped into spatial and/or temporal variation. We assembled studies that reported the total amount of spatial and temporal variation explained by their statistical models and found that only about 10–40% of community variation was explained (Table 2.1). The majority of invertebrate variation remained unexplained. When researchers find that explanatory power is weak, problems with sampling, design, or analyses are often blamed. However, it is possible that wetland invertebrate response to factors associated with spatial and temporal variation of habitats is weaker than suspected, and other kinds of control are stronger than suspected (but not addressed by studies). If so, we should think more creatively to gain a fuller understanding of invertebrate ecological controls.

Here we assess spatial and temporal variation of invertebrate assemblages in three large data sets, each with attributes that might permit greater explanatory abilities. Each of these

studies used the same basic approaches used by others (Table 2.1), by associating invertebrate variation with habitat variation among individual wetlands and across time periods (seasons and years); but these three studies tended to be more ambitious, or addressed a unique kind of wetland habitat, as compared to most previous efforts. The first study, from the prairie pothole region of North Dakota, USA, has monitored invertebrate assemblages in a complex of 17 prairie potholes seasonally for 24 years. To our knowledge, this data set (from the Cottonwood Lake Study Area: Mushet et al. 2017; McLean et al. 2020) is the world's largest compilation of invertebrate data from freshwater wetlands, particularly in terms of the temporal component. Because of the massive sample size, we predicted that high statistical power would enable a large portion of assemblage variation to be explained by spatial differences among the 17 wetlands, and temporal differences across the 24 years. As noted, most environmental factors considered important to wetland invertebrates, such as hydrology, water quality, plant communities, and predator complexes (Batzner and Wissinger 1996; Wellborn et al. 1996), are encapsulated in spatial (variation among wetlands) and temporal (variation among seasons and years) differences. The second data set was from a complex of seasonal rock pools in California. The assemblages in these rock pools were species poor due to harsh environmental conditions during the dry season. We hypothesized that perhaps the simplicity in assemblages, or the particularly strong environmental controls, might permit us to explain a greater amount of invertebrate variation across space and time. The third data set was from a complex of Carolina bay wetlands in Georgia, USA. Hydroperiods in these habitats did not vary as dramatically across space and time as the other data sets, and dry periods were relatively benign for invertebrates (basins always remain moist). We hypothesized that assemblages might thus be very predictable in Carolina bays, and much of the variation could be explained.

We used an assortment of statistical techniques to assess invertebrate assemblage variation in each of the three data sets. We predicted that in at least one of these wetland data sets, a large portion of the variation would be explained due to either the sheer size of the data set (prairie potholes) or the unique climatic conditions (California rockpools, Georgia Carolina bays), providing guidance towards a better understanding of invertebrate ecological controls in freshwater wetlands. If none of these efforts explained a large portion of variation, i.e. more than the 10–40% magnitudes suggested by other studies (Table 2.1), that would confirm that using traditional approaches to the study of wetland invertebrates (focusing on spatial and temporal variation in habitats) will yield limited amounts of explanatory power. This would suggest that new supplemental ways of study should be considered to significantly expand our understanding of the ecological controls of invertebrates in freshwater wetlands.

Methods

We assembled invertebrate data sets from 1) North Dakota prairie potholes, 2) California rock pools, and 3) Georgia Carolina bay depressional wetlands. Each data set addressed both spatial (among wetlands) and temporal (among years) variation. The prairie pothole data set included 17 wetlands over 24 years; within each year, monthly collections (April–September) were conducted (if the sites held water). The California rock pool data set included 28 pools with annual collections over three years, partitioned across four rock outcrops in a single region. The Georgia Carolina bay data set included a complex of 10 depressional wetlands, sampled seasonally (March, July, November) over five years. Details about the conditions at each location, and the sampling procedures used are provided in online Supplement 1.

Each data set included a range of hydroperiod conditions and habitat sizes, representative of the breadth of the environmental conditions that existed locally. (The original purpose for

each sampling effort was to relate invertebrate variation to environmental variation, to generate testable hypotheses about possible mechanisms of control.) Climatic conditions among the three habitat types varied greatly: with a cold-temperate, semi-arid climate in North Dakota; a warm, seasonally dry Mediterranean climate in California; and a warm, wet subtropical climate for Georgia. The three different wetland types also supported invertebrate assemblages of varying complexity: assemblages in the North Dakota potholes were taxonomically rich (159 taxa): assemblages in the California rock pools were simple (14 taxa): and assemblages in the Georgia Carolina bays were moderately rich (77 taxa). Taxa in all three efforts were classified to the lowest practical level, typically genus or family.

We selected widely used statistical analyses in the R statistical platform, vegan package (R Core Team 2020; R Studio 2020) that enabled partitioning of the variation in invertebrate assemblages in each data set into spatial and/ or temporal components (percentages explained), and that also yielded a measure of the percentage of unexplained variation. To assess variation in overall assemblage compositions, we used Permutational Multivariate Analysis of Variation (PERMANOVA) to directly partition variation of assemblages into spatial and temporal components, and Principal Coordinates Analysis (PCoA) to partition variation of assemblages across space and time to ordination axes, with the first two axes explaining the most variation. For both multivariate tests, we used the Bray–Curtis metric of $\log_{10}(x + 1)$ transformed abundance data as the community distance measure, with 1000 permutations being applied.

To assess variation in taxon richness, and in the total abundances and the abundances of individual taxa ($\log_{10}(x + 1)$), we used univariate analyses for each location, including Analysis of Variance (ANOVA) and Analysis of Covariance (ANCOVA; when a continuous within- year temporal measure was available). For all data sets, most individual taxa were absent from most

samples, so taxon-specific univariate analyses were rarely appropriate (large numbers of zero values violates assumptions of parametric tests and complicates rank non-parametric tests). Depending on the assemblages, we instead combined phylogenetically related lower taxa with numerous zero counts (> 50%) into higher level taxonomic groupings (e.g., Family, Order, Class), until zero values for samples were largely eliminated, and then conducted ANOVA or ANCOVA analyses to assess taxon-specific variation.

Researchers typically assume that any factor that can be statistically associated with a response variable (e.g., invertebrate assemblages) is important. Emerging opinion suggests that more focus should be placed on effect sizes (or variance explained) to identify the strength of the evidence (Cumming 2014; Wasserstein et al. 2019), rather than simple statistical significance. Thus, we focus on effect sizes as reflected by R² values for different components of the PERMANOVA, ANOVA, and ANCOVA test tables (spatial, temporal, unexplained), and % variation explained by the first two axes of the PCoA plots (which combined the influences of both spatial and temporal factors into the same axes). Because existing research suggests that the amount of explained variation is often small, for this paper we highlight the magnitude of the unexplained variation.

Results

North Dakota Prairie Potholes

Spatial differences among the 17 prairie potholes explained 11.8% of total assemblage variation, based on PERMANOVA (Table 2.1a). Temporal factors (annual, seasonal) explained 17.8% of total assemblage variation, with most of that (11.1%) being attributed to annual variation. The interaction between spatial and annual variation explained another 15.4% of variation. Most variation, 55%, was not explained by either spatial or temporal factors. The

PCoA ordination plot showed a largely random distribution among the 6847 samples (Fig. 2.1a), and the first two axes of the plot explained only 4% of variation. While data points did not distribute along either major axis, the PCoA plot clearly showed that there were sharp boundaries to variation extremes (Fig. 2.1a).

Using ANCOVA, we explained 48% of taxon richness and 36.7% of total invertebrate abundance, with most of that being attributed to seasonality; most variation in richness (52%) and abundance (63.3%), however, was unexplained (Table 2.2a). We could legitimately (i.e., meet assumptions) assess variation for only a few composite taxonomic groups (Cladocera, Other-Crustacea, Insects) and from 31.6–41.7% of taxon-specific variation in abundance was explained, again mostly due to either annual or seasonal factors. While effect sizes were generally small to moderate for spatial or temporal factors across all analyses, they were in all cases highly significant (all $p < 0.001$; Tables 2.1a, 2.2a).

California Rock Pools

Spatial differences among the 28 rock pools across 4 outcroppings explained 36.7% of total assemblage variation, based on PERMANOVA (Table 2.1b), with most (25.0%) being attributed to the pools. Annual variation among the 3 sample years explained only 8.1% of variation, and another 5.5% of variation was explained by the interaction between pools and years. Almost half of assemblage variation, 49.7%, was not explained by either spatial or temporal factors. The PCoA ordination plot for the rock pools showed some structure among the 136 samples (Fig. 2.1b), and the first two axes of the plot explained 24.7% of variation.

Using ANOVA (Table 2.2b), we explained 40.2% of variation in taxon richness with spatial factors, but annual variation was not significant; 57.7% of total variation in richness remained unexplained. For total abundance, spatial factors explained 42.4%, and annual

variation explained 15.2% of variation; 40.0% of variation remained unexplained. Only a single taxonomic group, pooled microcrustacean abundances (at 62.3% of variation explained), had more than 15% of variation explained. Again, while the effect size for spatial and temporal variation was only large ($> 50\%$) for a single response group (microcrustacean abundance), all the above reported R^2 values for spatial and temporal factors were associated with small p -values (all < 0.01 ; Tables 2.1b, 2.2b).

Georgia Carolina bay Wetlands

Spatial differences among the 10 Carolina bays explained 19.2% of the total assemblage variation, based on PER- MANOVA (Table 2.1c). Temporal factors (annual, seasonal) explained another 20.4% of the total assemblage variation, with most of that (11.4%) being attributed to annual variation. The interaction between spatial and annual variation was not significant, but because its effect size was comparatively large (18.8% of variation) we retained it in the PERMANOVA model. Residual unexplained variation comprised 41.7% of the total. The PCoA ordination plot showed minimal structure across the 137 samples (Fig. 2.1c), and the first two axes of the plot explained only 18.5% of variation.

Using ANCOVA, we were able to explain 50% of taxon richness and 26.5% of total invertebrate abundance, with most of that being attributed to the spatial dimension; 34.8% of variation in richness and 51.4% of variation in abundance was unexplained (Table 2.2c). In the Carolina bays, several individual taxa occurred in most samples, and thus could be assessed using ANCOVA. But spatial or temporal factors rarely explained more than 25% of variation (for two crustaceans, *Caecidotea* Assellidae and *Crangonyx* Crangonyctidae, 39% variation was explained by seasonal change, likely due to life histories). While effect sizes were generally

small for spatial or temporal factors across all analyses, they were highly significant (all $p < 0.003$; Tables 2.1c, 2.2c).

Discussion

We had reason to believe that a large portion of the variation in invertebrate assemblages might be explained in one or more of our three wetland data sets. However, effect sizes of analyses were small to moderate (typically 10–30% of variation explained), with the amounts of variation explained by either spatial or temporal components being roughly equal. Despite very different climatic conditions, assemblage complexities, study designs, and levels of sampling effort, each of the three geographic regions yielded remarkably similar results in terms of the amount of local spatial and temporal variation that was explained. The greatest amount of variation in each of the three data sets was consistently attributed to the unexplained residuals, with those amounts hovering around 50%. Our results are consistent with efforts elsewhere (see Table 2.3), suggesting that this is a general pattern (it seems unlikely that the overall phenomenon is spurious). Indeed, our findings might be the expected result for most studies of wetland invertebrates.

Perhaps closer scrutiny of each of our three data sets, and previous studies (Table 2.3), might indicate ways that explanatory rates could have been enhanced. Changes in study design, sampling approach, and/or statistical analyses might have somewhat improved results for a particular study (although our exploration of alternative statistical approaches that partition variation such as Redundancy Analysis and Joint Species Distribution Modelling did not yield increased explanatory power; unpublished analyses). To be clear, we are not advocating that the significant amounts of variation that were explained by local spatial and temporal variation be ignored. Those factors considered most important to wetland invertebrate ecology, such as

variation in hydrology, water quality, plant factors, and predator complexes (Wiggins et al. 1980; Batzer and Wissinger 1996; Wellborn et al. 1996; Euliss et al. 2004; McLean et al. 2021), undoubtedly contributed to much of the spatial and temporal variation that was explained. Teasing apart the mechanisms for that control is a worthwhile endeavor (we will attempt that for our data sets). However, our message is that we need to think more broadly by expanding alternative hypotheses that contribute to the large portion of unexplained variation ubiquitous in wetlands.

Because of the large size of the prairie pothole data set (almost 7000 samples), we had anticipated that the strongest explanatory patterns might emerge there. This was not the case. But the robustness of that data set may yield tangible clues on new approaches to explore. One possible control is suggested by the PCoA plot for the prairie pothole data set, where variation among individual wetlands across space or time was largely random (major axes explained only 4% of variation) but some sort of all-encompassing control seems to be operating to keep assemblages within a certain range of possibilities. This “cap” is probably imposed by a broad geographic factor, likely climate but also possibly geology (e.g., past glaciation, soils) or biogeography (available taxa pools) (e.g., Heino et al. 2017; McLean et al. 2022).

Recent meta-analyses of invertebrates in wetlands support the idea of broad controls on assemblages that transcend local factors. Batzer and Ruhí (2013) assessed invertebrate assemblages from 447 wetlands from 78 regions across the world and found that wetlands in each of the 78 regional data sets tended to cluster together (i.e., habitats at a location were similar), despite a wide range of environmental conditions within most of those data sets. Those authors did not, for example, find a fauna typical to temporary or permanent wetlands, or to forested or herbaceous wetlands; but they did find faunas typical for specific regions (e.g.,

Catalonia, Colorado, Minnesota, New Zealand, Switzerland). Thus, a range of overarching geographic controls appear to be operating. Rather than only trying to explain how invertebrates vary within a set of wetlands in Minnesota (e.g., Batzer et al. 2004), it might also be informative to explain why invertebrate assemblages in Minnesota vary from assemblages in South Carolina (Batzer et al. 2005). By including only 4 degrees of latitude in their study of wetlands in Michigan, Stewart and Schriever (2023) were able to explain an additional 7% of variation. Analyses that assess wetland invertebrates across continental (e.g., Stenert et al. 2020) or global (e.g., Epele et al. 2022) scales suggest that simple air temperature, which obviously varies minimally among wetlands at local scales, is an important control on invertebrate assemblage variation among different geographic regions.

Because depressional wetlands and rock pools exist as distinct habitat patches, the metacommunity approach has appeal (see Leibold et al. 2004), both to addressing explained and unexplained variation for those complexes. Most studies conducted thus far in wetlands tend to focus on the more deterministic components within a specific metacommunity, which revisits local spatial and temporal factors (e.g., DeClerck et al. 2011; Gascón et al. 2016). But broader controls that operate across entire metacommunities and vary among different metacommunities should also be considered (Heino et al. 2017). The PCoA plot for the prairie pothole assemblages, again, seems particularly noteworthy in that, while essentially no consistent variation was detected among samples, the edge of the spherical data cloud for the plot was crisp, not fuzzy, and absolutely no outlier assemblages were evident (Fig. 2.1a). It appears that the 17 individual pothole wetlands at the Cottonwood Lake Study Area likely exist within a tightly constrained metacommunity of all wetlands in the region (including other wetlands not included in the data set).

If complexes of wetlands are indeed within large invertebrate metacommunities, further research is needed to examine the factors that control variation within and among metacommunities. Environmental conditions over space and time only capture the “environmental sorting” perspective of metacommunity dynamics (Heino [2013](#)). Other perspectives capture ongoing dispersal and extinction dynamics. Patch dynamics, i.e., a wetland’s spatial configuration including proximity to other wetlands and transitions between terrestrial and aquatic phases, and habitat sizes likely contribute to colonization rates. Priority effects allow species to colonize and persist in perhaps sub-optimal systems, especially new patches formed due to environmental disturbances (e.g., extreme weather or land-use changes) (Chase and Knight [2003](#); Datry et al. [2016](#); Bohenek et al. [2017](#)). Mass effects, i.e. source-sink dynamics, might also help explain why the compositions of invertebrate species are not always explained by environmental features. If source wetlands for different taxa are habitats with environmental characteristics that allow invertebrates to persist and complete their life cycle, sink wetlands are typically habitats that can be readily colonized by species, but their environmental characteristics are unsuitable to sustain a population. When source and sink wetlands are nearby, colonization of taxa from source populations into sink populations would promote the occurrence of taxa in unsuitable habitats through rescue effects (Johnson et al. [2013](#)).

Besides looking at broader spatial scales, perhaps a more refined examination of the individual invertebrate taxa that occur may yield additional clues to unexplained variation. Trait-based analyses (e.g., Usseglio-Polatera et al. [2000](#); Moor et al. [2017](#)) is a way to assess how innate characteristics of the organisms are exerting assemblage control. Our analyses were taxonomically based and some traits in wet- land invertebrates are conserved within taxonomic

groups: predators are typically found in specific taxonomic groups (e.g., Odonata, aquatic Hemiptera, some beetle families) and consumers in others; responses to drying can be taxon specific (Wiggins et al. 1980). Thus, the influence of many traits in our data sets may already be reflected by the umbrella of spatial and temporal variation among wetlands. However, some traits of invertebrates may be largely independent of spatial and temporal variation among wetlands, such as life histories (including reproductive rates and strategies), some dispersal and colonization tendencies, and biogeographic distributions, among others, and thus may have contributed to unexplained variation. A broad assessment of traits might yet yield insights into both explained and unexplained variation.

Conclusions

We provide a list in Table 2.4 of factors that can operate independently of local spatial and temporal variation in controlling the structure of wetland assemblages, which may provide direction to exploring currently unexplained variation. Some factors relate to different scales across the landscape while others relate to characteristics of organisms. For example, some aspects of colonization, dispersal, or founder effects are likely under the umbrella of local spatial and temporal control (why or when do organisms select a wetland). Other factors may, however, be influenced by the regional species pool, which is likely dictated by broader climate, biome, geology, and biogeography, and thus less influenced by smaller spatial and temporal scales. Furthermore, the factors contributing to explained or unexplained variation may not be mutually exclusive. The use of strong inference (Platt 1964) with multiple alternative hypotheses (such as the possibilities listed in Table 2.4) can perhaps augment our understanding of wetland ecology. Novel ideas, beyond the few suggested here, are needed. Both classic and new approaches

should be combined to provide the most complete picture possible on what controls invertebrate assemblages in freshwater wetlands.

In our opinion, research on aquatic invertebrates in wet- lands requires rejuvenation. It appears that the traditional approach of assessing invertebrate variation across a single wetland complex will yield limited new information. Many location-specific studies have yielded ambiguous results (see Batzer [2013](#); Bird et al. [2013](#)). Typically, only a small part of the total variation is explained (Tables 2.1, 2.2 and 2.3). For the study of aquatic invertebrates in freshwater wetlands, the maxim “the definition of insanity is doing the same thing over and over again and expecting a different result” (anonymous) might be replaced with “the definition of insanity is doing the same thing over and over again and *getting the same result*”, especially if that result is unsatisfying and may not significantly advance the science. To move forward, we ask researchers to think “outside the box” and consider different ways that invertebrate assemblages in wetlands might be controlled (e.g., Heino et al. [2017](#)).

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Table 2.1. PERMANOVA tables partitioning total invertebrate assemblage variation among spatial (habitats) and temporal (years, seasons) factors, and to unexplained variation for: (a) North Dakota prairie pothole wetlands; (b) California rock pools; and (c) Georgia Carolina bay wetlands.

	% variation explained (R ² value)	DF	Sums of Squares	F value	P value
(a) Prairie potholes					
Wetland	11.8	16	152.8	87.2	<0.001
Year	11.1	23	143.0	56.7	<0.001
Month	6.7	5	85.9	156.8	<0.001
Interaction (wetland*year)	15.4	326	199.0	5.6	<0.001
Residuals	55.0	6477	709.9		
(b) California Rock pools					
Pool	25.0	27	2.70	1.8	0.002
Outcrop	11.6	3	1.26	7.6	<0.001
Year	8.1	2	0.87	7.9	<0.001
Interaction (outcrop*year)	5.5	6	0.60	1.8	0.035
Residuals	49.7	98			
(c) Carolina bays					
Wetland	19.2	9	3.17	4.4	<0.001
Year	11.4	4	1.89	5.9	<0.001
Season	9.0	2	1.49	9.3	<0.001
Interaction (wetland*year)	18.8	36	3.11	1.1	NS
Residuals	41.7	86	6.90		

Table 2.2. Amount of variation in invertebrate taxon richness and invertebrate total abundance explained by spatial (habitat) and temporal (years, seasons) factors, and amount of unexplained variation for: (a) North Dakota prairie pothole wetlands; (b) California rock pools; and (c) Georgia Carolina bay wetlands.

	% variation explained (R² value)	P value	% variation explained (R² value)	P value
(a) Prairie potholes				
	<i>Total Taxon Richness</i>		<i>Total Abundance</i>	
Wetlands (17)	6.1	<0.001	2.4	<0.001
Years (24) and Months (6)	26.5	<0.001	20.3	<0.001
Unexplained residuals	52.0		63.3	
(b) California Rock pools				
	<i>Total Taxon Richness</i>		<i>Total Abundance</i>	
Pools (28) and Outcrops (4)	40.2	<0.001	42.4	<0.001
Years (3)	NS		15.2	<0.001
Unexplained residuals	46.8		40.0	
(c) Carolina bays				
	<i>Total Taxon Richness</i>		<i>Total Abundance</i>	
Wetland (10)	31.9	<0.001	16.2	0.004
Year (5) and Season (3)	18.1	<0.001	10.3	0.003
Unexplained residuals	34.8		51.4	

Table 2.3. Amount of variation in invertebrate assemblages explained by spatial and temporal factors for other published studies from wetlands.

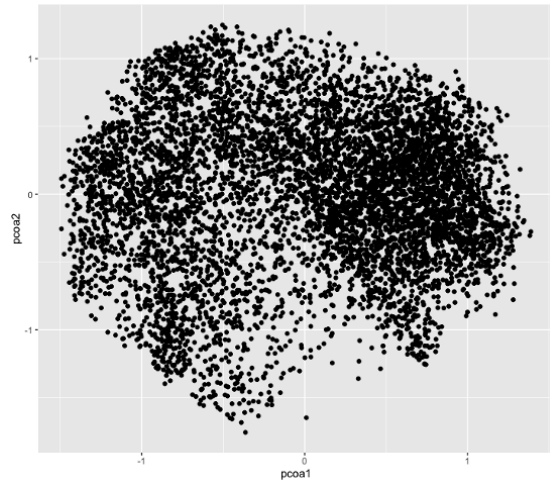
Habitat type	% community variation explained by spatial and temporal factors	Reference source
Prairie potholes, USA (North Dakota)	31%	Tangen et al. 2003
Seasonal woodland ponds, USA (Minnesota)	37%	Batzer et al. 2004
Temporary wetlands, France	24-52%	Waterkeyn et al. 2008
Temporary seasonal wetlands, Australia	37%	Sim et al. 2013
Constructed wetlands, USA (Maryland)	15%	Culler et al. 2014
Depression wetlands, USA (Oklahoma)	12-26%	Meyer et al. 2015

Table 2.4. Factors exerting control on invertebrate assemblages of depressional wetlands that can operate beyond local spatial and temporal variation, with supporting literature sources from wetlands, or related aquatic habitats.

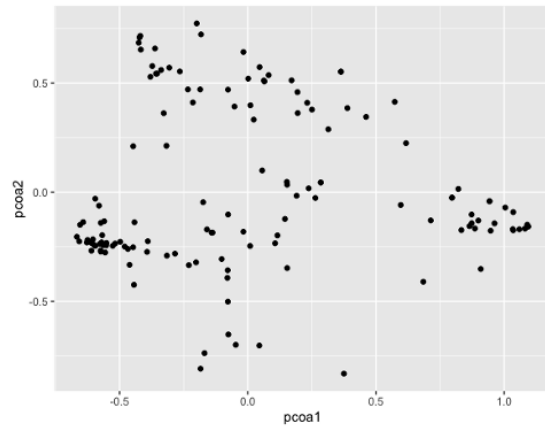
Factors	Operating through:	Literature support
<i>Organism characteristics</i>		
Organism life histories	Reproductive rates and strategies	Wiggins et al. 1980, Ruhí et al. 2013
Organism behaviors	Dispersal, colonization, and tolerance abilities	Wiggins et al. 1980, Ruhí et al. 2013, Kneitel 2018
Founder effects	Initial colonizer influence on others	Badosa et al. 2017
Neutral effects/Ecological drift	Stochastic assemblage drift from births, deaths, colonization, extinction	Chase 2007
<i>Landscape characteristics</i>		
Climate	Temperature and precipitation levels and variability across regions	Kneitel 2016, Dodds et al. 2019, Stenert et al. 2020, Epele et al. 2022
Geography	Soils and topography, broad patterns of inter-habitat connection or isolation	Tiner 2003
Biogeography	Pools of species available to a region	Dodds et al. 2019
Biome	Kinds of plants and other animals that co-occur	Wellborn et al. 1996, Dodds et al. 2019
Human land use	Current agricultural and other developmental practices across a region	Mantyka-Pringle et al. 2016
Legacy effects	Past history of geology and climate, historic human land use	Harding et al. 1998; Steinman et al. 2003; Anderson et al. 2020
Metacommunity	Local (species interactions and local conditions) and regional (dispersal and habitat heterogeneity) processes	Johnson et al. 2013, Gascon et al. 2016, Heino et al. 2017, Almeida-Gomes et al. 2020, Epele et al. 2021
Patch dynamics	Location influences colonization rates and disturbances	Declerck et al. 2011, Zamberletti et al. 2018
Mass-Effects	Complexes act as source or sink populations for different taxa	Zamberletti et al. 2018

Figure 2.1. Principle coordinates analyses plots of invertebrate assemblages in: (a) North Dakota prairie pothole wetlands (axis 1: 2.5%, axis 2: 1.5%); (b) California rock pools (axis 1: 16.7%, axis 2: 8.1%); and (c) Georgia Carolina bay wetlands (axis 1: 11.5%, axis 2: 7.0%).

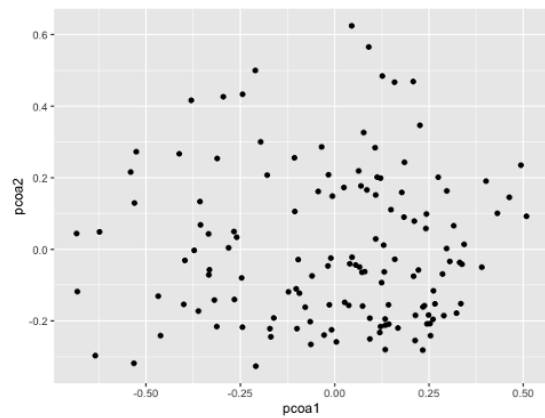
A



B



C



CHAPTER 3

SECONDARY SUCCESSION OF INVERTEBRATE ASSEMBLAGES IN
CAROLINA BAY WETLANDS²

² Reindl, S., *et al.* Secondary Succession of Invertebrate Assemblages in Carolina Bay Wetlands.
Submitted to peer-reviewed journal.

Abstract

1. We assessed patterns of secondary succession of aquatic invertebrate assemblages in a set of Carolina bay wetlands. Carolina bays are shallow, elliptical, precipitation-based depressional wetlands that occur across the Atlantic coastal plain of the Southeastern US. Some bays flood seasonally and others remain flooded continuously for multiple years. We hypothesized that assemblages of invertebrates in wetlands with longer hydroperiods would progressively change annually (i.e., undergo succession) and those in wetlands with shorter hydroperiods would not. We also hypothesized that in wetlands with significant annual variation, assemblages would diverge individualistically and organisms (mostly non-insects) that display site fidelity would be most responsible.
2. We sampled invertebrate assemblages of ten Carolina bays seasonally over six years. All ten wetlands were near pristine. We used Non-metric Multidimensional Scaling (NMDS) to visualize assemblage variation, and Permutational Analysis of Variance (PERMANOVA) to partition variance across time and space.
3. We found that annual variation was significant for only some of the wetlands, leading us to classify sites lacking annual change as “non-successional” and sites with annual change as “successional”. Non-successional wetlands tended to be smaller and drier, while successional wetlands were larger and wetter. We found that in those wetlands where annual variation was significant, assemblages changed individualistically following unique trajectories, rather than changing in similar ways. However, assemblages in all successional wetlands appeared to reach a compositional threshold, that once reached resulted in a return towards a regional central tendency in assemblage composition. Certain insects groups were associated with successional wetlands. Non-insect taxa, unlike as predicted, were not associated with either successional or non-successional wetlands.

4. We found that non-successional and successional wetlands occurred in close proximity spatially, and where succession was evident trajectories were inconsistent. Thus, even in this single wetland complex, predicting assemblage change will be difficult (some wetlands change, some don't, and those that change do not do so in parallel), complicating the use of these pristine habitats as reference sites for assessment purposes.

5. However, because variation was confined within a regional constraint, this range standard may serve as a useful guide when using invertebrate assemblages to assess wetland condition and potential non-natural changes, i.e., assemblages developing outside this natural range of variation are likely aberrant.

Introduction

Secondary succession refers to the changes in species composition that occur over time after a disturbance (Connell & Slayter, 1977; Odum, 1960). Early models of succession claimed a deterministic trajectory of communities toward an eventual climax, or semi-stable state (Clements, 1936). These models were met with criticism and gave way to more individualistic ideas about the constantly changing and specific environmental conditions of every community (an idea formulated earlier by Gleason 1917, 1927). Modern successional models support the Gleasonian idea that assemblages can either converge or diverge over time (del Moral, 2007; Matthews & Spyreas, 2010; Fig. 3.1). For freshwater systems, hydrologic disturbances (drought, floods) can alter community structure, and subsequent changes in assemblages may change directionally or non-directionally over time (Matthews et al., 2013). For fishes in streams, assemblages may successionally revert over time to a previous community state (Matthews et al., 2013).

Wetland habitats have long been useful model systems to test concepts about succession. Van der Valk (1981), for example, proposed a model of succession for freshwater wetland

vegetation based on species life history features including lifespan, propagule longevity, and propagule establishment requirements. In this model, the environmental conditions serve as a sieve that determines which species will be present in the wetland and which will be extirpated. As the environmental conditions change, the species composition of the wetland changes as well. Middleton (1998) successfully adapted this model for the vegetation of coastal monsoonal wetlands of Austral-Asia. Euliss et al. (2004) expanded on van der Valk's model for plants in prairie pothole wetlands, and extended it to address animals (invertebrates, amphibians, birds). Collectively, Euliss et al. (2004) coined the term the "wetland continuum" to address various successional patterns in prairie pothole wetlands. For the aquatic invertebrates of prairie wetlands, however, subsequent empirical testing (McLean et al., 2020; Reindl et al., 2023) indicated that this successional model may need modification to reflect the large natural variability typical for wetland invertebrates (Batzner, 2013).

For invertebrates in freshwater wetlands of Catalonia, Ruhi et al. (2013) hypothesized that primary succession (i.e., in newly created habitats), might be either convergent, with habitats following similar pathways over time, or divergent, following different pathways (Fig. 3.1). They also examined how strategies of invertebrate dispersal, represented by colonization (either nested or idiosyncratic) might confound successional changes in the macrofauna. Their empirical assessment found primary succession was different among different locations and that the means of invertebrate dispersal (who arrives first) complicates ideas about primary succession trajectories, yielding decreasing nestedness and divergent assemblage compositions over time. Both the plant-based and invertebrate-based studies conducted thus far suggest that the Gleasonian model of succession, where individualistic controls apply, is pervasive.

In our study, we evaluated aquatic invertebrate assemblages in a complex of established Carolina bay wetlands to determine if 1) secondary succession was occurring (Fig. 3.1; Non-

Successional, vs Successional) and if so, 2) were those successional assemblages converging, diverging, or following non-predictable individualistic trajectories (Fig. 3.1; Convergent, Divergent, or Individualistic). Conventional thought about succession (see above discussion of climax communities) suggests that we should observe convergent succession in this set of similar and adjacent wetlands, where assemblages all change in somewhat similar ways. Ruhi et al. (2013) already suggests that successional patterns for invertebrates in wetlands might be unconventional. We alternatively hypothesized that secondary successional patterns for invertebrates in wetlands might instead diverge or be individualistic, with either scenario leading to unique assemblages for individual wetlands, even among nearby habitats.

Methods

Study Sites

Carolina bays are depressional wetlands occurring on the Atlantic coastal plain of North Carolina, South Carolina, and Georgia (Kirkman et al., 2012). These wetlands can remain flooded over short time frames (seasons) or longer time frames (years) depending on rainfall patterns and rates of evapotranspiration. Carolina bays tend to have acidic waters ($\text{pH} < 5$), and support a diversity of plants, trees, aquatic vertebrates, and invertebrates (Kirkman et al., 2012). Ten Carolina bay wetlands were selected for this study, representing a range in habitat sizes (0.1 to 100 ha) and hydrologies (seasonal to semi- permanent). All occur within a 50 km² area of the Tuckahoe Wildlife Management Area (Georgia Department of Natural Resources), Screven County, Georgia. All 10 habitats were relatively undisturbed, possessing natural hydrologies and plant assemblages, and with intact natural forestland in the adjacent uplands. The 10 habitats had all dried completely in 2012-2013 during an intense drought but had all reflooded by 2015 when the study began.

Sampling

Invertebrate assemblages of the 10 Carolina bay wetlands were sampled using a D-frame aquatic net (30-cm width, 500- μ m mesh). Such nets are commonly used to sample and quantify invertebrate assemblages in wetlands because they collect a large sample that efficiently collects the complete range of organisms that occur in wetlands (Batzner et al., 2001). Four 1-m sweeps (~1.2 m² total areal coverage) were collected in each wetland on each sampling date. Sweeps, were partitioned to target the range of sub-habitats representative to each wetland, including open water, benthic and woody substrates, shallow edges, and plant stands. Samples were collected seasonally in March, July, and November (which encapsulated the normal growing season of the wetlands) for 6 consecutive years from 2015-2020, from all wetlands holding water. One hundred and fifty-six total samples were collected from the 10 wetlands over the 6 years, as some wetlands were dry during some scheduled samplings.

The 4 sweeps per wetland collections, including plant material, sediment, and organisms were pooled into one sample, transferred to a labelled plastic bag, and preserved with 95% ethanol. Because of the large volume of samples, subsampling was required. In the laboratory, samples were washed through a stacked 1-mm and 300- μ m sieve. To assess the microinvertebrate fauna, 1/16th of the material that passed into the 300- μ m sieve was sorted using stereoscopic magnification and identified to family. To assess the macroinvertebrate fauna, material remaining in the 1-mm sieve was halved and one half was randomly chosen for sorting. If these sub-samples did not include >100 organisms, a second sub-sample was processed. All organisms were removed by hand-picking under stereoscopic magnification. All picked specimens were preserved in 70% ethanol and identified to genus where possible, or family when not, using keys in Merritt et al. (2008) and Thorp and Covich (2010), up dated to reflect more recent taxonomic changes.

Analyses

Non-Metric Multidimensional Scaling (NMDS) is widely used by ecologists to compare assemblages of biota across space and time, including invertebrates from wetlands (Batzer & Ruhi, 2013; McCune & Grace, 2002). We developed a NMDS plot for all 156 samples, after eliminating rare taxa (i.e., <10 total individuals collected over the entire 6 years). We utilized the Bray-Curtis community similarity index as the ordination distance measure; samples close together in the ordination space have more similar invertebrate assemblages and those located further apart in the ordination space have less similar invertebrate assemblages (0-100% possible similarity). The ordination analysis determined axes that maximized the horizontal and vertical spread of the samples across ordination space. We used ellipses to visualize season and year as grouping variables within the plot. We conducted Permutational Multivariate Analysis of Variance (PERMANOVA) to partition variation across our samples in response to season, year, and site individually.

We then created ten individual NMDS plots for each study wetland and again overlaid ellipses corresponding with each of the six sample years. Wetlands whose NMDS plot with annual ellipses showed significant separation of assemblages among years (confirmed by PERMANOVA) were labelled as “successional” sites, while those showing no significant separation among years were labelled as “non-successional” sites.

We then recreated the NMDS plot and conducted a new PERMANOVA to partition variation across all 156 samples, now contrasting samples from “successional” and “non-successional” sites, creating new grouping ellipses for each category. To that plot, we further overlaid vectors corresponding to wetland size ranks (smallest to largest basin area) and wetland hydrology ranks (driest to wettest) to determine if these environmental vectors aligned with the differences between successional and non-successional assemblages. We then created yet

another NMDS plot of all samples, with grouping ellipses for samples from successional and non-successional sites, and overlaid vectors for the abundances of those taxa that varied the most ($P < 0.001$) across ordination space to determine if these taxa vectors aligned with the differences between successional and non-successional assemblages.

Finally, we assessed only “successional” wetlands. We created another more-limited NMDS plot and examined the year-to-year trajectories for each successional wetland to determine if assemblages changed similarly in each site from year to year (i.e., converged), or changed uniquely (i.e., diverged or changed individualistically (see Fig. 3.1).

All analyses were completed using the R statistical program (R version 4.1.2) with the vegan package (Oksanen et al. 2022). Data were natural log transformed to reduce undue bias from the most common taxa in the ordinations.

Results

Over the 6 years of sampling in the 10 wetlands ($n = 156$ samples), we collected over 300,000 invertebrates and 76 taxa. Season, year, and site each explained a portion of the variation in invertebrate assemblages (Table 3.1). In focusing on the effect of season, NMDS plot ellipses showed that the largest differences were between the spring invertebrate assemblages and the summer assemblages, with winter assemblages overlapping with the other two seasons (Fig. 3.2A). In focusing on the effect of year (i.e., succession), the NMDS with ellipses for years showed great overlap among all 6 years (Fig. 3.2B). But given that the effect of site explained the most variation (i.e., sites were unique from each other), individual NMDS plots were created for each wetland to examine potential differences among years on a site-by-site basis (Figure 3.3).

We found that the influence of annual variation was greater in some wetlands compared to others, which likely contributed to the lack of visible patterns among years in Figure 2B. The

NMDS plots and PERMANOVA analyses for bays 3, 5, 6, 7, 9, & 10 showed clear separation among year ellipses, indicating differences in the assemblages from year to year (Fig. 3.3A). We labelled these wetlands as being “successional”. The NMDS plots and PERMANOVA analyses of wetlands 1, 2, 4, & 8 did not show significant separation among year ellipses, indicating similarity in the assemblages from year to year (Fig. 3.3B). We labeled these wetlands as being “non-successional”.

We then recreated the NMDS plot of all 156 samples with two ellipses corresponding to “successional” and “non-successional” wetland samples (Fig. 3.4). PERMANOVA analyses indicated that “successional” or non-successional” wetlands had different invertebrate assemblages, although the effect size was small ($R^2=0.03$, $P < 0.01$). We then overlaid vectors corresponding to wetland size and hydrology to this plot and found both environmental variables were similarly and parallelly aligned along the non-successional to successional habitat gradient (Fig. 3.4A), suggesting that they were associated with the differences between the two habitat types.

We then used the same NMDS plot with all 156 samples, with ellipses corresponding to successional and non-successional samples, and overlaid vectors for the most responsive invertebrate taxa ($P < 0.001$) (Fig. 3.4B). The taxa vectors that were parallelly aligned along the non-successional to successional gradient were dominated by insects (Fig. 3.4B), suggesting these were the organisms most associated to the differences between the two habitat types. Non-insect invertebrates generally did not associate with either successional or non-successional wetlands (Fig. 3.4B).

We then looked at the annual trajectories of the 6 successional bays over the 6 years of study (Fig. 3.5), and none of them appeared to follow a similar path from year to year, suggesting that succession in each was individualistic, being neither convergent nor predictably

divergent. While this plot suggested that the invertebrate successional trajectory in each wetland was unique, the whole set of successional wetlands seemed to be constrained within a similar region of ordination space (i.e., they changed somewhat randomly but within a limited range of total variation).

Discussion

In this study, annual variation was not evident for all wetlands, leading us to classify some wetlands as non-successional and some as successional. This was surprising given that the environmental conditions in non-successional wetlands were in many ways (water quality, dominant plants) like those habitats that had invertebrate assemblages that changed annually. Other studies assessing biotic patterns over time have failed to detect annual change. Zingone et al. (2023) assessed changes in phytoplankton assemblages over a 25-year period in the Mediterranean Sea, and found negligible annual change, although seasonal patterns were strong. Other studies have shown that annual changes account for a relatively small amount of assemblage variation. Reindl et al. (2023) found that annual variation, in the same complex of Carolina bay wetlands used for our study, accounted for only 11.4% of the total assemblage variation. One of the many factors at work in wetlands is succession, but it may not operate in every wetland. Because our study suggests that non-successional and successional wetlands can co-exist in the same landscape, it complicates assessment of invertebrate community ecology across wetland complexes and may be a reason that patterns can appear intractable in many places (Batzner, 2013).

Non-successional Carolina bay wetlands were drier and/or smaller than most successional wetlands, and factors like size and hydroperiod may affect how invertebrate succession occurs in wetlands (e.g., Stenert & Maltchik 2007; Batzner et al., 2024). It may be that some habitat characteristics not only affect how succession progresses (see Euliss et al., 2004), but also affect

if succession occurs at all. Hall et al. (2004) suggest that periodic drying of playa wetlands of Texas may constantly “reset” the successional clock, and thus mature assemblages of invertebrates may never develop.

Even where successional changes in wetland invertebrates are apparent, the assemblages seem to respond individualistically. Ruhi et al. (2013) examined primary succession of wetland invertebrates (in newly created wetlands of Catalonia) and found divergence rather than convergence (i.e., the colonizing fauna was similar but then assemblage compositions diverged over time, in different wetland complexes). If wetlands with the same initial conditions (newly flooded) diverge, then it is not surprising that the wetlands in our study, each already possessing unique historical attributes developed over decades (and longer), would also diverge. What was surprising in our study was that each of our wetlands were individualistic in their responses, neither converging nor predictably diverging (Fig. 3.1). Reindl et al. (2023) found that community compositions of North Dakota prairie potholes were highly variable but were constrained within a defined range of variation. This suggests that while succession may be individualistic, it may not develop completely haphazardly, but instead converges within a predictable overall community composition determined by regional conditions (e.g., climate, biogeography). Our successional wetlands showed this “ricochet” effect (Figs. 3.5 and 3.6), where once communities reached some sort of compositional threshold, they returned towards an apparent regional central tendency. Successional assemblages were not static nor identical but did exhibit individualistic convergence (i.e., changed randomly but within a regional constraint). This idea of a boundary within which change occurs has already been used to describe individual populations (density vagueness *sensu* Strong, 1986) and may also apply to communities. The idea of a return tendency of assemblages after reaching a compositional extreme has also been described for stream fish assemblages (Matthews et al., 2013).

The kinds of invertebrates that contributed most to succession in our study were unexpected, however. We had predicted that non-insect taxa, lacking flight, would display site fidelity and should exhibit the greatest response to annual variation (i.e., populations in year 1 should affect populations in subsequent year 2, and so forth) (Hall et al., 2004; Moorhead et al., 1998). However, non-insect taxa were not associated with the successional to non-successional gradient in our set of wetlands. Instead, it was the insects, whose adults can readily fly from wetland to wetland, that were most positively associated with successional wetlands, even though they may lack site fidelity: i.e., populations in year 1 may not strongly affect populations in year 2, and colonists in year 2 might be comprised of adults that emerged from numerous other wetlands. Insects, it seems, were controlling succession of invertebrate assemblages in our study. The vagaries of aerial insect colonization may be responsible for the individualistic character of each wetland's succession, i.e., who shows up, and when, dictates assemblage compositions (Batzner, 2013).

The Gleasonian model of individualistic succession appeared a good fit for the aquatic invertebrate assemblages of Carolina bay wetlands (at least where succession was evident) (Gleason, 1927). However, the degree of individualism found in our wetlands was more extreme in comparison to the plant assemblages studied elsewhere (van der Valk, 1981). Previous research has indicated that wetland invertebrates are highly unpredictable, often displaying contradictory or difficult to reproduce results (Batzner, 2013). Bird et al. (2013) even suggest that the highly variable nature of macroinvertebrates make them an unreliable indicator of human disturbance in wetland bioassessment. The well-known variance of wetlands and wetland invertebrates in many places suggests that the individualistic convergence (Fig. 3.6) seen in our wetlands is likely pervasive (Batzner, 2013; Reindl et al., 2023).

Our results have important consequences for wetland monitoring and wetland restoration. All 10 of our sites were in near-pristine condition, seemingly making them useful reference sites for bioassessments of wetland impairment or target endpoints for restorations (Moorhead, 2013). However, the occurrence of non-successional and successional wetlands within the same landscape complicates selecting a reference standard against which impaired wetlands would be assessed. Fortunately, our results indicate that determining the central tendencies of natural wetlands in a region (identifying the boundary within which assemblages naturally vary) may prove more useful (Fig. 3.6). Wetlands with invertebrate assemblages that fall outside of this natural regional range of variation would likely be impaired or have not yet been successfully restored. Similarly, the natural range of variation across multiple sites and years may serve as a benchmark to assess the magnitude of future biotic alterations in a changing global environment.

Acknowledgements

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Declarations

Not applicable

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Table 3.1. Results of Permutational Multivariate Analysis of Variance (PERMANOVA) to partition variation across 156 samples, in response to season, year, and site individually, including R^2 and p values.

	% variation explained (R^2)	DF	Sum of Squares	F value	P Value
Season	0.12361	2	3.4786	14.2909	<0.01
Year	0.13261	5	3.7320	6.1328	<0.01
Site	0.14264	9	4.0142	3.6648	<0.01
Residuals	0.60114	139	15.9171		

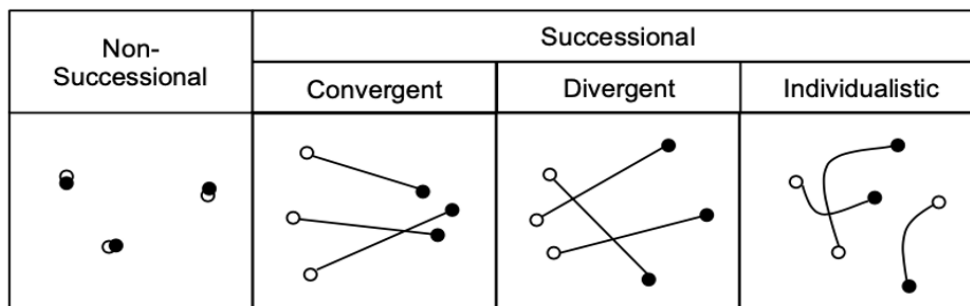


Figure 3.1. Modification of the model by Ruhi et al. (2013), showing the possible successional trajectories (lines) for aquatic macroinvertebrate communities during secondary succession: non-successional and successional (convergent, divergent, and individualistic). Open circles symbolize initial time points, filled circles symbolize later time points.

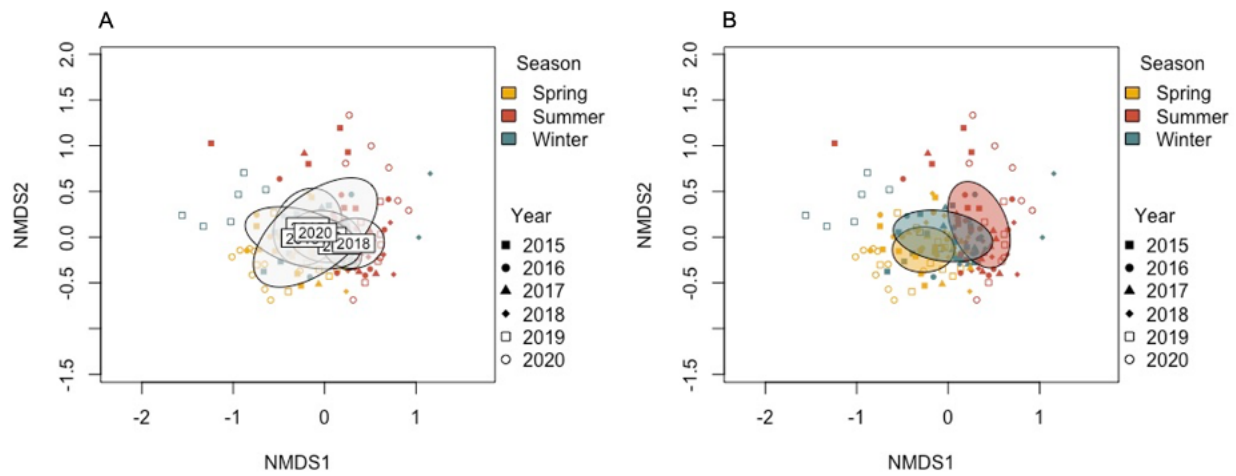


Figure 3.2. Non-Metric Multidimensional Scaling plots relating invertebrate assemblages collected from 10 Carolina bay wetlands over a 6-year period. Symbols represent sampling events of different years. The color of each symbol corresponds with the season of the sampling event. Ellipses in plot A group samples by season, while those in plot B group samples by year.

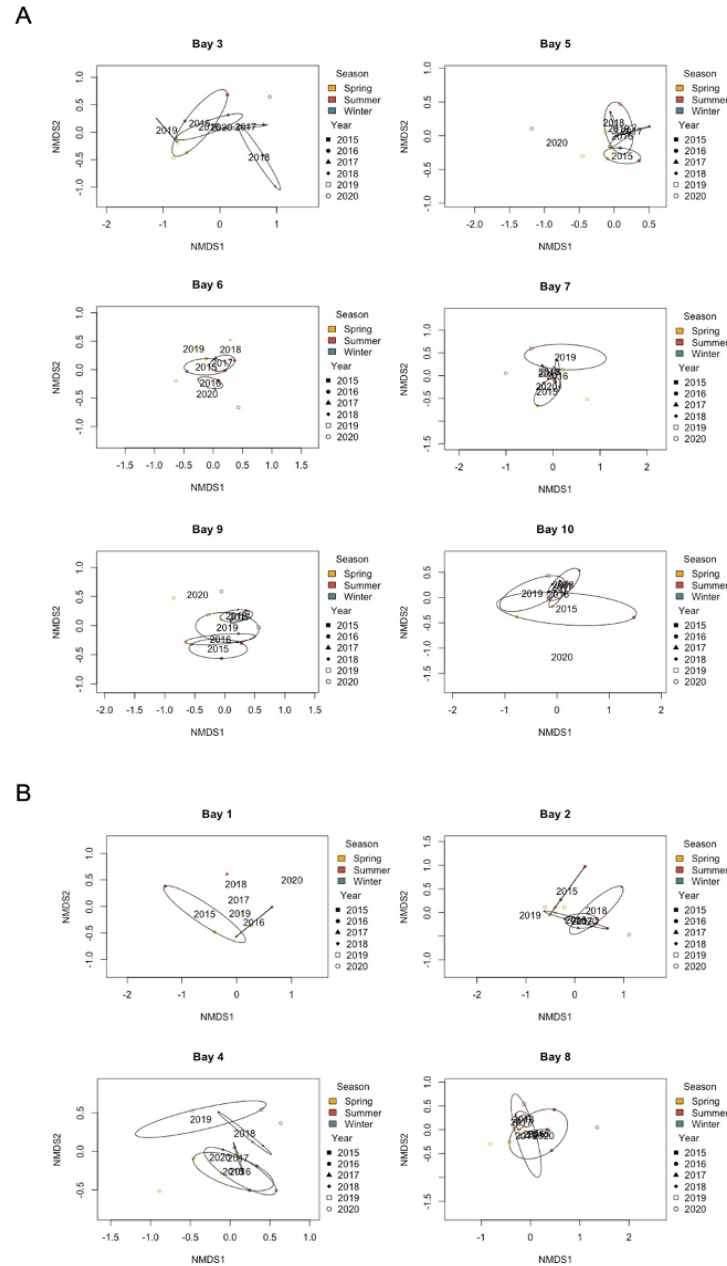


Figure 3.3. Non-Metric Multidimensional Scaling plots relating invertebrate assemblages collected from each of 10 Carolina bay wetlands over a 6-year period. Symbols represent sampling events of different years. The color of each symbol corresponds with the season of the sampling event. Ellipses group samples by year. The 6 plots in group A are considered successional wetlands (with significant invertebrate assemblage changes among years), while the 4 plots in group B are considered non-successional wetlands (without significant assemblage changes among years).

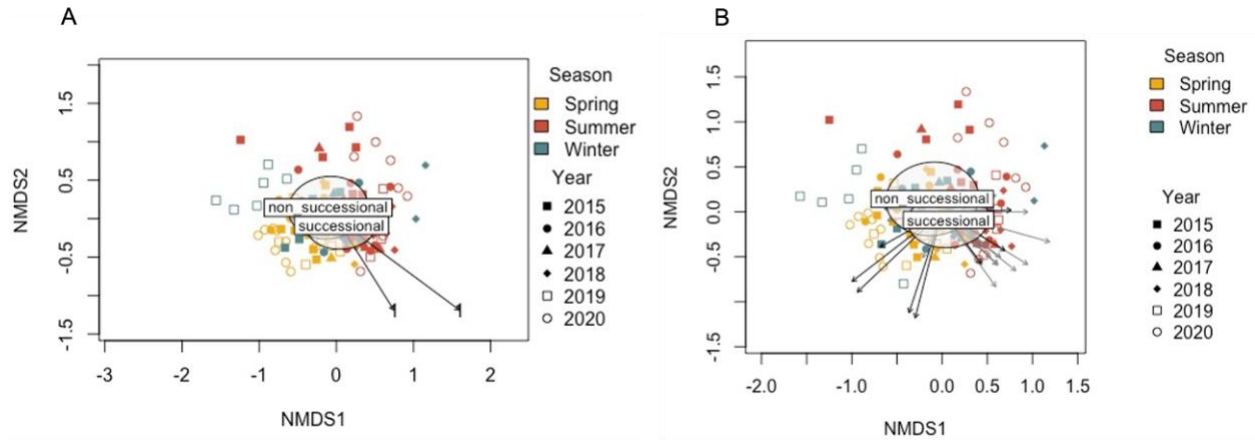


Figure 3.4. Non-Metric Multidimensional Scaling plot relating invertebrate assemblages collected from 10 Carolina bay wetlands over a 6-year period. Symbols represent sampling events of different years. The color of each symbol corresponds with the season of the sampling event. A) Ellipses represent groupings of successional and non-successional wetlands. Vectors represent increasing size and hydroperiod rankings, with both being aligned towards successional wetlands (lower right). B) Ellipses represent groupings of successional and non-successional wetlands. Vectors represent highly significant insect and non-insect taxa ($P < 0.001$), with vectors pointing towards the lower right and upper left being aligned with the successional and non-successional wetland gradient. The taxa for vectors pointing towards the lower right (indicators of successional wetlands) includes 9 insects: *Buenoa*, *Ceratopogonidae*, *Erythemis*, *Pachydiplax*, *Coenagrionidae*, *Pelocoris*, *Suphis*, *Tanyptodinae*, and *Tramea* and 2 non-insects: *Ferrissia* and *Ostracoda*. The taxa for vectors pointing towards the upper left (indicators of non-successional wetlands) includes only one insect: *Psorophora*. Taxa vectors not aligned with the successional to non-successional gradient, pointing either towards the upper right or lower left, includes 6 noninsects: *Cyclopoida*, *Crangonyx*, *Caecidotea*, *Calanoida*, *Daphniidae*, and *Naididae* and 7 insects: *Agrypnia*, *Agabus*, *Callibaetis*, *Chaoborus*, *Chironominae*, *Coptotomus*, and *Sminthuridae*. Note that some of the shorter vectors for taxa are not readily visible because they are covered by other plot elements.

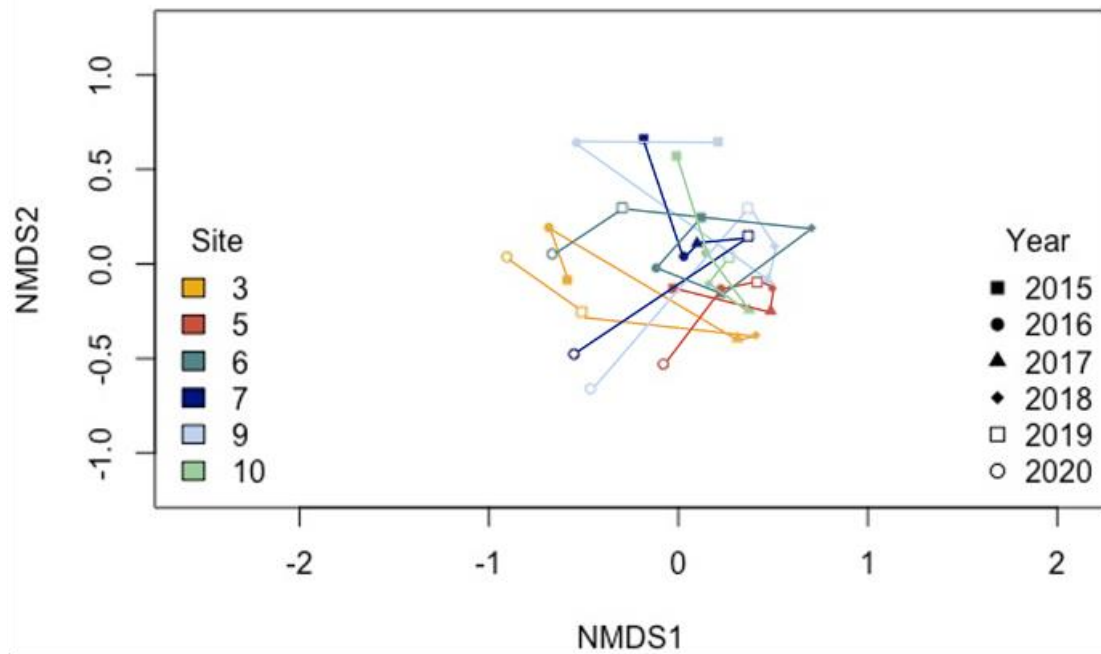


Figure 3.5. Non-Metric Multidimensional Scaling plot relating invertebrate assemblages collected from 10 Carolina bay wetlands over 6-year period. Symbols represent sampling events of different years. The color of each symbol corresponds with the wetland sampled. All samples displayed were collected in March, to avoid noise from seasonal changes.

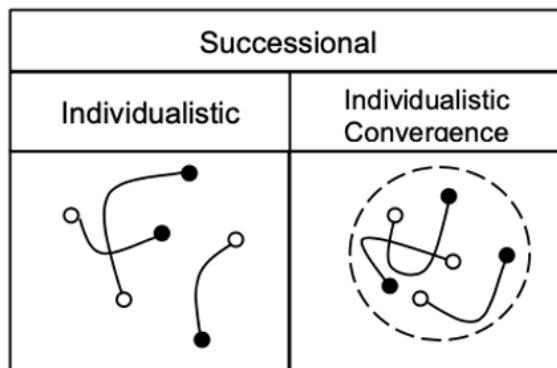


Figure 3.6. Possible individualistic successional trajectories (lines) for aquatic macroinvertebrate communities during secondary succession: Individualistic and Individualistic Convergence. Open circles symbolize initial time point, filled circles symbolize later time point, and the dotted line defines a constraint to variation.

CHAPTER 4

CONCLUSIONS

Aquatic invertebrates play a vital role in freshwater ecosystems. These organisms are instrumental in food webs, nutrient cycling, and as indicators of water quality (Batzner and Wissinger 1996; Batzner and Boix 2016). In the face of climate change, it is crucial that we understand how these organisms might be affected by environmental changes. Without greater understanding of the environmental controls acting on aquatic invertebrates, our attempts to act for their conservation, could be ineffective. If for example, we continue to simply attribute variation of wetland invertebrates to spatial and temporal factors, we will be ignoring unexplained variation of equal magnitude. It's possible that there are factors yet unidentified that are governing much of this unexplained variation, but if we don't first acknowledge what is unexplained in these systems, how can we ever hope to explain it.

Examination of patterns of secondary succession yielded some interesting insight about one complex of wetlands. Those wetlands where annual variation was significant, while individual in their trajectories, did appear to reach a regional central tendency of composition. In systems as variable as depressional wetlands, with organisms as variable as aquatic invertebrates, standards like a regional central tendency composition might be a far more reasonable metric for evaluating communities than the current norm of using a reference wetland.

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APPENDICES

APPENDIX A. CHAPTER 2 SUPPLEMENT

Supplement 1: Doing the same thing over and over again and getting the same result: assessing variance in wetland invertebrate assemblages

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Supplement 1 Title: Descriptions of the environmental conditions, field sampling procedures and laboratory methods used in Prairie potholes of North Dakota, Rock pools of California, and Carolina bays of Georgia, USA.

Prairie Pothole Study Wetlands: North Dakota

The Prairie Pothole Region is a wetland-grassland dominated landscape (~777,000 km²) extending from northwest Iowa, North and South Dakota and into central Alberta (Smith et al. 1964). The region is characterized by millions of individual wetland basins that can exhibit highly variable ponded-water dynamics (Gleason et al. 2011). The high spatial and temporal variability exhibited by prairie-pothole wetland ponds is a reflection of the high spatial and temporal variability in climate (Winter 2003; Liu and Schwartz 2012). Annual maximum and

minimum temperatures can reach 40 °C in the summer and -40 °C in the winter, respectively, and mean annual precipitation ranges from 30 cm/year to 90 cm/year.

Seventeen depressional prairie-pothole wetlands collectively known as the Cottonwood Lake Study Area (hereafter CLSA) were selected for this study using data from 24 sampling seasons (1992-2015). The CLSA is part of a 92-ha complex of natural prairie-embedded wetlands located on a U.S Fish and Wildlife Service managed Waterfowl Production Area in Stutsman County (ND). The 17 sampled wetlands ranged from being seasonally to permanently ponded, with hydroperiods varying annually (McLean et al. 2019).

Aquatic macroinvertebrates were sampled during the ice-free growing months (April to September) using vertically oriented, funnel traps (Swanson 1978) and deployed in wetlands for 24 h. Samples were collected once a month from random locations within each vegetation zone present (shallow marsh, deep marsh, open water) along three transects radiating from the center of each wetland. Traps are only placed in areas of the wetlands with surface water depth > 25 cm. A maximum of 54 samples are collected from a wetland a year if all three vegetation zones contain ponded water for all six sampling months.

Upon collection, trap contents are sieved through a 0.5-mm screen into a 475-ml plastic sample container, preserved with ethyl alcohol (80%) and stored in a U.S. Geological Survey laboratory in Jamestown (ND), until processed. Processing consists of rinsing samples through a stainless-steel beaker with a 0.5-mm screened side and then separating remaining aquatic macroinvertebrates (> 0.5 mm) from debris and identifying them to the lowest attainable taxonomic resolution (typically to genus, Mushet et al. 2017). Aquatic-macroinvertebrate sampling data for all seventeen wetlands was then summarized as mean-annual abundances of uniquely identified taxa per trap. Wetland macroinvertebrate-monitoring data from the CLSA are

openly available from the U.S. Geological Survey through the Missouri Coteau Wetland Ecosystem Observatory: <https://www.sciencebase.gov/catalog/item/52f0ffd9e4b0f941aa181fc6>.

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Study Rock Pools: California

Rock pools (Fig. 1) were sampled from four outcrops within a ~25 km² region in Contra Costa and Alameda Counties. The Mediterranean climate of this region is characterized by cool wet winters and hot and dry summer and falls. The hydrological cycle is highly variable across years because of the highly variable precipitation patterns. Nonetheless, because of the impermeable substrate, the first consistent rains lead to inundation, usually by November, and desiccate by May (Marr 2019). Each site had at least one sandstone rock outcrop with multiple pools on each outcrop.

Twenty-eight rock pools were sampled between November and April over three years (2016-2019), as part of a monitoring program of the endangered species *Branchinecta longiantenna* (Eng et al. 1990). Pools containing *B. longiantenna* were targeted along with adjacent pools. Pools are highly variable in size (depth (cm): mean = 12.4, SD = 9.5)

A large aquarium net (20.3 x 15.2 cm, 0.2 mm mesh) was used for a 10-s sweep capturing the depth and topography of the pool. Individuals were placed into a tray for identification and enumeration of each taxon. Voucher specimens were collected for further identification, using Thorp and Covich (2010). Observations (~1 min) of the pool are made for rare taxa not caught by nets to be recorded.

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Figure 1. Examples of rock pools and outcrops that were sampled in Contra Costa and Alameda Counties (Central Valley region of the state of California) (photos by J. Kneitel).

Carolina Bay Study Wetlands: Georgia

Ten Carolina bay wetlands were selected for study, located on a 30 km² portion of Georgia's Tuckahoe Wildlife Management Area (Screven County). Carolina bays are depression wetlands, common on the upper Atlantic Coastal Plain of the Southeastern US (Kirkman et al. 2012). None of the 10 wetlands existed in isolation, with multiple other Carolina bays being within a 1 km radius of each. Water budgets are dominated by inputs of rainfall and outputs of evapotranspiration; groundwater discharge into wetlands rarely occurs. The 10 study wetlands had near natural hydrologies, and waters were oligotrophic (pH 3.5-5.0; electrical conductivity <100µS/sec). Wetland vegetation was primarily forested (Fig. 2), although emergent and submersed vegetation was common; surrounding uplands were natural pine-hardwood forests. The sites were selected to cover the range of sizes (0.1-100 ha), and hydrologies (seasonal-permanent) that occurred naturally in the area. The local climate is humid, subtropical with hot summers and cool winters, with approximately 100 mm of rainfall occurring most months. Due to increased evapotranspiration in the summer and early fall, seasonally flooded habitats tended to dry then.

Each of the 10 Carolina bays was sampled seasonally (March, July, November) from 2015 through 2019. Samples were collected with a standardized sweep netting procedure (mesh size = 500 µm) that proportionally targeted all sub-habitats that occurred. Samples were not collected from completely dry habitats.

Field preserved samples (95% ethanol) were transferred to the laboratory for processing. Samples were rinsed in stacked sieves (1-mm and 500-µm), to split the organisms into macro- and micro-invertebrate components (small early-stage macroinvertebrates that washed through the 1-mm sieve were recombined with the larger individuals when quantified).

Macroinvertebrate portions were randomly split into halves, and one half was hand-picked using a stereomicroscope; if < 100 individuals were recovered from this subsampling, the entire sample was then picked. Microinvertebrate portions were randomly split so that 1/16 (or on occasion 1/8) of the sample was picked. Taxa were identified using keys in Merritt et al. (2008) and Thorp and Covich (2010), to levels where the research team was confident of reliability, and then quantified. A few key taxa names were confirmed by taxonomists at the Georgia Museum of Natural History.

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Figure 2. Example of a Carolina bay wetland in Screven County, Georgia (photo by D. Batzer).