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Changes in Zooplankton Community Structure in a Wetland Metacommunity Following Homogenization from Flooding

Kevin Peteroy

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Abstract

Wetlands in urban areas provide ecosystem services that benefit the environment and society. Through these services, wetlands are able to help mitigate consequences of human activities such as flooding by increasing permeable surface coverage and contaminant filtering from polluted runoff. One such type of wetland, vernal pools, provide important temporary habitat for many organisms, including myriad zooplankton species. For planktonic organisms, flooding events might be disruptive or allow them to disperse to areas that would be otherwise inaccessible. I observed changing abiotic conditions and zooplankton communities in 20 vernal pools at a wetland in Wayne, New Jersey, USA over a 4-month period, during which the wetland flooded twice. The primary goal was to understand how water quality parameters and zooplankton abundance, average family richness, and diversity change after a flooding event. The abiotic and zooplankton communities changed in response to flooding, although the abiotic changes did not predict changes to the biotic community. The first flood occurred in August and resulted in lower average taxonomic diversity and reduced compositional differences of zooplankton among pools. The second flooding event which occurred in late October increased zooplankton diversity. The increased diversity was likely caused by the flood water coming from warmer water in a large wetland and nearby river with more species of zooplankton than the smaller, colder, vernal pools at the time of flooding. This research suggests the potential importance of the action of seasonal flooding events in determining the effect on zooplankton diversity. In addition, the research indicates a lack of relationship between the zooplankton community and water quality conditions, and that even with the occurrence of flood events, zooplankton communities may be resilient.

MONTCLAIR STATE UNIVERSITY

Changes in Zooplankton Community Structure in a Wetland Metacommunity Following

Homogenization from Flooding

By

Kevin Peteroy

A Master's Thesis Submitted to the Faculty of

Montclair State University

In Partial Fulfillment of the Requirements

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Introduction

Urban wetlands serve as vital ecological sanctuaries that offer numerous environmental, social, and economic benefits (Alikhani et al., 2021; Jia et al., 2011; Zhang et al., 2023). Wetlands provide beneficial ecosystem services such as flow control, flood control, storing and removing pollutants, and managing heat flux (Jia et al., 2011; Palta et al., 2017; Shutes, 2001). Flow and flood control are particularly important in urban environments because impermeable surface coverage in urban areas increases the runoff from rainfall (Feng et al., 2021; Konrad, 2003). The presence of urban wetlands creates a buffer zone that can slow the water speed, as well as contain some of the runoff that would otherwise result in a flood (Alikhani et al., 2021; Konrad, 2003). Wetland buffer zones improve the water quality and store pollutants through the combined impact of the vegetation, soil filtration and rich associated microorganisms (Alikhani et al., 2021; Shutes, 2001). Additionally, the presence of wetlands in urban areas contributes to cooling, limiting the formation of an urban heat island (Alikhani et al., 2021; Jia et al., 2011; Palta et al., 2017). The degree in which urban wetlands are able to affect the surrounding environment might be limited when compared to their rural counterparts, due to their potentially limited size (Jia et al., 2011; Palta et al., 2017; Zedler, 2003; Zhang et al., 2023). Wetlands also improve cultural and recreational services (Palta et al., 2017; Pedersen et al., 2019). In some instances, urban wetlands are considered as a tool for conservation (Wang et al., 2022). Through successful management of wetlands in both urban and rural environments, managers can provide better quality of life for the people who live close to the wetland (Alikhani et al., 2021; Jia et al., 2011; Palta et al., 2017; Pedersen et al., 2019).

Vernal pools, also known as seasonal depressional wetlands or ephemeral pools, are created from low spots in the local topography that fill up with the rainwater (Zedler, 2003). The creation of vernal pools usually peaks in the spring (Keeley & Zedler, 1998). Vernal pools are separate from any larger water body, and therefore can appear and disappear independent of other nearby pools. The discrete nature of vernal pools makes them likely to contain communities reflective of the pool's distinct chemical and morphological profile (Zedler, 2003). Despite their temporary nature, vernal pools provide important breeding habitat for many amphibians (Lathrop et al., 2005). In New Jersey, seven species of reptiles and amphibians require ephemeral wetlands to reproduce, and six species found in ephemeral pools are listed as either endangered or threatened according to the state of New Jersey (Lathrop et al., 2005). The impact of vernal pools also stretches to other types of vertebrates (Dixneuf et al., 2021). For example, the areas around vernal pools in a boreal environment were determined to have a presence of both mammals and birds, sometimes with greater activity levels than around permanent wetlands (Dixneuf et al., 2021). Due to their significance, vernal wetlands are protected by both federal and state laws (Burne & Griffin, 2005).

Vernal pools are also host to myriad invertebrate taxa, most notably the orders Anostraca (fairy shrimp), Laevicaudata (clam shrimp), and Notostraca (tadpole shrimp). The presence of these groups can be indicative that a water body is temporary (Colburn et al., 2007). Copepods, ostracods, cladocerans, rotifers, flatworms, oligochaetes, mollusks, and numerous insect species can also be found in vernal pools, though they have less stringent requirements and often inhabit other types of freshwater environments.(Colburn et al., 2007). Zooplankton (copepods, cladocerans, rotifers, and ostracods) help to form the base of aquatic food webs, as their biomass provides food for many larger organisms such as fish and larger invertebrates. Different types of zooplankton serve as primary and secondary consumers, preying upon phytoplankton and other zooplankton respectively (Brandl, 2005b). As with larger organisms, the taxonomic community

within a vernal pool depends on various biotic and abiotic factors, which tends to result in unique, normally stable communities, even within close proximity to one another. Zooplankton are also able to affect both the water chemistry and phytoplankton community composition in the system (Declerck & de Senerpont Domis, 2022). This is done through their consumption of prevalent phytoplankton groups which sequester a greater amount of one of the key nutrients, helping to stabilize any discrepancies (Declerck & de Senerpont Domis, 2022). However, acute environmental factors have the potential to upset an otherwise stable state and change the community within the water body.

Flooding is a particular threat to urban areas, where increased coverage of impermeable surfaces has been tied to both increased frequency of flood events and increased flood intensity (Konrad, 2003). Blue and green infrastructure has been replaced with concrete and poorly draining soil. During periods of heavy rainfall, the impermeable surface causes more water than normal to become runoff (Feng et al., 2021). This runoff then travels along the surface, and through any series of gray infrastructure present in the urban area. Passage along the surface can lead to erosion of the remaining soil, disrupting the structure of wetlands through which the runoff passes. Although wetlands are able to store pollutants and slow the flow of water, rapid flooding can overload the system, causing damage to the wetland, resulting in a less resilient wetland less able to support vital ecosystem services (Alikhani et al., 2021). Flood events not only bring in contaminants from the surrounding land area, but an inconsistent water level can act as a disturbance event for resident taxa in the pools, and may even result in a regression to an earlier successional phase for the community (Zhou et al., 2023).

In this study, I examined a vernal pool metacommunity in Wayne, NJ, USA, to observe the changes in zooplankton community composition over a 4-month timespan in an area that

experiences seasonal changes, including flooding events. I aimed to determine whether any biological changes were caused by the stress of the flood events themselves or resulting water chemistry changes from the floods.

Methods

Study Site:

20 sites were observed at Walker Avenue Wetlands in Wayne, NJ, USA over a 4-month period of seasonal transition (Figures 1 and 2). These sites are located around a larger central wetland, which is connected to the Pompton River, which sometimes overflows into the main wetland, and the vernal pools nearby. These sites change in volume and surface area and their volume can vary significantly over days or weeks. On any given day, the pools range in size from 1.5 to 3 meters across at their widest, with some observation days showing surface areas up to 10 meters across. These sites were shallower than 30 centimeters deep in the center, and generally less than 10-15 centimeters. Many sites have vegetation in and around the pools, along with leaf matter from the nearby trees (Figure 2). While some pools have access to more sunlight based on their positions in the understory, all pools are located beneath the canopy. The pools, despite their apparent variety in structure, had similar exposure to the same natural and anthropogenic inputs.

Two flooding events occurred during this study period (Figure 3). The first flooding even occurred due to two storms, tropical storm Henri and Hurricane Ida which both occurred within 2 weeks. The first sampling event occurred prior to these storms and the second sampling event occurred after the flood water subsided as soon as each pond was discrete. The second flooding event occurred later in the fall, after the September and October sampling events but before November (Figure 3). The second flooding event washed warmer water over the berm separating

the primary wetland from the vernal pools, which were consistently close to ambient (cool) temperatures. The river water and primary wetland water remained warmer during this period, sustaining populations of zooplankton and phytoplankton. The extent of each flood could not be confirmed directly to ensure that all ponds were inundated with flood water. Therefore, we used chloride concentration as a proxy for detecting a flood event (Figure 4). The river and primary wetland water contain higher concentrations of anthropogenically-sourced chloride compared to each of the vernal pools, which are primarily filled by rain events and therefore have low chloride. Chloride concentrations increased after each flood event with relatively short retention time, as shown by the slight decrease between the September and October sampling events.

Sampling:

At each of the 20 sites, a 250 mL water sample was collected 1-2 times per month in August through November of 2021. Some bottles were reused between samples. Prior to reuse, these bottles were soaked with bleach for 10 minutes and rinsed with distilled water prior to reuse if they were not new bottles. Zooplankton samples were collected from as close to the center of the water column as possible so as to avoid collecting debris or organisms from the surface or bottom of the pools. In cases where the pool depth was too shallow to accommodate the mouth of the container, the 250 mL containers were filled with a backup 50 mL centrifuge tube. Samples were returned to the lab within 2 hours and treated with Lugol's iodine solution to preserve and stain the zooplankton.

In addition to the zooplankton samples, 50 mL water samples were taken in sterile and chemical-free centrifuge tubes for water chemistry analysis. The samples were taken from the approximate center of the water column and were closed and brought back to the lab and frozen

prior to analysis. Sampling size was standardized, regardless of site volume due to the instability in volume at any one site, as well as the differences in site volume among the 20 sites.

Laboratory Analyses*:*

Zooplankton were allowed to settle in the collection containers overnight. Once settled, the zooplankton samples were decanted, preserving both the zooplankton and debris at the bottom. The sample was then mixed and placed under a 100x magnification Zeiss Stemi 305 microscope (Zeiss, White Plains, NY 10601). The zooplankton were then observed and identified to Family based on the University of New Hampshire's Image-Based Key to the Zooplankton of North America (James F. Haney et al., 2024). Each individual was identified and counted using a gridded specimen dish, on a grid-by-grid basis, so as not to miscount. This process results in the abundance values of each present Family on each sampling date at each site. The sample was then dyed further with Lugol's Iodine Solution and stored in a 50mL centrifuge tube in case future analyses are required.

Water samples were analyzed with a HACH DR6000 UV-Vis Spectrophotometer (Hach, Loveland, Colorado 80539), following the procedures from the corresponding test kit from the HACH TNT line (Table 1). Samples were tested for chloride (Cl), total phosphorous (TP), total nitrogen (TN), nitrate (NO₃), calcium (Ca²⁺), and hardness. Details of each test designed by HACH can be found in Table 1. Each set of tests required that the UV-Vis spectrophotometer be given the baseline value through the zero vial. The testing protocols for chloride and nitrate were the least complicated, involving only the mixing of sample and solution, allowing for the listed time to pass, then placing the vial into the UV-Vis Spectrophotometer for a reading. The combined hardness, calcium, and magnesium tests required multiple reaction steps, with HACH's solutions A and B, as well as taking readings with the UV-Vis Spectrophotometer

throughout the process. Measuring total nitrogen requires the heating of sample solution in reaction tubes. Finally, the total phosphorous tests also require heating implements, and reaction of the sample with HACH's reagent and 2 Dosi-caps throughout the process. The results of these chemical analyses were recorded, and the remaining water samples were refrozen and stored.

Statistical Analyses:

All analyses and figures were completed using R (version 4.2.0; R Core Team, 2022). Taxa richness, abundance and diversity were calculated based on the counted zooplankton. These were then used for comparison with chemical presence, as well as to depict change over time. The packages used for data analysis in R included *ggplot2*, *cowplot*, *vegan*, *dplyr*, *ape*, and *sciplot* (Morales, 2020; Oksanen et al., 2022; Paradis & Schliep, 2019; Wickham, 2016; Wickham et al., 2022; Wilke, 2020). A Non-Metric Multidimensional Scaling analysis (NMDS) was performed using packages *vegan*, *dplyr*, and *ape* in order to describe the zooplankton community composition in the pools as it changed across the observational period. One-way ANOVAs were used to determine if zooplankton abundance, richness, and diversity changed from month to month during the study period. A Dunnett's post-hoc analysis was employed to determine if the abundance, richness, and diversity estimates were statistically different than the measurements collected in August. Dunnett's tests are often used when observing a change from a control in an experiment. In this case, I used Dunnett's test to determine if the zooplankton communities changed after the flood and determine if they returned to pre-flood conditions.

To understand how abiotic factors such as chloride, total phosphorus (TP), total nitrogen (TN), nitrate, and hardness (calcium and magnesium) affected the abundance of zooplankton along with taxonomic richness and diversity, I employed linear mixed-effects models with Month as a random factor to account for changes over time using the *lme4* package (Bates D,

Mächler M, Bolker B, 2015). All models were checked to ensure that the assumptions of mixed effects models were met with the current model structure (Figures 5-7). The LME models had chloride, TP, TN, nitrate, and hardness as fixed effects, and included random intercepts for months in order to account for temporal variability. By analyzing the standardized confidence intervals for the fixed effect estimates, we can gauge the strength of the relationship between each fixed effect and the corresponding abundance, average richness, and diversity.

Results

19 families of zooplankton were observed including 4 cladocerans, 5 copepods, 8 rotifers, and 2 miscellaneous arthropods (Table 2). The most abundant taxon in the zooplankton community was often cyclopoid copepods, being present in the hundreds to even thousands in every sampling period. *Daphniidae* was the most common of the cladoceran families observed, though with substantially fewer numbers, with 559 at their maximum. One family of note, despite lacking in numbers, was *Bosminidae*, which increased with each flood, and decreased or was not found entirely during other periods. *Cyclopidae*, *Daphniidae*, *Chydoridae*, and *Brachionidae* were found during every sampling period, though with severe declines from their former abundances after the initial flooding events.

Throughout the duration of the study, the water quality parameters remained unpredictive of the zooplankton communities' abundance, richness, or diversity (Figures 8-10, Table 3). The linear mixed effects model results indicate that total nitrogen was marginally associated with species richness (Estimate = 0.142 , SE = 0.060 , t = 2.388) and with total abundance (Estimate = 11.629, $SE = 4.24$, $t = 2.743$) suggesting a positive effect, but was not associated with taxonomic diversity. Other parameters, including Chloride, TP, Nitrate, and Hardness, did not show statistically significant effects with abundance, richness, or diversity (Table 3). Monthly

variance in species richness (Variance = 0.7071 , SD = 0.8409), abundance (Variance = 4034 , SD $= 63.51$), and diversity (Variance $= 0.05892$, SD=0.2427) were statistically significant indicating that the response variable relationships did change monthly.

The zooplankton community's composition underwent changes on a monthly basis (Figure 11). The communities in August serve as baseline values for their compositions and corresponding diversity metrics. In September, the community composition shifted, and became more uniform. In October, the community became more dissimilar than in either August or September. In November, the community shifted to a lesser degree, and became more uniform, but remained overall similar to the community observed in October. Another way to check for similar changes on a more individual basis, was through the examination of the relative abundances of the individual taxa at each site in each of the 4 months of observation (Figures 12- 15). In these charts, each site, and the corresponding zooplankton community is listed separately except in the cases where no zooplankton were observed due to an empty sample or a dry vernal pool. In nearly every instance, the community was dominated by *Cyclopidae*, with only a few occasions with higher numbers of a specific cladoceran or rotifer.

The changes in the zooplankton community were examined by quantifying abundance, richness, and diversity. Zooplankton abundance changed during the duration of the study period, $(F_{3,55}=5.173, p=0.003)$, and the Dunnett's post-hoc tests indicate that abundance was lower than August in October and November (p<0.05; Figure 16). Average family richness changed across the study period $(F_{3,55}=4.371, p=0.007)$, and was lower than August in September, October, and November (p<0.05; Figure 17). Any changes in diversity over the 4-month period were not statistically significant $(F_{3,55}=2.309, p=0.087;$ Figure 18).

Discussion

The purpose of this study was to observe a series of isolated zooplankton communities that experience acute flooding events so as to better understand the effects of both the flooding events themselves and any accompanying biological or chemical responses. Based on the differences between the sites, the idea of ecological succession, and the relative resilience of zooplankton communities, I predicted that the zooplankton community would tend to adjust to a state reminiscent of the former community, despite the acute changes presented by flood events, with few or minor lingering effects (Shin & Kneitel, 2019; Yampolsky et al., 2013).

A large shift in the zooplankton community occurred in September, following a flooding event in late August, resulting in a more uniform composition overall in the pools. By October, however, the zooplankton community in each of the pools started to revert back toward their August composition. This is based on the fact that the overlap between October and August is greater than that of August and September. A second flood then occurred in late October, which again altered the zooplankton community, resulting in a similar but not identical community to that observed in August (Figure 11).

Examining the temporal changes in the zooplankton community in terms of abundance, average family richness, and diversity offers insights into the effects of flooding on vernal pool zooplankton communities. A persistent decline in zooplankton abundance was observed after September (Figure 16). The decline is likely due to the original effects of the first flood, in combination with the normal seasonal changes in the zooplankton community. The observation occurred as the most productive part of the year was ending, so a corresponding decline in zooplankton is expected (Liu et al., 2022; Manickam et al., 2018). Average family richness declined in September and October, with November experiencing a slight rebound in average

richness values, though still reduced compared to the original values observed in August (Figure 17). These responses suggest that the flood event that occurred in late October provided an influx of new life from the main wetland. The larger and deeper main wetland is less prone to rapid temperature change, and the new material from the flood resulted in an otherwise unexpected recovery from seasonal declines (Junk et al., 1989; Manickam et al., 2018; Wantzen et al., 2008)

The linear mixed effects models of the water quality parameters and zooplankton response indicate that water quality was largely unrelated to zooplankton abundance, average family richness, or diversity (Figures 6-8). In every case, the confidence intervals overlapped with zero, making any relationship unlikely.

Two flood events occurred during our observation. This is shown by the USGS' water level measurements indicating on several instances that the water level reached eight feet, at which point every vernal pool would be inundated with water from the main wetland, as well as the river (Figure 3). The first occurred in early September, and the second in late October. These events were therefore accompanied by changes in the environmental conditions and community diversity metrics. The main indicator of a change besides the physical observation of the water body was its associated concentration of chloride, which is higher than those observed in nonflood circumstances in the vernal pools. The vernal pools experienced a corresponding increase following each flood event (Figure 4).

Floods, though generally considered as disruptive, may benefit the communities contained within vernal pools and similar small waterways. The flood in early September acted as an ecological disturbance (Thomaz et al., 2007). The homogenization of the previously distinct communities in the vernal pools caused declines in the measured diversity metrics in September (Figures 16-18). However, the continued decline in zooplankton abundance may be

explained in part due to the seasonal changes, as the increase observed in the richness of the zooplankton community in November was not present in abundance specifically (Liu et al., 2022; Manickam et al., 2018; Zhou et al., 2023).

Contrary to the effects observed in September, the flood event that occurred in late October enriched the community that was present. In this case, the floods impact more accurately aligns with those expected through the flood pulse concept (Junk et al., 1989; Wantzen et al., 2008). As the vernal pools were cooling and becoming less biologically active, the main wetland was buffered against this change, cooling slower due to its substantially greater depth (Shin & Kneitel, 2019). Because of this, the flood event itself was important for the otherwise declining vernal pools. When the main wetland flooded, this brought the vernal pools an influx of new taxa that were found in the primary wetland and river. However, further studies are needed to determine if these organisms were able to survive and reproduce.

Given the importance of wetland ecosystems in urban areas, and the potential for increased urban flooding events to negatively impact these systems, it is of critical importance to investigate the effects of urban flooding on wetland ecosystem structure and function. Wetlands have spatial and temporal heterogeneity that often leads to increased ecosystem function by selecting for species with unique traits in different areas of the wetland. However, flooding can homogenize these systems, which might further disrupt ecosystem structure and function. Additionally, zooplankton might act as top-down regulators of diversity and function in wetland ecosystems, especially those that lack fish. Thus, investigating how perturbations such as floods alter zooplankton communities while measuring changes to nutrients can provide insight into mechanisms that determine the resilience of freshwater ecosystems to human-induced changes.

Tables and Figures

Parameter	Test ID	Method name
Chloride	879	Mercuric Thiocyanate Method
Hardness	869	Metal Phthalein Colorimetric Method
Magnesium	849	Metal Phthalein Colorimetric Method
Nitrate	835-836	Dimethylphenol Method
Nitrogen (total)	826-827	Persulfate Digestion
Phosphorus (total)	843	Ascorbic Acid Method

Table 1: Summary of methods used to test each water quality parameter.

Table 2: Zooplankton families found across all observed sites in any given month, depicted in green. Most are family classification, however, nauplii, podocopida, and calanoid were unable to be reliably classified further. In addition, the total abundance of the zooplankton taxa across the vernal pools is included.

Table 3: Results of the linear mixed effects models showing the fixed effects on abundance, taxonomic richness, and taxonomic diversity. These results suggest that none of the measured water quality parameters had strongly positive or strongly negative associations with the measured response variables over the study period. Only total nitrogen (TN) showed some evidence of a positive relationship with abundance and richness.

Figure 1: A map of the study area, with sites 1-20 in distinct vernal pools along the line surrounding the main wetland.

Figure 2: Photo of site 1 as of the November sampling period, as an example of the vernal pools during a non-flooded period.

Figure 3: Water level recorded by the USGS at Walker Avenue Wetlands. The blue line indicates water level measured by the USGS from 1 January 2021 to 1 January 2022. Green vertical lines indicate the sampling events. The red dashed line indicates flood stage where all vernal pools are inundated.

Figure 4: Concentration of chloride during the study in the vernal pools showing low chloride concentrations in August and increasing chloride concentrations in September and November following flooding events.

Figure 5: Validation of model assumptions for the linear mixed effects model for abundance to ensure that the model structure was appropriate.

Figure 6: Validation of model assumptions for the linear mixed effects model for richness to ensure that the model structure was appropriate.

Figure 7: Validation of model assumptions for the linear mixed effects model for diversity to ensure that the model structure was appropriate.

Figure 8: Confidence intervals for each fixed factor of the mixed effect model exploring how measured abiotic factors affect the abundance (total number of individuals) of zooplankton across the duration of the study (August to November). All confidence intervals overlap with zero, indicating that there was no significant effect of each measured abiotic variable on total abundance.

Figure 9: Confidence intervals for each fixed factor of the mixed effect model exploring how measured abiotic factors affect the richness (total number of identified taxa) of zooplankton across the duration of the study (August to November). All confidence intervals overlap with zero, indicating that there was no significant effect of each measured abiotic variable on richness.

Figure 10: Confidence intervals for each fixed factor of the mixed effect model exploring how measured abiotic factors affect the diversity (ENSPIE) of zooplankton across the duration of the study (August to November). All confidence intervals overlap with zero, indicating that there was no significant effect of each measured abiotic variable on diversity.

Figure 11: Nonmetric Multidimensional scaling analysis of the zooplankton community composition as it varied throughout the observation period. The interlapping portions of the shapes indicate similarities between the communities present in the individual months. The sizes of the shapes help to display the differing levels of variability in the zooplankton communities, with months with larger shapes having greater variation in the types of zooplankton.

Figure 12: Relative abundances of zooplankton taxa at each site in August.

Figure 13: Relative abundances of zooplankton taxa at each site in September.

Figure 14: Relative abundances of zooplankton taxa at each site in October.

Figure 15: Relative abundances of zooplankton taxa at each site in November.

Figure 16: Zooplankton abundance as it changed each month. Colored points represent the mean value each month, error bars represent the standard error, black points represent the measured values from each vernal pool.

Figure 17: Average zooplankton family richness as it changed each month. Colored points represent the mean value each month, error bars represent the standard error, black points represent the measured values from each vernal pool.

Figure 18: Average Zooplankton Diversity as it changed each month. Colored points represent the mean value each month, error bars represent the standard error, black points represent the measured values from each vernal pool.

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