PULSED PREDATION DETERMINES FRESHWATER POND COMMUNITY ASSEMBLY

by

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July 2020

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Historically, community ecologists have assumed constant consumer pressure when modeling predator-prey interactions, however, we know that interactions in most natural systems are dynamic. Inconstant predation may account for some discrepancies between natural ecosystems and model predictions and recent theoretical work shows that episodic pulsed predation events can have strong, destabilizing effects on the persistence and equilibrium densities of prey populations. In this study we conducted an experiment modeled after natural systems (such as intermittent streams, temporary ponds, and periodically flooded riverine rock pools) that experience episodic introductions and removals of predators. Specifically we created 32 artificial freshwater ponds and applied one of four different bluegill sunfish predation treatments: no predation, constant predation, and two magnitudes of stochastically pulsed predation (one or five fish). Pulses consisted of 24-hour introductions of predators to pools, and by the end of the experiment constant predation and large pulses had experienced equivalent predator exposure. We compared both macroinvertebrate diversity and several metrics estimating microbial function to determine the effects of pulsed predation on assembly and structure of communities. We found that pulsed predation

resulted in communities with different overall abundance and diversity when compared to constant predation. In addition, the magnitude of predation pulses in environments appears to be key in determining their effect on communities, as the small pulse of fish resulted in communities more similar to control treatments while large pulse treatments resulted in communities more similar to constant fish presence. Microbial community function was high in all tanks, resulting in low free nitrogen in this experiment and thus we cannot conclusively link microbial community function to pulsed fish predation. Understanding how predation pulses structure ecosystems and invertebrate communities improves our general understanding of processes regulating consumer-resource interactions and can improve our ability to predict community responses to increasingly unpredictable environmental change.

PULSED PREDATION DETERMINES FRESHWATER POND COMMUNITY ASSEMBLY

A Thesis

Presented to the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment of the Requirements for the Degree

Master's in biology

by

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July 2020

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ACKNOWLEDGMENTS

I first need to thank my committee for their expertise and patience.

I would like to thank the McCoy lab (including McCoy Lab adopted members) for their edits on writing, help conducting my experiment, and their friendship. I also thank the students and faculty of the Department of Biology at East Carolina University for their guidance, knowledge, and emotional support through this process.

I thank National Science Foundation (Grant #1556743) and Sigma-Xi (Grant #4169124) for the monetary support they provided for this project.

Finally, at the risk of offending those unlisted, I would like to especially thank some of my scientific mentors and friends: Dr. Heather Waye, Dr. Pete Wyckoff, Dr. Tatyana Rand, Dr. Kelly Dorgan, Abby Mallek, and Lauren Agnew. Last but not least, for their tireless support of my career in Biology over the past decade: all four of my parents.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Introduction

Understanding the mechanisms that drive spatiotemporal variation in the structure and composition of ecological communities is a central goal of community ecology (Mittelbach 2012). Deterministic and stochastic processes are known to work in concert to structure ecosystems, but their relative importance may change across spatiotemporal scales (Chase 2003, 2007, Mittelbach 2012, Van Allen et al. 2017). For instance, some freshwater ecosystems (e.g. riverine rock pools, ephemeral wetlands, and intermittent streams) are characterized by dynamic hydroperiods that can lead to biotic community assembly “resets” as pools dry out and refill with water. The inconstant nature of these environments has made them models for understanding how variation in abiotic factors over different temporal scales leads to variations in local and regional patterns of community assembly (Jopp et al. 2010, Toth and van der Valk 2012, Haque et al. 2018, McLean et al. 2019).

The relative influence of deterministic and stochastic processes ultimately drives expected patterns of diversity at different spatial scales. At the local scale diversity is described as α-diversity, which is local species richness, while regional scale species richness is described as γ-diversity (Mittelbach 2012). The fraction of species in the regional species pool in local scale habitat patches, as well as the turnover of species across local sites is typically described by β-diversity (Jost 2007). Weak environmental filters and strong stochastic processes (such as random fluctuations in abiotic conditions and priority effects) are theorized to produce high β-diversity among sites as species occupying nearby habitat patches will be a random subset of the species in the regional species pool and thus could be very dissimilar. In contrast, strong

environmental filters and deterministic processes (e.g. niche partitioning, or presence of predation and keystone species) can lead to more homogeneous metacommunities as only species well suited to local conditions will persist and thus habitats will have similar filters and lower β-diversity (Jost 2007, Sokol et al. 2015). Freshwater habitats are understood to be a mix of stochastic and deterministic mechanisms, and spatial variation in habitat conditions result in dynamic ecosystems.

One of the primary processes affecting community assembly at both the local and regional scales is dispersal ability (Howeth and Leibold 2010, Heino et al. 2015). Species differ in dispersal modes, rates, and distances, leading to strong patterns in both the timing and spatial distributions of species among local patches within a metacommunity (Turner et al. 2020). While organisms with passive modes of dispersal may arrive in all patches within a given range, organisms that exhibit active modes of dispersal often rely on indirect cues of habitat quality and predation risk to choose the best habitat patch (Resetarits 2001, Turner and Chislock 2010). These two modes tend to generate different spatiotemporal patterns of diversity, and understanding the role of active dispersal may be particularly important in freshwater meta-communities that contain large numbers of species with complex life cycles and aquatic larval stages, where oviposition site choice has important consequences for the performance of offspring (Albeny-Simões et al. 2014). For example, female mosquitoes from the genus *Aedes* preferentially choose oviposition locations based on a variety of environmental cues including presence of conspecific larvae (Kalpage and Brust 1973, Maire 1985), predator absence (Lowenberger and Rau 1994, Vonesh and Blaustein 2010, Parrish et al. 2019), and abundance of food resources (Zahiri et al. 1997, Albeny-Simões et al.

2014). Active habitat selection behavior has been documented for a wide array of freshwater taxa including beetles (Resetarits 2001), dragonflies (Pierce 1988, McPeek 1990), and amphibians (Resetarits and Wilbur 1989, Hopey and Petranka 1994, Winandy et al. 2017). Thus, active habitat selection can strongly influence the distribution and density of species among habitat patches (Petranka et al. 1987, Jenkins et al. 1992, Resetarits 2001, Angelon and Petranka 2002, Vonesh and Blaustein 2010). However, animals rarely have perfect information about habitat quality, and chemical cues of habitat quality degrade and evaporate over time, so the effects of habitat selection behaviors on species distributions is temporally variable (Turner and Chislock 2010, McCoy et al. 2012).

After colonization of a habitat, post-colonization processes ultimately determine the diversity and abundance of biological communities. Abiotic habitat characteristics can function as strong filters by limiting the composition of the community to only those species that are tolerant to particular environmental conditions (Heino et al. 2015). Similarly, the presence of sufficient limiting resources can determine habitat suitability for different species as they may also be spatiotemporally variable (Weber and Brown 2013). Finally, species interactions, including competition and predation, determine community structure (Paine 1980, Steneck 2012).

The presence of keystone species or apex predators in particular has been shown to strongly affect the assembly, composition, and structure of communities (Paine 1980, Steneck 2012, Ripple et al. 2016, Piovia-Scott et al. 2017). For instance, in freshwater communities predatory fish function as strong environmental filters affecting the diversity and abundance of invertebrates over time through both direct indirect

predatory affects (Wiseman et al. 1993, Chase 2007, Van Allen et al. 2017). Habitat patches containing efficient predators have a lower β-diversity and more similar communities than predator free patches, which are expected to be more strongly driven by stochastic processes and thus have higher β-diversity (McPeek 1990). Selective predators like the Bluegill sunfish (*Lepomis macrochirus*) structure the prey communities by preferentially consuming particular subgroups of prey, which determine the prey species that can survive in the environment over time (Werner and Hall 1974, Butler 1989, Olson et al. 2003). Additionally, apex predators indirectly affect community structure by determining the distribution, behavior, and rate of growth of organisms within habitats (McCoy et al. 2012, Costa and Vonesh 2013).

In addition to changing the structure and composition of communities, predators can have large effects on mobilization and cycling of nutrients through ecosystems (Schmitz et al. 2010, Atkinson et al. 2017). Most studies have tracked the upstream effects that nutrient subsidies (e.g. leaf fall, tree masting, chemical fertilizers) eventually have on predator populations (Ostfeld and Keesing 2000, Davis et al. 2010). However, predators have been demonstrated to have strong effects on the concentration of nitrogen and phosphorus in freshwater environments though consumption and excretion (Schmitz et al. 2010, Atkinson et al. 2017). However, predators can also influence nutrient dynamics by reducing prey foraging activities or habitat ranges which in turn moves nutrients in the system to areas considered less optimal to prey species (Tronstad et al. 2015). While predators can mobilize nitrogen dynamics within and among habitats, predator effects on nitrogen cycles are largely mediated through microbial communities (Dessborn et al. 2016, Olsen et al. 2017). Thus, fluxes of nutrient

inputs and mobilization associated with pulses of predation could dramatically affect microbial processes and nutrient cycling in aquatic systems that ultimately affect patterns of community assembly of macro-organisms.

Most studies examining the effects of predators on community assembly, composition, and nutrients have considered predators as presses, either constantly present or absent (Glasby and Underwood 1996). However, we know that predation pressure is often spatially and temporally variable (Butler 1989, Maria et al. 2002). For instance, periodic flooding and drying, mass reproductive events, species introductions, and movements among habitat patches can all lead to variable or pulsed predation pressures on prey organisms over time (McCoy et al. 2009, Silliman et al. 2013). Even within a hydrologically stable environment, spatiotemporal variation in the abundance of fish predators (Butler 1989), seasonal preferences in prey (Olson et al. 2003), and preferred size of prey (Werner and Hall 1974, Howeth and Leibold 2010) potentially result in natural pulses in the effects that fish have on an ecosystem. Such variable and inconstant predation can drive community dynamics in different ways than constant or cyclic predation (Butler 1989, Willson and Womble 2006, Hamman and McCoy 2018). In fact, a recent theoretical study showed that repeated stochastic pulses of predation of different magnitudes and frequencies had stronger and more destabilizing effects on prey populations than constant or continuous predation (Hamman and McCoy 2018). In some scenarios, repeating predator pulses drove prey populations to extinction when constant predation of the same magnitude did not. The effects of pulsed predation in this study were found to be contingent upon the time between predation events and population dynamics of the prey, leading to potentially inconsistent effects on different

prey species. In our study we built upon this framework to investigate how constant and pulsed patterns of predation of different magnitudes (Figure 1) affect community assembly, composition, and ecosystem functions empirically in a simple freshwater system.

To our knowledge neither the effects of stochastic pulses of predation pressure on the assembly and composition of ecological communities nor the potential effects of such pulses on ecosystem functions have been investigated empirically.

In this study we ask **(1)** how does pulsed introduction of fish predators into freshwater ponds affect the composition and structure of macro-invertebrate communities **(2)** what is the relative importance of the timing versus the magnitude of predation on the macroinvertebrate community diversity and abundance, and finally **(3)** how does pulsed predation affect the nitrogen cycling and free chlorophyll of freshwater ponds?

Methods

Field Experiment

This experiment was conducted in 568-liter stock tanks (n=32) at East Carolina University's West Research Campus (WRC, Figure 2a) between June 12 and August 1 2019. Each individual tank was established to mimic small freshwater ponds or pools. Tanks were filled with approximately 500 liters of conditioned tap water (API© Quick Start), seeded with approximately 250g dry weight of hardwood leaf litter (primarily American Holly, Yellow Poplar, Ash, and American Sweetgum, see Figure 2b) and one liter aliquots of concentrated zooplankton and phytoplankton collected from nearby ponds (following protocols in Wilbur 1987 and previous McCoy lab research). We added three plastic aquarium plants to each tank to provide vertical structure for fish and invertebrates, and two bamboo sticks were added to provide structure for metamorphosing insects. All tanks were left uncovered and undisturbed for four days prior to the start of the experiment to allow leaf litter to settle and begin to decompose, and to allow bacterial and planktonic communities to become established. We added an additional 20 gallons of treated tap water to each experimental tank on July 7 to keep water levels safe enough for fish predators following a period of excessive heat and lack of rain that reduced water levels.

Our experiment consisted of four treatments: control, constant predation, small pulses of predatory fish, and large pulses of predatory fish (Figure 1). Large pulses consisted of five fish per tank and small pulses consisted of one fish per tank. We added predators to both pulsed treatments in the same block on the same experimental day (i.e. day 36) and then removed fish with a dipnet 24 hours later (i.e. day 37) nine

times throughout the experiment on a stochastic schedule (Table 1). Each of the four treatments was replicated in eight temporal blocks (for a total of 32 tanks) and every disturbance in one treatment within the block (e.g. sweeps for fish) was replicated for the other treatments. Predator manipulations lasted for 45 experimental days, and control treatments had no fish for the entirety of the experiment, while constant treatments had the same single fish present for all 45 days. This experimental design was devised so predation pressure in the large pulse treatment was equivalent to that of the constant treatment over the course of the entire experiment (i.e. five fish per pulsed day * nine pulsed days = 45 days of predator presence total in the large pulse treatment).

We collected Bluegill Sunfish and Shellcrackers *(Lepomis spp.)* from nearby ponds and the Tar River to use as pulsed fish predators in our experiment. When not in use in the experiment, fish were housed in 568-liter tanks in the same field as the experiment to reduce transportation stress and acclimatization time when introduced into experimental tanks. Fish added to constant fish treatments were added on June 16 and removed on July 31. Pulsed treatments had fish introduced and removed on predetermined schedules (Table 1) with three exceptions due to mistakes in the field. First, in the large pulse treatment of block eight an additional fish was mistakenly present from 20 June to 5 July, resulting in an additional 16 days of predator exposure for this tank and a period of time in which the pulsed treatment was essentially a constant predation treatment. Secondly, the final pulse of block three on 23 June tanks receiving large and small pulse treatments were accidentally switched, resulting in a grand total of 13 days of fish exposure for the small pulse and 41 days for the large

pulse for this block over the course of the experiment. We removed the tanks with changes from the fish schedule from all analysis occurring after the error since the predation pressure in these tanks were not equivalent to other replicates and could confound results. Therefore, the tank with an additional fish for 16 days was removed from all analysis, and the two tanks with a switch in fish were excluded from the final analysis.

Invertebrate Samples

Twice during the experiment, on experimental days 15 and 30, each tank was sampled with a subsampling protocol designed to establish species accumulation curves and estimate the abundance of taxa. This subsampling was conducted over four days, and all tanks in the same stochastic pulse schedule (four tanks, representing each treatment) were sampled on the same day, although on average only two randomly selected blocks were completed each day. To take these subsamples, we used aquarium dip nets (25cm x 16.5cm) to take nine standardized sweeps within the tanks, each time moving the dip net approximately 30 centimeters. The first three sweeps for each tank were water column sweeps, the second three sweeps scraped the sides of the tank, and the final three sweeps were benthic sweeps along the bottom of the tank. After each sweep we counted and identified individuals to the lowest taxonomic level possible. We immediately returned net contents to the tanks of origin after counting to avoid excessive death of invertebrates and skewing final community counts, although some predation was observed on trays as predators and prey were more densely clustered than in tanks. Loss of invertebrates due to sweep samples was assumed to be equivalent across all treatments.

On July 31 and August 1 2019 we drained all tanks through the same aquarium nets used for nine-sweep surveys and destructively sampled all macroinvertebrates in the tank for identification and enumeration. Five blocks were completed July 31, before sundown, and the final three blocks were completed the next day. We sorted samples, separating invertebrates from plant matter, and identified invertebrates to the lowest taxonomic level possible in the lab at East Carolina University with a dissecting microscope. Two complete blocks, three and eight, remain unsorted and unidentified as mistakes in fish additions led to deviations from the expected total number of fish days, so all analysis of the final community presented below uses 24 of the 32 tanks.

Nitrogen and Chlorophyll Samples

Nitrogen in the tanks was quantified in-situ with a YSI professional plus probe which sampled ammonia, nitrate, temperature, and pH from the tanks in the field. We only analyzed and reported the results for ammonia and nitrate, as the temperature would not be affected by predator treatments and pH was mainly used to calculate ammonia. Starting July 10, 2019 we sampled with the YSI at least once per week until the end of the experiment. The YSI probes were lowered into each tank about 45cm below surface level to ensure all sensors were immersed, and then allowed approximately 30 seconds for readings to stabilize before recording. We used an *Aqua*Fluor handheld fluorometer (Turner Designs Inc.) to obtain measurements of suspended chlorophyll-a and turbidity every week from 4 July 2019 to 31 July 2019 (n=7) by collecting a cuvette of water from the surface level of the center of each tank. Water samples used in the fluorometer were not returned to tanks after sampling.

Statistical Analysis

We analyzed all data in the R statistical programming environment version 3.6.3 (Team, 2020) and R studio version 1.2.5042 (© Rstudio Inc.). Each analysis included data tidying using the “tidyverse” package (Wickham et al. 2019) and visualization with package “ggplot2” (Wickham 2016). We fit linear mixed models (lmm) and generalized linear mixed models (glmm) using the “lme4” package (Bates et al. 2015). When random effect variance were not stable using lme4 we used Bayesian mixed models with weak Wishart priors using package “blme” (Chung et al. 2013) to improve numerical stability of our models and to appropriately account for among block variation. Candidate models were compared with the AICctab() function of package “bbmle” to determine the most parsimonious models for our data (Bolker 2020). We used Wald Chi-squared tests to conduct hypothesis testing for the statistical significance of fixed effects of the most parsimonious models using the “car” package (Fox and Weisberg 2019). Plots included in this thesis were arranged using the “patchwork” and “cowplot” packages (Pedersen 2019, Wilke 2019).

Abundance of Macroinvertebrates

We hypothesized that the abundance of macroinvertebrates (total number of individuals counted in each tank) would be consistently lower in treatments with the highest exposure to predators (45 days of fish exposure) due to the direct and indirect effects of predators on colonization and survival until sampling. We further hypothesized that the lowest abundance of invertebrates would be found in large pulse treatments in line with the expectation that pulses are more destabilizing to communities than constant predation. To analyze these data, we used two different datasets: one for the

abundance of invertebrates during assembly nine sweep surveys, and another dataset for the abundance of invertebrates from the final, destructive samples. For the assembly dataset we constructed five candidate generalized linear mixed models (glmms) with a poisson family error distribution with tank nested in block treated as random effects to account for non-independence of multiple samples from tanks over time. The five candidate models included 1. a null random effects only model, 2. a model that included treatment effects, 3. a model with only the fixed effect of sample time, 4. additive effects of treatment and time and 5. a model that includes interaction between fixed effects (Table 2). We performed model selection based on Akaikes Information Criterion (AIC) with a correction for low sample size using the “bbmle” package (Bolker 2020). To test differences in the total abundance of invertebrates between treatments in our final complete samples we did not need to consider sample time as a fixed effect, as there was only one sample. Therefore, we tested treatment as the fixed effect against a null model with block as a random effect in both candidate models (Table 2). The dataset was normal in error distribution, and therefore was best modeled by a standard gaussian generalized linear mixed model.

Diversity of Communities

To test whether the predation treatments affected the number of species present in the nine sweep subsamples, we estimated Chao diversity between treatments in non-destructive samples. The Chao index estimates total diversity of taxa from incomplete samples and corrects for sampling bias by considering the accumulation of new taxa after repeated sampling (Chao et al. 2014). While we present the results for the Chao index here, we calculated several estimates of diversity common in the literature (Chao,

jackknife, bootstrap) using the specpool() function of the “vegan” package as each estimate makes different assumptions about data distributions. All indices generated similar estimates and so we chose to use chao since it proved to be the most conservative index for our study (Oksanen et al. 2019). To test the hypothesis that the pulsed predation resulted in fewer species than constant predation we compared candidate models with the same five fixed effect structures consisting of the main effects of treatment, sample time, time and treatment, and their interaction, as well as a random effects null model. To analyze these data, we first attempted to fit glmms, but we did not have sufficient resolution to attain reliable estimates of random effect standard deviation. However, because each block in our experiment received a unique stochastic predation schedule, we felt it would be inappropriate to account for the random effects that were implicit in our block design. Thus, we used a Bayesian linear mixed model that imposed a weak Wishart prior to improve the random effects estimates. We assumed log normal errors for the main effects and used AICc to determine the most parsimonious model among the five candidate models.

Since final community analysis was based on complete samples of tanks collected after draining on July 31 and August 1 2019, it was unnecessary to extrapolate estimates of species in each tank (i.e. Chao Diversity) since we were able to comprehensively quantify both species richness and abundance of all taxa. We hypothesized that control tanks would be the most diverse, and large pulse treatments the least diverse. To determine if treatments differed in diversity, we compared species richness, Shannon-Weiner diversity, and Pielou’s evenness. All metrics of final diversity were calculated using the vegan package, and while Pielou’s evenness is not explicitly

encoded in the package it can be calculated from the Shannon-Weiner index. We used our three methods to access diversity because we wanted to directly compare the number of taxa present in the final sample to the assembling communities (Chao diversity and species richness), incorporate both richness and evenness when determining diversity of samples (Shannon-Weiner diversity), and determine if the taxa in treatments were even (Pielou's diversity).

All three metrics of final diversity were analyzed by comparing two candidate models: a model including treatment as a fixed effect and a null model. The blocked tank design was included as a random effect to control for the disturbance of nets in tanks. Due to singularity violations and model instability, we again used Bayesian mixed models that specify a weak Wishart prior for the random effect standard deviation. AICc was used to compare the candidate models.

Community Dissimilarity

To determine if the predator manipulations affected the structure and composition of the macroinvertebrate communities at different point during assembly, we examined Bray-Curtis dissimilarity between treatments on week three and five with PERMANOVA analysis using the *adonis* function in the "vegan" package. We constructed PERMANOVA's with both Bray-Curtis and Jaccard indices, as we were unsure which would best fit our data and we believed that the unevenness in species abundances that we observed among treatments may be better controlled by the Jaccard method. However, the results of these analyses for all datasets and timepoints which we analyzed were similar between the two indices (i.e. if treatment was significant in Bray-Curtis it was with Jaccard and vice-versa) and inference did not change based on

method, therefore we present the dissimilarity calculated using the Bray-Curtis index in our results.

To get a rigorous and unbiased examination of the composition and structure of the communities that formed in our different treatments we conducted separate PERMANOVA analyses for each time communities were sampled (samples at approximately 15 days, 30 days, and the final sample). PERMANOVA compares samples using rank-ordering of species, and then compares groups by calculating both how far apart in multidimensional space group centroids are and how dispersed the individual data points are around each group centroid(Oksanen et al. 2019). For this analysis, we used treatment as the grouping variable and each tank within the treatments as sample points. We used PCoA plots to visualize the results.

Ecosystem Function

Finally, to examine if correlates of ecosystem function differed by treatment, we analyzed chlorophyll and turbidity from the Aquafluor fluorometer, and nitrate and ammonia data from the YSI. To test hypotheses of treatment effects on ecosystem function, we compared two models for each ecosystem function: a null model, and a model with treatment as a fixed effect. Again, due to the structured nature of our experiment we included tank nested in blocks as random effects. Chlorophyll, nitrate, and ammonia were assumed to have normal error distributions and were analyzed with Bayesian mixed models to ensure robust estimation of random effects as above, and turbidity was analyzed with a glmm. All linear models for ecosystem function were compared against null models without fixed effects using AICc.

Results

Abundance of Macroinvertebrates

Invertebrate abundance changed significantly between all three sample times, and the relative abundance of invertebrates in treatments generally followed the same patterns over all three samples (Figure 3). In all analyses, models that included treatment as a fixed effect outperformed models without treatment effects (Table 2) and the analyses for the first two samples also included a time component that was also significant in post-hoc Wald Chi-squared tests (Table 2, X^2= 6.4364, $p < 0.05$). The significance of sample time is largely due to the overall decline in abundance across all treatments by an average of 61% between the first and second sample time. The large pulse treatment experienced the greatest decline (79%) of all the treatments (Figure 3A and 3B, X^2=450.66, $p < 0.001$). While some variation in the amount of fish predation was present due to the exact schedule of tank samples, all tanks were sampled approximately two weeks apart, with the first sample representing about a third of the total fish predation for the constant treatment, the second represented two-thirds, and the final represented the complete 45 days of predation. As expected, the final, complete sample contained far more invertebrates than the subsamples, nearly 2-3 times more invertebrates than the first sample (Figure 3A and 3C). Interestingly, the relative ranking of treatments besides the constant predation treatment changed between samples, however, the constant predation treatment always had the fewest macroinvertebrates (Figure 3). Total predation appeared to be an important factor, as the constant predator and large pulsed predator treatments had a similar abundance of invertebrates over time. The large pulse contained slightly more (4%) invertebrates than

the small pulse on the third week of the experiment, but the small pulse had more invertebrates in the samples taken during weeks five and seven (51% and 31% respectively). In the second nine-sweep sample, both control and small pulse treatments had more than double the total number of organisms as the large pulse and constant treatments (Figure 3A).

Diversity of Communities

The overall species richness in the final samples was consistent with estimates generated from the Chao diversity index that was extrapolated from the subsamples. The relative ranking of all treatments was mostly conserved between community assembly and final communities (Figure 4). The most parsimonious model for the Chao diversity included treatment (X^2= 20.566, $p < 0.001$), but not sample time, suggesting that there was no change in Chao diversity from weeks three to five. These results indicate that most species had arrived by experimental day 15 (Table 3). Treatment was also a significant predictor of species richness in the final dataset (X^2= 21.769, $p < 0.001$). Similar to the abundance of macroinvertebrates, species richness was lowest in constant treatments- both in the subsamples and in the final destructive sample (Figure 4). Large pulse treatments had 15% more species than the constant treatments in subsamples, but nearly the same number at the end. However, the small pulse treatments contained about 30% more taxa than large pulses, indicating the magnitude of pulses is important in community species assembly (Figure 4). In the final, destructive samples the control treatment contained about 1.5 times more species than treatments with 45 days of predator exposure (Figure 4).

We suspected our communities were uneven after preliminary data exploration, but we found that treatment was not a significant predictor of Shannon-Weiner diversity or Pielou's evenness in our study (Table 4). We were not able to resolve some taxa beyond the level of family or subfamily, so certain taxon likely included several different species in our dataset, and in the case of the Chironomidae and small Libellulidae we were concerned this may have disrupted the evenness of the communities. Shannon-Weiner weights the number of taxa and their rarity when determining overall diversity, and when we excluded two taxa we believed were overrepresented in our dataset (Chironomidae and small Libellulidae) the overall Shannon-Weiner diversity increased from 0.89 to 1.44, reflecting a rise in evenness overcoming the lower richness. However, our models indicated that there was a large amount of variation in Pielou's evenness within treatments, which caused the null model to be more parsimonious than the model including treatment as a fixed effect. Nonetheless, visual trends in the patterns of evenness indicate to us that these two taxa may still have been affecting treatment communities.

Community Dissimilarity

PERMANOVA results demonstrate that total predation was an important factor driving the structure of communities, and that pulsed predation affected communities differently than constant predation or no predation (Figure 5). Macroinvertebrate communities appeared to separate by treatment in the samples taken approximately two-thirds of the way through the experiment, but all treatments appeared to overlap in the beginning and end of the experiment (Figure 5). After three weeks of community assembly the treatment groups were not significantly dissimilar (Figure 5A, $r^2 = 0.1044$,

F = 1.0883, p = 0.353) but they were two weeks later during the second sample (Figure 5B, r^2 = 0.3018, F = 4.0337, p = 0.001). Treatments were again similar in the final dataset (Figure 5C, r^2 = 0.1200, F= 0.8634, p = 0.542).

We found that the composition of communities most strongly separated along the first PCoA axis for all three time points (Figure 5) while there was little separation along the PCoA 2 axis among treatments over time. PERMANOVA tests do not indicate which species most affect the rank-order of species, so we could not determine which species were responsible for the similarity in our communities.

Ecosystem Function

Aquafluor data were largely similar across treatments and time, however the treatment did improve the amount of deviance explained relative to the null (Table 5). Wald Chi-Squared test of relative chlorophyll concentrations was significant by treatment (X^2= 9.3198, p < 0.05) although a great amount of overlap in the large confidence intervals make visual interpretation of results tricky (Figure 7). Our free chlorophyll confidence intervals extended well below zero, which was possible on the fluorometer instrument we used and is not an artifact of an improperly fit linear model. Turbidity also differed among treatments (X^2= 14.70, p < 0.01). However, we again had very low resolution and high variation in our data as indicated by large confidence intervals (Figure 9). In both the free chlorophyll and turbidity, the pulsed predation treatments were lower than constant treatments. There was no detectable signal of treatment in the concentrations of nitrogen and ammonia as the null model outperformed the model including treatment in both sample (Table 6).

Discussion

The effects of nutrient or resource pulses into environments has been well examined by ecologists (Ostfeld and Keesing 2000, Tronstad et al. 2015, Dessborn et al. 2016, Bukaveckas et al. 2018), however explicit examinations of pulsed apex predators are rare (Schmitz et al. 2010, Tronstad et al. 2015, Atkinson et al. 2017). Previous studies have noted that both predator presence and the magnitude of predation risk for prey is often variable as a result of seasonality (Butler 1989), abiotic factors (Piovia‐Scott et al. 2019), migratory events (Willson and Womble 2006), or as an artifact of human intervention (Steneck 2012). Although some studies have examined the effects of pulsed removals of prey on population stability, few studies have investigated the implications of varied and pulsed predation events on ecological processes and community assembly (Howeth and Leibold 2010, Hamman and McCoy 2018). In this study we find that both the magnitude and timing of predation events is important for understanding how predation affects the composition and structure of communities. Understanding how ecosystems are affected by, and respond to, repeated pulsed events may provide insights about why some systems never seem to fully recover after perturbations.

Theoretical work has demonstrated that pulses can be more destabilizing than constant predation (Hamman and McCoy 2018). Our findings did not conform to this expectation. In this study assembly of communities was more strongly affected by the total amount of predation than by the pulsed timing of predation (Figure 5). Indeed α-diversity in the large pulse and constant predation treatments were most similar to each other, both having received 45 days of cumulative fish predation (Figure 4B). However, some effects of pulses of predation were evident in both the abundance of invertebrates

colonizing tanks (Figure 3C) and on ecosystem functions (Figure 7) when compared to constant and control treatments. The strong effects of total predation relative to the effects of pulsed predation events is consistent with many previous studies that also demonstrated that the magnitude of predation by fish predators drives the structure of freshwater communities (Werner and Hall 1974, Cross et al. 2013, Van Allen et al. 2017, Parrish et al. 2019).

Given the extensive literature showing that fish have strong effects on freshwater communities, it was surprising that predation experienced in small pulses did not have larger effects on the diversity of the communities (Figure 4). In fact, the small pulse treatment resulted in the highest abundance of invertebrates in the final sample, even larger than observed in the control treatment (Figure 3C). This finding could suggest that the well-studied effects of fish predators on freshwater communities via both consumptive and non-consumptive pathways can be dampened when predation events are periodic and small. This may have stemmed in part because the aggregate strength of predation signals were too weak or too infrequent to regulate prey populations, or the chemical signals that deter colonization by some species dissipated over time (Angelon and Petranka 2002, McCoy et al. 2012, Trekels and Vanschoenwinkel 2019). Alternatively, we may have not seen a strong effect of the small pulse treatment as a result of “spatial contagion”, whereby the presence of high fish densities of fish in nearby pools affected colonization of predator free and small pulsed pools.

Spatial contagion in metacommunities occurs when habitat patches near risky or low reward habitats experience lower colonization as a result of assembling species’ avoidance (Trekels and Vanschoenwinkel 2019). Several species that were common in

our study area did not colonize any of the experimental mesocosms, regardless of treatment, which may have been a result of the spatial proximity of tanks with and without fishes. For example, our study site historically hosts large amphibian populations, and frogs have commonly deposited eggs into experimental mesocosms at this site in years previous, especially those without fish (Michael McCoy, personal comm.). With the exception of a few tadpoles sporadically observed in tanks during subsampling, amphibians were completely absent by day 45. The absence of amphibians may be an example of spatial contagion, as amphibians were observed at the site but are known to detect and strongly avoid fish predators (Hopey and Petranka 1994, Turner et al. 2020).

Spatial contagion may have similarly affected colonization by invertebrate species, since we know that many taxa are able to recognize predator cues in environments and may have avoided our experimental array altogether (Resetarits 2018). While species likely differ in their sensitivity to predator cues, recent studies have found spatial contagion to affect oviposition behaviors in adjacent habitats as far as five meters away for mosquitoes (Trekels and Vanschoenwinkel 2019). In our design, all mesocosms were within five meters of a tank containing one or more fish. Spatial contagion may explain why our mesocosm tanks, including the control and small pulse treatment tanks, had relatively low alpha and beta diversity overall (Figure 4) and why we did not see the expected larger differences between fish present and fish absent treatments (Figure 5).

Another explanation for the increase in the abundance and diversity of small pulse tanks relative to the control (Figure 3C and Figure 4A) is that disturbance to

assembling communities through small pulses of predation may have enhanced the diversity via intermediate disturbance effects (Connell 1978, Thorp and Cothran 1984, Hubbell 2001). Taxa may find habitat patches with predators favorable, as predators can reduce competition, increase resource availability, or consume meso-predators through trophic cascades (Albeny-Simões et al. 2014). The taxa Chironomidae provide an interesting example of this behavior, as they assemble rapidly in freshwater environments and are readily preyed upon by a variety of freshwater meso- and apex predators, including dragonflies and fish used in this study (Werner and Hall 1974, Olson et al. 2003, Kraus and Vonesh 2010, Togashi et al. 2010). This taxon represented a majority of invertebrates numerically, and likely provided an important food source for the assembling communities. When fish are present, they have been found to reduce dragonflies and predatory diving beetle assembly through direct predation and chemical cues that deter colonization, but can increase Chironomid assembly (Kraus and Vonesh 2010). Future research should more closely examine taxon specific responses as a test of this hypothesis as this may explain similar patterns of richness and diversity between large pulse treatments and constant treatments, but the differences between the two lie in the abundance of individual taxa.

It is important to note in our study that community assembly data collected from the final survey has a greater taxonomic resolution than the data collected during subsamples. Most groups of taxa identified in the nine-sweep surveys were not identifiable in the field to lower levels of classifications, although several groups identified in the field had no lower classification (i.e. all Nepidae water scorpions found in the final tanks were *Ranatra sp.,* and the vast majority of the *Dytisicidae* beetle larvae

were *Agabetes acuductus*). Dragonflies during subsamples were only classified as members of the family *Libellulidae* during the nine-sweep surveys but were further classified to the genera *Pachidiplax*, *Erythemis,* and *Pantala* in the final samples. Mosquitoes were also identified to the genus-level in the laboratory. The overall species richness of treatments in the final sample (Figure 4B) matched closely to the estimated richness from Chao diversity (Figure 4A) therefore we believe the lack of taxonomic resolution in our subsamples does not strongly affect our inference.

Treatment effects on ecosystem functions were most noticeable in the tank turbidity and chlorophyll measurements, where the difference between constant and pulsed predation was strongest (Figure 7). These findings align with previous work indicating that bluegill sunfish are responsible for increased turbidity and suspended chlorophyll, although previous studies were conducted with constant bluegill presence (Breukelaar et al. 1994, Dantas et al. 2019). For generalist, benthic-dwelling fish such as bluegill, increase turbidity due to foraging in the leaf litter benthos. Additionally, fish activity in the benthos can increase chlorophyll concentrations by mobilizing nutrients from sediments and leaf litter into the water column, promoting growth of chlorophyll (Breukelaar et al. 1994, Dantas et al. 2019). These results may also suggest a trophic cascade effect, as the *Lepomis spp*. used in our experiment are unlikely to directly feed on phytoplankton, but will consume zooplankton grazers, like the daphnia, ostracods, and copepods present in our mesocosm array (Olson et al. 2003). The consumption of zooplankton consumers could therefore lead to an increase in suspended primary producers.

We propose two main hypotheses for the low nitrogen in our tanks: fish did not excrete as much nitrogenous waste as expected or microbial communities were more robust than expected. Nitrogen, in addition to being a critical and often limiting nutrient in freshwater environments, has been previously shown to respond strongly and quickly to fish predators (Vanni 2002, Weber and Brown 2013, Tronstad et al. 2015). However, the number of fish per tank in our experiment was determined by the number of fish which could be safely housed in 568-liter stock tanks long-term, and this number may not have affected the nitrogen in our mesocosms as strongly as we had hoped. We also assumed in our study that nitrogen came mainly from two sources: microbial decomposition of leaf litter and biological waste from invertebrates and fishes. The nitrogen in our tanks may have followed several pathways, but we only explicitly quantified the free nitrogen in the water column, assuming this metric would reflect treatment level changes in nitrogen. However, nitrogen released from tanks into the air through denitrification overnight as oxygen levels decreased, and free nitrogen uptake into other primary producers (algal and periphyton, invertebrate biomass) were not explicitly examined in this study. Undetected differences in treatments may have been present in the release of dinitrogen gas and bio uptake as has been found in other freshwater studies (Hessen et al. 1997, Vanni 2002, Cross et al. 2006). Furthermore, all YSI measurements of free nitrogen were at or near the lower detection limit for our equipment, posing further challenges in determining treatment effects as detection near calibrated limits is more challenging than near the middle of calibration curves.

Further research is needed to determine the response of variable environments to pulsed predation, as our study suggests pulsed predation may affect invertebrate

pond communities and internal nutrient cycles. To better understand the effect of pulsed predation on ecosystem processes will likely require a more holistic examination of nutrients. Some of our findings may have stemmed from the logistical limitation of available equipment and sampling protocols rather than a lack of any effects of pulsed predators on primary producers and nutrient cycling. We also believe that spatial contagion had important affects on the outcome of this study, however, we are unable to discern whether dissimilarity in communities was due to pre-colonization signals of predation or post-colonization direct effects of predators. Regardless, the results of this study were not consistent with theoretical expectations for destabilizing effects of pulsed predation events.

In summary, our results indicate that pulsed predation may have important but subtle effects on freshwater environments by differentially affecting prey species and the assembly processes for communities. However, we found that total predation was a much stronger driver of community assembly in our system (Figure 5). Predators emit strong top-down structuring forces in metacommunities (Butler 1989, Walls et al. 1990, Chase et al. 2010, Steneck 2012, Van Allen et al. 2017) and predation often occurs in short intense, repeated pulses. Pulsed predation is already documented for many systems (Willson and Womble 2006, Piazza and Peyre 2012, Silliman et al. 2013) and it is likely that the effects of apex predators will become increasingly more pulsed over time in response to fragmentation, species introductions, and other anthropogenic effects on ecosystems (Blanchard et al. 2011, Parrish et al. 2019). Our findings further highlight the need for recognizing the dynamic and variable nature of ecosystems , and the value of understanding when and in what ways those dynamics are important. This

study provides an important first step in unravelling how predator pulses might affect the structure, function, and composition of ecological communities.

Figures and Tables

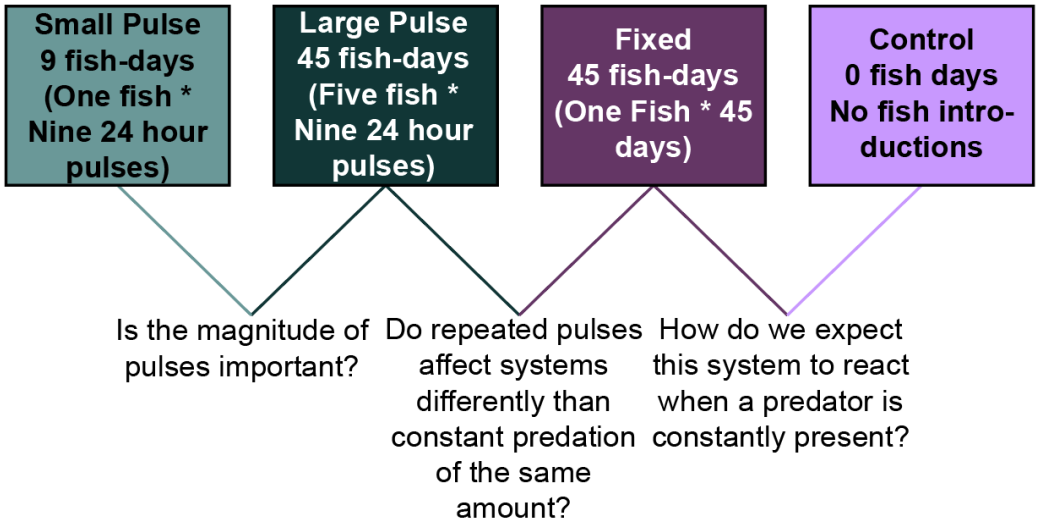

Figure 1: Conceptual map of experiment, showing the four predation treatments included in each of the eight experimental blocks. Predation treatments represented varying magnitudes and temporal schedules of predator exposure to assembling freshwater invertebrate communities. Below treatments the questions which pairwise comparisons of treatments may answer are included.

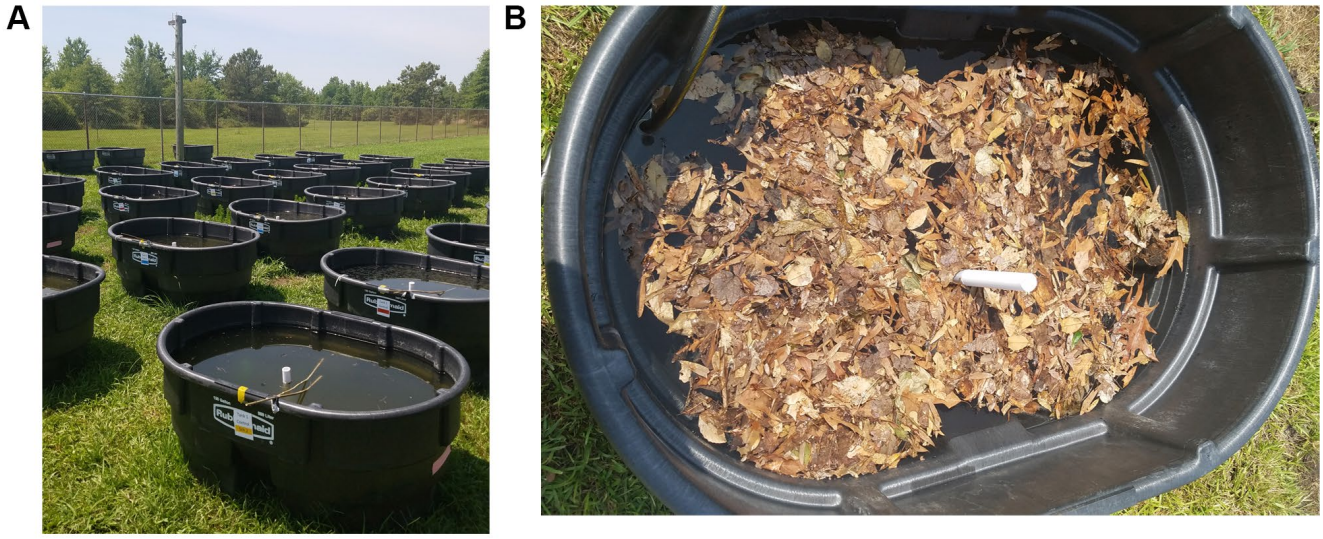

Figure 2: Photographs of 568 liter stock tanks used for this experiment arranged in a grid pattern in the field on 22 June 2019, including three of the eight extra tanks used to house fish not in pulsed treatments along the fence line (A) and photograph of a tank on the date of experimental setup, June 16 2019, containing water and dried leaf litter (B).

Table 1: Schedule used to determine the pulsed 24-hour fish additions and net disturbances to tanks for each block. While all eight experimental blocks experienced nine days of pulses, the timing of the pulses or disturbances differed between each block.

Block	Experimental Days with fish additions to pulsed treatments
1	4, 8, 16, 19, 22, 26, 37, 40, 41
2	1, 6, 11, 15, 17, 35, 37, 44, 45
3	10, 11, 19, 20, 21, 33, 39, 40, 44
4	5, 8, 15, 19, 20, 22, 28, 36, 38
5	10, 15, 18, 21, 26, 29, 33, 34, 39
6	6, 11, 12, 19, 20, 31, 33, 34, 37
7	1, 2, 6, 19, 23, 27, 28, 36, 43
8	1, 5, 8, 14, 23, 27, 28, 31, 33

Table 2: Comparison of candidate models constructed for the abundance of species in sample tanks. Assembly subsample models were run on data gathered during tank assembly, while final sample models used a dataset constructed from the final destructive tank samples. The most parsimonious models with the lowest ΔAIC were chosen and are signified in bold.

Sample Type	Fixed effects of model	ΔAIC	D.F.	AIC weights
Assembly Subsamples	**Sample time * treatment**	**0.0**	**10**	**1**
	Sample time + treatment	681.6	7	<0.001
	Sample time	683.1	4	<0.001
	Treatment	5324.5	6	<0.001
	Null model	5326.0	3	<0.001
Final Samples	**Treatment**	**0**	**6**	**1**
	Null model	34.9	3	<0.001

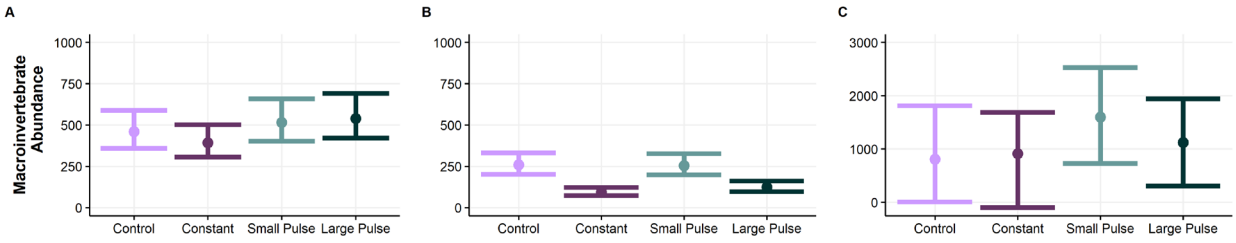

Figure 3: Effects of treatment on the abundance of all macroinvertebrates in samples. Subsamples of full tanks were taken on the third (A) and fifth (B) week of the experiment, and final, destructive tank samples were collected at the end of the experiment (C). Values for each treatment are calculated through a poisson distributed glmm, and each estimate includes a 95% confidence interval.

Table 3: AICc table comparing candidate models of Chao diversity and species richness. Chao models were run on assembling communities, while species richness models were run on the final, complete communities. The most parsimonious models with the lowest ΔAIC were chosen and are signified in bold.

Diversity Metric	Fixed effects	ΔAIC	D.F.	AIC weights
Chao Diversity	**Treatment**	**0.0**	**6**	**0.732**
	Sample time + treatment	2.9	7	0.170
	Null model	4.5	3	0.079
	Sample time	7.6	4	0.016
	Sample time * treatment	11.8	10	0.002
Species Richness	**Treatment**	**0**	**6**	**0.9985**
	Null Model	13	3	0.0015

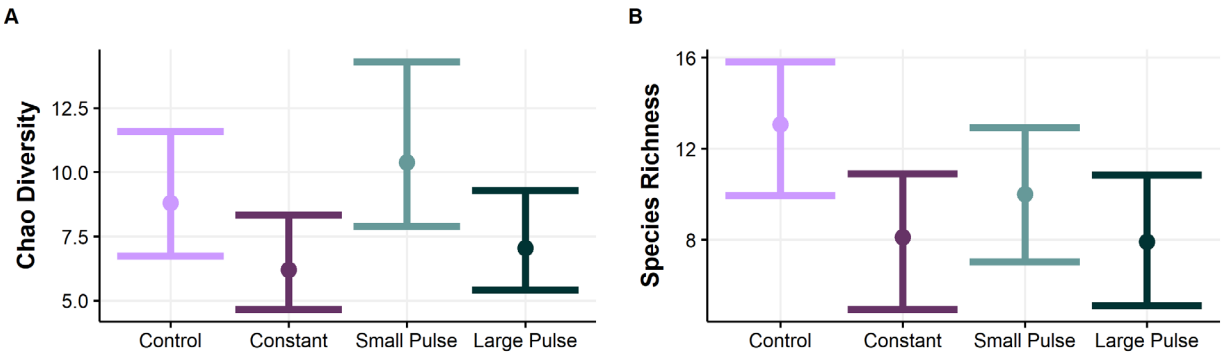

Figure 4: Species richness (α-diversity) of treatments, both during assembly (A) and of final, complete samples (B). Chao Diversity estimates of the species richness and final species richness were both modeled with Bayesian lmm with weak Wishart priors, and estimates include 95% confidence intervals.

Table 4: Diversity models run on final, complete dataset in which the null model was more parsimonious than models including treatment as determined by ΔAIC.

Diversity Metric	Fixed Effects	ΔAIC	Degrees of Freedom	AIC Weights
Shannon-Weiner	**Null model**	**0**	**3**	**0.82**
	Treatment	3	6	0.18
Pielou's Evenness	**Null Model**	**0**	**3**	**1**
	Treatment	16	6	<0.001

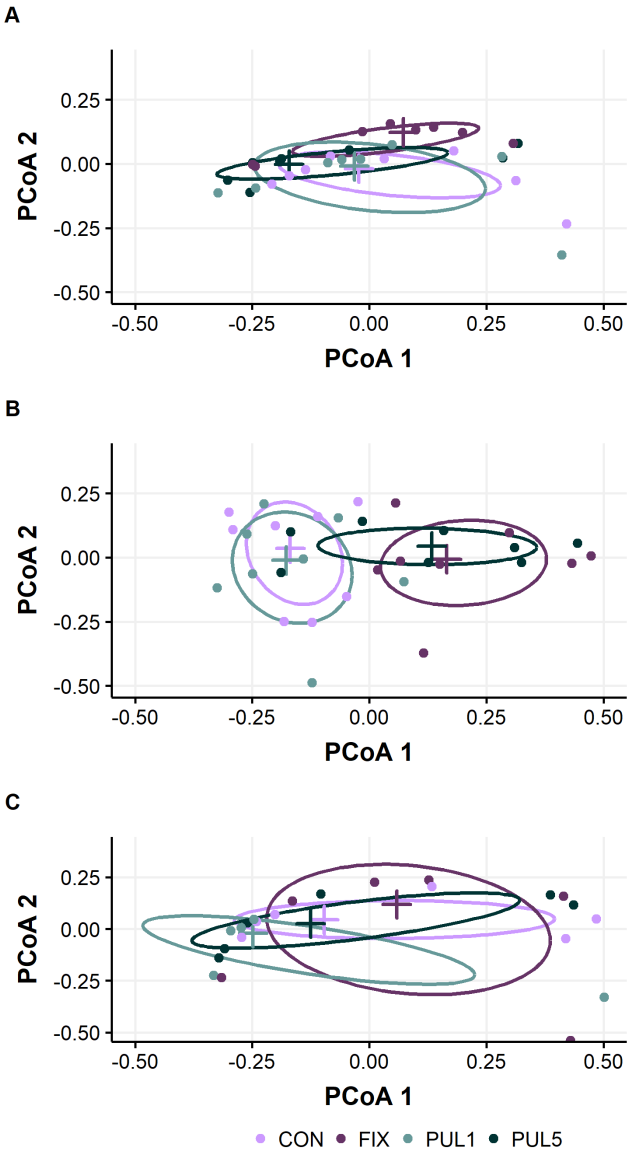

Figure 5: Visualization of PERMANOVA tests run on datasets including all species. Three PERMANOVAS were run, for each timepoint representing the third week of the experiment (A) the fifth week of the experiment (B) and the final community samples (C). Treatment groups represent control treatments without fish (CON) constant predation treatments (FIX) and two different magnitudes of pulsed predation: one fish (PUL1) and five fish (PUL5).

Table 5: AICc table comparing candidate models for measurements taken with Aquafluor fluorometer: free chlorophyll and turbidity. Turbidity was modeled with a lmm, while chlorophyll used Bayesian lmm with a weak Wishart prior. The most parsimonious models with the lowest ΔAIC were chosen and are signified in bold.

Aquafluor Measurement	Fixed effects of model	ΔAIC	Degrees of Freedom	AIC weights
Chlorophyll	**Treatment**	**0.0**	**8**	**0.79**
	Null model	2.6	5	0.21
Turbidity	**Treatment**	**0.0**	**8**	**1**
	Null model	22.2	5	<0.001

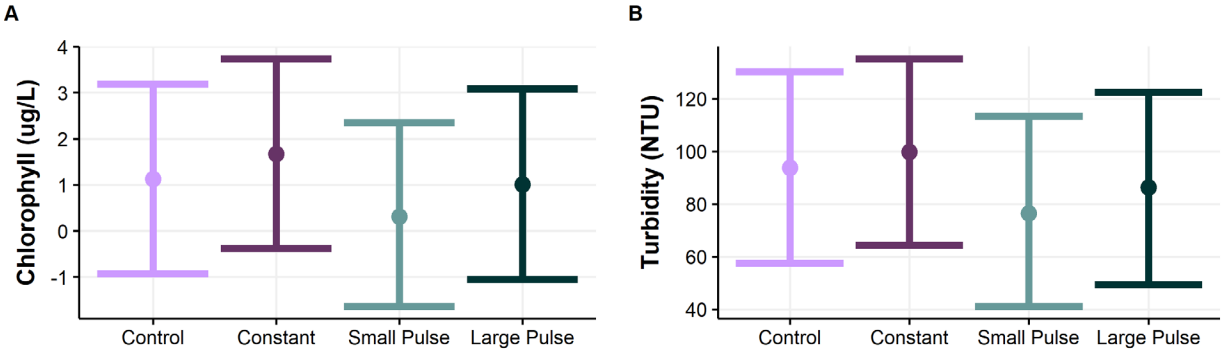

Figure 6: Effects of predation treatment on ecosystem measures of primary productivity and turbidity. Predicted free chlorophyll (A) and turbidity (B) of tank water predicted from lmm, estimates of treatment effects and 95% confidence intervals are displayed.

Table 6: Models of Nitrogen in tanks including treatment as a fixed effect did not outperform null models in ΔAICc testing.

Nitrogen Measurement	Fixed effects of model	ΔAIC	Degrees of Freedom	AIC weights
Ammonia	**Null model**	**0.0**	**5**	**0.959**
	Treatment	6.3	8	0.041
Nitrate	**Null model**	**0.0**	**5**	**0.9972**
	Treatment	11.7	8	0.0028

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