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WATERFOWL IMPACTS TO ZOOPLANKTON COMMUNITIES IN WETLAND META-ECOSYSTEMS

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WATERFOWL IMPACTS TO ZOOPLANKTON COMMUNITIES IN WETLAND META-ECOSYSTEMS

by

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Dream big. We're taught from a young age to reach for the stars--to find our edge, our limit, and then surpass it. As I grew, I began to understand that a dream realized is not only the accomplishment of one single person. Rather, the contributions, support and encouragement of those around us allow us to surmount and surpass our limitations and obstacles.

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WATERFOWL IMPACTS TO ZOOPLANKTON COMMUNITIES IN WETLAND META-ECOSYSTEMS

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The meta-ecosystem concept is an attempt to combine metacommunity, ecosystem and landscape ecology. In meta-ecosystems, both organismal dispersal and material movement between patches can have important effects on communities. This concept provides a more realistic framework of natural systems by considering both processes jointly.

My dissertation presents a case-study of natural metaecosystems by studying the role of waterfowl in structuring zooplankton communities in prairie pothole wetlands in South Dakota. I use observations of natural wetlands, microcosm and mesocosm experiments to show how dispersal of materials and organisms by waterfowl can affect zooplankton abundance and community composition. Waterfowl are conspicuous, behaviorally adaptable, highly mobile and economically important members of wetland habitats. They are thought to have possible effects on zooplankton communities either by dispersing zooplankton propagules among wetlands or by moving nutrients into (via defecation) or out of (via consumption of macrophytes and invertebrates) wetlands.

In this dissertation, I show evidence that waterfowl disperse a limited subset of locally rare zooplankton species between wetlands. I also provide experimental evidence that these dispersed species may have impacts on zooplankton community assembly.

I also show how input of waterfowl excreta may sometimes have strong impacts on the local community. Very large inputs of goose excreta promote abundance and diversity of zooplankton. However, inputs at more modest levels, such as those routinely found in nature, are rarely detectible. Additions of excreta at levels five-times that typically found in nature produce a possible shift in zooplankton community structure away from both no-excreta communities and communities fertilized with comparable amounts of nitrogen and phosphorus. I postulate that most excreta quickly sinks to the benthos and only a small fraction becomes available for use by zooplankton. On the time scales used in my dissertation, it is only with very large additions of excreta that shifts in the zooplankton community become apparent.

My dissertation is one of the first to apply the meta-ecosystem concept to a natural system. It also shows that waterfowl impacts on the zooplankton community may be most important in small wetlands or early in community assembly.

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Introduction

Ecologists have long realized that organismal dispersal and material movement between habitat patches can have profound effects on the ecosystem. Though the literature is rich in research concerning organismal and material dispersal, less understood is how these processes interact. Much of the focus of previous work in metacommunity ecology has been on simple food web models and simple dispersal between patches in aquatic systems. Patterns and flows in resources, upon which organisms depend, has generally been the purview of ecosystem and landscape ecology. Both organismal dispersal and material movement between patches are important to communities, and a realistic framework of natural systems requires that we consider both processes jointly. In 2003, Loreau and others formalized this idea that both organismal dispersal and material flow between patches are important to community and ecosystem processes in their "metaecosystem" framework. The term "metaecosystem" hearkens back to ideas of metapopulation and metacommunity ecology which consider movement of organisms between habitat patches. As ecologists become increasingly aware of the importance of abiotic conditions to community processes, the next logical step in "meta" ecology is to consider material flows between habitats alongside organimal dispersal.

My research is motivated toward understanding how the joint processes of organismal dispersal and material movement affect community structure and diversity in a case-study of natural metaecosystems by studying the role of waterfowl in prairie pothole wetlands.

I sought both realism and feasibility in my model system. Much metacommunity research has been conducted in lakes and ponds due in part to their discrete nature in the landscape, and these wetland habitats are particularly suited for metaecosystem studies as

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well. Dispersing organisms can arrive to aquatic systems not only by direct waterflows but also via vertebrates and wind (Magnusson and Magnusson 2000). Significant material inputs can arrive into wetlands via wastes from passing vertebrates (Manny et al. 1994).

Waterfowl are an ideally suited focal vector for studies of organismal dispersal and material movement between wetland patches. Although their residence time within a wetland patch is relatively short in comparison with organisms which spend their entire lives in a patch, waterfowl are nonetheless important members of the biota. They are dispersal vectors for plants and invertebrates, though the magnitude and species identity of their contribution is still largely unknown (Figuerola et al. 2005, Charalambidou and Santamaria 2005). Their effects on nutrient loading of wetlands can be severe and can have large effects on the biota therein (Post et al. 1998, Manny et al 1995, Olson et al. 2005).

Background

The meta-ecosystem concept is an attempt to combine metacommunity, ecosystem and landscape ecology. Metacommunity ecology (Leibold 2004) has made great strides toward understanding how organismal dispersal affects the biota in networks of communities. Even modest amounts of dispersal can have severe consequences for the native biota (Howeth and Leibold 2008). Invasive species which fundamentally alter local communities and cause local extinction are extreme examples of the effects dispersal can have on a community. Additionally, organisms can be the vectors for material transport when they leave or arrive in new habitat patches. Especially if organisms go to new patches to feed or breed, they directly alter species composition and abiotic conditions. This idea, that organisms fulfill multiple functions in communities and

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ecosystems within a landscape of interconnected patches, finds home in the emerging discipline of metaecosystems (Loreau et al. 2003).

To date, relatively few studies have embraced the metaecosystem framework. Study systems most suited for this research commonly involve a long-distance dispersing animal vector which moves materials and organisms between habitats. For example, fish that feed in areas separate from those where they spawn (and often die) are excellent subjects for studying large-scale organismal-mediated material movement (Varpe et al. 2005). Salamander habitat shift at metamorphosis exports materials to surrounding forest habitat and causes a shift in planktonic and invertebrate community composition (Regester 2008). Spiders and other arthropods feeding on aquatic insects in riparian areas contribute nutrients to terrestrial habitats (Paetzold and Tockner 2005, Baxter et al 2005, Burdon and Harding 2008). Birds are also ideal vectors. They have also been known to be strong ecological actors for dispersing other organisms and materials (Sekercioglu 2006). Changes in plant community composition due to propagules arriving from dispersing gulls have also been recorded on islands (Magnusson and Magnusson 2000). Waterfowl such as ducks and geese have long been known to be important dispersers of seeds and zooplankton resting eggs, and recent research has shown they may be responsible for patterns of genetic structure in zooplankton in the major American flyways (Charalamidou and Santamaria 2005).

Study system

The Prairie Pothole Region (PPR) of north-central North America is a network of small ponds and wetlands ("prairie potholes") created by receding glaciers during the last ice age. The Prairie Coteau, situated largely in eastern South Dakota, was created from glacial grinding and contains large numbers of ponds and wetlands. Biologically, it is a

globally unique ecosystem composed of tallgrass prairie with thousands of glacial wetlands dotting the landscape. In the summer, the PPR becomes home to thousands of breeding waterfowl from throughout the central flyway. Many conservation initiatives have focused on the PPR as an important site of waterfowl breeding and wetland habitat conservation. These ecologically important wetlands are facing multiple challenges. Agricultural use, habitat fragmentation and habitat destruction all threaten their persistence and continued existence. Global climate change predictions indicate many wetlands within the prairie pothole region will have shorter persistence times and dry faster (Johnson et al. 2005). Understanding these wetlands, including the interactions between the organisms that inhabit them, is key for making informed policy decisions regarding their future.

My research focuses on the effects of waterfowl on the plankton community within wetlands. Plankton are key players in aquatic ecosystems (Downing and Leibold 2002). They are numerically dominant, conspicuous members of the wetland metacommunity. They have fast generation times which makes them ideally suited for study. This, in concert with their low position in the food web, makes plankton readily responsive to changes in abiotic conditions, such as nutrient concentrations. Unlike macroinvertebrates which often have a highly mobile flying life stage, zooplankton rely on passive dispersal by wind, rain or some animal vector.

The target dispersal vector, waterfowl, including geese and ducks, are conspicuous, behaviorally adaptable, highly mobile and economically important members of wetland and terrestrial habitats. This role makes them unique members of the community. Many previous studies focus on how the environment impacts duck populations—what habitat characteristics make the most suitable breeding grounds and feeding grounds (e.g. Patterson 1976, Duebbert and Frank 1984). But, the effects

waterfowl themselves have on their surroundings have been much less studied and almost never in the meta-ecosystem context. Most of these studies focus on the nutrient loading effects of nesting waterfowl in wetlands. Oftentimes, only one or very few wetlands are highlighted in these studies (Post et al. 1998, Manny et al. 1995).

My dissertation research focuses on the ways in which waterfowl act as vectors for both the dispersal of organisms and materials in the metaecosystem, and how this dispersal impacts diversity in natural zooplankton communities. In Chapter 1, I provide evidence that zooplankton are transported within waterfowl in hatching experiments and surveys of newly created wetlands. In Chapter 2, I present results from a pilot microcosm experiment and mesocosm experiment, I show how dispersal of materials and organisms via an avian vector can influence the planktonic community. In Chapter 3, I present results from a mesocosm experiment which illustrate that patchiness in waterfowl distribution, and differential input of excreta into wetlands can have a significant impact on the planktonic community.

Chapter 1: Evidence for waterfowl-mediated zooplankton dispersal

ABSTRACT

Zooplankton are small ubiquitous organisms in aquatic habitats and their dispersal and colonization into new wetlands is often the subject for study. Here, I provide evidence that zooplankton are dispersed via waterfowl, and this mode of transport, though idiosyncratic, is most effective for a small subset of zooplankton species in the community. I present evidence from a series of excreta hatching experiments and anecdotal observations from newly created wetlands. Fresh samples of goose excreta were incubated in water, and the resulting zooplankton populations which hatched were observed and enumerated. Excreta hatching experiments reveal that only a small subset of zooplankton species found in surrounding areas are viable after gut passage. I also found that zooplankton populations established soon after wetlands were created, and the species present were similar to those found in excreta hatching experiments. Establishment of zooplankton populations could not be attributed to egg banks or aboveground waterflows with other established wetlands.

INTRODUCTION

In order for organisms to colonize a new habitat, they must arrive in that habitat and establish populations. Colonization into a new habitat is the first major hurtle that must be overcome in order to establish a population. Passive dispersal can often be idiosyncratic and unpredictable, but many organisms arrive in habitats in this way.

Zooplankton lend themselves well to models of dispersal and colonization. They are small, ubiquitous, and easily transported in minute amounts of water, either via aboveground waterflows or on the wet exterior of large-bodied organisms—on feet, feathers or fur. Many have desiccation-resistant life stages which allow the organisms to persist in ponds and wetlands that undergo annual or seasonal drying cycles. Resting eggs ("ephippia") are small and are covered by a protective coating which serves to protect the embryo. These desiccation-resistant life stages are easily transported aboveground via wind and rain. Vector organisms also serve to move zooplankton resting eggs between patches, either externally or internally. Resting eggs may adhere to vector organisms' feet, feathers or fur. Additionally, zooplankton are also thought to be dispersed through animal vectors via their resting eggs passing unharmed through an animal's digestive tract.

Previous studies which have sought to determine the source for colonization of zooplankton populations have done so most often in tank experiments covered with various sizes of mesh screen to exclude particular size classes of dispersal vectors. The largest size classes of dispersal vectors, which include birds and other vertebrates, are often not included in such experiments. It is thought that their size and relative infrequency of dispersal events is difficult to measure, especially with relatively small-sized experiments. The relative frequency of dispersal events via an animal vector is much lower than those expected for wind and rain. However, though vertebrate dispersal

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events may be relatively rare, when it does occur, it may be of a significant type or magnitude to alter the trajectory of community assembly, especially if timing or identity of dispersers is important. In one previous dispersal experiment, where treatments were "closed", bedelloid rotifers became established (Caeceres 2002). This "closed" treatment was covered by very small mesh which was open to only dispersal via wind and rain and the inclusion of the occasional input via bird droppings left on the mesh. Interestingly, in this study, no real difference between the various mesh sizes was observed.

Early research in phytoplankton and zooplankton dispersal has shown that waterfowl may be important vectors of zooplankton dispersal. Vernon and Proctor (1965) and Maguire (1963) documented hatching success of zooplankton following gut passage. However, in order for waterfowl to successfully transport zooplankton resting eggs internally, they must first injest them. Geese are behaviourally flexible organisms and may feed near wetlands or in adjacent fields depending on food availability. Geese and many ducks typically feed on plant matter. Ephippia, zooplankton resting eggs, must be produced and cling to plant matter in order for them to be injested and transported internally. Some ephippia may be buoyant and more likely to float, while others may settle to the bottom of the pond or wetland and become incorporated into the egg bank. It is unclear why some ephippia float and others sink, but it is likely that genetic and environmental factors determine this facet of ephippial production (Caceres 2007).

In Eurpose, some experiments provide evidence for waterfowl-mediated dispersal via internal transport. Studies have observed a large suite of organisms dispersed in this manner including Nematoda, Rotifera, Copepoda, Ostracoda, Insecta (Tipulidae), as well as the cladocerans *Daphnia* and *Moina* (Frisch et al. 2007). However it is unclear if these same species are transported in North American waterfowl. Experiments on survivability

of zooplankton gut passage through ducks and geese have also been conducted, most recently and extensively in Europe (Green and Figuerola 2005).

Internal transport within waterfowl bodies is proposed as an important vector in the transport of zooplankton. Hatching live zooplankton from field-fresh excreta collections demonstrates the ability of zooplankton resting eggs to persist within waterfowl digestive systems, thus providing support for the role of waterfowl as zooplankton dispersal vector. Though similar experiments have been conducted in the past and in Europe, further experiments of this kind help establish the types, species and frequency of zooplankton gut passage. Experiments using native geese in the region also serve to establish the link between zooplankton species incidence in highly ephemeral areas or newly-created areas.

Observational evidence from newly created wetlands also provides evidence that waterfowl may play a significant role in zooplankton dispersal and community assembly. Rapid colonization following creation and filling of new wetlands may indicate a role for long distance organismal dispersal, especially when inputs from an egg-bank or aboveground waterflows can be ruled out. Observation of colonization following wetland creation is well-established. Recent research (Louette and DeMeester 2004, Louette et al. 2008) shows that zooplankton tend to establish early in wetlands, and there is a possible role for colonization via a waterfowl vector. Typically after colonization, a few zooplankton species arrive and increase quickly, but establishment of other species later leads to species turnover and a new community of zooplankton. Colonizers in waterfowl excreta may tend to arrive faster than other methods of passive dispersal. Poor competitors arriving in such a fashion may have the ability to grow and reproduce before being outcompeted.

METHODS

This study was conducted in the Prairie Coteau region of eastern South Dakota. This area is a part of the larger Prairie Pothole Region (PPR) of the northern United States. This area is characterized by numerous kettle lakes and wetlands which were formed during the last ice age as glaciers and ice sheets moved across the landscape. The Prairie Coteau itself is a large, easily distinguished elevated landform drained by the Big Sioux River and the Cottonwood River. It contains numerous glacial lakes and wetlands. In the summer, waterfowl in the central flyway migrate to the PPR and breed. The abundant waterfowl moving across the landscape at this time makes the region an ideal setting to study avian-mediated dispersal of small invertebrates.

Hatching experiments

In the summer of 2008 and again in 2009, I conducted experiments to demonstrate the ability of zooplankton to survive and hatch following gut passage via an avian vector. In these experiments, I hatched zooplankton from field-fresh Canada goose (*Branta canadensis*) excreta. Goose fecal material is compact, easy to obtain and easy to measure and so it holds advantages over other avian excreta. Also, geese roost sites are easy to find and fresh fecal material is abundant and easy to collect in these areas. These experiments were designed to assay which species of zooplankton were commonly transported in goose bodies.

In June 2008, I conducted a small hatching experiment at the Oak Lake Field Station in eastern South Dakota. I added a 5g slurry of field-fresh goose excreta to each of ten 5-gal buckets. Excreta was collected the day of inoculation from fresh droppings at three known goose roosting sites. Buckets were filled with tap water and covered with 2 mm mesh screen covers to reduce outside contamination into the buckets. After three

weeks, I checked for presence of zooplankton, and I collected and preserved all hatched zooplankton by destructively sampling the buckets.

In 2009, I repeated the hatching assay using excreta from additional roosting sites. Both in April and August, I collected excreta from five roosting sites, and deposited 5g field fresh goose excreta (in the form of a slurry) each to ten 5-gal buckets. Zooplankton were collected weekly and preserved in acid Lugol's solution. I also included four buckets containing water only (no excreta added) to act as a control against possible wind-dispersed organisms. Assays in both April and August were conducted over a six week period.

New wetlands

Rapid colonization of wetlands following their creation can also give clues and insights about the time scale and efficacy of dispersal, particularly if the circumstances of their creation are known.

In the spring of 2008, I observed first colonization of two newly-created wetlands. The EcoSun Prairie Farm near Brookings, South Dakota, was created with the goal of becoming a sustainable, restored prairie farm. Particularly in the tallgrass prairies of the United States, sustainable farming initiatives often reclaim deserted farmland and attempt to restore it to native prairieland. In South Dakota, wetlands are a crucial component of the landscape and provide important habitat function. Therefore, sustainable farming initiatives often allow wetlands to fill rather than tile or drain them. Other times, new wetlands are created by plugging areas which were previously drained and tiled. These wetlands then become filled with snowmelt runoff and spring rains.

In 2007-2008, two areas of the prairie farm were plugged so that new wetlands would fill the following spring 2008. Previous to this project, the property had been tiled

and had been used as an active corn and soybean farm for several years. These two small artificially created wetlands filled that spring, and were used by a number of migrating waterfowl during the spring migration. Workers at the site observed Canada geese and ducks visiting the wetlands shortly after they were filled. The largest wetland measured 12 acres at full pool. These wetlands were previously dry areas and not connected to other wetlands via aboveground waterflows. At its creation, the wetland was muddy and deplete of wetland plants. Prairie wetland grasses were planted that first year and in subsequent years.

I sampled these wetlands using a zooplankton net three weeks after their creation and until the wetlands dried three weeks later. Zooplankton was collected with a small zooplankton net and preserved in acid Lugol's solution. A single pass with a zooplankton net sampled .2 m^3 open water. Ten passes with a zooplankton net sampled 2 m^3 open water. Zooplankton samples were collected weekly, preserved, and later identified.

RESULTS

Hatching experiments

In both 2008 and 2009, all buckets containing excreta produced algal blooms. In 2009, I included buckets which did not contain excreta, and these buckets showed no evidence of algal blooms or other organisms throughout the experiment.

In 2008, I destructively sampled the experiment after three weeks. I collected all zooplankton by filtering the water in the buckets through an 80μm mesh screen. Zooplankton were preserved in Acid Lugol's solution and identified. Only two species presented themselves in this bucket experiment--the cladoceran *Moina* (average individuals per bucket 4.8 +/- 5.18 s.d.) and *Synchaeta* rotifers (average individuals per bucket 7.1 +/- 10.08 s.d.) (Figure 1.1).

In 2009, I collected zooplankton from the bucket experiment at weekly intervals. I attempted to collect all zooplankton each week using a small net. However, since I did not destructively sample the experiment until the conclusion of the experiment, it is possible that some juveniles or small individuals were not counted. The assay conducted in April 2009 produced no hatched organisms during the entire six week period. Zooplankton did hatch from excreta in the assay conducted later in the season, in August, though most of the buckets showed no zooplankton emergence. Only two of the ten buckets showed any degree of hatching success over the experiment time period. Eighteen small cyclopoid copepods were the first to emerge three weeks after inoculation in Bucket One. Rotifers, including *Synchaeta* and *Keratella*, became apparent a week later (four weeks from the start of the experiment). *Synchaeta* was present in one bucket (Bucket One: 11 individuals); *Keratella* was present in both buckets (Bucket One: at least 15 individuals; Bucket Two: at least 4 individuals). Specimens of the cladoceran *Moina* were present 5 weeks after inoculation in the bucket containing both *Synchaeta* and *Keratella* (Bucket One: 5 individuals).

New wetlands

The newly formed wetlands were shallow, muddy bottomed pools which contained very few plants (Figure 1.2). A former cornfield, the area contained residual decomposing cornstalks from previous years. The nearest wetland to the site was found in adjacent field, and no aboveground waterflows connected this wetland to the wetlands at the site. The only plants near the wetlands (cordgrass) were those which had been planted by researchers during the course of the restoration project. When the wetlands were first surveyed three weeks after filling, wetland depth at the deepest point was 40 cm in Wetland 1 and 30 cm in Wetland 2. Over the course of the successive three weeks, both

wetlands dried. *Moina* cladocerans, *Synchaeta* rotifers, and a small number of copepod nauplii were found in Wetland 1 in the first sample. *Keratella* rotifers, in addition to *Moina*, *Synchaeta*, and few nauplii, were harvested in Wetland 2 that first week. Successive sampling in the following weeks (at weekly intervals) did not produce additions to the species list. Zooplankton densities increased as the wetlands dried and wetland water volume decreased.

DISCUSSION

Zooplankton are thought to be dispersed most commonly via abiotic means. For example, wind and rain may provide consistent levels of dispersal between patches due to their recurring nature, but these means of dispersal may only account for a small fraction of total dispersal. Aboveground waterflows may connect patches of habitat, but dispersal in this case is restricted to watersheds or between adjacent watersheds.

Zooplankton can also be dispersed via transport in the bodies of waterfowl such as geese. Hatching experiments, outlined here, show that zooplankton resting eggs can survive gut passage and hatch. However, only a small subset of the local zooplankton community became established in hatching experiments. Two species of zooplankton were shown to hatch from fresh excreta in both years that hatching experiments were performed, though other species of rotifers and copepods hatched in the second year only. Interestingly, these same species also hatch readily from waterfowl excreta collected in Europe (Frisch et al. 2007).

An advantage of this study is that field fresh excreta from local wetlands in South Dakota was used, and hatching experiments were performed immediately after excreta collection. This more closely mimics what would occur during a typical summer in a wetland. For transport to be effective early in the season, zooplankton must be able to

hatch soon after they are deposited. Otherwise, establishment of colonizers may be hampered by the difficulties faced by invasion to an established population. Invasion success into an established zooplankton population is unlikely if colonizers are poor competitors. After community establishment in the summer, it is less likely that inputs via excreta will be important in shaping community composition.

The evidence provided by the rapid colonization of the new wetlands shows that early establishment free of competitors can lead to numerical dominance of only a few species. Zooplankton established early in this system, and their density grew to very high levels three weeks after the filling of the wetland. Once they arrived in the wetland, they quickly established, and grew to huge numerical abundance. There were no other apparent sources for zooplankton colonization which could have caused such rapid establishment; these wetlands were not connected via aboveground waterflows and other abiotic means could not have produced such high densities at that time scale. Typically, wind dispersal or dispersal by flying invertebrates is thought to disperse only small numbers of individuals. Wind dispersal has been implicated in the colonization of zooplankton in microcosm experiments, but this dispersal is stochastic, infrequent and happens over the course of several months (Caceres 2002). Rapid colonization and high population densities are potentially more indicative of colonization via an animal vector which can disperse many propagules at one time. Hatching experiments presented here show that even modest amounts of avian fecal material can produce sizeable zooplankton densities after only a few weeks.

These two lines of evidence suggest that waterfowl can serve as dispersal agents for zooplankton via internal gut passage through geese, but they do so in highly biased ways that favor specific taxa. Extensive suverys of natural wetlands in the Prairie Coteau region during the past two years indicate that *Moina* and *Synchaeta* are very uncommon

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in the local zooplankton assemblage. Their persistence in the wetland complex may be highly dependent on waterfowl dispersal between wetland patches.

These two species, the cladoceran Moina and the rotifer Synchaeta, are rare in local wetlands. Field surveys of the Prairie Coteau show that these species are rare in wetlands with open water. These species may persist instead primarily in small wetlands or perhaps as "fugitive species." That is, they may be poor competitors and be easily outcompeted by other zooplankton species. Their dispersal mediated by waterfowl may introduce them to habitats early in the season, before local communities become established, or introduce them to habitats which have low levels of dispersal via other vectors like wind and rain. Though a rare occurrence, this means of dispersal may bring ephippia propagules to novel habitats which competitively poor species are able to exploit before the later arrival of other species.

The dispersal of these species early in the season or to new habitats may have consequences for the trajectory of community assembly in these wetlands. Invasion order has been shown to be important for later community composition. Evidence suggest that the identity of the first colonizers to a patch may have a significant effect on subsequent colonization and community structure of the patch in a process of community assembly. DeMeester et al (2008) have shown empirically that the first colonizers to ponds dominated the community in subsequent years, but whether that is due to other species' dispersal ability or because of a competitive advantage of the resident species is unknown.

The role and importance of waterfowl in structuring zooplankton populations is little understood in natural settings. Insights to this process provide a larger context for the importance of migratory waterfowl in community dynamics. Waterfowl do not often have long residence times within a patch and they may be numerically rare in the habitat,

but their role as dispersers of other organisms, including aquatic invertebrates and plants, has important implications for community structure and trajectory, as well as ecosystem functioning.

Chapter 2: Zooplankton abundance and community compositional changes due to goose fecal inputs

ABSTRACT

The role of waterfowl in the assembly and maintenance of zooplankton metacommunities has been little studied. This study provides insights for the involvement of large-bodied vertebrates in transporting materials and organisms to aquatic microinvertebrate communities. This study shows that large amounts of waterfowl excreta can increase local diversity in a relatively short period of time. It also provides experimental evidence, through a mesocosm experiment, that differential transport of organisms via animal vectors can have consequences for local species assemblages. These experiments have aimed to provide more realism in both amount and timing of excreta additions than previous experiments (Pettigrew 1998).

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INTRODUCTION

Aquatic communities in nature are rarely isolated entities, rather they receive material and organismal inputs from a variety of sources. Ponds and wetlands, for instance, can be connected via waterflows and exchange nutrients, particulate matter, and organisms. Animal vectors can also facilitate transfer of materials and organisms into aquatic communities. Many vertebrates are able to move large numbers of nutrients and organisms relative to their abundance in the community, and many provide a link between different habitat types. Alteration of habitat characteristics by animal vectors has been shown to have significant effects on local communities. Bears move nutrients from aquatic systems into terrestrial ones by feeding on salmon and defecating in riparian forest (Helfield and Nairman 2001). These inputs are in turn used by a variety of organisms within the habitat. In addition, vertebrates are often vectors for organism dispersal either on or within their bodies. Many vertebrates have long migrations as a feature of their life history, and there is evidence to suggest that in some passively dispersing species, animal vectors have provided a positive role in their widespread distribution (Figuerola et al. 2005).

Waterfowl are such an animal vector with roles in dispersing nutrients and organisms. Waterfowl, despite their relative small abundance compared with other organisms, are crucial components of the meta-ecosystem (Loreau et al. 2003, Massol et al. 2011), dispersing not only nutrients such as nitrogen and phosphorus, between and within habitat types, but also organisms, including plants and zooplankton, between patches.

Waterfowl, such as geese and ducks, are well-known for their ability to alter local nutrient regimes via excreta input. As mobile omnivores, they are often responsible for moving materials between terrestrial and aquatic habitat types. For example, during

summer months, geese in midwestern states are often found feeding during the day in farmland and return at night to a roosting site near a waterbody (Rave 1999, Dwyer et al 1979), thereby effectively moving nutrients from field and farm to water. In extreme cases, large populations of waterfowl can significantly alter nutrient regimes of wetlands. For example, Wintergreen Lake in Michigan is a well-documented example of eutrophication caused by an avian vector (Manny 1994). Goose populations in the United States are large, and it is estimated that they can contribute up to 40% of allocthonous nitrogen and 75% of allocthonous phosphorus to a lake annually (Unckless 2007).

Besides contributing to the nutrient loading of a lake or wetland, waterfowl have been shown to be vectors for dispersing organisms as they move between waterbodies. It has been well established that waterfowl are an important dispersal vector for seeds and plants (Charalambidou and Santamaria 2005). Such dispersal, especially if it is mediated via gut passage, can facilitate long range dispersal of organisms (Figuolera et al. 2005). Most research concerning gut passage of organisms within waterfowl has focused on the ability of plants or small organisms to remain viable following gut passage (Figuolera et al. 2003). These studies indicate a positive role for waterfowl transport of zooplankton, rotifers, nematodes and other small animals. These studies consist largely of fecal incubation and growth of organisms obtained from collected excreta samples. The literature to date has focused on the potential ability of waterfowl to transport organisms, but it is less well understood how dispersal of these aquatic organisms impacts local communities. Population establishment of dispersers and interaction with the local community are processes which occur after dispersal, but which are highly dependent on the local community assemblage and the metacommunity.

Metacommunity theory has established that timing, frequency, and species identity of dispersing organisms has consequences for both local communities and the set

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of local communities connected by dispersal (the metacommunity) (Leibold et al. 2004). Disperser identity, in conjunction with dispersal timing and frequency, is important for immigration success and population establishment. Immigration to a local community by a competitively superior species often has unfavorable outcomes for similar, competitively poor species, and in this way dispersing organisms can alter the process of community assembly. The fate of those competitively poor species is often local extinction. Such competitively poor species may nonetheless persist within the metacommunity by dispersing to depauperate or recently disturbed patches.

Waterfowl fecal input effects on the community are not limited to inputs of nitrogen and phosphorus, but also by dispersal of organisms by waterfowl. These factors may have significant consequences for community assembly and community composition. Here, I present two experiments which examine the contributions waterfowl make to local communities via their excreta. The first examines the effect addition of untreated, field fresh goose excreta has on an established zooplankton community. The second experiment attempts to tease apart the factors present in goose excreta and their effects on the zooplankton community. In this second experiment, I separately examine the effects of wholesale excreta addition, dissolved nitrogen and phosphorus addition, and addition of zooplankton immigrants to the community.

METHODS

Bucket Experiment

I conducted a bucket experiment in the summer of 2007, at Oak Lake Field Station in eastern South Dakota. It was designed to easily assay the immediate effects of adding goose excreta to a hyperdiverse zooplankton community. Fifteen 5-gallon buckets were filled with water filtered with 80-micron mesh from Oak Lake. Water was filtered

with this size screen to limit the number of organisms disproportionately coming from Oak Lake. Each bucket was then inoculated with a hyperdiverse cocktail of phytoplankton and zooplankton from 15 surrounding wetlands. After one week, treatments were imposed on each bucket, either 1) a control treatment (no excreta addition), 2) a low goose excreta treatment (2.5g wet weight), 3) a high goose excreta treatment (5 g wet weight). No other nutrients or excreta innoculations were added to the buckets besides this one-time pulse. Each treatment was replicated in 5 buckets. Goose (*Branta canadensis*) excreta for the treatments was collected field-fresh and frozen for 24 hours in an attempt to kill microorganisms. The excreta was then mixed with lake water to create a slurry which was innoculated into the 5-gallon buckets. After four weeks, buckets were destructively sampled.

Water samples were collected and frozen after collection. Water samples for dissolved nutrients were filtered through Whatman GF/F glass microfiber filters immediately after collection. These filters exclude particles more than 0.7 microns from filtered samples, thereby reducing contribution of any particulates in dissolved nutrient assays. After filtration, samples were then frozen for later analysis.

Nitrogen concentrations were quantified by digesting samples with potassium persulfate and then using the second-derivative spectroscopy method (Crumpton et al. 1992, Bachmann and Canfield 1996). Phosphorus concentrations were assayed by first digesting samples with potassium persulfate and then treating samples with a mixed reagent which quantitatively reacts with phosphorus allowing samples to be read easily by spectrophotometer (APHA 1980, Prepas and Rigler 1982).

The entire zooplankton assemblage from each bucket was harvested and preserved in acid Lugol's solution. Cladocerans in bucket samples were enumerated and identified to genus. Rotifers, calanoid and cyclopoid copepods were identified to group.

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Differences between treatments in total and dissolved nutrient concentrations were analyzed using one-way ANOVA in R. Measures of zooplankton diversity were calculated with EstimateS (Colwell 2009). Differences between treatments in total diversity and amongst different genera were analyzed using one-way ANOVA in R.

Mesocosm Experiment

Zooplankton community changes in response to excreta additions may be the product of several different factors in the excreta addition. As outlined above, goose excreta additions contribute a variety of components to an aquatic habitat. This experiment aims to separate the effects on the community of several components of excreta. Among these are the effects caused by nitrogen and phosphorus addition, other nutrients and carbon, microbial fauna, and dispersed zooplankton.

This mesocosm tank experiment was conducted in summer 2008 at the Oak Lake Field Station. 70-gallon cattle tanks were filled with tap water, and particulates were allowed to settle out of the water for four days. A hyperdiverse array of phytoplankton from 15 surrounding wetlands was inoculated into the mesocosms, and three weeks later a hyperdiverse array of zooplankton from those same wetlands was inoculated into the tanks. The zooplankton and phytoplankton communities were allowed to establish over the course of a month before experimental treatments were imposed.

All tanks received a small weekly subsidy of dissolved nitrogen ($0.173g$ tank^{-1}) and phosphorus (0.009 g tank^{-1}), in the form of sodium phosphate and sodium nitrate. This was done to maintain target levels of nutrients and to offset 5% day^{-1} loss of nutrients to the bottom substrate (Leibold, unpublished data).

After one month following zooplankton and phytoplankton inoculation, I began adding treatments to tanks. I had four total treatment conditions as follows

1) Control treatment

2) Fresh goose excreta addition treatment

3) Nitrogen and Phosphorus nutrient addition treatment

4) Zooplankton dispersal treatment.

Each treatment was replicated by five separate mesocosm tanks, and I had a total of 20 mesocosm tanks in this experiment. The control treatment consisted of no further additions to tanks besides the small nutrient subsidy that was added to all tanks.

Fresh excreta additions were composed of field fresh Canada goose excreta mixed with a small amount of water to create a slurry. The slurry was then applied to the water surface. Excreta was added in amounts proportional to what is found in nature as described by Unckless and Marakewicz (2007). Every three days, 2.418g wet weight excreta was added to the fresh excreta addition treatment tanks.

Nitrogen and phosphorus nutrient addition treatments were designed to mirror the average contribution of nitrogen and phosphorus by waterfowl excreta. Nitrogen and phosphorus are known to be among the most common limiting nutrients to aquatic ecosystems, and they may have a disproportionately large role in shaping community interactions. Nitrogen and phosphorus were added as sodium phosphate and sodium nitrate at levels found in goose excreta (Unckless and Makarewicz 2007). Analyses show that nitrogen and phosphorus levels found in goose excreta reported by Unckless and Makarewicz (2007) are similar to those found in collections of field-fresh excreta in this experiment. Every three days, 0.43g sodium nitrate and 14.7g sodium phosphate were added to treatment tanks.

Zooplankton dispersal treatments mimic the process of immigrants hatching from excreta into an established community. Zooplankton collected from buckets incubated with waterfowl excreta (see Chapter 1) were added to treatment mesocosms twice

weekly. 20 *Synchaeta* rotifers and 10 *Moina* cladocerans were added to each tank twice weekly.

This experiment was begun on July 11, 2008 and was ended five weeks later. The final sample was collected on August 17, 2008. Treatments were first imposed on tanks following an initial sample of all tanks. During the course of this experiment, water samples and zooplankton samples were collected every five days. Dissolved oxygen readings were taken with a YSI probe three times for each sample date—the evening previous to sampling, the morning of sampling, and the evening of the sampling day. These three readings are used as an indirect measurement of algal primary production. Water samples were also collected for analysis with a fluorometer which can give measures of relative concentrations of algal density.

Zooplankton samples were collected and preserved in acid Lugol's solution. Following collection, zooplankton were enumerated and identified. Cladocerans were identified to genus. Calanoid copepods, cyclopoid copepods and rotifers were identified to taxonomic group.

Total and dissolved nitrogen and phosphorus samples were collected and analyzed similarly to the bucket experiment, above. Water samples were collected for total nitrogen and phosphorus analysis and immediately frozen. Water samples for dissolved nitrogen and phosphorus analysis were filtered using Whatman GF/F glass microfiber filters and then frozen for later analysis. Nitrogen concentrations were quantified by digesting samples and using second-derivative spectroscopy as outlined above. Phosphorus samples were also digested with potassium persulfate and treated with the mixed reagent as outlined above.

Statistical analyses using parametric tests were conducted in R. Diversity estimates were performed with EstimateS (Colwell 2009).

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RESULTS

Bucket Experiment

Nitrogen levels and phosphorus levels ranged from 558 μg/L to 2396 μg/L and 29 μg/L to 131 μg/L, respectively. These values are within range of those found in surrounding wetlands as obtained from a field survey. Total nitrogen levels significantly increased across treatments (ANOVA, $F_{2,8}=4.964$, p=0.04, Figure 2.1), though this trend disappears in measurements of dissolved nitrogen. Total phosphorus levels tended to be higher in high-excreta treatments (ANOVA, $F_{2,8}=3.220$, p=0.094, Figure 2.2), but this trend disappears in measurements of dissolved nutrients.

Fifteen common zooplankton genera were identified in bucket samples. Rotifers, cyclopoid and calanoid copepods were not broken into separate genera in this study. Some buckets could sustain large populations of zooplankton with densities reaching into the thousands for some species, such as *Chydorus*.

This experiment shows positive evidence that waterfowl excreta additions can affect local community composition. Total species diversity, as measured by Shannon's diversity index, was highest in "high excreta addition" treatments; diversity was lowest in control treatments which received no additional inputs (ANOVA, $F_{2,12}=6.630$, p=0.01, Figure 2.3). There is evidence that a few key groups may be driving this pattern. Ostracod abundances were significantly higher in both excreta addition treatments (ANOVA, $F_{2,12}=19.05$, p=0.0002, Figure 2.4). High goose excreta treatments also contained significantly greater abundances of Ceriodaphnia (ANOVA, $F_{2,12}=5.459$, p=0.02, Figure 2.4), and Rotifers (ANOVA, $F_{2,12}=4.719$, p=0.03, Figure 2.4), and marginally greater abundances of Pleuroxus (ANOVA, $F_{2,12}=3.069$, p=0.08, Figure 2.4).

Mesocosm Experiment

There are many components within a sample of waterfowl excreta which may influence zooplankton diversity—a variety of nutrients, dispersing organisms, and other, unknown factors. A second experiment was performed to separate the potential effects caused by these various components. Experimental treatments were begun July 11, 2008, after the one-month period following zooplankton inoculation. This experiment continued until August 17, 2008.

There was no significant difference between treatments in nitrogen concentrations at the conclusion of the experiment. Neither total nitrogen (ANOVA, F=0.7151, p=0.59) nor dissolved nitrogen (ANOVA, F=1.15, p=0.38) concentrations significantly differed between tanks. Additions of dissolved phosphate significantly increased phosphorus concentrations in this experiment. Phosphate levels were significantly higher in these treatments relative to all other tanks in both total phosphorus (ANOVA, F=146.7, p<0.0001, Figure 2.5) and dissolved phosphorus (ANOVA, F=149.8, p<0.001, Figure 2.6). However these concentrations were not outside the range found in surveys of surrounding wetlands. Other tanks in the experiment contained lower concentrations of phosphorus and were indistinguishable from each other.

Experimental treatments were introduced into tanks on July 11, 2008. At the start of the experiment, there were no significant differences between tanks in total cladoceran abundance (ANOVA, $F_{3,16}$=0.2237, p=0.92.), copepod abundance (ANOVA, $F_{3,16}$=0.2885, p=0.88), nor rotifer abundance (ANOVA, $F_{3,16}$=1.195, p=0.34). Additionally, there were there not any significant differences between treatments in the abundance of individual genera.

At the conclusion of the experiment, zooplankton generic diversity as measured by the Chao 1 statistic tended to be lower in the biotic dispersal treatment (ANOVA,

$F_{3,16}$=2.54, p=0.072, Figure 2.7). Total cladoceran (ANOVA, $F_{3,16}$=0.5606, p=0.65), copepod (ANOVA, $F_{3,16}$=1.343, p=0.20), and rotifer (ANOVA, $F_{3,16}$=0.5696, p=0.64) abundance did not differ between experimental treatments.

Individually, some genera responded very differently to treatment conditions. In particular, *Pleuroxus* (ANOVA, $F_{3,16}$=4.327, p=0.021, Figure 2.8), *Simocephalus* (ANOVA, $F_{3,16}$=7.121, p=0.026, Figure 2.9), and calanoid copepod (ANOVA, $F_{3,16}$=2.695, p=0.081, Figure 2.10) log-transformed abundance showed marked differences between treatments. Addition of dissolved nutrients depresses *Pleuroxus* abundance relative to the control treatment or addition of fresh excreta. Though neither *Simocephalus* nor calanoid copepods were added to dispersal treatments, they both show increases in abundance in this treatment.

DISCUSSION

The preceding two experiments show that waterfowl are able to impact zooplankton communities via their excreta inputs. However, the relationship between excreta additions and the biota is complex. Waterfowl are highly mobile organisms, and may spend little time in one particular area. Even at average levels of input to waterbodies, excreta additions can have perceptible effects on local communities. Waterfowl excreta is a bulky, particulate matrix of partially digested waste, some of which is fibrous in nature and contains insoluble compounds. In order to be used by organisms, easily dissolved nutrients must be taken up my microbes or algae, and large particulates must be broken down further.

I expected to see a spike in nitrogen and phosphorus following treatment additions of waterfowl excreta. However, the experiments presented here did not show evidence of such a spike. Other experiments reported it the literature also fail to detect a post-excreta

addition nutrient pulse. One such experiment (Unckless and Makarewicz 2007), similar to this one in excreta addition timing and amount, did not find significant differences in water quality immediately after waterfowl excreta was added. The authors postulate that particulate matter may have sunk and become incorporated into the benthos quickly. Dissolved nitrogen and phosphorus deriving from excrata may have also been taken up by the biota (benthic organisms, bacteria or algae) quickly before it could be detected in water samples. Another possibility is that nutrients may be tied up in an unusable form within excreta, and may require breakdown by decomposers before it is available. The first experiment presented here lasted a total of 5 weeks, which is a similar timescale to that of Unckless and Makarewicz. An experiment at this scale may not be long enough to observe the effects of inputs of nutrients from excreta. In the second experiment presented here, excreta was applied at a higher frequency, but increases in nutrient levels in the water column were not observed. This may be because of long residence times of the nutrients within the benthos, or this may be because nutrients were taken up by other organisms whose abundance was not quantified, such as the periphyton.

Dissolved nutrient additions to tank mesocosms did, however, have an impact on water quality. Phosphorus levels in dissolved nutrient addition treatments were significantly higher than in other tanks. However, nitrogen levels in these same treatments did not differ from other tanks. This may indicate that nitrogen may be incorporated into organismal tissues more readily than phosphate in this experiment, and thereby be the first to disappear from the water column. It may also be a result of large inorganic phosphorus additions relative to nitrogen additions. Organisms within tanks may not be phosphorus limited, but instead may have been provided with an excess of phosphorus, and so excess phosphorus remained in the water column.

Experiments presented here also examine the impact of excreta additions on the local microinvertebrate community. This study differs from previous studies in that it aims for greater realism in mimicking timing and rate of inputs by waterfowl to a waterbody. Greater realism in input timing is especially important when examining the impact on zooplankton communities because zooplankton generation times are often on the scale of weeks. Also, large, infrequent additions of goose excreta to the water column may quickly become unusable to the pelagic community because goose excreta contains many particulates which readily sink to the benthos. After the addition of very large amounts of excreta, much of the nutrient content may be locked in the benthos until decomposers release nutrients to the water column. The effects on the algal producers and planktonic microinvertebrates may be greatly delayed if there is a long residence time in the benthos for excreta nutrients. This study adds realism in that excreta was applied at shorter intervals throughout the duration of the experiment as compared with previous studies.

For example, an experiment by Pettigrew et al. (1998) added pulses of excreta to mesocosms at four-week intervals, and examined the microinvertebrate community post-addition. The authors found few detectible changes except for a transient peak in algal abundance immediately following excreta addition which quickly disappeared. Over the three month period of the experiment, authors observed cyclical fluctuations in cladoceran and copepod density. They did find that cladoceran density significantly increased after very large excreta additions (115.1 g DW m^2), but this observed pattern was driven by one replicate having very high densities of the cladoceran *Chydorus*.

Experiments presented here show that excreta additions do have impacts on the microinvertebrate community. In the first experiment, planktonic diversity is greatest following large inputs of excreta. The abundances of several genera are significantly

higher following excreta additions. This experiment indicates that it is possible for excreta addition to impact the local zooplankton community on a relatively short timescale.

One of the goals of the second experiment is to understand how the various components of waterfowl excreta contribute to the patterns observed in nutrient concentration and microinvertebrate community composition. This was done by using an array of treatment conditions which sought to mimic possible sources of important inputs from excreta. Interestingly, when organisms hatched from fresh excreta were added to treatment tanks, measures of zooplankton diversity (Chao 1) tended to be lower than other treatments, including controls. This pattern is largely driven by a few species. Other treatments which examined nutrient input, microorganism input, or other particulates did not show any difference from controls. Treatments did have some effect on individual genera abundance within tanks. Excreta addition may have idiosyncratic food web effects within tanks, and prediction of these effects is difficult. This pattern suggests that organisms hatching from excreta may play a large role in structuring zooplankton community interactions. Experiments presented here give positive evidence that it may be the organisms dispersed within excreta which have the most immediate effects on the local community rather than inputs from nutrients.

The trends in zooplankton abundance from the bucket experiment may also have been the result of successful establishment of zooplankton hatching from the excreta. In previous experiments, zooplankton resting eggs hatched and established populations from goose excreta within three weeks (Chapter 1). Therefore, it is possible that some individuals may have hatched in the bucket experiment. Though *Moina* and *Synchaeta* were not present at the conclusion of the bucket experiment, other species which have been shown to have the ability to pass through waterfowl digestive systems were present

in higher abundances at the conclusion of the experiment, namely *Ceriodaphnia* and rotifers (Figuolera et al 2007).

The second experiment shows that even modest levels of dispersal may be important in shaping community assembly and trajectory. In the dispersal treatment, the dispersing organisms in this experiment, *Moina* cladocerans and *Synchaeta* rotifers are rare within the landscape (as evidenced from field surveys of surrounding wetlands), but readily hatched from goose excreta. Interestingly, they remained very rare in experimental tanks, but these dispersers had strong impacts on some key zooplankton groups, and caused unexpected changes in community composition. This evidence may indicate a role for these organisms as potential facilitators or competitors of other zooplankton. Complex competitive interactions in the zooplankton community are little-studied and difficult to quantify. These interactions are hard to predict, but evidence presented here suggests that they are repeatable. Such unexpected effects may indicate that the zooplankton community is a complex assemblage, and immigration by rare species can have significant impacts on the assembly process. Additionally, these immigrants may be poor competitors within the zooplankton community, but persist in the landscape due to high rates of dispersal. Relatively little is known about *Moina* or *Synchaeta* life history and ecology. However, their ability to pass through waterfowl digestive tracts increases their potential rate of dispersal, though they are rare in the local assemblage.

Additionally in this experiment, addition of these dispersers to tanks produced changes in abundance of other zooplankton, even though disperser abundance remained very low. It is likely that a suite of competitive interactions occurred in the zooplankton community which resulted in a few key species increasing their abundance in the

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community. These interactions are hard to predict, but the evidence presented here suggests that the trends are repeatable among replicates.

Chapter 3: Waterfowl fecal input effects on pond zooplankton communities not solely driven by Nitrogen and Phosphorus input

ABSTRACT

Although it has been long recognized that waterfowl can have very strong effects on lake and pond ecosystems, the contributing mechanisms that underlay these effects have yet to be well resolved. Waterfowl largely affect such ecosystems via fecal inputs but these inputs can affect ponds in various ways including inputs of nutrients (especially nitrogen and phosphorous), inputs of carbon, and by facilitating the dispersal of organisms between wetland patches. I conducted a mesocosm experiment to try to resolve the various roles of these possible factors. My experiment compared a control treatment (no inputs) with fecal additions, nutrient additions that correspond to fecal additions but consist only of $NaHPO_4$ and NH_4NO_3, and sterilized fecal additions (to reduce/remove the effect of facilitated dispersal). I found that fecal inputs affected zooplankton species abundance. This research also suggests that fecal inputs produce a different zooplankton community than nutrient inputs alone. I also found that these effects were different than the additions of nutrients alone but similar to additions of sterilized fecal inputs. This indicates that the effect of fecal inputs was distinct from the addition of nutrients and that facilitated dispersal was not a major factor. Instead results suggest that the effects of fecal inputs are more likely due to material inputs other than phosphorus and nitrogen, such as carbon inputs.

INTRODUCTION

Waterfowl have commonly been shown to have important effects on limnetic ecosystems. These effects often involve either facilitating the dispersal or organisms among ecosystems, or the movement of materials. When ecosystems exchange materials and dispersing organisms in a landscape they may be thought of as meta-ecosystems (Loreau et al. 2002, Massol et al. 2011). Thus waterfowl may be important contributors to the spatial dynamics of an ecosystem. Here I examine the possible ways that they may do this.

While the effects of waterfowl can be large, they can be attributed to a number of possible factors. First, they may be due to the inputs of nutrients such as phosphorous and nitrogen that are associated with fecal inputs. Previous studies have shown that such nutrient inputs can be substantial compared to other inputs or to internal loading. Wintergreen Lake in Michigan is a well-known example of this phenomenon, and this lake and its bird population has been studied extensively. Migrant geese, among other waterbirds, have been shown to contribute significant nutrients to the lake (Manny et al. 1975), including nearly 70% of carbon and phosphorus inputs (Manny et al. 1994). Though significant amounts of POC are contributed by migratory waterfowl, it has been thought that the resultant effects on the zooplankton community have been due to high phosphorus loading. However, cases such as Wintergreen Lake represent the extreme on the scale of waterfowl densities found in nature. Less is known about how more modest levels of inputs impact the wetland community. Previous studies which have focused on average waterfowl inputs to ponds and wetlands have found conflicting results. Particularly in small wetlands, the effects of geese roosting near a wetland can increase nitrogen levels significantly (Kitchell et al. 1999) and thereby promote primary production within the wetland (Bazely and Jeffries 1985). Other studies have found more

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modest increases in nitrogen and phosphorus concentrations in the water column, and much of this seems attributable to nutrient loss to the benthos (Pettigrew et al. 1998).

Secondly, waterfowl are also likely dispersal vectors for limnetic organisms and could thus affect pond communities by altering community assembly and composition. Waterfowl have been implicated as potentially important dispersal vectors for seed plants, planktonic algae, and zooplankton. Much research to date has been conducted on long-distance dispersal and gut passage survivorship of seed plants (Soons et al. 2008, Mueller and van der Valk 2002, Figuerola et al. 2002, Figuerola et al. 2003). Studies have also shown that seeds readily adhere to feet and feathers, and this too may be an important mode of dispersal (Vivian-Smith and Stiles 1994). Experiments also indicate that waterfowl may play a key role in long distance dispersal of algae and small invertebrates, such as zooplankton (Charalambidou and Santamaria 2005, Green et al. 2007). Indeed, algal dispersal by waterfowl has long been suspected and studied (Proctor 1959, Schlicting 1960). While phoretic dispersal on feathers and skin are involved, fecal dispersal of ingested material is also likely. Not only is it likely, but it has been implicated a major driver in long distance dispersal of zooplankton and other small invertebrates (Green and Figuerola 2005, Figuerola et al. 2005). However, recent experiments in Europe indicate that not all zooplankton can survive gut passage; only a limited subset of zooplankton are viable in duck feces (Frisch et al 2007). In such cases, the trajectory of community assembly may be affected if that limited subset of biota are the first colonizers to a wetland (Louette and DeMeester 2004).

Finally fecal inputs may also affect limnetic ecosystems due to the input of other materials such as organic carbon in various forms. Allochthanous carbon inputs are believed to have a larger effect on consumers in small, humic lakes as opposed to large, clearwater lakes (Pace et al. 2007). In small, humic lakes, bacteria process DOC via the

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microbial loop, and those bacteria are in turn consumed by zooplankton. In these small lakes, a large percentage of the zooplankton diet are these bacteria, rather than the rarer phytoplankton. In large, clearwater lakes, zooplankton feed primarily on algae, which use DIC as their carbon source (Perga et al. 2006). Indeed, in such small lakes with high bacterial activity and low primary production, zooplankton populations take up a large percentage of allocthanous carbon (Karlsson et al. 2003). Experimental studies have also shown that in humic lakes, much of the carbon propagating up the food chain has come from allochthanous POC (Carpenter et al. 2005, Cole et al. 2006). Therefore, small wetlands, especially those which are humic or have rich bacterial and decomposer populations, have the potential to be most affected by carbon introduced through waterfowl feces.

These three factors may contribute significantly to the aquatic community, but the magnitude of these effects is in proportion to the contribution made by waterfowl. Waterfowl are patchily distributed across the landscape, and their average contribution across the landscape is low in proportion to their effect within frequented feeding or roosting sites. In places where their densities are high, waterfowl effects on aquatic communities can be extreme, as noted above. Near wetlands that are prime roosting sites, waterfowl effects are likely to be more extreme than in wetlands which are visited rarely. This variation in waterfowl behavior creates a patchy landscape of frequently visited versus non-visited wetlands, and this contributes a degree of complexity. To give a more realistic picture of waterfowl contributions in light of this patchiness, this experiment incorporates different treatment levels to mimic waterfowl inputs during the growing season in "average" wetlands and in more highly-impacted wetlands.

In this experiment, I attempt to tease out the effects of different components of waterfowl excreta and their contributions to zooplankton community structure. This

mesocosm experiment shows that excreta affect local zooplankton community structure, and this effect is not due solely to nitrogen and phosphorus loading. This experiment is similar to the one conducted by Pettigrew (1998), but uses more realistic amounts of excreta input and timing according to reported average inputs during the growing season. This experiment also aims to illustrate the differential effect of high and low excreta additions on the zooplankton community.

METHODS

In the summer of 2009, a mesocosm experiment using cattle tanks was conducted to examine the effects waterfowl excreta inputs have on zooplankton communities. Waterfowl excreta may contribute to aquatic communities via nutrient inputs or act as dispersal vectors for organisms. This experiment separates these different contributions, and quantifies their effect on local zooplankton assemblages within experimental mesocosms.

In May 2009, twenty 70-gallon (265 L) cattle tanks were filled with tap water and particulates were allowed to settle for 5 days. A hyperdiverse cocktail of phytoplankton obtained from 15 surrounding wetlands was added. Algae were allowed to establish within the tanks for three weeks. Afterwards, a hyperdiverse cocktail of zooplankton from those same surrounding wetlands was added to the tanks and allowed to establish for one month before experimental treatments were begun.

All tanks received a small weekly subsidy of dissolved nitrogen (0.173g tank^{-1}) and phosphorus (0.009 g tank^{-1}), in the form of sodium phosphate and sodium nitrate. This was done to maintain target levels of nutrients and to offset 5% day^{-1} loss of nutrients to the bottom substrate (Leibold, unpublished data).

Waterfowl impacts to wetlands via excreta come in the form inputs of nutrients and dispersing organisms, which includes bacterial, plant and microinvertebrate dispersal. Mesocosms were subjected to one of seven treatment types. First, a control treatment in this experiment has no additional added nutrients or excreta for the duration of the experiment, aside from the weekly nutrient subsidy described above. Second, this experiment incorporates three types of dispersal addition treatments at two different treatment levels (high and low). The three types of additions are: 1) field fresh excreta additions, 2) alcohol sterilized excreta additions, and 3) inorganic nitrogen and phosphorus additions. Field fresh goose excreta additions contribute the full suite of nutrients and living organisms available. In the sterilization treatment, goose excreta was incubated in 200mL of 70% alcohol for 24 hours in an attempt to kill microorganisms. To account for effects that may have been caused by the alcohol, other tanks which did not have a sterile-excreta treatment instead received a 200mL input of alcohol directly. The third treatment type consisted of nitrogen and phosphorus additions in proportion to published values of nutrient content of goose excreta (Unckless and Makarewicz 2007). These nutrient additions are also similar in nutrient content to goose excreta collected in the local area. Nitrogen and phosphorus are often cited as the most important nutrients contained in excreta that act to increase productivity in the food web.

Each of the three addition treatments was replicated at both a "high" and "low" input level. The first of which mimics average levels of excreta input as reported by the literature (Unckless and Makarewitz 2007); the second, high-level addition, is an input five-times the average level, more closely mimicking a wetland used frequently by geese during the growing season. There was a total of twenty mesocosms used in this experiment and seven treatment conditions: a control, a low fresh excreta addition, a high fresh excreta addition, a low sterile excreta addition, a high sterile excreta addition, a low

dissolved nutrient addition, and a high dissolved nutrient addition. Each treatment was replicated in three mesocosm tanks except for one treatment, the low addition of dissolved nutrients treatment, which was replicated twice.

The experiment was sampled just before the first treatment additions and twice more at three week intervals. The first treatments were added 27 July 2009 and the experiment was terminated 18 September 2009. Zooplankton were preserved in Acid Lugol's solution and were enumerated. Cladocerans were identified to genus. Copepods and rotifers were classified according to major groups.

RESULTS

Water chemistry was performed on samples taken the final day of zooplankton sampling. Treatment tanks which included phosphorus additions showed significantly higher levels of total phosphorus (ANOVA, $F_{6,13}=101.7$, $p<0.0001$, Figure 3.1). This pattern is strongly driven by treatment additions of inorganic phosphorus. Soluble reactive phosphorus (SRP) in the water column did not significantly differ between treatments (ANOVA, $F_{6,13}=2.516$, $p=0.088$, Figure 3.2). However, additions of sterile excreta tended to depress SRP levels in mesocosms, below levels found in control tanks.

There were no significant differences in zooplankton abundance at the start of the experiment. Zooplankton abundance tended to fluctuate only slightly throughout the course of the experiment except in some key genera. Some key genera of zooplankton peaked towards the conclusion of the experiment. *Daphnia* increased numerically during the course of the experiment (Figure 3.3). *Daphnia* populations were also significantly higher in excreta addition treatments, both fresh and sterile ($F_{6,13}=4.468$, $p=0.016$). Log-transformed *Daphnia* abundance was significantly higher in excreta treatments (both fresh and sterile additions) than for other treatments ($F_{6,13}=3.73$, $p=0.029$, Figure 3.4).

Simocephalus populations show a similar trend with variable, increased abundance in excreta treatments ($F_{6,13}$=2.628, p=0.08, Figure 3.5), but the trend disappears after log-transformation (Figure 3.6). Cyclopoid copepods also had a slight tendency to increase in the presence of high fresh excreta additions (ANOVA, $F_{6,13}$==2.147, p=0.13, Figure 3.7).

Overall estimates of the zooplankton community using diversity estimators indicate that treatments produced divergent communities. Shannon's diversity index is based on the number of species in a community and their relative abundance. Values of this index increase with the number of species present and with species evenness, that is, with similar abundance values. Shannon diversity estimates were significantly different among treatments in the final zooplankton sample (ANOVA, $F_{6,13}$=3.846, p=0.026, Figure 3.8). Low additions of sterile excreta and nutrients produced the lowest and most variable diversity estimates. Low additions of fresh excreta also had a slightly depressed diversity.

Principal components analysis is a convenient way to visualize the relative composition of zooplankton communities in the experimental mesocosms. The first two principal component axes of the zooplankton log-transformed abundances from the final September sample were generated using R. (Figure 3.9). Using that analysis, tank scores and their associated treatments were plotted (Figure 3.10). Mesocosms with the same treatment sometimes tended to clump, but there is a large amount of variation within treatments. Though not statistically significant (ANCOVA, p=0.08, Figure 3.11), there was a slight tendency for high inputs of excreta or nutrients to produce differing communities. High and low additions of nutrients and excreta appear to produce divergent communities.

However, when nauplii, a group composed of immature cyclopoid and calanoid copepods, are excluded from the analysis, the pattern shown in Figure 3.11 disappears

(Figure 3.12; Figure 3.13). When nauplii are excluded, principal components analysis of the zooplankton community using log-transformed zooplankton abundance returns a different pattern than above (Figure 3.14). Nauplii, however, do not show significant differences between treatments (ANOVA $F_{6,13}$=0.3946, p=0.84, Figure 3.15). Zooplanton genera largely sort into two groups along PC1. Many of the larger bodied cladocerans abundances' have a positive relationship with PC1.

DISCUSSION

These results indicate that goose excreta influences a wetland ecosystem in a way that extends beyond bulk contributions of phosphorus. The absence of a spike in phosphorus concentrations following addition of fecal material may indicate rapid uptake of those nutrients into the food web or a loss of those nutrients to the benthos. Zooplankton abundance and composition was positively impacted by excreta addition, so this gives an indication that not all inputs via the excreta were lost to the benthos. Additionally, levels of soluble reactive phosphorus were remarkably low in sterile excreta treatment tanks. This may indicate that unintended interactions may have occurred between the biota/dissolved phosphorus and the alcohol. It was believed that a small volume of alcohol (ca. 200mL) would have an insignificant effect on 265L mesocosms. However, these results suggest small volumes of alcohol may have perceptible effects.

High levels of excreta input, both fresh and sterile, have a positive effect on key zooplankton genera. In particular, Daphnia and Simocephalus were positively impacted by excreta addition, and these impacts tend to be greater than those of other treatments. These species are large bodied, typically numerically abundant zooplankton species. This experiment demonstrated that zooplankton were affected by several types of input to the wetland ecosystem, not solely by nitrogen and phosphorus. The effects of nitrogen and

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phosphorus additions tended to be much smaller than large additions of excreta, even though nitrogen and phosphorus levels in nutrient addition treatments were high at the conclusion of the experiment.

Additionally, there are indications that the zooplankton communities varied depending on the treatment type. This experiment, though suffering from low sample size, suggests that zooplankton community trajectory varies depending on how materials are added, whether by bulk additions of excreta or by inorganic nutrient additions. This result has implications for our understanding of community assembly and the factors which contribute to assembly in situ.

In the literature, it is often the extreme cases of waterfowl impacts which are reported. Cases such as Wintergreen Lake or coastal wetlands, which are home to huge breeding colonies of waterbirds, are extreme cases which have produced their own unique effects on the local environment and biota. This study suggests that waterfowl impacts on local communities may not be linear and predictable. Low levels of excreta input tend to produce different communities than high levels of input, and these in turn are different than communities which receive only inorganic nitrogen and phosphorus.

Zooplankton communities are affected by the amount, type and frequency of additions provided from outside sources. Zooplankton species are sensitive to environmental characteristics, and different zooplankton communities tend to arise based on the ability of different species to 'sort' to a particular environmental template. Zooplankton community dynamics are affected by a mixture of complex interactions between the environment and the constituent species.

Because of the dynamic nature of zooplankton communities, detection of waterfowl effects can be problematic. Wetlands which are very strongly impacted by waterfowl (such as Wintergreen Lake) are often swamped by an enormous nutrient load,

and communities respond strongly to that condition. However, effects to the zooplankton community in wetlands which receive intermediate levels of input via waterfowl may be more difficult to detect. When material inputs of nutrients are much smaller, the relative effect of dispersing organisms, including algae, bacteria, and zooplankton ephippia, may be more important to the resident biota. The community resulting from such interactions may not appear qualitatively or numerically similar to a very highly impacted community.

The scale at which waterfowl excreta inputs are incorporated into the food web after deposition is also an important consideration to identifying waterfowl effects on ecosystems. Once deposited, fecal inputs via waterfowl sink to the benthos and remain undissolved, locked within the benthos, until an event such as lake turnover stirs the waterbody. If ponds and wetlands are not sufficiently large enough to become stratified, processes such as lake turnover and mixing may not ever send benthic materials into the water column. However, not all fecal inputs are applied by waterfowl in the same way. For example, geese often deposit fecal material near the margins of wetlands where they roost. Following a rainstorm, nutrient and material inputs are contributed via runoff, and such inputs are more likely to be detected in the water column. This mode of input can contribute both dissolved and particulate matter to waterbodies which may be unaffected by lake turnover.

In this study, there are indications that community compositional shifts following waterfowl excreta addition can be detected two months after excreta addition. However, significant patterns which are readily detectible may require studies conducted on longer time scales. Generation times of zooplankton are generally on the order of weeks, and sufficient time following application of treatment conditions is necessary to detect effects. At the conclusion of this experiment, immature copepods and nauplii were highly

abundant and were strong drivers of observed community compositional patterns. The presence of these immature forms may indicate the beginnings of a larger shift in zooplankton community composition. Their presence may indicate a shift in the type of materials and nutrients available to the zooplankton community. Following an addition of excreta, microbes and other decomposers must first process the excreta into a form usable by zooplankton. Copepods are thought to be more tightly linked to the microbial loop than cladocerans. Their foraging habits place them in the benthos and on macrophytes, where they are more likely to come into contact with the products of decomposition and bacteria, as well as in the open water. Though cladocerans also feed on bacteria in addition to algae, they are more efficient feeders of algae in the open water. This study has shown that goose excreta has a variety of effects on local biota. This study provides further indication that the role of waterfowl in the ecosystem is complex. Goose excreta contains many living and dead components which can shape local zooplankton assemblages, often on time scales greater than what has been observed in most studies, including the present study. Further studies which examine excreta impacts, particularly how excreta is decomposed and percolates up the food web, are required to understand the role of waterfowl in local wetlands.

Conclusion

My dissertation has shown several lines of evidence indicating that waterfowl effects on the meta-ecosystem may be important. Zooplankton communities respond strongly to very large inputs of waterfowl excreta (Chapter 2). However, such large inputs into wetlands are rare. More realistic levels of excreta input by waterfowl produce more modest effects on zooplankton communities (Chapters 2 and 3). Even at levels of excreta five times the average found in nature, effects on zooplankton community composition from excreta additions are modest, though highly suggestive (Chapter 3). And further, the effect on zooplankton community composition at high levels of excreta addition is driven by few key community members. In chapter 3, zooplankton compositional changes following inoculation of tanks with excreta five times average levels were strongly driven by populations of immature copepods--nauplii and copepodites. The presence of many immature forms may indicate the beginning of larger community compositional changes. Responses of cladocerans and copepods to changes in the environment differ slightly due to their differing reproductive biology. Copepod development is marked by a number of immature stages, whereas cladocerans are essentially born as tiny adults (Gillooly 2000). Additionally, copepods sexually reproduce few times per year, but cladocerans are able to reproduce parthenogenically in a matter of weeks (Allan 1976). Inputs into the detritus from sunken excreta are more likely to affect copepod populations rather than cladocerans due to differences in feeding habits and life history. Some copepod species use detritus as an important food source (Ustach 1982, Mauchline 1998), and copepods are thought to be more highly tied to the "microbial loop" (Steele 1998). Experiments presented herein may indicate that zooplankton community responses to such excreta additions may only be apparent on longer time

scales than those of a few weeks or a couple of months, except at extremely large input levels.

My dissertation also examines waterfowl as a vector of zooplankton dispersal. Most previous research to date has focused on the ability of waterfowl to serve as a vector for microbes, plants, and small invertebrates. However, few studies have examined the consequences to local zooplankton communities of immigration by waterfowl-dispersed zooplankton. My research (Chapter 1) has illustrated that dispersal by waterfowl is feasible and repeatable at least for a limited subset of zooplankton. My research (Chapter 2) has also shown that immigration into local communities by these species can cause unexpected shifts in the zooplankton community. These results suggest that the zooplankton communities are complex, and interactions between species are often indirect and mediated by food-web interactions. I also suggest that waterfowl-mediated dispersal may be especially important during community establishment and early in community assembly. Evidence from newly created wetlands indicates that zooplankton dispersal by waterfowl allows zooplankton species invasion opportunities at a much faster time scale than other modes of dispersal such as wind or rain (Caceres and Soluk 2002).

The impact of waterfowl in structuring zooplankton communities is complex and highly dependent on the abundance and frequency of waterfowl movement in the meta-ecosystem. When waterfowl are not highly concentrated, their effects on local communities are modest. It is when waterfowl are highly abundant and have long residence times that waterfowl effects become extreme. However, in small wetlands, or in newly established wetlands, the presence of few waterfowl can have significant effects on community trajectory.

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Figures

Figure 1.1: In 2008, the cladoceran *Moina* and the rotifer *Synchaeta* hatched readily from samples of goose excreta incubated in ten 5-gallon buckets for three weeks. Here, I show the per-bucket average number of individuals and the associated standard deviation from two genera which hatched after three weeks. *Synchaeta* were generally more abundant than *Moina* in buckets, but there was high variation between replicates. Following this experiment, hatched individuals were used as immigrants in a mesocosm experiment.
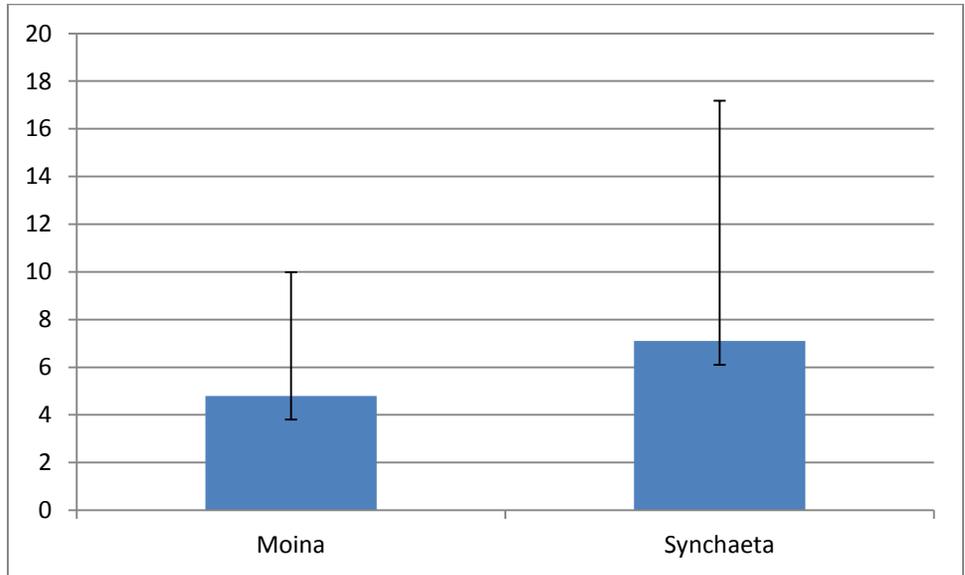
Figure 1.2: Two wetlands at the EcoSun Prairie farm were sampled for zooplankton in June 2008 and until they dried three weeks later. This photograph shows Wetland 2 while workers were planting cordgrass during that summer. *Moina* cladocerans and *Synchaeta* rotifers were identified early in these newly created wetlands.

Figure 2.1: In bucket experiments conducted in 2007, total nitrogen concentration at the conclusion of the experiment was significantly higher in replicates which received high amounts of added goose excreta (ANOVA, $F_{2,8}$=4.964, p=0.04). Shown are the average concentrations of total nitrogen and the associated standard deviation for each.
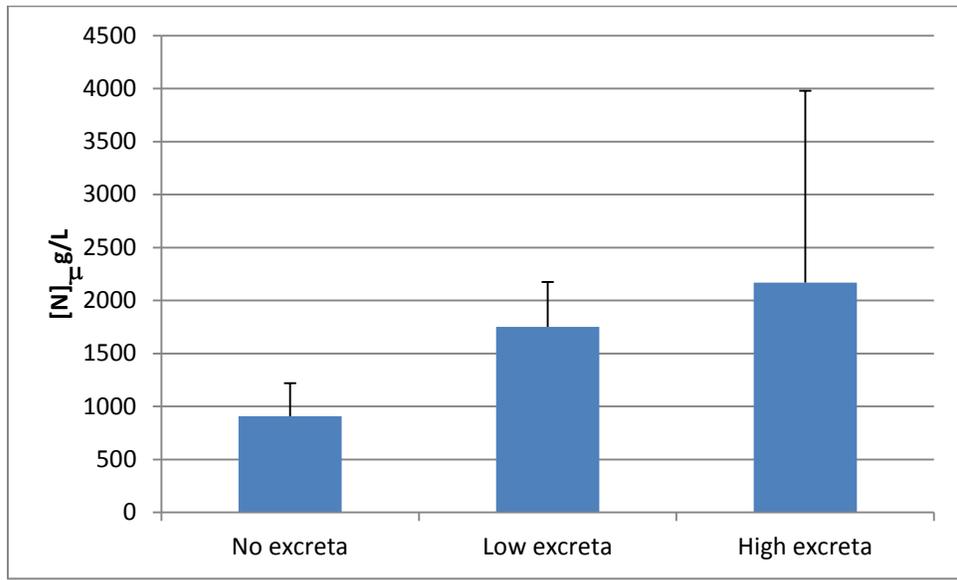
Figure 2.2: In bucket experiments conducted in 2007, total phosphorus concentration at the conclusion of the experiment showed no significant trends among treatments (ANOVA, $F_{2,8}=3.220$, $p=0.094$). Low excreta treatment additions, however, did tend to have slightly increased phosphorus concentrations relative to high excreta additions. Shown are the average concentrations of total phosphorus and the associated standard deviation for each.
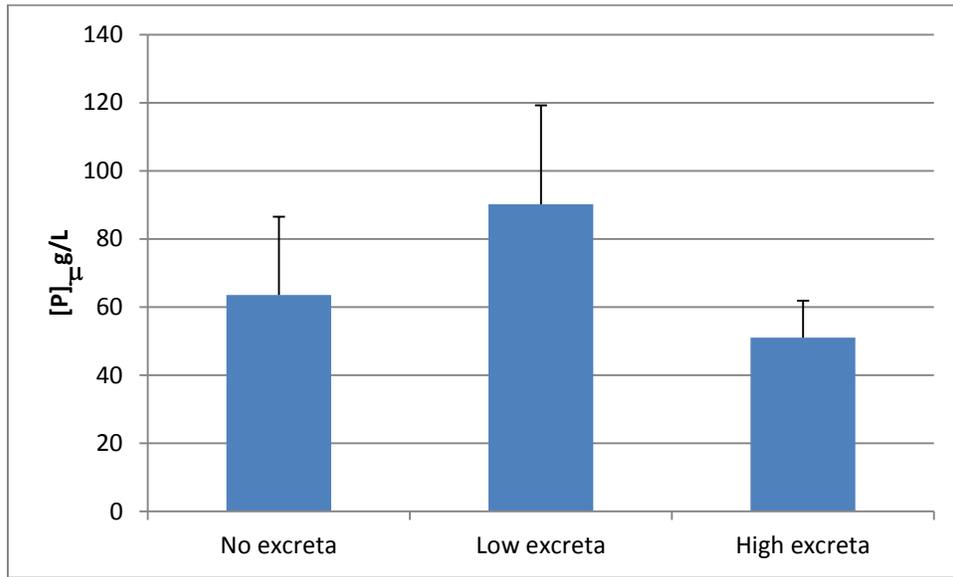
Figure 2.3: Zooplankton diversity was positively impacted by high excreta additions in the 2007 bucket experiment as measured by the Shannon diversity index (ANOVA, $F_{2,12}=6.630$, p=0.01). Zooplankton diversity was calculated using EstimateS (Colwell 2009). Control and low excreta additions were not significantly different. Shown are the average Shannon diversity indices and the associated standard deviation for each.
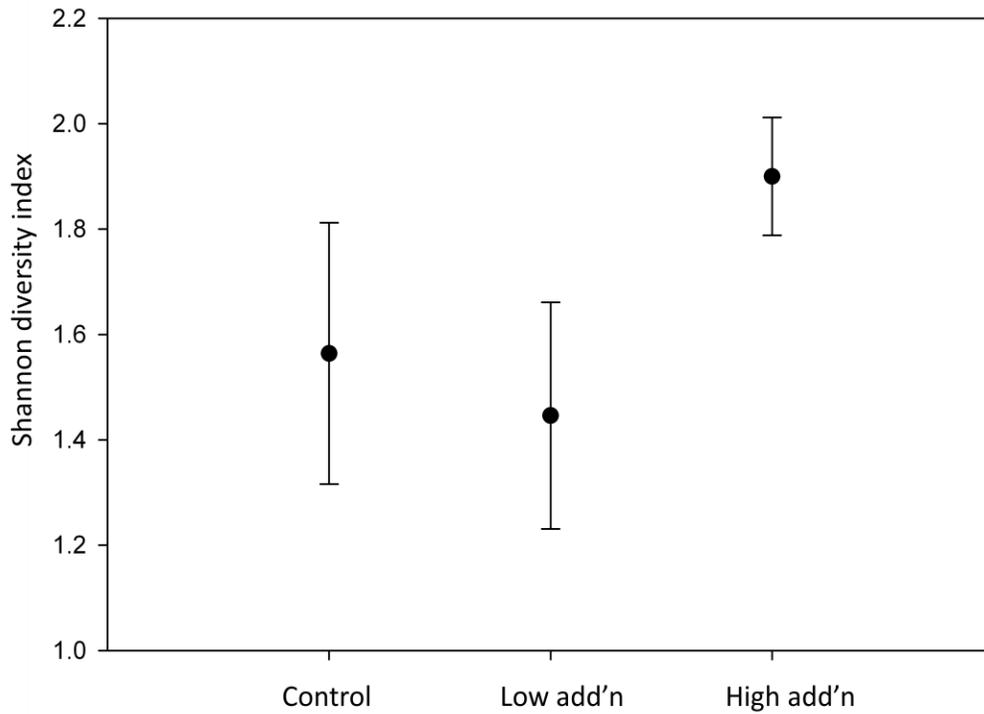
Figure 2.4: Some key genera showed differential response to treatment regimes. Shown are the average abundances and the associated standard deviation for ostracods, *Pleuroxus*, *Ceriodaphnia* and rotifers. Ostracods, *Ceriodaphnia* and rotifers had significantly higher abundances following high additions of waterfowl excreta.
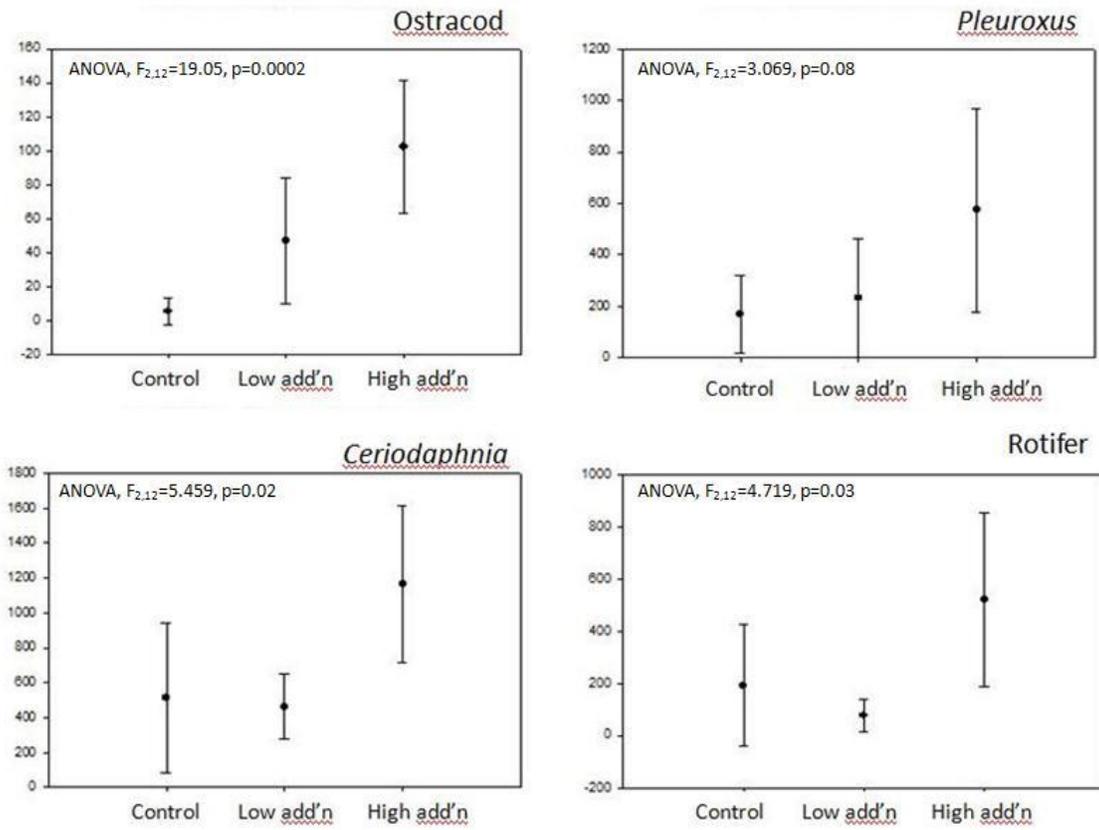
Figure 2.5: In the 2008 mesocosm experiment, total phosphorus concentrations were significantly higher in dissolved nutrient addition treatments compared with all other treatments (ANOVA, F=146.7, p<0.0001). Shown are the average concentrations of total phosphorus and the associated standard deviation for each.
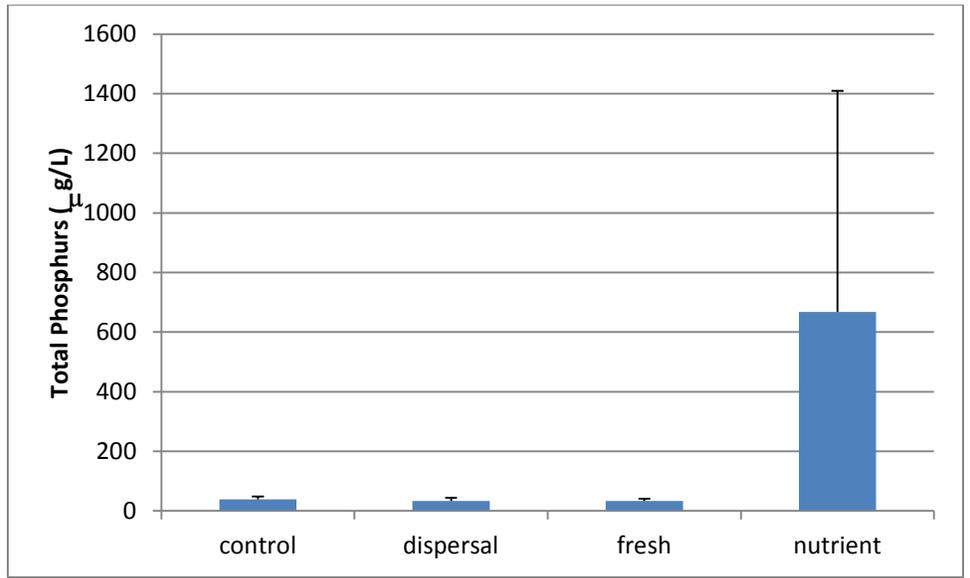
Figure 2.6: In the 2008 mesocosm experiment, total dissolved phosphorus concentrations were significantly higher in dissolved nutrient addition treatments compared with all other treatments (ANOVA, F=149.8, p<0.001). Shown are the average concentrations of total dissolved phosphorus and the associated standard deviation for each.
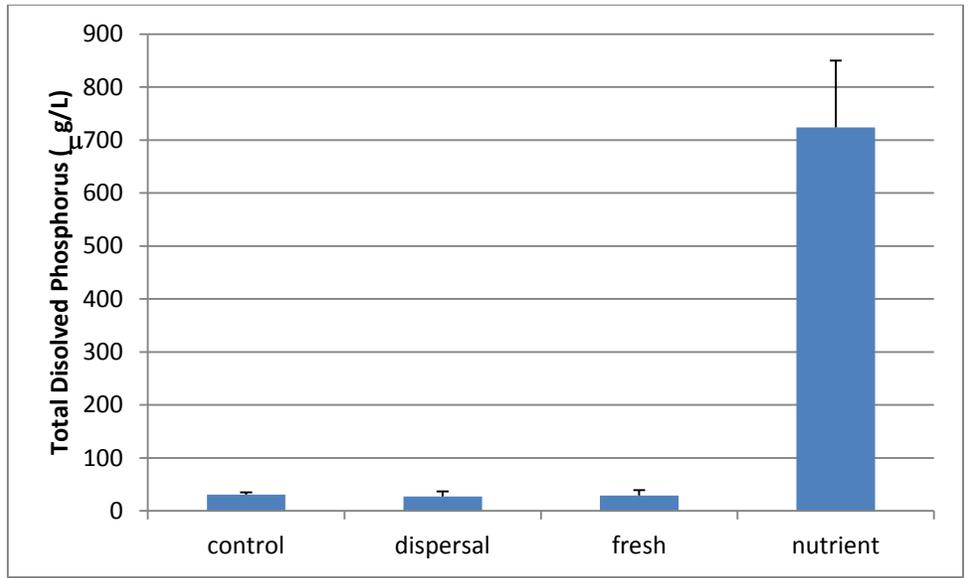
Figure 2.7: In the 2008 mesocosm experiment, Chao 1 diversity of zooplankton tended to be lower in dispersal treatments (ANOVA, $F_{3,16}=2.54$, $p=0.072$). Diversity estimates were calculated using EstimateS (Colwell 2009). Control, fresh excreta additions, and dissolved nutrient additions produced similar average zooplankton diversity. Shown are the average Chao 1 diversity indices and the associated standard deviation for each.
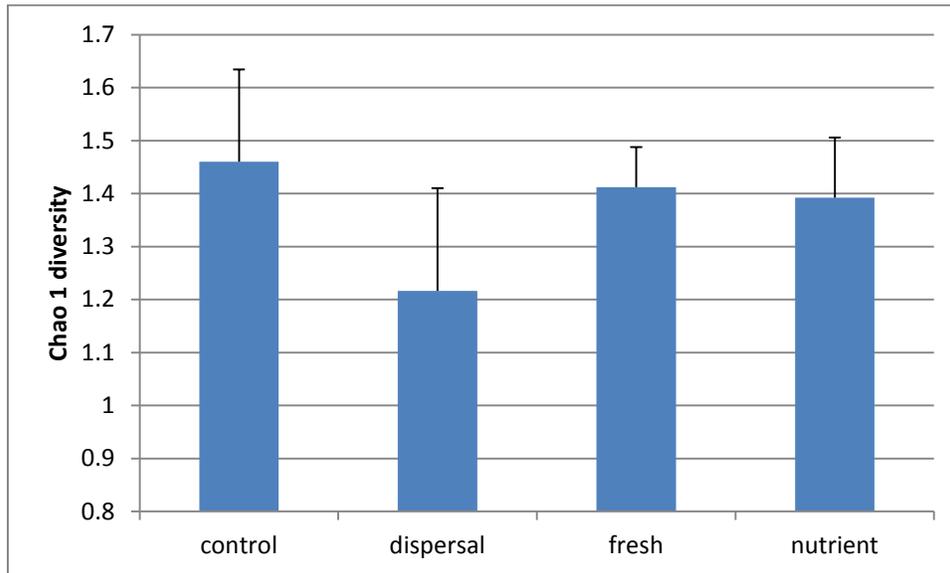
Figure 2.8: In the 2008 mesocosm experiment, log-transformed abundance of *Pleuroxus* cladocerans significantly differs among treatments (ANOVA, $F_{3,16}$=4.327, p=0.021). *Pleuroxus* abundance is reduced following additions of dissolved nutrients and immigrants. These observed patterns may be the result of *Pleuroxus* being a weak competitor in the zooplankton community. Shown are the average log-transformed abundances and the associated standard deviation for each.
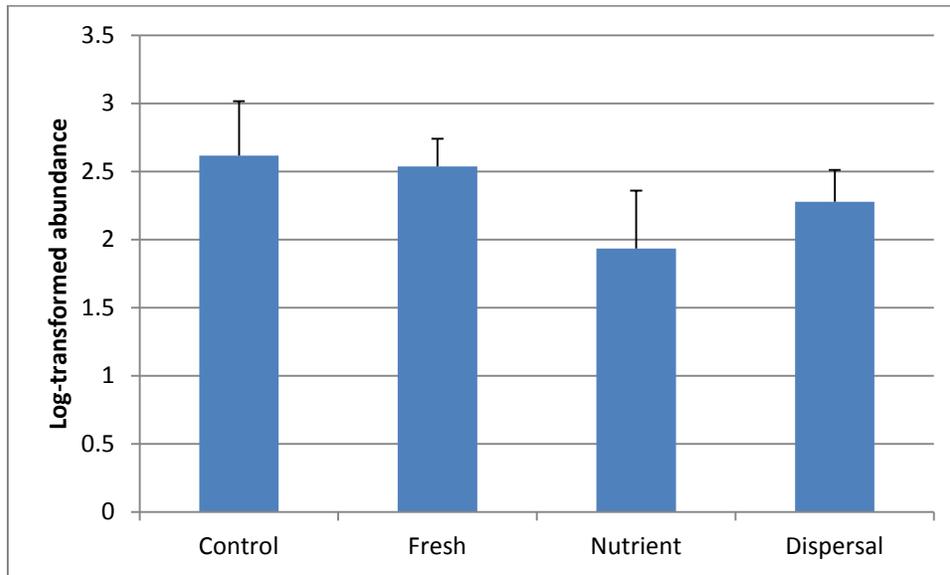
Figure 2.9: In the 2008 mesocosm experiment, log-transformed abundance of the cladoceran *Simocephalus* is significantly higher following additions of fresh excreta or additions of immigrants compared with controls (ANOVA, $F_{3,16}$=7.121, p=0.026). *Simocephalus* are large bodied cladocerans that, like *Daphnia*, are dominant grazers of algal primary producers. Their positive responses to treatment conditions may be indicative of their ability to exploit available resources, or indicative of the effects of competition within the zooplankton community. Shown are the average log-transformed abundances and the associated standard deviation for each.
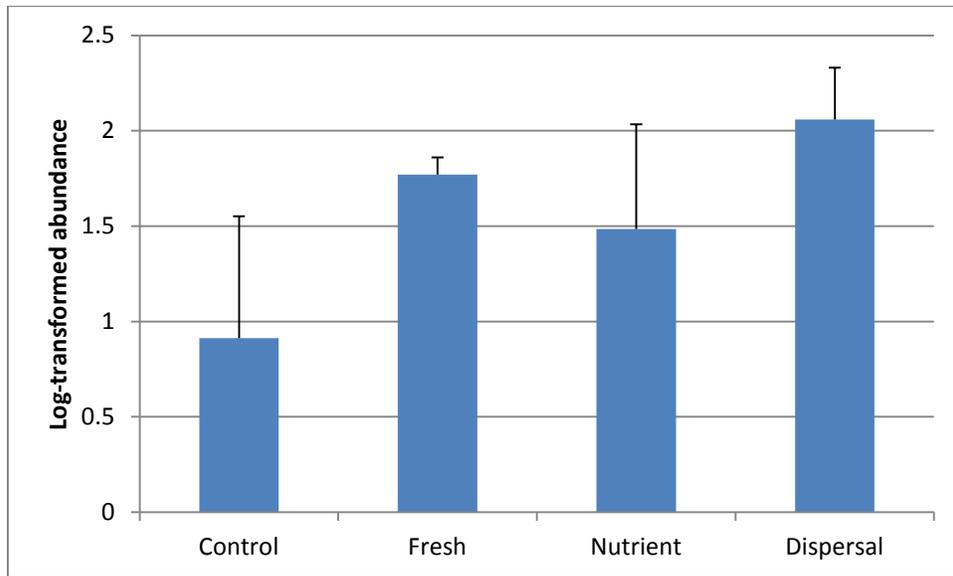
Figure 2.10: In the 2008 mesocosm experiment, log-transformed abundance of calanoid copepods abundance tended to be higher following dispersal of immigrants into mesocosms (ANOVA, $F_{3,16}=2.695$, $p=0.081$). There was high variation in the number of calanoid copepods present in each mesocosm replicate which contributed to the difficulty in identifying a clear pattern. Shown are the average log-transformed abundances and the associated standard deviation for each.
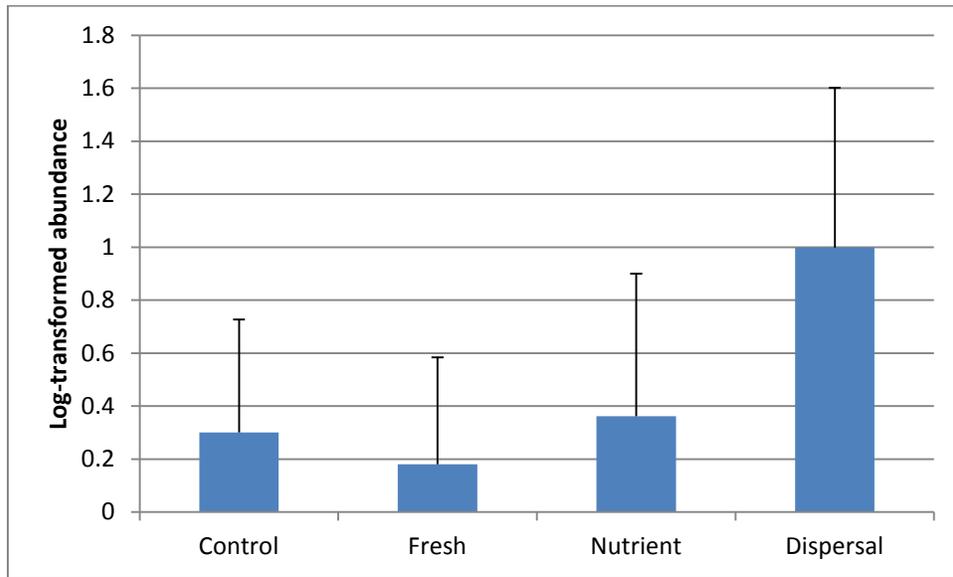
Figure 3.1: Final concentrations of total phosphorus were significantly higher in nutrient addition treatments (ANOVA, $F_{6,13}=101.7$, $p<0.0001$). Shown are the average concentrations of total phosphorus and the associated standard deviation for each.
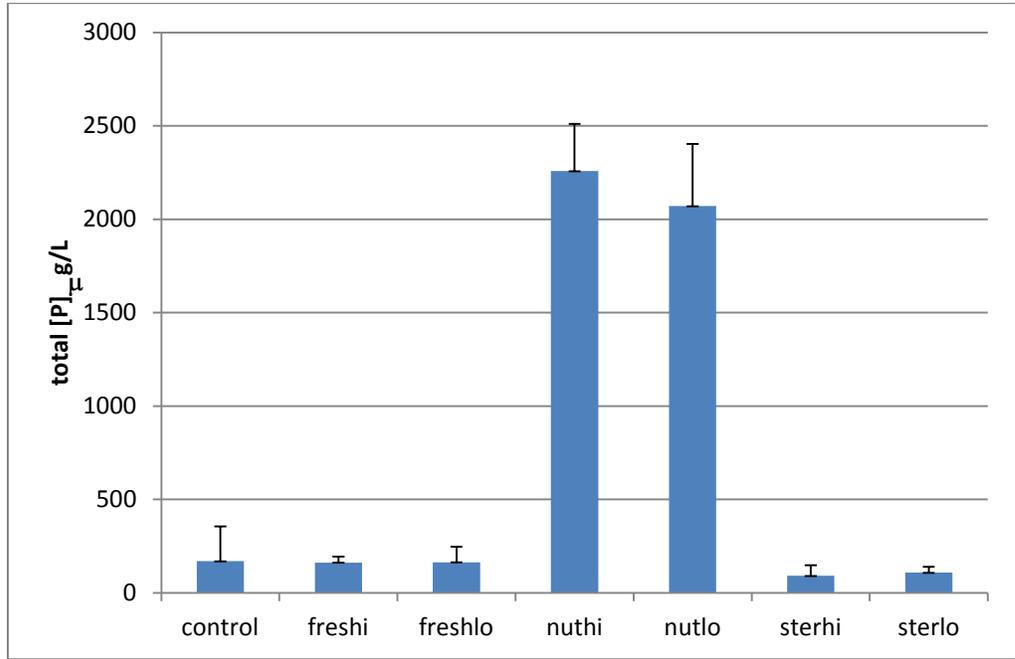
Figure 3.2: Soluble reactive phosphorus (SRP) concentrations showed a strong tendency to differ between treatments at the conclusion of the experiment (ANOVA, $F_{6,13}=2.516$, p=0.088). Sterile excreta additions showed depressed levels of SRP, below that of control treatments. Shown are the average concentrations of SRP and the associated standard deviation for each.
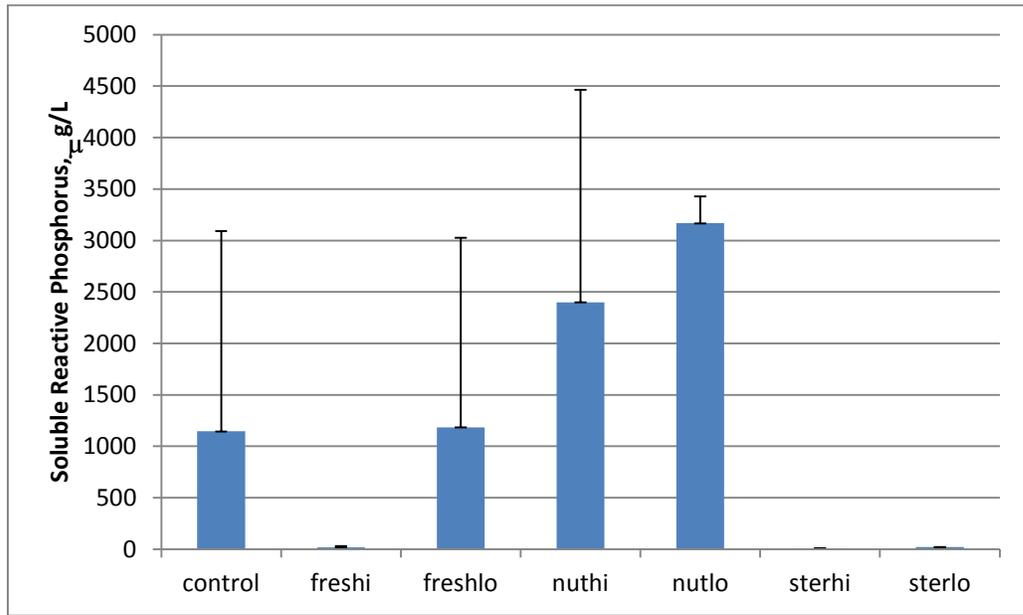
Figure 3.3: *Daphnia* abundance in treatments containing high amounts of excreta addition (both fresh and sterile) were significantly higher in the September sample ($F_{6,13}$=4.468, p=0.016). Shown are average *Daphnia* abundances and the associated standard deviation for each.
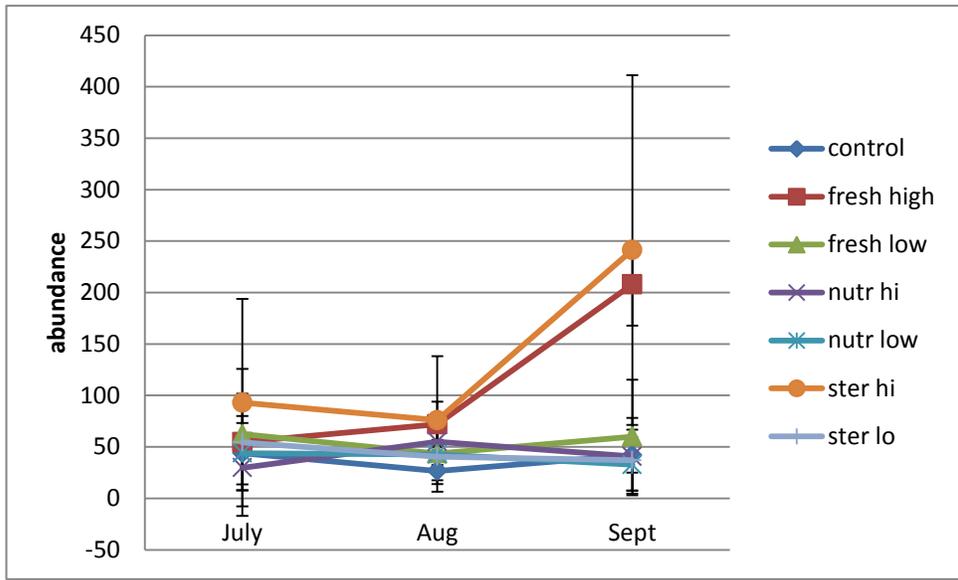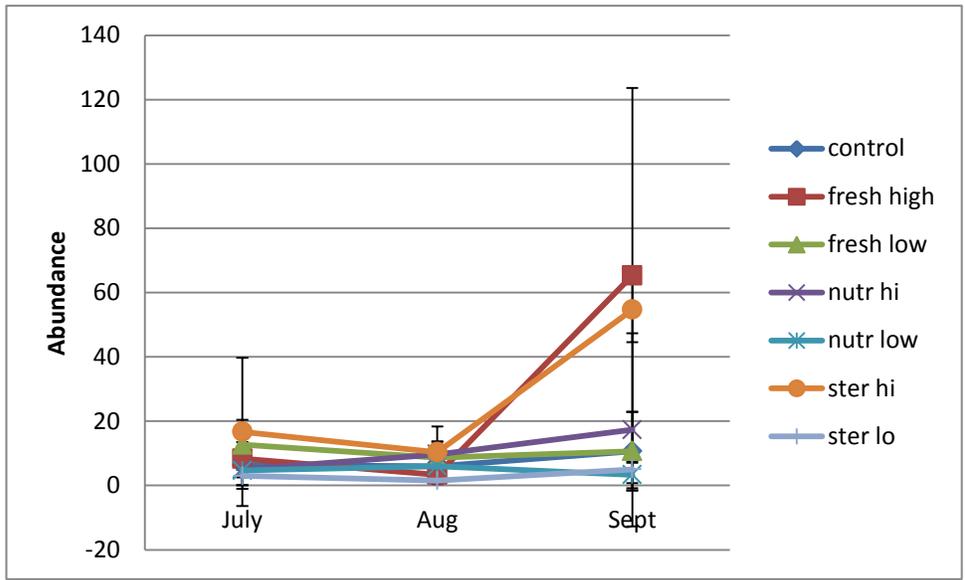
Figure 3.4: *Daphnia* log-transformed abundance in treatments containing high amounts of excreta addition (both fresh and sterile) were significantly higher in the September sample ($F_{6,13}$=3.73, p=0.029). Shown are average *Daphnia* abundances and the associated standard deviation for each.
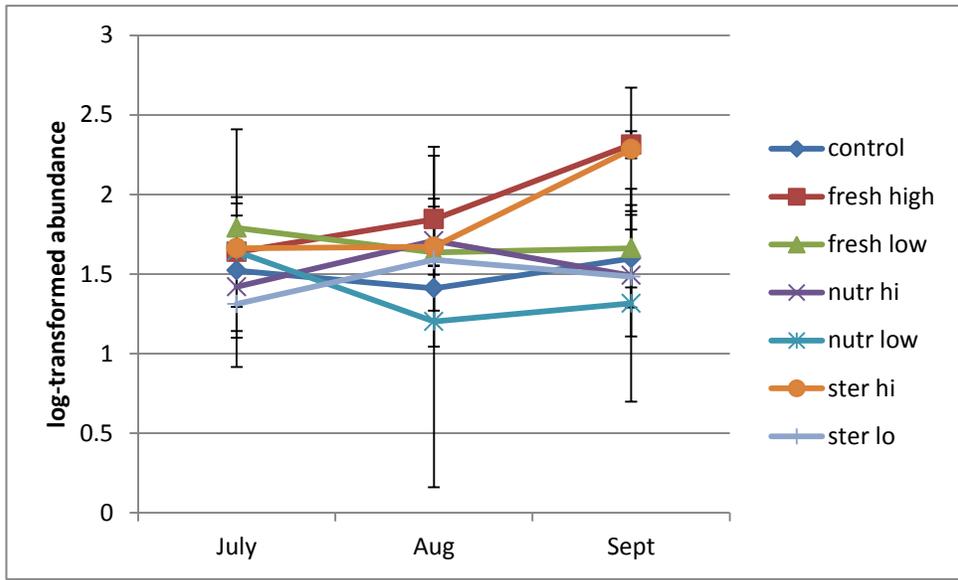
Figure 3.5: *Simocephalus* abundance in treatments containing high amounts of excreta addition (both fresh and sterile) tended to be higher in the September sample ($F_{6,13}$=2.628, p=0.08). Shown are average *Simocephalus* abundances and the associated standard deviation for each.

Figure 3.6: *Simocephalus* log-transformed abundance does not significantly differ between treatments in the final September sample. Shown are the average log-transformed *Simocephalus* abundance and the associated standard deviation for each.
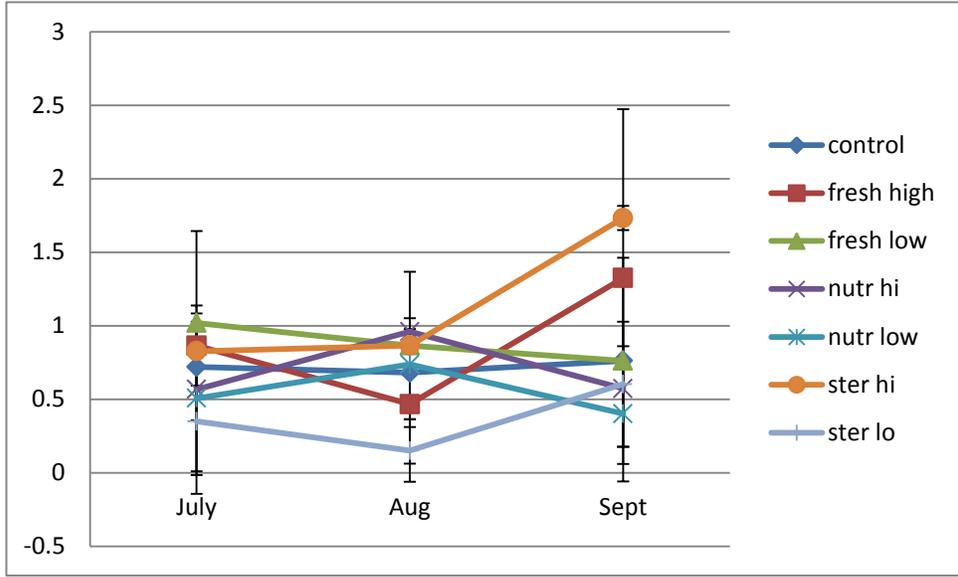
Figure 3.7: Cyclopoid copepod had a slight tendency to increase after high additions of fresh excreta (ANOVA, $F_{6,13}=2.147$, p=0.13). Shown are the average abundance of cyclopoid copepods and the associated standard deviation for each.
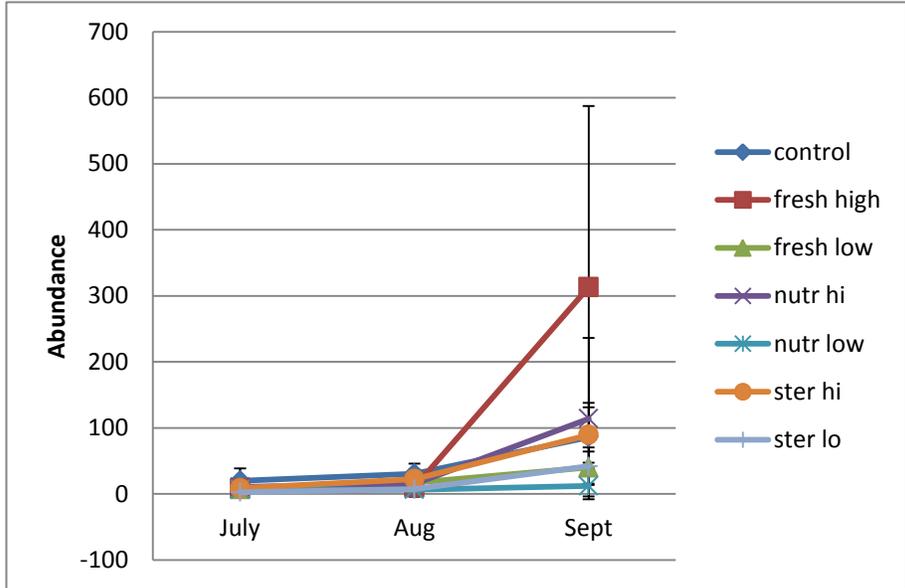
Figure 3.8: Zooplankton diversity was highly impacted by treatment additions as measured by the Shannon diversity index (ANOVA, $F_{6,13}=3.846$, $p=0.026$). Additions of low amounts of sterile excreta or low amounts of dissolved nutrients suppress diversity, though there is a remarkable amount of variation between mesocosm replicates. High additions of fresh excreta, sterile excreta or dissolved nutrients do not differ from patterns observed in control mesocosms. Zooplankton diversity was calculated using EstimateS (Colwell 2009). Shown are the average Shannon diversity indices and the associated standard deviation for each.
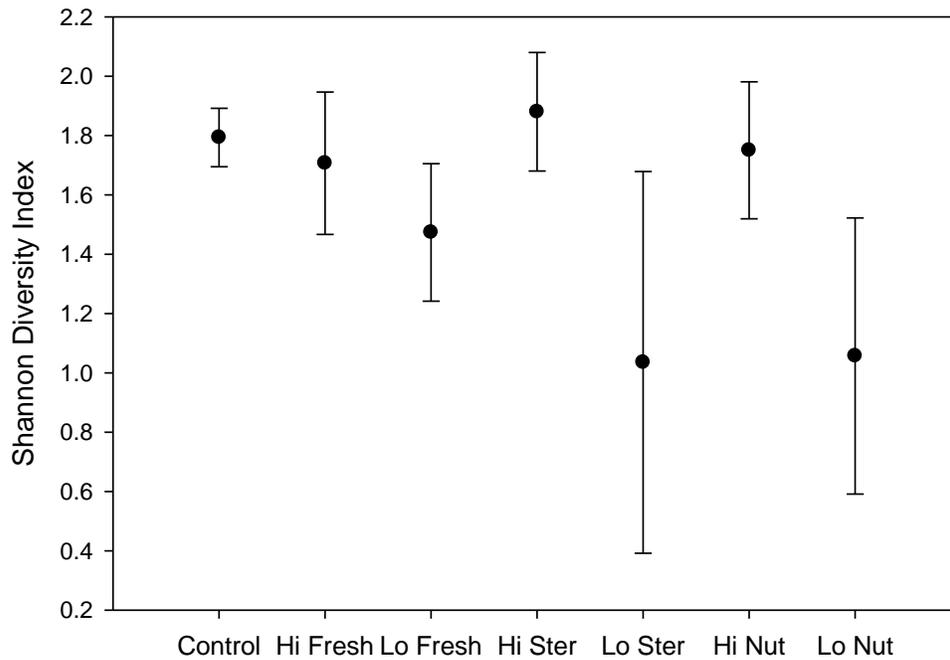
Figure 3.9: Principal components analysis of zooplankton from the final September sample. PC1 and PC2 are plotted on the x-axis and y-axis, respectively. Cyclopoid copepods and nauplii load negatively on both axes. Large-bodied cladocerans such as *Daphnia* and *Simocephalus* load negatively on PC1 and positively on PC2. Codes are as follows: *Alona*, *Allonella*, Cala=calanoid copepods, Cerio=*Ceriodaphnia*, *Chydorus*, Cyclo=cyclopoid copepods, Daph=*Daphnia*, *Moina*, nauplii, Ostr=ostracods, Pleur=*Pleuroxus*, Simo=*Simocephalus*, Scaph=*Scapholeberis*, rotifers.
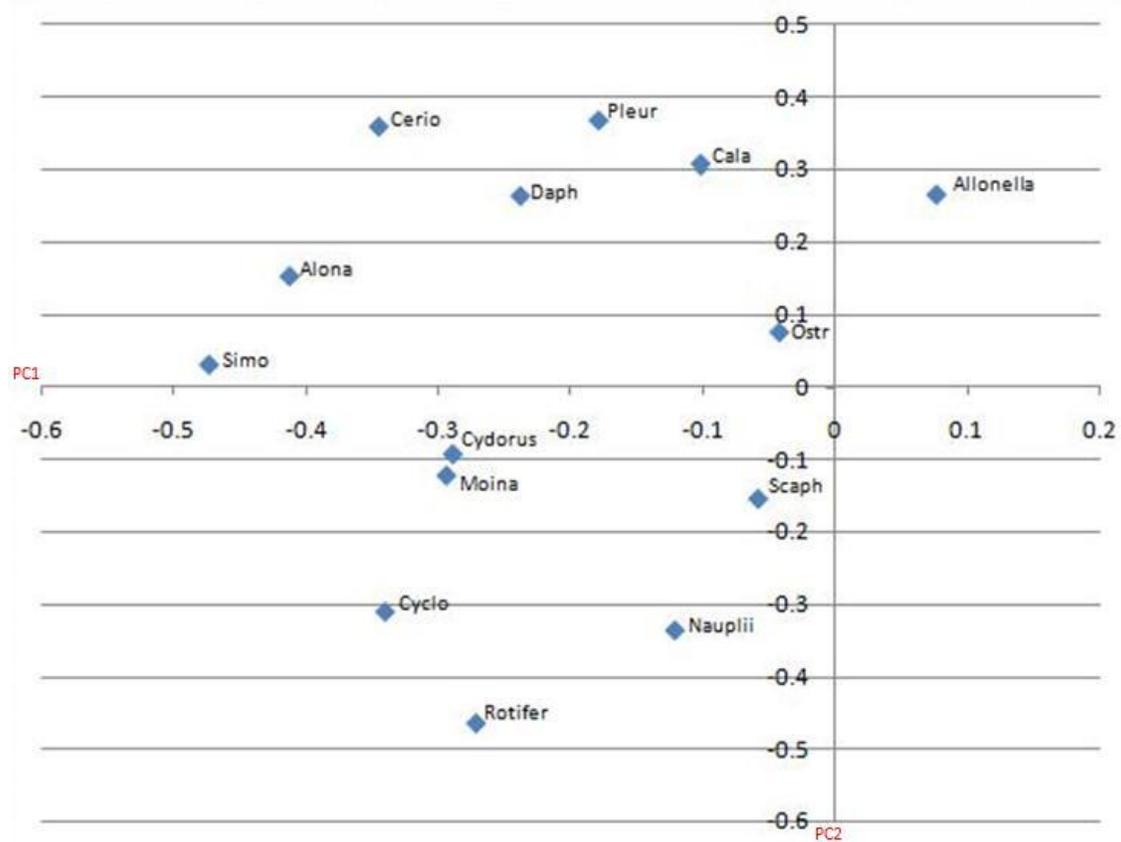
Figure 3.10: Principal components analysis showing the mesocosm treatments of each replicate. PC1 is shown in the x-axis. PC2 is shown in the y-axis. Control treatments group the most tightly and have the most similar zooplankton communities. Codes are as follows: Control, FrHi=high additions of fresh excreta, FrLo=low additions of fresh excreta, SterHi=high additions of alcohol-incubated excreta, SterLo=low additions of alcohol-incubated excreta, NutHi=high additions of dissolved nitrogen and phosphorus, NutLo=low additions of dissolved nitrogen and phosphorus.
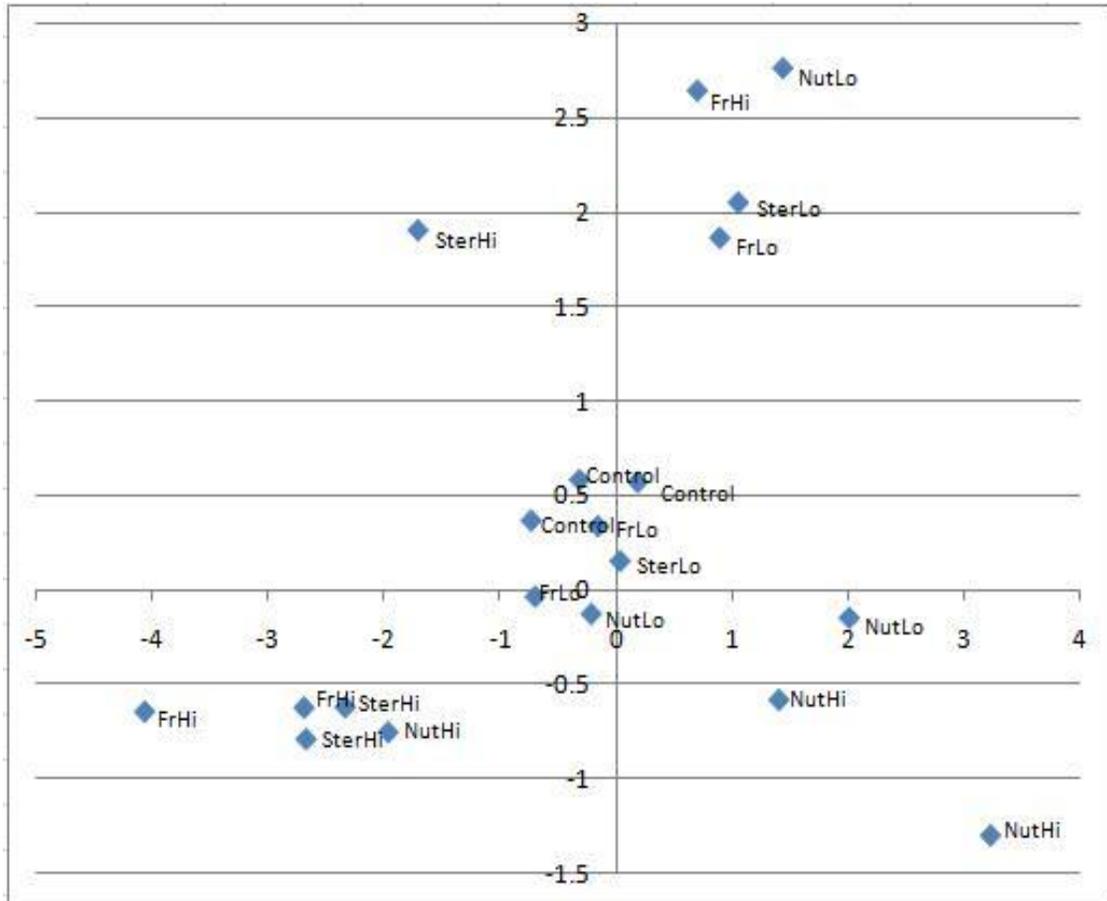
Figure 3.11: Principal components analysis showing the mesocosm treatments of each replicate. PC1 is shown in the x-axis. PC2 is shown in the y-axis. There is a tendency for similar treatments to cluster (ANCOVA, p=0.08). High additions of fresh and sterile excreta produce somewhat similar zooplankton communities. Control treatments group the most tightly and have the most similar zooplankton communities. Communities which are the result of high additions of dissolved nutrients may sometimes appear different than any other treatment. Average PC scores for treatments and their associated standard deviations are plotted here. Codes are as follows: Control, FrHi=high additions of fresh excreta, FrLo=low additions of fresh excreta, SterHi=high additions of alcohol-incubated excreta, SterLo=low additions of alcohol-incubated excreta, NutHi=high additions of dissolved nitrogen and phosphorus, NutLo=low additions of dissolved nitrogen and phosphorus.
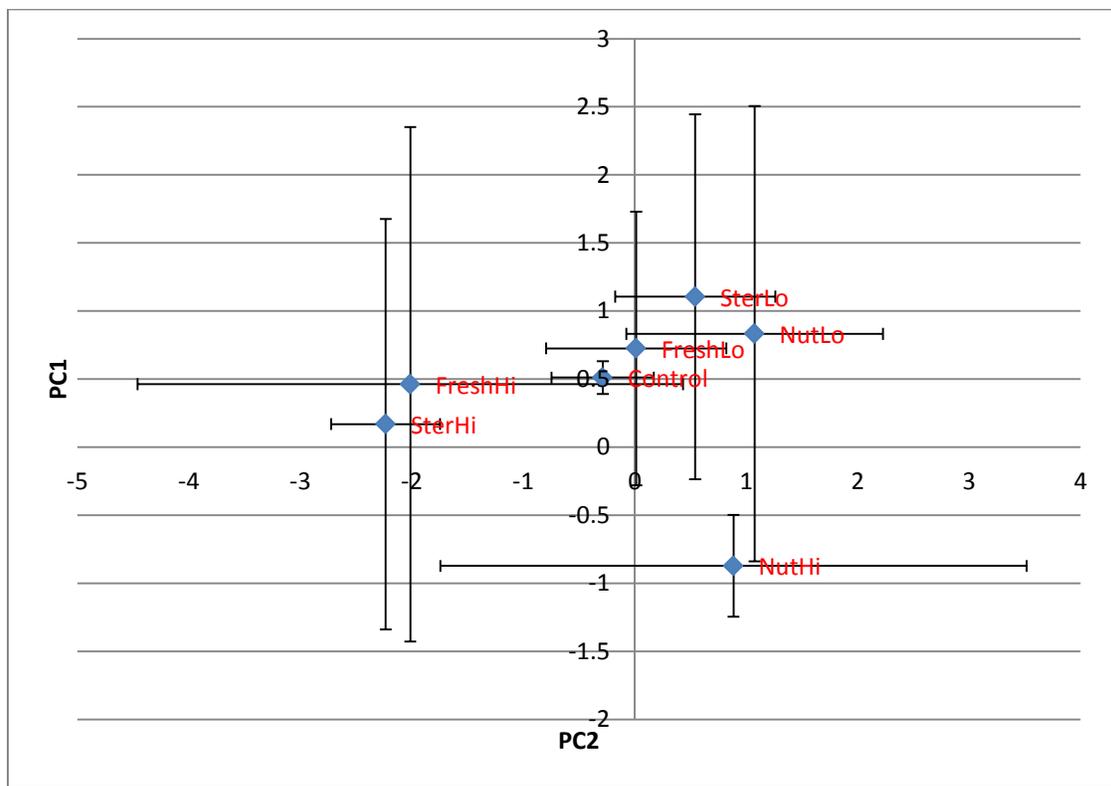
Figure 3.12: When nauplii are removed, principal component anlaysis of the zooplankton community results differ. Shown are the treatments in relation to PC1 (x-axis) and PC2 (y-axis) following removal of nauplii from the analysis. Less grouping of treatments is apparent. Codes are as follows: Control, FrHi=high additions of fresh excreta, FrLo=low additions of fresh excreta, SterHi=high additions of alcohol-incubated excreta, SterLo=low additions of alcohol-incubated excreta, NutHi=high additions of dissolved nitrogen and phosphorus, NutLo=low additions of dissolved nitrogen and phosphorus.
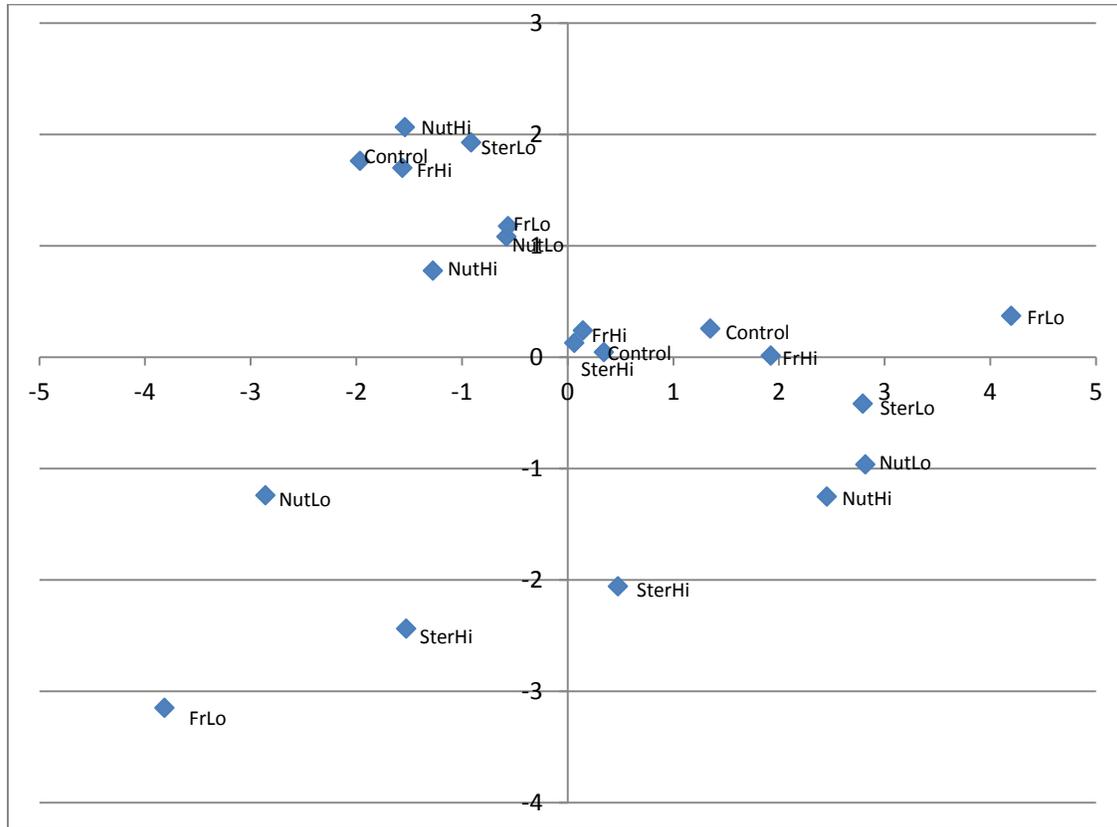
Figure 3.13: Principal components analysis showing the mesocosm treatments following exclusion of nauplii from the analysis. PC1 is shown in the x-axis. PC2 is shown in the y-axis. Average PC scores for treatments and their associated standard deviations are plotted here. When nauplii are removed from the analysis, zooplankton communities appear much more similar across treatments. Codes are as follows: Control, FrHi=high additions of fresh excreta, FrLo=low additions of fresh excreta, SterHi=high additions of alcohol-incubated excreta, SterLo=low additions of alcohol-incubated excreta, NutHi=high additions of dissolved nitrogen and phosphorus, NutLo=low additions of dissolved nitrogen and phosphorus.
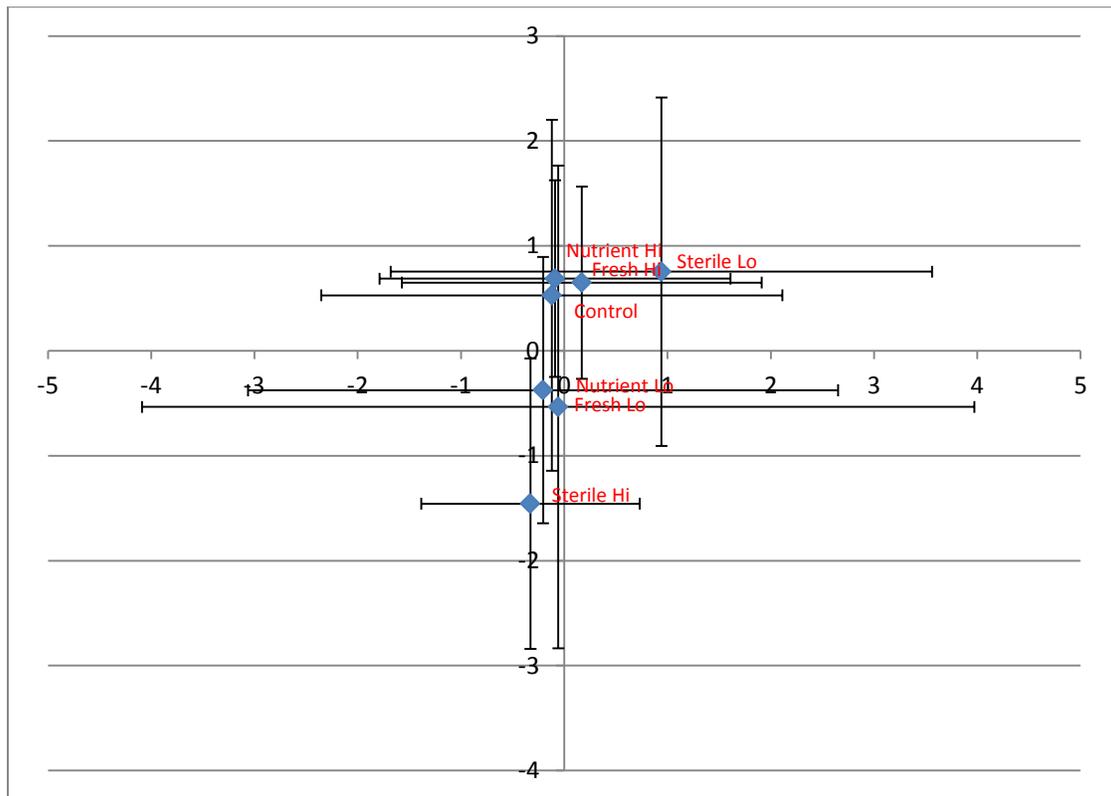
Figure 3.14: Principal components analysis of zooplankton from the final September sample. PC1 and PC2 are plotted on the x-axis and y-axis, respectively. Zooplankton may cluster into two groups in this anlaysis, though the relationship is weak.
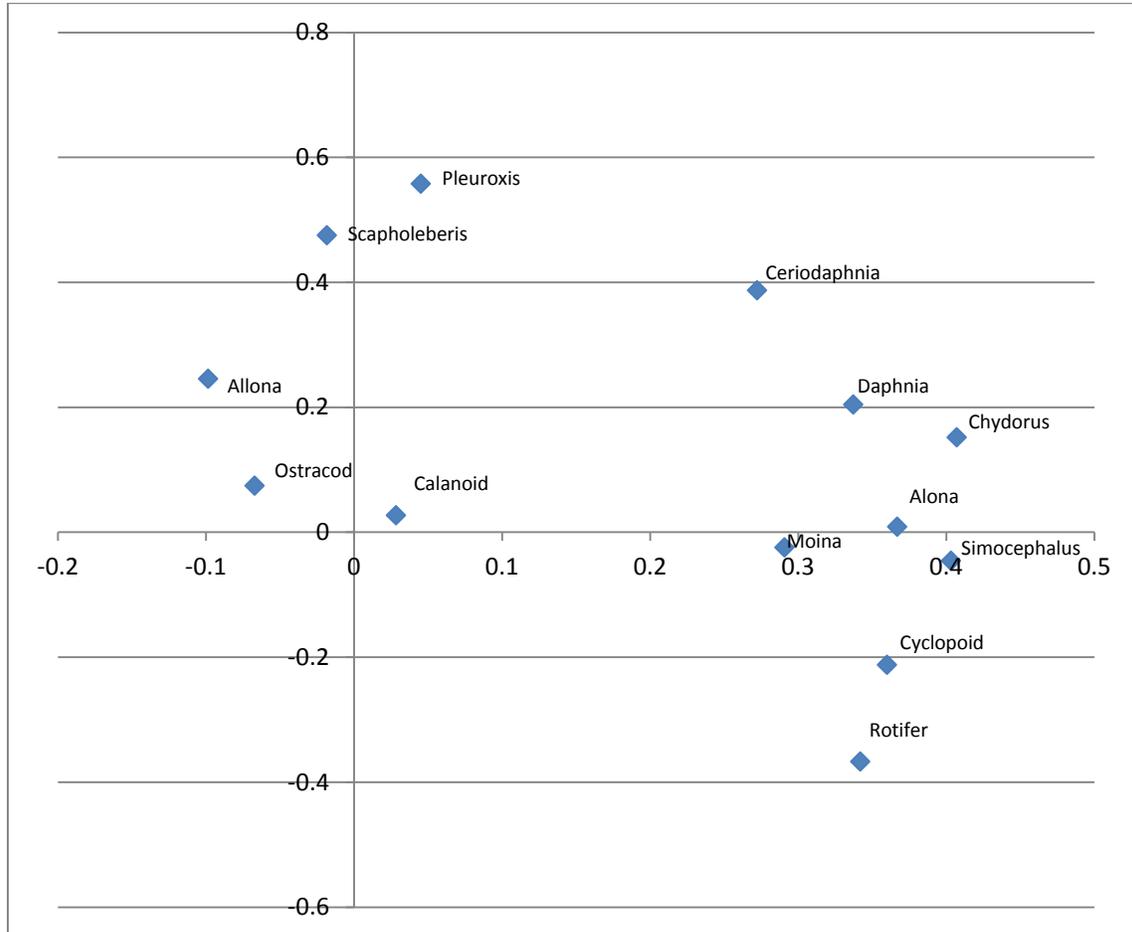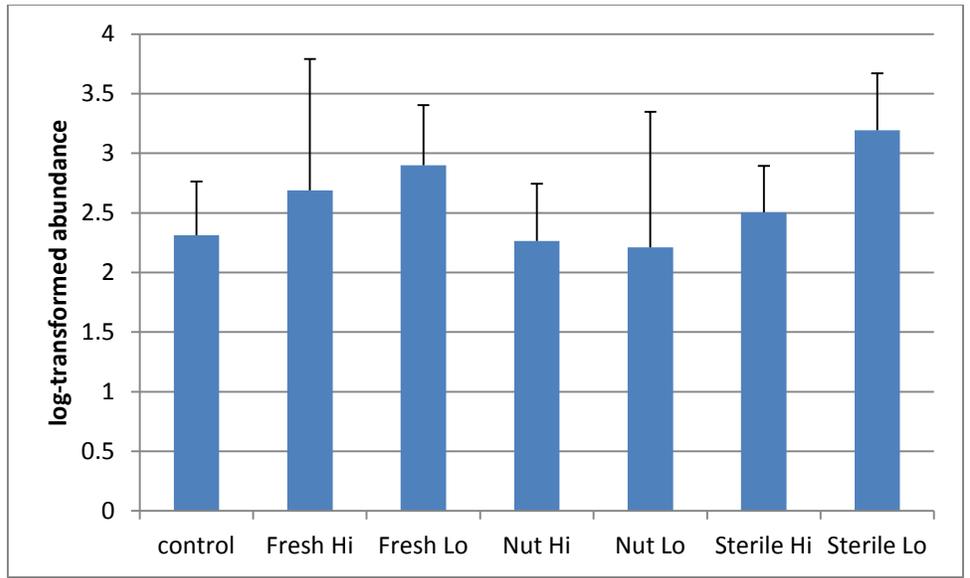
Figure 3.15: Nauplii abundance does not differ significantly between treatment groups though nauplii presence may strongly influence overall zooplankton community composition (ANOVA $F_{6,13}=0.3946$, $p=0.84$). Shown are the average abundances of nauplii in each treatment and the associated standard deviation for each.

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Vita

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