FRESHWATER ZOOPLANKTON COMMUNITIES AS INDICATORS OF HABITAT QUALITY:

TESTING RESPONSES TO MULTIPLE DISTURBANCES

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Abstract

Zooplankton can be effective bioindicators of water quality because they are common to most aquatic ecosystems, can be rapidly sampled, and different zooplankton taxa respond differentially to environmental change. During the summers of 2014 and 2015, 106 samples were collected from 53 ponds in the San Francisco Bay Area to evaluate how zooplankton communities respond to disturbances in their environments, such as eutrophication and presence of non-native fishes. We found that zooplankton communities were sensitive to greater nutrient concentrations, such as phosphorus, which had a negative effect on zooplankton species richness and the density of certain zooplankton taxa, notably, cladocerans such as *Simeocephalus vetulus* and *S. serrulatus.* The presence of non-native fish had a strong, negative effect on zooplankton density and average body size, but did not affect species richness. We found that ponds with a greater degree of turbidity had lower cladoceran species densities, but had slightly higher densities of copepod taxa. Overall, these results suggest that common forms of environmental alteration, such as invasive species and nutrient runoff, have differential impacts on zooplankton species riches, average body size, and species density, highlighting their potential value as bioindicators within pond ecosystems.

Introduction

Considering the importance of access to fresh water in supporting most forms of life, freshwater ecosystems are highly vulnerable to climate shifts, non-native species introductions, and land use changes (Dudgeon et al. 2006, Woodward et al. 2010). While freshwater habitats make up only 0.1% of all of the planet's water resources relative to marine systems and ice, they support almost 6% of all described species (Gleick 1993, Gleick & Palaniappan 2010, Collen et al. 2014). Freshwater ecosystems are especially susceptible to the negative consequences of global environmental change drivers (e.g., climate change, elevated nutrient deposition, and land cover/land use change), because of their tendency to be fragmented in space, situated in geographical low points, and their association with human settlements (Dudgeon et al. 2006, Woodward et al. 2010). Conservative estimates project that the average global air temperature will increase by 1.5°C or more by the end of this century (Pachauri et al. 2015), subsequently increasing water temperatures and evaporation rates in most aquatic ecosystems and, therefore, altering the characteristics of aquatic habitats (Thomas et al. 2004, Parmesan 2006).

One of the greatest threats to freshwater ecosystems is the degradation of habitat quality through inputs of excess nutrients (Schindler 1974, Smith et al. 1999, Tilman et al. 2001). Much of the pollution within aquatic ecosystems occurs indirectly and occurs from external sources as nitrogen and phosphorus used in agriculture contaminate aquatic environments through surface run off or atmospheric deposition, thereby increasing the risk of eutrophication (Tilman et al. 2001). Eutrophication is defined as the process by which bodies of water are made nutrient rich (Smith et al. 1999). In extreme cases, eutrophic waters are degraded because of excess plant and algae growth, which decrease the amount of dissolved oxygen (Schindler 1974). This decrease is linked to a loss of competent species, such as large plankton or fish, and the ensuing loss of ecosystem services (Moss, 1990, Smith et al. 1999). It is likely that rates of eutrophication will increase as a consequence of the resource demands made by the addition of an estimated 2 billion people to the global population in the next 30 years (McKinney et al. 2006).

Presently, landscapes are shaped by economic activities and will be used in ways that maximize their profitability when used for urban or agricultural development (De Marco et al. 2014). Changes to existing environments can facilitate colonization from non-native species (Johnson et al. 2013). Relative to grasslands and agricultural areas, wetlands in urban areas have 60% lower richness of amphibians and aquatic reptiles and 33% lower richness of aquatic insects, mollusks and crayfish (Johnson et al. 2013). Biodiversity has been shown to improve water quality through niche partitioning, as communities with more species take greater advantage of niche opportunities and capture a greater proportion of biologically available resources, like nitrogen or phosphorus (Cardinale 2011). Previous research has shown that non-native species can negatively influence native species through predation and competition (Kupferberg et al.1997, Ricciardi & Rasmussen 1998). Determining the implications of non-native species is challenging, especially when these invaders occur simultaneously with other types of environmental change. For example, Preston et al. (2012) recently showed that invasive species can have both positive and negative effects on zooplankton communities within pond ecosystems. Specifically, they documented that the presence of mosquito fish, *Gambusia affinis,* a planktivorous fish that hunts prey visually, nearly eliminates daphnia populations and thus triples the density of phytoplankton communities, creating reduced water clarity, but also allowing surviving zooplankton refuge from predation. (Preston et al. 2012). Overall, securing the stability of freshwater ecosystems is important to future ecological conservation efforts.

Background

I. Significance of studying ponds

Ponds are ideal study systems to investigate how freshwater systems may be affected by environmental change or disturbance because they are relatively common geographically, provide valuable ecosystems services, and act as refuge for many species (De Meester et al. 2005, Céréghino et al. 2014). Ponds here are defined as small (1 to 5 ha), shallow water bodies (less than 7 meters deep) (De Meester et al. 2005) and represent 90% of the global 304 million standing water bodies, or 30% of standing water by surface area worldwide (Downing et al. 2006, Céréghino et al. 2014). Ponds are widely distributed across continents and have global significance as areas of habitat and in material processing (e.g., carbon (C), nitrogen (N), water, sediment, nutrients) (Downing et al. 2006, Woodward et al. 2010). In contrast to larger water bodies, such as lakes, ponds show greater biotic and abiotic variation and consequently provide a unique opportunity to study how specific characteristics of aquatic ecosystems, such as size, level and type of disturbance, and relative proximity to other aquatic systems, affect biotic communities (Dudgeon et al. 2006, Céréghino et al. 2008, Howeth et al. 2010). Ponds can also be biodiversity hotspots in terms of both species composition and biological traits (Woodward et al. 2010, Céréghino et al. 2014). For example, a study of farm ponds in France discovered that 37 ponds captured 40% of the region's species pool of Odonata (dragonflies), including both 7 rare and 26 common species (Ruggiero et al.

2008). Many freshwater ecosystems are experiencing rapid declines in biodiversity, but compared to other surface waters, ponds receive very little legal protection (Dudgeon et al. 2006, Céréghino et al. 2014). In areas where natural wetlands are becoming scarce, constructed agricultural ponds could be important alternative habitats for native taxa (Knutson et al. 2004, De Marco et al. 2014).

II. The potential of zooplankton as bioindicators

Historically, researchers have sought to link certain species or functional groups of species with specific environmental characteristics in order to gauge the effects of certain environmental dynamics to perturbations (Parmesan et al. 2006, Primo et al. 2015). These species or groups are termed bioindicators (Parmesan et al. 2006, Primo et al. 2015). Bioindicators here are defined as species whose presence or abundance reliably indicates a particular suite of environmental conditions (Wilson et al. 1994). One important group of bioindicators in aquatic ecosystems are zooplankton. Zooplankton are microscopic crustaceans that are crucial components of aquatic food webs; as primary consumers, they respond strongly to environmental change. Thus, they can to be used to assess the conditions in aquatic ecosystems (Ricciardi et al. 1998, Hanazato et al. 2001, Brito et al. 2011, Primo et al. 2015). They represent an important food source for small fish and predatory invertebrate diets (90% calories) (Hairston et al. 1993). Zooplankton communities often include cladocerans, which are distinguishable by their rounded body shape and non-segmented thorax, as well as copepods, which have elongated body thoraxes with segmented rear appendages (Berner & Horning 1986). The taxa of zooplankton present in aquatic ecosystems can have large impacts on the trophic transfer efficiencies from phytoplankton to zooplankton, and from zooplankton to fish (Pace et al.

1981, Hairston et al. 1993). Previous research has found strong evidence that zooplankton are capable of controlling the transfer of energy through trophic levels by exerting a top down control on phytoplankton, limiting the amount of primary production with in a body of water (Pace et al. 1981, Hairston et al. 1993, Hairston et al. 1996, Jeppesen et al. 1997). Changes in zooplankton community composition can affect the degree of top down control on phytoplankton communities, influence the amount of nutrient processing, and determine the capacity of aquatic ecosystems to uptake carbon dioxide (Brooks et al. 1965, Brucet et al. 2010, Adamczyk et al. 2015). Zooplankton communities often vary in composition as certain species are highly sensitive to changes in nutrient cycling, temperature, and variable environmental conditions (Hanazato et al. 2001, Vadadi-Fülöp et al. 2012, Primo et al. 2015). Previous research has demonstrated that zooplankton richness decreases in systems with increasing amounts of phosphorus, a nutrient commonly associated with eutrophic processes, and that certain species of cladocerans are especially sensitive to increased phosphorus (Jeppesen et al. 2011). Due to their trophic significance, as well as their unique responses to certain environmental dynamics, zooplankton are effective bioindicators that can be used to measure the impact of disturbance in aquatic ecosystems.

III. Focal organisms

The zooplankton communities of freshwater ponds of the San Francisco Bay Area of California, including Contra Costa, Alameda, Santa Clara and San Mateo counties, include a variety of cladocerans, copepods, and other invertebrate taxa. Differences in the post-abdominal claw, along with other morphological traits, are used to identify a specimen (Berner et al. 1986). One of the most common species is *Ceriodaphnia reticula,* a small-bodied cladoceran known for its short generation times (Berner et al. 1986). Other common larger-bodied cladocerans include *Daphnia mendotae*, known for their ability to survive eutrophic conditions (Hanley et al. 2016), *Simocephalus vetulus,* and *Simocephalus serrulatus.* The common copepods are from the two orders Cyclopoida*,* capable of eating other smaller zooplankton species (Fryer et al. 1957), and Calinoida*,* a predominantly herbivorous species (Kleppel et al 1993). Most of these species are vulnerable to changes in pond water chemistry, amount of water, and interspecies interactions (Hanazato et al. 2001, Brucet et al. 2010, Vadadi-Fülöp et al. 2012).

IV. Current study and objectives

My research addresses how zooplankton communities respond to changing environmental conditions by assessing the following questions: How are zooplankton in aquatic ecosystems affected by disturbances, such as nutrient runoff and nonnative fish species? And which subsets of zooplankton populations and communities (average size, species richness, abundance, or fecundity) are most affected by these disturbances (Fig. 1)? Using data collected across a gradient of pond sizes and land use types with varying hydroperiod and permanence, we tested the individual and combined effects of introduced fishes and nutrient concentrations (nitrogen and phosphorus) on zooplankton in freshwater ponds. Specifically, we analyzed the effects of nutrient concentrations (nitrogen and phosphorus) and the presence of introduced fishes on zooplankton species' richness, abundance, average size, and fecundity from 53 freshwater ponds over 2 years.

We hypothesized that disturbances in the form of nutrient runoff will have multiple effects on zooplankton. Because previous literature has identified phosphorus as a driver in the process of eutrophication and promotes the growth of large phytoplankton species, we hypothesize that ponds with higher concentration of total dissolved phosphorus (TDP) will have smaller densities of *Simocephalus vetulus* and *Simocephalus serrulatus,* as well as lower species richness, as *Simocephalus* sp. and other cladocerans are unable to consume large food particles, like cyanobacteria (Schindler 1974, Jeppesen et al. 1997). We hypothesized that total dissolved nitrogen (TDN) may have a positive effect on both average individual zooplankton size, as well as zooplankton density, as larger quantities of TDN may facilitate the growth of phytoplankton species that are more palatable to zooplankton (Elser et al. 2000). We also hypothesized that disturbances in the form of introduced fishes will have negative effects on zooplankton average individual size and density due to the predation pressure from fish, excess nutrients from cattle excrement, and loss of shoreline structure from the trampling actions of livestock. Certain environmental aspects, such as habitat size and habitat connectedness, could also affect zooplankton, therefore, we hypothesized that wetland area within 1 kilometer of site location could have a positive effect on zooplankton species richness as it could help connect communities of zooplankton and that pond size could have a positive effect on zooplankton species richness and zooplankton density.

Methods and Materials

I. Field surveys for environmental conditions

During the summers of 2014 and 2015, we sampled 53 freshwater ponds distributed along a 130 km stretch of the South San Francisco California Bay Area, including parts of Contra Costa, Alameda, Santa Clara and San Mateo counties (Fig. 2). All ponds were located in similar drainage networks and of comparable depth and surface area to assess zooplankton communities. Each pond was sampled twice per summer: once in May-June to collect zooplankton samples and then again in July-August to gauge the change in pond size. Many of these ponds were artificially constructed to support livestock grazing but still provide essential habitat for amphibians, invertebrates, birds, and terrestrial mammals (Joseph et al. 2016). Consistent with the methods outlined in Joseph et al., we used Google Earth imagery to assess the consistent presence of water year round and confirm pond permanence (Joseph et al. 2016). Using a YSI MDS 550 probe held 30 cm below the surface, we measured temperature, pH, and salinity. We measured water clarity using LaMotte 2020i turbidity meter and pond perimeter was calculated using the tracking function on a handheld GPS unit (Garmin GPSMAP 60) as the researcher walked the shoreline of the pond. From 2012 to 2014, California experienced drought considered by some to be the single most arid case in the last 1200 years (Marty et al. 2005, Griffin et al. 2014) and evaporation rates were calculated as the proportional difference in pond area between the two different sampling times within each year ([Time 1– Time 0] / Time 0). The field team collaborated to estimate the percentage of vegetated shoreline around each pond and identified the relative species' portions of the vegetated shoreline between the three most common species, *Juncus* sp., *Typha* sp., and *Schoenoplectus* sp.

In each pond, if present, fish were identified to the taxonomic level of species and measured as being absent or present based off of visual observations from all other sampling procedures, such as habitat-stratified dipnet sweeps, hook and line, and 3 to 5 habitat-stratified seine hauls. We estimated the extent of cattle impact by recording the number of cow paddies within 3 meters of shoreline and recorded the perimeter of the shoreline to quantify cow paddy density at each pond. We observed whether cow were present or absent. Finally, water samples were collected in washed high-density polyethylene (HDPE) Nalgene bottles from the pond's surface, filtered through Whatman 47 mm GF/F filters (0.7 µm pore size) into new acid washed Nalgene bottles, and frozen before processing for both TDN or TDP by Arikaree Laboratory using standard methods (https://instaar.colorado.edu/research/labs-groups/arikaree-environmental-lab/free-play).

II. Zooplankton analysis

Zooplankton samples were collected using an 80 µm Wisconsin net (1.35 m long) towed horizontally through the water during the initial visit from May to June. Samples of captured zooplankton were preserved in 250 mL of 80% ethanol solution and analyzed using an Olympus SZX10 stereo dissection microscope. To provide a homogenized sample, we inverted each sample and its contents. Immediately following the inversion, we used a Hensen Stempel pipette to measure 10 mL of the homogenized sample into a gridded, plastic Petri dish for analysis. We moved through every dish examining the contents in each section, starting at the top left corner (A1) then continuing across the row to (A6), and then moving down the column to the row below (B1) until finally examining the entire tray (A1-F6). To calculate average individual size, we measured the length of the carapace, or the chitinous body structure, of the first 50 adult zooplankton of every species. Adult zooplankton were differentiated from the larval zooplankton, based on size, as larval cladocerans, neonates, look similar to adults but are 10% or less of the average adult size (Nandini & Sarma 2006). We determined zooplankton species richness and abundance at each pond by counting and identifying all zooplankton within the first 50 mL of every sample. Zooplankton were identified to the species level for all large-bodied cladocerans, using a compound microscope Olympus BX51 as well as an online taxonomic key (Haney et al. 2016). All other organisms, copepods, ostracods, rotifers, mites, and other insects were identified to family or lowest taxonomic level possible (Ryther et al. 1980, Pace et al. 1981, Berner et al. 1986, Dodson 1989, Havel et at. 2004, Haney et al. 2016).

III. Statistical analysis

I used general linear models, generalized linear models, and generalized linear mixed models to analyze the effect of TDP, TND, water clarity, cow presence, fish presence, wetland area, and pond size, in our research sites over time in relation to the measured qualities of zooplankton communities. The measurements for zooplankton abundance were scaled to account for the amount of zooplankton in a liter of pond water. Fecundity was estimated as a proportion of gravid individuals in each sample relative to the total amount of zooplankton in the same sample. Nitrogen to phosphorus (N:P) ratios were calculated by finding the molar ratio of TDN to TDP in each pond. Highly skewed continuous variables were transformed to help normalize distributions. Average individual zooplankton size data as well as abundance data were log10 + 1 transformed for analysis. Fecundity estimates were highly skewed proportions and transformed by taking the arcsine of the square root. Pond area, cow paddy counts, TDN, TDP, salinity, turbidity and pH were also log10-transformed. Wetland area within 1 kilometer of site location was transformed by taking the arcsine of the square root.

Prior to model development, correlation analysis of all the predictor variables were run to test for collinearity. Salinity and conductance (uS/cm) ($r = 0.799$), as well as TDN and TDP ($r = 0.613$), were identified as being highly correlated. I also found marginal correlations between turbidity and TDN ($r = 0.521$), turbidity and TDP ($r =$

0.444), and turbidity and pond size (r = -0.452), so I excluded using both of these variables within the same model in order to reduce standard error. Afterwards, I ran univariate analysis with all of the predictor variables that described habitat quality against the response variables for zooplankton community responses. After testing the univariate effects of each predictor on the response variables, I built multivariate models using generalized linear mixed effects models fit with pond identity as a random effect; because sites were sampled repeatedly? over the course of two years, observations from the same site cannot be considered independent. I made comparisons among the models using an AIC-based selection approach (AIC package, R Development Core Team 2008).

Results

I. Results from field study

Sampled ponds included both 41 permanent and 12 temporary bodies of water that ranged from 0.1 to 5 m in depth. Of the 53 total sites, 39 sites lacked fish and 14 contained fish. Within the 14 sites with fish there were 11 observations of *Gambusia affinis,* 7 observations of bluegill sunfish, *Lepomis macrochirus*, 4 observations of largemouth bass, *Micropterus salmonides,* and 1 observation of goldfish, *Carassius auratus*. In 2015, two sites lost fish due to the ongoing drought and one site gained fish (or fish were detected in 2015 but not 2014). Overall, 38 of the sites had evidence of cattle activity while the remaining 15 lacked cows.

II. Zooplankton species richness

Ponds had varying richness and community composition. Total zooplankton species richness per pond averaged 5.84 ± 0.22. The highest richness was 10 taxa, which was observed at 4 different ponds (CA-MUD67, CA-SF79, MUDEF, and SF41), while the lowest was 1 taxa detected, which was observed at 2 different ponds (BNPND005 and VPPND001). Specific zooplankton taxa commonly found across many sites included the copepod groups, Cyclopoida (found in 96% of samples), and Calanoid (found in 72% of samples), as well as the small-bodied cladoceran Chydoridae (found in 89% of samples). Other cladoceran species such as *C. reticula* (found in 63% of samples), *D. mendotae* (found in 32% of samples), *Simeocephalus* sp. (found in 63% of samples), *Diaphanosoma brachyurum* (found in 9% of samples), *Scapholeberis mucronata* (found in 26% of samples), and Bosmina (found in 4% of samples), were less abundant across samples and years. Ostracods (found in 83% of samples) were also found in many sites, while mites were less common (found in 25% of samples) (Fig. 3). Zooplankton abundance ranged from 0.013 to 26.99 individuals per L, with a mean of 5.76± 0.97 zooplankton per L. Average individual zooplankton size for the first 50 adult zooplankton per pond ranged from 0.200 mm to 1.26 mm, with a mean size of 0.58 mm overall across all sample sites. Fecundity ranged from 0 to 0.33 gravid individuals.

Based on the univariate general linear model (GLM), the concentrations of TDP (μM/L), TDN (μM/L), turbidity, pH, fish presence, percent shoreline vegetation, and nearby wetland area all had significant relationships with one or more zooplankton responses (Fig. 1). We used a generalized linear mixed models with wetland area and zooplankton species richness as a Poisson-distributed response, wetland area and TDP as fixed effects with site as a random effect. Wetland area had a strong positive effect on total zooplankton species richness (β: 0.51± 0.22, $P < 0.05$, $n = 79$) (Fig. 4) and TDP (μM/L) had a negative effect on zooplankton species richness (β: -0.48± 0.21, $P < 0.05$, n= 79) (Fig. 5). Two univariate GLMs with TDP and TDN on zooplankton density showed that TDP had no significant effect on copepod densities, but was positively associated with *D. mendotae* density (GLM, β: 0.05± 0.02, P< 0.05, n= 79). However, TDP (µM/L) had a negative effect on *Simeocephalus vetulus,* and *Simocephalus serrulatus* densities (GLM, β: -0.02± 0.01, P< 0.05, n= 79). TDN (µM/L) had a positive effect on copepod density (GLM, β: 0.09± 0.04, P< 0.05, n= 79) as well as on *D. mendotae* density (GLM, β: 0.08 ± 0.02, P< 0.001, n= 79). Interestingly, univariate GLMs on calculated N:P ratios, showed that sites with N:P ratios < 16 had greater amounts of zooplankton species richness (GLM, β: 0.92± 0.53, P< 0.09, n= 79) (Fig. 6). More univariate GLMs comparing turbidity and species richness showed that higher levels of turbidity were negatively associated with zooplankton species richness (GLM, β: -0.10± 0.35, P< 0.005, n= 79). Curiously, turbidity had a positive effect on copepod species density (GLM, β: 0.09±0.04, P< 0.05, n= 79), but no significant negative effect on cladoceran density (Fig. 7).

III. Average individual zooplankton size and fecundity

Overall zooplankton population traits, average individual zooplankton size and fecundity were sensitive to wetland area, chemical concentrations, water quality and fish presence. We used generalized linear mixed models with zooplankton fecundity as a Poisson-distributed response, wetland area a fixed effect, and site as a random effect. Wetland area near sampling sites had a positive effect on overall zooplankton fecundity (β: 0.04±0.02, P< 0.005, n= 79)(Fig. 8) and on cladoceran fecundity. Univariate GLMs on TDP and turbidity both had negative effects on cladoceran fecundity (GLM β: -0.08±0.04, P<0.05, n= 79)(Fig.9)(GLM, β: -0.10±0.03, P< 0.005, n= 79).

Two different generalized linear mixed models, one with average individual size as a Poisson-distributed response, the other with zooplankton density as a Poisson-distributed response and fish presence as a fixed effect with site as a random effect, showed that the presence of non-native fishes was negatively associated with average individual zooplankton size (β: -0.03±0.01, P< 0.05, n=49) and zooplankton density (β: -0.34±0.12, P< 0.005, n=49)(Fig. 10). For instance, sites with fish had, on average, 57% fewer zooplankton per L and an average individual zooplankton size that was 16% smaller. Similarly, the 3 sites that transitioned from containing fish to fishless between 2014 and 2015 demonstrated that fish had a strong negative effect on zooplankton density but no notable effect on average zooplankton size. In these sites, zooplankton density increased by 68% individuals per L. Among fish species, a univariate GLM based off 23 observations of mosquito fish, *G. affinis,* showed that *G. affinis* had a negative effect on zooplankton abundance (GLM, -0.27± 0.10, P< 0.005, n=106).

Discussion

Our results revealed that characteristics of zooplankton populations and communities exhibited different responses to factors associated with eutrophication, including higher phosphorus concentrations and turbidity, as well as the presence of invasive fishes. In general, the ponds within our study system had high levels of TDN and TDP. Higher concentrations of nitrogen and phosphorus tended to have negative effects on zooplankton species richness, the density of cladocerans (e.g., *Simeocephalus* sp. and *C. reticula*), and cladoceran fecundity. For instance, average individual zooplankton size increased by 0.91 (mm) in sites that had N:P ratios >16. These findings (Fig. 6) are consistent with previous research that found many cladoceran species decline with increases in nutrient loading (Jeppesen et al. 2000, Conley et al. 2009) ,whereas copepods are either less sensitive to phosphorus or even exhibit increases (Jeppesen et al. 2011). Total phosphorus is typically higher in shallow, non-stratified bodies of water, as sediments that store phosphorus are being constantly released back into the water (Jeppesen et al. 1997, Oliver & Ganf 2000). The mechanisms for negative effects on cladocerans likely involve changes in the types and sizes of phytoplankton available for grazing (Jeppesen et al. 1997, Oliver & Ganf 2000). Large, filamentous phytoplankton thrive with greater phosphorus concentrations, which can be challenging for zooplankton to consume, thereby weakening the top-down control of zooplankton grazing pressure. Excess phosphorus has also been linked to blooms of cyanobacteria, which are a poor source of food for zooplankton and can exacerbate eutrophication by fixing more nitrogen (Schoenberg & Carlson 1984, Oliver & Ganf 2000, Conley et al. 2009). In contrast to other cladoceran species, *D. mendotae* is one of the larger species of cladocerans found in the ponds and was less sensitive to the effects of higher nutrient concentrations, both in this study and previous research (Schoenberg & Carlson 1984, Haney et al. 2013).

Our results also indicated that the presence of non-native fishes had strong, negative effects on both average individual zooplankton size and abundance. Many warm water game fishes, such as bluegill sunfish, *Lepomis macrochirus*, and largemouth bass, *Micropterus salmonides,* are introduced to freshwater ecosystems for sport fishing or in the case of mosquitofish, *Gambusia affinis,* to help control vector populations (Pyke, 2008). Fish frequently exert strong, top down control of zooplankton, in some cases leading to trophic cascades with resultant blooms of phytoplankton (Brooks & Dodson

1965, Pace et al.1999, Sommer et al. 2002). Previous work has suggested that in shallow bodies of water fish exert stronger pressure on zooplankton, as zooplankton are unable to seek refuge in deeper depths where they are less visible to fish. Consistent with this previous body of work, we found fish had a negative effect on body size and zooplankton density. Sites with fish had an individual average zooplankton size 0.13 mm smaller and 4.02 individuals per L fewer than sites without fish. In our study, pond permanence and fish presence were moderately correlated (r= 0.49), as many of the smaller ponds were ephemeral and are not able to sustain a population of fish without constant restocking. Based on similar findings (Jeppesen et al. 1997), it appears that removal of fishes from shallow bodies of water could be an effective way to increase the zooplankton populations and the grazing pressure they exert upon phytoplankton communities (Schoenberg & Carlson 1984, Moss 1990).

The effects of livestock grazing in this study were less clear. Although most of the sampled ponds were originally constructed to support livestock, our data did not indicate any association between the presence of cows or the density of cow paddies around the perimeter on dissolved phosphorus and nitrogen concentrations within the ponds. Previous research has demonstrated that cow excrement can be a significant source of nitrogen and phosphorus into aquatic habitats (Marty, 2005, Conley et al. 2009), which could lead accelerate the rate of eutrophication. Sites with cows present had higher amounts of turbidity ($\bar{x}$ =109.49±40.60) compared to those without ($\bar{x}$ =72.53±18.96), likely as a result of sediment disturbance by cattle or the associated loss of aquatic shoreline vegetation. Turbidity negatively impacted cladoceran abundance per liter, but positively affected copepod abundance per liter, however, this relationship could be attributed to sediment suspension from trampling or loss of water clarity from phytoplankton groups (Jeppesen et al. 2011). Although turbidity, nitrogen concentrations, and phosphorus concentrations were all positively correlated, we did not detect a direct relationship between grazing intensity and nutrient concentrations. Overall, this lack of effect could stem from the long history of nutrient loading in and around these sites. Even for ponds without active grazing during the study period, decades of previous nitrogen and phosphorus inputs from nearby cattle activity have likely contributed to higher nutrient concentrations.

Intuitively, our results also found that zooplankton species richness increased with wetland area, or habitat connectedness. These findings are consistent with the principles established by MacArthur & Wilson in 1967 of island biogeography, where the larger the habitat, the more species the area is capable of supporting. Zooplankton are limited in their dispersal abilities, but cladocerans are capable of producing resting egg sacks which can be transported by the wind, water flow, or animals (Havel & Shurin 2004), and so have the potential to increase in richness, abundance, and size once action is taken to mitigate disturbances.

Zooplankton showed sensitivity to disturbances within shallow bodies of water. Overall, zooplankton responded very strongly to mechanisms of eutrophication such as high concentrations of phosphorus. Eutrophication appeared to have a negative effect on species richness, cladoceran fecundity, and certain densities of cladoceran species. Zooplankton also responded really strongly the disturbance of nonnative fish. Because zooplankton responded strongly to disturbances, in this instance, they were successfully able to signify whether a pond was disturbed. However, no experimental manipulations were preformed so we were unable to identify the accuracy with which zooplankton responded to each disturbance. With further manipulations, it may be possible to assess the magnitude of each disturbance and response in order to create standardized thresholds of disturbance based on zooplankton responses. Future research would also benefit from incorporating phytoplankton samples in order to quantify the size of phytoplankton within each pond and determine how effectively zooplankton are able to exert a top down control on primary production. In summation, this study demonstrated that zooplankton are sensitive to environmental disturbances and have the capacity to be used as bioindicators to monitor both water quality and invasive species in aquatic environments. Therefore, the continued monitoring of zooplankton could be a beneficial management strategy for future conservation efforts of freshwater resources.

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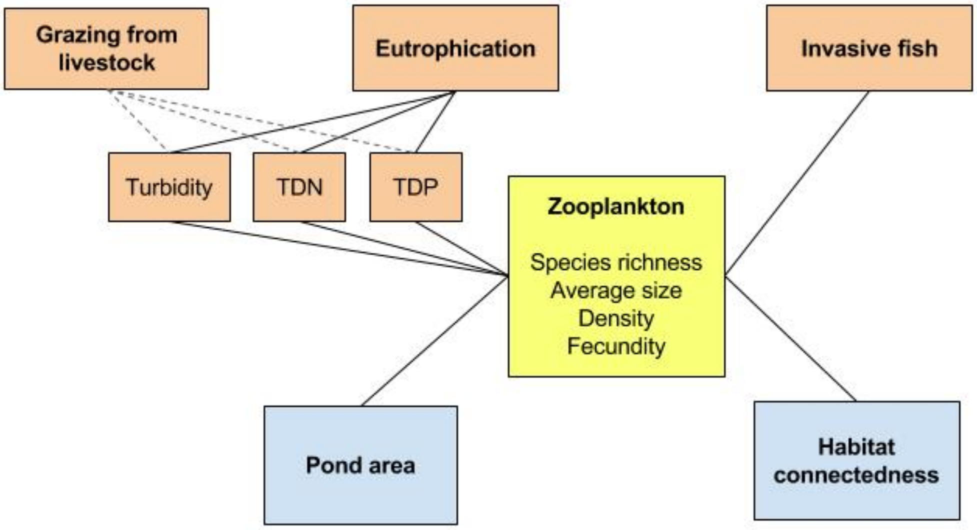

Fig. 1. Conceptual model of the factors that effect zooplankton within our study system ponds. The orange boxes represent the potential measured disturbances to zooplankton, like invasive fish and eutrophication. Turbidity, total dissolved nitrogen (TND), and total dissolved phosphorus (TDP), are potential mechanisms of eutrophication. Many study sites were heavily used by livestock and this grazing pressure could alter the level of turbidity, as well as the concentrations of nutrients (TND and TDP) with in a pond via excrement. The blue boxes represent aspects of habitat quality like pond size and pond area that could also effect zooplankton.

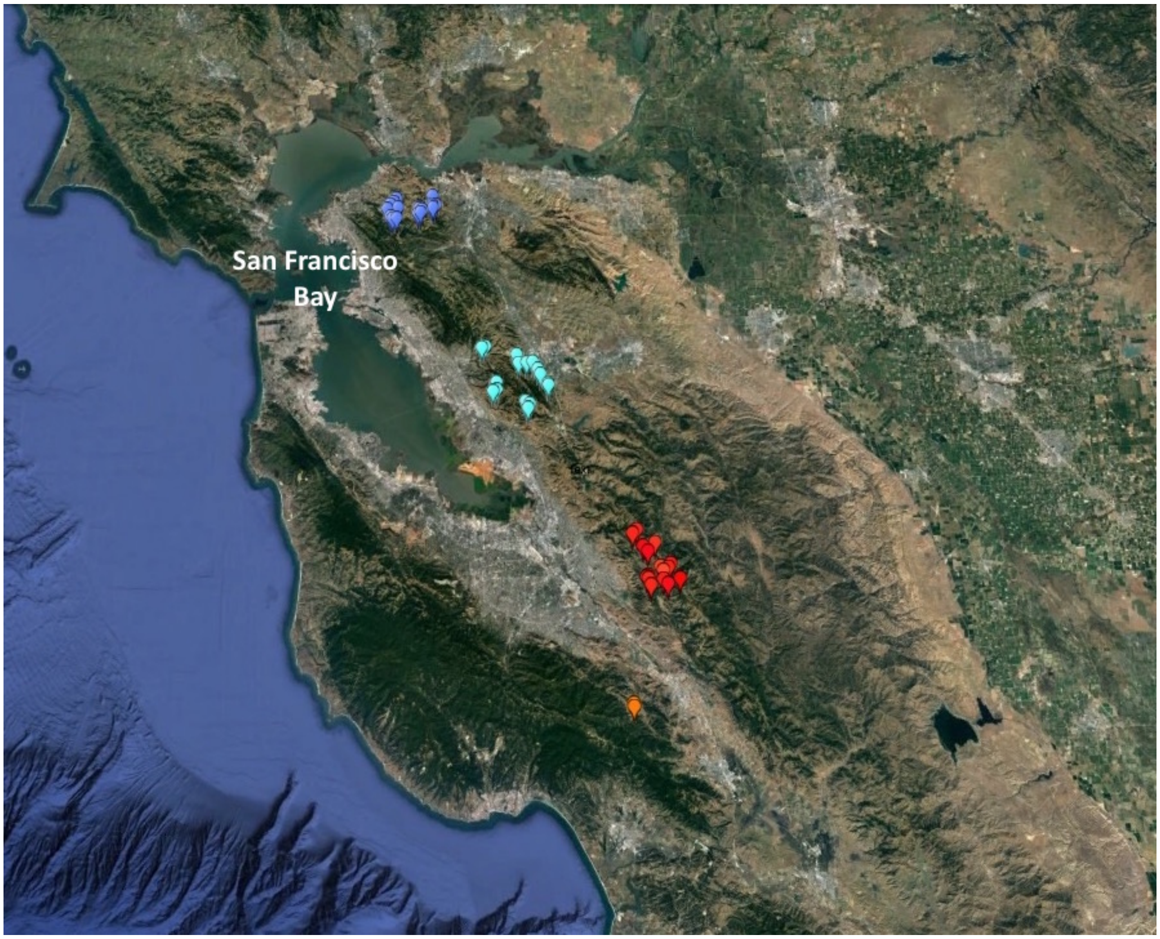

Fig. 2. Map of sampled freshwater ponds in the California Bay Area during 2014 and 2015 field season. Study sites are depicted as aggregated and colored in certain different regions. Blue pins represent sites in Briones metacommunity (19), turquoise pins represent sites in Pleasanton (15), red pins represent sites in Grant (15), and orange pins represent sites in Silver Oaks (4).

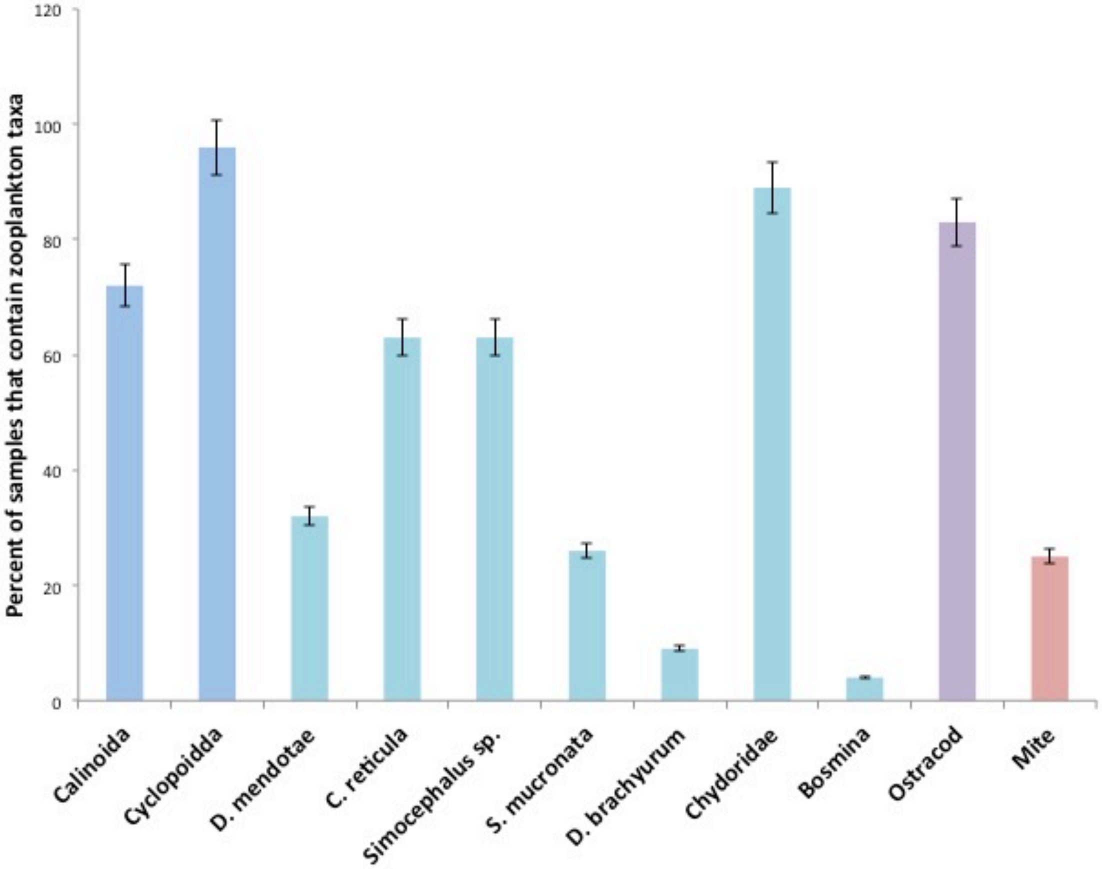

Fig. 3. The distribution of zooplankton taxa with each sample from the 53 sites sampled in California Bay Area between 2014 and 2015. Copepod geniuses Cyclopoida (97% of samples), Calanoida (73% of samples) are on the left and depicted in blue. Cladoceran species *D. mendotae* (32% of samples), *C. reticula* (63% of samples), *Simocephalus sp.* (63% of samples), *S. mucronata* (26% of samples), *D. brachyurum* (9% of samples), Chydoridae (89% of samples), and Bosmina (4% of samples) are in the middle and depicted in light blue. The other two groups Ostracods (83% of samples) and mites (25% of samples) are on the left and are depicted in purple and red respectively. The error bars represent 95% limits around the estimate of sites that contained each species.

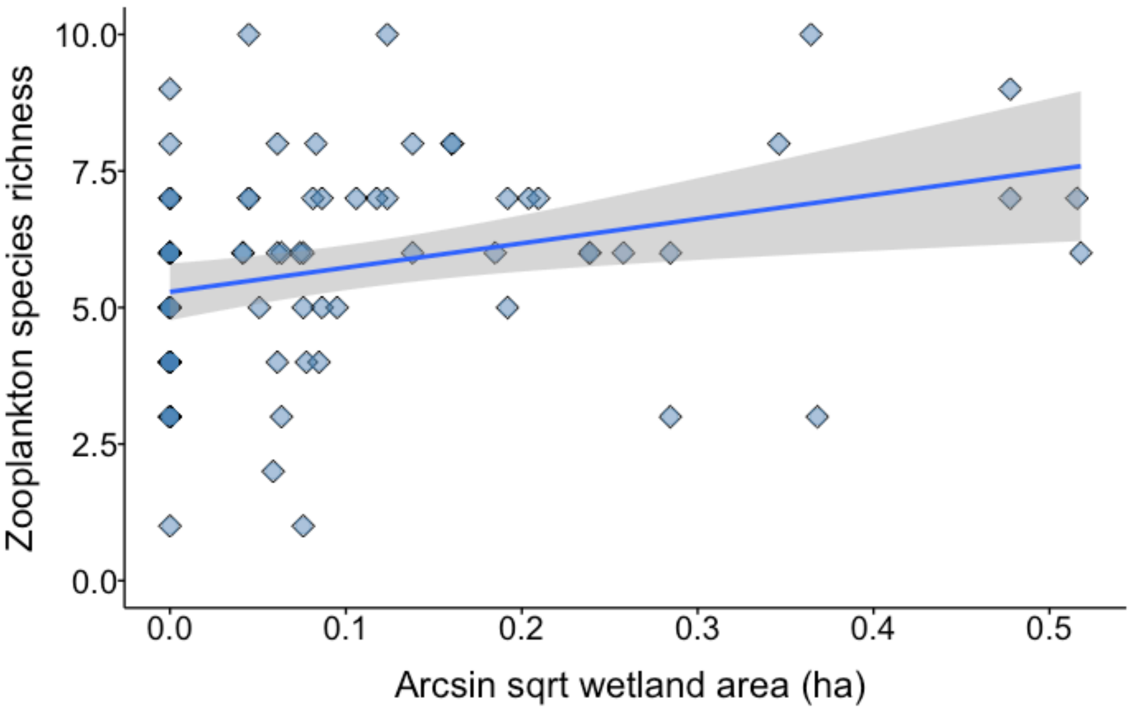

Fig. 4. The relationship between zooplankton species richness and wetland area (ha) described by a generalized linear mixed models with wetland area and zooplankton species richness as a Poisson-distributed response, wetland area and TDP as fixed effects with site as a random effect for 79 observations from both 2014 and 2015 sampling seasons. Wetland area had a strong positive effect on total zooplankton species richness (***β***: 0.51± 0.22, $P < 0.05$, n = 79). Zooplankton species richness raged from 1 to 10 species and wetland area ranged from 0 to 0.245 ha. The grey shading demonstrates standard error around the linear regression.

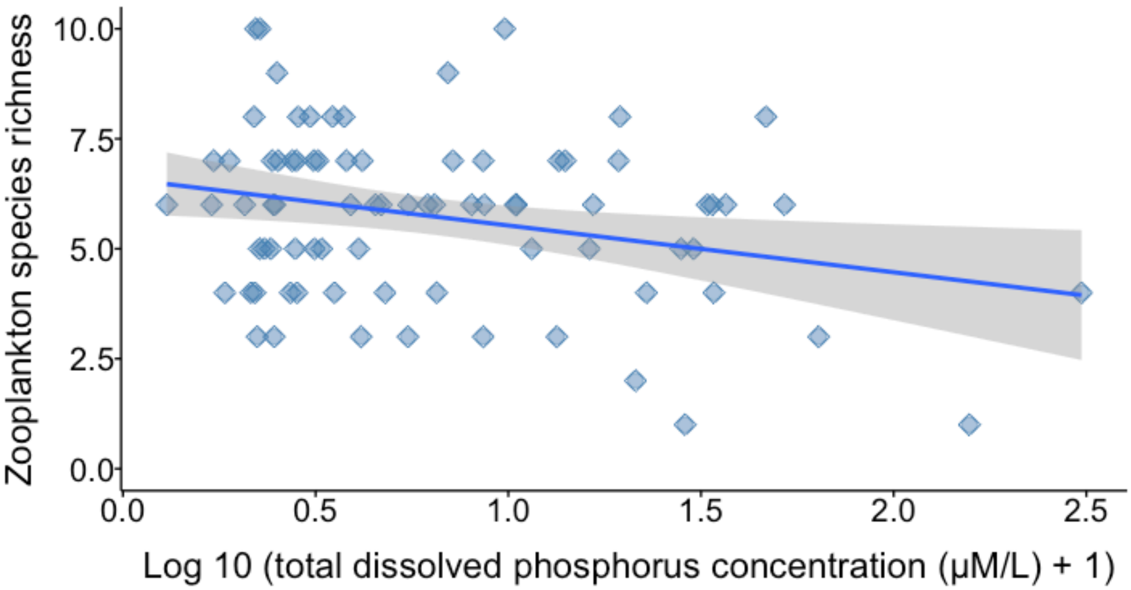

Fig. 5. The relationship between zooplankton species richness TDP (**µ**M/L) described by a generalized linear mixed models with wetland area and zooplankton species richness as a Poisson-distributed response, wetland area and TDP as fixed effects with site as a random effect for 79 observations from both 2014 and 2015 sampling seasons. TDP had a negative effect on zooplankton species richness (***β***: -0.48± 0.21, P< 0.05, n= 79). Zooplankton species richness ranged from 1 to 10 species present and phosphorus concentration ranged from 0.3 to 306.0 (**µ**M/L). The grey shading demonstrates standard error around the linear regression.

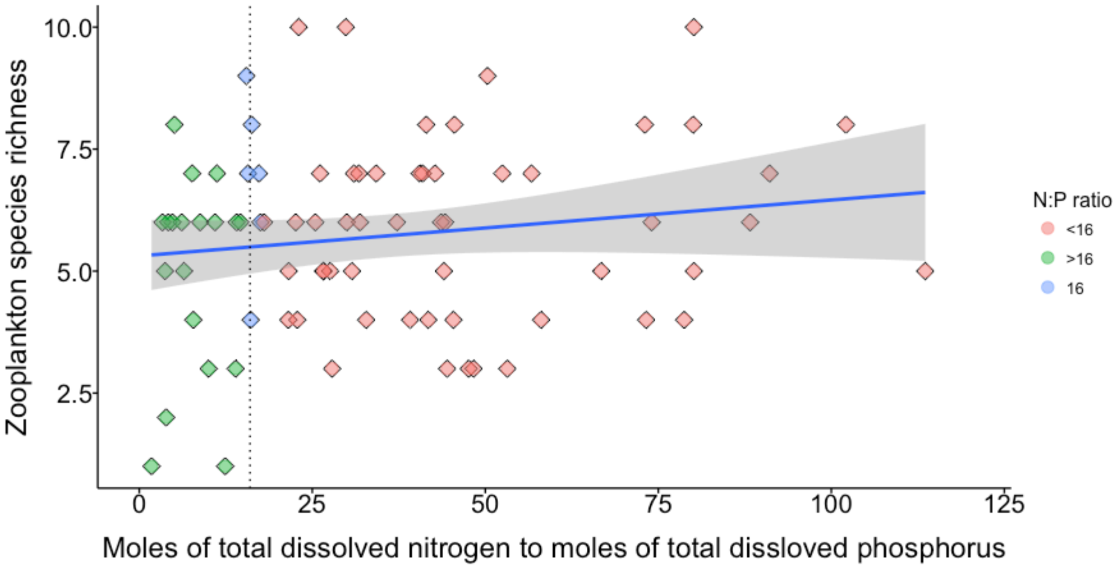

Fig. 6. The relationship between zooplankton species richness and the ratio of moles of total dissolved phosphorus and moles of total dissolved phosphorus (N:P ratios). Univariate GLMs on calculated N:P ratios, showed that sites with N:P ratios > 16 had greater amounts of zooplankton species richness (***β***: 0.92± 0.53, P< 0.09, n= 79). Zooplankton species richness ranged from 0 to 10 species present, and N:P ratios ranged from 1.76 to 510.10 (**µ**M/L). The grey shading shows the standard error around the linear regression.

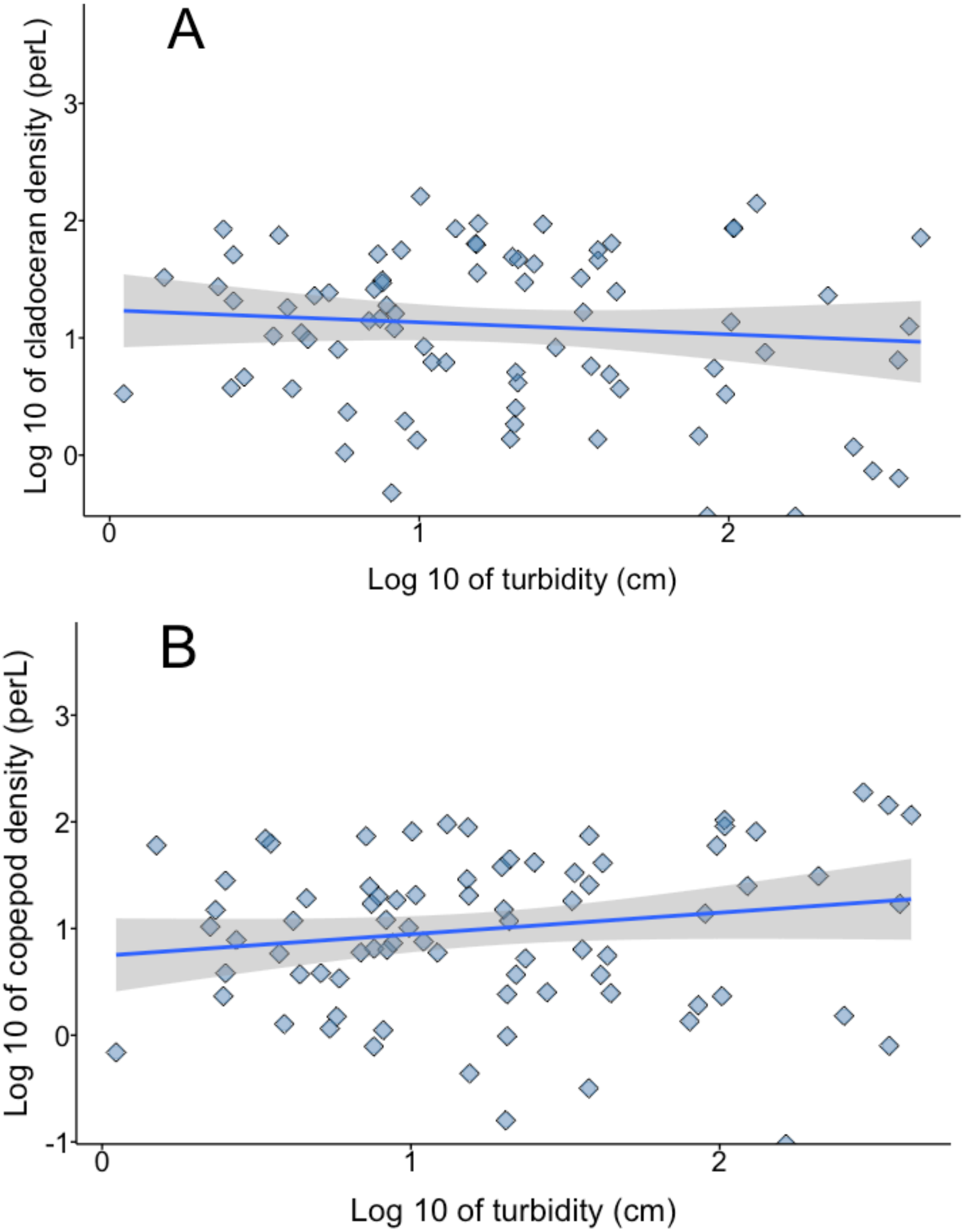

Fig. 7. The relationship between both cladoceran and copepod density with turbidity (cm). The grey shading shows the standard error around the linear regression. Univariate GLMs comparing turbidity and species richness showed that higher levels of turbidity were negatively associated with zooplankton species richness ($\boldsymbol{\beta}$: -0.10± 0.35, P< 0.005, n= 79). **Fig. 7A.** Cladoceran density per liter and turbidity (cm) from 79 observations from both 2014 and 2015 sampling seasons. Cladoceran abundance ranged from 0 to 16 individuals per liter and turbidity ranged from 1.11 to 417 cm. **Fig. 7B.** Univariate GLMs comparing turbidity and copepod density demonstrated that higher turbidity had a positive effect on copepod species density (GLM, $\boldsymbol{\beta}$: 0.09±0.04, P< 0.05, n= 79) from 79 observations from both 2014 and 2015 sampling seasons. Copepod abundance ranged from 0 to 18. 91 individuals per liter and turbidity ranged from 1.11 to 417 cm.

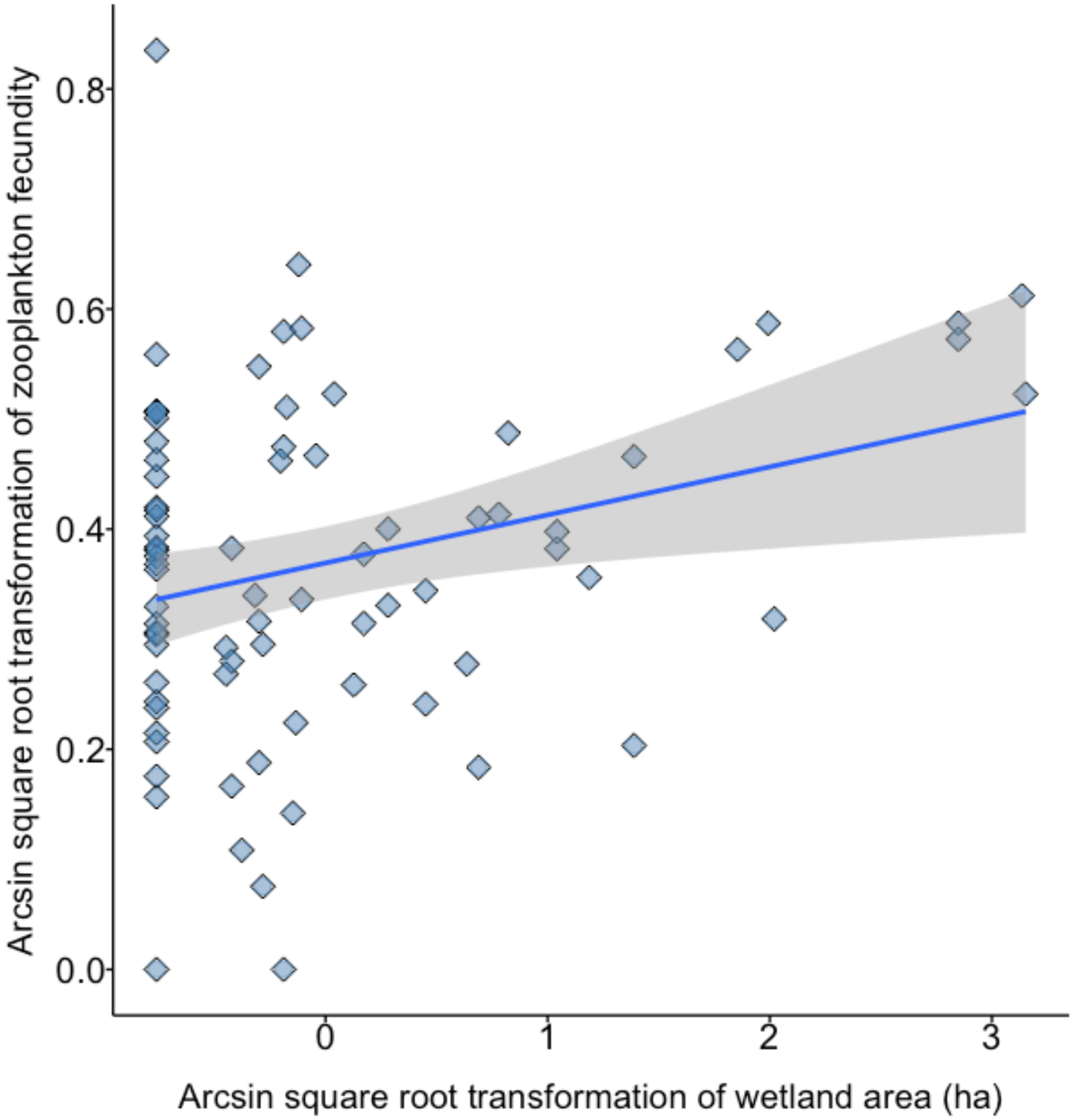

Fig. 8. The association between zooplankton fecundity and wetland area. A generalized linear mixed model with zooplankton fecundity as a Poisson-distributed response, wetland area a fixed effect, and site as a random effect from 79 observations from both 2014 and 2015 sampling seasons. Wetland area near sampling sites had a positive effect on overall zooplankton fecundity ($\boldsymbol{\beta}$: 0.04±0.02, $P< 0.005$). Zooplankton fecundity ranged from 0 to 54.7% of the population containing eggs and wetland area ranged from 0 to 0.245 ha. The grey shading represents the standard error around the linear regression.

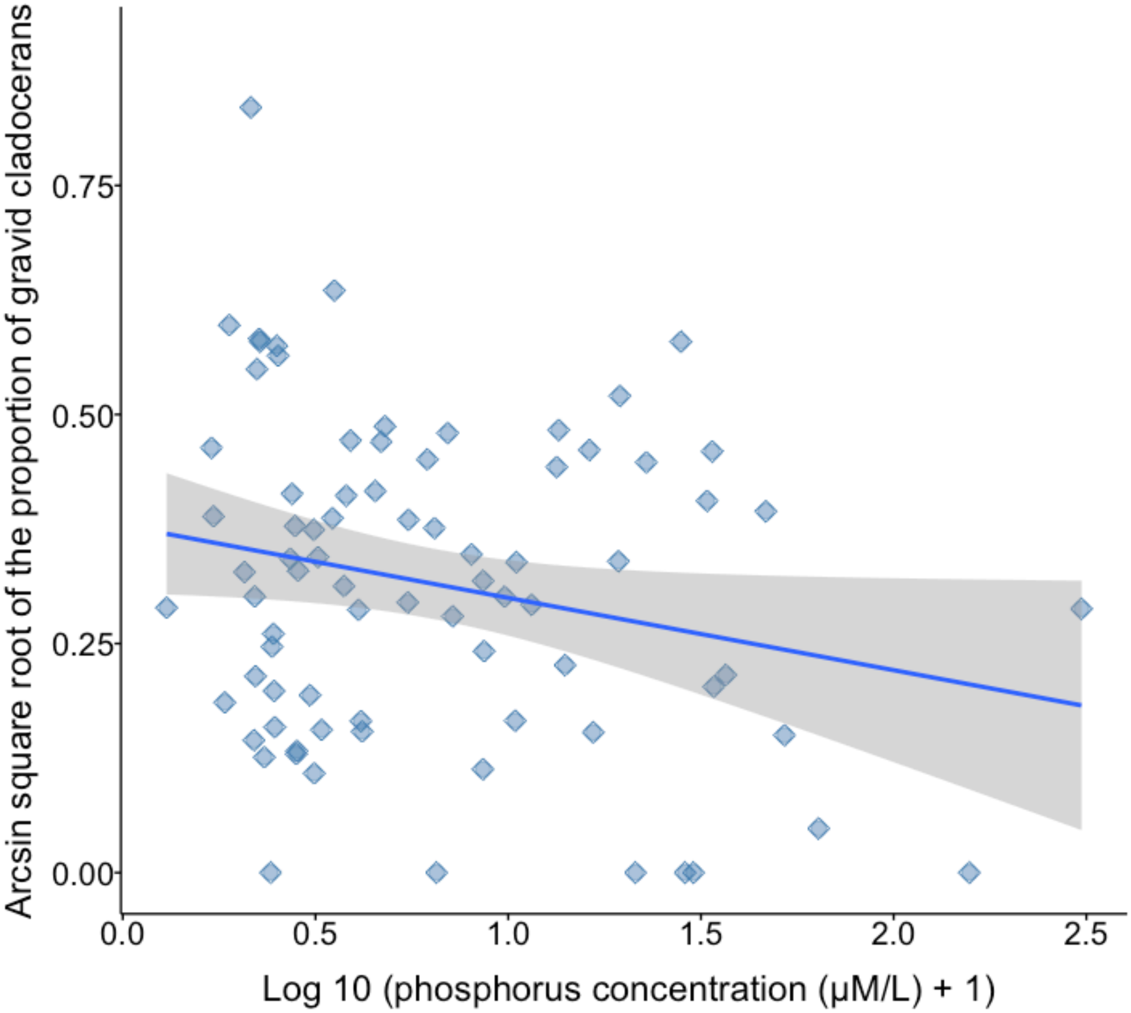

Fig. 9. Zooplankton fecundity as function of TDP concentration (***μ***M/L). A univariate GLM on TDP and cladoceran fecundity 79 observations from both 2014 and 2015 sampling seasons. TDP had a negative effect on Cladoceran fecundity (***β***: -0.08±0.04, $P<0.05$). Cladoceran fecundity ranged from 0 gravid individuals to 2289 gravid individuals and phosphorus concentration ranged from 0.3 to 306.0 (***μ***M). The grey shading demonstrates standard error around the linear regression.

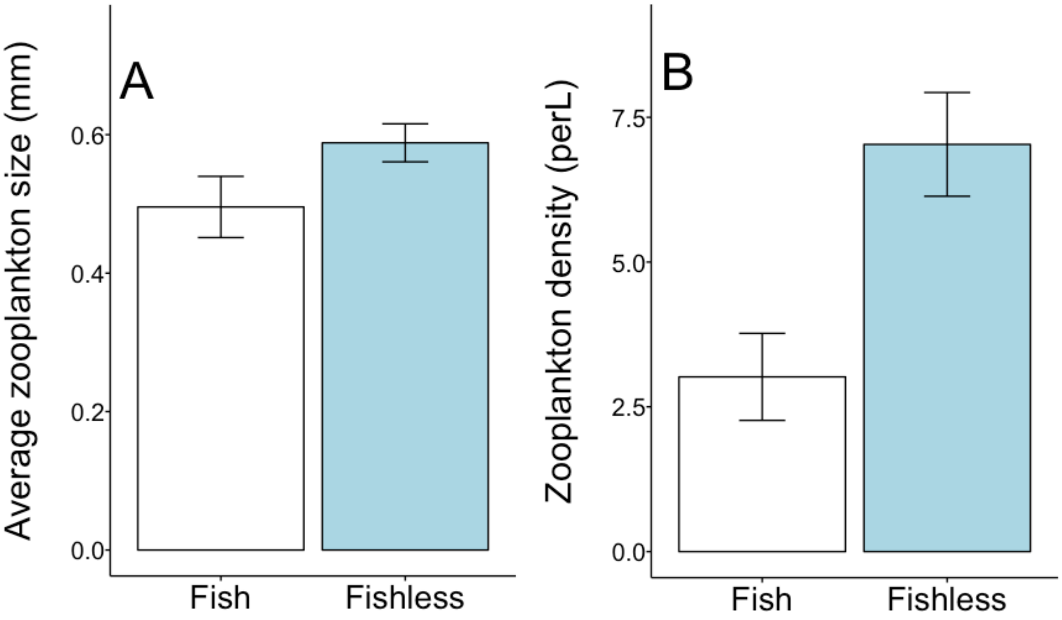

Fig. 10. Average size in relationship to zooplankton density as function fish presence with standard error bars on each column for 79 observations from both 2014 and 2015 sampling seasons. The blue column represents the 39 sites that lacked fish, while the white column represents the 14 sites that contain fish. In a general comparison between the two collected years, in 2015 two sites lost fish due to drought and one site was stocked with fish. **Fig. 10A.** A generalized linear mixed model with average individual size as a Poisson-distributed response and fish presence as a fixed effect with site as a random effect, showed that the presence of non-native fishes was negatively associated with average individual zooplankton size ($\boldsymbol{\beta}$: -0.03±0.01, P< 0.05, n=49) Average individual size ranged from 0.20 to 1.16 mm. **Fig. 10B.** A generalized linear mixed model with zooplankton density as a Poisson-distributed response and fish presence as a fixed effect with site as a random effect. The presence of non-native fishes was negatively associated with zooplankton density ($\boldsymbol{\beta}$: -0.34±0.12, P< 0.005, n=49). Zooplankton abundance ranged from 0.020 to 25.01 individuals per liter.

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