Response of Zooplankton Biodiversity to Nutrient Pulses in Experimental Mesocosms

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Preface

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ABSTRACT

Delineating the response of species to disturbance is a large part of ecological and environmental research. Freshwater ecosystems are essential for supporting life on earth and are also highly vulnerable to disturbance. With predicted increases in the intensity and frequency of disturbance associated with climate change understanding the impact of disturbance regimes on components of the ecosystem is important. The Intermediate Disturbance Hypothesis (IDH) predicts greater species diversity at a moderate intensity and frequency of disturbance than at a high intensity and frequency of disturbance. To test this postulate we assessed the response of zooplankton diversity to two nutrient pulse regimes; a moderate intense frequent pulse and a high intense infrequent pulse in four mesocosms. Higher zooplankton diversity was observed in the moderate intensity pulse in half the experimental group while higher zooplankton diversity was observed in the high intensity pulse. One-way ANOVA showed an effect of the nutrient pulse regime on chlorophyll a concentration but not on zooplankton diversity. Correlations between chlorophyll a concentration and zooplankton diversity were non-significant. The intermediate disturbance hypothesis may not account for zooplankton diversity within freshwater ecosystems. Alternative theories and mechanisms could better explain the trends in zooplankton diversity measured across experimental groups.

INTRODUCTION

Earth's biodiversity is at threat. Several anthropogenic factors stress ecosystems and contribute to biodiversity decline (Wilcove et al., 1998; Thomas et al., 2004, Richter et al., 2003). Of particular importance is maintaining the integrity of freshwater ecosystems. Freshwater ecosystems make up 0.8% of the earth's surface and support over 100,000 of the 1.8 million described species on the planet (Dudgeon et al., 2006). Not only do freshwater ecosystems support aquatic life but they are also an essential resource that humans and other terrestrial species depend on (Vorosmarty et al., 2010). Therefore conservation of freshwater ecosystems is a major priority (Dudgeon et al., 2006).

Freshwater ecosystems are highly vulnerable to anthropogenic stressors and biodiversity loss within these ecosystems is greater than in terrestrial ecosystems (Sala et al., 2000; Dudgeon et al., 2006; Vorosmarty et al., 2010). Water pollution, climate change, habitat degradation, and flow modification are among the major factors that threaten biodiversity in freshwater ecosystems (Dudgeon et al., 2006; Woodward et al., 2010). These threats can act solitarily or synergistically to cause species declines. Much of the attention on threats to freshwater ecosystems over the past few decades has focused on water pollution because of the possible direct impacts to humans, terrestrial and freshwater biota. For example, inputs of substances like phosphorus, nitrogen, carbon and sulphur from agriculture, industrialization, mining, urbanization and atmospheric deposition have been investigated (Schindler, 1977, Neary and Dillon 1988, Smith et al., 1999, Vorosmarty, 2004).

More recently the impact of climate change on freshwater ecosystems has become a prominent issue (Hauer et al., 1997, Ficke et al., 2007, Woodard, 2010). Changes in temperature, precipitation, atmospheric CO_2 along with increases in the intensity and frequency of drought and

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flood events are expected to occur (Milly et al., 2004, Barnett et al., 2006, Ficke et al., 2007, Woodward, 2010). Such changes in climatic variables are expected to exacerbate the effects of stressors like pollutants on freshwater ecosystems (Hauer et al., 1997, Ficke et al., 2007). Delineating responses of species to anthropogenic and climatic disturbances is essential to our understanding of the ecological interactions in aquatic systems.

Perturbation can be described as the response elicited by a disturbance in the structure and function of an ecological system that results in the departure from a stable state (Odum et al., 1979, Rykiel, 1985). Alternatively, ecologists have also used the term perturbation when referring to factors that cause a shift in the stability of a system (Rykiel, 1985). To reduce ambiguity with the use of the word perturbation, authors refer to disturbance as being the cause of perturbation and response being the effect on the system to disturbance (Rykiel, 1985, Bender, 1984,Glasby and Underwood ,1996, Lake, 2000). Disturbances can either occur naturally or be induced by human activity and vary in intensity, frequency and duration (Lake, 2003, Michener and Haueber,1998;Turner and Dale, 1998). Responses of organisms to a disturbance have been classified as press, pulse or ramp (Bender et al., 1984, Lake 2000). Press disturbances arise sharply but last over a prolonged period of time while ramp disturbances gradually increase in intensity over time (Lake, 2000). Pulses are sharply defined disturbances that last a short period of time (Bender et al., 1984, Lake 2000). For example, natural disturbances like floods and hurricanes would be classified as pulse disturbances to aquatic ecosystems.

Intensity and frequency of pulse disturbances are two features that can greatly influence community structure. A prominent, albeit controversial, postulate to the response of a community to disturbances of varying frequency and intensity is given by the intermediate disturbance hypothesis (IDH) (Connell 1978). According to the IDH, higher biodiversity is maintained within

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systems experiencing disturbances of intermediate intensity and frequency (Connell, 1978). The IDH assumes a trade-off between competition and dispersal (Roxburgh et al., 2004). When the intensity and frequency of disturbance is high, sensitive species can go extinct and the species best able to survive in such a system are those with high dispersal and growth rates. Alternatively when the intensity of a disturbance is low and infrequent competitive exclusion may take place where the species that is best able to utilize resources after a disturbance event or persist through disturbance events eventually dominates the system (Connell, 1978).

Numerous studies have provided support for the IDH (Sousa, 1979, Sommer, 1995). However, there is still a discrepancy in the literature surrounding the validity of the IDH (Owen, 1988, Mackey and Currie, 2001). A criticism often made against the IDH is that competitive exclusion is only likely to be a factor if two species are directly competing for the same resource in space and time (Hutchinson, 1961; Richarson, 1970; Fox, 2013). However if conditions change so rapidly that no single species can maintain competitive superiority over others for long enough to eliminate other species then we can expect high diversity (Hutchinson, 1961). Furthermore if species are specialized to exploit different niches then this could also allow for high diversity (Richerson et al., 1970, Connell, 1978).

Nutrient loading is a feature associated with pulsed inflow events that can influence freshwater ecosystems (Flodder and Sommer 1999, Smith and Nekola, 1999, Cottingham and Schindler, 2000; Buyukates and Roelke, 2005). Nutrient pulses exert bottom-up effects on trophic levels within freshwater ecosystems by influencing the structure and abundance of phytoplankton species which in turn can influence higher organisms (Cottingham and Schindler, 2000). The response of phytoplankton communities to nutrient pulses has been modelled and well-studied in field and laboratory experiments (Roelke et al., 1999; Hambright and Zohary,

2000, Roelke et al., 2003). Due to their ability to act as limiting factors within freshwater ecosystems, the effects of nitrogen and phosphorous have been the focus of much of the research (Schindler, 1977, Smith, 1982, Guildford and Hecky, 2000). Inorganic and organic nitrates and phosphates cause eutrophication in lake and marine ecosystems because of their ability to nourish autotrophic phytoplankton (Smith, 1982, Guildford and Hecky, 2000). However, the responses of zooplankton communities to nutrient pulses are important given the role zooplankton play within lake ecosystems.

There are three major groups of zooplankton; Rotifera, Cladocera and Copepoda (Wetzel, 1975). In freshwater systems, most zooplankton species are herbivorous, grazing on phytoplankton. Phytoplankton community structure can therefore influence zooplankton communities (Buyukates and Roelke, 2005). Furthermore, by exerting top down control, zooplankton can buffer phytoplankton against the effects of nutrient pulses (Jeppesen et al., 1999, Cottingham and Schindler, 2000; Buyukates and Roelke, 2005). Zooplankton are an important food resource for fish and aquatic invertebrates (Piasecki, 2004). Zooplankton can be parasitic and act as vectors for pathogens that infect fish and other aquatic organisms (Piasecki, 2004). Zooplankton can also be used as bio-indicator species in environmental assessments (Gannon and Stemberger, 1978). Given the important ecological role zooplankton play within freshwater ecosystems, describing the responses of zooplankton to nutrient pulses can provide important insights into our understanding of how aquatic ecosystems respond to disturbance.

The objective of this study was to assess how zooplankton biodiversity responds to variation in the frequency of nutrient pulses in experimental mesocosms, much like the pulses lentic water systems would experience due to rainfall events, while holding the total level of nutrient inputs constant. The indoor mesocosms (hereafter termed the Limnotron) were designed

to mimic freshwater lentic systems (lakes and ponds) and contain a host of phytoplankton and zooplankton species as well as other aquatic micro-invertebrates. Mesocosms are useful proxies for aquatic systems and have been widely used in ecological risk assessments (Graney et al, 1993). The mesocosms were subjected to sequential variation in the nutrient pulse regimes over the course of the study: moderate intensity and frequent pulse regime (so-called Phase 1) and a high intensity and low frequency pulse regime (Phase 2). The IDH predicts that zooplankton diversity and abundance should be more enhanced in the moderate intensity and frequent pulse regime than in the high intensity and low frequency pulse regime.

MATERIALS AND METHODS

Mesocosm design

Four tanks served as the mesocosms for this experiment. Each mesocosm was a double-walled stainless steel cylindrical tank with a capacity of 28,000 litres (diameter 4 m, depth 5 m) (Fig. 1). All four tanks were part a group of six tanks that make up the Limnotron experimental facility housed in the Biodiversity Institute of Ontario at the University of Guelph (43°32′ N, 80°13′ W, Guelph, ON, Canada). Data used in this study were collected over a nine month period between May 1st 2013 and January 30th 2014 as part of an ongoing experiment that begun in 2011 on the spatial and temporal dynamics of zooplankton and phytoplankton within the limnotron. At the start of the experiment in 2011 each tank was filled with 24 000 L of water from a well. Well water was filtered through a five μm filter to remove debris before being transferred into each tank. All mesocosms were closed experimental systems with no additional water being added after the tanks were filled. Each mesocosm was kept under a 12 hr light and 12 hr dark cycle.

Each tank had 18 sampling ports, divided into three sampling stations around each tank, from which water samples were drawn (Fig. 1). Ports enabled sampling from different heights within the water column of the tanks. Furthermore, sampling pipes connected to each port penetrated each water column at lengths of 25 cm (Short), 50 cm (Medium) and 100 cm (Long). This was to ensure all water columns were sampled in both vertical and horizontal planes.

All tanks were re-inoculated in 2011 with algae (*Chlorella vulgaris*) from the Canadian Phycological Culture Centre. *C. vulgaris* is a single celled, fast-growing phytoplankton species commonly found in freshwater ecosystems (Liang et al., 2009). By the time of the trials outlined in this paper, other blue-green algae had also invaded the mesocosms (Turgeons, pers. Comm.).

Experimental treatments and Sampling design

From May to August 2013, 50 ml of nutrient solution was added to each mesocosm every two to seven days (Table 1) for a total of eight times per month (except in July where we had 9 additions). This was the moderate intensity and frequent nutrient pulse regime (Phase 1). From September 2013 to January 2014, 400 ml of nutrient was added to each tank once at the beginning of every month. This was the high intensity and infrequent nutrient pulse regime (Phase 2). In both treatments Miracle-Gro Liquafeed® (Scotts Canada Ltd) with an N:P ratio of 12:4 was used as the nutrient input. As phosphorous had the lower concentration within the N:P ratio it was considered as the limiting nutrient. Nutrient was poured into the mesocosms from inlets located at the top of each tank (Fig. 1). Zooplankton were sampled according to the schedule presented in Table 1. Estimates of phytoplankton and zooplankton abundance were based on 100-500ml water samples collected from each of the 18 individual sampling ports on each tank. Before collecting water samples for zooplankton identification and enumeration all

ports were drained of water that may have accumulated within the pipes. This was done to ensure water samples obtained were drawn from within the mesocosm. Drained water was then poured back into the tanks.

Water samples were drawn into a one litre flat-bottomed flask. Water samples were passed through a filter funnel with a 20 μm Nitex® fine cloth filter to separate the zooplankton. The filtrate was analysed for chlorophyll a content using a fluorometer (Aquafluor ® Turner Designs) with a detection range of 0.3 - 300 μg/L. Chlorophyll is a good indicator of phytoplankton abundance (Lorenzen, 1966, Platt, 1971). Residue left on the filter funnel and on the filtering cloth was thoroughly rinsed into a tissue culture plate. Zooplankton were identified and counted under a light microscope (Stemi 2000 C Carl Zeiss). Zooplankton densities were calculated per litre of water sampled. Zooplankton were categorized as Rotifers *(*Rotaria and Brachionus), Cladocera (*Daphnia sp.*) and Ostracods. Freshwater micro-invertebrates also present in the mesocosms were classified as Flatworms (*Planaria sp.*) and Clearworms (*Nematoda spp.*). Zooplankters difficult to identify were categorized as either "mini", "micro" or "eggs".

Statistical analysis

The Shannon index (Shannon and Wiener, 1949) was used to assess differences in faunal biodiversity between the mesocosms within and across nutrient pulse regimes. The Shannon index was chosen over other measures of biodiversity because of its usefulness in assessing alpha diversity, however the index may be more sensitive to changes in species abundance than richness (Levine, 2009).

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Spearman's rank correlation and linear regression analysis were used to assess associations between chlorophyll *a* concentration and zooplankton diversity within each mesocosm. Spearman's rank correlation is useful in eliminating the confounding effect of multico-linearity between predictor and response variables. Although mesocosms were originally designed to be identical and exact replicates of each other there were differences in terms of the zooplankton assemblages found within each tank and therefore each mesocosm was treated as its own lentic system and analysed separately. For each tank, a one-way, repeated measures ANOVA was used to check for differences in both zooplankton diversity and chlorophyll a concentration between the nutrient regimes. Because all tanks received the same nutrient treatments they could be considered as replicates for this analysis. A repeated measures analysis was used because data were collected on the same mesocosms but across two treatments. Furthermore a one-way ANOVA is a useful statistical test commonly used to check for differences between experimental treatments. All statistical analyses were conducted using SPSS 21.0 (IBM Armonk, NY, USA).

RESULTS

The composition of zooplankton assemblages differed across mesocosms over time and nutrient regimes. *Rotaria* was the most abundant identifiable genus sampled during the first phase of the experiment (94.2), followed by Planaria (28.2), *Daphnia* sp. (22), Ostracods (2.5),*Brachionus* (0.2) and Nematods (0.04). For indiscernible forms of zooplankton "mini" were the most abundant (50.6) followed by "micro" (2.5) and "eggs" (0.4). *Daphnia* outnumbered all other species in the second phase of the experiment (12.4), followed by *Rotaria* (8.4), Planaria (1.6)*Brachionus* (0.7), Ostracods (0.2) and Nematods (0.2). For indiscernible species "mini" were the only fauna counted (8.9).

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To monitor changes in zooplankton diversity Shannon's diversity index was calculated for each mesocosm during each of the nine months the study was conducted. Zooplankton diversity varied considerably within each mesocosm across time (Fig. 2). Furthermore changes in zooplankton diversity were observed between the two nutrient regimes. In Tank 2 higher zooplankton diversity was recorded during phase 1 ($H^1 = 1.18$) than in phase 2 ($H^1 = 1.07$). Similarly, higher zooplankton biodiversity was recorded in Tank 3 during phase 1 ($H^1 = 1.24$) than in phase 2 ($H^1 = 0.95$). In contrast, lower biodiversity was recorded in tank 4 during phase 1 ($H^1 = 0.55$) than during phase 2 ($H^1 = 1.20$). As in tank 4, lower biodiversity was recorded in tank 5 during phase 1 ($H^1 = 1.05$) than during phase 2 ($H^1 = 1.24$).

Changes in chlorophyll *a* concentration in each mesocosm were monitored throughout the study and were used as a proxy for phytoplankton abundance. As with zooplankton diversity, chlorophyll a concentration varied over the duration of the experiment (Fig. 3). To assess if there was an association between zooplankton diversity and phytoplankton abundance, Spearman's correlational analysis and linear regression analysis were carried out on each of the mesocosms. Positive correlations between zooplankton diversity and chlorophyll concentration were observed in Tank 3 and Tank 5. Within Tank 3 (Spearman $\rho = 0.550$, $P = 0.125$, $r^2 = 0.348$) there was a stronger positive correlation of zooplankton diversity to chlorophyll concentration than in Tank 5 (Spearman $\rho = 0.083$, $P = 0.831$, $r^2 = 0.063$). In Tanks 2 and 4 biodiversity was negatively correlated to chlorophyll concentration. In Tank 2 biodiversity was moderately correlated with chlorophyll concentration (Spearman $\rho = -0.450$, $P = 0.224$, $r^2 = 0.029$). However, in Tank 4 the correlations observed were much weaker (Spearman $\rho = -0.067$, $P = 0.865$, $r^2 = 0.004$). No correlations between chlorophyll concentration and zooplankton diversity were significant.

A one way repeated measures ANOVA was used to check for differences in zooplankton biodiversity between the nutrient regimes (Fig. 4). No significant differences in biodiversity were observed between phase 1 (M = 0.83, SD = 0.36, n= 16) and phase 2 (M= 0.72. SD= 0.41, n=16) ($F_{0.403}$ =0.974, P= 0.535). We did observe significant variation in chlorophyll concentration between the first phase (M = 73.69, SD = 42.32, n =124) and the second phase of the experiment (M=23.68, SD = 33.33, n = 124) ($F_{86.74} = 0.586$, $P < 0.0001$; Fig. 4)

DISCUSSION

We conducted this study to test if intermediate levels of nutrient pulse disturbance within the aquatic mesocosms could account for increases in zooplankton biodiversity. Our predictions were that moderate intensity and frequent nutrient pulse regime would support higher zooplankton diversity than the high intensity and low frequency pulse regime.

Species Abundance and Sampling

Higher zooplankton abundance was observed in phase 1 than in phase 2. This was true for most zooplankton species, except for *Brachionus* and "mini" which had higher abundance in phase 2. The effect of the treatments may offer an explanation for some of the variance in species abundance between the two nutrient pulse regimes but it is does not account for the overall trend in higher species abundance seen in phase 1. The most parsimonious explanation for the trend observed may be related to differences in the sampling effort between phase 1 and phase 2 of the experiment. Because zooplankton counts were made based on volumes of water collected from individual ports in phase 1 this led to higher volumes of water being sampled in phase 1 of the experiment and therefore a higher chance of encountering zooplankton when compared to phase 2 where volume of water sampled was less because zooplankton counts were made on all ports at the same height. Sampling effort affects the accuracy and precision of community attributes

estimates (Angermeier and Smogor 2009, Magurran, 2004). Small sampling effort may lead to an underestimation of rare species, reduced estimates of species number and an overestimation of species variability across space and time (Angermeier and Smogor, 2009, Mackenzie and Kendall, 2002).

Higher sampling effort may give a better representation of the population being sampled. (Angermeir and Smogor,2009). In this study a higher sampling effort was used in the first phase of the experiment than in the second phase resulting in higher counts (evenness) of individual zooplankton species from the populations within the mesocosms. However, because the Shannon-Weiner diversity index is a composite of both richness and evenness and is less sensitive to variations in species evenness (Levine, 2009) the measure of diversity used was comparable between treatments.

Biodiversity and Species behaviour

As predicted, higher zooplankton diversity was observed during phase 1 of the experiment when compared to phase 2 of the experiment, but only in two of the four mesocosms (tanks 2 and 3). The relationship between zooplankton diversity and primary productivity within natural lakes is complex and depends on predator-prey interactions, competitors, seasonal variability, and alternative resources (Barnett and Beisner, 2007). Although the mesocosms used in this study were controlled systems some of these factors did play a role in influencing the results of this experiment. For example different food preferences in zooplankton species could have resulted in less interspecific competition and therefore reduced competitive exlusion of some species. An assumption built into the model of this experiment was that zooplankton feed upon a single food source (i.e., phytoplankton). However, zooplankton have a variety of food sources not limited to phytoplankton but also feed on detritus and bacteria (Walz, 1995,

Gentleman et al., 2003). Feeding selectivity and efficiency can be influenced by size of the predator, and the predator's ability to detect, capture, handle and assimilate prey items (Pourriot, 1977, Demoot 1986, Walz, 1995). Furthermore food choice can also be influenced by the quantity, nutritional value and palatability of the food item. Therefore food preferences may vary with species (Ricci, 1985, Demoot, 1986). The mesocosms contained two genera of bdelloid rotifers, *Rotaria* and *Brachionus*. Most bdelloid rotifers are microphagous and are limited in the size of food particles they can ingest which results in the exclusion of algae for smaller sized food (Pourriot 1977, Ricci, 1985). For example, the population growth of *R.rotatoria* was found to be slow and unresponsive to cultures inoculated with algae when compare to cultures inoculated with bacteria and yeast (Ricci, 1985). However, rotifers have also been shown to be inefficient at ingesting low densities of bacteria and require a mixed diet of both bacteria and alga to optimize egg production (Snell et al., 1983, Seaman et al., 1986).

Daphnia are larger and do not discriminate between food choice based on size. *Daphnia* are able to ingest both small (e.g. bacteria and protozoa) and large (e.g. alga and fungi) food particles (Demoot, 1982, Demoot, 1986, Walz, 1995). However, both the quality and quantity of food can influence grazing. For example, bacteria have a lower nutritional value to *Daphnia* than algae (Walz, 1995). Furthermore, not all algae are palatable and beneficial to the survival of *Daphnia* with green alga conferring more benefits than blue-green alga, which were also present in the mesocosms (Arnold, 1971). Planarians are generally omnivorous and have a varied diet feeding off of living and dead zooplankton, detritus and algae (Pickavance, 1971, Calow et al., 1981). Therefore their role as scavengers of other zooplankton species within the mesocosms cannot be overlooked and should be taken into account when considering the trophic interactions within the mesocosms. Information on the diet of freshwater nematodes is sparse but their diet

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has been found to consist mainly of bacteria and algae (Traunspruger, 2000). The overall effect of the different species within the experimental mesocosms having such a variety in diet means that although phytoplankton abundance may have changed between phase 1 and 2 of the experiment (Fig. 4) drawing a relationship between the functional response of zooplankton diversity and phytoplankton abundance is difficult and can explain the non-significant correlations we found between zooplankton diversity and chlorophyll a concentration.

Preferences in the diet of different zooplankton species could also explain why large zooplankton such as *Daphnia* exert stronger top down effects on phytoplankton and have been found to be more effective at buffering against nutrient pulses, however this is not always the case (Cottingham and Schindler, 2000 Buyukates and Roelke, 2005). Endogenous population responses to seasonal changes in photoperiod, light intensity, temperature and food are known to occur in phytoplankton and zooplankton and this could have played a role in the trends in diversity. For example circadian rhythms associated with decreases in light intensity, photoperiod and temperature are known to be responsible for differences in phytoplankton abundance between the summer and autumn (Prezelin and Sweeney, 1977, Falowski 1984, Gibbs and Vant, 1997). This may explain the declining trends in chlorophyll abundance seen in all our mesocosms as we moved from summer to autumn (Fig. 3) and also explain the difference in mean chlorophyll a concentration seen between the nutrient regimes (Fig. 4)

Life history strategies are a product of an organisms metabolic demands and abiotic and biotic constraints in the organisms environment (Walz, 1995). According to Gause's principle of competitive exclusion, species occupying the same niche and utilizing the same resources cannot invariably compete which can lead to the extinction of one competitor (Hardin, 1960). However, as Hutchinson (1961) notes, plankton species are able to coexist even in the presence of

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seemingly strong competitor species due to differences in resource utilization over space and time and the inability of a competitor to establish dominance given the rapid succession of one organism over another within the same habitat (Hutchinson, 1961; Richerson, 1970).

Zooplankton have evolved different life history strategies to cope with resource constraints. Rotifers and Cladocerans are both considered to be r-strategists compared to copepods (Allan, 1976). Both *Daphnia* and rotifers reproduce asexually under normal conditions, have short lifecycles and develop large populations (Allan, 1976, Walz, 1995), However there are a few differences between the two taxa. *Daphnia* for example have slower growth times and live longer. Development to maturity can be anywhere between 6 to 34 days in with their lifespan reaching 85 days (Allan, 1976). Rotifers on the other hand have shorter development times. It takes 1.25 to 7 days for rotifers to reach reproductive maturity and their survivorship can be as long as 20 days (Allan, 1976, Walz, 1995). Planarians also reproduce parthogenetically but have longer development times than *Daphnia* and rotifers (Reynoldson et al., 1965, Calow et al., 1981). These subtle differences in development times, lifespans and attributes of the organism should be considered in relation to the disturbance regime (Turner and Dale, 1998). For example, in this experiment the moderate intensity and frequent nutrient additions were frequent enough to punctuate the lifecycle of the rotifers but the time between the nutrient additions in the high intensity/low frequency additions may have been so large that it did not have consequences on the lifecycle of rotifers. The nature of the disturbance has to be taken into account when considering the ability of a disturbance to remove individuals from the community. There was no evidence to suggest that the nutrient pulse additions in our experiment were toxic and caused direct mortality of zooplankton. Although pulses did not directly remove individuals by causing death the nutrient pulses did alter the abundance of phytoplankton prey items for certain

zooplankton (Fig 4) which could have led to the indirect removal of individuals due to a lack of sufficient food resources.

Limitations and Recommendations

The results of this experiment attest to the complexity and often controversial debate around the intermediate disturbance hypothesis and plankton dynamics (Hutchinson, 1961, Fox, 2013). Although we recorded increases in biodiversity within two mesocosms with a change in nutrient pulse treatment this change did not occur across all four experimental systems as predicted by the IDH. A conceptual problem with the IDH is defining what level of disturbance can be defined as "intermediate" in frequency and intensity. Determining what an intermediate level of disturbance would require sampling across a broad range of disturbance frequencies and intensity (Fox, 2013).

Given that the experiment was conducted under controlled laboratory conditions generalizing the findings to natural lake ecosystems is difficult. As the tanks were inoculated with zooplankton the founder populations within the mesocosms may not be genetically or phenotypically as diverse as natural populations therefore we cannot extrapolate these findings to natural lakes. The nutrient pulse disturbances may not mimic pulsed inflow events associated with floods or point source disturbances which may have other potential disturbance mechanisms such as turbulence associated with flow events. For example the storms or hurricanes may actually aid in flushing out nutrients from a water body (Paerl and Huisman, 2008). Furthermore the concentration poured into the tanks may have been too high. In this experiment 50 ml of nutrient input was used as the moderate intensity pulse regime. Assuming equal mixing within the mesocosms this would translate to circa 0.35 mg of P dissolved per liter of water in the mesocosms, well above the 100 μg/L of P considered typical for extreme precipitation events

within natural lakes (Cottingham and Schindler, 2000). Finally the mere act of removal sampling method used could have acted as a disturbance to the zooplankton assemblages within the mesocosms.

References

Allan JD, 1976. Life history patterns in zooplankton. Am Nat: 165-180.

Angermeier PL, Smogor RA, 1995. Estimating number of species and relative abundances in stream-fish communities: effects of sampling effort and discontinuous spatial distributions. Can. J. Fish. Aquat. Sci. 52: 936-949.

Arnold DE, 1971. Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of blue-green algae. Limnol. Oceanogr. 16*:* 906-920.

Barnett A, Beisner BE, 2007. Zooplankton biodiversity and lake trophic state: explanations invoking resource abundance and distribution. Ecology. 88: 1675-1686.

Barnett TP, Adam JC, Lettenmaier DP, 2005. Potential impacts of a warming climate on water availability in snow-dominated regions. Nature. 438: 303-309.

Bender EA, Case TJ, Gilpin ME,1984. Perturbation experiments in community ecology: theory and practice. Ecology. 65: 1-13.

Boose ER, Foster DR, Fluet M, 1994. Hurricane impacts to tropical and temperate forest landscapes. Ecol. Monogr. 64: 369-400.

Buyukates Y, Roelke D, 2005. Influence of pulsed inflows and nutrient loading on zooplankton and phytoplankton community structure and biomass in microcosm experiments using estuarine assemblages. Hydrobiologia. 548: 233-249.

Calow P, Davidson AF, Woollhead AS, 1981. Life-cycle and feeding strategies of freshwater triclads: a synthesis. J. Zool. 193: 215-237.

Connell JH, 1978. Diversity in tropical rain forests and coral reefs. Science, 199: 1302-1310.

Cottingham KL, Schindler DE, 2000. Effects of grazer community structure on phytoplankton response to nutrient pulses. Ecology. 81: 183-200.

DeMott WR, 1982. Feeding selectivities and relative ingestion rates of Daphnia and Bosmina. Limnol. Oceanogr. 27: 518-527.

DeMott WR, 1986. The role of taste in food selection by freshwater zooplankton. Oecologia. 69: 334-340.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Richard AHP, Soto D, Stiassny MLJ, 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81: 163-182.

Falkowski PG, 1984. Physiological responses of phytoplankton to natural light regimes. J. Plankton. Res. 6: 295-307.

Ficke AD, Myrick CA, Hansen IJ, 2007. Potential impacts of global climate change on freshwater fisheries. Rev. Fish. Biol.Fisher. 17: 581-613.

Flöder S, Sommer U, 1999. Diversity in planktonic communities: an experimental test of the intertmediate disturbance hypothesis. Limnol. Oceanogr. 44: 1114-1119.

Fox JW, 2013. The intermediate disturbance hypothesis should be abandoned. Trends. Ecol. Evol. 28: 86-92.

Gannon JE, Stemberger RS, 1978. Zooplankton (especially crustaceans and rotifers) as indicators of water quality. T. Am. Microsc. Soc. 16-35.

Gentleman W, Leising, A, Frost B, Strom S, Murray J, 2003. Functional responses for zooplankton feeding on multiple resources: a review of assumptions and biological dynamics. Deep. Sea. Res. Pt. II, 50: 2847-2875.

Gibbs MM, Vant WN, 1997. Seasonal changes in factors controlling phytoplankton growth in Beatrix Bay, New Zealand. New. Zeal. J. Mar. Fresh. 31: 237-248.

Glasby TM, Underwood AJ ,1996. Sampling to differentiate between pulse and press perturbations. Environ. Monit. Assess 42: 241-252.

Guidford SJ, Hecky RE. 2000. Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? Limnol. Oceanogr. 45: 1213 -1223.

Graney RL, 1993. Aquatic mesocosm studies in ecological risk assessment. CRC Press.

Hambright KD, Zohary T, 2000. Phytoplankton species diversity control through competitive exclusion and physical disturbances. Limnol. Oceanogr. 45:110-122.

Hardin G, 1960. The competitive exclusion principle. Science, 131: 1292-1297.

Hauer FR, Baron JS, Campbell DH, Fausch KD, Hostetler SW, Leavesley GW, Leavitt PR, McKnight DM, Stanford JA, 1997. Assessment of climate change and freshwater ecosystems of the Rocky Mountains, USA and Canada. Hydrol. Process. 11: 903-924.

Hutchinson GE, 1961. The paradox of the plankton. Am. Nat. 95: 137-145.

IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

Jeppesen E, Jensen JP, Søndergaard M, Lauridsen T,1999. Trophic dynamics in turbid and clearwater lakes with special emphasis on the role of zooplankton for water clarity. Dev. Hydrob. 143: 217-231.

Lake PS, 2000. Disturbance, patchiness, and diversity in streams. J. N. Am. Benthol. Soc. 19: 573-592.

Lake PS, 2003. Ecological effects of perturbation by drought in flowing waters. Freshwater. Biol. 48: 1161-1172.

Liang Y, Sarkany N, Cui Y, 2009. Biomass and lipid productivities of *Chlorella vulgaris* under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnol. Lett. 31: 1043-1049.

Lorenzen CJ, 1966. A method for the continuous measurement of *in vivo* chlorophyll concentration. Deep-Sea. Res. 13: 223-227.

Levin SA, Carpenter SR, Godfray HCJ, Kinzig AP, Loreau, M., Losos JB., Walker B, Wilcove DS, 2009. The Princeton guide to ecology. Princeton University Press.

MacKenzie DI, Kendall WL, 2002. How should detection probability be incorporated into estimates of relative abundance?Ecology: 2387-2393.

Mackey RL, Currie DJ, 2001. The diversity-disturbance relationship: is it generally strong and peaked? Ecology: 82 3479-3492.

Magurran AE, 2004. Measuring biological diversity. Blackwell Publishing. Oxford.

Michener WK, Haeuber RA, 1998. Flooding: natural and managed disturbances. BioScience. 48: 677-680.

Milly PC, Dunne KA, Vecchia AV, 2005. Global pattern of trends in streamflow and water availability in a changing climate. Nature. 438: 347-350.

Neary BP, Dillon PJ, 1988. Effects of sulphur deposition on lake-water chemistry in Ontario, Canada. Nature. 333: 340-343.

Odum EP, Finn JT, Franz EH, 1979. Perturbation theory and the subsidy-stress gradient. BioScience, 29: 349-352.

Owen JG, 1988. On productivity as a predictor of rodent and carnivore diversity. Ecology: 1161-1165.

Paerl HW, Huisman J, 2008. Climate - Blooms like it hot. Science, 320: 57-58

Platt T, 1972. Local phytoplankton abundance and turbulence. Deep. Sea. Res. 19: 183-187.

Piasecki W, Goodwin AE, Eiras JC, Nowak BF, 2004. Importance of Copepoda in freshwater aquaculture. Zool. Stud. 43: 193-205.

Pourriot R,1977. Food and feeding habits of Rotifera. Arch. Hydrobiol. Beih. 8: 243-260.

Pickavance JR,1971. The diet of the immigrant planarian *Dugesia tigrina* (Girard): II. Food in the wild and comparison with some British species. J.Anim. Ecol. 40: 637-650.

Prézelin BB, Sweeney BM, 1977. Characterization of photosynthetic rhythms in marine dinoflagellates II. Photosynthesis-irradiance curves and in vivo chlorophyll a fluorescence. Plant Physiol. 60: 388-392.

Reynoldson TB, Young JO, Taylor MC, 1965. The effect of temperature on the life-cycle of four species of lake-dwelling triclads. J. Anim. Ecol. 34: 23-43.

Ricci C, 1984. Culturing of some bdelloid rotifers. Hydrobiologia. 112: 45-51.

Richerson P, Armstrong R, Goldman CR, 1970. Contemporaneous Disequilibrium, a New Hypothesis to Explain the "Paradox of the Plankton". P. Nat. Acad. Sci. USA. 67: 1710-1714.

Richter BD, Braun DP, Mendelson MA, Master LL, 1997. Threats to imperiled freshwater fauna. Conserv.Biol. 11: 1081-1093.

Roelke DL, Eldridge PM, Cifuentes LA, 1999. A model of phytoplankton competition for limiting and non-limiting nutrients: implications for management of dynamic environment. Estuaries 22: 92–104.

Roelke DL, Augustine S. Buyukates Y, 2003. Fundamental predictability in multispecies competition: The influence of large disturbance. Am. Nat. 162: 615–623.

Roxburgh SH, Shea K, Wilson JB, 2004. The intermediate disturbance hypothesis: patch dynamics and mechanisms of species coexistence. Ecology. 85: 359-371.

Rykiel EJ, 1985. Towards a definition of ecological disturbance. Aust. J. Ecol.10: 361-365.

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R,Sanwald EH, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT. Walker BH. Walker M, Wall DH, 2000. Global biodiversity scenarios for the year 2100. Science. 287: 1770-1774.

Schindler DW, 1977. Evolution of phosphorus limitation in lakes. Science.195: 260-262.

Seaman MT, Gophen M, Cavari BZ, Azoulay B, 1986. Brachionus calyciflorus Pallas as agent for the removal of E. coli in sewage ponds. Hydrobiologia. 135: 55-60.

Shannon CE, Weaver W, 1949. The Mathematical Theory of Communication. University of Illinois Press, Urbana.

Sousa WP,1979. Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. Ecology. 60: 1225-1239.

Smith VH, 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: an empirical and theoretical analysis. Limnol. Oceanogr. 27: 1101-1112.

Smith VH, Tilman GD, Nekola JC,1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut.100: 179-196.

Snell TW, Bieberich CJ, Fuerst R, 1983. The effects of green and blue-green algal diets on the reproductive rate of the rotifer *Brachionus plicatilis*. Aquaculture. 31: 21-30.

Sommer U, 1995. An experimental test of the intermediate disturbance hypothesis using cultures of marine phytoplankton. Limnol.Oceanogr. 40: 1271-1277.

Traunspurger W, 2000. The biology and ecology of lotic nematodes. Freshwater. Biol. 44: 29-45.

Turner MG, Dale VH, 1998. Comparing large, infrequent disturbances: what have we learned? Ecosystems. 1: 493-496.

Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Sullivan CA, Liermann CR, Davies PM, (2010). Global threats to human water security and river biodiversity. Nature. 467: 555-561.

Walz N,1995. Rotifer populations in plankton communities: Energetics and life history strategies. Experientia. 51: 437-453.

Wetzel RG 1975. Limnology, Saunders. Philadelphia, PA, 743.

Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E, 1998. Quantifying threats to imperiled species in the United States. BioScience. 48: 607-615.

Woodward G, Perkins DM, Brown LE, 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Phil. T. R. Soc. B. 365: 2093-2106.

Tables

Table 1: Sampling schedule for moderate intensity pulse and high intensity pulse regime

Moderate intensity pulse sampling schedule from May 2013 to August 2013				High intensity pulse sampling schedule from September 2013 to January 2014				
Tanks	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan
T2/T5	03,10,14, 17,22, 24,28,31	04,07,11, 14,18, 21,25,28	03,05,09, 12,16,19 23,26,30	02,07,09, 13,16, 23,27	12,19, 26	03,10, 17 22,25	01,05,08, 12,15,19, 22,26,29	03,06,10, 13,17,20, 24,30	03,07,10, 14,17,21, 23,24,28
T3/T4	02,09,13, 16,21, 23,27,30	03,06,10, 13,17, 20,24,27	02,04,08, 11,15,16 ,22,25,29	01,06,08, 12,15, 19,22,26	10,17, 24	01,08, 15,21, 24,31	07,11,14, 18,25,28	02,05,09, 12,16,19, 23,26,27	03,06,09, 13,16,20, 27,30

FIGURES

Fig. 1: From left to right: Lateral view of tanks used as mesocosms; Sampling station with six sampling ports; Overhead view of "empty" tank; Overhead view of mesocosm.
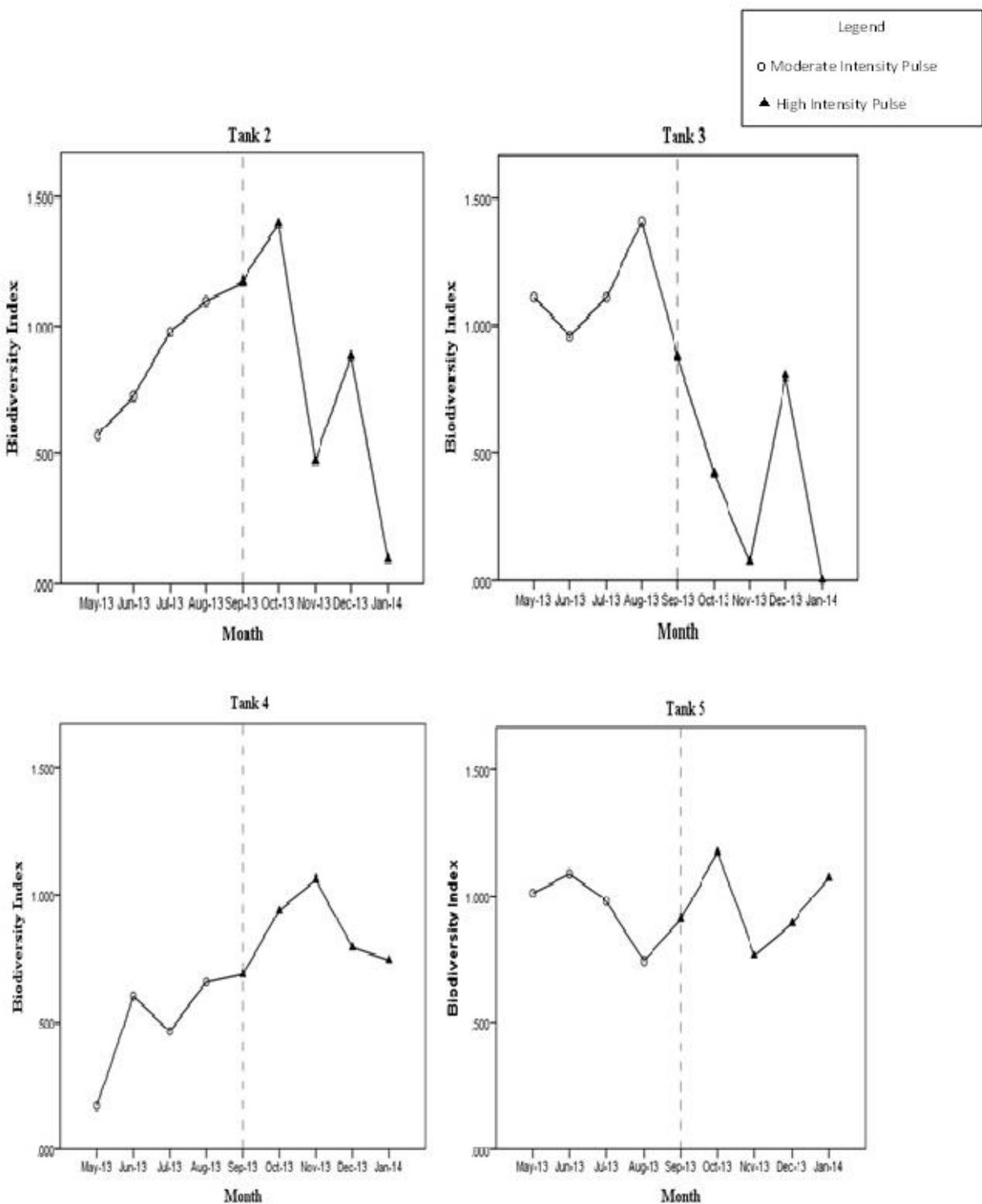
Fig. 2: Biodiversity time series plots for each mesocosm. The Biodiversity index was based on monthly calculation of Shannon -Wiener biodiversity index.
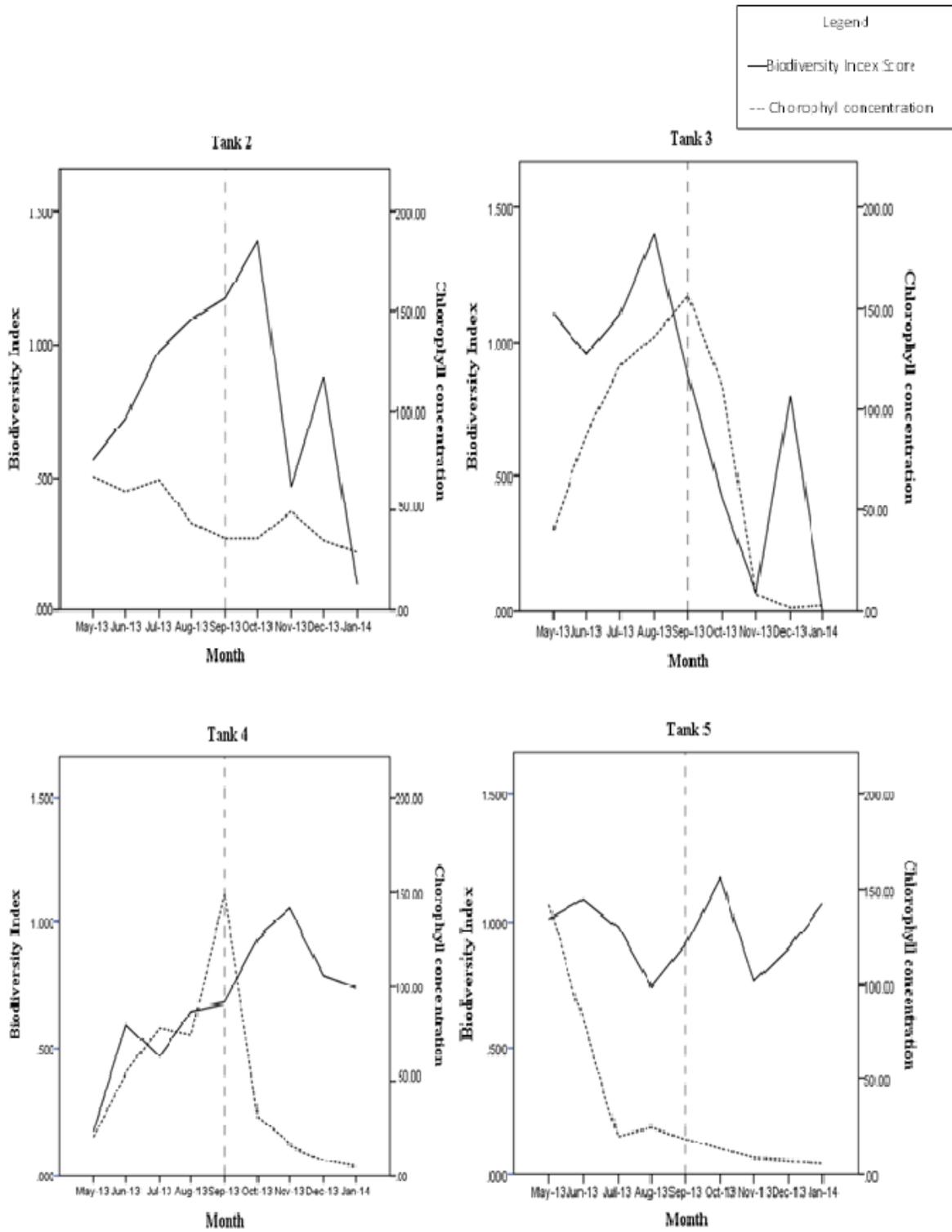
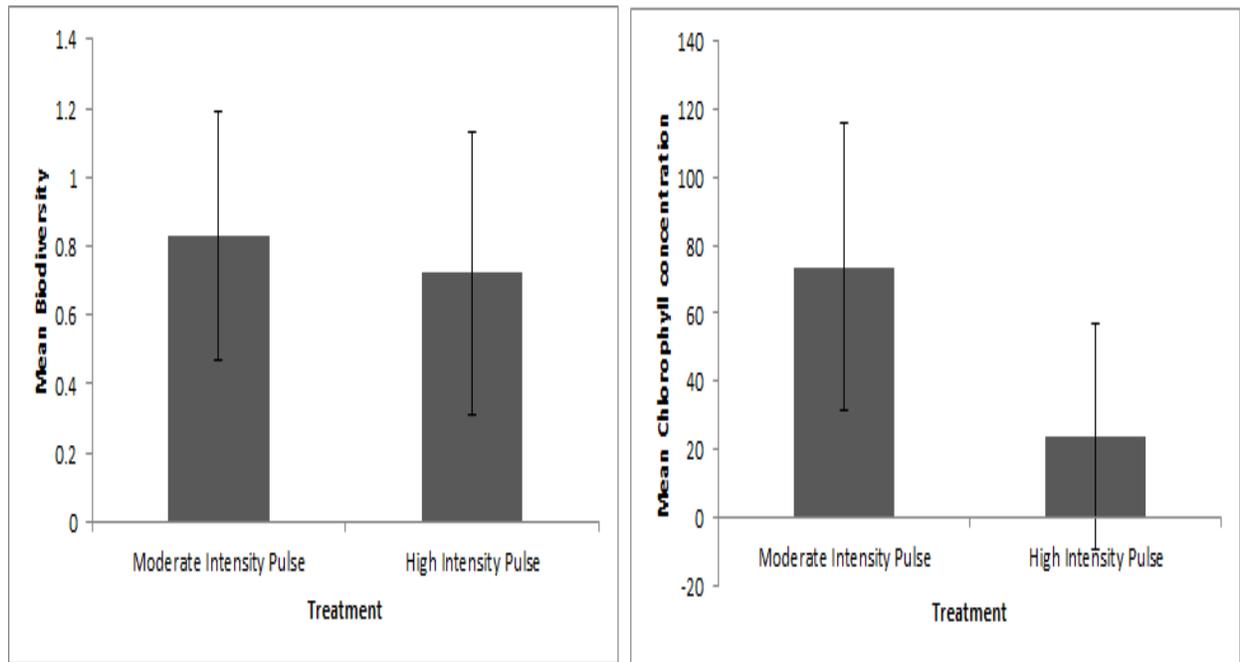Fig. 3: Biodiversity and chlorophyll concentration (µg/L) time series plots.

Fig. 4: Differences in mean biodiversity and mean chlorophyll concentration (µg/L) between nutrient pulse regimes.