A Comparative Analysis of the Effects of Zequanox® on Benthic Substrate Macroinvertebrates and Emergent Insect Populations in an Open Freshwater System

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University of Michigan Biological Station
EEB 482
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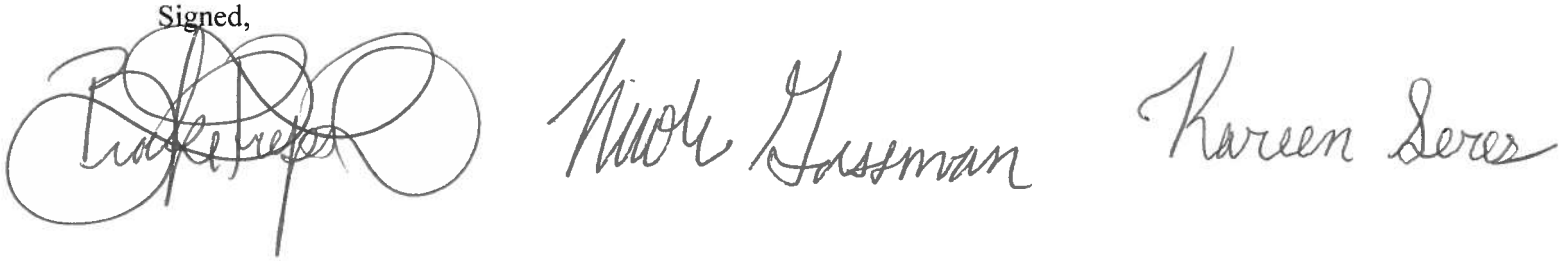

Title A Comparative Analysis of the Effects of Zequanox on Benthic Substrates Macroinvertebrates and Emergent Insect Populations in an Open Freshwater Ecosystem

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Abstract

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Introduction

The zebra mussel, *Dreissena polymorpha,* is an invasive species of bivalve mollusk that is becoming an increasingly important issue in regards to the health and sustainability of freshwater ecosystems (Minchin et al., 2002). As no one component of an ecosystem can exist in isolation, the introduction of an invasive mollusk species is capable of producing immense pressure on the biodiversity and community structure of the system in which it inhabits. Zebra mussels create a severe ecological impact on native mussels, in both direct and indirect ways, and subsequently the ecosystem as a whole. Zebra mussels encompass the ability to firmly attach to any hard surface in which they encounter (Beaver et al., 1995). All mussel species contain proteinaceous fibers produced within the shell of the mussel that aid in movement and attachment that are known as byssal threads (Schmitt et al., 2015). The atypical adhesive qualities of byssal threads of zebra mussels are one of the main factors that give them their tremendous invasive capabilities as the advanced anatomical feature allows them to adhere tightly to nearly any solid surface (Dodds & Whiles, 2010). Adhesion of zebra mussels to native mollusks causes natives to experience immobility and reduction in bivalve motion, directly killing the native species (Beaver et al., 1995).

Although the danger of zebra mussels may appear most prominent as a result of direct contact in the form of adhesion to native mollusks, the greatest harm is exerted on native mollusks in the zebra mussel's indirect ability to outcompete natives for resources via the mussels' immense filtering capacity (Vanderploeg et al., 2011). Both native mollusks and zebra mussels are filter feeders and feed primarily on phytoplankton in the water column (Hulsmann & Galil, 2002). An increase in zebra mussels results in a direct decrease in phytoplankton able to be eaten by natives and ultimately results in a decrease in total phytoplankton populations. Changes in phytoplankton populations may result in algal blooms which would create mass disturbance to the overall ecology of the freshwater system (Hallegraef, 1998). Since the zebra mussels consume the phytoplankton at a greater volume than the zooplankton in the water does, fish and native mollusk species are subsequently affected which will also create substantial disturbance (Nalepa & Schloesser, 1993).

Favoring slow moving waters, the mollusks are most commonly found in lakes and low-velocity rivers across the United States and Europe (Minchin, Lucy, & Sullivan, 2002). A native of Eastern Europe, the zebra mussel first appeared in Western Europe in the late 1700s and began rapidly spreading after the construction of major canal systems (Nalepa & Schloesser, 2013). This rapid dispersal only escalated with time as the invasive species traveled across the Atlantic via freshwater ballast stowaway in commercial vessels as transportation of goods via ship increased with economic demand (Dextrase & O'Neill, 1994). Transportation across the Atlantic ultimately resulted in the establishment of zebra mussels in North America in 1989 with the first known discovery being in Lake St. Clair in Saint Clair Shores, MI (Murawski, 2016). Due to their aggressive dispersal rates, zebra mussels have spread to all 5 Great Lakes and

surrounding inland lakes and have been estimated to total in numbers as high as 750 trillion mollusks in the state of Michigan alone (Lavey, 2017).

Due to their ability to firmly attach to nearly any hard surface, zebra mussels have created more than just ecological disturbance, but have also cost humans, particularly the water industry, millions of dollars in damage. Zebra mussels adhere to water pipes causing increased sedimentation and corrosion to the pipes as well as restricting or completely stopping water flow (Whitledge et al., 2014). The Great Lakes have taken a substantial economic hit with restoration and treatment costs totaling $267 million between the years 1989-2004 (Nalepa & Schloesser, 2014). Maintenance of clogged pipes costs power industries an estimated $60 million per year and is predicted to cost the United States in total $3.1 billion over the next 10 years (French, 2017). Zebra mussels first fell under public scrutiny in the early 1990s when water pumping abilities were compromised and consequently water shortages occurred in numerous cities in southeastern Michigan (Dextrase & O'Neill, 1994).

As ecologic and economic issues have arisen and advanced in frequency and degree, Marrone Bio Innovations created a product called Zequanox® in an attempt to aid in the abolition of zebra mussels from freshwater ecosystems. Zequanox® is composed of dead cells from a naturally occurring nonpathogenic saprophyte, *Pseudomonas fluorescens,* that colonize soil and water and plant surface environments (Ganeshan & Kumar, 2005). The bacterium is recognized by both native and invasive mussels as a source of food and is readily consumed (Whitledge et al., 2014). The dead bacterium is a highly selective toxic substance which specifically attacks and destroys the digestive linings of zebra mussel's but is non-toxic to a wide variety of non-target organisms such as fish, daphnids, ciliates, and native mussels (Sousa et al.,

2012). The destruction of the mussel's digestive system results in the death of the mussel soon thereafter (Whitledge et al., 2014). There is no known literature as to how Zequanox® works to specifically target the digestive linings of zebra mussels while simultaneously leaving the digestive systems of non-target organisms unharmed. Zequanox® is the only molluscicide registered by the EPA with no known effects on native mollusks (Claucherty, 2017).

Although created with intentions to cease mass spread of zebra mussels in larger, more problematic bodies of water, small inland lake zebra mussel quantities have become of increasing importance since the early 2000s (Whitledge et al., 2014). The invasive species has initial explosive population growth typically followed by a decline to more stable densities (Whitledge et al., 2014). With noted presence of almost 20 years, inland lake zebra mussel populations are increasing in stability which may indicate a good time to seek treatment of the issues the mussels have created. In less than 20 years, the impact zebra mussels have made on inland ecosystems has created a sense of urgency in regards to the rehabilitation of the native mussel populations and biodiversity of the systems as a whole. Northern Michigan inland lakes, such as Round Lake (45.4089° N, 84.8925° W) in Emmet County, MI have suffered noticeable damage to their native mussel populations due to the invasion of zebra mussels (Whiteledge et al., 2017). For this reason, Round Lake was chosen as the location for study. Round lake was also chosen because of lake size, low flow input, and low flow output within the watershed. Round Lake is a recreational lake with an area of 1.35 km^2 and is approximately 16ft at its deepest point (Round Lake, 2017).

The study took place via coordination with Tip of the Mitt Watershed Council, the United States Geological Survey (USGS), and the Environmental Protection Agency (EPA). The core of

the study was led by Tip of the Mitt under the leadership of Matt Claucherty, project manager. The study aimed to analyze the effects that Zequanox® produces on the biodiversity and overall health of the freshwater ecosystem in which the product is applied. This study was the first time Zequanox® had ever been applied to an open water system (Claucherty, 2017). Although there have been no published reports that state Zequanox® produces any effects on native mollusks, there is still very little known in regards to the product's effects on other aspects of the ecosystem. Previous studies have found that, when administered in a closed system and at EPA approved dosages, Zequanox® does not produce effects on macroinvertebrate, specifically amphipoda, species richness (Waller et al., 2016). Nor has Zequanox® been shown to produce effects on chironomidae species richness at benthic substrate levels (Meehan et al., 2014). Our study analyzed the effects that Zequanox® produces on the pelagic water column by analyzing the effects on macroinvertebrates' metamorphic and hence emergence timing. emergent abilities. To align our hypotheses with previous findings, we hypothesize that Zequanox® will not affect benthic substrate macroinvertebrate richness and evenness and therefore, will not affect emergent insect population richness or evenness in Round Lake, Emmet County, MI.

Methods

Trap Construction

This study is a comparison of control and treatment plots looking at data collected pre-treatment and post-treatment. Two different traps were used in attempt to survey the biodiversity of both macroinvertebrates at the benthic substrate and emergent insects at the surface.

For macroinvertebrate sampling, Hester Dendy traps were constructed. A Hester Dendy trap was comprised of a series of seven pressboards 10cm x 10 cm held together by six steel washers, one eyebolt, and a wingnut (Figure 1). Stability of the Hester Dendy trap was ensured by a cement block attached with rope and two zip ties to the bottom of the trap. A 30 cm size cylindrical polyethylene foam was tied to the eyebolt on the top of the Hester Dendy trap with rope. Traps were placed in each plot, totaling 8 total Hester Dendy traps. The plots were approximately 1 meter deep. The traps were placed approximately 15 cm off the bottom of the lake and 70 cm from the water's surface.

Figure 1: Hester Dendy Trap

Figure 1 displays an image of a replica of one of the Hester Dendy Traps used in this experiment.

For emergent insect sampling, floating emergent insect traps were constructed using 1 m x 1 m mesh fabric, 60 cm x 60 cm polyvinyl chloride (PVC) frame material, and 60 cm x 60 cm pool noodles on the exterior to allow the trap to float. The traps were designed into a square pyramid shape according to the directions in the paper "Low-cost floating emergence net and bottle trap: comparison of two designs" in the *Journal of Freshwater Ecology* by Cadmus et. al. (Figure 2). Equipment used in this process included a handheld saw, Amazing Goop ® , power drill, and Marson 39000HP2 Rivet Tool. Some small alterations were made on their design, such that only clips

Figure 2: Emergent Insect Traps

Figure 2 shows the emergent insects traps, with appropriate modifications, used in this study.

were included that held the noodle. Clips that held the net were not included in our design.

There was an external collection bottle made by two 500 mL polystyrene bottles in the trap filled with 200 mL of 90% ethanol. The trap was designed to funnel insects toward the bottle opening at the top of the net for capture. Cement blocks were attached with rope to the trap to create anchorage. Two traps were placed in each plot, ultimately producing 16 total emergent insect traps.

Study Sites and Application

Four sites of the lake were chosen to be studied and surveyed by USGS. Pre-treatment analysis of native mollusk and zebra mussel abundance was conducted within each treatment and control plot to ensure homogeneity (Claucherty, 2017). Substrate and bathymetry, wind fetch, access, and public use were deciding factors in plot locations (Claucherty, 2017).

Two square plots, 55 m x 55 m, were made in each of the four sites (Figure 3). Flag buoys were placed at the corner of each plot and GPS coordinates were recorded approximately 30 days prior to treatment. Within each of the four areas there was one control plot and one treatment plot, totaling eight plots in the entire study. Treatment and control plots were paired close together to reduce spatial bias. Plots 2, 3, 6, 8 were control plots. And plots 1, 4, 5, 7 were treatment plots. Control plots were spaced at least 25 meters to assure that the control plot is not affected by Zequanox® drift (Claucherty, 2017).

Figure 3 : Study Plots on Round Lake, Emmet County, MI

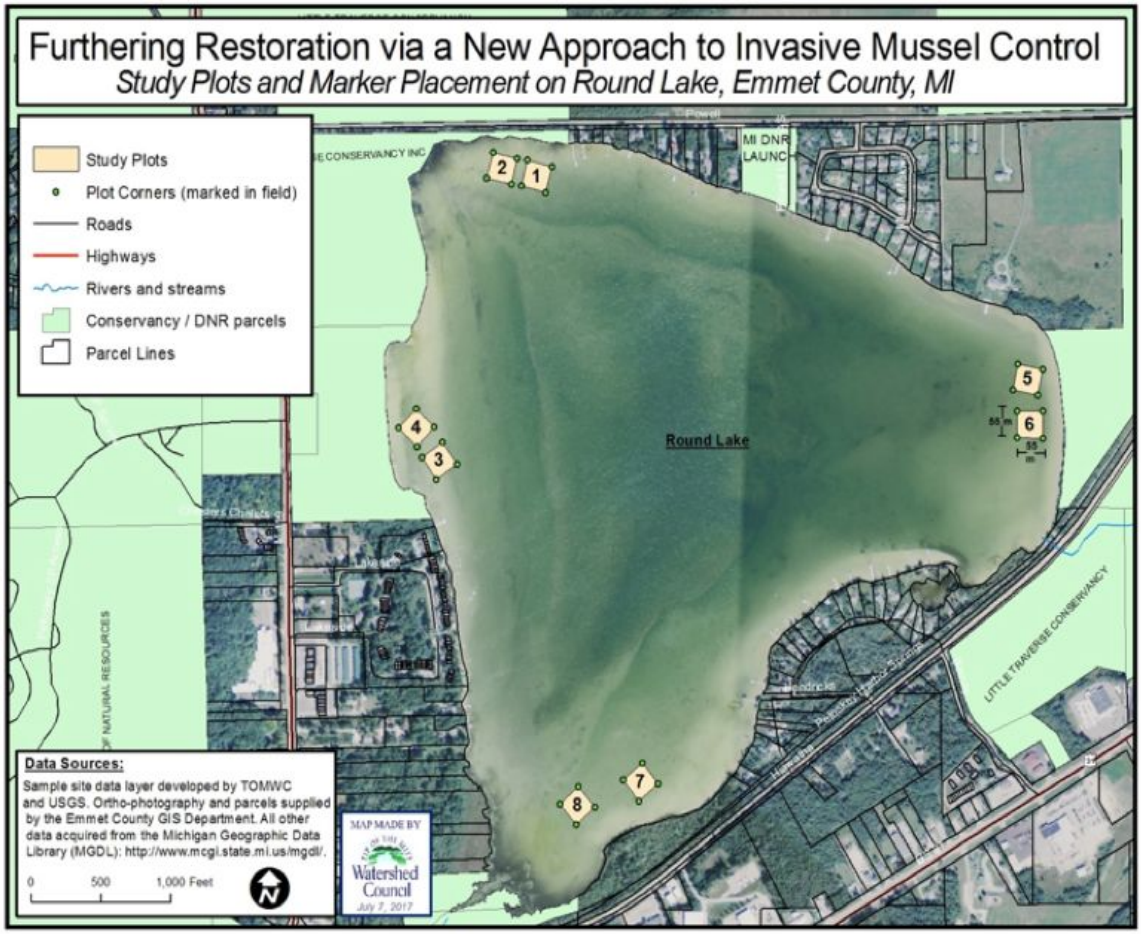

Figure 3 shows a satellite image of Round Lake, Emmet County, MI with study plots.

One week before Zequanox® was sprayed, on 7/20/17 to 7/21/17, pre-treatment data was collected. Two emergent insect traps were placed for 24 hours at the center of each of the eight plots, totaling 4 traps per site, and 16 total traps for the entire pre-treatment portion of the study. Traps were placed inside the parameters of the plots and were aimed for the center to ensure both visibility and Zequanox® exposure. The insects caught in the trap were placed into scintillation vials containing ethanol.

One Hester Dendy trap was also placed on 7/20/17 to 7/28/17 for a one week time period at each of the eight plots. The noodle and rope attached to the Hester Dendy trap was tied around the flag at the corner of each of the plots. It was ensured that the Hester Dendy trap was not touching the bottom of the lake, but was floating in the benthic zone. The Hester Dendy trap was untied from the flag buoy on 7/28/17.The wingnut was unscrewed and the seven pressboards were taken apart and laid in an open metal container. Tweezers were used to scrape macroinvertebrates into a 250 mL polystyrene bottles containing ethanol that was labeled according to plot number.

Physical application was dependent upon a variety of abiotic factors, most notably weather. Spraying was scheduled to be halted due to conditions such as rain or wind, as such events could cause disturbance in the water and/or heightened dispersal rates of Zequanox® (Claucherty, 2017). Site exposure is listed in the order, with corresponding dates, in which they were sprayed (Table 1). Zequanox® was applied to plot four on 7/27/17, plot five on 7/29/17, plot one on 7/31/17, and plot seven on 8/2/17. A Zequanox® stock solution of approximately 20% weight/volume was prepared using Zequanox® and lake water mixed in the application system, which was then immediately applied underwater to ensure that a 100 mg/L concentration of the active ingredient reached the bottom 0.75 m of the water column (Claucherty, 2017). The application process was executed by USGS Upper Midwest Environmental Sciences Center and involved spraying treatment plots via a custom-built application system and a flat-top work barge (Claucherty, 2017). For sake of continuity, application boats transversed both control and treatment plots to create equal levels of disturbance amongst the plots as to ensure physical disturbance did not affect the study in any way.

Table 1: Site Exposure and Application Dates

Site	Application Date	Control	Treatment
1	7/27/17	Plot 3	Plot 4
2	7/29/17	Plot 6	Plot 5
3	7/31/17	Plot 2	Plot 1
4	8/1/17	Plot 8	Plot 7

Table 1 displays site numbers with corresponding application dates for each, as well as which plot within the site was control and which was treatment.

One day after Zequanox® was applied to a plot, post-treatment data was collected. Insect traps were placed for a 24 hour period at the center of each of the eight plots. Post-treatment data was collected for plots four and three from 7/28/17 to 7/29/17, plots five and six from 7/30/17 to 7/31/17, plot one and two from 8/1/17 to 8/2/17, and plots seven and eight 8/3/17 to 8/4/17. The insects caught in the trap were placed into scintillation vials containing ethanol

One day after Zequanox® was applied, a Hester Dendy trap was placed for a one week time period at each of the eight plots. Post-treatment data was collected for plots four and three from 7/28/17 to 8/4/17, plots five and six from 7/30/17 to 8/6/17, plots one and two from 8/1/17 to 8/8/17, plots seven and eight from 8/3/17 to 8/10/17. The macroinvertebrates were collected in a 250 mL polystyrene bottle as described above.

Insect and Macroinvertebrate Classification

Insects collected in the polystyrene bottles of the traps were placed into scintillation vials containing ethanol and taken back to The University of Michigan's Biological Station (UMBS)

where the samples would later be recorded, analyzed under a Leica EZ4 dissection microscope, and categorized. Insects were analyzed under a dissection microscope and categorized by family according to a taxonomic key that Dr. Pillsbury and Cassidy Carroll created (Table 2). Categorization was determined by distinct visual differences in anatomical features such as body length, antenna detail, noticeable bodily patterns, differences in legs, eyes, wings, etc. Drawings of distinct features were constructed and photos of insects from each newly discovered taxa were taken and included in the identification key for sake of continuity during the classification process. Insects were classified into 15 different taxa with the intention of analyzing any differences in richness and evenness before and after Zequanox® applications.

Table 2: Emergent Insect Classification Key

Emergent Insect Classification Key

Taxa	Description
Taxa A	3-5mm, ocelli, claspers on males, furry antennas for females, hairy wings, hump with 4 distinct lines that do not touch, long legs relative to body size
Taxa B	4 mm, segmented antenna, segmented abdomen with horizontal dark stripes on each of the segments, abdomen diverges to a sharp point
Taxa C	2 mm, spotted beaded nose, some hair on the wings, eyes are touching, tergite has blotches of setae, there is an enlarged portion of the antenna near the head
Taxa D	Similar to taxa A, but with longer, more slender bodies, feathery antennae, purple and long extending front legs
Taxa E	Is around 1.5mm large with very little pigment, ocelli
Taxa F	3mm, similar to Antennae have bristles, ocelli, and its legs are long, have no stripes, comb like hairs, and purple rings, hair on the edges of the wings, legs extend to the top of the abdomen
Taxa G	2 mm, has striped legs, 2 dark stripes on back and a white spot in middle, rounded eyes, hairy and blotchy wings
Taxa H	3mm, large external segment on head, round eye shape, beaded antennas
Taxa I	Really big wings, antenna protrude out from side of head at 90 degree angles, beaded antennas
Taxa J	Similar to D, but smaller and more slender. J has defined, dense, armored hunch.
Taxa L	Hairy wings with eyes touching
Taxa N	2 sets of wings, red eyes, long protruding V-shaped antennas
Beetle	Has two antennae, compound eyes, a thorax, two pairs of wings, three pairs of legs, and an abdomen
Damsel Fly (Coenagrionidae)	Similar to dragonflies, but with smaller and slimmer bodies with wings that fold along its body
Wasp	Two pairs of membranous wings, a stinger (only in females), antennae, smooth bodies without hair, 3 pairs of yellow legs, is 1.5-2cm in length, black, brown, red, yellow, or blue in color

Table 2 gives brief descriptions on how emergent insect taxa were constructed and classified.

Macroinvertebrates caught in the Hester Dendy traps..The Hester Dendy traps were collected and macroinvertebrates were scraped into a 250 mL collection bottle containing ethanol. The macroinvertebrates were taken back to UMBS to be

analyzed under a microscope and categorized by family according to the Aquatic Insects of North America by Merritt and Cummins (Merritt, 1984).

Physical and Chemical Data

Physical and chemical data were collected on 8/2/2017 for Round Lake to analyze the ecology of each of the plots in the lake (Table 3). The eureka HydroLab Manta 2 Model: sub 3 Amphibian 2 probe was used at each of the plots. The HydroLab read the temperature, pH, dissolved oxygen, conductivity, and turbidity, and was recorded for each of the plots.

Table 3: Hours Post Zequanox Treatment in which Chemical Data was Obtained

Hours Post Zequanox Treatment	Plot #	Control/Treatment
0	7	Treatment
0	8	Control
24	1	Treatment
24	2	Control
48	5	Treatment
48	6	Control
72	3	Control
72	4	Treatment

Table 3 depicts the number of hours post Zequanox application in which each plot had chemical data obtained at.

Data concerning sediment was collected at each of the eight plots using an Ekman grab. The Ekman grab was lowered in the relative center of the plot to pull up substrate that was released into a 13 liter bucket to be analyzed. This was done twice to fill the bucket up about 6 liters full. The substrate was then placed in a Fisher Scientific USA Standard Testing Sieve with six gradations and shaken for five minutes. The sediments were distributed according to size.

The top layer held sediment 2 mm or greater. The next layers held sediment 1-2mm in size, 0.5-1mm in size, 0.25-0.5mm, 125-0.25mm in size, and 0.025-0.125mm in size. The six gradients of the sediment sieve were separated and displayed, to estimate a relative percentage of how much sediment each layer contained.

Water was collected for chemical analysis at the center of each of the eight plots and the center of the lake. Water from Round Lake was used to rinse the polystyrene bottle and a 50 mL filtered syringe to prevent cross contamination of the water sample. A piece of 0.45 μm Hawp type micron filter paper was retrieved with metal tweezers and placed on the filter attachment of the syringe. The filtered syringe was filled with water from Round Lake and the filter adaptor was attached to the mouth of the syringe. The plunger was used to filter the water into the polystyrene bottle. The filter adaptor was removed from the syringe and the plunger was taken out. This process was repeated for a second time, so that there was approximately 150 mL of water in the polystyrene tube. This water was sent to the UMBS Analytical Facilities to be analyzed for nitrate, ammonia, total nitrogen, phosphate, total phosphate, and silicon dioxide. The filter paper containing phytoplankton was removed from the filter syringe with tweezers and wrapped in aluminum foil to test the paper for chlorophyll A.

Statistical Analyses

For the chironomids and amphipod family of macroinvertebrate as well as the total number of macroinvertebrate, a 2 x 2 fully factorial MANOVA test was conducted between the pre-treatment and post-treatment macroinvertebrate in the control and treatment plots. For taxa A and taxa J insects as well as the total number of insects, a 2 x 2 fully factorial MANOVA test

was conducted between the pre-treatment and post-treatment insects in the control and treatment plots. Both of the 2 x 2 MANOVA test was run for multiple dependent variables including time and exposure.

Results

When physical and chemical data was taken Zequanox® had been present in some plots longer than others. The difference in time of Zequanox® presence is due to Zequanox® being applied to certain plots on certain days *(Table 4)*. To account for this, the data is arranged by time since Zequanox® application. Time since application is measured in hours, with time = 0 meaning Zequanox® was applied the day of sampling. A difference in the pH between control and treatment plots was observed 24 hours since Zequanox® application *(Figure 4)*. Dissolved oxygen peaked 48 hours after Zequanox® application for both the control plots and treatment plots *(Figure 5)*.

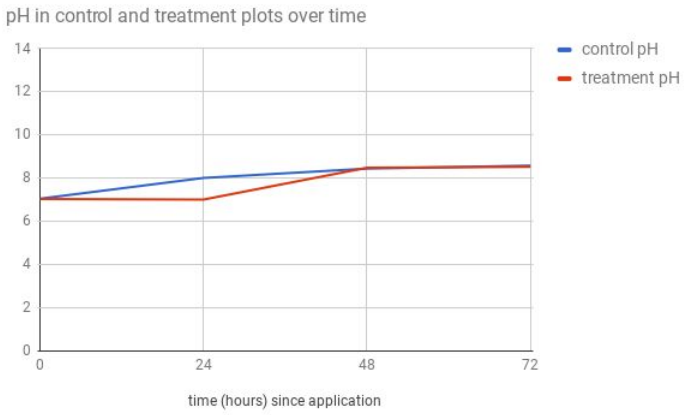

Figure 4*. pH increased in the control plots 24 hours after Zequanox® application.*

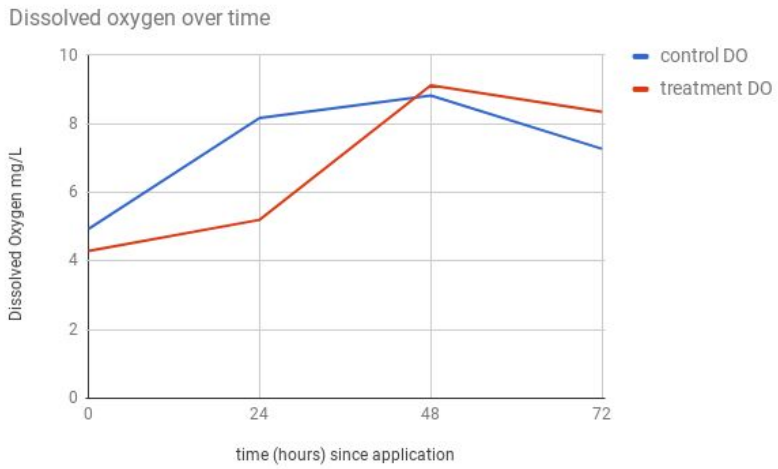

Figure 5. *Dissolved oxygen increased in both the control and treatment plots between 0 and 48 hours since application. Dissolved oxygen decreased in both the control and treatment plots after the 48 hours mark.*

Concentrations of nitrate, phosphate, ammonium, and chlorophyll-A were observed over time in the control and treatment plots. In the treatment plots, nitrate decreased between 0 hours and 24 hours after Zequanox® application, and then rapidly increased after 48 hours since Zequanox® application *(Figure 6)*. In the control plots, nitrate increased between 0 hours and 48 hours since Zequanox® application, thereafter the concentration plateaued at a concentration of 0.13 mg/L *(Figure 6)*. Phosphate concentrations decreased between 0 hours and 24 hours since Zequanox® application in both the control plots and the treatment plots *(Figure 7)*. Concentrations of ammonium in the treatment plots rapidly decreased between 0 hours and 24 hours since Zequanox® application, and then began to increase after 48 hours after Zequanox® application *(Figure 8)*. In the control plots, ammonium concentrations peaked at 48 hours since Zequanox® application with a concentration value of 30.3 ug N/L *(Figure 8)*. In both the control and treatment plots Chlorophyll-A concentrations decreased between 0 hours and 24 hours since Zequanox® application, whereafter concentrations increased *(Figure 9)*.

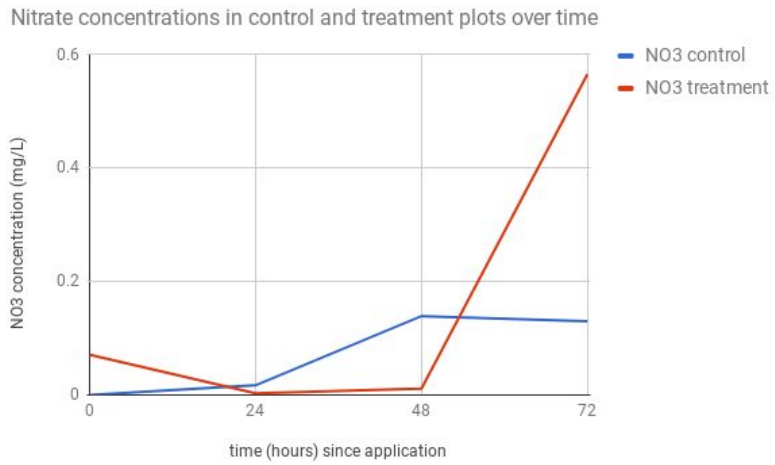

Figure 6. *The trend of increase of NO3 concentrations in treatment plots was not observed in the control plots.*

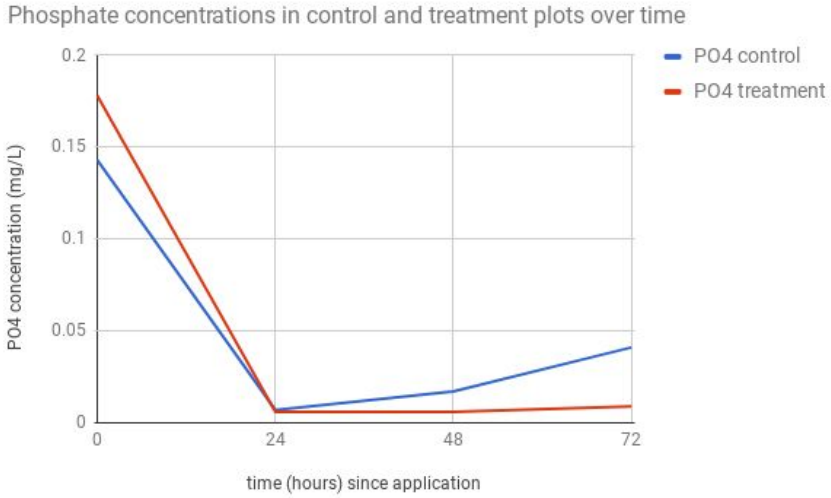

Figure 7. *The trend of decrease in PO4 concentration in the control and treatment plots was similar.*

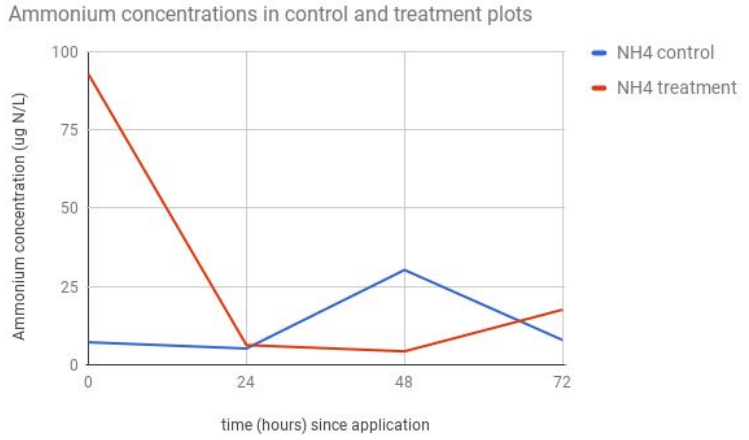

Figure 8. *The peak in ammonium concentration took place in the treatment plots prior to the peak in the control plots. The treatment plot peak concentration was noticeably higher than the concentration in the control plots.*

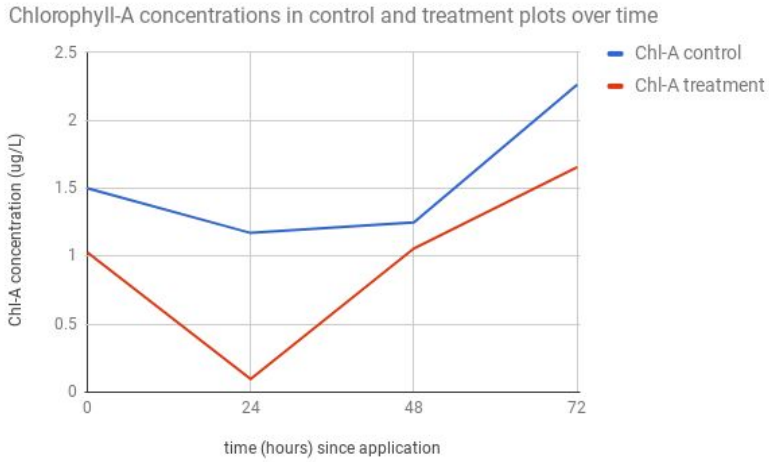

Figure 9. *The vertex of decrease for both control and treatment plots occurred 24 hours since Zequanox® application.*

The time factor for total macroinvertebrates was tested first. A time effect was observed for total macroinvertebrates between pre-treatment and post-treatment. There was a significant difference in the number of total macroinvertebrates collected between pre-treatment and post-treatment (Wilks lambda=.09435, F(6, 7)=11.199, p=.00274) (*Figure 10).* Corresponding to the trend in total macroinvertebrates, there was also a significant difference in both the number

of chironomids and amphipods collected between pre-treatment and post-treatment (Wilks lambda=.09435, F(6, 7)=11.199, p=.00274) *(Figure 11) (Figure 12)*.

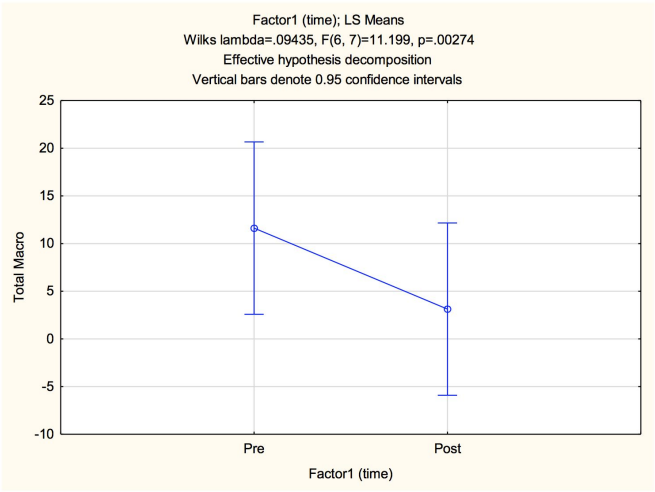

Figure 10 *There was a difference in the number of total macroinvertebrates from pre-treatment to post-treatment. The total number of macroinvertebrates decreased from pre-treatment to post-treatment.*

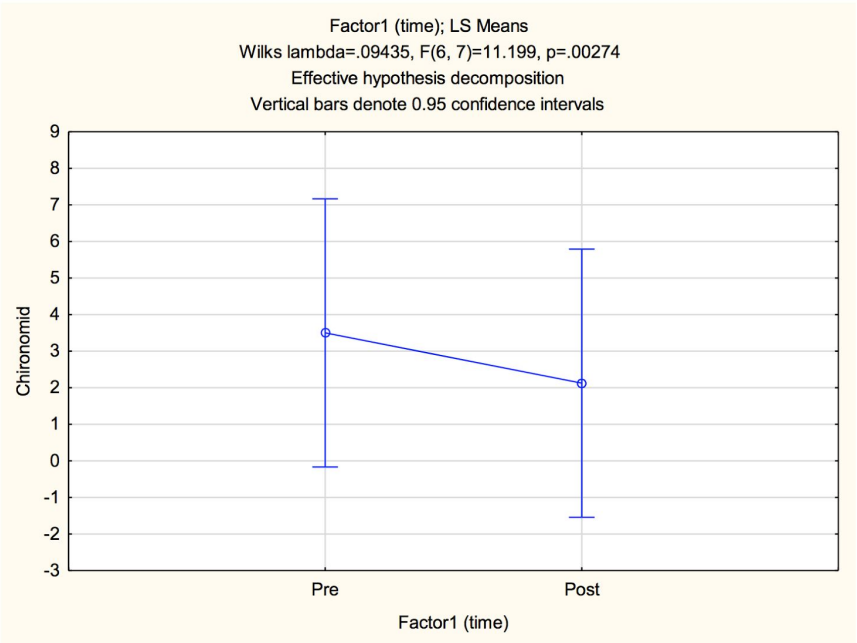

Figure 11 *There was a difference in the number of chironomids between pre-treatment and post-treatment. There was a decrease in number of chironomids from pre-treatment to post-treatment.*

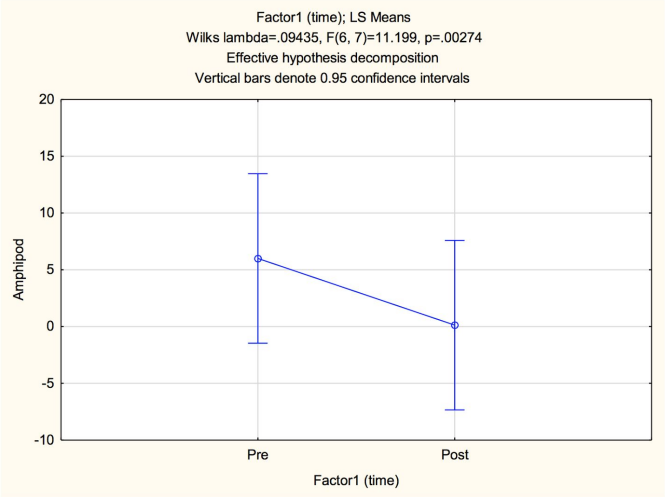

Figure 12 *There was a decrease in the total number of amphipods between pre-treatment and post-treatment.*

The time effect was also observed in similar fashion for the emergent insects between pre-treatment and post-treatment. There was a significant difference in the number of total emergent insects collected between pre-treatment and post-treatment (Wilks lambda=.09435, F(6, 7)=11.199, p=.00274) *(Figure 13)*. Total Taxa J between pre-treatment and post-treatment also follows this decreasing trend although not at a level of statistical significance *(Figure 14)*.The number of total Taxa A between pre-treatment and post-treatment was significantly different. The standard error bars do not overlap *(Figure 15)*.

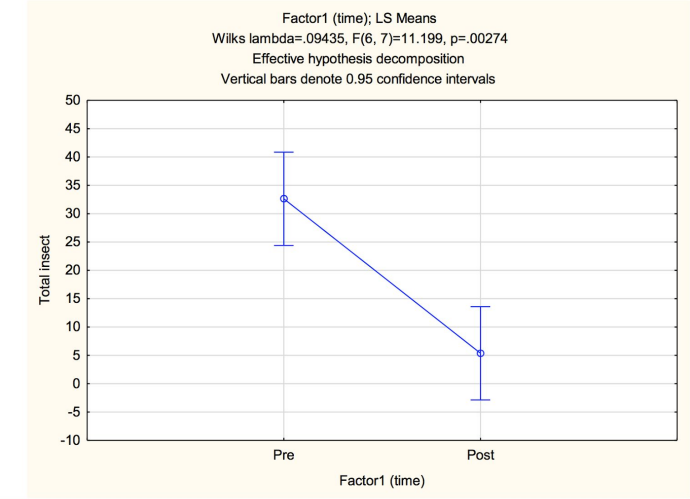

Figure 13 *There was a decrease in the number of total emergent insects over time.*

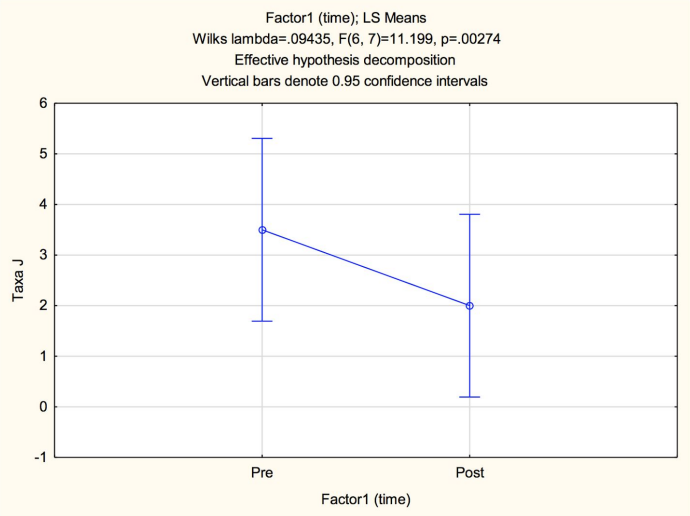

Figure 14 *There was a decrease in the number of total Taxa J insects over time.*

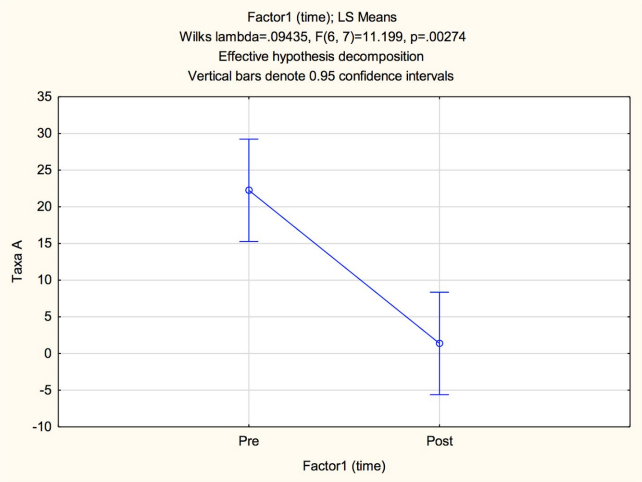

Figure 15 *There was a decrease in the total number of Taxa A insects between pre-treatment and post-treatment times.*

The second factor tested was exposure between control and treatment plots. There was no exposure effect observed between control and treatment plots for any of the variables. There was no significant difference for total macroinvertebrates between control and treatment plots (Wilks lambda=.50854, F(6, 7)=1.1275, p=.43350) *(Figure 16)*. The difference between control and treatment plots for chironomids was also not significant (Wilks lambda=.50854, F(6, 7)=1.1275, p=.43350) *(Figure 17)*. Total amphipods between control and treatment plots followed this trend and was not significant (Wilks lambda=.50854, F(6, 7)=1.1275, p=.43350) *(Figure 18)*.

There was no exposure effect observed for emergent insects. The difference of total emergent insects between control and treatment plots was not significant (Wilks lambda=.50854, F(6, 7)=1.1275, p=.43350) *(Figure 19)*. There was no significant difference for total Taxa A (Wilks lambda=.50854, F(6, 7)=1.1275, p=.43350)*(Figure 20)* or Taxa J (Wilks lambda=.50854, F(6, 7)=1.1275, p=.43350)*(Figure 21)*.

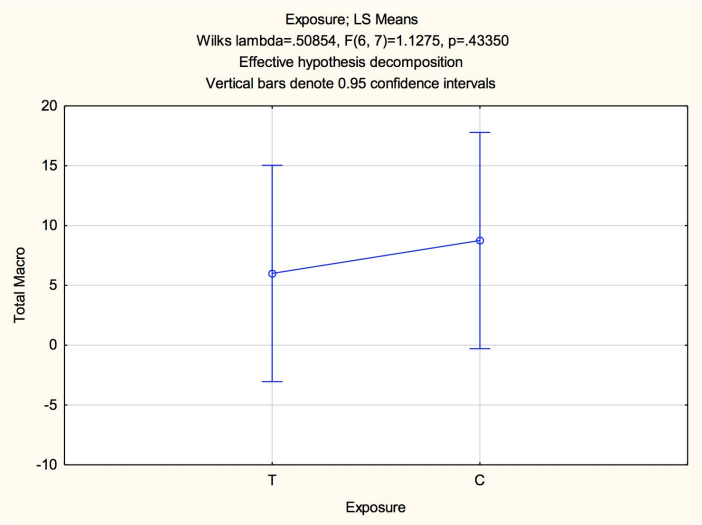

Figure 16 *There was a lower number of total macroinvertebrates found in the treatment plots than there was in the control plots.*

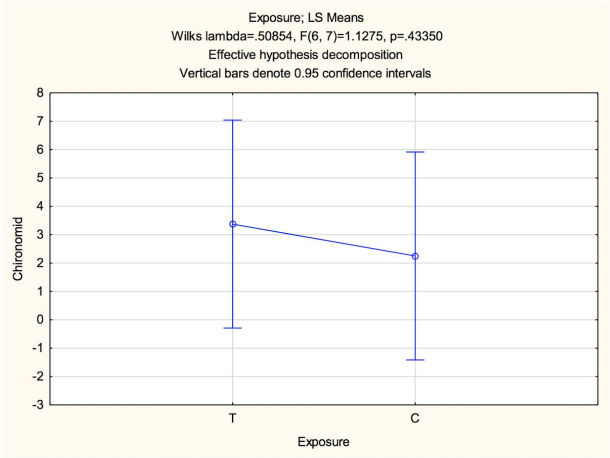

Figure 17 *There was a higher total number of total chironomids in the treatment plots than in the control plots.*

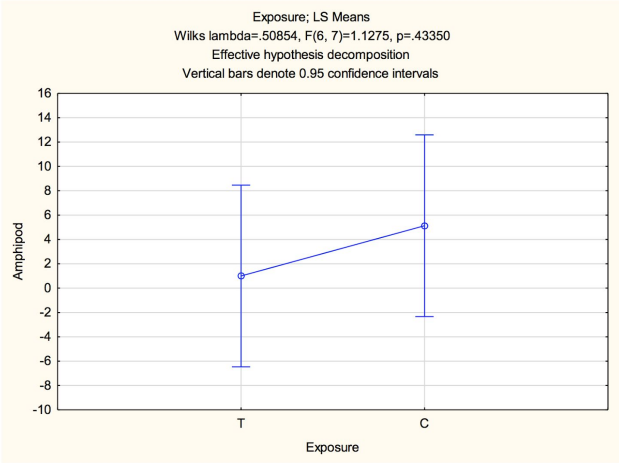

Figure 18 *There was a lower total number of amphipods found in the treatment plots than there were found in the control plots.*

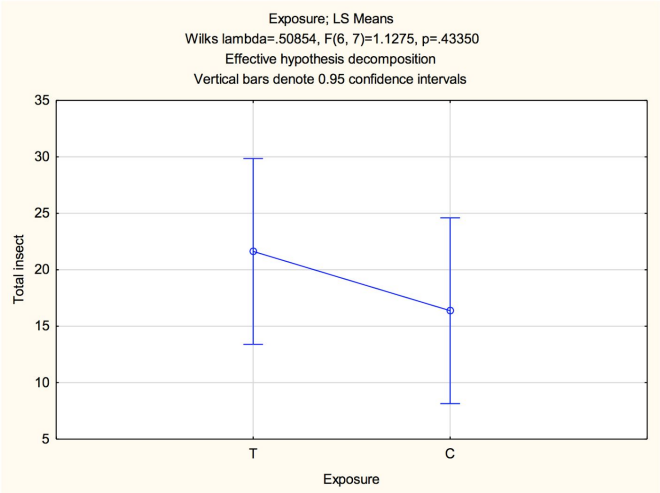

Figure 19 *There was a higher total number of emergent insects found in the treatment plots than in the control plots.*

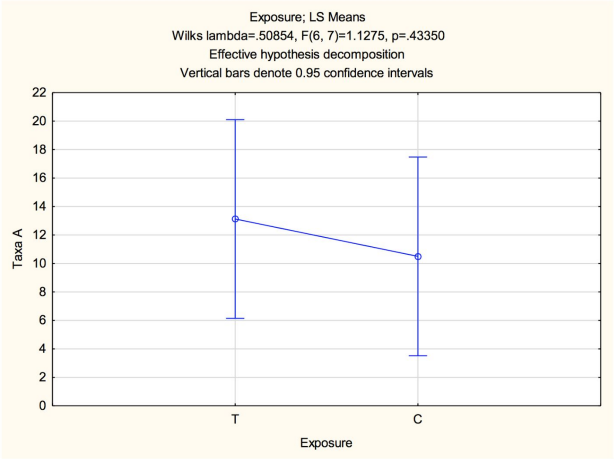

Figure 20 *There was a higher number of total Taxa A found in the treatment plots than in the control plots.*

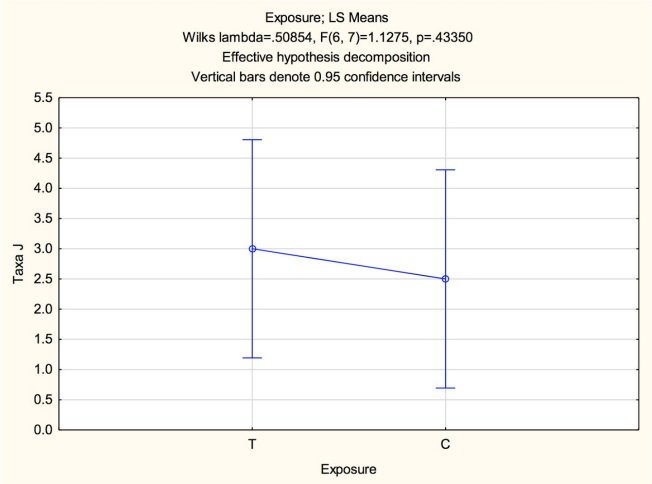

Figure 21 *There was a higher number of Taxa J found in the treatment plots than in the control plots.*

The interaction effect for macroinvertebrates cross-compared both the time and exposure factors. There was not a significant difference for total macroinvertebrates between pre-treatment and post-treatment for either the control or treatment plots (Wilks lambda=.53989, F(6,

7)=.99425, p=.49462) *(Figure 22).* An interaction trend was observed for chironomids; however this was not a statistically significant difference (Wilks lambda=.53989, F(6, 7)=.99425, p=.49462)*(Figure 23).* There was not a significant interaction trend for amphipods either (Wilks lambda=.53989, F(6, 7)=.99425, p=.49462) *(Figure 24).*

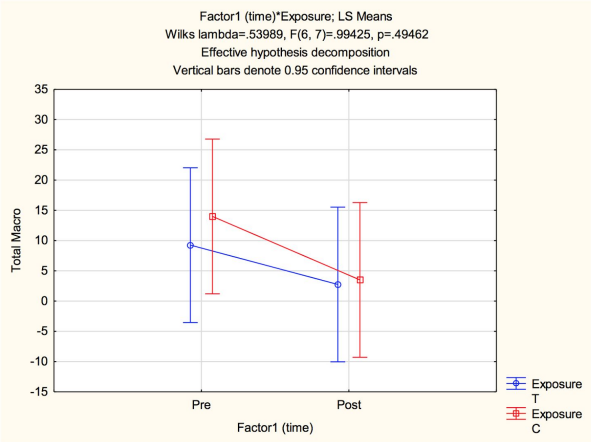

Figure 22 *The total number of macroinvertebrates decreased between pre-treatment and post-treatment for both the treatment plots and the control plots.*

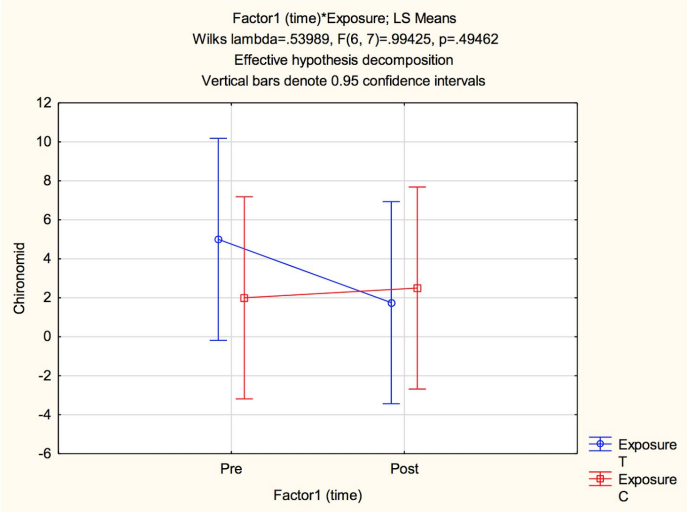

Figure 23 *The number of chironomids between pre-treatment and post-treatment decreased in the treatment plots and increased in the control plots.*

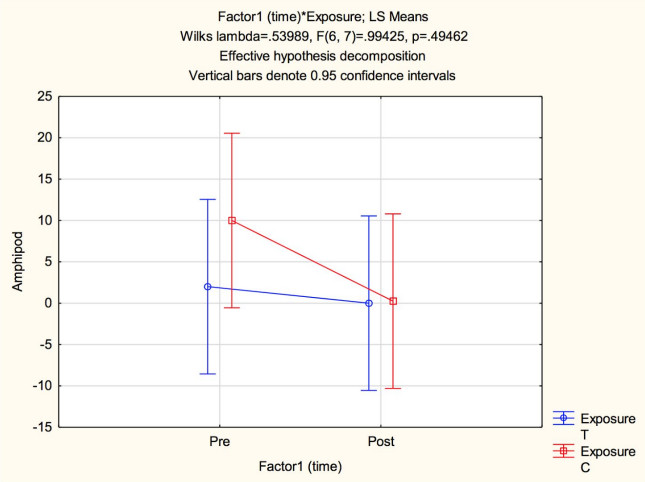

Figure 24 *The number of amphipods decreased between pre-treatment and post-treatment in both the control and treatment plots.*

The number of emergent insects were more affected by time than exposure, and therefore an interaction effect was not noticeable. The total number of insects was significantly different between pre-treatment and post-treatment, but was not significant between the control and treatment plots between these times (Wilks lambda=.53989, F(6, 7)=.99425, p=.49462) *(Figure 25).* There was not a significant interaction effect for total taxa A between pre-treatment and post-treatment and control and treatment plots *(Figure 26).* There was a slight interaction trend for taxa J however it is not statistically significant *(Figure 27).*

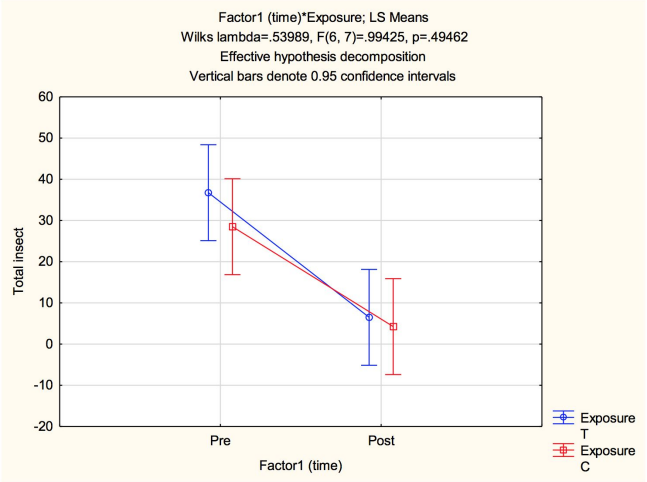

Figure 25 *The trend of decrease for the number of total emergent insects between pre-treatment and post-treatment was observed in both the control and treatment plots.*

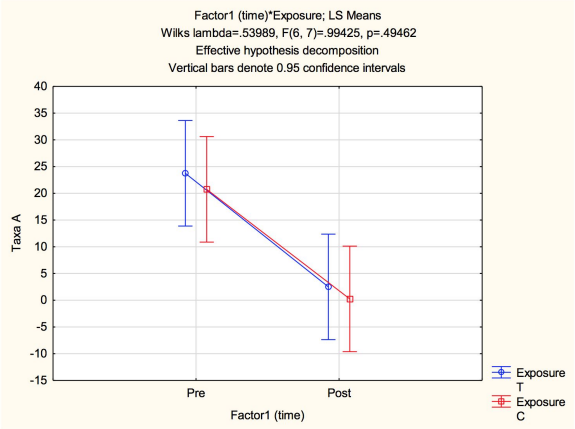

Figure 26 *The trend of decrease for the number of total Taxa A between pre-treatment and post-treatment was observed in both the control and treatment plots.*

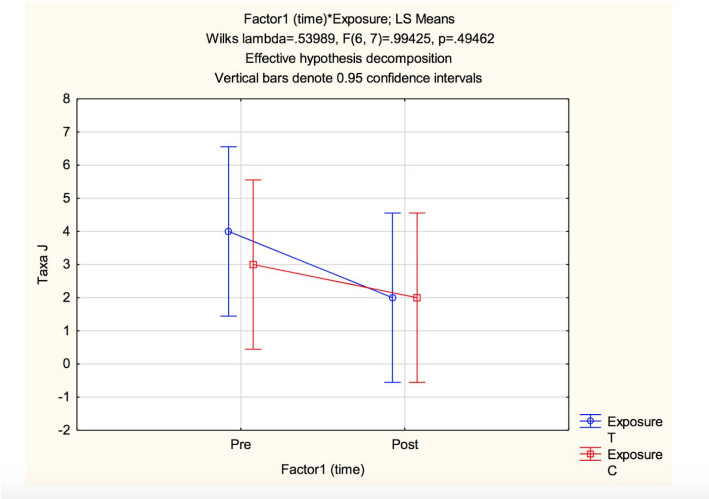

Figure 27 *The trend of decrease for the number of total Taxa J between pre-treatment and post-treatment was observed in both the control and treatment plots.*

Discussion

The data here supports the hypothesis that Zequanox® does not have an effect on the biodiversity of macroinvertebrates. The variable of time had an effect on the total number of macroinvertebrates and time also had an effect on the number of chironomids and amphipods, *refer to figures 10, 11, 12.* Since the p values are all less than 0.05 the null hypothesis is rejected and the the significant difference is accepted. The change in numbers as function of time could be driving the change in macroinvertebrate numbers. This change could be attributed to the metamorphosis of larval and nymph forms of insects that would no longer be present in the Hester Dendys.

The variable of exposure appears to have no significant effect on the total number of macroinvertebrates and no significant effect on the numbers of chironomids and amphipods, *refer to figures 16, 17, 18* . Since the p values are all greater than 0.05, the null hypothesis is not

rejected. The third set of graphs, *figures 22, 23, 24*, show an interaction effect of the two variables, time and exposure, in which they both have an effect. However, it illustrates there is a greater effect from time than from exposure.

Physical and chemical data and sediment samples were taken to help in describing the ecology of the lake. None of this data was used as a variable in determining the effects of Zequanox®. In each of the four study areas the sediment composition in the control and treatment plots was quite homogenous. Statistical analysis was not done comparing number of macroinvertebrates in each area in regards to sediment composition.

The Zequanox® itself may not be causing the change in macroinvertebrate numbers. The Zequanox® appears to have a direct result on the pH and DO, *refer to figures 4, 5,* which could be affecting the macroinvertebrate numbers. This would illustrate an indirect effect of Zequanox® on macroinvertebrates.

Follow up research is planned by the Tip of the Mitt Watershed Council of Petoskey, MI. They will conduct physical and chemical testing of Round Lake and take samples of the molluscs and macroinvertebrates 30 days from the treatment and one year from the treatment. Macroinvertebrate numbers will be an important piece of data as they exhibit great resilience from environmental disturbances due to their short lifespan and high reproduction rates (Niemi, C.J. 1990).

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