The effect of trisprintec and metformin and their doses on the mortality and reproduction of *Ampullariidae, Daphnia magna,* and *Lemnoideae* & the heart rate of *Daphnia magna* in aquatic ecosystems

Sarayu Das

Spring Valley High School, Columbia SC, 29223

In recent years, an increasing level of pharmaceuticals has been detected in rivers and streams. The purpose of this experiment was to test effect of two of the most common drugs found at Congaree National Park in South Carolina, metformin and tri-sprintec, on three prevalent aquatic species, snails, plants, and crustaceans. This experiment was done in two stages: phase-1 to study effect of pharmaceuticals on the organisms and phase-2 to find maximum threshold of pharmaceuticals affecting the lifespan of these species. It was hypothesized that when dosage of pharmaceuticals increased, the *Daphnia magna* would experience increased heart rate, and mortality and reproduction of these organisms would be negatively affected. This was achieved by placing the organisms in separate habitats, and three different doses of these pharmaceuticals were added to their environments over three weeks. ANOVA and linear-regression tests were done to analyze vitals of these organisms and found pharmaceuticals did have significant impact. Furthermore, LD50 curves were drawn and results indicated that 3 ppt is the lethal dosage that eliminated at least 50% of the entire population for each drug.

Abbreviations and Acronyms

ppt: Parts Per Trillion
NIR: Natural Increase Rate
LD50: Median lethal dose
ANOVA: Analysis of Variance
M: Mean
SD: Standard Deviation
F: Ratio of 2 mean square values
R: Correlation coefficient
R2: Coefficient of determination
DF: Degrees of freedom
Adj SS: Adjusted sums of squares
Adj MS: Adjusted means of squares

INTRODUCTION

Significance

Since the 1990s, medical consumption has increased vastly, with over 3.4 billion prescriptions filled in the year 2006 ("Protecting the great lakes from pharmaceutical pollution," 2010). With an increased amount of pharmaceuticals being prescribed, a large amount of these drugs are ending up in the aquatic environment. Every year, two billion dollars worth of unused pharmaceuticals are thrown away by American consumers (Gorenstein, 2014), with over 100 different types of pharmaceuticals detected in rivers and streams across the world (Donn, Mendoza, and Pritchard, n.d.). The most common way that pharmaceuticals are disposed of is by placing them in the trash or flushing them down the toilet (Gorenstein). Even though filtration systems are efficient at removing waste from the water system, 93% of pharmaceuticals are not detected by these filter systems, and ultimately, these pharmaceuticals accumulate in aquatic ecosystems (Gorenstein). The seven most prevalent classes of pharmaceuticals found in rivers and streams are steroidal estrogens and progestogens, nonsteroidal anti-inflammatory drugs (NSAIDs), antidepressants, azoles, fibrates, beta blockers, and antibiotics ("A critical review of the evidence for health effects in fish," n.d.). These drugs affect different organs in aquatic organisms, ultimately causing varied mutations within these organisms.

Congaree National Park

Recently at the Congaree National Park in South Carolina, a dilemma has arisen dealing with mutations within crucial fish populations present in the water systems (Fretwell, 2014). Scientists tested the water sources and discovered tremendous amounts of pharmaceuticals in the rivers and streams of this park (Fretwell). Two of the most prevalent drugs discovered were tri-sprintec and metformin (Fretwell). Tri-sprintec is the brand name of the generic drug ethinyl estradiol, which is used to prevent pregnancy ("Tri-sprintec"). Metformin is the generic and brand name of the drug used to treat type 2 diabetes ("Metformin").

Previous Research

Previous research examined the effects of the seven most recurrent classes of pharmaceuticals found in rivers and streams on the anatomy of aquatic organisms. For instance, one study tested the effects of levonorgestrel and promethazine on *Daphnia magna* (Furuhagen, Fuchs, Lundström Belleza, Breitholtz, Gorokhova, 2014). This study concluded that promethazine enhanced the development of *Daphnia magna* (Furuhagen, Fuchs, Lundström Belleza, Breitholtz, Gorokhova). Another case study tested the effect of fluoxetine, an antidepressant, on *Pimephales promelas* (fathead minnow) (Margiotta-Casaluci et al., 2014). The results concluded that the fish exposed to the antidepressant became more active and aggressive than the control, fathead minnow without

a dose of fluoxetine (Margiotta-Casaluci et al.). Furthermore, Stancova et al. (2014) tested the effect of ibuprofen, diclofenac, and carbamazepine on the mortality, growth, early ontogeny, and histopathological changes in *Tinca tinca*.

Ampullariidae, Lemnoideae,* and *Daphnia magna

Three of the most common species found in most rivers and streams were used in this study. *Ampullariidae* is the most common aquarium snail ("Apple snails"). This organism has a remarkable capability to thrive in freshwater ecosystems. *Daphnia magna* is a freshwater flea primarily used in scientific research as a model organism (Elenbaas, 2013). *Daphnia magna* has a brief lifespan and an ability to propagate rapidly. *Lemnoideae* is an aquatic plant that floats on the surface of freshwater systems ("Common Duckweed"). They have a simple anatomy, fundamentally lacking stems or leaves and reproducing asexually ("Common Duckweed"). This plant has a rapid procreation rate and is grown easily.

Purpose

The purpose of this study was to determine if pharmaceuticals would have an impact on ecosystems, and consequently, to test the maximum threshold of pharmaceutical doses capable of sustaining the equilibrium of the ecosystem. This experiment provides insight concerning pharmaceutical discharge and hopefully establishes a realization that pharmaceuticals are affecting the environment. Thousands of drugs are affecting aquatic organisms, causing aggression, variations in propagation, cardiovascular disease, and unknown complications in the world of aquatic sciences. The first step of this project involves the assessment of the vitals of *Daphnia magna* and reproduction and mortality of *Ampullariidae*, *Daphnia magna*, and *Lemnoideae*. A controlled environment was also created in parallel to the treatments to compare the variables to the doses of pharmaceuticals. Subsequently, the dosages of the pharmaceuticals were increased to find the maximum threshold of these pharmaceuticals affecting the lifespan of these species.

Hypothesis

It was hypothesized that if tri-sprintec is used on *Daphnia magna*, *Ampullariidae*, and *Lemnoideae*, then it would affect the reproduction of these organisms, because tri-sprintec is a birth control used to prevent pregnancy in humans. If metformin was used on *Ampullariidae* and *Lemnoideae*, then the mortality of this species would increase, because metformin inhibits glucose production. And finally, if metformin was used on *Daphnia magna*, then this medicine would increase the heart rate of this organism, because one of the side effects of using metformin in humans is increased heartbeat.

MATERIALS AND METHODS

Metformin ($C_4H_{11}N_5$) (bought from Wal-Mart pharmacy)
Tri-sprintec ($C_{43}H_{55}NO_5$) (bought from Wal-Mart pharmacy)
Ampullariidae (Apple snails) (bought from Amazon)
Daphnia magna (bought from Carolina Biological)
Lemnoideae (Duckweed) (bought from Carolina Biological)
pond water (bought from Carolina Biological)
473.4 g bottles of water (Deer Park Natural Spring Water)
510.3 gram clear plastic cups
timer
Pipette (bought from Carolina Biological)
plastic container
masking tape
black sharpie
magnifying glass
microscope slide (bought from Carolina Biological)

Phase One:

90 clear plastic cups were each filled with 50 mL of pond water. Then, using a pipette, one *Daphnia magna* was placed in each of the 90 cups. 30 more cups were each filled with 50 mL of pond water and one apple snail was randomly placed in each cup. Finally, 90 more cups were filled with 50 mL of pond water, and four *Lemnoideae* were placed in each cup. Samples of *Daphnia magna* and *Lemnoideae* were grouped by 30 cups each and *Ampullariidae* were grouped with 10 cups each. All of these three groups were marked with control, treatment-1 (tri-sprintec), and treatment-2 (metformin) and numbered randomly. The organisms were allowed to settle into their habitat for one to two days. Afterward, 1 part per trillion (ppt) of tri-sprintec and metformin were formulated using a serial dilution process. Then, 1 ppt of each pharmaceutical was pipetted out and added to the samples correspondingly.

The mortality and reproduction count of all of the organisms were observed for five days and recorded. The heart rate of the *Daphnia magna* was recorded for six seconds for all three groups (control, tri-sprintec, and metformin). This heart rate was multiplied by 10 to calculate beats per minute (bpm) of each sample and this data was recorded for 5 days.

Phase Two:

After five days of experimentation with the 1 ppt treatments in phase one, two more ppt of each drug were added to the one ppt cups, making it three ppt. The same types of data were observed and collected for next 5 days. Two more ppt of each drug were added into the 3 ppt contaminated organisms (using the same dilution process). Once again, the cups were labeled accordingly and data was collected for another five days.

Figure 1. Phase One Experimental Design Diagram

Independent Variable: The different types of drugs tested on the species of aquatic organisms.

0 parts per trillion of each pharmaceutical in organism habitat (Control)

Levels of Independent Variable:	*Ampullariidae*	*Daphnia magna*	*Lemnoideae*
No pharmaceuticals	1 per cup (total 10 cups)	1 per cup (total 30 cups)	4 per cup (total 30 cups)

1 parts per trillion of each pharmaceutical in organism habitat

Levels of Independent Variable:	*Ampullariidae*	*Daphnia magna*	*Lemnoideae*
Trisprintec	1 per cup (total 10 cups)	1 per cup (total 30 cups)	4 per cup (total 30 cups)
Metformin	1 per cup (total 10 cups)	1 per cup (total 30 cups)	4 per cup (total 30 cups)

Dependent Variable: The effect of the organisms' lives in the ecosystem. (measured by mortality, reproduction, and heart rate (*Daphnia magna*))

Constants: water temperature, cup size, species of organisms, testing period, number of organisms in each cup, number of organisms in each species, amount of pharmaceuticals in each cup

Control: Habitats with no drugs present in the water.

Figure 2. Phase Two Experimental Design Diagram

Independent Variable: The different concentrations of drugs present in the water. (measured in parts per trillion)

0 parts per trillion of each pharmaceutical in organism habitat (Control)

Levels of Independent Variable:	*Ampullariidae*	*Daphnia magna*	*Lemnoideae*
No Pharmaceuticals	1 per cup (total 10 cups)	1 per cup (total 30 cups)	4 per cup (total 30 cups)

3 parts per trillion of each pharmaceutical in organism habitat

Levels of Independent Variable:	*Ampullariidae*	*Daphnia magna*	*Lemnoideae*
Trisprintec	1 per cup (total 10 cups)	1 per cup (total 30 cups)	4 per cup (total 30 cups)
Metformin	1 per cup (total 10 cups)	1 per cup (total 30 cups)	4 per cup (total 30 cups)

5 parts per trillion of each pharmaceutical in organism habitat

Levels of Independent Variable	*Ampullariidae*	*Daphnia magna*	*Lemnoideae*
Trisprintec	1 per cup (total 10 cups)	1 per cup (total 30 cups)	4 per cup (total 30 cups)
Metformin	1 per cup (total 10 cups)	1 per cup (total 30 cups)	4 per cup (total 30 cups)

Dependent Variable: The effect of the lifespan of *Daphnia magna, Lemnoideae,* and *Ampullariidae*. (measured by mortality, reproduction, and, for *Daphnia magna* only, heart rate)

Constants: water temperature, cup size, species of organisms, testing period, number of organisms in each species, number of organisms in each cup

Control: Habitats with no drugs present in the water.

RESULTS

The experiment was carried out in two phases, the first phase tested the effect of tri-sprintec and metformin on the mortality, reproduction, and heart rate of *Daphnia magna* and *Lemnoideae*. The second phase tested the effect of the different doses and found the maximum threshold of these pharmaceuticals affecting the lifespan of these species.

For 1 ppt, the control (M=126.25, SD=7.18) and tri-sprintec (M=135.95, SD=1.38) had a slightly lower heart rate for the *Daphnia magna* than metformin (M=142.63, SD=2.32). However, when dose increased to 5 ppt, the control (M=119.59, SD=6.45) had a significantly higher heart rate of the organisms than of tri-sprintec (M=86.70, SD=79.1). Additionally, when 5 ppt of metformin was introduced to the organisms, the whole population became extinct. The effect of 1 ppt of tri-sprintec and metformin had a significant impact on *Daphnia magna* ($F(2,8)=15.88$, $p=0.002$). However, there was not a significant impact from time ($F(4,8)=0.76$, $p=0.582$) on the retrogression of the *Daphnia magna* populations. Furthermore, when the dosages of the pharmaceuticals increased to 5 ppt ($F(2,8)=9.51$, $p=0.01$), there was a greater and significant effect on the organisms, but there was not a significant impact from time ($F(4,8)=1.14$, $p=0.40$) (table 1) in relation with treatment and the deterioration of the vitals of *Daphnia magna*. Interaction plots (figures 3 and 4) also portray a significant interaction between treatment and heart rate. In both figures, there was a clear indication that when the dosage of the pharmaceuticals increased, so did the heart rate of the *Daphnia magna* samples.

For 1 ppt of tri-sprintec (M= 3.0, SD= 7.55), both organisms had a significantly higher natural increase rate than that of its counterpart, 5 ppt of tri-sprintec (M= -21.80, SD= 9.18). See annexes. However, with a metformin dosage of 3 ppt used on the *Daphnia magna* populations, the test resulted in a negative population growth (M= -17.60, SD= 7.70). See annexes. Besides that, metformin dosage of 5 ppt on *Lemnoideae* indicated a moderate population growth (M=5.60, SD= 5.18). As dose increased, there was a population decline on the two organisms. Figure 5 shows a scatterplot of the NIR of *Daphnia magna* with tri-sprintec for 1 ppt. The data points are close to the fitted regression line, suggesting that there is a correlation between the number of births and deaths when 1 ppt of tri-sprintec was added to the *Daphnia magna* populations. A regression test was run for this data set, and it indicated that $R(3)=0.97$, $p<0.01$. This indicates that there is a relationship between NIR and tri-sprintec. A scatterplot was generated for the NIR of *Daphnia magna* with tri-sprintec for 5 ppt. In figure 6, there is a significant correlation between NIR and tri-sprintec. A regression test was run for the data, such that $R(3)=0.96$, $p<0.01$. Since the p-value is less than the alpha value of 0.05, the null hypothesis was rejected. Another scatterplot was created for 3 ppt of metformin. The data points of figure 7 are significantly close to the line of best fit, once again suggesting a correlation between treatment and NIR. In continuation, a regression test was run and it was found that $R(3)=0.96$, $p<0.01$. Since the p value is less than the alpha of 0.05, the null hypothesis was not supported. The NIR of *Lemnoideae* was also calculated. In figure 8, the data points are quite close to the fitted regression line. A regression test was run, and it was found that $R(3)=0.88$, $p<0.04$. This means that the p value is less than the alpha value, thus indicating that the null hypothesis was rejected. The tests of tri-sprintec of 1 ppt and 5 ppt and metformin of 3 ppt for *Daphnia magna*, and metformin of 5 ppt for *Lemnoideae* supports the claim that increased doses of pharmaceuticals affects mortality, reproduction, and heart rate of these two organisms. Furthermore, LD50 curves were generated, and it was discovered that the lethal dosage rate that killed at least 50 percent of the population for both drugs was 3 ppt (figure 9 and 10).

Heart Rate of *Daphnia magna*

Table 1: General Linear Model of Heart Rate of *Daphnia magna*

		1 ppt	1 ppt	1 ppt	1 ppt	5 ppt	5 ppt	5 ppt	5 ppt
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Time	4	64.57	16.14	0.76	0.582	9165.00	2291.00	1.14	0.40
Treatment	2	678.38	339.19	15.88	<0.002	38162.00	19081.00	9.51	<0.01
Error	8	170.85	21.36			16057.00	2007.00		
Total	14	913.80				63384.00			

Table 1 indicates that there is an interaction between treatment and the *Daphnia magna*, such that $F(2,8)=15.88$, $p=0.002$ for 1 ppt and $F(2,8)=9.51$, $p=0.01$ for 5 ppt. However, the time did not have a significant impact on either the 1 ppt ($F(4,8)=0.76$, $p=0.582$) or 5 ppt ($F(4,8)=1.14$, $p=0.40$) doses.

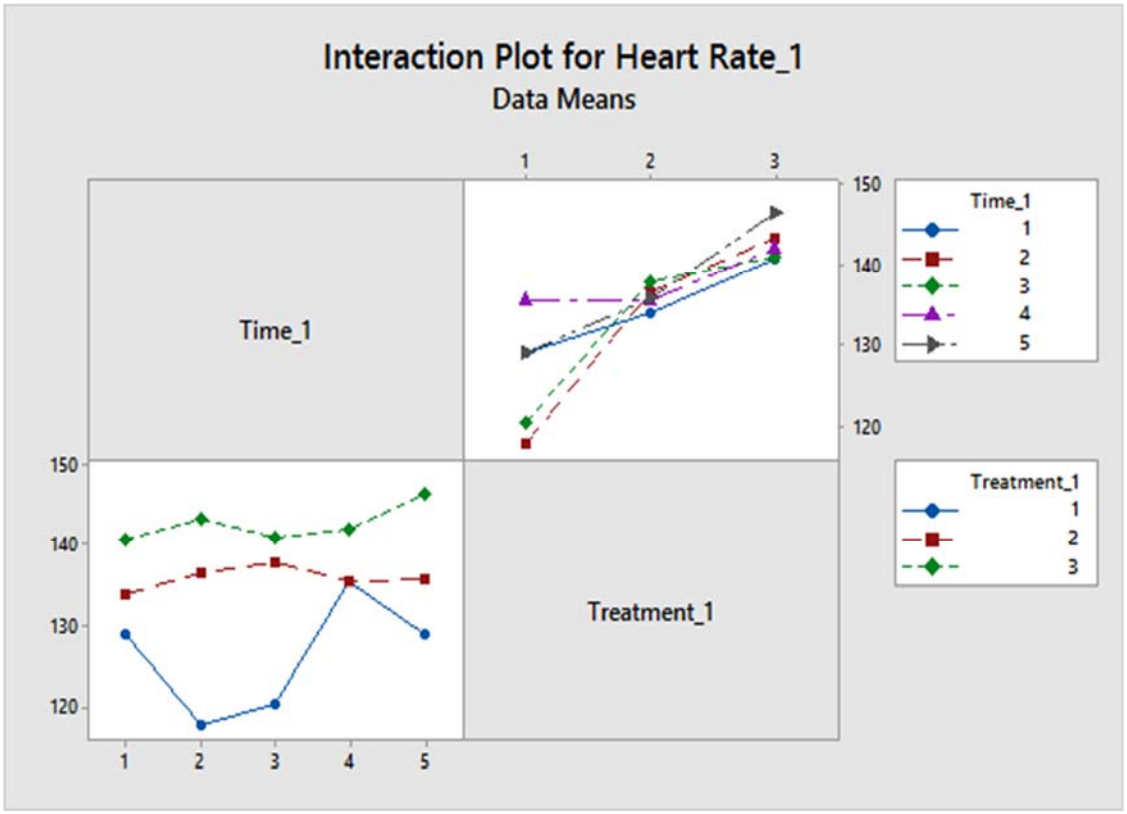


Figure 3: Interaction Plot of Heart Rate of *Daphnia Magna* for 1 ppt. This indicates that there is an interaction between time and heart rate. The heart rate of *Daphnia magna* increased with each treatment over time.

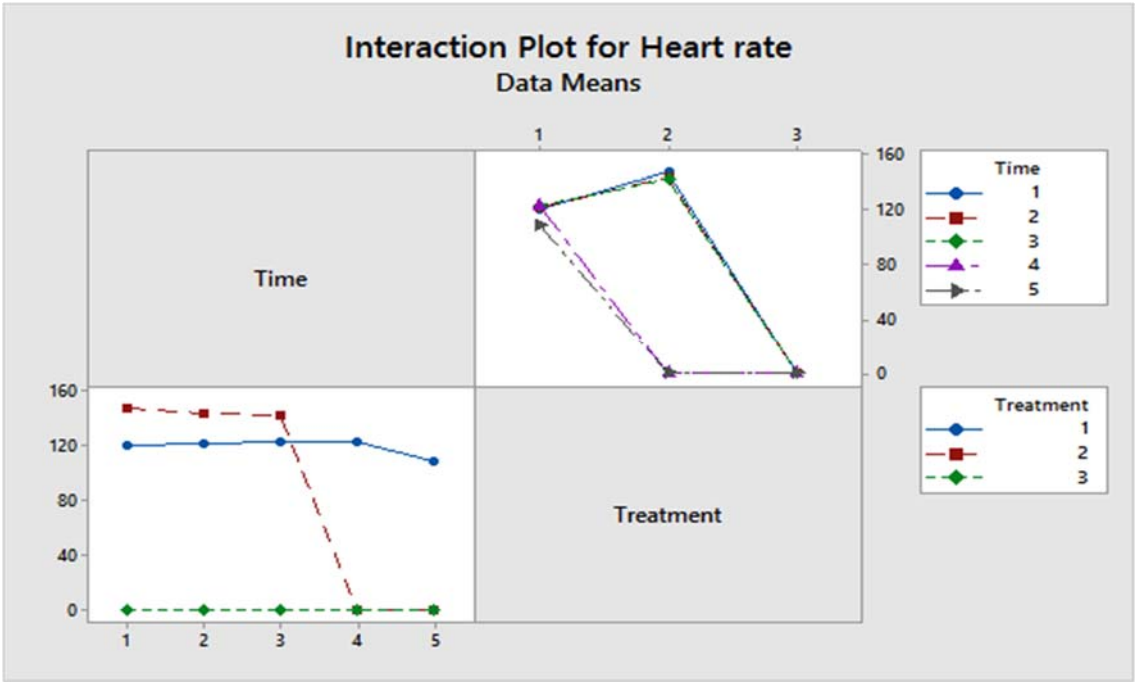


Figure 4: Interaction plot of Heart Rate of *Daphnia magna* for 5 ppt. This figure indicates that there is an interaction between time and treatment. The heart rate of the *Daphnia magna* increased with each treatment over time.

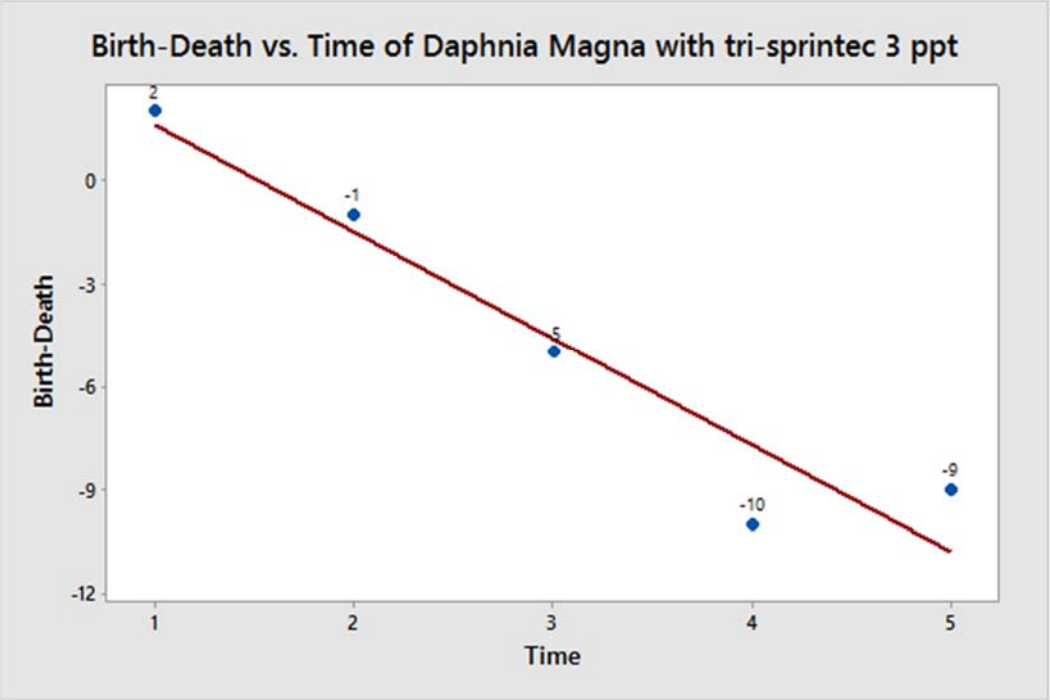


Figure 5: Scatterplot of Birth minus Death of *Daphnia magna*- Trisprintec for 1 ppt This figure suggests that there was a strong, negative linear relationship between tri-sprintec and *Daphnia magna*, such that R(3)=0.97, p<0.01. A regression equation was derived from the scatterplot (Birth-Death = 15.30 - 4.10 Time) and an R^2 value of 0.91 was calculated, thus indicating that 91% of the NIR variation is attributed to the trisprintec sample used.

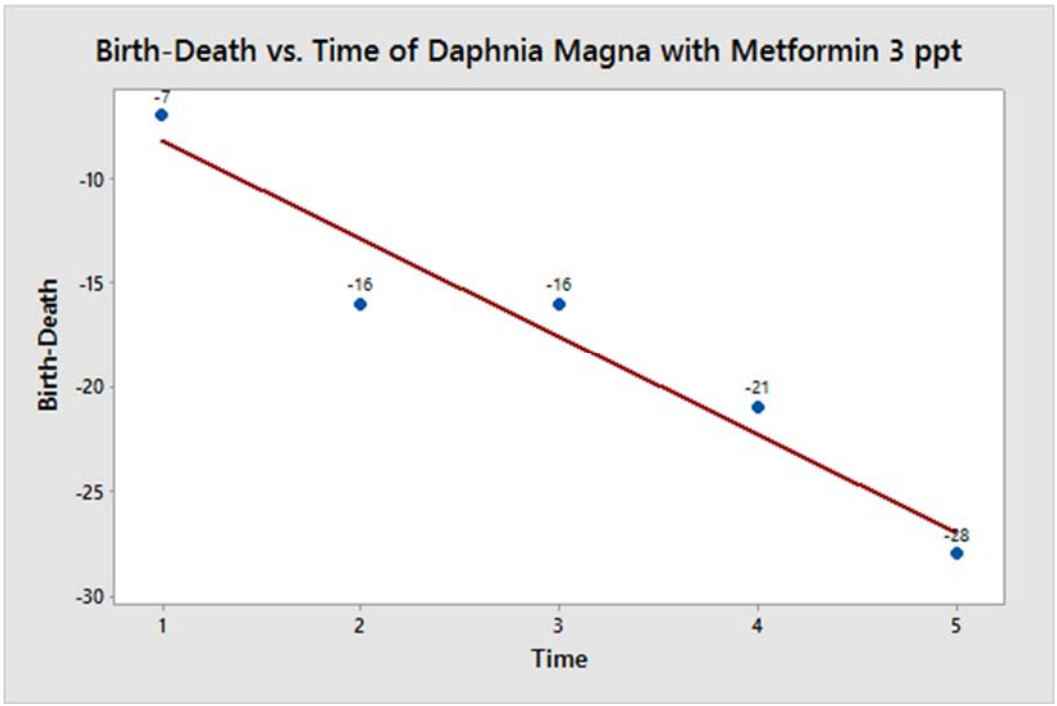


Figure 6: Scatterplot of Birth minus Death of *Daphnia magna*- Metformin for 3 ppt This figure suggests that there is a strong, negative linear correlation between metformin when 3 ppt was added onto the *Daphnia magna* populations, such that R(3)=0.96, p<0.01. The regression equation was derived from this scatterplot (Birth-Death = -3.50 - 4.700 Time) and an R^2 value of 0.93 was also calculated, thus indicating that 93% of the NIR variation is related to the metformin treatment.

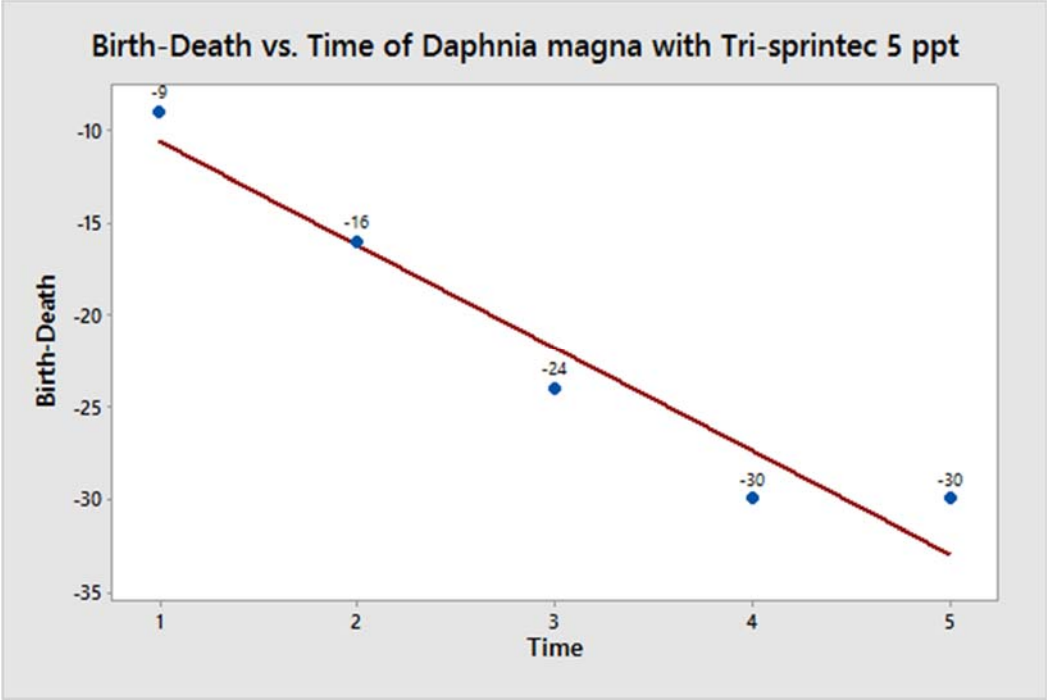


Figure 7: Scatterplot of Birth minus Death of Daphnia magna- Tri-sprintec for 5 ppt. Figure 7 indicates that there is a strong, negative relationship between tri-sprintec with a dosage of 5 ppt and *Daphnia magna*, such that R(3)=0.96, p<0.01. A regression equation (Birth-Death = -5.00 - 5.600 Time) was taken from the data set to calculate an R^2 value of 0.93, thus indicating that 93% of the NIR variation within this data set is due to the trisprintec treatment.

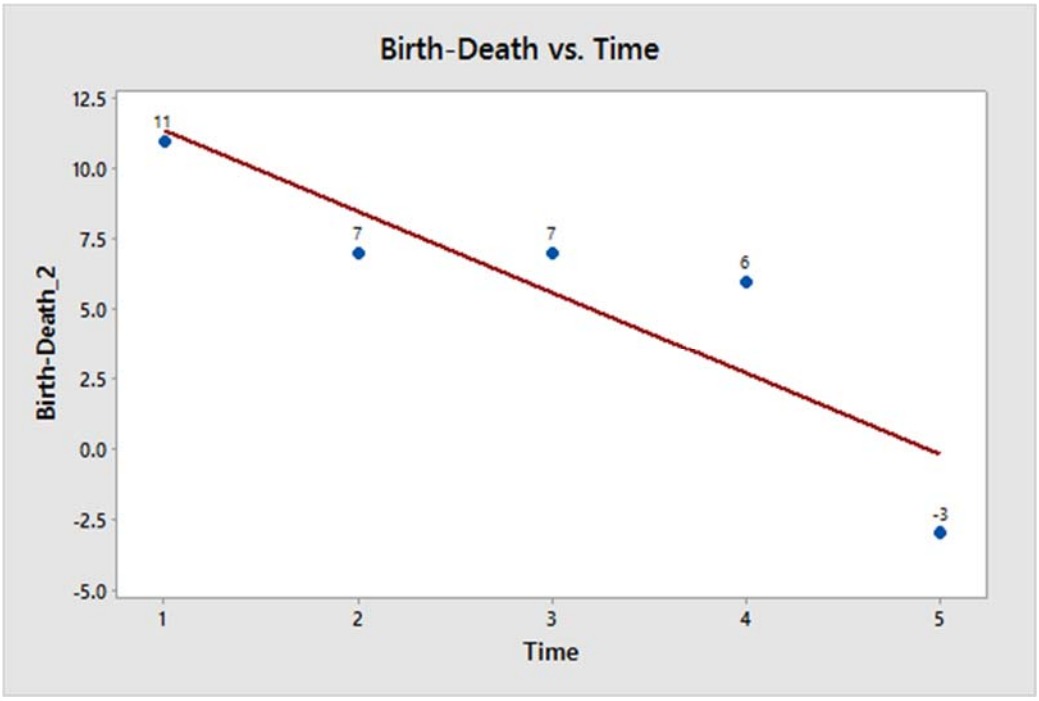


Figure 8: Scatterplot of Birth minus Death of *Lemnoideae*- Metformin for 5 ppt. Figure 8 shows a medium, negative correlation between metformin with a dosage of 5 ppt and *Lemnoideae*, such that R(3)=0.88, p<0.04. A regression equation (Birth-Death= 14.30 - 2.900 Time) was derived from the scatterplot to calculate an R^2 value of 0.79, thus indicating that 79% of the NIR variation within this data set is due to the metformin treatment.

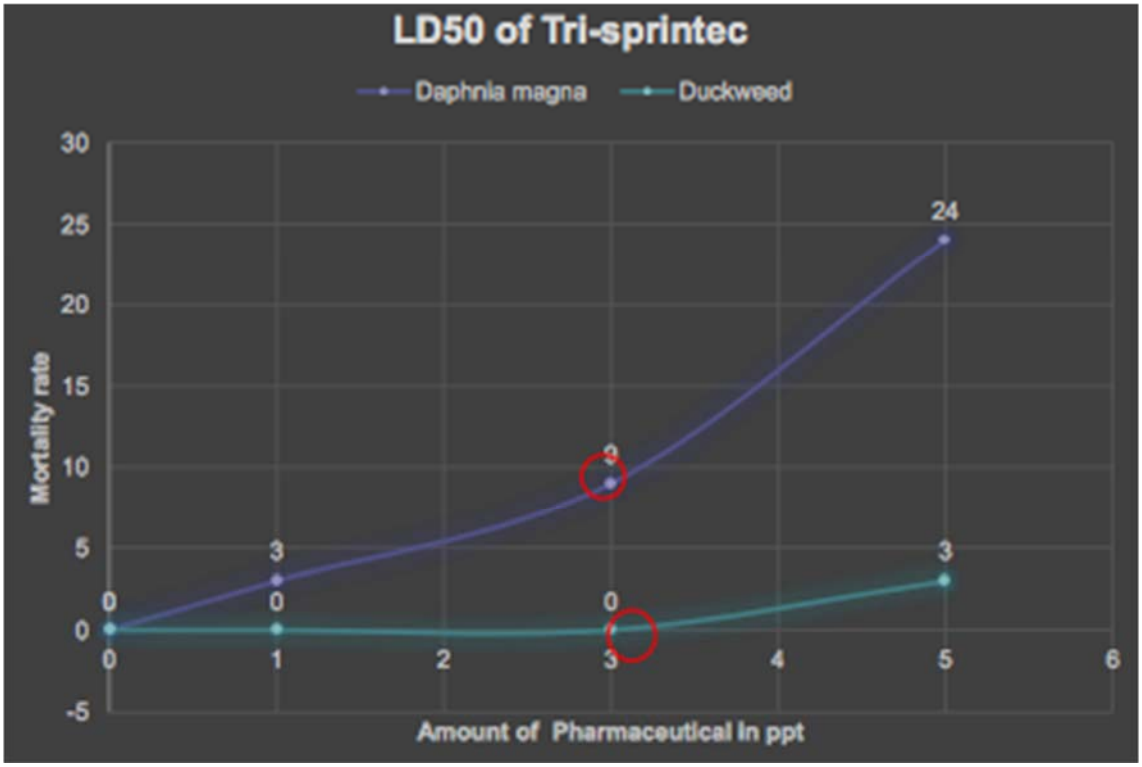


Figure 9: LD_{50} Curve of Tri-sprintec. As shown marked in red, the lethal dose that killed 50% of the population for both organisms was 3 ppt for the drug, tri-sprintec.

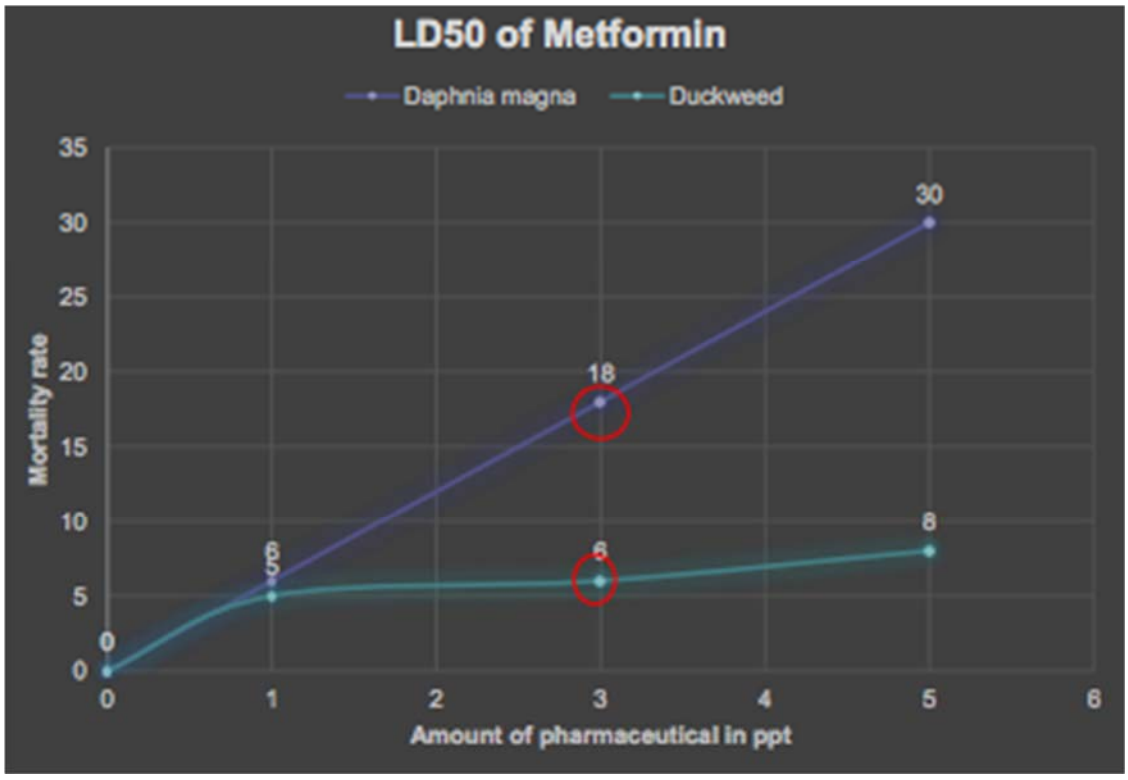


Figure 10: LD_{50} Curve of Metformin. As shown marked in red, the lethal dose that killed 50% of the population for both organisms was 3 ppt for the drug, metformin.

DISCUSSION AND CONCLUSIONS

When pharmaceuticals are disposed of, there is not much thought given to the fact that these pharmaceuticals ultimately end up in aquatic ecosystems ("Pharmaceuticals in Coastal Waters"). The purpose of this study was to bring awareness about how much pharmaceuticals are impacting organisms in aquatic environments. This was achieved by testing the effects of two prevalent drugs in the river systems of South Carolina, tri-sprintec and metformin, on three common aquatic species: *Ampullariidae*, *Lemnoideae*, and *Daphnia magna*. Even with minimal amounts of tri-sprintec and metformin used, there were major impacts on the heart rate, mortality, and reproduction of *Lemnoideae* and *Daphnia magna*. However, there was not a significant impact on *Ampullariidae*. The reason why the snails were not affected was probably due to their larger relative size. A higher dosage of tri-sprintec and metformin may be necessary to have an extensive effect on *Ampullariidae* populations. Since the data collected for *Ampullariidae* was not significant, further analysis was not completed on *Ampullariidae*. However, the pharmaceuticals did have a radical influence on the *Daphnia magna* and *Lemnoideae* populations.

There have been several studies conducted in the past to find the effects of pharmaceuticals on fish populations. For example, Cook (2014) sought to determine the effects of anti-anxiety drugs on young European Perch. In order to sustain equilibrium in the aquatic ecosystem, not only the fish population need to survive, but also the plants and other organisms to support that equilibrium. This warranted a study including the aquatic plant and other organisms in this experiment. In this study, *Lemnoideae*, along with other organisms, were exposed to the pharmaceuticals, and there was a notable decrease of the population sizes of *Daphnia magna* and *Lemnoideae*. For 1 ppt of tri-sprintec, both organisms had a significantly higher natural increase rate than 5 ppt of tri-sprintec. However, with a metformin dosage of 3 ppt used on the *Daphnia magna* populations, the test resulted in a negative population growth. The metformin dosage of 5 ppt on *Lemnoideae* indicated a moderate population growth. A pattern was configured, as dose increased, there was a population decline on the two organisms. An ANOVA test of $F(2,8)=15.88$, $p=0.002$ for heart rate of *Daphnia magna* and a linear regression t-test of $R(3)=0.97$, $p<0.01$ for the natural increase rate (NIR) of *Daphnia magna*, showed that the pharmaceuticals did have a significant impact on these organisms. Furthermore, a linear regression t-test ($R(3)=0.88$, $p<0.04$) was run for *Lemnoideae* with metformin for 5 ppt, and this indicated that increased doses of pharmaceuticals did have a greater impact on the organisms. Furthermore, as dose increased, the mean number of births and deaths of both organisms plunged, while the heart rate of *Daphnia magna* escalated. Given these points, even minimal doses of pharmaceuticals could have immense impacts on aquatic organisms. Therefore, the hypothesis that pharmaceuticals do affect aquatic organisms and higher doses of pharmaceuticals have a greater impact on the organisms was supported.

There were certain areas of uncertainty that may have had an influence on the results.
One of the predominant areas of uncertainty was the ages of the *Ampullariidae* and *Daphnia magna*, which were not known. Older, weaker organisms are prone to have an increased death rate with the addition of the drugs compared to the younger, stronger organisms, who may be able to withstand the chemicals (Lovett, 2012). An improvement would be to buy a specific batch of *Daphnia magna* or *Ampullariidae* based on age. Another area of uncertainty was some of the *Daphnia magna* were already pregnant when the batch arrived. This could have caused the reproduction data to be skewed. An improvement would be to examine each *Daphnia magna* under a high powered microscope and group them based on whether the females carry any eggs. Even though the organisms were given sufficient nourishment, a larger ecosystem and better care could have been given for these organisms to get more accurate results. All of these procedural improvements could have influenced the probability of gathering more and better data. However, these improvements and sectors of uncertainty can be fixed in subsequent experiments.

It is known that pharmaceuticals in the aquatic environment have a tremendous effect on fish populations, such as mutations (Corcoran, Winter, & Tyler, 2010). A further study could be conducted to examine the effects of tri-sprintec and metformin on various mutations that transpire in fish or *Daphnia magna*. As mentioned before, there are seven recurrent classes of pharmaceuticals found in water systems in America (Corcoran, Winter, & Tyler, 2010). Further research could test the effects of taking one drug out of each of the seven classes of pharmaceuticals found in aquatic systems and exposing it to different species of aquatic organisms to see which of the drugs causes reduced appetite and lethargy within the population. This experiment can be improved further by expanding the sample size of each organism along with introducing different classes of pharmaceuticals to study the effect on vitals. A more effective technological tool or process can also be engineered to filter out pharmaceuticals disposed into the water bodies.

What are the challenges and limitations our society facing? The Clean Water Act gives EPA authority to set “best available” technology standards for treatment of waste for discharges into the water system (“Protecting the great lakes from pharmaceutical pollution,” 2010). However, this does not regulate or measure the amount of pharmaceuticals excreted to the waters. Furthermore, the FDA allows for consumers to dispose of their pharmaceuticals by placing them in the trash or flushing them down the toilet if there are no recycling services in the area (“Protecting the great lakes from pharmaceutical pollution,” 2010). This should instead be reconsidered, and more community-based take-back programs should be funded and introduced. The FDA has also authorized the states in the US to regulate rules concerning proper pharmaceutical disposal (“Protecting the great lakes from pharmaceutical pollution,” 2010). However, only 30 out of the 51 states in the US actually have pharmaceutical take-back programs for proper disposal, and most of these programs are not currently operational (“Protecting the great lakes from pharmaceutical pollution,” 2010).

What can we do, as consumers of pharmaceuticals and scientists of our era? After experimentation was conducted, the researcher felt obligated to share the results and bring awareness to the community. A brochure was created (see the Apendix) and circulated to nearby neighborhoods. Many were surprised by the amount of pharmaceuticals ending up in the aquatic environment and the tremendous effect it has on aquatic populations. Furthermore, 75 % of these population actually admitted to flushing pharmaceuticals down the toilet, and many were unaware of the proper way to dispose of pharmaceuticals, which is returning the unused medicines to a pharmacy for proper disposal or get it to the drug buy-back facilities. This problem will not go away on its own without proper awareness.

This study should be an eye-opener to the public that pharmaceuticals are having an enormous impact on aquatic populations and the aquatic ecosystem. Even though this study was based off of the rivers and streams of the Congaree National Park in South Carolina, this is a global problem. Unless the public is brought to attention about this issue and proper disposal techniques, the uncontrollable effects to our waters will be catastrophic in the next decade or two.

ACKNOWLEDGEMENTS

I would like to thank Dr. Richard Mann for prescribing the pharmaceuticals and Dr. Sindhu Raveendran RPh for dispensing the pharmaceuticals and supervise me while handling drugs during my experimentation. I would also like to thank Mr. Das Kesavapillai for helping me do the serial dilution process. Finally, I would like to give a big thank you to my science teacher, Mr. Dale Soblo of Spring Valley High School, for assisting me with my data analysis.

Notes and references

A critical review of the evidence for health effects in fish. *Critical Reviews In Toxicology*, *40*(4), 287-304. doi:10.3109/10408440903373590

Apple snails. (n.d.). Retrieved September 4, 2015, from http://applesnail.net/

Common Duckweed. (n.d.). Retrieved September 4, 2015, from http://aquaplant.tamu.edu/plant-identification/alphabetical-index/common-duckweed/

Cook, B. (2014). H 2 whoa?!!: An examination of the presense of pharmaceuticals in America's waters. *Journal Of Legal Medicine*, *35*(1), 211-234. doi:10.1080/01947648.2014.885342

Corcoran, J., Winter, M.J., & Tyler, C.R. (2010). Pharmaceuticals in the aquatic environment: A critical review of the evidence for health effects in fish. *Critical Reviews In Toxicology*, *40*(4), 287-304. doi:10.3109/10408440903373590

Daphnia magna. (n.d.). Retrieved September 4, 2015, from http://animaldiversity.org/accounts/Daphnia_magna/

Donn, J., Mendoza, M., & Pritchard, J. (n.d.). The associated press: Pharmaceuticals found in drinking water, affecting wildlife and maybe humans. Retrieved May 6, 2016, from http://hosted.ap.org/specials/interactives/pharmawater_site/day1_01.html

Fretwell, Sammy. "EXCLUSIVE: Chemicals and medicines tainting Congaree National Park waters." *Thestate*. TheState.com, 25 Oct. 2014. Web. 21 Nov. 2015. <http://www.thestate.com/news/local/article13903487.html>.

Furuhagen, S., Fuchs, A., Lundström Belleza, E., Breitholtz, M., & Gorokhova, E. (2014). Are pharmaceuticals with evolutionary conserved molecular drug targets more potent to cause toxic effects in non-target organisms? *Plos ONE*, *9*(8), 1-8. doi:10.1371/journal.pone.0105028

Gorenstein, D. (2014, December 10). The shocking cost of wasted prescription pills. Retrieved September 4, 2015, from http://www.marketplace.org/topics/health-care/shocking-cost-wasted-prescription-pills

Lovett, Richard A. "Human drugs make fish flounder." *Nature.com*. Nature Publishing Group, 16 Nov. 2012. Web. 01 Dec. 015.<http://www.nature.com/news/human-drugs-make-fish-flounder-1.11843>.

Margiotta-Casaluci, L., Owen, S. F., Cumming, R. I., de Polo, A., Winter, M.J., Panter, G. H., &...Sumpter, J. P. (2014) Quantitative cross-species extrapolation between humans and fish: The case of the anti-depressant fluoxetine. *Plos ONE, 9(10), 1-18. doi: 10.13171/journal.pone.0110467*

Metformin. (n.d.). Retrieved September 4, 2015, from http://www.drugs.com/sfx/metformin-side-effects.html

"Pharmaceuticals in Coastal Waters." *NOAA*. USA.gov, n.d. Web. 01 Dec. 2015. <http://www.noaa.gov/features/protecting_1208/pharmaceuticals.html>.

"Protecting the great lakes from pharmaceutical pollution." (2010). Retrieved May 6, 2016, from http://www.greatlakes.org/Document.Doc?id=810

Stancova, V., Plhalova, L., Bartoskova, M., Zivna, D., Prokes, M., Marsalek, P., & ... Svobodova, Z. (2014). Effects of mixture of pharmaceuticals on early life stages of tench (Tinca tinca). *Biomed Research International*, 1-10. doi:10.1155/2014/253468

Tri-Sprintec. (n.d.). Retrieved September 4, 2015, from http://www.drugs.com/cdi/tri-sprintec.html

APPENDICES

Table 2: Descriptive Statistics: Birth minus Death for *Daphnia magna*-Tri-sprintec for 1ppt

Variable	Mean	StDev	Median
Birth-Death	3.00	7.55	0.00

Table 3: Descriptive Statistics: Birth minus Death of *Daphnia magna*- Tri-sprintec for 5 ppt

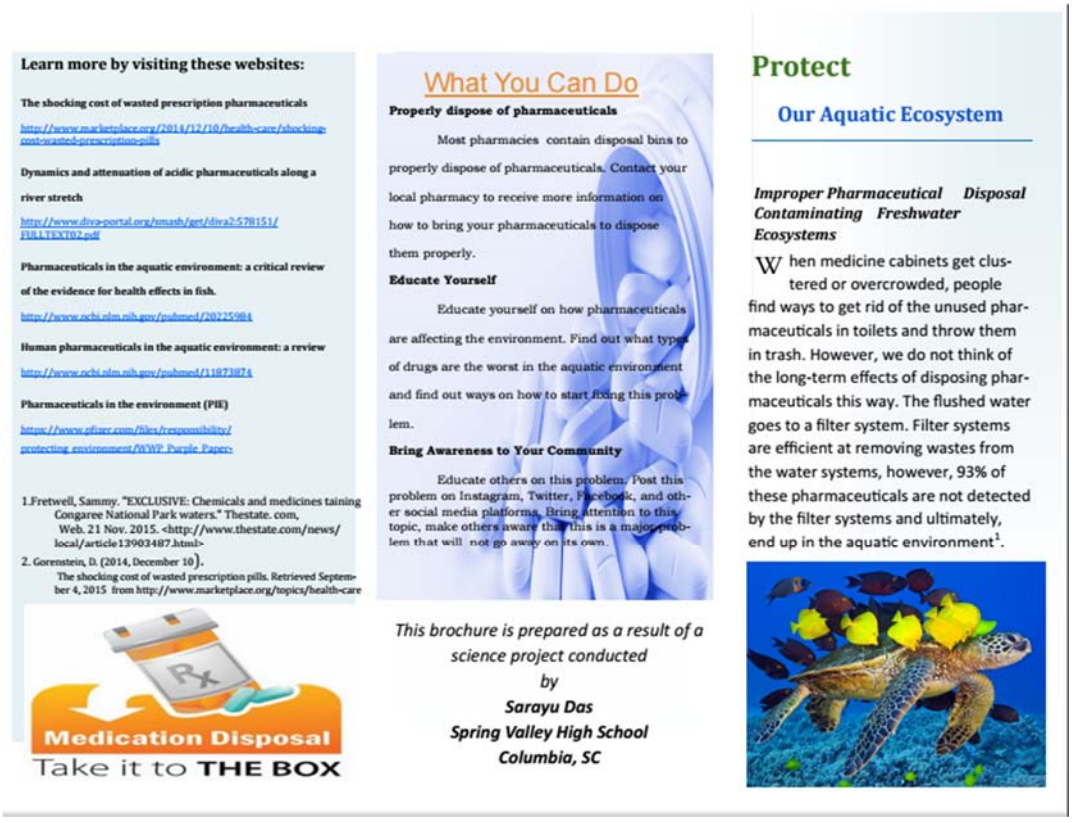
Variable	Mean	StDev	Median
Birth-Death	-21.80	9.18	-24.00

Table 4: Descriptive Statistics: Birth minus Death of *Daphnia magna*- Metformin for 3 ppt

Variable	Mean	StDev	Median
Birth-Death	-17.60	7.70	-16.00

Table 5: Descriptive Statistics: Birth minus Death of *Lemnoideae*- Metformin for 5 ppt

Variable	Mean	StDev	Median
Birth-Death	5.60	5.18	7.00



Aquatic Ecosystems are Valuable

If one organism in an aquatic food web is taken out, there could be devastating effects on the aquatic environment. There are tens and thousands of pharmaceuticals littering many freshwater ecosystems in the world, causing problems such as mutations within fish populations. Scientists studying the various issues of the fish populations in the world are finding shocking evidence of dying species mainly linked to pharmaceutical exposure. At present, there has not been any filtration devices engineered to prevent pharmaceuticals accumulating in the aquatic environment and hence affecting our environment and waters negatively.

The Concern with Congaree National Park

Recently at the Congaree National Park, there has been a high mortality rate among the fish populations there[2]. Scientists tested the waters, and discovered high levels of pharmaceuticals contaminating the rivers and streams of this park[2]. It was not only regular pharmaceuticals such as birth control found, there were other drugs found that are only used on agricultural farms[2]. There were pharmaceuticals found on the parts per million level (ppm), which is a high enough dosage to take out multiple fish populations at this park[2]..

Eventually Affecting Humans?

There are many people in this world who consume seafood. There are some mutations in fish populations that are external, but there are also many more which are internal, affecting the organs of these fish. Fish are nowadays mass-caught and mass-shipped, so there is nobody present between the time the fish is caught and shipped to determine whether there are mutations within each individual fish. When we as tertiary consumers eat these fish, it may cause unknown mutations within us, because the contaminated fish that we just ate probably contains hundreds and thousands of pharmaceuticals mixed together. Even though there is no evidence to support this yet, if pharmaceuticals can affect aquatic populations heavily, it can also eventually affect humans.