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Benthic Macroinvertebrate Composition In A Rural Zambian Stream

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BENTHIC MACROINVERTEBRATE COMPOSITION IN A RURAL ZAMBIAN STREAM EXPOSED TO LAUNDRY DETERGENTS

By

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Glossary of Terms

AOM- Allochthonous Organic Matter; that organic matter which originates outside the stream.

Coartem- Anti-parasitic medicine commonly used to treat Malaria in Zambia.

EC50- Median effective concentration; a term used in toxicology for the concentration at which 50% of a population becomes immobilized.

EPA- Environmental Protection Agency

FAO- Food and Agriculture Organization of the United Nations

FFG- Functional Feeding Group; grouping system for macroinvertebrates which classifies them based on the resources consumed and how they are collected.

HIV-Human Immuno-deficiency Virus; the sexually transmitted virus which is a precursor to Auto Immune Deficiency Syndrome (AIDS)

LC50- Median lethal concentration; a term used in toxicology for the concentration which kills 50% of the population.

Maize- another term for corn

Mpasa- Reed Mat in Nyanja

NASS- Namibian Scoring System; a rapid bioassessment protocol designed for the Southern African nation of Namibia

NGO- Non-Governmental Organization

Nsima- a corn based food which is the staple of the Zambian diet.

PCMI- Peace Corps Masters International

PCV- Peace Corps Volunteer

RAP- Rural Aquaculture Promotion

RBP- Rapid Bioassessment Protocol

SAFRASS- Southern African River Assessment Scheme

SASS- South African Scoring System

Tuck Shop- Zambian colloquial term for a small village shop typically “tucked” away in someone’s house or other building

USAID- United States Agency for International Development

Vidoyo- the word meaning: pest, virus, bacteria, insect, and macroinvertebrate in Nyanja

WHO- World Health Organization

ZMW- International currency symbol for the rebased Zambian Kwacha. Prior to 2012 it was ZMK

Preface

The field research conducted for Chapter One of this document was done collaboratively with the members of Kalichero Community, Chipata, in Eastern Province, Zambia. Lackson Ngoma and Paul Phiri assisted in the collection of benthic macroinvertebrates and recording of stream metrics. The families of Paul Phiri, Lackson Ngoma, Abram Ngoma, and Martha Ngwira were also involved in the field work by washing clothing and promptly reporting the cessation of their washing activities to me for sampling purposes. Detergents were shipped from Zambia by Eleanor Brasfield and Boom was graciously donated by Heather Robinson of Peace Corps-Zambia.

At Michigan Technological University, Drs. Casey Huckins, Amy Marcarelli, Nancy Auer, and Kari Henquinet aided in the proof reading and preparation of this document for final submission.

Acknowledgements

Writing a thesis as one might expect, is no easy task. The same can be said for conducting research in a developing country. However, because you're reading this today, I made it and to toss out a bit of my rusty Nyanja skills, "ndasaliza ndi ndalema angako, kwambili, maningi", meaning: I have finished and I am very very tired. Getting to this

point in my research and even in my life could not have been done without a host of amazing people who have supported me along the way.

First off, I would like to thank my family. My parents, sister, grandparents, aunts, uncles, and more have all been there to support me from the first day of Kindergarten up through now. I've put them through the stress of packing up some suitcases and moving across the globe for two years and through stories of mishaps that almost lead to injury, close encounters with snakes, and trips to the hospital with mysterious illnesses which never were diagnosed. Spectacularly, my family supported me and kept me sane through it all. The same can be said for them since my return as well. While I compiled the data for this document there were innumerable stresses but talking to them helped me stay focused and got me through. There is no amount of writing that could appropriately thank my family for all of their love and support so I may as well wrap this part up.

Now I must extend my gratitude to the United States Peace Corps staff in Zambia from Tom Kennedy, the country director when I arrived in Zambia to Cleopher Bweupe my program manager to Fraser Kaseya and Donald Nyamushi; you all supported me in so many ways during my service and I cannot thank you enough. To Ba Donald, thank you for giving me the inspiration for this project. I still remember the day we walked down to the stream and you mentioned something about detergents in streams which triggered this whole ordeal. To Ba Heather, you really deserve your own paragraph for carrying a box of that awful smelling "Boom" detergent over to the US in your suitcase for me and for shipping it to me without charge. Having that detergent show up was the motivation I

needed to get going on the toxicity study which I must say, turned out to be a great learning experience for me even if the results don't manage to find their way into a publication.

To Kalichero community: Zikomo Kwambili! Thank you very much. You were an amazing host to me for my service in Zambia and I will always view Zamanga village as my home away from home. Each and every one of you did and still do inspire me in so many ways. I am eternally indebted to you for your hospitality and lessons on life, hard work, and happiness.

Mr. Lackson Ngoma, Mr. Paul Phiri, and Mr. Acren Moyo: The three of you were by far the most influential people of my time in Zambia and especially this work. Your thirst for knowledge about the world around you and especially your water resources and the variety of life they support is one of the reasons I embarked upon this study. Thank you for everything that you did including helping out in the stream when I could not be there or just needed an extra hand because it was early and the stream was cold. You will always be my brothers as well as a father figure. Thank you.

Finally to my advisor Dr. Casey Huckins and my thesis committee members, Drs. Amy Marcarelli, Kari Henquinet, and Nancy Auer.: Thank you for your patience, guidance, answers to rookie level questions and support along the way. All that you have done for me from moral support, to pushing me for deadlines and ultimately for reading over this document to help make it sound professional is greatly appreciated and the knowledge you have imparted through this first round will surely be carried on as I start up my PhD.

Summary

The Peace Corps Master's International (PCMI) program at Michigan Technological University is one of many such programs across the country that allow a student to serve in the United States Peace Corps while working on their Master's degree. This program combines the rigors of coursework and research with the cross-cultural exchange of Peace Corps. Through this program I was able to spend 27 months in Zambia working as a Rural Aquaculture Extension Agent in Kalichero community in Zambia's Eastern Province. During my service I worked to introduce and improve fish farming practices within my community as well as to be an ambassador of American culture, all while conducting research on the impacts of washing detergent on benthic macroinvertebrates at washing sites in Chisitu stream, a tributary of the Msandire River in Chipata District Zambia. Research conducted in the following project sought to determine if laundry detergent use in Chisitu stream impacted the macroinvertebrates inhabiting it and the toxicity of these detergents. The outcomes of this research can potentially influence the behaviors of Kalichero community when they wash clothes and has the potential to improve the health of Chisitu stream. Despite numerous setbacks and thanks to the community partnerships I developed, this endeavor was a success and the relationships formed along the way will be lasting bonds that I carry with me for the rest of my life.

Abstract

Across the globe, synthetic detergents are commonly used in everyday life (Azizullah et al. 2011). In rural Zambia, washing often occurs directly in the streams where each individual washing effort becomes a point source of pollution; discharging a suite of chemicals as well as particulates into the waterways. Although organophosphates have been banned for use in laundry and dish detergents throughout much of the developed world; many parts of the developing world have yet to follow suit. A system of weak environmental regulations and a lack of enforcement of those that do exist have left water quality in much of the Southern African nation of Zambia, a country the size of Texas, essentially unregulated. Further, the use of non-phosphate based detergents may also pose a risk to aquatic communities as components such as surfactants, the foaming agents in soaps and detergents, are known to be toxic to some organisms and have yet to have significant legislation enacted to regulate their use. In a survey of Kalichero community, Eastern Province, Zambia, 56.7% of respondents stated they use nearby water bodies to wash their clothing. Since detergents are known to be toxic in aquatic environments, the goal of this study was to understand possible impacts to benthic aquatic macroinvertebrates in Chisitu stream. Field data collected in 2014 from Chisitu stream suggest a relationship between the number of macroinvertebrate families present at a washing site and the distance downstream from the nearest upstream washing site (r^2= 0.49). Comparison of taxa richness from pre and post washing sampling revealed a reduction (-0.9 ± 0.27) in the number of families present in the wash reach of the Phiri site. In the laboratory at Michigan

Technological University, acute toxicity tests were conducted using two detergents, Omo and Boom, which are readily available in Kalichero community. These tests produced median lethal concentrations (LC_{50}) of 17.87 mg/L and 25.19 mg/L for Omo and Boom, respectively. These estimated thresholds can be used as baselines for further research in this area and in the compilation of a recommendation for Kalichero community to reduce the amount of detergent entering Chisitu stream.

Chapter One: Benthic Macroinvertebrate Composition In A Rural Zambian Stream Exposed to Laundry Detergents

Introduction

Across the globe, synthetic detergents are commonly used in everyday life (Azizullah et al. 2011). Despite the widespread use of detergents and their potential adverse effects on the aquatic environment (Warne 1999), there has been relatively little work focusing on toxicity to aquatic organisms in Africa. Within Zambia, the majority of this work has focused on the impacts of copper mining to aquatic ecosystems (Norrgren et al. 2000; Nakayama et al. 2010). Specific studies aimed at understanding the toxicity of detergents to benthic macroinvertebrates have not shown up in extensive literature searches for Southern African freshwater systems. A study by Chukwu and Odunzeh (2006) conducted in Nigeria, compared the relative toxicities of a detergent and used engine oil on marine benthic macroinvertebrates and found that invertebrates are highly susceptible to both used engine oil and detergent; with one test species being 8.46 times more susceptible to the detergent than to the oil.

Lewis and Suprenant (1982) investigated the impacts of detergents on freshwater benthic macroinvertebrates and determined that the severity of the toxic effects of detergents to midge and mosquito larvae was dependent on the type of surfactant, a type of chemical used in soaps and detergents as a foaming agent. In light of their potential to be a toxic agent to aquatic life, some leading detergent brands in the United States, such as Tide $_{TM}$ (Proctor and Gamble), have begun using a biologically degradable surfactant in an

effort to be more environmentally friendly; much like they did in the late 20th Century with the removal of Phosphates (Kehoe 1992).

In developing countries such as Zambia, where infrastructure has not yet provided electricity and modern appliances to the majority of the population, detergents are used in the washing of clothing which often occurs either near streams or in many cases, directly in the streams. In Kalichero community, Zambia, where I conducted the field work for this study, large proportions of the community use the stream for washing, which may potentially pose problems for aquatic ecosystems as many components of detergents can interact with the aquatic environment and contribute towards toxicity (Azizullah et al. 2011). This study sought to investigate the impacts of washing clothing in ephemeral streams to macroinvertebrate communities.

Human settlements have historically been centered on water bodies (Strayer 2006) and have utilized them in various manners from irrigation to drinking water and for washing clothing. As populations have increased water has become a more coveted resource and increased use has led to concentrated human impacts on aquatic systems (Strayer 2006). Through the use of water bodies for laundering purposes, large quantities of detergents have entered the environment (Warne 1999) where they have had toxic effects on fishes and other aquatic organisms (Abel 1974).

Impacts to aquatic ecosystems as a result of human settlement in close proximity to water can, through mechanisms such as toxicity lead to alteration in ecosystem processes (Schafer et al. 2012). In addition to their importance in the aquatic food web and ecosystem

function, macroinvertebrates in aquatic systems also serve as indicators of overall ecosystem health (Minshall 2003) and in some instances can function much like a canary in a coal mine.

Intermittent or ephemeral streams lose connectivity of water flow at some point throughout the year and as a result, generally support different communities of aquatic orgamisms (Watson and Dallas 2013). Bunn and Arthington (2002) outlined a series of key principles linking hydrology and aquatic biodiversity in ephemeral rivers which can be linked to the success of organisms, especially macroinvertebrates in intermittent streams. The first principle outlined by Bunn and Arthington (2002) focuses on the influence of flow on physical habitat and its resultant influence on biodiversity. This is particularly important in the ephemeral streams of Zambia where large, flashy spates of water frequently occur as a result of heavy rains during the “rainy season” months of November through February. Bunn and Arthington (2002)’s second principle deals with the evolution of life histories by macroinvertebrates to compensate for the flow regimes associated with life in an ephemeral river. This principle can potentially have implications for the macroinvertebrates in Zambia through the potential for macroinvertebrates to have developed a life history that leaves young larvae and eggs in the streams during months of adequate but not excessive flow, a time period which consequentially overlaps with the time when the stream is most heavily utilized for washing clothes.

Chisitu stream in Kalichero community is located in rural Zambia where there are four major brands of laundry detergent available. These brands are all made on the African

continent by a variety of companies such as Unilever. Because of their manufacture in developing countries they may not be as regulated in terms of chemical composition in the same way as the detergents in the United States. South Africa is known to have some regulations in place for the protection of their aquatic ecosystems. Zambia however, tends to be less strict in its environmental regulations. This coupled with the lack of a water treatment system throughout its predominately rural area, leaves the aquatic ecosystems in that country facing significant alteration unless changes are made and poses the question of how exactly do certain events such as washing clothing in a stream impact organisms within that system.

There are numerous ways to quantify toxicity to aquatic organisms; however, the use of acute toxicity testing produces easily repeatable results due to most tests lasting no more than 96 hours (Johnson and Finley 1980). Acute toxicity tests can be effective in determining both the effective concentration (EC_{50}), which is the concentration at which half of the sample population is immobilized over the course of 48 hours, and the median lethal concentration (LC_{50}), the concentration at which death results in 50% of the population. The static toxicity technique involves keeping organisms exposed to a concentration of the test material dissolved in water for a period of time, generally 96 hours, without renewing the test material.

Objectives

Field work for this project was conducted during my service as a volunteer with the United States Peace Corps in Kalichero community, Zambia and sought to understand how

washing clothing in streams may impact the benthic macroinvertebrates of Chisitu stream. Field and laboratory studies aimed to understand how the use of laundry detergents in Chisitu stream impacted the benthic macroinvertebrates living there. The field study sought to test hypotheses predicting spatial and temporal change in macroinvertebrate richness and diversity in the wash and downstream reaches following the use of laundry detergents in the stream. In terms of spatial change, I predicted that there would be a decrease in macroinvertebrate richness and diversity in the wash and downstream reaches with respect to the upstream reach. I also hypothesized that there would be a reduction in diversity and richness in these same reaches after washing occurred. Laboratory studies were conducted to determine the toxicity of two detergents, readily available in the community (Omo and Boom), in comparison with a detergent manufactured and available in the United States (Tide). Acute toxicity testing of these detergents was done using a model benthic macroinvertebrate, *Chironomus tentans.* For these tests, I predicted that the detergents with the lowest median lethal concentrations, LC_{50}s, would be those manufactured in the countries with the least stringent water quality regulations. From this it was expected that, Boom, which is manufactured in Zambia would be the most toxic, followed by Omo from South Africa and Tide from the United States.

Methodology

Peace Corps and the PCMI Experience

The United States Peace Corps is a program of the United States government, which works in collaboration with host country governments to implement development initiatives. Peace Corps also works in collaboration with many colleges and universities across the United States to form a program called Peace Corps Masters International (PCMI; United States Peace Corps 2016). It is through this program, that I was able to serve as a Peace Corps Volunteer (PCV) in Zambia and was provided with the opportunity to conduct scientific research as well as volunteer in the realm of international development.

During my Peace Corps service, I was stationed in Zamanga Village, a village of about 50 households within Kalichero community. Kalichero was located in Chipata District of Eastern Province, roughly 600 km from the capital, Lusaka. Here I participated in an initiative called Rural Aquaculture Promotion (RAP). RAP promoted the development and management of small scale sustainable fish farms within local communities, an initiative aimed at improving food security and promoting positive lifestyles for people living with Human Immuno-Deficiency Virus (HIV).

In part due to my working as a Rural Aquaculture Extension Agent through the RAP program, I was given a firsthand perspective to many of the issues facing the local aquatic ecosystems and water resources. One issue that I became especially fascinated with during my service was how the local community used the available water resources. Within

my community certain water bodies were given specific functions while others were much more multipurpose. Chisitu stream was a seasonal stream which flowed through Kalichero community, and was multipurpose in its function for the community. From collection of drinking water to irrigation for gardens and small fields and even washing clothes and kitchen utensils, Chisitu stream was truly the lifeblood of Kalichero.

As I observed the community's interactions with the aquatic ecosystems, I began to wonder just how these activities, particularly the washing of clothing, which was a weekly affair for every family, impacted the system. Did it hurt the fish populations in the streams? What about the benthic macroinvertebrates? With these questions in mind, I decided to embark upon one of these two lines of questioning for the research. As I began talking with members of the community and learning a little more about this stream, I came to realize that the fish populations were rather sparse in this stream as a result of various factors, not the least of which was "pesticide fishing." Pesticide fishing is a technique used by some members of the community in which pesticides are dumped into the stream to kill off fish as they are carried downstream by the current. These fish are then collected, dried, and consumed. Knowing this, I felt inclined to focus my research on how these "pesticide fishing" events impacted fish populations. However, I was quickly informed by my community counterparts that this was unsafe and I was forced to reconsider. After some more thought about how ecosystems function and what organism play important roles in these functions, I turned my focus to how the detergents for washing impacted the benthic macroinvertebrates.

Study Design

In an effort to understand the use and potential impacts of laundry detergents in Chisitu stream, I conducted a social survey, a field study, and laboratory testing. The social survey provided insights into the washing habits of the community and aided in the planning of field data collection and the determination of concentrations used in toxicity testing. Work in the field involved sampling water chemistry and macroinvertebrate communities at four washing sites along Chisitu stream within Kalichero community, Zambia. To determine an estimate of the toxicity of the detergents, laboratory acute toxicity tests using a model invertebrate, the chironomid *C. tentans,* were conducted at Michigan Technological University in Houghton, MI.

Study Region

Field work for this study was conducted in Kalichero community located 20 kilometers outside of Chipata in Zambia's Eastern Province. Despite low annual rainfall, estimated by the Food and Agriculture Organization of the United Nations (FAO) to be less than 1000mm annually, the community is predominately agricultural. The majority of the agricultural land surrounds water sources such as seasonal streams, dambo (wetlands), and shallow wells.

The community is located around a seasonal stream called Chisitu that flows south to north and is roughly 11.5 km in length (Figure 1). This stream is used for agricultural,

drinking and washing purposes and also provides a source of food in the rainy season when fish migrate into the area for spawning. The community primarily utilizes the stream for the purposes of laundry during the months of March-August when streams are at their peak flows. During washing, many members of the community carry their laundry up to 1km from the village to the stream where they soak, lather, and rinse their clothes either directly in the stream or in a basin nearby which is then dumped into the stream.

Social Survey of Washing Habits

Prior to sampling benthic macroinvertebrates in Chisitu stream, a social survey was conducted in Kalichero community in order to better assess washing habits and frequency within the community. Community members were invited to participate in the survey through communication with the village headmen. For each village, an invitation, written in the local language chiNyanja, was delivered. At the time of delivery of the written invitation, the headman of each village was also invited verbally in chiNyanja. During the survey, participants from Zamanga and Mkule villages gathered at the Zamanga Village football grounds to participate in the survey. These villages were chosen based on their close proximity to and heavy reliance on the stream for household and agricultural purposes. The survey was conducted orally in local language (Chinyanja) on a voluntary basis and individual responses were recorded by hand. Survey questions included where household clothing was washed, the type of laundry detergent used and the frequency of washing and are displayed in Table 2. In the event of a response indicating the washing of

laundry in or near Chisitu stream, further questioning sought to determine if washing (lathering and rinsing of clothes) occurred directly in the stream or in a basin near the stream. A further line of questioning was also given when respondents indicated use of a basin so as to determine where the water was discharged from the basin in relation to the stream.

From Zamanga and Mkule villages, a total of 38 responses were recorded from either the head of the household or the person in charge of washing. This return constituted roughly 40% of the total number of households in the two villages. On the recommendation of Paul Phiri, a community counterpart who is actively involved in community mobilization, survey participants were thanked for their participation and rewarded with a piece of candy as a token of appreciation.

Site Selection

Based on the social survey results four sample areas along Chisitu stream were chosen as they were highlighted as areas of high washing incidence (Figure 2). In each of the four sampling areas, a family and their corresponding washing site were chosen. Families were selected based on their reliability, as regular participation was crucial to the study. Within each washing site three reaches of about 10m in length were created and labelled “wash,” “upstream,” and “downstream” based on their proximity to where the washing occurred.

Field Data Collection

Sampling of benthic macroinvertebrates (benthos) occurred on a weekly basis from the end of May through August 2014, a time period which extended from the end of the rainy season through the cold season (mid-May to July) and into the beginning of the dry season (August-November). with the exception of site number 4 which dried up after only two weeks of sampling due to damming of the stream for agricultural purposes in June.

Benthic macroinvertebrates were collected at each site 30 minutes prior to washing, permitting the collection of invertebrates without immediate influence of detergents, and within one hour following the conclusion of washing. Sampling began with the downstream area and progressed upstream so as to not disturb benthos and send them downstream into other reaches. During the sampling, benthos were sampled in each of the three reaches at a washing site using a D-Frame net (Farias et al. 2012) which was 30 cm in diameter and constructed of 1000µm nylon mesh as recommended by Lowe et al. (2013). Sampling technique involved gentle kicking in gravel substrates and a scooping motion in areas sand, silt and wood debris (Lowe et al. 2013). All samples were collected by starting at the bottom of the reach and working in an upstream direction. Benthos collections occurred randomly in the stream channel every 1 to 2 meters. After each sample was taken from the stream, it was placed in a bucket to prevent loss of benthos in other sampling areas within the site. Collected organisms were sieved through a 500µm kitchen sieve and then transferred to a repurposed Coca Cola bottle, labelled, and preserved in 99% ethanol. This procedure was conducted weekly from late May 2014 through the first week in August

2014. After August, stream water levels dropped below where the community typically used the stream for washing.

Preserved samples were picked to separate any benthos from sediments and particulate organic matter that remained after sieving the sample. Picked samples were placed into scintillation vials and covered in 99% ethanol for storage until identification could occur. Identification was done using a 10x magnification hand lens and a Sun King Pro, solar powered light. Benthic Macroinvertebrates were quantified and classified to Family using *A Photographic Guide to Aquatic Macroinvertebrates of Zambia* (SAFRASS, 2012) and *Aquatic Invertebrates of South African Rivers* (Gerber and Gabriel, 2002).

Data on substrate was collected during this study and is displayed in Table 1 alongside stream metrics such as wetted width, depth, water temperature, and pH from before and after washing at all sites. Depth was measured using a meter stick and wetted width using a measuring tape. Both measures were recorded to the nearest hundredth of a meter. pH was measured using an Oakton ecoTestr pH 2 probe inserted near to the shore due to this area being the most disturbed by humans during washing activity. The pH meter was calibrated weekly using Hydrion capsule form pH buffers (4.00, 7.00, and 10.00) that were mixed with 100mL distilled water. As with the collection of benthic macroinvertebrates, these data were collected weekly at the upstream, downstream, and wash reaches at each of the four sites.

Acute Toxicity Testing

Owing to the widespread acceptance of LC_{50} as a measure of acute toxicity (Johnson and Finley 1980; Baek et al. 2014), I estimated LC_{50} to assay the toxicity of detergents to a model aquatic macroinvertebrate, the Chironomid or midge larvae. Commonly known as "bloodworms" in the aquarium industry, Chironomids are prevalent throughout Zambia (SAFRASS 2012) and are easily cultured in aquaria; thus making them a prime candidate for use in this study. While toxicity testing can be accomplished using various methodological outlines, a single methodology was used to standardize experimental protocol (Harless et al. 2011). I utilized ASTM International's *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians* (ASTM E729-96). From this manual, I conducted a static toxicity test, which places organisms in a fixed amount of water with no circulation or renewal of the test materials.

In accordance with the ASTM manual, *Chironomus tentans* larvae were acquired from *Environmental Consulting and Testing*,Superior, WI and shipped overnight to Michigan Technological University. Upon delivery, larvae were placed in 10 gallon aquaria filled partially with a moderately hard media water (Mike Nagel, Personal Communication; Weber 1991). This water was a combination of Potassium Chloride, Magnesium Sulfate, Calcium Sulfate, and Sodium Bicarbonate and deionized water as outlined in Weber (1991). Mixing of the media water involved the addition of 1.2 g Magnesium Sulfate, 1.92g Sodium Bicarbonate, and 0.08g Potassium Chloride to 19L of deionized water in an appropriately sized plastic carboy. Following the addition of the salts

to the water, the carboy was aerated overnight. The following day, 1.2 g of Calcium Sulfate was dissolved in 1L deionized water using a stir plate and then added to the carboy (Weber 1991). Water in the carboy was then mixed and aerated for 24 hours before use in aquaria or for dilution of test materials in acute toxicity testing. Aquaria were filled and heated to maintain a constant temperature around 22 degrees Celsius (ASTM E729-96) for the entirety of the rearing and acclimation processes. Partial water changes occurred every two days during rearing but were not conducted during the acclimation period prior to toxicity testing. Following a water change, larvae were fed a slurry of pureed trout chow (Hein and Madhu 1992) with the amount of food given varying based on the density of larvae in the tank and the consumption rate (Batac-Catalan and White 1981). Feeding did not occur during the 48 hour acclimation period prior to testing per ASTM E729-96 recommendation. Larvae used in toxicity testing were in the 3rd instar.

Static toxicity tests were conducted for three detergents by placing 10 larvae into glass petri dishes with 50 mL of media water (Johnson and Finley 1980) where they were exposed to a range of concentrations of test material (detergents). Two detergents tested, Boom and Omo, are readily available in Kalichero community (Lackson Ngoma, Personal Communication) and for this study one package of each was shipped from Zambia. The third detergent, Tide, a *Proctor and Gamble* product was included in the assay for comparison. These three detergents came in two forms, paste (Boom) and powder (Omo and Tide) (Figure 3) and were manufactured in Zambia, South Africa, and the United States, respectively.

Concentrations used in the toxicity tests were calculated to bracket concentrations estimated to be in the stream during the washing of clothes. Estimated concentrations in the stream during washing were calculated by using a rough estimate of the volume of water present at each washing site from available data of stream depth(h), wetted width(r), and the length(l) of each washing site using the formula for volume of a partial cylinder: $V = l[r^2 cos^{-1}\left(\frac{r-h}{r}\right) - (r-h)\sqrt{2rh - h^2}]$. This volume was then divided by the average amount of detergent used as calculated from social survey data to give a g/L concentration. Detergents were diluted in the media water outlined above and 50ml of diluted detergent water was added to the petri dish prior to introduction of the organism (Petterson et al. 2000). During the first 24 hours of testing, organisms were checked for mortality at 3, 6, 12, and 24 hours. Following this first 24 hour period, larvae were checked for mortality after every 24 hours. Mortality was determined to be the lack of a response to gentle prodding of the larvae and deceased organisms were removed from the experiment (ASTM E729-96). Throughout the testing water temperatures were maintained around 22°C to mimic conditions in the rearing and holding tanks. Maintenance of this water temperature was used as a mechanism to reduce stress on the organisms. At the conclusion of the experiment, all organisms were removed from the test chambers and euthanized.

Data Analysis

Statistical analysis of field data was conducted using JMP Pro 12.0. Analysis of diversity and family richness data from before and after washing events was conducted using a paired t-test, or the matched pair analysis feature of JMP. Diversity indices for each site were calculated using the Shannon Index: $H = -\sum_{i=1}^{s} p_i ln p_i$ where s is the number of

families and p_i is the proportion of individuals per family divided by the total number of individuals. Regression analysis using the bivariate fit function in JMP was used for analysis of relationships between cumulative and mean family richness and the distance downstream from the uppermost washing site. Following the plotting of the bivariate fit, a linear trend line was fitted to the data for all comparisons to facilitate the determination of potential strength of relationships.

Data from acute toxicity tests were analyzed to calculate an LC50 using the probit model (Stephan 1977). There was at least 10% mortality in every control replicate so the Schneider-Orelli 1947 formula was used for correction of the data to compensate for mortality in the control (Stephan 1977). The Inverse Predictor function in JMP 12.0 was used to estimate median lethal concentrations and the corresponding upper and lower 95% confidence intervals.

Results

Social Survey

Analysis of survey data on the washing habits of members of Zamanga and Mkule villages indicated that 47.3% of the households surveyed use the stream for washing their clothes. Of this subset of the population, 33 of 38 respondents acknowledged the use of

detergent in two major forms: paste and bar. Paste detergents were distributed throughout Zambia under several major brands and were used as a general cleaning agent in laundry. The bar detergents on the other hand were made by two brands, Boom and Chapa, and were primarily used in removing tough stains. Every respondent who indicated use of the stream for washing also indicated that whether or not they used a basin for the actual washing of clothing, their waste water was dumped into or within a few meters of the stream which, in most sites sampled, is still within the bankfull height of the stream. In response to questions about the frequency of washing, participants ranged in frequency from once every two weeks for the small households to daily washing for the bigger households. The average frequency of washing was determined to be 2.5 times per week. During these washing events, respondents indicated an average use of 1.4 "big boxes" (400g box) of Boom detergent paste per event, which equates to 562.8 grams of Boom paste. Individual responses ranged from a low of 0.5 box (200g) per week to 4 boxes (1600g) per week. The high response corresponds to washing 7 times per week.

Macroinvertebrate Survey: Taxa Richness

Data collected during this study began to demonstrate the potential impacts that the use of detergents may be having on the benthos in this system. Analyses of macroinvertebrate taxa richness indicated that there was a range of 42-144 invertebrates (Figure 4) and 7-17 families (Figure 4b) found across the four sites certain families being present in higher densities (Figure 5a-d). Table 3 displays the distribution of taxa across the four washing sites.

When cumulative taxa richness for each site (before and after counts combined) was analyzed using linear regression against the distance downstream from the uppermost washing site, Site Phiri, a strong relationship (r^2=0.78) appeared with distance downstream seemingly playing a large factor in the number of macroinvertebrate families found (Figure 6a). Using the same regression technique, analysis of the mean taxa richness ± standard error for each site with the distance from the uppermost washing site did not indicate a similar relationship (r^2= 0.10, Figure 6b). Together these data indicate that the farther away from the most upstream washing site, the fewer families were present.

Analysis of richness and biodiversity data from the wash and downstream reaches of each site indicated no temporal change in the composition of benthic macroinvertebrates across sites as was hypothesized. In looking at the richness data from the Mfumu and Ngoma sites, it was noticed that there were "gaps" in the data or weeks in which no macroinvertebrates were collected. These "gaps" which can be seen in Table 4 and Table 5 indicate the potential for influence from a factor which was not measured in this study. One such factor are "pesticide fishing" events which will be discussed in depth later. Analysis of pre and post wash cumulative richness data from the downstream reach of all sites shows no significant difference among sites (p=0.18). The data do however show a mean difference of -1.5±0.87 families which may be indicative of a loss of taxa. Just a few meters upstream, the wash reaches had a mean decrease of -1.75 ± 1.7 families between pre and post washing periods and like the downstream reach, this difference was not statistically significant (Figure 7, p=0.38). The cumulative number of families collected from the wash and downstream reaches, respectively (Figures 8 and 9) highlight some of

the local variation in macroinvertebrate richness. Comparison of the mean richness data shows similar trends to the cumulative richness (Figure 7) analysis indicates a decrease of -0.5± 0.30 in the mean loss of species with the wash reaches. Contrary to the other three analyses, before and after comparisons of mean family richness in the downstream reaches show a mean increase of 0.1±0.15 (Figure 10) indicating the potential for there to be a slight increase in the number of families present at certain sites.

Comparisons of upstream and downstream reaches showed some large differences in the number of macroinvertebrate families present. When mean richness data was considered, there was a mean difference of -3.87±2.83 families (Figure 11) and when the cumulative richness of the sites were compared, the mean difference was -0.25±2.2 families (Figure 12). Calculation of Shannon's Index showed no temporal or change in diversity among the sites in the wash and downstream reaches across sites (Figures 13 and 14). There was also no significant spatial change in diversity across sites pre and post washing (Figure 15; t=0.34 pre- and t=0.66 post-).

Toxicity Testing of Detergents

Acute toxicity tests for all detergents exhibited high initial mortality (first 24 hours) and then began to plateau for the duration of the study as can be seen in Appendix A. Tide laundry detergent, which was made in the United States, resulted in the greatest mortality across the range of concentrations tested and reached at least 50% mortality in all replicates within 48 hours of the start of the test. Data from all three replicates were pooled for the calculation of the median lethal concentration (Figure 16). The resultant LC_{50} was 9.43

mg/L with an upper 95% confidence interval of 36.85 mg/L and a lower 95% confidence interval which was incalculable.

Tests conducted with Omo detergent, a South African manufactured product, produced the most replicates with long term survival of all three detergents tested. Similar to the Tide trials, in the highest concentrations, 100% mortality was recorded following the 6 hour checkup in replicates A and B and after the 3 hour check in replicate D (Appendix A). In all three replicates of Omo detergent, high mortality (>10%) was recorded in the control groups. Pooled data for this detergent (Figure 17) showed high mortality in the control and had a median lethal concentration (LC_{50}) of 25.19 mg/L. The upper 95% confidence interval for Omo was 75.63 mg/L while the lower limit was incalculable.

In tests conducted with Boom, the Zambian made detergent, there was 100% mortality in all replicates after the three hours of exposure to the high concentration of 88.725 mg/L. Replicates A and D also experienced high mortality in the 60.489 mg/L chambers after three hours (Appendix A). Figure 18 displays the pooled data from the Boom replicates. Boom was found to have an LC_{50} of 17.87 mg/L with an upper confidence interval of 90.93 mg/L. The lower 95% confidence interval for this detergent was incalculable.

Discussion

Washing of clothing was a common occurrence within Zamanga and Mkule villages, with frequencies ranging from once every two weeks to nearly every day for each household. While the results of this field study are not unequivocal evidence that detergent use during clothes washing was impacting macroinvertebrate biodiversity in Chisitu stream; there is some indication of negative effects on the stream communities. The need to better understand how these detergents impact the aquatic life is of importance to the maintenance and possibly improvement of stream health. Going forward, the data collected in this survey can serve as a useful foundation to better understand the washing habits of rural based Zambians and should guide further questioning as to the frequency, location, and duration of washing events. All of this information will help guide future endeavors into better understanding the impacts to benthic macroinvertebrates and all aquatic life these detergents are having.

Field Study

The overall number of taxa collected from each site, (Table 3) indicates a general decreasing trend in family richness following washing. However some samples taken from the Phiri and Ngwira sites showed an increase in the number of taxa present post washing. This likely indicates that there are other factors such as substrate type, dissolved oxygen (DO) content, flow rate of the stream, and toxic effects from runoff of fertilizer and pesticides used in agriculture which surrounds the stream, that could be influencing the richness of macroinvertebrate taxa. The Phiri, Mfumu, and Ngoma sites had more coarse

substrate materials and they appeared to have higher macroinvertebrate family richness (Figure 9). This is potentially attributable to cobble and bedrock having large surface areas which the macroinvertebrates are able to use for habitat and refuge. Substrates with large surface area can also potentially increase DO as water moving across these substrates can easily become turbulent and mix with the atmosphere; the primary mechanism through which oxygen enters the aquatic environment. Additionally, these large substrates are less prone to physical disturbance because they are more stable and generally more embedded.

While no sites showed a statistically significant reduction in the number of taxa during pre- and post-washing periods (Wash, t=0.38; Down t=0.18), some did exhibit trends in this direction (Figures 8-12). One factor that was not appropriately accounted for in this study was the potential for macroinvertebrates to drift, a mechanism used for both dispersal and avoidance of danger, out of the impacted area during exposure to detergent. Taking this into consideration makes it pertinent to look at spatial differences in macroinvertebrate richness in addition to the temporal aspect. Data from before washing indicated that there was no significant difference (t=0.92) in the number of families between the upstream and downstream reaches of the four sites in contrast to the initial hypothesis that there would be spatial differences in macroinvertebrate richness along short scales.

Distance from the uppermost washing site (Phiri) had the greatest detected impact (r^2=0.78) on the number of families found in a particular site indicting a spatial trend as was hypothesized; albeit at a much greater scale than was initially expected.

Potential reasons for this apparent decrease in richness could be the accumulation of any toxic components of the detergents however data to support such a hypothesis was not collected during this study. If in fact this decrease in richness is attributable to the detergent and not to other unquantified factors, then there is the potential for ramifications in the aquatic food web as well as in other natural processes and functions of the stream. Analysis of diversity data comparing the distance from the Phiri site, the most upstream washing site included in this study, indicates that there is a strong positive relationship between the distance downstream and an increase in diversity in the upstream reach (Figure 19). This is in conflict with the decreasing richness found as one moves farther downstream and warrants further investigation in future studies.

No macroinvertebrates were collected in the weeks of June 21 and 28 at the Mfumu site and the week of June 7 at the Ngoma site (Table 4, 5). The lack of macroinvertebrates detected at multiple sites across several weeks seems to indicate that a factor which is unaccounted for in this study may be impacting macroinvertebrate richness and diversity at the Mfumu and Ngoma sites. One possible explanation lies in the life cycles of the macroinvertebrates. Since sampling occurred over a period of ten weeks it is likely that several life stages of invertebrates were captured during sampling. Emergence of certain families over the course of the sampling period is one possible explanation for these weeks in which few to no macroinvertebrates were collected, however this remains to be investigated. Another potential explanation is "pesticide fishing," the release of pesticides into the stream in order to poison the fish. After release, fish are collected, dried and consumed; a serious health concern for both humans and the aquatic environment. During

the study, I received information from counterparts within the community about "pesticide fishing" events. Two of these reports lined up with days when sampling at the Mfumu and Ngoma sites collected few, if any macroinvertebrates. While there is no recorded evidence to link pesticide fishing to the absence of macroinvertebrates during these sampling efforts, anecdotal evidence suggests that this may be a cause. A third potential explanation is that there is a cumulative effect of the detergents in the Mfumu and Ngoma sites as these are the most downstream sites of the four sampled in this study. More detergent entering the stream from the major washing sites upstream of Mfumu and Ngoma coupled with a long uptake distance leaves the potential for higher impacts to the macroinvertebrate communities farther downstream. Such impacts were not investigated in this study but would be a useful tool in better understanding how detergents impact macroinvertebrates in Chisitu stream.

Toxicity Testing

High mortality in the control chambers across all replicates resulted in the need for correction of the data. For all three detergents tested, LC_{50} values fell within or below the range of concentrations used in the toxicity test. Data from the tests reject the initial hypothesis that Boom which is made in Zambia, would be the most toxic. Tide, the detergent manufactured in the United States was the most toxic of the three detergents tested followed by Boom and then Omo. These detergents were not analyzed for composition and ingredients lists were not available for those manufactured outside of the United States. In the United States, detergents can no longer contain Phosphates and as a result, other synthetic surfactants have been added to increase the effectiveness as a

cleaning agent (Kehoe 1992). Many of these surfactants are of unknown toxicity to aquatic organisms and their presence in Tide could explain its toxicity. To further investigate this, it would be pertinent to conduct chemical analyses of the detergents. Analysis of the components for similarities and of the known toxicity of the components could aid in further understanding why the detergent made in the country with the most stringent water quality laws was the most toxic to chironomid larvae in these tests.

Across all detergents, the two highest concentrations of detergent had 100% mortality in at least two replicates in the first 24 hours indicating that in the very short term, these detergents are extremely toxic to macroinvertebrates. Depending on the frequency with which washing occurs at a site, this could potentially have catastrophic impacts on the macroinvertebrate populations near these washing sites. Chironomid larvae are considered to be in the mid-range of sensitivity for benthic macroinvertebrates. As such, data from these tests should be considered appropriately as these LC_{50} values could be an overestimate of toxicity for less sensitive macroinvertebrates such as snails and potential underestimates for the more sensitive macroinvertebrates such as mayflies.

To better understand how short term toxicity may factor in to the survival of benthic macroinvertebrates, it would be pertinent to extend field studies to include the collection of more data on the frequency with which each of these major washing sites are used. Additionally, the collection and identification of macroinvertebrates to a finer taxonomic scale would be an appropriate next step so that each genus or species can be assessed for its relative sensitivity to pollutants and other disturbances. Understanding the ability to and

rate at which recolonization and migration occurs with regards to the benthic macroinvertebrates in Chisitu stream would be a useful tool in better assessing how the toxicity of these detergents may play into overall impacts to macroinvertebrate richness, community composition, and ecosystem functions.

All of the analyses conducted with field data collected during this study were limited by small sample sizes. Future work in this area would be well suited to encompass a longer period of sampling and to include other commonly used washing sites along Chisitu stream. This study primarily occurred during the cold season in Zambia when the ephemeral streams were flowing at some of their highest, most consistent discharge of the year due to the rains which fall from November through April. Data collection across all seasons would be beneficial in order to better understand the macroinvertebrate communities of Chisitu stream as well as to gain an understanding of when the various taxa are in each phase of their life cycle. Understanding this information could potentially prove beneficial in the development of a management plan or recommendation for the local community with regards to their washing habits.

To further improve community recommendations it would be prudent to consider the use of a rapid biomonitoring protocol to assess ecosystem health in Chisitu stream. Bioassessment protocols are a widely accepted methodology for determining river health or condition (Dickens and Graham 2002) and are largely recognized as an initial step towards protecting biological diversity (Karr and Chu 1999). These protocols replace older methods which relied primarily on the collection of chemical and toxicological parameters

(Ogren 2014). In some cases these protocols can provide a faster assessment of a system (Dickens and Graham 2002). Additionally the use of bioassessment protocols can detect issues with water quality which may not be indicated through chemical and toxicological parameters but show up in biological parameters (Ogren 2014).

As a result of varying diversity in community assemblages and habitat types, no one biomonitoring protocol can be effective throughout the world (Diamond et al. 1996). To combat this issue in Southern Africa, collaboration between several Universities, Non-Governmental Organizations (NGOs), conservation groups, and governments have begun to develop a set of bioassessment or biomonitoring protocols for the region (Dallas et al. 2010). Following the examples of the United States Environmental Protection Agency's (EPA) rapid bioassessment protocols (RBPs), the South African Scoring System-SASS (Dickens and Graham 2002) and the Namibian Scoring System-NASS (Palmer and Taylor 2004) a project termed the Southern African River Assessment Scheme, SAFRASS has been under development in Zambia to create an equivalent suite of RBPs to be used in place of high cost physical and chemical assessments of Zambian streams (Dallas et al. 2010). The SAFRASS project has also focused heavily on outreach and the development of assessment tools and materials which facilitate capacity building in the country (Lowe et al. 2013b). Implementation of the SAFRASS protocol, with modifications to better assess an ephemeral stream would be highly beneficial to both the stream and the community.

Other aspects of future studies that would be beneficial for better understanding how detergents impact benthic macroinvertebrates in Chisitu stream would involve estimation of discharge, which would be beneficial in making more accurate estimates of the environmental concentration of detergents in the stream. The collection of data on the transport of detergent downstream would also be useful in clarifying why there was a relationship between the distance downstream from the nearest washing site and the number of families present at a site. Additionally, this information could serve to potentially develop particular locations where washing in the stream may be more acceptable as its impact to aquatic life would be the most minimal.

Chapter Two: Zambia and the Peace Corps Experience

Situated 15.5 degrees south of the equator in the heart of Southern Africa, lies the nation of Zambia. Zambia is bordered by the Democratic Republic of Congo, Tanzania, Malawi, Mozambique, Zimbabwe, Botswana, Namibia, and Angola. Throughout its history Zambia has become vastly diverse in language and culture. Reminiscent of colonial days when it was known as Northern Rhodesia, Zambia today lists English as its national language; however according to Ethnologue has 46 living languages spoken within its borders. These languages are largely grouped based on locality and the ethnic group residing in that area. Due to emigration as a result of employment, natural disaster, and

proximity to cities, several areas of the country such Lusaka, Kitwe, and Ndola have become melting pots for traditional languages and cultures. While western culture and the English language is common in these larger cities, it is still quite common to find traditional languages and practices in these areas as well. Despite this infiltration of western culture in the urban areas, much of rural Zambia remains proudly traditional (Gertrude Mwanza, Personal Communication).

Culture of Zambia

Understanding Zambian culture is no simple task even if fully immersed in it for an extended period of time. The numerous ethnic groups that make up the country inevitably contribute to a mosaic of cultures varying from region to region. From the Lozi in the West to the Chewa and Ngoni in the east, Zambia's diversity in culture is a direct reflection of the vast area the country covers and ultimately the climate and topography of each region which drives its economy. Despite the differences amongst the ethnic groups of Zambia in terms of culture, a few commonalities still exist. The most obvious commonality and largest income generation activity for the majority of the population is agriculture. From maize, or corn as it is known in the United States, to cassava, sugar cane, and rice, agriculture on both the commercial and subsistence levels can be found in

throughout Zambia's 10 provinces: Central, Copperbelt, Eastern, Luapula, Lusaka, Muchinga, Northern, Northwestern, Southern, and Western.

As is the case for many cultures and countries, including the United States, food plays a central role in Zambian culture. In times of plenty, meals serve as a happy reminder of the hard work that went into planting and maintaining fields and gardens and even can sometimes be used as a status symbol. In times of scarcity and famine; however the story unfolds in quite the opposite fashion. Regardless of the amount of food available, Zambians love sharing food and readily invite friends, family, and revered guests to join them on the mpasa (reed mat in Nyanja) to share a meal family style. Maize forms the base of Zambian diet and is the major field crop cultivated in Zambia (MAFF 2001). In Zambian cuisine, maize is utilized in several ways. It can be ground for making Nsima, a thickened porridge that is formed into lumps and eaten with the right hand, or the maize can be freshly boiled or roasted for consumption as a snack or meal replacement when food is scarce (Gertrude Mwanza, Personal Communication). Outside of the kitchen, maize can be sold to the government where it is stored in reserve for distribution during droughts and famines. In 2015 maize sold to the Zambian government earned roughly $10 for 50Kg; however, depreciation of the national currency, the Zambian Kwacha (ZMW), coupled with rapid inflation, the value of maize came out to be quite low (Paul Phiri; Lackson Ngoma; Acren Moyo, Personal Communication). Other aspects of Zambian cuisine vary depending on region. For example, the Bemba people who inhabit the northern and central portions of the country grow cassava while the Tonga's to the south grow maize and sugar cane. The Chewas in the east grow maize, peanuts, and sweet potatoes, and the Kaonde's, Lunda's,

and Lozi's in the western portion of the country focus on rice, fish, and pineapples (Cleopher Bweupe, Personal Communication).

Another, universal aspect of Zambian culture is the love of song and dance. Zambian people often enjoy any opportunity which presents itself to song and dance. This song and dance is often linked to another powerful aspect of Zambian culture today-religion. Throughout the country there are two major religions: Christianity and Islam with some traditional religions holding on tenuously in some parts of the country. These two religions coexist in harmony bringing about the reputation that many Zambians like to tout of a "peaceful (read peace-uh-full) nation."

Climate

Much of the agricultural production in Zambia is heavily influenced by rainfall. According to the FAO, Zambia's climate can be described as tropical. However Zambia has several microclimates which influence the rainfall patterns throughout the country. The FAO has divided Zambia into three major rainfall regions based on the amount of precipitation received annually (MAFF 2001). Much of Southern province, the southern portions of Western province and the Luangwa River Valley in Eastern and Central Zambia to form rainfall region I. Region I is characterized by less than 700mm of rainfall annually and frequently experiences high temperatures with some provinces experiences upwards

of 35°C in the hot season months of October and November. Rainfall region III encompasses the northern half of the country including much of Luapula, Northern, Muchinga, Copperbelt, and Northwestern Provinces. These areas receive an average of 1000-1500mm of rain annually (MAFF 2001) and as a result are home to some of Zambia's better rice, cassava and fruit producing regions. The Eastern Province, which was my home, received 800-1000mm of rainfall annually (MAFF 2001) and was considered Rainfall region II. This region also includes Central, the northern reaches of Southern, Lusaka and Western provinces.

Hindrances to Development

While many western luxuries can be obtained in parts of Zambia, the majority of the country is still developing. Problems ranging from illiteracy to energy production, food security and access to clean drinking water plague the nation. This coupled with a high rate of HIV has drastically hindered the nation's development. In an effort to promote sustainable development within Zambia, numerous NGOs and similar organizations such as the United States Peace Corps are working on a variety of projects in Zambia to aid the country in its development.

One of the most prominent hindrances to development in Zambia is the high rate of malaria. With numerous endemic species of mosquito, the country harbors three

different malaria strains including *Plasmodium vivax* and *Plasmodium falciparum*, the most prevalent strains in Africa (W.H.O. 2016). Malaria is an easily preventable malaise through the regular use of prophylaxis, however these medications are often quite expensive and therefore out of reach of the average Zambian's income (Paul Phiri, Personal Communication). Further methods of malaria prevention, which are commonplace throughout Zambia are sleeping under insecticide treated mosquito nets. These nets are distributed by the Zambian government through a collaboration with USAID, the United State Agency for International Development, have a lifespan of 5 years and are the preferred measure for the prevention of the transmission of malaria (W.H.O 2016). Despite the presence of these nets, malaria is still regularly contracted by many Zambians, largely as a result of the misuse or disuse of mosquito nets. It is a common belief that mosquito nets inhibit air flow during the hot season, making sleeping unbearable and as such are often not used. By not using these nets while sleeping, Zambians are placing themselves at a high risk of contracting malaria as the peak hours for mosquito activity in the country are from 10PM until 2AM. Thankfully, if detected soon enough, malaria is easily treated and the medication Coartem, is readily available at many village stores or "tuck shops" or can be obtained free of charge at government health clinics and hospitals (W.H.O 2016)

Challenges of Conducting Research in a Developing Nation

Embarking upon a field study in a rural community in a developing nation is challenging. Zambians are a very curious people, so the initiation of something that involved a white person going to the stream and using tools they had never seen came as quite a shock to people in the community. This is where the Peace Corps experience became beneficial to me as a researcher. Since I had lived in this community for over a year at the start of data collection and because of the trusting relationship and local language skills I had acquired during that time, it was relatively easy for me to explain what I was doing and why. Looking back on this, I am not sure that coming into Zambia as purely a researcher I would not have been nearly as successful as I was. Similarly, the experience of conducting research in Zambia led to the furthering of many skills that were useful to my Peace Corps service as well. Trying to explain my research forced me to expand my vocabulary in Nyanja and when I was unable to find a word, use alternative forms of communication to express my point. Conducting research in Zambia also provided me with the opportunity to teach the people I worked with and encountered along the stream while sampling a little bit about aquatic ecology and how the organisms in the stream function. This proved to be and still is an especially important aspect of my research, when I learned that the closest translation of benthic macroinvertebrate, the organisms I was sampling, was "vidoyo," which literally translates to pest. In Nyanja, there is no distinction between insects in their larval stage and those that are adults. There is also no distinction between the aquatic and terrestrial insects. To the people of Kalichero village,

there simply was “vidoyo”, pests. The only insects they knew or really cared about were those that consumed their crops and caused them to lose precious income or worse, food. Through interactions with the people I met while out in the stream sampling however, I believe that I was able to create a distinction and it was through these efforts that I realized another goal for this project. From this point forward, not only was I working to conduct my research to the best of my abilities, but I was also now tasked with informing Kalichero community on how to better care for their environment.

To ignore the challenges involved in working in Zambia would be a disservice to many of the toughest lessons I learned along the way. The most prominent issue I faced while working in Zambia was obtaining research equipment. Items such as meters for measuring water chemistry (pH, Dissolved Oxygen, turbidity, etc.) are not readily accessible to the rural based researcher. Obtaining these items would have involved having them shipped from the United States, a costly and time consuming endeavor when one factors in shipping costs as well as customs tariffs. Since ethanol is rarely used for household purposes so it was not available in the markets of Chipata, my local city. I did however manage to find a pharmaceutical dealer who imported it from South Africa and kept it in stock for $30 for two liters. When I talked to my counterparts about this cost, and explained its use, they recommended that I use the local village whiskey next time.

A further obstruction to conducting research in rural Zambia was the availability of labs to process samples. For my particular study, I had hoped to collect water samples from before and after washing. However, after some research, I came to the realization that the

nearest lab that could process these samples was across an international border, in Malawi. Getting the samples there would have required extensive paperwork and be costly in transport. Domestically, Zambia had a lab at the University of Zambia which could have processed the samples; but again this lab was quite far away in the capitol of Lusaka. On a good day, when the road was not under construction transit from Kalichero to Lusaka would take roughly 7 hours and cost upwards of $30, thus making frequent trips prohibitively expensive. Even had there been a lab nearby, the storage of water samples was out of the question as there was limited reliable electricity in the community to keep samples cold in a refrigerator or freezer.

Other hindrances to conducting research in the developing world are not necessarily obvious from the start. One such example of this that I encountered, was the availability and reliability of field assistants. As a result of working both as a researcher and a PCV, I was sometimes expected to leave my site to attend provincial meetings, load up on food and supplies or even attend trainings. During these times, I often wished to continue data collection so that I could get a continuous data set for the duration of the time that these families would be relying heavily on Chisitu stream for washing their clothes. To accomplish this, I worked with two of my more educated counterparts in the community to train them in data collection, use of the pH meter, and how to collect the macroinvertebrates and preserve them properly. The training of my counterparts seemed to go really well. To test their skills I spent a day observing them collecting data as a practice run and everything went smoothly. They worked great as a team, kept the samples organized, and made sure to write everything down in the log book. When I set them out on their own however, things

changed. I came back from a visit to the US for vacation to find that stream metrics had not been fully recorded at all locations and some pH data was missing. To my relief though, all of the sample bottles I left for them had been filled and labelled. When I went to pick samples and identify the macroinvertebrates collected, I found that several bottles contained the same label and others no label at all. In the end, I only lost one week of samples to this incident but it definitely has left me aware that human error is entirely possible, especially when working with assistants who have never been involved in research in aquatic ecology before.

One final challenge that I faced during my time working on this study in Zambia that is particularly pertinent to conducting research in aquatic ecology in the developing world was the influence of people. In two different ways, activities by humans caused a loss of data for this project. The first human-induced interference I encountered came just a few weeks into the study. After the first week of sampling I was told that I had to take the back way to get to the site because my providing soap to Martha Ngwira, one of the people whose washing site I monitored, as part of the study, was causing other people in the village to become jealous. Then on the fourth week, when I went to sample at the site where Martha washes her clothes, I found the site to be completely dry. Upon return to the village to tell Martha that her site was dry and to inquire about why and when this occurred, people seemed shocked and indicated that the stream had probably been dammed just above there. Whether this was for agriculture or out of jealously, no one would say for sure. I just had to let things go and move on from that site. The other human induced interference I had to deal with was people going "fishing" with pesticides. In these "fishing" events,

pesticides would be dumped into the stream and as they moved downstream, they would kill off the fish and make it easy for people to come in and collect. The fact that this methodology was used to catch fish for consumption is another concern. In the weeks following "pesticide fishing" incidences, sampling efforts would produce no macroinvertebrates. There's no telling what long term impact this may have had on the system, but I know for sure in the short term, it wreaks havoc.

Closing Remarks

Living, working, and conducting research in Zambia was a life-changing experience for me. Each day I learned new lessons, found new ways of doing things, and most importantly, was shown a new perspective from which to view something. Serving in the Peace Corps, much like working on a Master's thesis, is not for everyone. However, for those with the sense of adventure and the drive to make an impact in someone's life, it is an experience like none other. I cannot be sure that any of the work I did in Zambia will have an impact on Kalichero community and I do not know if my presence there changed anyone's lives. What I do know is that their influence on my work, research, and daily routine influenced my life, and for that I am eternally grateful.

Chapter Three: Community Recommendation

To Kalichero Community,

One year ago I left you with a promise that I would go back to the United States and finish up the research that I started in 2014. What follows is a summary of my research and my recommendations to you based off of the results.

Macroinvertebrates, the large invertebrates such as insects that live in streams and other water bodies are very important to the environment. When leaves fall into the streams and make the water look dirty, these are animals that eat the leaves and feed other animals. Macroinvertebrates are also a favorite food of some fish and birds. These animals vary in size from the size of a termite (nyelele) to about the size of a caterpillar (vincuala). They are unable to move long distances unless they use what we call drift, which is when they let go of the surface they are living on and let the water carry them away. As you can imagine, when the water in Chisitu stream is low, this is very difficult for them.

During my research in Zambia I collected macroinvertebrates to try and understand how washing in the stream with Boom and other detergents may be impacting them. Once I returned to the United States, I conducted some toxicity tests with Boom and Omo to see if these detergents would be toxic to macroinvertebrates. From these tests I found that Boom and Omo are both toxic to macroinvertebrates. In fact, only 18mg of Boom and 25mg of Omo in one liter of water can kill half of the population for one type of invertebrate which was used in testing. This amount is very small, it is about 10 times smaller than a kernel of maize.

From the data I gathered from talking to several of you in the survey back in 2014, I estimated that you are using about 1.5 big boxes of Boom paste for each family when washing. When this amount of detergent is put into the stream, it equates to roughly 89 mg/L, which is over 3 times higher than the amount needed to kill half of the macroinvertebrates in an area. While I did not collect data on how these detergents impact fish, research conducted by other scientists on this topic have shown that these detergents are also very toxic to fish.

Since the majority of people I interviewed during the survey indicated that they are already washing their clothes in the village or in a basin by the stream; making some changes to how and where you wash your clothes to protect the stream should be very simple. One option for keeping these detergents out of the stream is to move your washing to the village. By doing this and dumping the water out in the village you will reduce the amount of detergent entering the stream and help make Chisitu stream a better place for drawing drinking water and fishing. Another possibility, is to use the water to deter pests in your garden. If you decide to use this water in your garden, it is best to dilute it. Diluting the detergent water can be done by filling your watering can half way with soapy water and half with clean water. Doing this will reduce the amount of clean water you are putting in the garden and also safely prevents the soapy water from entering the stream. If you are unsure about trying this method, know that I used it in my garden in Kalichero. Alicia knows a lot about this type of pesticide and will be able to tell you more information about this. For those of you who currently wash your clothing in the stream and do not want to carry water back to the village for washing there is another option. If you continue to wash at the stream, it is recommended that you use a basin. By washing in the basin you are keeping the water with detergent in it out of the stream. When you are done washing, the soapy

water should not be dumped back into the stream. Instead, if you dump the water 20-30 meters away from the stream, some of the toxins in the detergents will be filtered out by the ground before the water re-enters the stream.

Overall, the results of this research are quite important to the health of Chisitu stream. With some small changes in how and where you wash your clothes, you can make a difference. There are many other factors which may also be impacting Chisitu stream, but by removing the detergents from the water, you can take a solid first step towards making Chisitu a healthier stream for your children and grandchildren.

Thank you for letting me conduct research in your community. The lessons I learned from you about life in Zambia and about conducting research will stay with me for the rest of my life. You are all family to me and I wish the best for all of you.

Zikomo Kwambili,

“Uncle” Wells

Tables

Table 1. Primary and secondary substrates along with mean ± SE for wetted width, depth, water temperature, and pH observed at each of the four sites where sampling occurred.

Site	Phiri	Ngwira	Mfumu	Ngoma
Primary Substrate	Exposed Bedrock	Silt	Exposed Bedrock	Gravel
Secondary Substrate	Silt and LWD	Submerged and Emergent Aquatic Vegetation	Gravel, Sand, LWD, Detritus	Sand, LWD, Boulders
Wetted Width-Mean±SE (m)	2.78±0.06	3.81±0.90	2.86±0.09	2.5±0.07
Depth-Mean±SE (m)	0.73±0.02	0.27±0.05	0.31±0.03	0.34±0.04
Water Temp.-Mean±SE (°C)	19.03±0.2 2	20.4±1.81	17.34±0.19	17.05±0.21
Before pH-Mean±SE	7.6±0.06	8.3±0.03	7.8±0.06	7.9±0.06
After pH-Mean±SE	7.5±0.08	8.2±0.03	7.7±0.07	7.9±0.06

Table 2. Social survey questionnaire issued to members of Kalichero community to better understand their washing habits. While the questions specifically reference Boom, information was collected no matter the detergent brand used.

Social Survey to Understand "BOOM" use and washing habits near Chisitu Stream

1) How much soap do you utilize when washing clothes for your family?
    a. Do you use BOOM bars or paste?
2) Where do you wash your clothes?
    a. Do you wash in a basin or directly in the stream?
    b. If you wash in a basin, where do you dump the water?
3) How frequently do you wash your clothes?
    a. Do you always wash in the same place?

Table 3. Matrix displaying presence/absence data for all Families of macroinvertebrates collected across the four sites sampled combining before and after washing sampling efforts. Sites are divided into Upstream (Up), Wash, and Downstream (Down) reaches and listed in downstream order. Wash site data is shown in grey for presentation clarity.

	Phiri			Ngwira			Mfumu			Ngoma		
	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down
Aeshnidae		X										
Ancylidae					X							
Baetidae		X	X	X	X	X				X	X	X
Coenagrionidae	X	X									X	
Dytiscidae												
Elmidae		X	X									
Gerridae					X							
Gomphidae		X	X	X	X		X				X	
Gyrinidae		X										X
Heptageniidae										X		X
Hydraenidae				X								
Hydropsychidae	X	X			X	X	X	X	X	X	X	X
Leptoceridae			X					X	X			
Lymnaeidae			X	X	X	X	X					X
Nepidae	X	X	X									
Physidae	X	X		X	X	X				X	X	X
Pisuliidae	X	X				X					X	X
Planariidae						X						
Planorbidae	X	X	X	X	X	X	X	X	X	X	X	X
Polymitarcyidae						X					X	
Pyralidae											X	
Sericostomidae	X						X					
Tabanidae		X			X	X						
Teloganodidae											X	
Thiaridae	X	X										
Tipulidae							X				X	X

Table 4. Matrix displaying presence/absence data for all Families of macroinvertebrates collected from all reaches during the first five weeks of sampling at Mfumu site.

	5/24/14			6/7/14			6/14/14			6/21/14			6/28/14			7/5/14			7/12/14			7/19/14			7/26/14			8/2/14		
	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down
Dytiscidae																														
Gyrinidae			X																											
Elmidae																														
Hydraenidae																														
Tabanidae																														
Tipulidae			X		X																									
Polymitarcyidae		X																												
Teloganodidae		X																												
Heptageniidae	X		X																											
Baetidae		X	X		X												X								X					
Gerridae																														
Nepidae																														
Pyralidae								X												X										
Aeshnidae																														
Gomphidae																										X				
Coenagrionidae								X															X						X	
Hydropsychidae	X	X	X			X		X								X				X			X			X				X
Leptoceridae																														
Sericostomidae																														
Pisuliidae		X				X																	X							
Ancylidae																														
Lymnaeidae																											X			
Physidae		X	X					X															X		X				X	
Planorbidae	X	X	X	X		X	X	X									X	X					X	X	X	X	X		X	X
Thiaridae																														
Planariidae		X																												

Table 5. Matrix displaying presence/absence data for all Families of macroinvertebrates collected from all reaches during the first five weeks of sampling at Ngoma site.

	5/24/14			6/7/14			6/14/14			6/21/14			6/28/14			7/5/14			7/12/14			7/19/14			7/26/14			8/2/14		
	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down	UP	Wash	Down
Dytiscidae																														
Gyrinidae																														
Elmidae																														
Hydraenidae																														
Tabanidae																														
Tipulidae													X																	
Polymitarcyidae																														
Teloganodidae																														
Heptageniidae																														
Baetidae																														
Gerridae																														
Nepidae																														
Pyralidae																														
Aeshnidae																														
Gomphidae	X																													
Coenagrionidae																														
Hydropsychidae	X			X							X	X	X	X			X	X			X					X				
Leptoceridae		X	X																											
Sericostomidae													X																	
Pisuliidae																														
Ancylidae																														
Lymnaeidae																												X		
Physidae																														
Planorbidae		X							X		X			X											X			X	X	
Thiaridae																														
Planariidae																														

Table 6. Median Lethal Concentrations (LC_{50}) and the Upper and Lower 95% confidence limits for each detergent and those replicates which were still usable following correction for mortality in the controls. The symbol ∞ represents values which were unable to be converted from $\log_{10}$ transformations due to excessively high standard errors.

Detergent	LC_{50} (mg/L)	Upper 95% Confidence Limit (mg/L)	Lower 95% Confidence Limit (mg/L)
Tide	9.43	36.85	∞
Omo	25.19	75.63	∞
Boom	17.87	90.93	∞

Figures

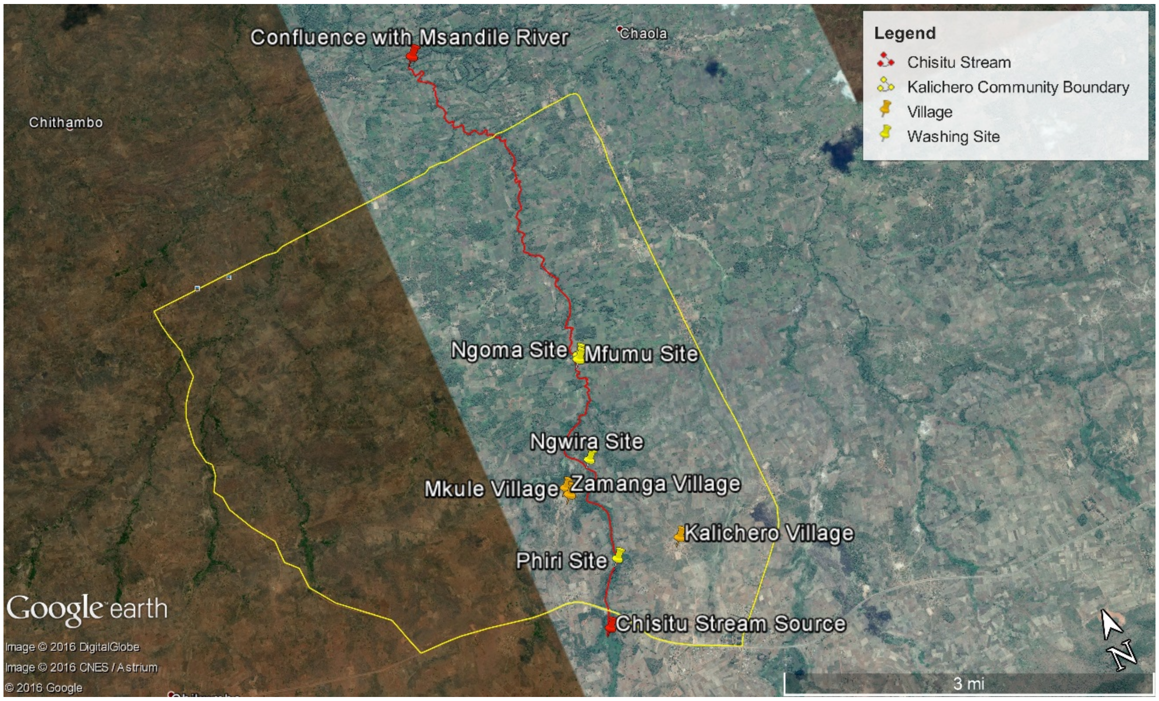

Figure 1. Satellite Imagery of Chisitu stream (in red) and Kalichero Community (Google Earth 2016). Chisitu stream flows from the bottom of the image to the top (South to North). Kalichero is designated by the yellow boundary.

Figure 2a. The “dambo” area where Chisitu stream originates (Google Earth 2016). This area is commonly used for watering cattle and for drawing water for various activities such as brick making thanks to its maintenance of water year-round.

Figure 2b. Phiri site and the surrounding area (Google Earth 2016). The dark boxes just upstream of Site 1 are fish ponds maintained by Mr. Paul Phiri and utlize the water from the stream through inlet and outlet canals.

Figure 2c. Ngwira site and the surrounding area (Google Earth 2016). To the left of the stream are Zamanga and Mkule villages and to the right, the main agricultural area for Kalichero village, which is not pictured but roughly 1.5km away.

Figure 2d. Mfumu and Ngoma sites and their surrounding areas (Google Earth 2016). In this area, the stream is bounded on both sides by agriculture in the form of fields and small gardens. On the right of the stream at a distance of about 0.5-0.75 km is my home.

Figure 2e. Confluence of Chisitu stream and the Msandire River about 7-8 km north of the northern-most study area, Site 2 (Google Earth 2016).

Figure 3. Photo of the detergents used in toxicity testing. Boom detergent is Zambian made and was used in its paste form which is sold in 400 g boxes. Omo detergent from South Africa was in powder form and is sold most frequently in 1kg bags (shown here).

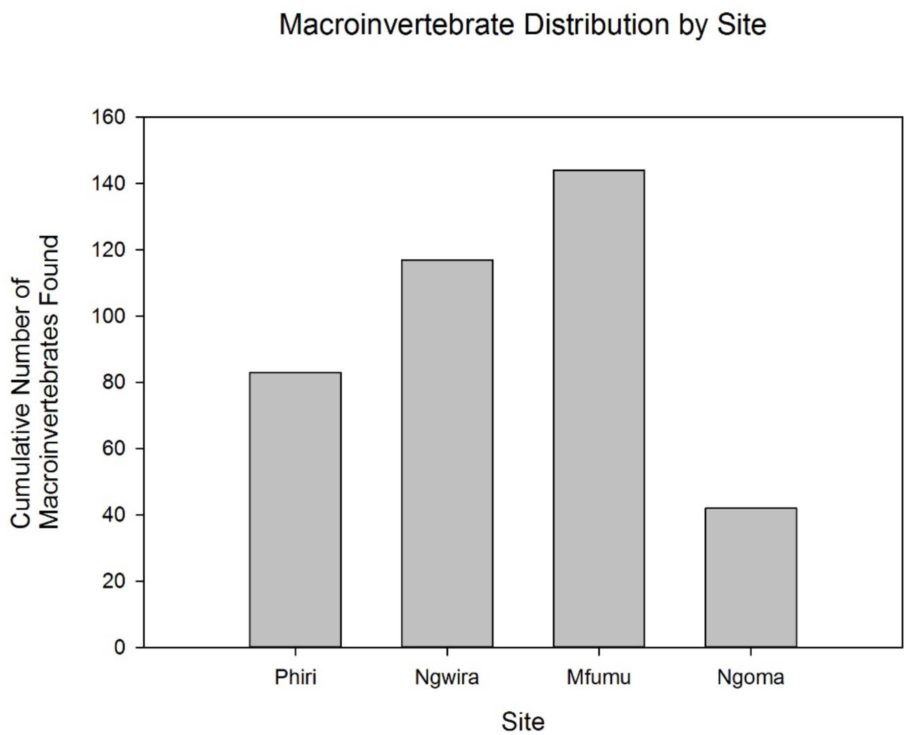

Figure 4a. Number of macroinvertebrates collected in total within each of the four sampling sites. Sites are listed in upstream to downstream order.

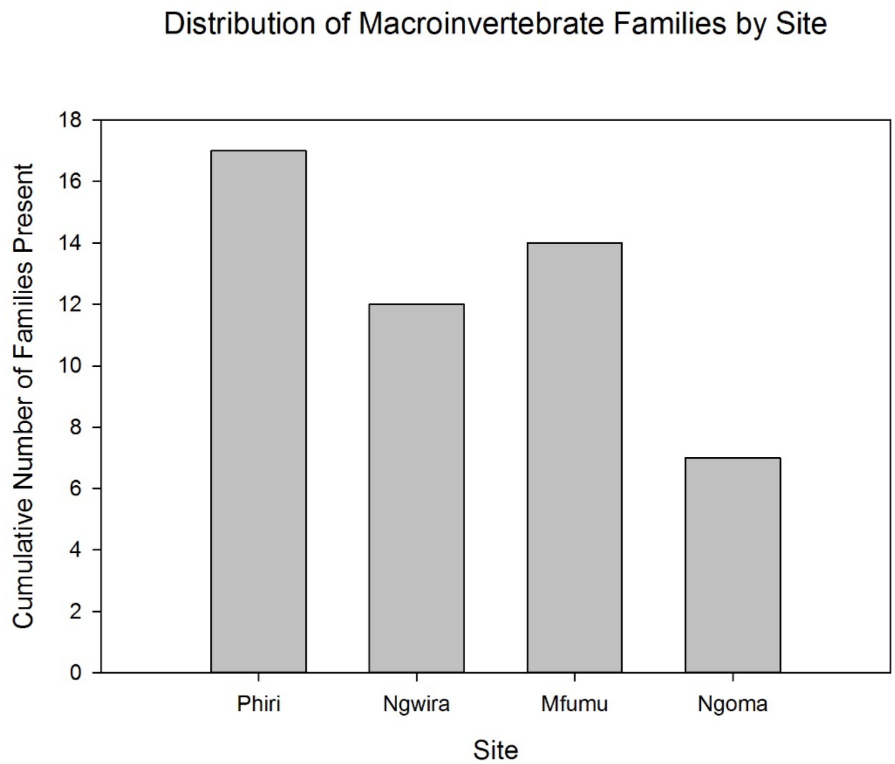

Figure 4b. Number of macroinvertebrate families collected in total within each of the four sampling sites. Sites are listed in upstream to downstream order.

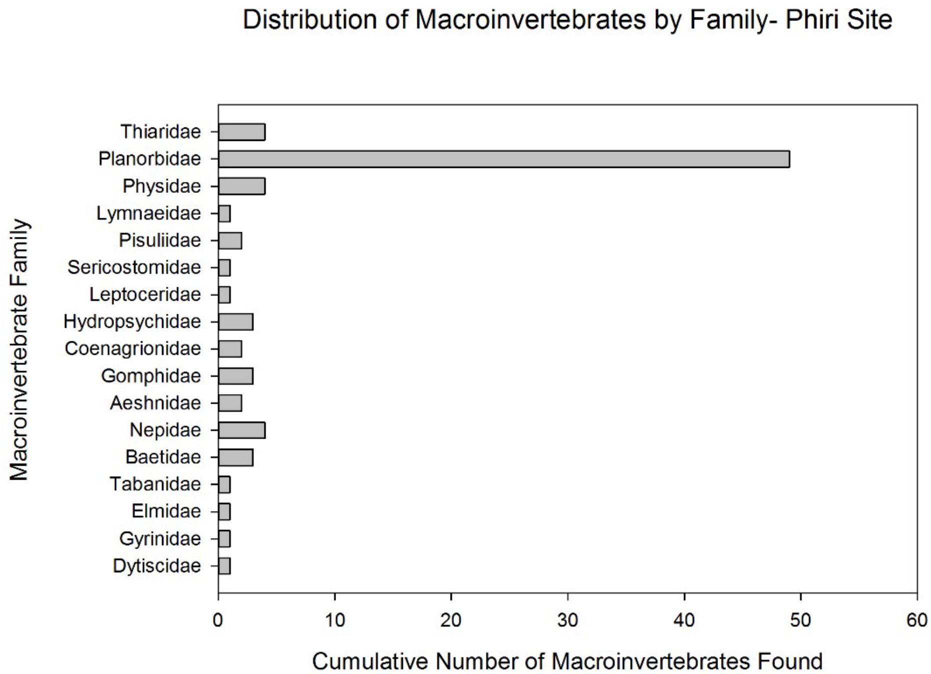

Figure 5a. Cumulative macroinvertebrate abundances for the families found at the Phiri site.

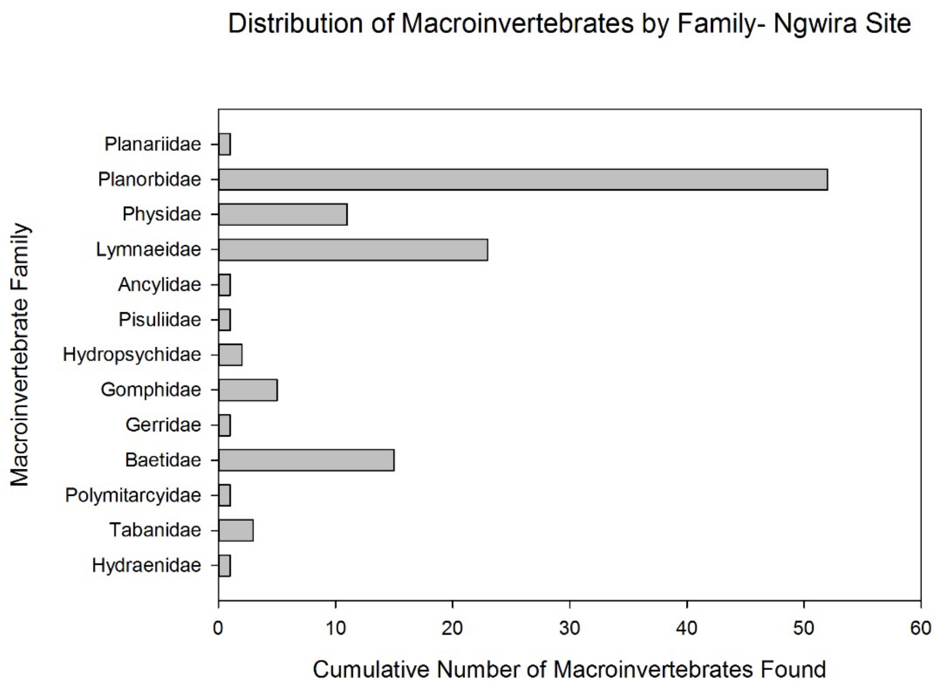

Figure 5b. Cumulative macroinvertebrate abundances for the families found at the Ngwira site.

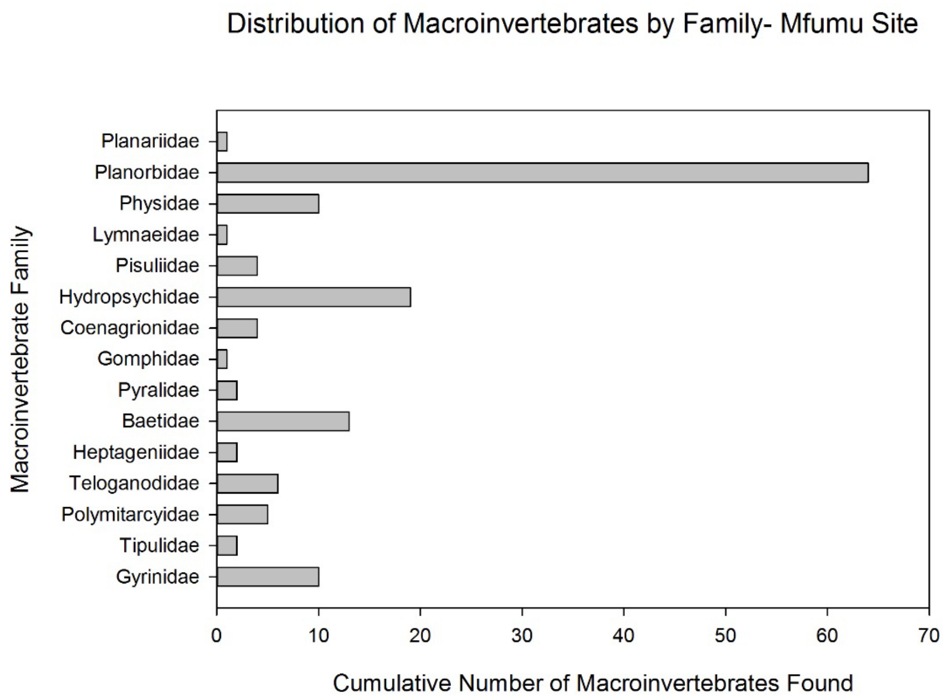

Figure 5c. Cumulative macroinvertebrate abundances for the families found at the Mfumu site.

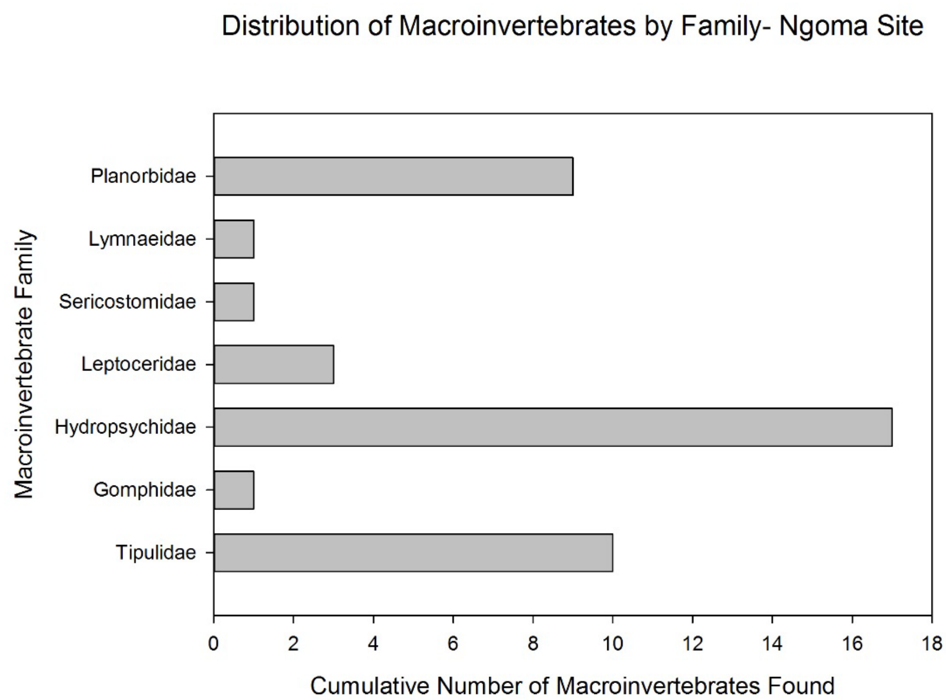

Figure 5d. Cumulative macroinvertebrate abundances for the families found at the Ngoma site.

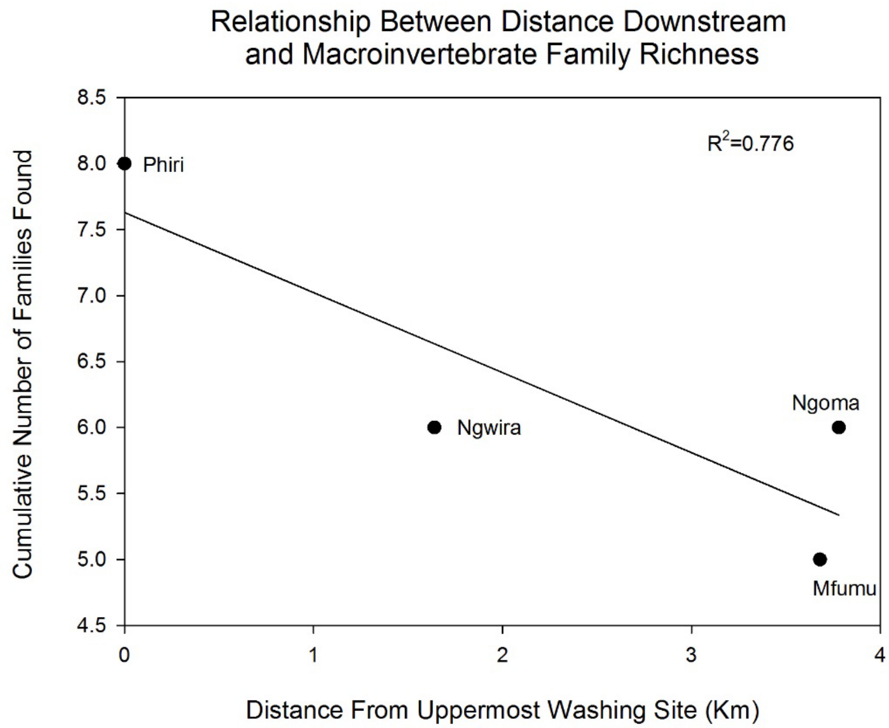

Figure 6a. Cumulative number of families detected from a site relative to the distance downstream from the uppermost washing site.

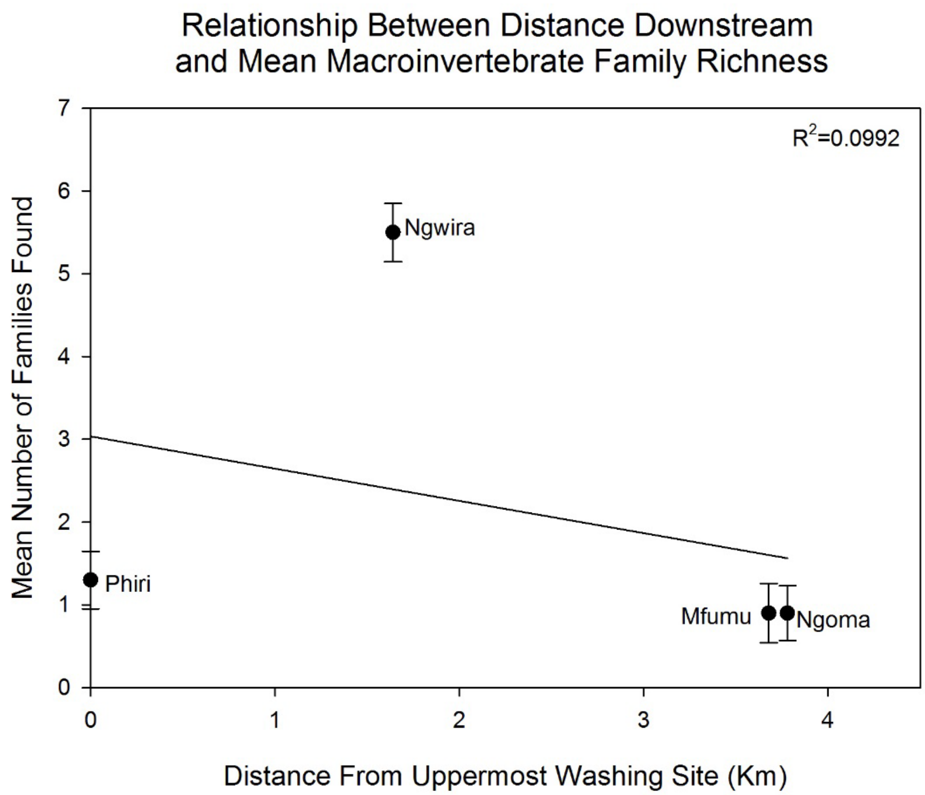

Figure 6b. Mean number of families detected from a site relative to the distance downstream from the uppermost washing site.

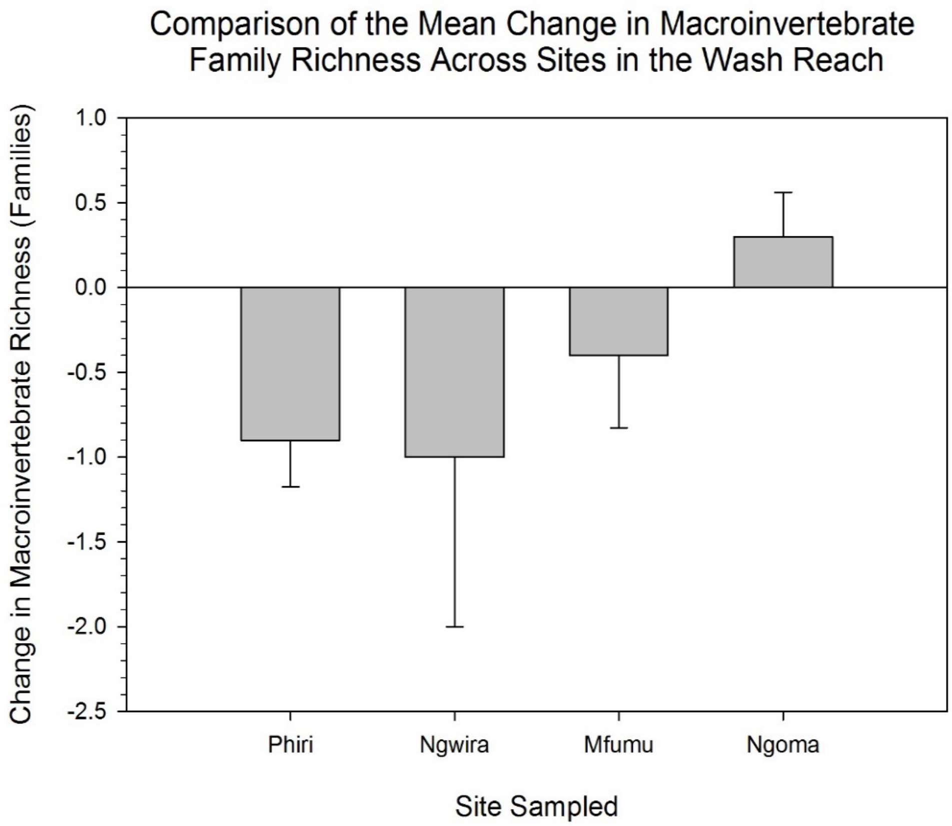

Figure 7. Mean change in macroinvertebrate richness at each of the four sites in the wash reach. Error bars are expressed as one standard error. Sites are arranged in upstream to downstream order.

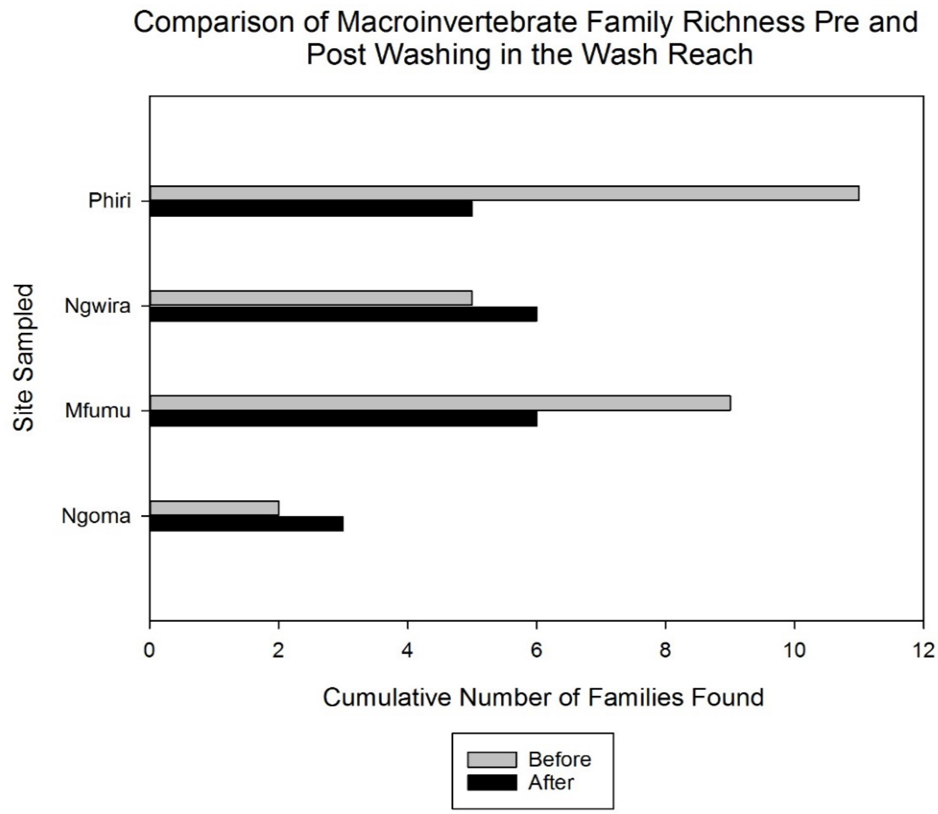

Figure 8. Cumulative macroinvertebrate family richness within the washing reaches before and after washing at each of the 4 sites. Sites are arranged from top to bottom in upstream to downstream order.

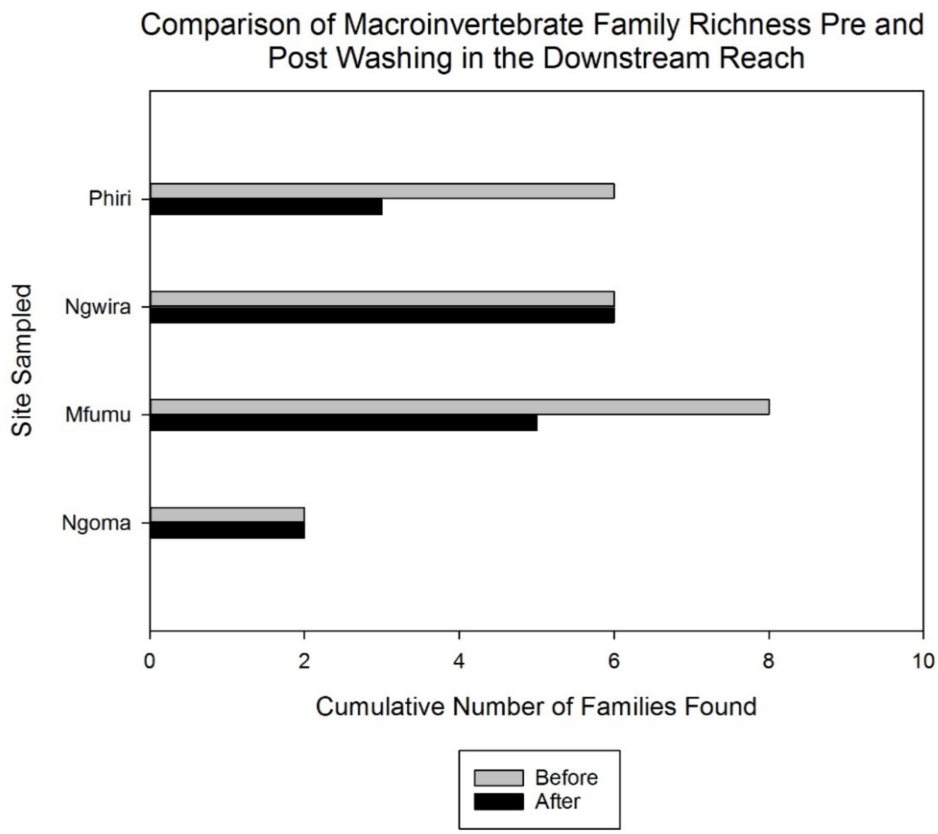

Figure 9. Cumulative macroinvertebrate family richness within the downstream reaches before and after washing at each of the 4 sites. Sites are arranged from top to bottom in upstream to downstream order.

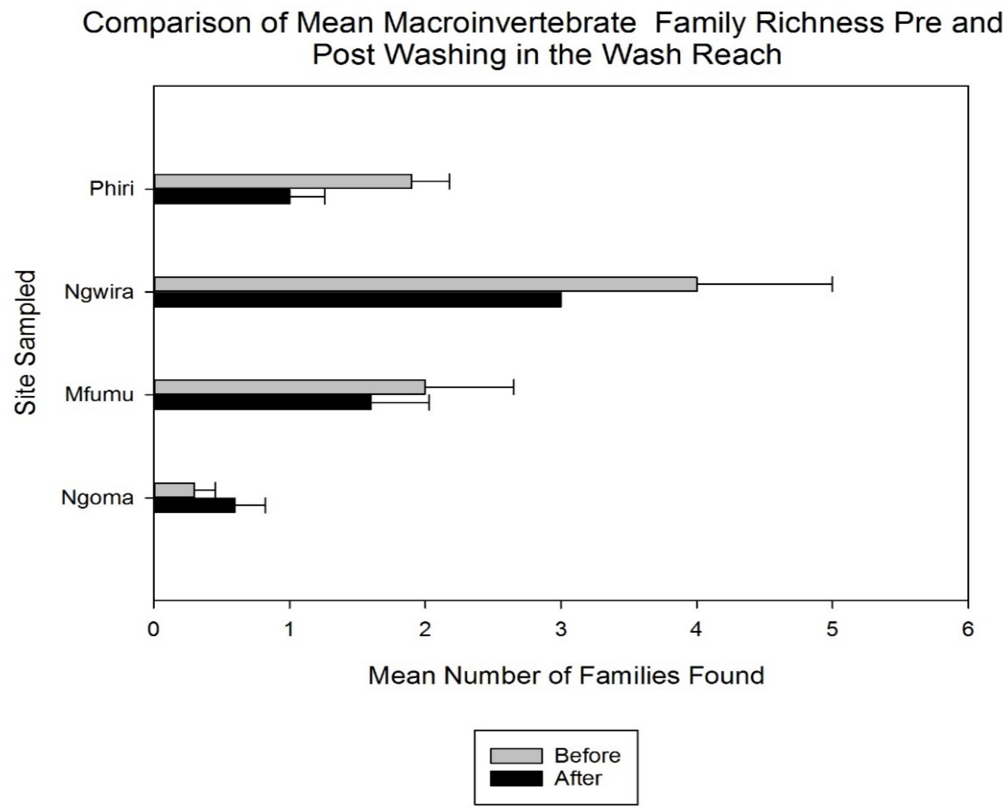

Figure 10. Mean macroinvertebrate family richness within the washing reaches before and after washing at each of the 4 sites. Sites are arranged from top to bottom in upstream to downstream order.

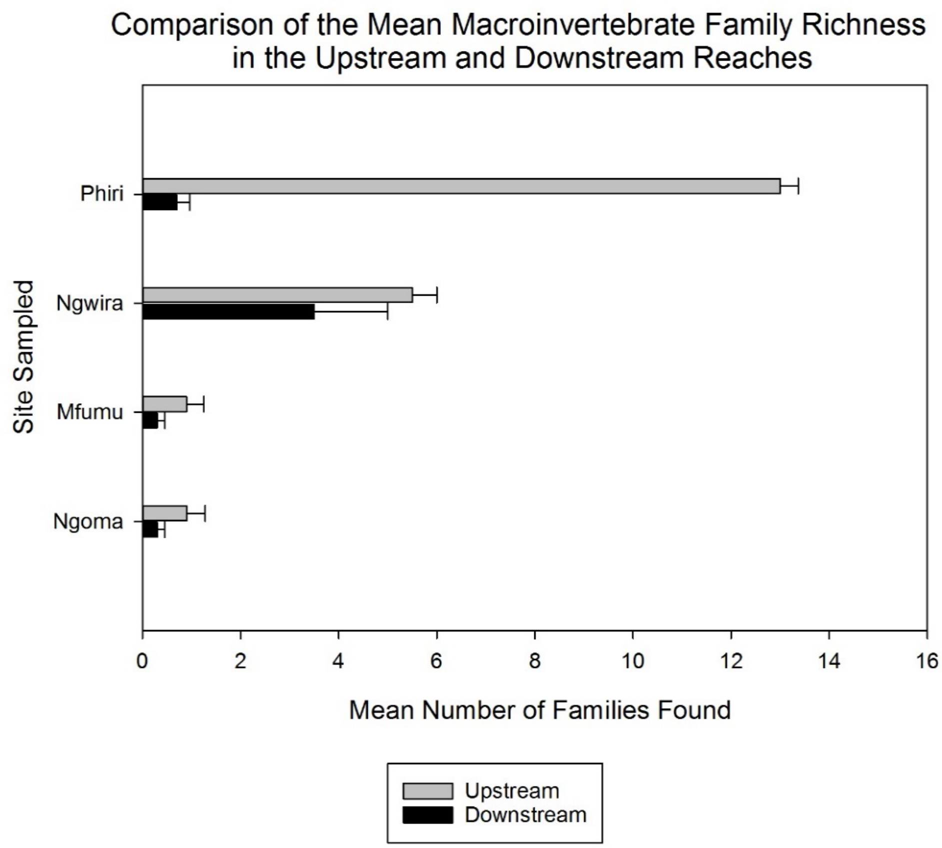

Figure 11. Mean macroinvertebrate family richness within the downstream reaches before and after washing at each of the 4 sites. Sites are arranged from top to bottom in upstream to downstream order.

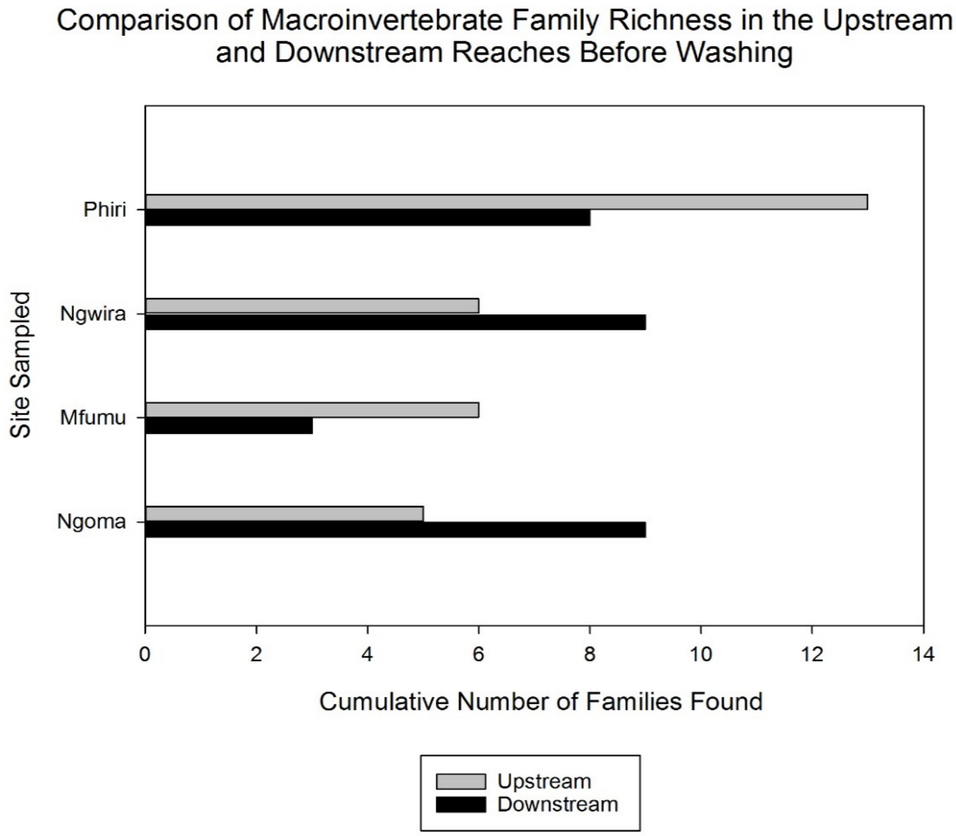

Figure 12. Cumulative macroinvertebrate family richness within the upstream and downstream reaches before and after washing at each of the 4 sites. Sites are arranged from top to bottom in upstream to downstream order.

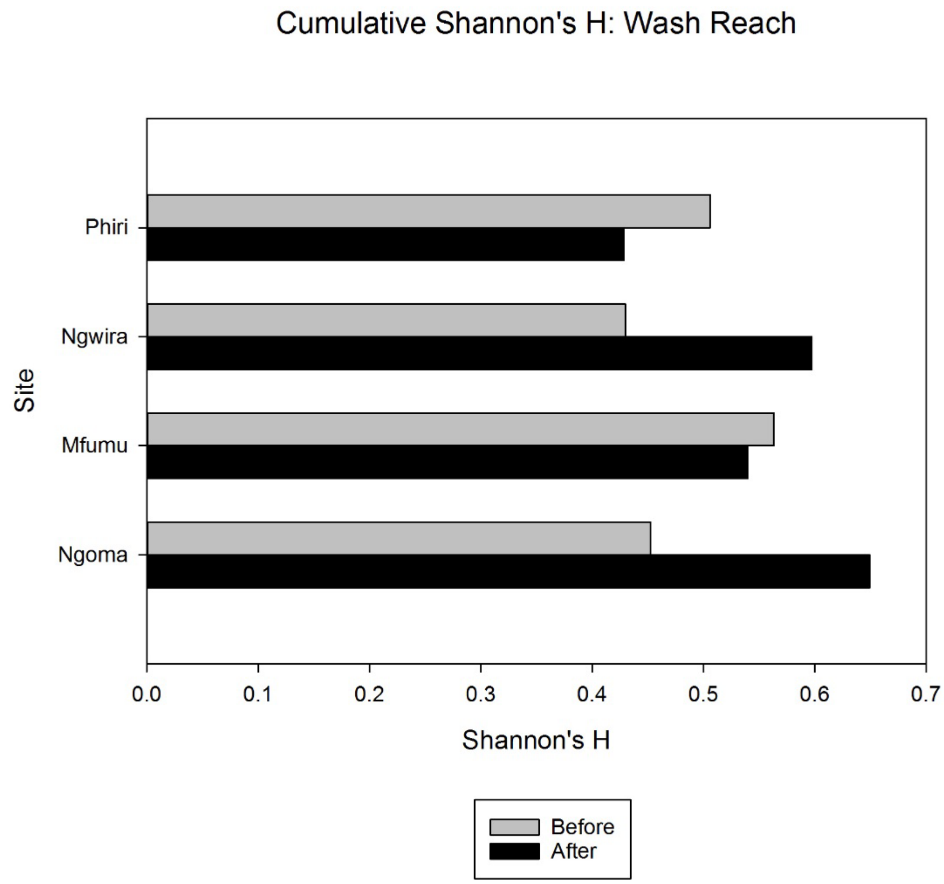

Figure 13. Change in diversity in the wash reach across the four washing sites sampled, pre- and post- washing. Diversity was calculated using the Shannon Index (H).

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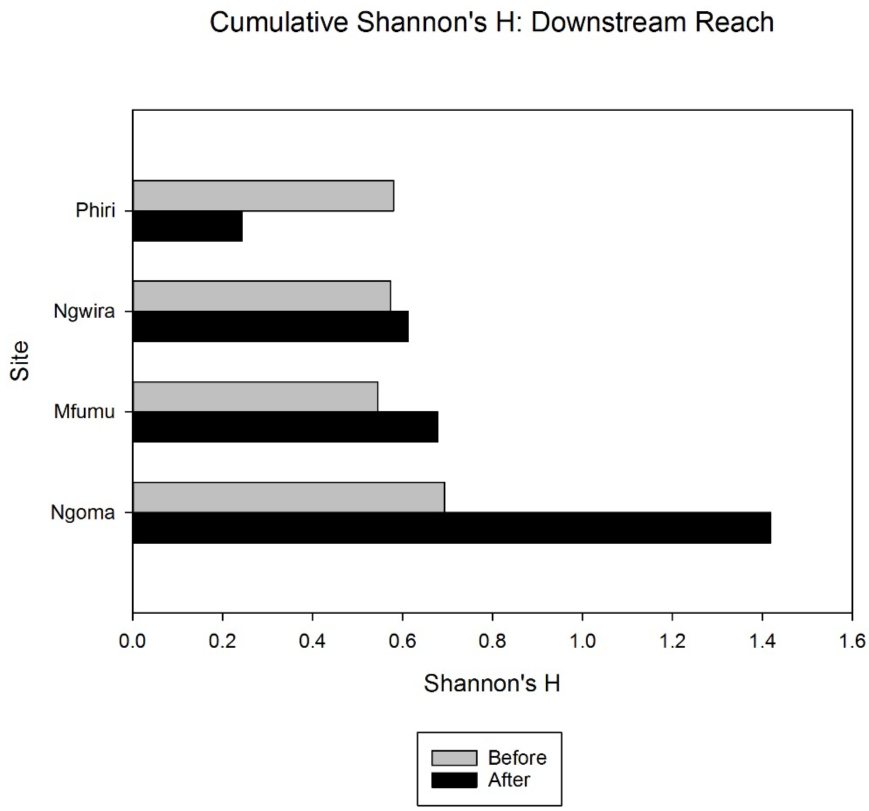

Figure 14. Change in diversity in the downstream reach across the four washing sites sampled, pre- and post- washing. Diversity was calculated using the Shannon Index (H).

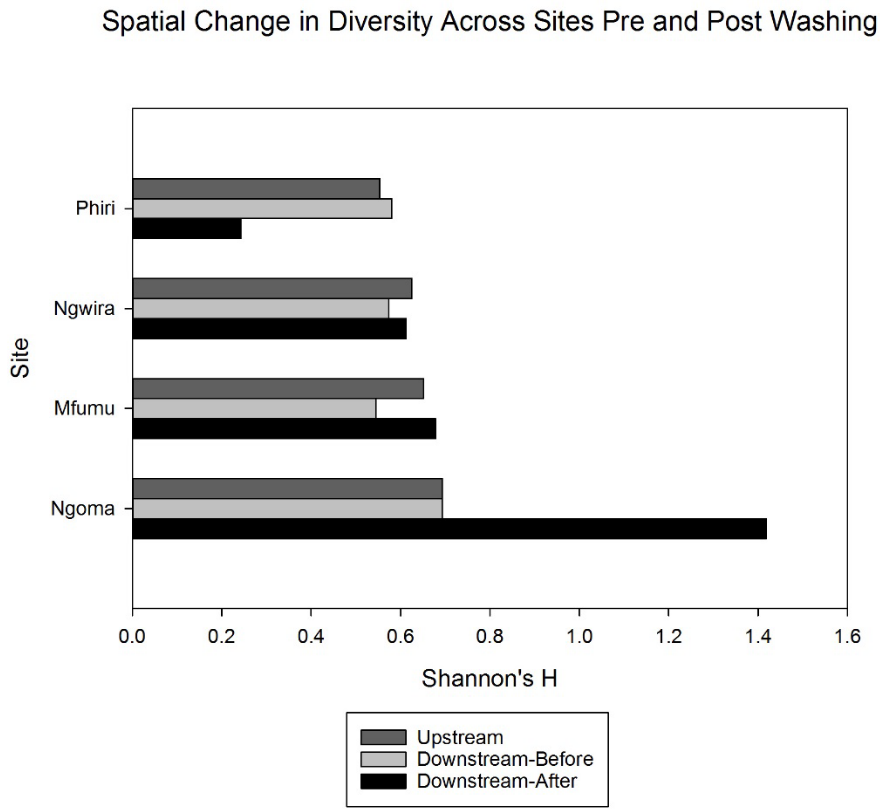

Figure 15. Spatial change in diversity between the upstream and downstream reaches across the four washing sites sampled. Diversity was calculated using the Shannon Index (H).

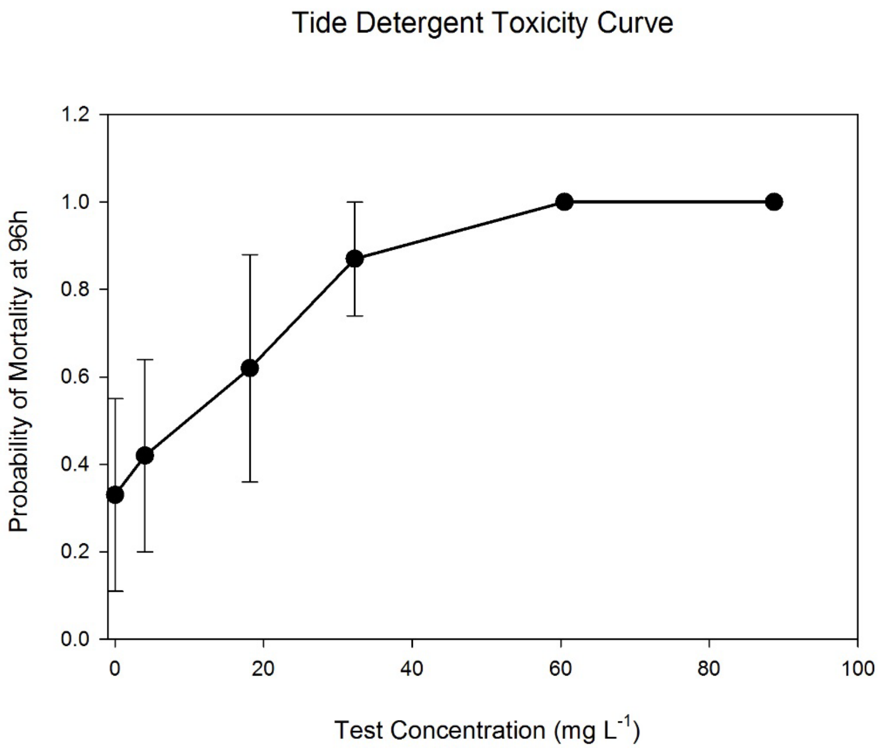

Figure 16. Dose-Response curve from acute toxicity test for Tide. The LC_{50} for this replicate was 9.43 mg/L with an Upper 95% confidence limit of 36.85 mg/L and the lower limit unable to be calculated.

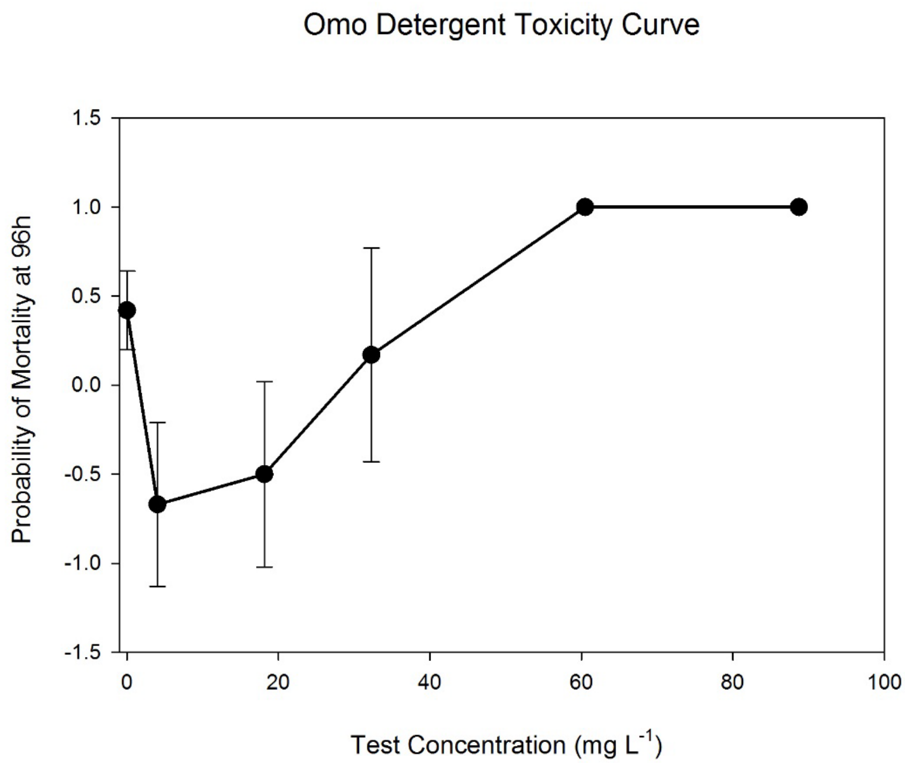

Figure 17. Dose-Response curve from acute toxicity test for Omo. The LC_{50} for this replicate was 25.19 mg/L with an Upper 95% confidence limit of 75.63 mg/L and the lower limit unable to be calculated.

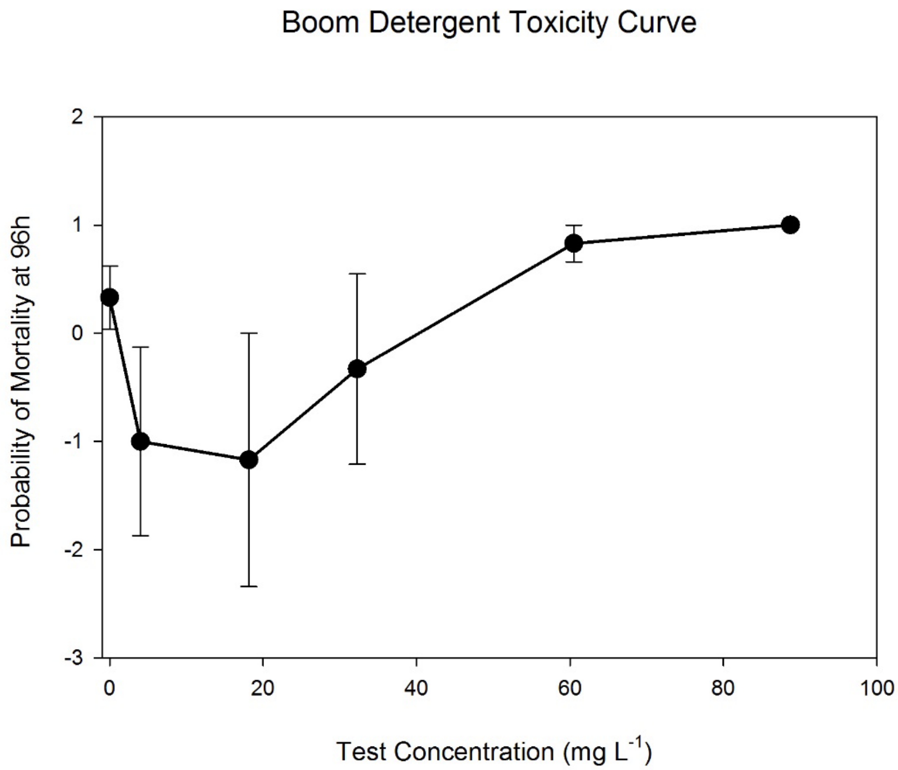

Figure 18. Dose-Response curve from acute toxicity test for Boom. The LC_{50} for this replicate was 17.87 mg/L with an Upper 95% confidence limit of 90.93 mg/L and the lower limit unable to be calculated.

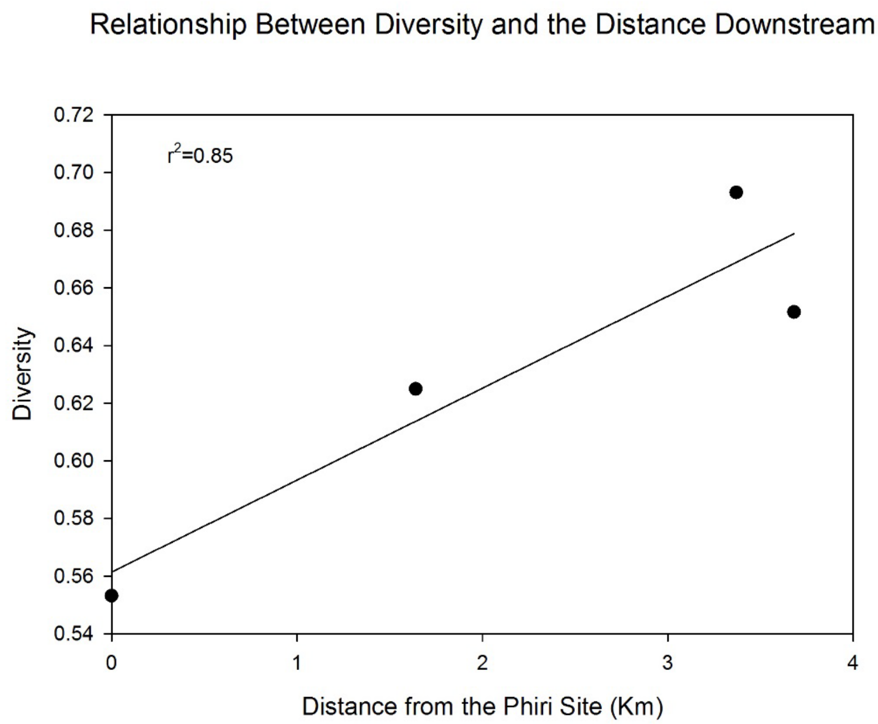

Figure 19. Comparison of the downstream distance from the uppermost washing site to the cumulative diversity (Shannon's H) calculated for each site.

References

2007. ASTM E729-96. ASTM International.

(WHO), W. H. O. 2016. Malaria.

Abel, P. D. 1974. Toxicity of synthetic detergents to fish and aquatic invertebrates. Journal of Fish Biology **6**:279-298.

Azizullah, A., P. Richter, and D.-P. Häder. 2011. Toxicity assessment of a common laundry detergent using the freshwater flagellate Euglena gracilis. Chemosphere **84**:1392-1400.

Baek, M., T. Yoon, D. Kim, C. Lee, K. Cho, and Y. Bae. 2014. Effects of Road Deicer Runoff on Benthic Macroinvertebrate Communities in Korean Freshwaters with Toxicity Tests of Calcium Chloride (CaCl2). Water, Air, and Soil Pollution **225**:1-14.

Batac-Catalan, Z., and D. S. White. 1981. Entomological news. American Entomological Society, [Philadelphia].

Bunn, S. E., and A. H. Arthington. 2002. Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental management **30**:492-507.

Chukwu, L., and C. Odunzeh. 2006. Relative toxicity of spent lubricant oil and detergent against benthic macro-invertebrates of a West African estuarine lagoon. Journal of Environmental Biology **27**:479-484.

Corps, P. 2016. Peace Corps Master's International.

Dallas, H., M. Kennedy, J. Taylor, S. Lowe, and K. Murphy. 2010. SAFRASS: Southern African River Assessment Scheme. WP4: Review of existing biomonitoring methodologies and appropriateness for adaptation to river quality assessment protocols for use in southern tropical Africa.

Dickens, C. W., and P. Graham. 2002. The South African Scoring System (SASS) version 5 rapid bioassessment method for rivers. African Journal of Aquatic Science **27**:1-10.

Farias, R., L. Carvalho, and E. Medeiros. 2012. Distribution of Chironomidae in a semiarid intermittent river of Brazil. Neotropical entomology **41**:450-460.

Finney, D. J. 1952. Probit analysis. JSTOR.

Gerber, A., and M. Gabriel. 2002. Aquatic invertebrates of South African rivers field guide.

Google Earth. 2016. Chisitu Stream.

Harless, M. L., C. J. Huckins, J. B. Grant, and T. G. Pypker. 2011. Effects of six chemical deicers on larval wood frogs (Rana sylvatica). Environmental Toxicology and Chemistry **30**:1637-1641.

Hein, J., and N. M. Madhu. 1992. Rearing & Maintaining Midge Cultures (Chironomus Tentans) for Laboratory Studies. The American Biology Teacher **54**:236-240.

Johnson, W. W., and M. T. Finley. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebrates : summaries of toxicity tests conducted at Columbia National Fisheries Research Laboratory, 1965-78. Report 137.

Karr, J. Chu., EW, 1999. Restoring life in running waters. Better Biological Monitoring. Island Press, Washington, DC.

Kehoe, T. 1992. Merchants of pollution?: The soap and detergent industry and the fight to restore Great Lakes water quality, 1965-1972. Environmental History Review:21-46.

Lewis, M., and D. Suprenant. 1983. Comparative acute toxicities of surfactants to aquatic invertebrates. Ecotoxicology and environmental safety **7**:313-322.

Lowe, S., H. Dallas, M. Kennedy, J. Taylor, C. Gibbins, P. Lang, J. Day, H. Sichingabula, K. Saili, and F. Willems. 2013a. The SAFRASS biomonitoring scheme: general aspects, macrophytes (ZMTR) and benthic macroinvertebrates (ZISS) protocols. Produced for the ACP Science and Technology Programme.

Lowe, S., H. Dallas, M. Kennedy, J. Taylor, C. Gibbins, P. Lang, H. Sichingabula, K. Saili, C. Ntobolo, and K. Kabangu. 2013b. Assessment of performance of the SAFRASS pilot river biomonitoring scheme.

MAFF. 2001. Agriculture Commercialization Programme 2002-2005.

Minshall, G. W. 2003. Responses of stream benthic macroinvertebrates to fire. Forest Ecology and Management **178**:155-161.

Ogren, S. A. 2014. Using indicators of biotic integrity for assessment of stream condition.

Palmer, R., and E. Taylor. 2004. The Namibian Scoring System (NASS) version 2 rapid bio-assessment method for rivers. African Journal of Aquatic Science **29**:229-234.

Pettersson, A., M. Adamsson, and G. Dave. 2000. Toxicity and detoxification of Swedish detergents and softener products. Chemosphere **41**:1611-1620.

SAFRASS. 2012. Photographic Guide to the Aquatic Macroinvertebrates of Zambia. Pages 1-16.

Schäfer, R. B., M. Bundschuh, D. A. Rouch, E. Szöcs, C. Peter, V. Pettigrove, R. Schulz, D. Nugegoda, and B. J. Kefford. 2012. Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services. Science of the Total Environment **415**:69-78.

Schneider-Orelli, O. 1947. Entomologisches Praktikum: Einführung in die land-und forstwirtschaftliche Insektenkunde. Sauerländer Aarau,, Switzerland.

Stephan, C. E. 1977. Methods for Calculating an LC50. Aquatic Toxicology and Hazard Evaluation, ASTM STP 634 **634**:65-84.

Strayer, D. L. 2006. Challenges for freshwater invertebrate conservation. Journal of the North American Benthological Society **25**:271-287.

Warne, M. 1999. Toxicity of Laundry Detergent Components to a Freshwater Cladoceran and Their Contribution to Detergent Toxicity. Ecotoxicology and environmental safety **44**:196-206.

Watson, M., and H. Dallas. 2013. Bioassessment in ephemeral rivers: constraints and challenges in applying macroinvertebrate sampling protocols. African Journal of Aquatic Science **38**:35-51.

Weber, C. I. 1991. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Environmental Monitoring Systems Laboratory, Office of Research and Development, US Environmental Protection Agency.

Williams, K. A., D. W. J. Green, D. Pascoe, and D. E. Gower. 1986. The Acute Toxicity of Cadmium to Different Larval Stages of Chironomus riparius (Diptera: Chironomidae) and Its Ecological Significance for Pollution Regulation. Oecologia **70**:362-366.

Appendix A

Mortality data for acute toxicity tests with Tide. Cells were left blank once total mortality had been reached. 11 chironomid larvae were inadvertently placed in the following test chambers: replicate A, 32.253 mg/L.

Time Checked (Hours Post Start)	Replicate	4.0175 mg/L	18.135 mg/L	32.253 mg/L	60.489 mg/L	88.725 mg/L	Control (0 mg/L)
3	Tide A	0	0	0	3	10	0
3	Tide B	1	0	0	7	9	2
3	Tide D	1	1	1	4	10	6
6	Tide A	0	1	2	7		0
6	Tide B	0	5	3	3	1	0
6	Tide D	2	3	0	4		2
12	Tide A	0	1	7			0
12	Tide B	0	3	4			0
12	Tide D	0	3	6	2		0
24	Tide A	0	1	2			0
24	Tide B	1	0	1			1
24	Tide D	0	0				0
48	Tide A	2	0				0
48	Tide B	0	2	2			1
48	Tide D	2	0				0
72	Tide A	4	0				0
72	Tide B	0					0
72	Tide D	0	0				0
96	Tide A	2	0				2
96	Tide B	0					0
96	Tide D	1	1				0

Mortality data for acute toxicity tests with Omo. Cells were left blank once total mortality had been reached. 11 chironomid larvae were inadvertently placed in the following test chambers: replicates A and B, 60.489 mg/L and replicate D, 32.253 mg/L.

Time Checked (Hours Post Start)	**Replicate**	**4.0175 mg/L**	**18.135 mg/L**	**32.253 mg/L**	**60.489 mg/L**	**88.725 mg/L**	**Control (0 mg/L)**
3	Omo A	0	0	0	5	9	0
3	Omo B	0	1	0	6	9	1
3	Omo D	6	4	0	8	10	3
6	Omo A	0	0	1	4	1	1
6	Omo B	0	0	0	4	1	0
6	Omo D	0	0	2	1		1
12	Omo A	0	0	3	1		0
12	Omo B	0	0	0	1		1
12	Omo D	0	1	4	1		1
24	Omo A	1	0	0	1		0
24	Omo B	1	1	1	0		2
24	Omo D	0	1	4			0
48	Omo A	1	0	3			2
48	Omo B	0	0	0	0		1
48	Omo D	0	1	1			1
72	Omo A	0	0	1			3
72	Omo B	0	1	1	0		2
72	Omo D	1	0				2
96	Omo A	0	0	0			0
96	Omo B	0	2	0	0		2
96	Omo D	0	0				0

Mortality data for acute toxicity tests with Boom. Cells were left blank once total mortality had been reached. 11 chironomid larvae were inadvertently placed in the following test chambers: replicate D, 18.135 mg/L and 32.253 mg/L.

Time Checked (Hours Post Start)	Replicate	4.0175 mg/L	18.135 mg/L	32.253 mg/L	60.489 mg/L	88.725 mg/L	Control (0 mg/L)
3	Boom A	2	0	0	7	10	0
3	Boom B	0	0	0	1	10	1
3	Boom D	1	0	1	7	10	0
6	Boom A	0	0	4	3		4
6	Boom B	0	0	1	7		0
6	Boom D	0	4	4	3		2
12	Boom A	1	1	3			0
12	Boom B	0	1	2	0		0
12	Boom D	0	3	0			4
24	Boom A	2	1	1			0
24	Boom B	0	1	0	1		3
24	Boom D	3	2	0			2
48	Boom A	0	0	0			0
48	Boom B	0	2	1			1
48	Boom D	3	2	4			0
72	Boom A	0	0	0			4
72	Boom B	1	1	0			4
72	Boom D	1		2			0
96	Boom A	1	0	0			0
96	Boom B	2	0	0			0
96	Boom D	1		0			1