EFFECT OF CONTROPHIC SPECIES ON NATURAL POPULATION DYNAMICS OF MALARIA MOSQUITO LARVAE ON RUSINGA ISLAND

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented for a degree or any other award in any other University.

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DEDICATION

This work is dedicated to my loving parents, the late Dr. Lambert Isaac Mirieri and Mrs. Miriam Mirieri together with my siblings Christopher, John and David.

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ABSTRACT

Controphic species are organisms that share the same trophic levels within an ecosystem. Ecological theory and empirical research show that controphic species affect mosquito larval populations through predation or competition. The goal of this study was to identify controphic species that may serve as potential biological control agents for malaria mosquito larvae. A baseline survey of the abundance and diversity of malaria mosquito larvae (target species) and assemblages of their controphic species in breeding sites present on Rusinga Island was conducted. The effects of physicochemical factors on mosquito larval dynamics were also evaluated. The Renyi, Inverse Simpson and Shannon diversity indices established the presence of 35 controphic species in all habitat types studied. Redundancy analysis showed a positive correlation between the *Anopheles* species abundance with oxygen availability and levels of conductivity, salinity, pH and turbidity in decreasing order of importance .*Anopheles* species abundance had a negative correlation with depth, volume of water and distance of the larval habitat from the nearest house. Competitors contributed largely to the variation seen in the abundance of L1/L2 *Anopheles* larvae ($R^2 = 77.2\%$). The predators had a negative effect on abundance of the L1/L2 *Anopheles* larvae ($R^2 = 24.76\%$). The study demonstrates that assemblages of controphic species modulate the population dynamics of *Anopheles* mosquito larvae and are also affected by the physicochemical environment in the mosquito larval habitats.

ACRONYMS

ACTs:	Artemisinin-based Combination Therapies
Bs:	*Bacillus sphaericus*
Bti:	*Bacillus thuringiensis var. israelensis*
DDT:	Dichlorodiphenyltrichloroethane
DO:	Dissolved Oxygen
IRS:	Indoor Residual Spraying
ITNs:	Insecticide Treated Nets
LLINs:	Long-lasting Insecticidal Nets
LSM:	Larval Source Management
MPHS:	Ministry of Public Health and Sanitation (currently Ministry of Health)
PCA:	Principal Component Analysis
PCR:	Polymerase Chain Reaction
PPF:	Pyriproxyfen
RDA:	Redundancy Analysis
SIT:	Sterile Insect Technique
WHO:	World Health Organization

1. CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Vector control is of great importance in combating malaria. Since the year 2000, the World Health Organization (WHO) embraced vector control as a major tool for combating malaria. This has been done by financially facilitating increased availability of insecticide-treated bed nets (ITNs) from 3% to 50% by the year 2011 and increased indoor residual spraying (IRS) from 5% to 11% by the year 2010 in Sub-Saharan Africa (WHO, 2011). This shows that in Kenya and other Sub-Saharan African countries where Malaria is endemic while there have been massive investments towards provision and access to Artemisinin-based Combination Therapies (ACTs), efforts have been largely towards adult mosquito control. Adult mosquito control has been achieved using ITNs and IRS, which both use chemical insecticides. In contrast, there has been little adoption of larval control strategies.

In 1939, Dichlorodiphenyltrichloroethane (DDT) and other conventional insecticides were discovered leading to a neglect of the biological methods of control because of their effectiveness in killing insect pests such as mosquitoes. Consequently, Rachel Carson (1962) brought to the attention of the world that, the continued indiscriminate use of chemical insecticides was harmful to the environment. Since the commencement of the use of chemical insecticides, continued indiscriminate, uncontrolled and unexamined use of these insecticides for about a century has had major impacts on human health and the environment by causing resistance in mosquitoes and affecting non-target organisms (Zhang *et al*., 2012). Due to these concerns, it has become necessary to find new intervention tools other than the harmful conventional use of chemical insecticides. There is renewed interest in the use of biological control methods such as the use of bacteria like *Bacillus sphaericus (Bs)*, *Bacillus thuringiensis var. israelensis (Bti),*the use of fungus as well as predators and competitors (hereafter termed controphic species of the mosquito larvae).These control methods target the larval stages of mosquito vectors (Blaustein and Chase, 2007; Becker *et al,* 2010; Mwangangi *et al.,* 2011; Scholte *et al.,* 2004).

In their review, Walker and Lynch (2007) concluded that targeting larvae can significantly reduce their numbers especially in man-made habitats and particularly in urban areas where the larval habitats are limited. The use of *Bti* is one of the most studied, most successful, and one of the best environmentally benign larval control methods since it does not affect non-target organisms (Kahindi *et al*., 2008; Shililu *et al.*, 2003). However, it can be costly especially to resource-poor communities where malaria is endemic. It is more useful in areas where malaria transmission is low or moderate and where mosquito breeding sites are contained and well defined (Worral and Fillinger, 2011).

Controphic species may play a major role in reducing mosquito larval populations. The mosquito life cycle exists in four stages of egg, larva, pupa and adult. The first three stages are aquatic thus giving an opportunity for larval control. A large number of invertebrate taxa share the same larval habitats as mosquitoes and interact with their larvae through predation, competition and mutualism (Duquesne *et al.*, 2011; Elono *et al.*, 2010). These invertebrate taxa are generally found in the phylum Arthropoda with exceptions such as the Anuran larvae and the Mollusca. The Athropods include the larvae of Diptera, Coleoptera, Hemiptera, Zygoptera, Hydrachnidiae, Anisoptera and Crustaceans among others. Members of these invertebrate taxa have been shown to influence the fitness of mosquito larvae by increasing mortality of immature stages of the mosquito through prolonged development time and reducing the number of offspring by causing delayed reproduction and low fecundity in the adults (Fischer *et al.*, 2012). Potential predatory insects include those belonging to the orders Odonata, Coleoptera and Diptera.

The controphic species of the mosquito larvae have been explored as a possible opportunity for mosquito control with more studies concentrating on predation (Marten and Reid, 2007) and intraspecific competition between mosquito larvae (Costanzo *et al.*, 2011). The interaction between mosquito larvae and Anuran larvae (tadpoles of frogs and toads) has also received a great deal of attention (Blaustein and Margalit, 1994; Mokany and Shine, 2002; Mokany and Shine, 2003). One advantage of using predators is that they can reach mosquito larvae in some habitats such as tree holes, (phytotelmata) and other water bodies that are difficult to reach using other biological measures such as larvicides (Shalaan and Canyon, 2009). Therefore, controphic species are a potential biological control method of the *Anopheles* mosquito larvae, and consequently an ecological approach towards malaria control.

Studies on the population dynamics of malaria mosquitoes in larval habitats may provide knowledge about interactions and hence the effect of controphic species on mosquito populations. This knowledge may be harnessed to control and manage *Anopheles* mosquito larval populations (Blaustein and Chase, 2007; Duquesne *et al,* 2011; Munga *et al.,* 2006). Few studies have been conducted on the use of predators and even fewer on the use of competitors to control mosquitoes particularly the *Anopheles* larvae. Little has been done in the natural larval habitats and also little has been published on the effect of controphic species on malaria mosquito larvae in these habitats. The aim of this thesis therefore, was to do an observational study(Field and Hole, 2008) of the different taxa and densities of controphic species available in malaria mosquito larval habitats on Rusinga Island, determine their effects on the malaria mosquito larval population dynamics and to assess the effect of physicochemical factors on controphic species within these habitats. The understanding of their effect will add knowledge to the potential use of controphic species as a potential control method for malaria mosquito larvae.

1.2 Literature Review

1.2.1 The Malaria Situation

Vector borne diseases are a major cause of torment to humanity. Malaria alone, caused by parasites of the genus *Plasmodium* and transmitted by the bite of female *Anopheles* mosquitoes, annually affects 250 million people and kills approximately another one million, the majority of whom are the children in Sub-Saharan Africa (WHO, 2011).It mainly affects countries in the tropics, subtropics and those around the equator and is endemic in over 100 countries in the world. The countries affected are largely in Sub-Saharan Africa where malaria is generally associated with poverty and is a hindrance to economic development.

In Kenya, malaria has been recognized as a health and socio-economic burden by the government. Nearly 28 million Kenyans live in areas of malaria risk and majority of them are children under the age of 5 years. In 2010, clinically diagnosed malaria accounted for 34% of outpatient hospital visits (Malaria control Division, MPHS, 2010).According to WHO, in the year 2010 alone1.8 billion dollars were allocated to malaria research and control. However, amounts of dollars such as these that are often allocated substantially fall short of resources required for malaria control.

Tremendous efforts and collective interventions have been made. For example, the use of ITNs of which approximately 289 million had been secured for Sub- Saharan Africa by the year 2010,the use of IRS and the use of inexpensive, rapid and quality assured diagnostic kits have been made to combat malaria. However, malaria still remains to be a scourge to the human population.

In the year 2010 Morocco and Turkmenistan were certified as malaria free and no single case of *P. falciparum* was reported in Europe in 2009. However, resurgence has been observed in about three African countries. This could be because of natural variation or lapses in control measures. Treatments of monotherapies of Artemisinin instead ACTs could also contribute to parasite resistance and also threaten the therapeutic life of ACTs. Therefore, there is a need to keep vigil and find new methods of combating malaria (WHO, 2010). The WHO has recently recommended community-based case management of malaria, which has been shown to be effective in reducing mortality and morbidity in Ethiopia starting from the low to moderate areas of transmission which already have a high coverage of LLINs. Intervention has been made by the use of Rapid Diagnostic Kits for quick diagnosis and deployment of Artemether-lumefantrine drugs at a community level in Ethiopia. The results showed a crude parasite prevalence of 7.4% in the intervention district and 20.8% in the non-intervention district (Lemma *et al.*, 2010; WHO, 2012).

1.2.2 Malaria mosquito vectors

More than 60 mosquito species have been incriminated in the transmission of malaria parasites which consist of *Plasmodium falciparum* (the deadliest*)*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium vivax* among others. All these parasites are transmitted by the female *Anopheles* mosquito especially the *Anopheles gambiae sensu lato* and *Anopheles funestus* which rank among the most efficient vectors in Africa (Takken and knols, 1999; Cross, 2004).However, in more recent studies, *Anopheles arabiensis* has been shown to be more ubiquitous in Africa (Sinka *et al.,* 2012; Okara *et al.,* 2010). Some species are more significant than others, as vectors. The significance is due to variations in susceptibility to the parasite or the propensity of the mosquito to bite humans and to enter houses when looking for a blood meal (Cross, 2004).

Dominant malaria vectors in Africa (Figure 1) have been documented (Sinka *et al.*, 2012). A study by Bigoga *et al.,* (2007) in Cameroon has shown that in Africa, *An. gambiae, An. arabiensis* and *An. funestus* are co-dominant across much of the continent. *Anopheles arabiensis* and *An. funestus* have also been reported to be the widely distributed malaria vectors in Kenya with *An. arabiensis* showing the most ubiquitous distribution nationally (Okara *et al.*, 2010).

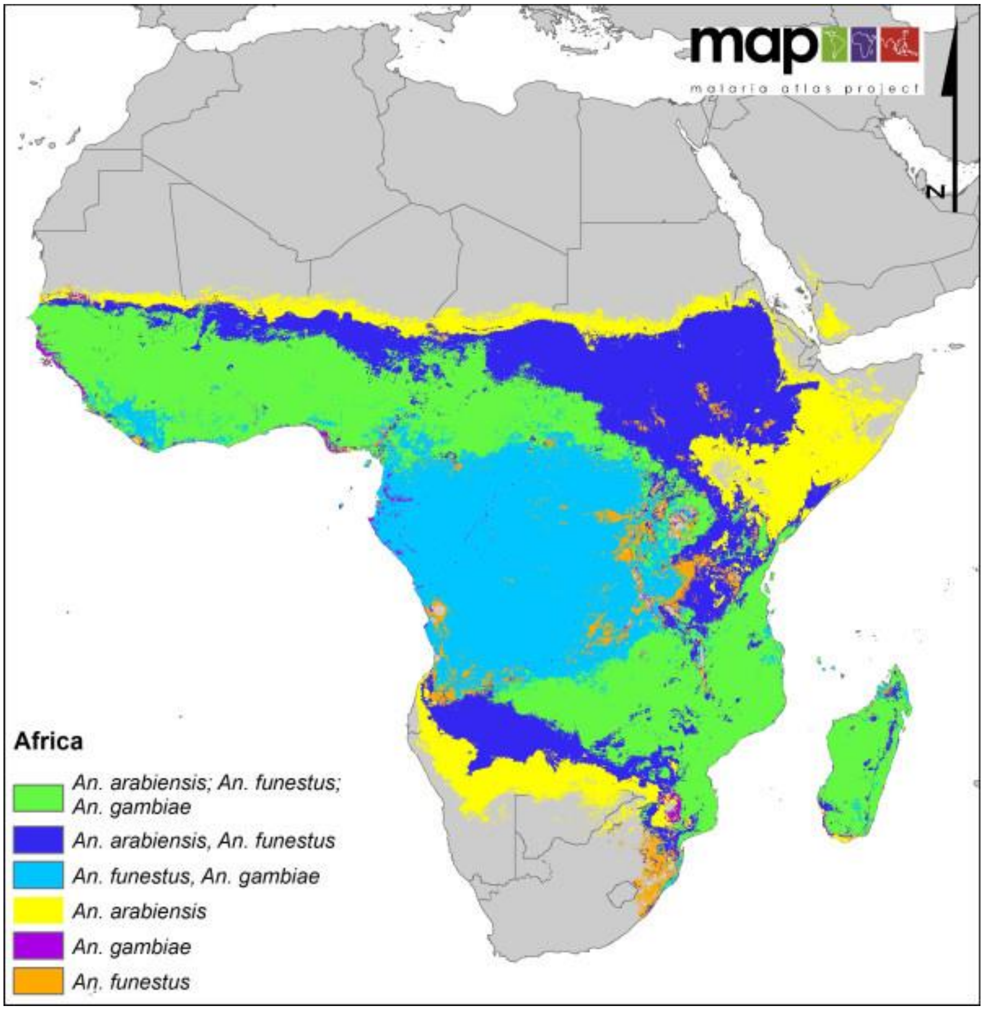

Figure 1: A map of Africa showing dominant malaria vector species (Source: Sinka *et al*., 2012).

1.2.3 Mosquito lifecycle

Understanding the lifecycle of mosquitoes could better help in strategizing mosquito control methods. The mosquito has four developmental stages the egg, the larvae, the pupa and the adult (figure 2). Some species lay eggs in stagnant water, on water edges and on aquatic plants. The *Anopheles* female adults lay eggs on water surfaces where they float due to their air filled chambers formed from the outer layer of the egg while species such as the *Aedes* females lay their eggs on the moist substrate of breeding habitats such as soils (Becker *et al*., 2010). Others breed in temporary pools while others breed in marshes including salty ones according to species and preference (Wigglesworth, 1932). Some species prefer phytotelmata (natural reservoirs on plants) like the tree holes and some on pitcher plants.

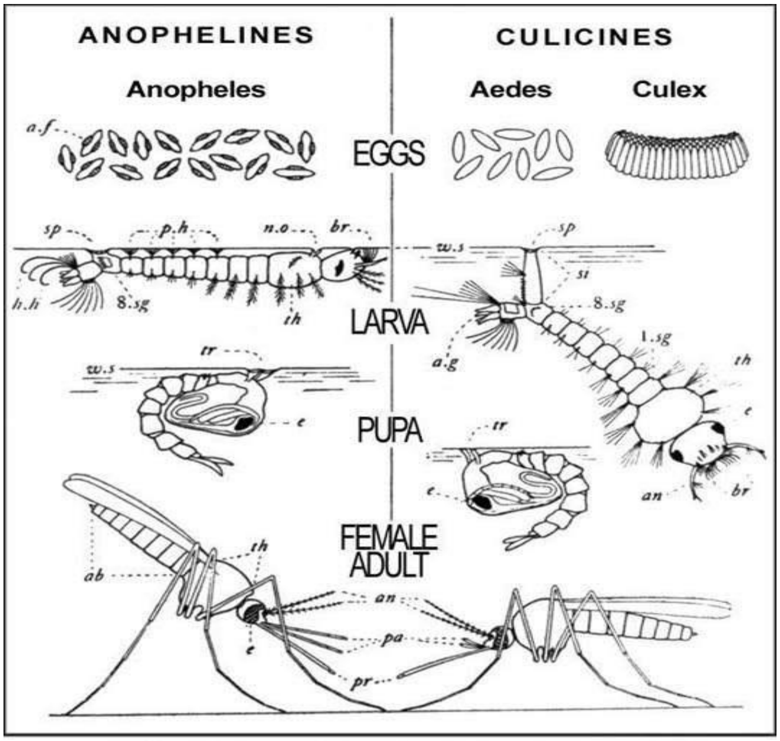

Figure 2: Mosquito life cycle (Source: WHO, 1996)

The first three developmental stages are aquatic and last 5-14 days depending on the species and their adaptation to different temperatures (Becker *et al*., 2010). Mosquitoes living in areas where some part of the year is freezing or waterless spend part of the year in diapause and continue with life when the seasons are favorable (Crans, 2004; Jonida, 2008; Minakawa *et al.*, 2001).

1.2.4 The mosquito larva

The mosquito larva is the second stage of the mosquito cycle and knowledge of their development, feeding mechanisms and ecological influence will guide in better larval control strategies. The mosquito larva molts four times before reaching the pupa stage. The larva's development is dependent on temperature with great variation in optimum developmental temperature between different mosquito species (Becker *et al.*, 2010).

Different species of mosquitoes use different feeding modes such as filtering, suspension feeding, browsing and interfacial feeding and previous studies have catalogued different species as obligate or facultative feeders (Pucat, 1965; Surtees, 1959). According to Harbach (1977) the mosquito feeding categories can be defined as predators, plankton feeders, surface feeders, bottom feeders and scavengers. An Anopheline larva lies horizontally in water and feeds by rotating its head at 180^0C. It then feeds using the interfacial method with physiological and environmental factors such as larval age, temperature and starvation influencing the ingestion rate of particulate matter suspended in water (Becker *et al.*, 2010). Sometimes mosquito larvae (such as the Culicines) display a natural crowding behavior but for other species, it is perhaps a defense mechanism against predation enhancing their survival (Rashed and Mulla, 1989; 1990).

Laboratory studies have suggested that vector competence varies with the quality of the larval environment (DeFoliart, 1987). Other studies suggest that larval stress caused primarily by food limitation within habitats not only produces small adults but adversely affects larval survival, development rates and adult fitness (Hawley, 1985; Yee, *et al*., 2004). An understanding of the spatial and temporal distribution of the dietary resources available to mosquito larvae in their natural habitats could clarify the relationships among food availability, vector competence and mosquito fitness (Merrit *et al*., 1992).

In a study by Imbahale *et al*., (2011), the larvae of *Anopheles gambiae s.l.* were shown to exist in both temporary and permanent habitats. Man-made habitats and grassy habitats showed an increasing abundance while the rainfall intensity was shown to affect the larval abundance either positively or negatively depending on the locations studied which included two village highlands and a peri-urban area. The highland areas showed increased abundance in early instars of Anopheline larvae exhibiting two times more abundance with rain compared with no rain. In the peri-urban area there was a decrease in larval abundance with heavy rains.

1.2.5Mosquito vector control

According to Takken and Knols (2009) current vector control methods include IRS, LLINs, slow killing entomo-pathogenic fungi and viruses, spatial repellents, removal trapping and confusion techniques, *Bti*, environmental modification, predatory fish, Sterile Insect Technique (SIT) and genetically engineered mosquitoes. In recent years, control of mosquitoes by genetic methods like sterile male technique, cytoplasmic incompatibility, chromosomal translocations, sex distortion and gene replacement have been explored, and are still on research phase (Macgraw and O'Neill, 2013).Since malaria vectors have not been eradicated yet, there is need for novel methods and an integrated approach to mosquito control as no one method can eradicate malaria on its own.

1.2.5.1 Adult mosquito control

The use of chemical insecticides for IRS and ITNs reduces malaria transmission by killing adult mosquitoes entering houses and sleeping areas (Hamel *et al.*, 2011; Pleus *et al.*, 2010). According to WHO (Undated article on the Global Malaria Program position statement), the use of ITNs is important. According to the this article, if used by the total population, the ITNs have been shown to lower transmission by 90%, malaria incidence by 50% and lower all-cause child mortality by 18%. This is because ITNs reduce the number of mosquitoes that come into contact with the sleeping human being especially during the active hours of feeding of the adult mosquito (Mutuku *et al.*, 2011) and have a mass-killing effect which reduces the mosquito population size in an area of high density ITN coverage.

However, it has been shown that repeated washing of this insecticide treated nets can quickly render them in effective in preventing mosquito vectors from feeding (Atieli *et al.*, 2010). There is also evidence of changing biting behavior in vectors due to mass coverage of LLINs (Moiroux *et al.*, 2012). Because of environmental concerns and the development of resistance in the vectors (Curtis *et al.*, 2003), mosquito control is slowly moving from the use of broad spectrum, persistent chemicals to more specific control materials such as microbial insecticides, parasites and pathogens. These methods have been scaled up to complement the use of ITNs and IRS leading to a reduction in the amounts of chemical insecticides used. Other methods of adult mosquito control limit exposure to malaria mosquitoes by reducing bites and hence infection.

These methods include the use of door and window screens on homes, insecticide-treated protective clothing and the use of repellants and attractants in mosquito traps as explored in some studies (Becker *et al*., 2010; Njiru *et al.,* 2006; Obermayr, 2012; Okumu *et al.*, 2010).

1.2.5.2 Larval mosquito control

Larval control could be one of the most effective methods of mosquito control having been used in other parts of the world, but has little application in Africa because *An. gambiae* preferentially oviposit in habitats with transient waters. This makes it impossible to locate all breeding sites and the use of larvicides or environmental management is cumbersome and expensive because it requires identifying and treating a high proportion of breeding sites (Fillinger and Lindsay, 2011). Larviciding involves the use of contact poisons, growth regulators, surface films, stomach poisons, and biological agents such as fungi, nematodes, copepods and fish (Walker and Lynch, 2007). The historical and recent success of larval source management (LSM) have been highlighted by Fillinger and Lindsay (2011) in their paper. They have discussed early pre-DDT practices such as removal of stagnant water which was used alone in Egypt as well as integrated with other control methods in Brazil to eradicate malaria. Thereafter, DDT was used but had big repercussions such as neglect of research studies on mosquito control and environmental degradation.

More recent successful larviciding methods are such as the use of larvicides like Pyriproxyfen (PPF) (Sihuincha *et al.,* 2011). The PPF is a larvicide that relies on the use of adult female mosquitoes as a disseminating vehicle to transfer it between resting and oviposition sites but has limitations because it relies on survival, reproduction and abundance of the mosquito vector (Devine and Killeen, 2010).Biological control agents such as *Bti,* have been in existence as early as 1901 (Roh *et al.*, 2007). The *Bti, Bs* and fungi (*Lagenidium giganteum*) larvicides have been largely studied and successfully applied for many years to control mosquito larvae and other insects (Batra *et al.*, 2000; Fillinger *et al.,* 2003; Kumar *et al.,* 2009; 2003 Majori *et al.*, 1987).However, the effectiveness of these agents depend on ingestion by the mosquito larvae (Laird *et al*., 1990; Aly, 1983).

The limitations of *Bti* include; reduced large scale use because of high costs of production, reduced potency lasting for short periods due to ultraviolet rays, specificity, shorter shelf life and the need for special formulations for applications. The *Bs* formulations have also shown some resistance in some mosquito species for example, *Culex quinquefasciatus Say* and *Culex pipiens Linnaeus* (Lacey *et al*., 2007). In contrast, controphic species maybe cheaper, readily available, environmentally friendly and unlikely to cause harm to non-target organisms and could be well integrated into existing control tools to complement them. A study carried out by Imbahale *et al.*, (2012) on integrated Larval Source Management (LSM) in Western Kenya, combined various methods such as drainage, land leveling, filling ditches with soil and *Bti* treatment. Significant results were observed with the reduction of abundances of larvae in the intervention habitats compared to the non-intervention habitats. This shows that various tools can be integrated in malaria interventions and controphic species could potentially be one of them.

1.2.5.2.1 Controphic species

Controphic species have been described as species that share the same trophic levels but not necessarily the same functional foods (Blaustein and Chase 2007). In this study, they are defined as species that share the same trophic level as the mosquito larvae and will compete with them or predate on them. Examples of controphic species of the mosquito larvae include; the Coleoptera adults and larvae, the Anisoptera and the Chironomid larvae as predators and zooplankton such as Cyclopoids and Ostracods as competitors.

In their review, Blaustein and Chase (2007) are of the opinion that ecological theory and empirical research show that controphic species have large direct and indirect effects on target species (*Anopheles* larvae in this study) through predation and sharing of resources. In their view the roles of controphic species in affecting mosquito larval populations has received little attention which is also scarcely published. The published empirical evidence suggests effects on the mosquito larvae by the zooplankton and anuran larvae. They conclude that more studies are needed to elucidate the roles of controphic species in predicting mosquito populations and thus better management of mosquitoes. Modeling, observational studies and experimental approaches carried out for decades have led to the conclusion by ecologists that controphic species often play important but diverse roles in affecting each other(Chase *et al.*, 2002; Connell, 1983;

Lord,2007;Schoener, 1983). This is because mosquito larvae and their controphic species may both share common resources, common predators and common habitats.

Various interactions exist in ecological communities, among them interspecific competition, predation and mutualism which occupy the interface between population and community ecology and interact with each other to structure communities (Abrams, 2002; Gimnig *et al.*, 1999; Gurevitch *et al*., 1992). Figures 3 is a graph illustrating competition where species Y completely excludes species X and figure 4 is a graph illustrating predator prey relationship, where species X is predating on species Y. These graphs show some of the main expected interaction patterns in this study which may explain or be explained by the population dynamics of the mosquito larvae and their controphic species (Verberk, 2010).

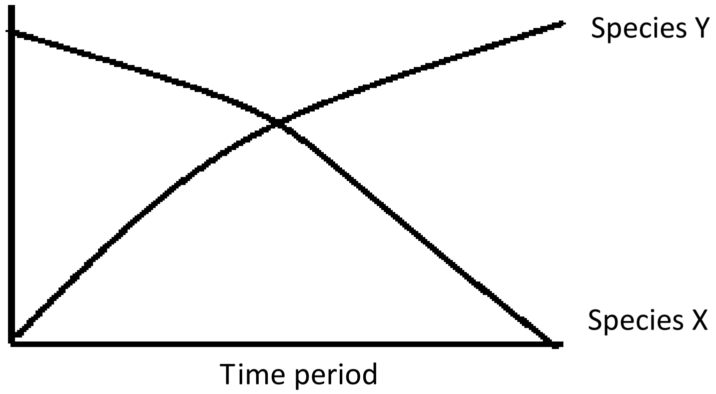

Figure 3: Competitive interaction between species X and Y. Species Y eliminates species X.

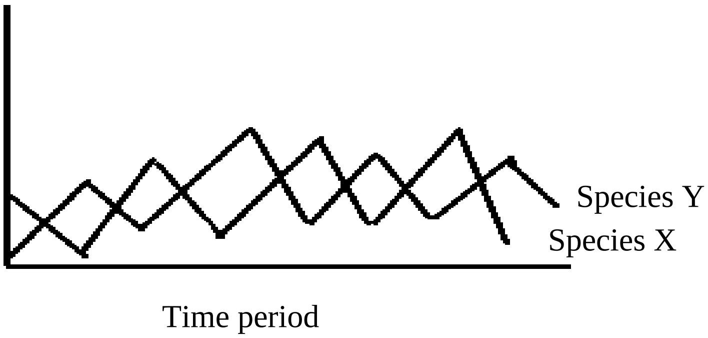

Figure 4: Predation of species X on Y. An increased abundance of species Y leads to an increase in species X which declines when the predator Y is in large numbers as the cycle continues.

Modules by which controphic species interact indirectly include apparent competition, apparent mutualism and indirect mutualism among others (Blaustein and Chase, 2007). In apparent competition two prey species share the same predator and have mutually negative effects on one another. Here, the controphic species serve as an additional food resource to the predator leading to an increase in predator densities and as a result, there is more predation on the target species. In apparent mutualism (two prey species share the same predator), the controphic species satiates the predator allowing a relief on predation of target species and thus benefiting it. In indirect mutualism, for example, the *Anopheles* and *Culex* species that are filter feeders benefit when the controphic species can feed on alternative food e. g periphyton instead of phytoplankton while in the case of *Aedes* and *Culiseta* which feed on periphyton, would benefit if the controphic species were phytoplankton feeders e.g. the Daphnia (Blaustein and Chase, 2007; Blaustein 1996; Stav *et al.*, 2005)

Alternative prey (controphic species) may reduce predation on mosquitoes depending on the predator's preference for the different prey, the controphic species and mosquito larvae abundance ratios and the interaction between the two. Preference for mosquito larvae by the predator relative to controphic species depends on; the species of mosquito and the species of the predator (Blaustein, 1998) and also the relative size of the predator, mosquito and the alternative prey (Kumar and Rao, 2003).Various studies have shown that predation and competition influence the development and survival of mosquitoes (Blaustein and Margalit, 1994; Knight *et al.*, 2004) which in turn may affect the populations of mosquitoes. Mosquitoes have been shown to avoid oviposition in areas with potential predators and competitors (Munga *et al*., 2006).

1.2.5.2.1.1 Predation

The role of predators on reducing mosquito populations is relatively a widely studied subject (Lundkvist *et al*., 2003). In a study done in Western Kenya on 330 predators (Ohba *et al.*, 2010) using PCR to confirm predation on mosquito larvae, 54.2% of the predators showed positivity of predation. The Odonata, Hemiptera, Amphibians and Coleoptera were among those showing high predation abilities. Early and recent studies done have also showed that many other insects including Notonectids, Dytiscids larvae and adults, Naucorids, Hydrometrids, some Neuroptera, and Hydrophilid larvae prey on immature mosquitoes (Blaustein, 1998; Quiroz-Martinez *et al.*, 2005). The larvae of Hydrophilid beetles of the genus *Tropisternus* are especially important predators. Hixson (1943) cited in Howard (1951) presented data indicating that in Florida larvae of *Tropisternus spp.* were the principal predator of Anopheline larvae in an association in which no minnows were present. A study done in India showed that even in the presence of alternative prey such as Chironomids and tadpoles, predators in the orders Hemiptera and Odonata exhibit a preference for mosquito larvae (Saha *et al*., 2014).

The other arthropods that also prey on mosquito larvae are the copepods of the genus *Cyclops.* They are abundant in quiet waters and have been observed to seize the 1st and 2nd instar larvae of Culicine and Anopheline mosquitoes (Howard, 1951).These copepods have been used successfully in Vietnam to control disease-bearing mosquitoes such as *Aedes aegypti* that transmit dengue fever and other human parasitic diseases. Their success has been enhanced by the preference of mosquitoes to breed in container habitats allowing these habitats to be easily identified and the Cyclops added (Marten and Reid, 2007).Other predators of mosquito larvae that have also been researched on are the *Gambusia affinis* fish that are commonly introduced to rice fields and in appropriate stock rates are said to have almost 100% control (Hoy *et al.,* 1972). However the efficacy of mosquito fish in the control of mosquitoes can vary because of different environmental conditions such as vegetation type and density as well as different secondary productiveness of controphic species (Linden and Cech, 1990).

Mosquitoes and controphic species may also be intraguild predators (prey on each other). In addition to consuming the same resources and competing with one another, *Culex* and tadpole larvae have been seen to mutually prey on one another depending on the relative sizes of the individuals which in turn depends on which individuals colonized the pool first (Blaustein, 1996; Blaustein and Margalit, 1996). Some mosquitoes can also prey upon early stages of cladocerans (Wallace and Merit, 2004) while some commonly prey on protozoans. However, protozoans also compete with the mosquito larvae for bacterial resources (Cochran-Stafira and von Ende, 1998; Kneitel and Chase 2004)) and could therefore benefit or harm mosquito populations.

1.2.5.2.1.2 Competition

In competition, controphic species can interact directly through physical or chemical interference (Mokany and Shine, 2003) or indirectly through exploitation and depletion of shared resources (Morin *et al.*, 1988; Steiner, 2003) thus affecting the abundance and distribution of these species in shared lentic habitats (Wilbur, 1997).Intraspecific competition between mosquitoes is an extensively explored subject. Examples include the effects of intraspecific larval densities on the longevity of adults maintained under relatively harsh environmental conditions which was tested in the laboratory by measuring the longevity of adult *Aedes aegypti* and *Aedes albopictus* (Reiskind and Lounibos, 2008).

A further example is of a study where competition of the same species of *Aedes aegypti* and *Aedes albopictus* was done to determine their susceptibility to dengue fever infection (Alto *et al.*, 2008). More and more studies have been done on intraspecific competition among mosquito larvae (Hardstone and Andreadis, 2012; Hawley, 1985). Interspecific competition among mosquito larvae is also well explored (Ho *et al.*, 1989).Much research has been carried out on the impact of interspecific competition on mosquito larvae but the work still remains preliminary because; first, most of the experiments take place in artificial ponds which are unlike the natural environments and secondly, experiments that show negative results are unlikely to be published and thirdly there is a general scarcity of studies in this area (Blustein and Chase, 2007).

Detritus type has been shown to cause interspecific competition between *Aedes aegypti* (L.) and *Aedes albopictus* (Murell and Juliano, 2008). Additionally, in a study carried out on feeding habits, Chironomid larvae ate different matters such as algae, fungi, pollen, leaf and wood fragments, animal remains, detritus and silt making them possible competitors of the mosquito larvae (Henrique s*et al*., 2003). Marten (1986) suggests that controphic species for example the Cladocerans may influence mosquito larvae by reducing the amount of edible algae available to the mosquito larvae. Mosquitoes may prefer to oviposit where the controphic species are present if food resources levels are adequate to reduce predation on mosquito larvae (Blaustein and Chase, 2007).

Studies of competition between mosquito larvae and other controphic species are few.One of these studies is by Elono *et al.*, (2010) whose findings in the field showed that the abundance of *Aedes* spp. was negatively associated with the abundance of competitors for food, and to a lesser extent with those of intraguild predators and strict predators. Another study is by Duquesne *et al.*, (2011) who in a semi-field set up showed a potential in Cladocerans to control *Culex pipiens* mosquitoes. Yet another study is by Carver *et al.,* (2010) of natural populations of mosquitoes and their controphic species in ephemeral bodies fed by rainwater in the wheat belt of Australia. The results of the study demonstrated that the density and richness of mosquitoes generally declined in association with increased richness of predators and density of all other taxa.

1.2.5.2.1.3 Abiotic factors affecting controphic species

In his PhD thesis findings, Paaijmans (2008) demonstrated that biotic and abiotic factors can significantly affect the growth, development and survival of malaria mosquito larvae in a significant way. He suggested that future studies on malaria epidemiology and malaria control should consider these factors so that they could improve malaria risk predictions and successfully incorporate the larval stages of the mosquitoes in malaria intervention strategies.

Biotic interactions involving mosquito larvae are context dependent with effects of interactions on populations altered by ecological conditions (Juliano, 2009).In mosquito-controphic species food webs one often observed scenario is that the presence of controphic species at least initially results in lower predation. This is probably due to turbidity as the benthic controphic species e.g. clam and fairy shrimps and predatory fishes forage and thus reduce detection of the larvae by predators (Benfield and Minello, 1996; Blaustein and Margalit, 1991; Luzier and Summerfelt, 1997). Relative impacts of competition and predation change across the gradient of habitat size and permanence. Hydro-period lengths (the period when a wetland is covered by water) also play a role in the interactions between controphic species (Gaff *et al.*, 2000). Notwithstanding how predators become part of the community either through colonization (predatory insects), arising from drought resistant stages (e.g. Cyclopoids), or introduction to pools (e.g. mosquito fish), overall predation intensity on prey usually increases with the hydro-period length (Well borne *et al.*, 1996).

In wetlands with short hydro-periods, interspecific competitors such as zooplankton can become dense and may reduce the density of larval mosquitoes in addition to increasing their development time and exposing them to desiccation. The competitors can also reduce fitness by reducing size at metamorphosis according to studies done by Juliano and Stoffregen (1994). In intermediate habitats (those that dry intermittently), predation density increases for both the larvae and the controphic species. This may benefit mosquitoes due to reduced predation intensity. In longer hydro-period habitats, predators can become dense and exhibit numerical responses reducing the numbers of both the mosquito larvae and their controphic species considerably (Well borne *et al*., 1996).

Investigations of context-dependent interactions among mosquito larvae will yield greater understanding of mosquito population dynamics and provide useful model systems for testing theories of context dependence in communities (Juliano, 2009). The interactions and the factors affecting mosquito survival and their controphic species discussed above among other abiotic parameters such as temperature, pH, salinity and dissolved oxygen, may assist in explaining the change in mosquito populations when observed in their natural habitats.

PROBLEM STATEMENT

Conventional malaria mosquito vector control techniques, which use chemical insecticides for example ITNs and IRS, have been utilized for decades causing a negative impact on the environment, non-target organisms, human health and resistance in vectors. This has necessitated the need to find other novel, affordable and environmentally benign methods of control or methods that can be integrated with each other to minimize chemical insecticide use. The use of *Bti* and other bio-pesticides has been explored as an environmentally friendly method but when used on a small scale, are costly in production and application. This bio-pesticide is also not readily available especially to resource poor communities where malaria is endemic. Consequently, there has been further need to explore more biological methods. The use of controphic species on mosquito larvae especially the *Anopheles spp.* and their roles in affecting mosquito larval populations in their natural environment remains poorly studied and applied. Additionally, most studies are carried out in controlled environments and focus on single predator or competing species. The diversity, distribution and effect of controphic species on *Anopheles* larvae in natural habitats of the Rusinga Island where Malaria is endemic are unknown. Furthermore, effect of competitors unlike the predators of mosquito larvae is scarcely published.

JUSTIFICATION AND SIGNIFICANCE OF THE RESEARCH

Malaria control using ITNs and IRS which use long-lasting chemical insecticides is increasingly failing, as mosquito vectors develop resistance against these insecticides which also cause harm to non-target organisms and human health. In addition, promising biopesticides such as *Bti* and fungus are expensive to use in resource poor communities where malaria is rampant. This calls for the use of alternative methods. Controphic species are likely to have a strong negative impact on mosquito populations and may likely be an effective management tool that is low cost and environmentally benign in controlling malaria mosquito populations and subsequently, malaria infections. The knowledge acquired from this study can be used for better management of mosquitoes.

OVERALL OBJECTIVE

To assess the diversity of controphic species, their effect on malaria mosquito population dynamics and the effect of physicochemical factors on the presence of controphic species in malaria mosquito larval habitats.

SPECIFIC OBJECTIVES

1. To determine the diversity of controphic species present in malaria mosquito larval habitats on Rusinga Island.

2. To assess the effect of physicochemical factors on the presence of controphic species in malaria mosquito habitats.

3. To determine the effect of controphic species on the population dynamics of malaria mosquito within their larval habitats.

HYPOTHESIS

Controphic species assemblages modulate the population dynamics of the *Anopheles* mosquito larvae and are affected by physicochemical factors within the larval habitats.

2. 0 CHAPTER TWO: MATERIALS AND METHODS

The research done in this study took place in natural malaria mosquito breeding habitats found within the Town zone of Rusinga Island, Lake Victoria, in Western Kenya. Figure 5 shows a map of Rusinga Island with the Town zone area encircled.

2.1 Study site

Rusinga Island (0°35'–0°44' South; 34°11'–34°22' East and altitude of 1,100 m) is approximately 42 km^2 in area and is the second largest island in Lake Victoria. It is an isolated, underdeveloped and disadvantaged area with a growing human population. The predominant tribe on the island is the Luo community. The average annual rainfall in this area is between 800-1152mm with the long rainy season starting from March through May and the short rainy season between October and November though the exact timing of the seasons varies from year to year. The temperatures are between 17.9 -33.6^{0}c. The majority of mosquito larval habitats on Rusinga Island originate from human activities. The three species of *Anopheles* species of mosquitoes sustaining malaria transmission throughout the year are: *An. gambiae, An. funestus and An. arabiensis*.

Figure 5: A map of Rusinga Island showing the Town zone sampled for mosquito larval habitats (Courtesy of the Solarmal Project, Rusinga Island)

2.2 Study design

This study was observational in design. A profile of the diversity and abundance of controphic species was made and their effect on the malaria mosquito larvae populations was established through weekly sampling.

2.2.1 Preliminary Survey

A preliminary survey was carried out for three days to sample the mosquito larval habitats in the Town Zone area of Rusinga Island. A total of 60 potential larval habitats (11 types) were sampled to determine the available types of mosquito larval habitats and the presence of mosquito larvae and their controphic species. The mosquito larval breeding habitats were randomly sampled and categorized as abandoned boats, ditches, swamps, lake shore pools, tyre tracks, cement pits, un-cemented pits, water puddles, fish bait mines, fish ponds and waste water run-offs (see some of these on Plate1).

Plate 1: A pictorial view of the different types of larval habitats sampled in the Town zone area of Rusinga Island. A- Cemented pit, B-Uncemented pit, C-Swamp, D-Ditch.

Five or more dips were made at different points of the habitats to scoop at least one liter or more of water for observation of the controphic species. The following criteria were used to determine the number of dips that would be made at each larval habitat depending on the type and size of the larval habitat:

1. A minimum of five dips were made at each cemented pit, un-cemented pit and water puddle.
2. A minimum of ten dips were made at the ditches.
3. A minimum of 20 dips were made at different points of the swamp.

The malaria mosquito larvae together with controphic species that were visible to the naked eye were counted as individuals per liter on a white tray (22cmx17cmx4cm) in the field. The diversity of the different controphic species available in the sampled water was recorded during that visual survey. The collected samples were preserved in 70% alcohol for further identification of the controphic species in the laboratory. At the laboratory the species were counted one by one on a counting glass slide and identified using diverse keys including the Ward and Whipple key (1959),the Macan key (1994) and the Bouchard key (2004).

2.2.2Diversity of controphic species present in malaria mosquito larval habitats

A total number of 30 mosquito larval habitats (which had their GPS coordinates recorded and later used to estimate the distance of the habitats from the nearest house) were randomly picked for a cross- sectional diversity study from the 60 habitats sampled in the preliminary survey. This exercise was carried out for one day. Five or more dips (according to the size and type of habitat) as described in the preliminary survey were made at the larval habitats. The water was randomly sampled from different areas of each larval habitat to ensure that all the species within each habitat had an equal chance of being sampled. The diversity of controphic species per liter of water was determined by use of a standard 350ml dipper (diameter, 11.5cm; height 5.5cm) to scoop water from each habitat. A composite of the water from as many dips as described in the preliminary survey, was made in a 20 liter plastic bucket (Plate 2-A). The water was stirred and thereafter scooped from the bucket in small portions using the dipper into a 1.5L calibrated water plastic jug (plate 2-B). From each habitat, only one liter of water was sampled. The controphic species were afterwards sieved through a 50 µm plankton mesh (Plate 2-B) and preserved in 70% ethanol in labeled sample bottles (12ml screw top clear bottles)(Plate 2D). The bottles had a label showing the date of collection, the larval habitat type from where it was picked, the name of the habitat and a number ranging between 1 and 30 to denote different habitats for easier tallying during identification. The remaining water was replaced back to the habitat then the bucket was rinsed with clean water after sampling of each habitat, in readiness for the sampling of the next larval habitat. The sample was then transported to the laboratory where identification of the different controphic species from the larval habitats was done under a dissecting microscope (Plate 2-D).

To identify the controphic species, the samples were extracted from each bottle using a pipette and placed on a counting glass slide. The slide was then examined under a dissecting microscope using a pin to move the sample in the counting glass slide and a counter to keep track of the high numbers of some of the species. The organisms were morphologically identified as described during the preliminary survey to the species level, where it was possible. Some of the organisms could not be identified to the species level but to the order, family and genus levels (also known as morphospecies and also termed as ‘species’ in this study) (Kindt and Coe, 2010).After identification, each sample was returned to its preservation bottle using a pipette. Between the examination of one sample and the next the counting glass slide was cleaned with distilled water from a wash bottle.

Plate 2: A pictorial view of different methods and materials. A- sampling of water using a dipper into a 20 liter bucket, B- Sieving using a jug and a planktonic sieve, C-measurement of depth using a wooden stick, D- identification of species from sample bottles using the dissecting microscope and the counter.

2.2.3 Effect of physicochemical factors on the presence of controphic species in malaria mosquito habitats.

Abiotic factors play a significant role in the preference of habitats by the *Culex* and *Anopheles* larvae (Dejenie *et al.*, 2011; Muturi *et al.*, 2007; Okech *et al.*, 2007). Therefore, abiotic parameters were also measured during the species monitoring and sampling exercise as described in section 2.1.2. The physicochemical parameters that were measured and recorded for each larval habitat included the water temperature (^{0}C) and the dissolved oxygen (ppm/ (g/l)) determined using an electronic Oxymeter (HANNA, Woonsocket, U.S.A.).The salinity (ppm), conductivity (µs) and pH were determined using a multipurpose electronic pH/conductivity meter(ExStik II EC500, Extech, Walthman, U.S.A.) while the turbidity (NTU) was determined using a Turbidity Meter (Turbiquant 350 IR, Merck, Darmstadt, Germany). Environmental factors such as the depth of the water was assessed by getting the mean value of two to five random measurements taken at different points, lengthwise or widthwise depending on the size of the pond. This was done using a wooden stick (Plate 2-C).Rainfall (mm) data for the 11 week study period was acquired from the Rusinga Island meteorological station. An approximate surface area of the larval habitats was roughly estimated by recording the length (m) and the width (m) for habitats that had more regular looking shapes (resembling rectangles or squares) while the diameter (m) measurements were recorded for the circularly shaped larval habitats. Up to five random measurements of width and the same for length were made to get the means that were used to calculate the surface area of the habitat. The volume (m^3) of the water was approximated each week by measuring the depth of the water in the different habitats multiplied by the surface area of the habitat that had been recorded at the beginning of the study. The distance (m) of the nearest house to the larval habitat was approximated using the GPS, by standing at the nearest house to each larval habitat and making use of the already mapped GPS coordinates of the larval habitats.

To measure the different physicochemical parameters of the water, one scoop of water from the 20 liter bucket was made using the 350 ml dipper after the one liter of controphic species water sample had been made in section 2.1.2. Afterwards, it was poured into the calibrated 1.5 liter plastic jug that was used to measure the water sample containing the controphic species earlier. The Oxymeter and pH/conductivity meters were placed in the plastic jug and up to five minutes were allowed to record different parameters by switching from one to the other as necessary. After recording the measurements, the instruments were rinsed with distilled water in readiness for measurements in the next larval habitat. A sample of water from the bucket was also collected in a brown colored preservation bottle for the turbidity measurement which was carried out within the same day of collection in the laboratory, using the Turbidity Meter.

2.2.4Effect of controphic species on the population dynamics of malaria mosquito larvae

Controphic species (the taxa and the densities) present in the mosquito habitats identified in section 2.2 were monitored through a longitudinal study for a period of 11 weeks. Four categories of habitats (total habitats = 16) were selected to study the effect of controphic species on the population dynamics of malaria mosquito larvae. These included ditches, swamps, un-cemented pits and cemented pits. These 16 habitats were selected randomly from the 60 habitats identified during the preliminary survey on the basis of (a) abundance of mosquitoes (majorly the *Anopheles spp.*), (b)the presence of controphic species and (c) the availability and dominance of the larval habitats in the study area.

The following criteria were used to select experimental larval habitats (a) those with 20 or more mosquito larvae (*Anopheles* and *Culex)* per liter of water sampled with or without controphic species, and (b) those with a diversity of more than four species (totaling to 200 or more individuals) with or without mosquito larvae (both *Anopheles* and *Culex*). Monitoring and sampling was carried out once a week for a period of 11 weeks. Sampling for controphic species was repeated on the same habitat each week hence pseudo-replicating the sites over time. The sampling process and the criteria for deciding on the number of scoops for each habitat were as described in the preliminary survey.

During laboratory identification of controphic species (see section 2.1.2), the *Anopheles* and *Culex* mosquito larvae were identified morphologically from each other by observing the absence or presence of the respiratory siphon, respectively, and by counting the number of setae and spicules using the Highton (1983) key. The mosquito larvae were then divided into two groups i.e. early (L1 / L2) and late instars (L3 / L4). The information on the species counts was recorded in a data sheet in the laboratory.

2.3Data analysis

Data was entered into Excel spreadsheets in preparation for analysis. For all statistical tests the significance level was set at $p<0.05$. Preliminary analysis was done using MS Excel. Prior to all ordination analysis, the counts of larvae and controphic species were subjected to Log(x+1) transformation in order to diminish the influence of the dominant species (Kindt and Coe, 2005).

A pairwise comparison between the L1/L2 and L3/L4 *Anopheles* larvae with all the controphic species was done using the Spearman's rank correlation test to define the significant negative and positive correlations. All data analyses in this study were computed in the R statistical package called Biodiversity R (Kindt and Coe, 2005). Diversity indices such as the Shannon, the Inverse Simpson and the Renyi profile used to analyze the diversity of controphic species provided a summary of richness and evenness. The Shannon, the Inverse Simpson and Renyi profile are larger statistics each of them combining richness and evenness (Kindt and Coe, 2005; Renyi, 1961). Each of these indices make different assumptions on diversity and use the Jacknife estimates to show how the species accumulate beyond the sampled larval habitats. The Jacknife population estimate was used to extrapolate the expected number of species in the survey area (Legendre and Gallagher, 2001).The Shannon and the Inverse Simpson indices were used to analyze the diversity in all the habitats sampled in the cross-sectional study (n=207). The Renyi profile was used to analyze the diversity in the habitats sampled in the longitudinal study (n=193).Only Renyi diversity profiles provide sufficient information to conclude that one site is more diverse than another by ordering sites from low to high diversity (Renyi, 1961). The profile value for α=infinity provides information on the proportion of the most abundant species. Consequently, profiles that are higher at α =infinity have a lower proportion of the dominant species. A larger evenness means a lower proportion of the dominant species.

The species accumulation curves were used to show the rate of accumulation of new species during sampling. They were used to estimate the effort required to reach a satisfactory level of completeness (Karl *et al*, 200). The PCA (Principle Component Analysis) method was used to show the relationships between the taxa (controphic species) that were identified during the study. The PCA ordination graphs were used to determine similarity in species composition between different larval habitat types and the correlations in abundances among controphic species. The associations that were detected in the PCA analysis were tested for significance using the stepwise multiple linear regression model. The stepwise regression chooses the variables that should remain in the model. The R^2 which is the coefficient of determination shows the amount of variability in different groups. The model was used to assess the partial contribution of competitors, predators and other species that could neither be classified as predators or competitors predicting the influence of controphic species with respect to the abundance of *Anopheles* mosquito larvae. Competitors, predators and other species were entered in that order in three blocks for the analysis. The individual contribution of each competitor, predator or other species was also determined.

The controphic species were classified as competitors if they were known to share similar foods or feeding mode with the mosquitoes while predators were composed of known predatory species. The remaining species were classified as others if they were not known to predate on, or share same foods and/or similar feeding modes. An RDA ordination graph was also used to determine the effect of controphic species on the population dynamics of malaria mosquito larvae. The effect of the abiotic parameters on the abundances of mosquito larvae and their controphic species was assessed using redundancy analysis (RDA), which is related to PCA but which is constrained by environmental variables (Kindt and Coe, 2005). Outliers of controphic species were detected during the PCA analysis when included in the ordination graph and were omitted to give final ordination graph that is presented in the results. The broken stick distribution method was used to find out how many principal components axes should be analyzed to get a good ecological picture of the total variance of the dataset during the PCA analysis. Significant axes, were selected when the % variance was larger than the corresponding % variance of the broken stick-distribution. The equilibrium circle was used to assess the species that contributed largely to the first and second principal components.

3.0 CHAPTER THREE: RESULTS

This study was carried out for a period of 11 weeks. The number of mosquito larval habitats studied was 207 for the cross-sectional survey (section 2.1.2) and 193 for the longitudinal survey (section 2.1.3).In the graphs containing rainfall data over time (Figure 6) the lowest peaks (week two, three and six) in the abundance of *Anopheles spp.* mosquito larvae coincided with the highest peaks of rainfall while the highest peaks of the abundance of the *Anopheles* larvae coincided with the absence of rain. There was a considerable increase in the abundance of *Anopheles* larvae just after the rains in week eight.

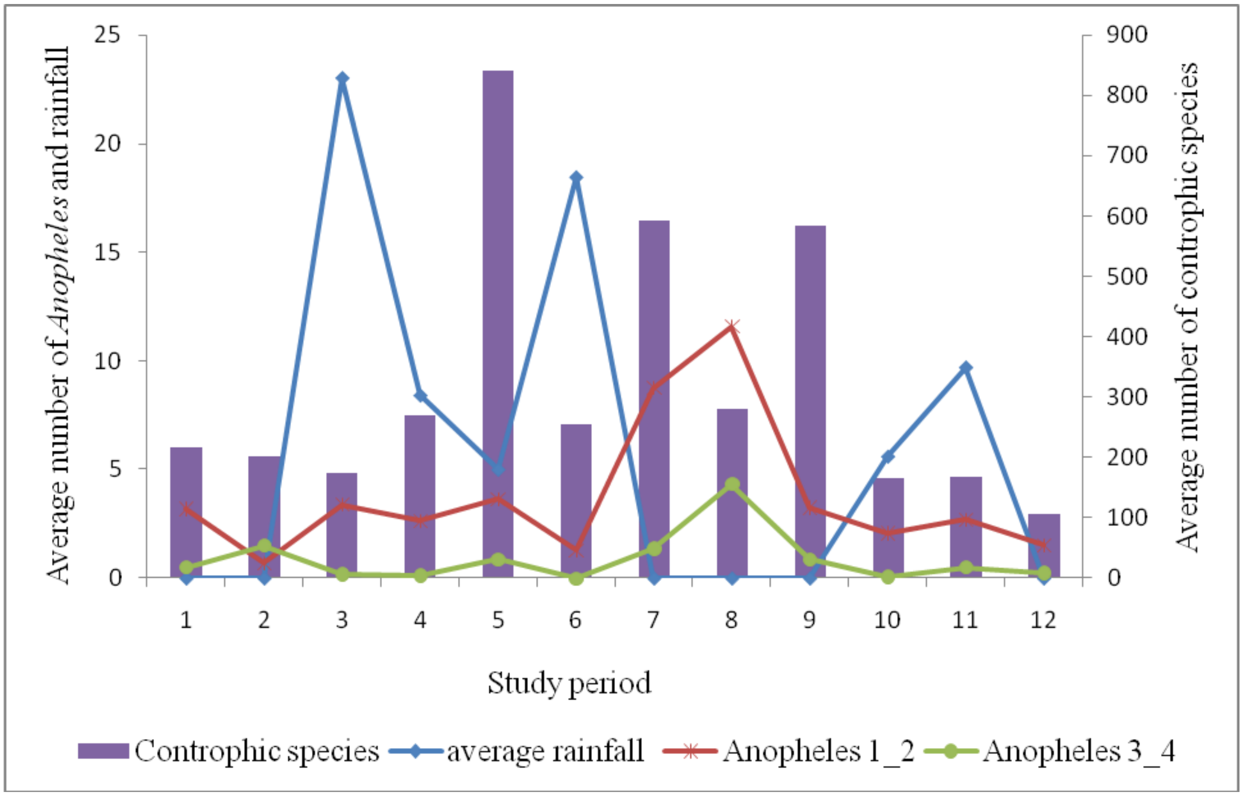

Figure 6: The dynamics of *Anopheles* larvae and their controphic species as influenced by rainfall (without the outlier). High peaks of rainfall read to decreased abundances of the *Anopheles* larvae and their controphic species.

The Ostracoda, Rotifera and Moinidae were often counted in hundreds and in thousands. The Rotifera were in exceptionally high abundance hence an outlier as shown in figure 7 and the omission in some of the analysis. In one cement pit at week five a total of 90,000 individuals were observed. The estimation of individuals was made by measurement of the area under the field of view and multiplying it by the rest of the unfocussed field of view after it was established that they were evenly distributed on the counting glass slide. According to data not shown the abundances of Rotifera were much lower at other weeks. Therefore, the three species mentioned were excluded from the analysis in the PCA ordination graph because they caused crowding and illegibility of effect from other species (figure 7). However, they were included in all other analysis.

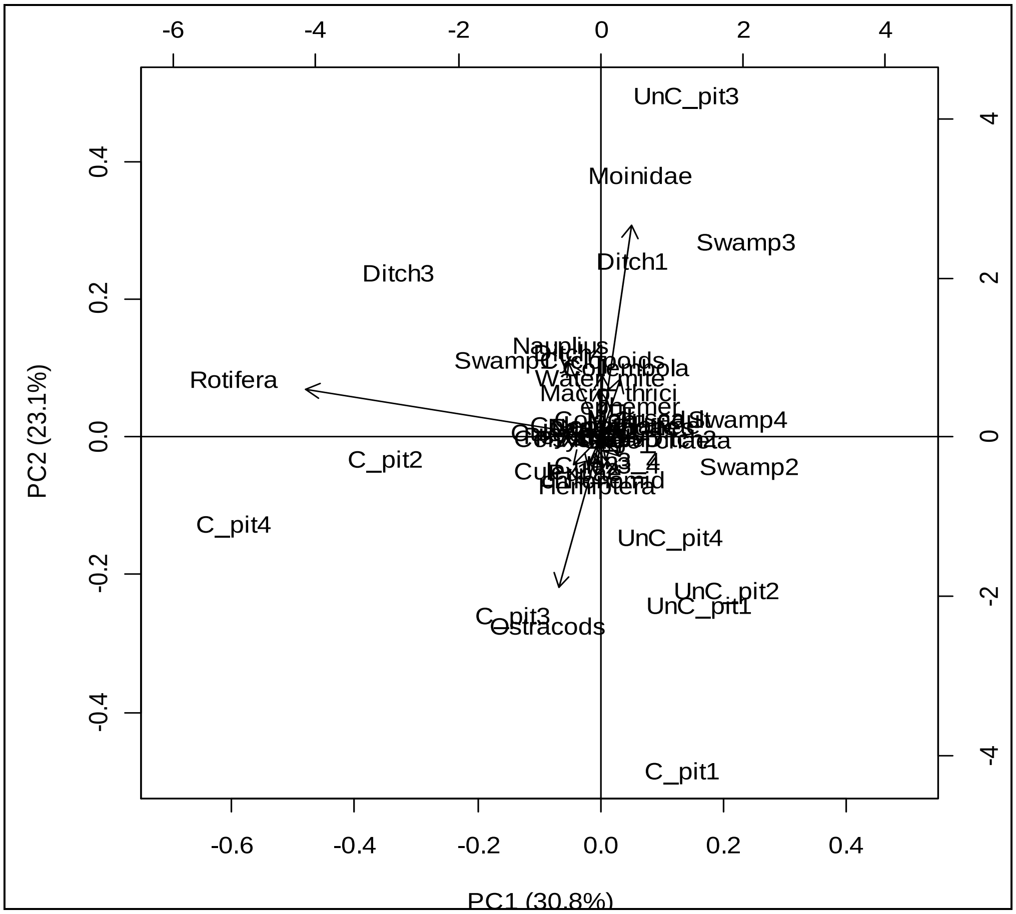

Figure 7: A PCA biplot showing the outliers during the analysis. Because of the exceptionally high abundance of Moinidae, Ostracoda and Rotifera there was crowding of the remaining species at the center, hence their deletion in the final analysis and display.

For the PCA significance table (Table 1) results reported four axes as significant: axis 1 with 30.8% >22.1% of the broken stick distribution, axis 2 with 23.1% >15.5%, axis 3 with 14.7% >12.1% and 11.8% >9.9%. These four axes explained the most variation in the data since their variance was greater than that of the broken stick percentage.

	1	2	3	4	5	6	7
Eigen value	9.96144	7.48541	4.74833	3.84275	1.60447	1.37381	0.81241
Percentage of variance	30.7929	23.139	14.6781	11.8788	4.95976	4.24672	2.51131
Cumulative percentage of variance	30.7929	53.9319	68.6099	80.4887	85.4485	89.6952	92.2065
Broken-stick percentage	22.1215	15.4549	12.1215	9.8993	8.23264	6.8993	5.78819
Broken-stick cumulative %	22.1215	37.5764	49.6979	59.5972	67.8299	74.7292	80.5174

Table 1: A PCA significance table. These first four axes explained the most variation in the data since their variance was greater than that of the broken stick percentage

3.1 Preliminary survey

A total of 60 larval habitats of 11 categories (types) were sampled. According to the categorization (Figure 8) the dominant habitat types were the ditches (=24%), the swamps (=19%), the cement pits (=15%), the water puddles (=12%) and the un-cemented pits (=9%) making 79% of the randomly surveyed habitats. The least were the tyre trucks (=2%) and lakeshore pools (<2%) totaling to less than 5%.Two of the waste water run-off habitats are excluded from the output as they were outliers.

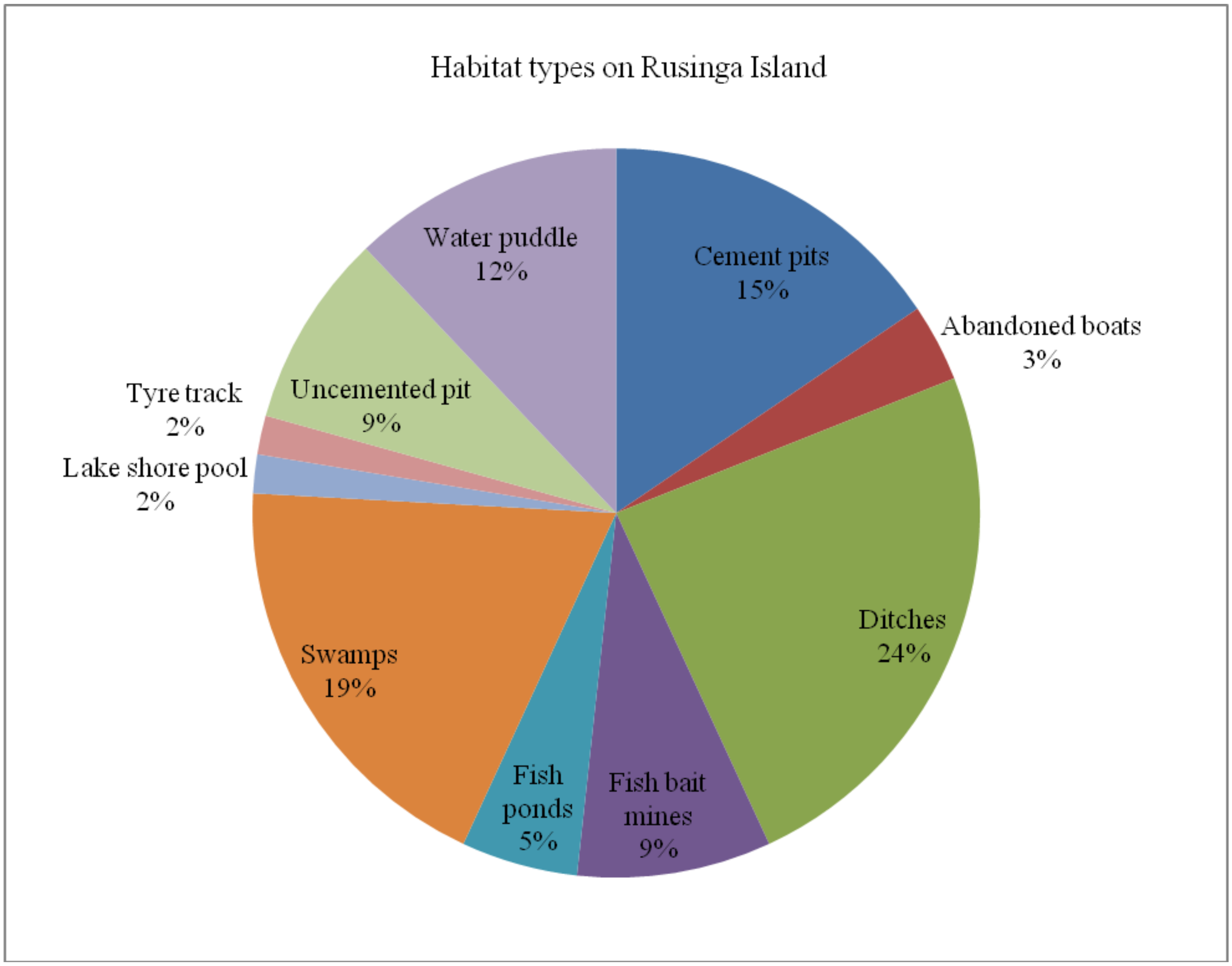

Figure 8: Larval habitat types sampled during the preliminary survey. There were 11 habitat types sampled in total with the dominant habitat types being the ditches, swamps, cement pits, water puddles and un-cemented pits.

The average numbers of mosquitoes (*Anopheles spp.* and *Culex spp.*) were recorded as shown on figure 9. The habitats containing the highest average numbers of *Anopheles* larvae were the un-cemented pits (=11) and the water puddles (>10) while those containing the lowest numbers were the ditches (<4), the cement pits (<2), the lakeshore pools (<2) and the swamps (<1).The 16 mosquito larval habitats that were used for longitudinal study were therefore selected randomly on the basis of abundance of mosquitoes (majorly the *Anopheles spp.*), the presence of controphic species (data not shown) and the availability and dominance of the larval habitats as mentioned in section 2.1.4.

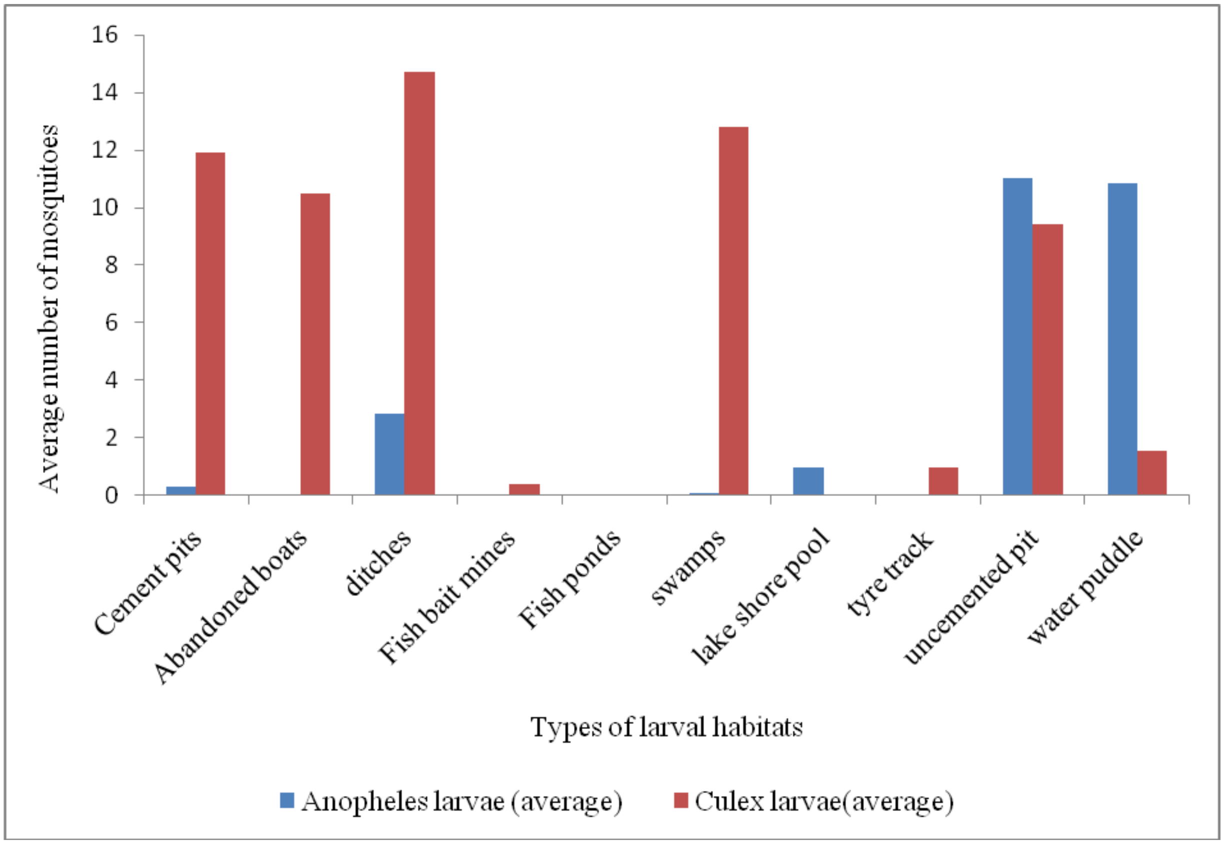

Figure 9: The averages of mosquitoes as spread across different habitats types collected in the preliminary study.

3.2 Diversity of controphic species in malaria mosquito larval habitats on Rusinga Island

Data analysis using the Spearman's rank correlation test (Table 2) showed a positive significant association ($p<0.05$) between L1/L2 *Anopheles* larvae and L1/ L2 *Culex spp.* (<0.0001), Aranae (=0.01), Chydoridae (=0.04577), Hemiptera (=0.0975) and Collembola (=0.00656). Therefore, as the abundances of *Anopheles* larvae increased or decreased, those of the controphic species also increased or decreased respectively. There was also a significant positive correlation between the L3/L4 *Anopheles* larva and the L1/L2 *Culex* larvae (=0.0002), L1/ L2 *Culex spp.* larvae (=0.02277), Hydrachinidae (<0.0001), Coleoptera larvae (=0.003819) and Hemiptera (=0.001968). There was also a positive correlation with the Psychodidae larvae (=0.4377) and Nauplius (=0.1993) although it was not significant.

Table 2: Spearman's rank correlation coefficients of pairwise associations between *Anopheles* larvae and their controphic species. Sampling was pseudo-replicated 207 times (ns: not significant).

	Controphic Species	Early instar (L1/L2) *Anopheles*		Late instar (L3/L4) *Anopheles*	
		Coefficient	P value	Coefficient	P value
1	Chironomid	-0.06	ns	0	ns
2	Chydoridae	-0.14	0.046	-0.08	0.299(ns)
3	Coleoptera adult	-0.11	0.137(ns)	-0.05	ns
4	Coleoptera larvae	0.11	0.124(ns)	0.21	0.004
5	Collembola	0.2	0.007	0.12	0.925(ns)
6	Corixidae	0.11	0.141(ns)	0.07	ns
7	*Culex spp.*1_2	0.31	<0.0001	0.26	0.0002
8	*Culex spp.*3_4	0.06	ns	0.16	0.0228
9	Cyclopoida	0.05	ns	0.06	ns
10	Damsel flies (Zygoptera)	0.02	ns	0.08	ns
11	Dragonflies (Anisoptera)	0.16	0.292(ns)	0.09	0.235(ns)
12	Ephemeroptera	0	ns	0.06	ns
13	Hemiptera	0.19	0.010(ns)	0.22	0.002
14	Hydrometridae	0.02	ns	-0.01	ns
15	Macrothiricidae	-0.02	ns	-0.13	ns
16	Moinidae	0.12	ns	0.14	ns
17	Mollusca	-0.02	ns	0.08	ns
18	Naucoridae	-0.1	ns	-0.02	ns
19	Nauplius	0.08	ns	0.17	0.020
20	Notonecta	-0.05	ns	0.04	ns
21	Oligochaeta	0.03	ns	-0.12	0.106
22	Ostracoda	0.09	ns	0.1	ns
23	Psychodidae pupae	0.12	0.100(ns)	0.12	0.100(ns)
24	Psycodidae larvae	0.16	0.227(ns)	0.15	0.044
25	Rotifera	0.02	Ns	0.12	0.087(ns)
26	Spider (Aranae)	0.18	0.01	0.06	ns
27	Stratiomyidae	-0.09	Ns	0.01	ns
28	Tadpoles (Anura)	0.04	Ns	0.07	ns
29	Water mite (Hydrachnidiae)	0.34	<0.0001	0.24	<0.0001

The Macrothiricidae, Chironomids, Coleoptera adults, Naucoridae, Notonecta, Mollusca and Stratiomyidae showed a negative correlation with the L1/L2 *Anopheles* larvae (though not significant). A negative correlation was exhibited when an increase in the abundance of the controphic species led to decreased abundance of the *Anopheles* larvae or on the contrary a decrease in controphic species led to an increase in *Anopheles* larvae abundances. A positive correlation was exhibited where the abundances of correlated species increased or decreased simultaneously. The Chydoridae had a significant negative correlation ($p<0.05$) with the L1/L2 *Anopheles* larvae. The Macrothiricidae, Oligochaeta, Coleoptera adults, Naucoridae and Hydromitidae showed a negative correlation (not significant) with the L3/ L4 larvae (Table 1)

A total of 35 controphic species were distinguished on the basis of morphological features in the laboratory as described in section 2.1.2.The total of 27 species presented on the rank abundance table (Table 3) were the most encountered and the remaining eight controphic species (not shown in the table) were encountered in low abandunces (≤10). These included the Ceratopogonidae (n=2), Shrimps (Crustacea) (n=2), plecoptera (n=1), hydrozoa (n=2) Ephydridae (n=10), Athericidae (n=1), Lepidoptera larvae (n=1) and a collembolla like species without springtails (n=2) which could not be identified further. Rotifera accounted for 65.5% of the total count of controphic species showing exceptional abundance and therefore was considered an outlier in this study. The first four controphic species which included the Rotifera, Cyclopoida, Moinidae and Ostracoda dominated the area surveyed amounting to 90.5% of the abundance of all controphic species collected.

Table 3: Abundances and frequencies of controphic species collected from the different categories of larval habitats on Rusinga Island. A total of 207 larval habitats were surveyed.

Group	Rank	Total Abundance	Mean Abundances (Individuals/liter)	Proportion
Rotifera	1	126,772	660.27	65.5
Cyclopoida	2	16,741	87.19	8.6
Moinidae	3	16,447	85.66	8.5
Ostracoda	4	15,272	79.54	7.9
Culex spp.	5	6,729	35.05	3.5
Nauplius	6	3,092	16.1	1.6
Ephemeroptera	7	1,535	7.99	0.8
Collembola	8	1,093	5.69	0.6
Anophelesspp.	9	915	4.77	0.5
Hemiptera	10	839	4.36	0.4
Coleoptera	11	790	4.11	0.4
Hydrachnidiae	12	663	3.45	0.3
Corixidae	13	502	2.61	0.3
Chironomidae	14	472	2.45	0.2
Oligochaeta	15	367	1.91	0.2
Macrothiricidae	16	357	1.86	0.2
Anisoptera	17	324	1.69	0.2
Mollusca	18	253	1.32	0.1
Psychodidae	19	158	0.82	0.1
Zygoptera	20	106	0.55	0.1
Naucoridae	21	56	0.29	0
Aranae	22	55	0.28	0
Stratiomyidae	23	26	0.14	0
Chydoridae	24	25	0.13	0
Anura	25	18	0.09	0
Hydrometridae	26	16	0.08	0
Notonecta	27	16	0.08	0

Mosquito abundance and presence was evident in all the four types of larval habitats studied (Table 4) albeit in low abundance for the *Anopheles spp.* larvae compared to the *Culex* larvae. The *Culex* species were the more abundant of the two. The mean abundances of *Anopheles* mosquito larvae were the highest in un-cemented pits (8.48 ±2.4) which was greater than in the cement pits (6.56± 3.78), swamps (2.04 ±0.61) and ditches (1.29±0.39) in descending order, while the highest mean abundances of the *Culex spp.* were in cement pits (60.84±15), swamps (44.14±21.05), un-cemented pits (21.27±8.1) and ditches (9.62± 4.52) in descending order (Table 3).

Table 4: The Mean abundances (±SE) of controphic species across four habitats types namely ditches, swamps, un-cemented pits and cemented pits selected for the longitudinal study

Controphic Species	Ditches		Swamps		Un-cemented pits		Cement pits	
Rotifera	347.35	±312.44	38.5	±27.19	1.17	±0.81	2253.68	±1883.75
Cyclopoida	65.00	±10.17	66..35	±12.60	143. 60	±49.66	55.79	±41.9
Moinidae	26.45	±8.74	41.17	±12.34	220.9	±159.43	0.45	±0.26
Ostracoda	3.56	±0.73	7.15	±1.75	70.21	±26.29	236.94	±61.48
Culex spp.	9.62	±4.52	44.14	±21.05	21.27	±8.1	60.84	±15.46
Nauplius	14.54	±4.41	11.04	±3.74	13.27	±5.20	20.29	±12.08
Ephemeroptera	12.38	±3.75	5.75	±1.12	6.13	±2.65	1.14	±0.33
Collembola	2.85	±0.73	5.13	±1.08	12.81	±8.080	1.75	±0.51
Anopheles spp.	1.29	±0.39	2.04	±0.61	8.48	±2.4	6.56	±3.78
Hemiptera	4.02	±1.2	1.33	±0.22	5.91	±1.70	6.04	±1.34
Coleoptera	2.59	±0.39	5.65	±0.94	4.00	±1.07	2.17	±0.71
Hydrachnidiae	2.77	±0.61	2.58	±0.47	6.58	±4.08	1.54	±0.56
Corixidae	1.19	±0.30	2.7	±0.56	1.1	±0.26	5.13	±2.47
Chironomidae	0.85	±0.20	2.13	±0.99	0.27	±0.14	6.17	±2.10
Oligochaeta	1.33	±0.30	5.15	±0.91	0.52	±0.12	0.38	±0.13
Macrothiricidae	5.29	±4.64	2.08	±0.80	0	0	0	0
Anisoptera	3.48	±0.56	1.13	±0.25	1.52	±0.33	0.38	±0.14
Mollusca	2.48	±0.66	1.85	±0.47	0.85	±0.23	0.02	±0.020
Psychodidae	0.21	±0.09	1.31	±0.70	0.54	±0.27	1.4	±0.27
Zygoptera	1.16	±0.48	0.56	±0.15	0.36	±0.17	0	0
Naucoridae	0.25	±0.07	0.75	±0.12	0.04	±0.03	0	0
Aranae	0.21	±0.07	0.52	±0.24	0.15	±0.06	0.21	±0.07
Stratiomyidae	0.125	±0.06	0.21	±0.08	0.02	±0.02	0.02	±0.02
Chydoridae	0.13	±0.08	0.40	±0.38	0	0	0	0
Anura	0.02	±0.02	0.15	±0.13	0.10	±0.05	0.10	±0.07
Hydrometridae	0.06	±0.04	0.17	±0.06	0.10	±0.07	0	0
Notonecta	0.33	±0.15	0	0	0	0	0	0

The analysis focused on the *Anopheles* species because they are the malaria vectors. The *Culex spp.* were considered as controphic species as they are not only likely to be competitors for the same resources in the mosquito larvae habitats but also intraguild predators (Muturi *et* *al.,*2010).The distribution and abundances of controphic species across habitats was further confirmed by the PCA ordination graph computed in section 3.3.Here, habitats which are close together imply that they have similar species in them (Appendix 2).The abundances of controphic species could also be traced to particular larval habitats using the PCA (Appendix 2).For example: The Ostracoda were in higher abundance in cement pit 3, 4 and 2 than in swamps 3 and 4 since they are in the same direction as these cement pits than the swamps. Others like the Moinidae, had a large abundance in swamp 3 and ditch 1 than in the cement pits while un-cemented pit 1 and 2 were very similar in their species composition than they are with cement pit 1 or swamp 2.

A total of 27 controphic species were collected from the malaria mosquito larval habitats and a further extrapolation by the Jackknife also predicted a richness of 28 species (Table 4). The Jackknife estimate predicted higher richness for the ditches (=28) and the swamps (=27), the fish bait mines (=24.7), un-cemented pits (=24) and cement pits (=23). The lowest richness was estimated in the tyre track (=6) and the lake shore pool (=10) which had one larval habitat each. The Jacknife extrapolation predicted exceptionally more species than those observed from the data collected. The Jacknife predicted n=24.7 species in fish bait mines, n=19.3 in the fish pond and n=23 in cement pits. These were higher than the (n=20), (n=16) and (n=21) species observed in these mosquito habitats respectively.

At the scale of α=1(Shannon index) swamps were more diverse than the fish bait mines>un-cemented pits>lakeshore pools>fish pond>tyre trucks> boats>ditches>puddles> cement pits (Table 5). The Jackknife estimated higher diversity in the fish bait mine, un-cemented pits and the swamps. Ranking at scale of α=2 (Inverse Simpson), the swamp was more diverse than the fish bait mines>lake shore pool>un-cemented pit> tyre truck>boat> fishpond> ditch> boat> puddle> cement pits (see Table 4). The jackknife estimates for the sites with n=1 could not be calculated (output= NaN meaning Not a Number) because of the small sample size. However, the Jackknife predicted a higher diversity than sampled for the fish bait mines, the swamps and the un-cemented pit from the observed diversity.

Table 5: Richness and diversity as shown by the Shannon and Inverse Simpson Indices with their Jacknife estimates, 207 sampling points were used.

Habitat type	No. of sampling points	Total richness	Jacknife estimate	Shannon	Jacknife estimate	Inverse Simpson
Boat	1	12	12	1.478	1.478	2.92
Cement pit	48	21	23	0.643	0.211	1.38
Ditch	49	27	28	1.319	1.012	2.11
Fish bait	3	20	24.7	1.974	2.171	5.16
Fish pond	3	16	19.3	1.581	1.494	2.69
Lakeshore pool	1	10	10	1.627	1.627	3.64
Puddle	4	11	14	0.711	0.711	1.49
Swamp	49	26	27	2.218	2.295	6.34
Tyre track	1	6	6	1.496	1.496	3.57
Un-cemented pit	48	24	24	1.649	1.714	3.58

The Renyi diversity profile (Figure 10) indicates that the swamps were more diverse (had a high diversity of controphic species) than un-cemented pits, the ditches and the cement pits, in descending order. It also shows that the species were not evenly distributed or the profile would be inclined to a near horizontal profile. This profile at a scale of (α =infinity) shows that swamps had the least numbers of the dominant species while the cement pits had the highest numbers of the dominant species (Rotifera).

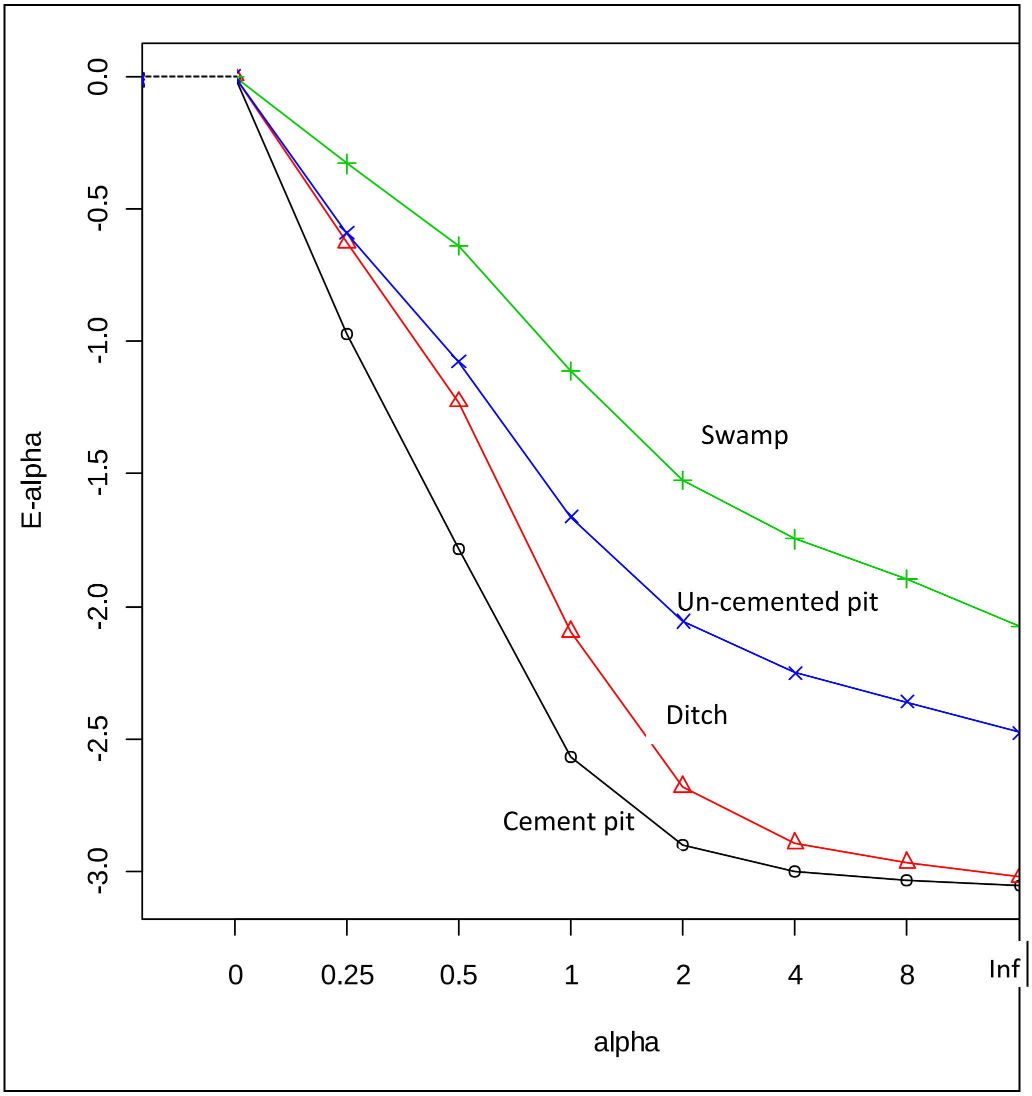

Figure 10: Renyi evenness profile for the swamps, un-cemented pits, ditches and cement pits. A total of 193 sampling points were used.

The species accumulation curves showed that after the sampling of approximately 75 habitats (sampling points), all the 27 controphic species that were analyzed for their diversity and effects had been acquired. Sampling beyond this point would not yield new species from the habitats used in this study (Figure 11).

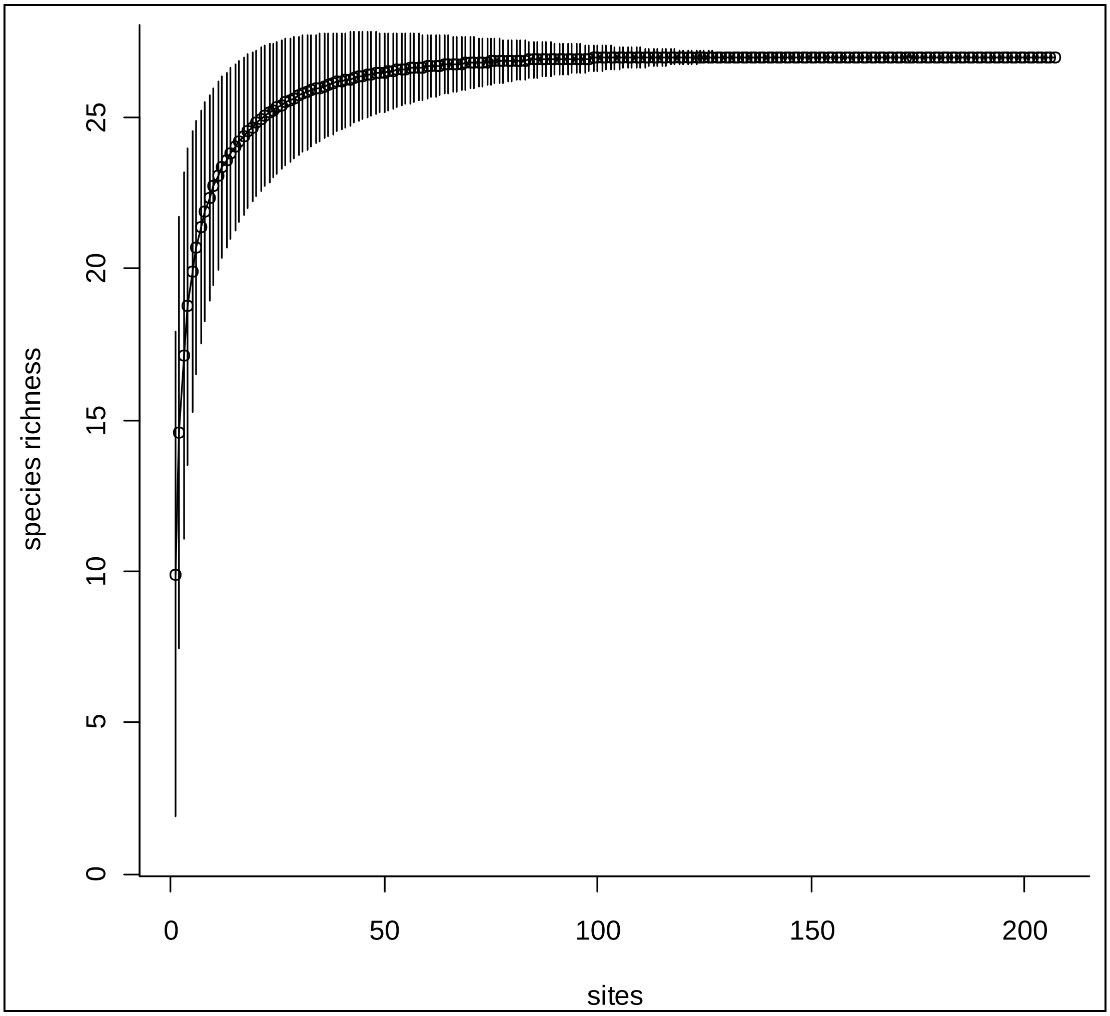

Figure 11: A species accumulation curve showing the richness in n=207 sampling points. The species accumulation curve reached a plateau with about 27 species at approximately 75 sampling points showing no need to sample beyond this as no new species would be acquired.

3.3Effect of physicochemical factors on the presence of controphic species in malaria mosquito habitats

In the RDA ordination graph (Figure 12), sites with high Oxygen had high conductivity, salinity, pH and turbidity in order of decreasing importance. Oxygen had the strongest influence on the presence and abundance of *Anopheles* mosquito larvae. Oxygen also had a strong influence on the associations (correlations) of these larvae with the other controphic species. The *Anopheles* larvae (all stages) were negatively correlated with Mollusca, Anisoptera, Coleoptera flies, Naucoridae, Oligochaeta, Moinidae, Collembola, Cyclopoida, Chironomidae, Zygoptera, Naucoridae and

Macrothiricidae in larval habitats with high Oxygen, conductivity, turbidity, salinity and pH in descending order. The temperature was not correlated with the abundance of *Anopheles spp.* (of least importance).The Mollusca, Anisoptera and Coleoptera adults which were positively correlated to each other in turn showed positive correlations with distance, volume, and depth which were also positively correlated with each other. The water mite (Hydrachnidiae) and Psychodidae pupae were positively correlated (in high abundances) with high salinity sites. The water mite (Hydrachnidiae) showed a strong positive correlation with conductivity.

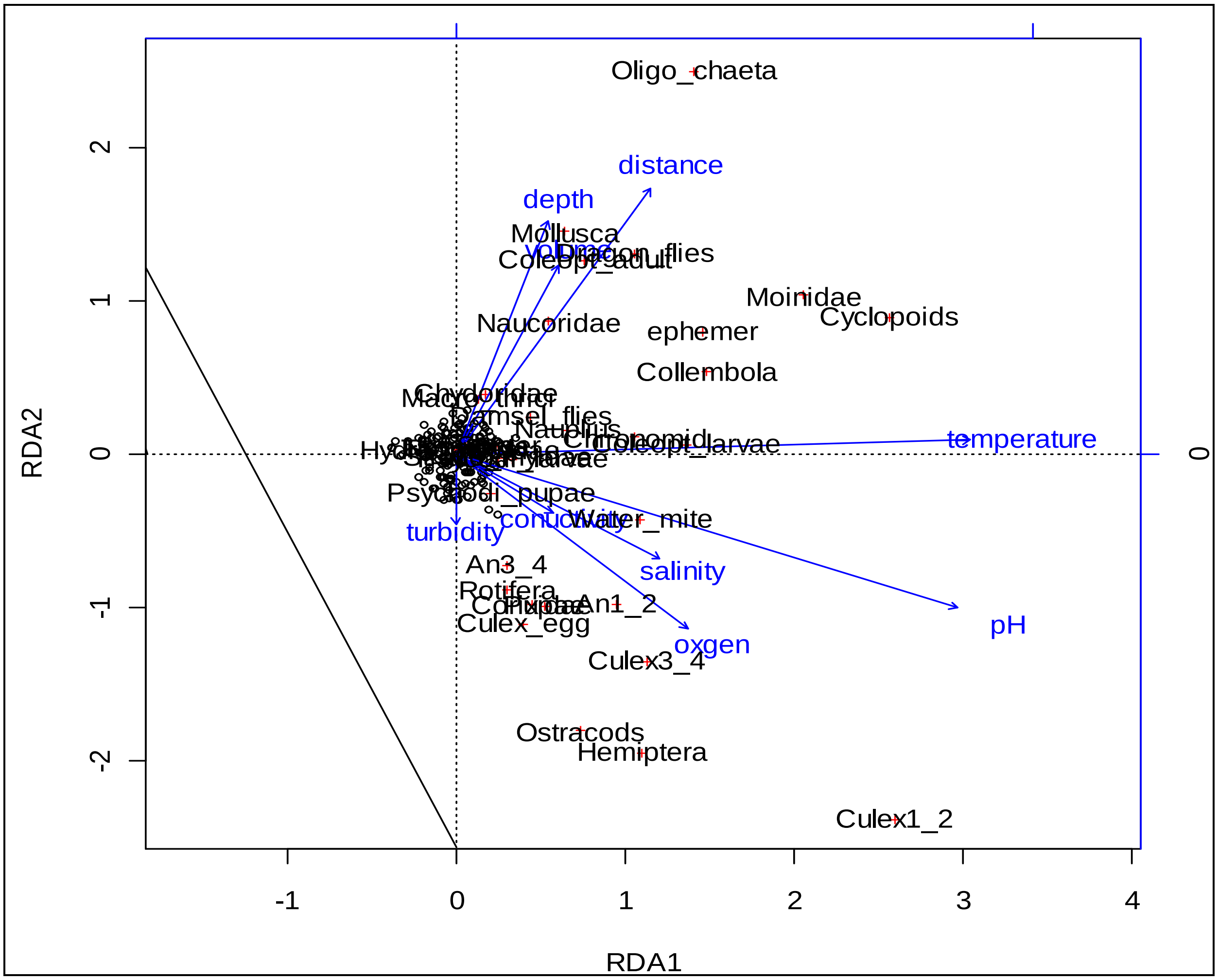

Figure 12: RDA ordination graph (as produced by Biodiversity R package) showing the effect of physicochemical factors on associations and abundances of controphic species larvae within malaria mosquito larval habitats.

Environmental factors such as the depth, volume and distance of the nearest house from the larval habitat were negatively correlated with the abundance of *Anopheles* larvae. An increase in depth, volume and distance of houses from larval habitat caused a decreased abundance of the *Anopheles* larvae and some of their controphic species while a decrease in these environmental factors caused an increase in the abundance of the *Anopheles* larvae.

3.4 Effect of controphic species on the population dynamics of malaria mosquito larvae.

Some species were omitted from the PCA analysis either as outliers (Figure 7) with low abundances or those with high abundances. The results of the principal component analysis (Figure 13) showed that the abundances of the larvae of the *Anopheles spp.* were negatively correlated with the abundances of controphic species such as the Chironomidae, Oligochaeta, Ephemeroptera, Anisoptera, Mollusca, Macrothiricidae and the Coleoptera adults. Strong positive correlations were found between the *Anopheles* larvae and the *Culex* larvae, the Hemiptera, the Collembolla and the Water mite (Hydrachnidiae) in addition to weak positive association with the Cyclopoida, the Coleopteran larvae and the Nauplius. The Cyclopoida, *Culex spp.* and Nauplius were in the highest abundances as shown by the length of the arrows.

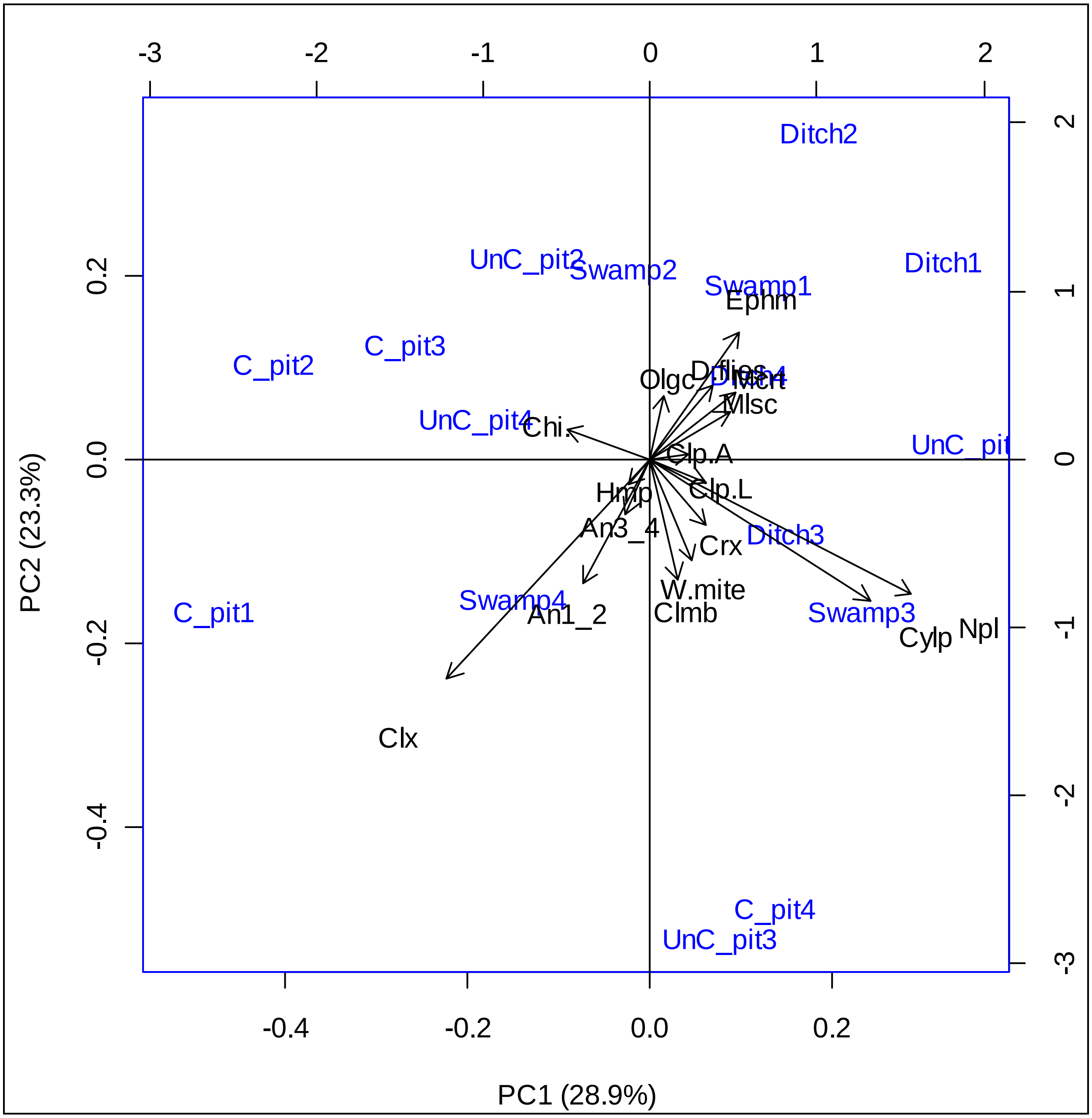

Figure 13: PCA ordination graph without outlier but with *Anopheles* larvae stages separated as produced by the Biodiversity R package: C_pit- Cement pit,UnC_pit-Un-cemented pit, Clx-Culex, An-*Anopheles*, Cylp-Cyclopoids, W.mite- Water mite, Npls- Nauplius, Olgc-Oligochaeta, Clmb-Collembola, Mlsc- Mollusca, Crx- Corixidae, Clp A-Coleoptera adult, Clp L-Coleoptera larvae, Hmp- Hemiptera,, D-flies- Dragon flies, Chi- Chironomids, Mcrt-Macrothiricidae .

The partial contribution (R^2) of the abundance of pooled competitors (with the outliers) explained 77.2 % of the variation observed in the L1/L2 *Anopheles* mosquito larvae (Table5). The explained variation was significant at $p< 0.05$.The competitors also explained 84.64% ($p<0.001$) of variation observed in the L3/L4 *Anopheles spp.* The predators explained 24.8% of variation in L1/L2 and 36.9% in L3/L4 while ‘others’ explained 62.2% on L1/L2 and 37.4% in L3/L4 which was not significant. The predictors that remained in the model were the Chironomidae, Cyclopoida, Moinidae, Nauplius with the effects of the Cyclopoida and L3/L4 *Culex spp.*showing significance at levels of ($p< 0.05$). The partial contribution (R^2) of the abundance of pooled competitors explained 84.64% of the variation observed in L3/ L4 *Anopheles spp.* mosquitos at a significance level of ($p =0.0008164$). The predictors; Chironomidae, Cyclopoida and L3/L4 *Culex spp.* and Ostracoda remained in model with the Chironomidae, Cyclopoida and L3/ L4 *Culex spp.* significantly contributing to the model at the levels of ($p<0.05$), ($p<0.05$) and ($p<0.001$) respectively. The partial contribution of the predators and ‘other’ controphic species of the variation observed in L1/ L2 and L3/L4 was not significant. However, the Hemiptera and water mites (Hydrachnidiae) which remained in the model as predator and ‘other’ respectively, were positively correlated with the L1/ L2 *Anopheles spp.* and their correlation was significant ($p<0.05$).The Rotifera and Ephemeroptera as ‘others’ showed no significant correlation with the L3/ L4 larvae (Table6).

Table 6: Stepwise multiple regressions showing the individual and partial contribution of competitors, predators and others to the variation observed in the L1/L2 and L3/L4 *Anopheles* larvae. The adjusted R^2 is in brackets.

Anopheles larval stage	Classification	Controphic Species (Predictor)	R^2	Regression Coefficient		t value	P value
L1, L2	Competitor	Chironomid	0.772(0.5725).	-0.466	±0.211	-2.213	0.058
		Cyclopoida	p=0.03846	-0.571	±0.193	-2.954	0.018
		Moinidae		0.149	±0.089	1.683	0.131
		Nauplius		0.321	±0.185	1.741	0.12
		*Culex spp.*1_2		-0.234	±0.210	-1.119	0.296
		*Culex spp.*3_4		0.823	±0.297	2.768	0.024
		Mollusca		-0.588	±0.297	-1.983	0.083
L3,L4	Competitor	Chironomid	0.8464(0.7696)	-0.233	±0.085	-2.74	0.021
		Cyclopoida	p=<0.0001	-0.129	±0.056	-2.309	0.044
		Mollusca		0.203	±0.106	1.914	0.085
		Ostracoda		0.054	±0.034	1.59	0.143
		*Culex spp.*3_4		0.468	±0.082	5.687	<0.0001
L1, L2	Predator	Hemiptera	0.2476(0.1318)	0.507	±0.307	1.651	0.123
		Anisoptera	p=0.1574	-0.553	±0.361	-1.534	0.149
L3,L4	Predator	Hemiptera	0.3694(0.2724)	0.395	±0.155	2.54	0.025
		Zygoptera	p=0.4991	-0.444	±0.286	-1.554	0.144
L1, L2	Others	Oligochaeta	0.6223(0.4335)	-0.366	±0.298	-1.225	0.249
		Rotifera	P=0.05124	-0.087	±0.068	-1.284	0.228
		Ephemeroptera		-0.257	±0.187	-1.374	0.199
		Hydrachnidiae		0.754	±0.265	2.852	0.017
		Stratiomyidae		-2.581	±1.608	-1.606	0.139
L3,L4	Others	Rotifera	0.3739(0.2776)	-0.072	±0.036	-2.009	0.066
		Ephemeroptera	p= 0.4767	-0.229	±0.109	-2.105	0.055

The abundance of *Anopheles* mosquito larvae increased to more than 11 and more than4 individuals/ liter during the eighth week of the study for both the L1/L2 *Anopheles* and the L3/L4 *Anopheles* respectively (see Figure 14). There was a steady mean abundance of all larvae with time with the L1/L2 larvae always maintaining a higher profile than the L3/ L4 larvae.

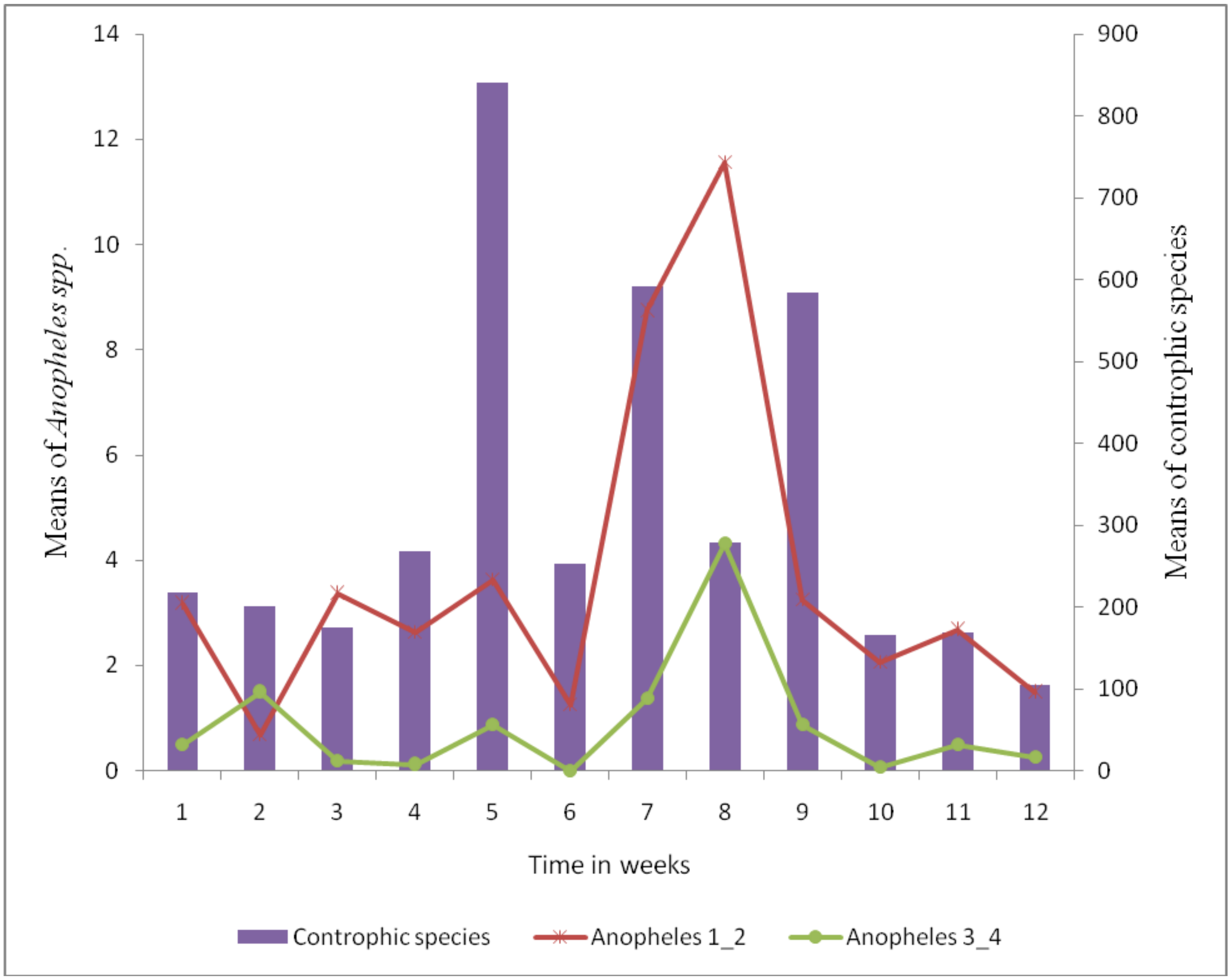

Figure 14: The dynamics of L1/ L2, and L3/L4 *Anopheles* larvae and their controphic species during the 11 week study period.

An average increase in the abundance of controphic species exhibited a decreased abundance of the *Anopheles* larvae (Figure 14). There was an increase in mean abundance of *Anopheles spp.* between weeks 6, 7 and 8 and a decline at week 9.The species that contributed to the abundance observed in figure 14 were established on the RDA graph (figure 15). The Rotifera were the most abundant on the 5th week. The *Anopheles* larvae were negatively correlated with Collembola, water mites, Coleoptera adults, Rotifera and Macrothiricidae between weeks 7 and 8.

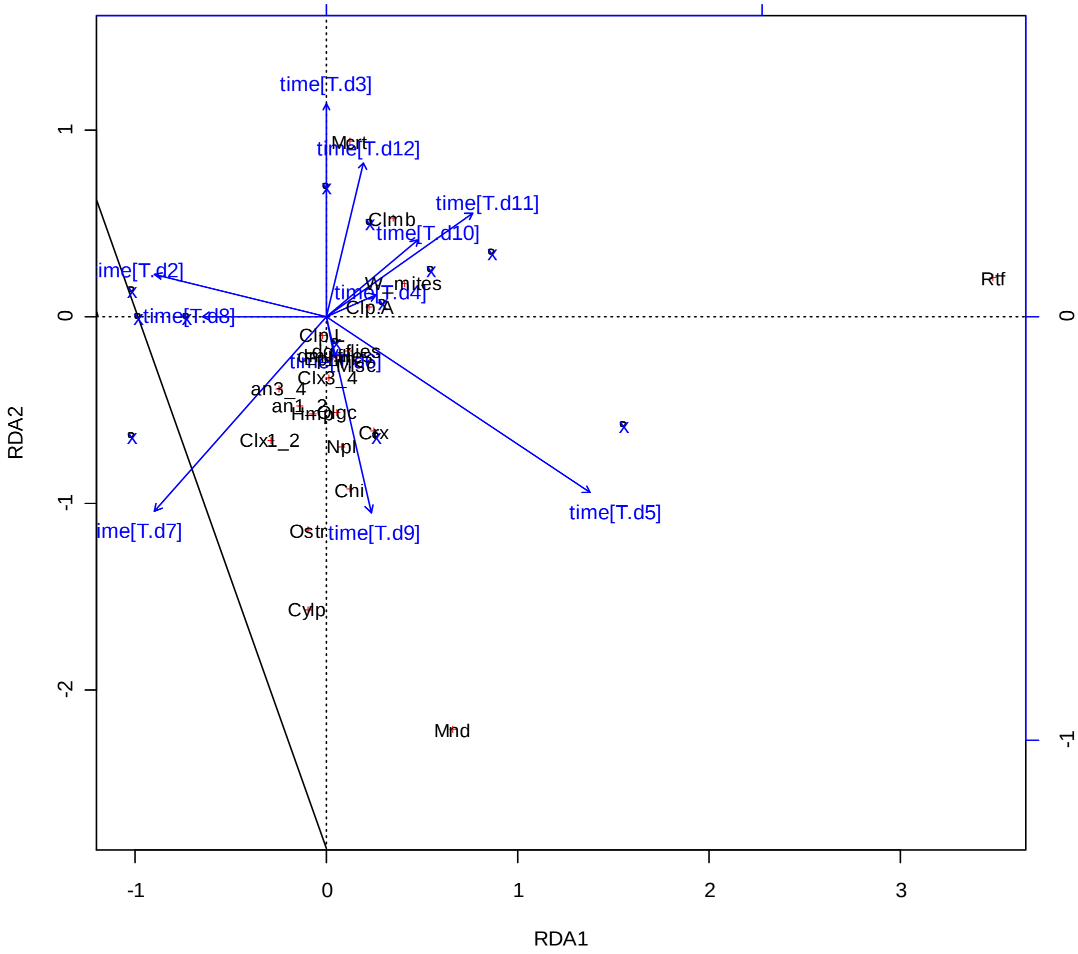

Figure 15: RDA ordination graph (as produced by Biodiversity R) showing species that contributed to the population dynamics observed in the *Anopheles* larvae during the 11 week study period.

4.0 CHAPTER: DISCUSSION

This study found that there is a large diversity and abundance of controphic species in the malaria mosquito larval habitats on Rusinga Island. The study also found that physicochemical factors such as Oxygen and environmental factors such as distance of larval habitats from the nearest house influenced the abundance of *Anopheles spp*. positively and negatively, respectively. The controphic species that were analyzed as predators or competitors had a negative influence on the abundance of the *Anopheles* larvae. This is in agreement with the hypothesis that controphic species assemblages modulate the population dynamics of the *Anopheles spp.* and are affected by the physicochemical factors within malaria mosquito larval habitats.

The presence and abundance of *Anopheles spp.* and controphic species was evident throughout the spatial and temporal frame of the study. The abundance of *Anopheles* larvae was higher in cement pits and un-cemented pits. This may be attributed to their small surface area and their more ephemeral nature compared to swamps and ditches. It may also be attributed to an attempt by the mosquito larvae to avoid the predators which were generally found in permanent habitats with larger surface areas. Studies by Gimnig (2007), found that the *Anopheles spp.* preferred small aquatic habitats with little or no vegetation. The *Culex* species also showed a high abundance of about six-fold that of the *Anopheles spp*. throughout the study period and across all habitat types. These abundances might be due to the more generalist nature of the Culicines that can live in a wide range of habitats unlike the *Anopheles spp.* which are specialists preferring newly made pools. According to Verbak, (2010) there is a tendency of widespread species in geographical areas to be also abundant. This concurs with the study by Minakawa *et al.,* (2004*)* who also collected more Culicines than Anophelines from a study done in Western Kenya close to the study area. The richness of controphic species collected from the study area is within the range of records of controphic species in mosquito larvae habitats on Rusinga Island and other parts of Kenya and the world as shown in studies by Ohba *et al.,* (2010) and Kweka*et al.,* (2011).They collected similar predator species from Rusinga Island and a nearby area respectively, to carry out laboratory experiments while Carver *et al.,* (2010) similarly collected predators and competitors in analogous field studies but in highly salinized areas in Australia.

The occurrence, abundance, richness and diversity of controphic species between the ten larval habitat types in the cross sectional study was highly variable, conspicuously so because of the unequal sample sizes but was relatively variable for the longitudinal study which had almost equal sample sizes. The results showed that the controphic species were the most abundant in the cemented and un-cemented larval habitats which are temporary in nature with small surface areas. This could be attributed to the large abundance of competitors which were counted in large abundances (unlike the predators) in these habitats. This demonstrates that competitors were of importance in reduction of *Anopheles* mosquito larvae which also abounded in these habitats and therefore potential *Anopheles* vector control tools. This conforms to studies by Chase and Knight (2003), who found competitors were of more importance during droughts when only temporary habitats can be found.

The 27 species that were sampled from the different types of larval habitats are close to the highest Jackknife estimate of 28 species though other habitats have large differences in their Jackknife estimates compared to the number of species collected. This demonstrated that overall sampling was well done and only one species was not sampled. Therefore, if all habitats in the study area were sampled, only one more species would be acquired. Extrapolation using the Jacknife estimate improved the controphic species inventory especially in the habitats that had fewer replications (Verbak, 2010).This shows that there was incomplete sampling of certain habitat types such as the lakeshore pools and the abandoned boats in the area sampled. If more habitats had been sampled, the difference between the collected species and the Jacknife estimate would have been smaller. The habitats with more sampling points such as the cement pits, ditches, swamps and un-cemented pits had little difference between the actual numbers of collected species and the Jacknife estimate. Therefore, diversity increased as sample size increased. The species accumulation curve indicated approximately 75 sampling points as adequate to recover at least 27 species implying 207 sampling points as oversampling. This demonstrates that at 75 sampling points (number of habitats), all the 27 species in this study had been acquired and therefore the sampling of each habitat was well done and that the sample size was agreeable. The species accumulation curve estimated the completeness of biodiversity and the minimum effort needed to collect all species in the sampled area (Kindt and Coe, 2005; Moreno and Halfter, 2000; Jefwa *et al.,* 2012; Karl *et al.,* 2003).

In the cross sectional study, the diversity of controphic species was higher in swamps than in the lake shore pool, fish bait mines, un-cemented pits, and fish ponds in descending order probably due to the proximity of these habitats to each other. Similarities in the dense vegetation, large surface areas, source of water, permanent nature of the habitats and the soils may also have influenced the diversity in these habitats. This perhaps explains the low abundance of *Anopheles* larvae in these habitats in an attempt to avoid predators which were in high abundance in these habitats (Munga *et al.,* 2006). In the longitudinal study, the Renyi profile showed that the swamps, un-cemented pits and ditches were more diverse (richness and evenness) than the cement pits in descending order (Figure 10). This is perhaps due to the size and permanence of the first three habitats. These habitats had larger surface areas and were well supplied with water during the study. This relates to some studies which show that species composition and richness are a function of habitat size (Gouagna *et al.,* 2012). The most encountered species over time (data not shown) and ubiquitous in habitats were the Rotifera, Cyclopoida (whose immature stages are Nauplius), Moinidae, *Culex spp.* and the Ostracoda. This may be attributed to the fact that these are zooplanktonic animals that are normally microscopic in size with high reproduction and growth rates and therefore a larger distribution (Verbak, 2010). The predators were encountered in low abundances possibly because of their tendency to occupy permanent habitats with large surface areas (mostly collected in swamps and ditches), their slow reproductive rates and their consumption rate of mosquito larvae being high. Therefore, unlike competitors fewer numbers may still have a negative effect as shown in the results (Becker *et al*., 2010).

Effects of physicochemical parameters on the abundance and relationships of *Anopheles* larvae and controphic species assessed in this study were apparent. The results agree with the hypothesis that controphic species within malaria mosquito larvae habitats are affected by physicochemical parameters and environmental factors that were measured. The results showed that sites with high Oxygen had a strong positive correlation with the abundance of *Anopheles spp.* and some of the controphic species. *Anopheles* larvae and controphic species had strong negative correlations in these sites. This shows Oxygen was the best predictor variable to the abundance of *Anopheles* larvae and their interactions with other controphic species. Other parameters like the salinity, pH, conductivity and turbity showed a weaker positive correlation with *Anopheles spp.* and these controphic species and their relationships (Carver *et al.,* 2010). The negative correlation between

Anopheles larvae and its controphic species was evidently much stronger in these larval habitats. Other studies have also found, turbidity, pH, water temperature and vegetation cover to influence mosquito abundances (Teklu *et al.,* 2010) while others have found only Oxygen was of importance (Elono *et al.,* 2010). Temperatures did not contribute to the variability and interactions seen in the mosquito larval habitats probably because the environmental temperatures were likely to be uniform in all habitat locations. Environmental factors such as rainfall, depth, volume and distance from the larval habitats that were investigated also affected the presence and abundance of controphic species. An increase in depth, volume and distance of houses from larval habitats caused a decreased abundance of the *Anopheles* larvae and some of their controphic species. This shows that *Anopheles* larvae have a preference for shorter distances to houses from the larval habitat, shallow pools of water and smaller volumes of water. Wanji *et al.*, (2009) have shown significant relations between physicochemical factors such as conductivity, distance of breeding habitat from the house, type of habitat and predators with Anopheline species in Cameroon. Similarly, Minakawa *et al.*, (1999) have also shown distance to be an important factor suggesting the preference of mosquito to lay eggs in habitats that are close to houses inhabited by humans.

The rainfall results showed that the highest peak of *Anopheles* larvae abundances were during the absence of rainfall but soon after the heavy rains. The lowest peaks were during the high rainfall weeks. During the low peaks, the heavy rains may have reduced the numbers of mosquitoes especially the early instars by killing them. During the highest peaks after the rain, the mosquito reproduction and re-colonization increased faster perhaps due to the fast reproductive cycle, the reduced disturbance by the rain and also the ambient temperatures on the Island. This concurs with studies that have shown the heavy rainfalls do kill mosquito larvae (Paaijmans *et al.,* 2007) and also an upsurge of mosquito larvae soon after the rain. Similarly, the rains also affected the abundance of controphic species with the high peak leading to a decrease in controphic species concurring with studies by Carver *et al.,* (2010) where increasing rainfall negatively affected quantities of aquatic fauna. A slight increase in the *Anopheles* larvae was noted as the controphic species numbers declined. At week 8 there was an absence of rainfall, an increase in mosquito larval populations and a decrease in controphic species. This may be due to the fact that mosquito colonize habitats faster than the controphic species, soon after the rainfall. Therefore, the influence of controphic species on the abundances of *Anopheles spp.* is affected by physicochemical factors which consequently affect the interactions between the controphic species.

There is clear evidence that the *Anopheles* larvae populations were negatively correlated with that of controphic species that were either termed as competitors, predators or others. This could be attributed to the contribution by competitors through negative or positive correlations with the *Anopheles* larvae. Negative correlations where abundances were probably due to predation or exclusion competiton (depletion of resources) while positive correlations of *Anopheles* with other species such as the *Culex* larvae were probably due mutualism or apparent competition where either species competes for and limits resources but it does not deplete it for the other or both species share common predators respectively (Blaustein and Chase, 2007). Several studies demonstrate these predator-prey and competition relationships in different habitats and with other species such as *Aedes Aegypti* (Gouagna *et al.,* 2012; Howard, 1951; Kweka *et al.,* 2011; Munga *et al.,,* 2006). Those grouped as competitors contributed largely to the variation seen in the mosquito larvae compared to the predators. This may be due to the differences in surface area and the permanence of habitats as *Anopheles spp*. and competitors are in larger abundances in them (Juliano, 2009; Elono *et al.,* 2011). Cyclopoida which are known to be intraguild predators were analyzed as competitors in this research. Known strict predators such the Zygoptera, Hemiptera and Anisoptera had the least effect probably due to the low abundances collected in this study(Duquesne *et al.,* 2011; Elono *et al.,* 2010; Martein and Reid, 2007).The effect of these controphic species was more pronounced when grouped rather than when analyzed as individuals implying that the more diversity of invertebrate taxa, the larger the effect and that one species may not be as effective in reduction of *Anopheles* larvae when solely relied on.

Conclusion and Recommendations

All larval habitat types in this study were potential breeding sites for malaria mosquito vectors and had a large diversity and abundance of controphic species of these mosquitoes. The results showed that controphic species reduce the suitability of these habitats as mosquito breeding habitats. The competitors majorly affected population dynamics of mosquito larvae in temporary habitats with a small surface area while the effect by predators was observed in permanent habitats with larger surface areas. This shows that the competitors are as important as the predators in the management of *Anopheles* vectors. Physicochemical factors involving the water quality and environmental factors also had an impact on the abundances, distribution and interaction of the mosquito larvae and their controphic species. Knowledge of the effect of controphic species on *Anopheles* larvae, their distribution in different habitats and how they are affected by physicochemical factors within larval habitats will assist in customization of mosquito larvae control by targeting habitats appropriately as well as in integrating them with the correct LSM strategies. This knowledge may be applied on Rusinga Island and other areas where habitats are akin in their composition of *Anopheles* larvae, controphic species, physicochemical and environmental variables. Further work needs to be done to confirm the effect of these controphic species as individuals and as groups (especially the competitors) on *Anopheles* mosquito larvae in controlled experimental settings. The controphic species may also be tested for their efficacy while integrating them with various LSM practices such as the addition of *Bti* as well as during short and long hydro period lengths.

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