PREDATION EFFICACY OF ANOPHELES FUNESTUS LARVAE BY AQUATIC PREDATORS IN RURAL SOUTH EASTERN –TANZANIA

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A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Master of Science in Public Health Research of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

The study aimed to examine the impact of 3 common predator on *Anopheles funestus* larvae. Specifically, (a) The impact of predator on larval and adult density (b) The impact of aquatic predation on fitness traits of *Anopheles funestus* mosquitoes (wing size, larval and adult survivals) in the semi-field system. Three selected predator families (Aeshnidae, Coenagriondae and Notonectidae) and *Anopheles funestus* group larvae were collected from the natural aquatic habitats in rural south eastern Tanzania and transferred to the semi-field system (Mosquito city) at Ifakara Health Institute. *Anopheles funestus* larvae were exposed to artificial habitats with predators. The number of surviving *Anopheles funestus* larvae were counted after 24 hours. Remaining larvae were monitored until all they are consumed or developed into pupae stage. An emerged trap was placed at the top of artificial habitats to capture an emerging mosquito. Emerged mosquitoes were provided 10% glucose solution-soakes cotton wool and their 24 hours mortality were recorded. Wings of died female mosquitoes were measured and used as a proxy for their body sizes. All predators were significantly reduced the *Anopheles funestus* density, affect the survival and wing sizes of emerged mosquitoes. Coenagrionidae were most efficient predators followed by Notonectidae while Aeshinidae were least efficient predators on *Anopheles funestus* larvae. The current study suggest that these aquatic predators may play an important role as complementary tool in reducing *Anopheles funestus* larval population and hence contribute to the reduction of the malaria vectors in Southern eastern Tanzania. Further investigations should be done in a real natural aquatic habitat.

Key words: *Anopheles funestus* group, predators, aquatic habitats, efficacy, malaria transmission, and biological control.

DECLARATION

I, Herieth Mahenge do hereby declare to the senate of the Nelson Mandela African Institution of Science and Technology that this dissertation titled *"Predation efficacy of Anopheles funestus larvae by aquatic predators in rural south-eastern –Tanzania"* is my original work and has never been or intending to be submitted for a degree award in any other institution.

Herieth Mahenge Date

The declaration is confirmed by:

Dr. Emmanuel W. Kaindoa Date

Dr. Halfan S. Ngowo Date

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CERTIFICATION

The undersigned certifies that they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology a dissertation titled *"Predation efficacy of Anopheles funestus larvae by aquatic predators in rural south-eastern –Tanzania"* in partial fulfilment of the requirements for the Degree of Master of Science in Public Health Research at the Nelson Mandela African Institution of Science and Technology Arusha, Tanzania.

Dr. Emmanuel W. Kaindoa Date

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DEDICATION

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

ATSB	Attractive Toxic Sugar Baits
Bs	*Bacillus sphaericus*
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EC	Electro conductivity
GLMEM	Generalized Linear Mixed Effect Model
GM	Genetically-modified GM
LLINs	Long-lasting insecticide-treated nets
IHI	Ifakara Health Institute
IRS	Indoor Residual Spraying
PBO	Piperonyl butoxide
PPF	Pyroproxyfen
NIMR	National Institute of Medical Research
NMCP	National Malaria Control Programme
MRCC	Research Coordinating Committee
SNP	School Net Program
TDS	Total dissolved solids
TNVS	Tanzania National Voucher Scheme
UCC	Universal Coverage Campaigns
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Malaria continue to be one of major public health challenges in Africa despite significant efforts made in reducing its burden (Tizifa *et al*., 2018). For example, there were 247 million malaria cases and 619 000 death globally, whereby Sub-Saharan Africa contributed to about 95% and 96% of these cases and deaths respectively (WHO, 2022). Though the core vector control tools notably long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS), effective diagnosis and treatment have significantly reduced malaria cases and deaths across Africa since 2001 (Loha *et al*., 2019), previous Malaria World report shows that malaria transmission has stalled due to major control limitations associated with COVID-19 disruptions (WHO, 2021), insecticide resistance (Eba *et al*., 2021), behavioural change in mosquitoes (Killeen & Chitnis, 2014) as well as human behaviours and activities (Barreaux *et al*., 2021; Finda *et al*., 2019) which affect the efficacy of vector control interventions. Therefore because of these challenges, achieving malaria elimination will be difficult, unless additional of novel methods that complement existing interventions (Kelly *et al*., 2012).

Larval control interventions as a part of integrated malaria vector control approach have additional impacts on controlling of disease transmitting mosquitoes (Killeen *et al*., 2002). Such interventions are effective, inexpensive and safe to non-target organisms (Fillinger & Lindsay, 2011; Imbahale *et al*., 2012; Ingabire *et al*., 2017), they are, however, underused. In a review of studies conducted between 1987 and 2019 discussing the use of bacterial larvicides (Derua *et al*., 2019), they were only able to describe 32 field based studies of these techniques. They suggest that larviciding should become more important as a vector control tool. It is important to have a thorough understanding of larval biology in order to effectively utilize these techniques. Specifically, the role of predators on the population of mosquito larvae has not been thoroughly researched.

Many aquatic invertebrate predator species, including Aeshnidae (Mandal *et al*., 2008), Notonectidae (Mandal *et al*., 2008) and Dytiscidae (Chandra *et al*., 2008) coexist with mosquito larvae (Kweka *et al*., 2011). Isolating these predators and distinguishing them from other organisms can be done by direct observation of their behaviour, visual examination of their midgut contents, molecular methods or electrophoretic methods (Morales *et al*., 2003; Ohba *et al*., 2010; Schielke *et al*., 2007). A single aquatic habitat may contain several species

of invertebrate predators. Such predators have been shown to be effective biocontrol agents against mosquitoes in different larval habitats (Aditya & Saha, 2006; Kweka *et al*., 2011; Mandal *et al*., 2008). For example, the mosquito larval mortality attributed to predators ranges between 54% and 90% depending on the environment, predator species diversity and density (Kweka *et al*., 2011). Although, aquatic predators may directly, or indirectly, influence mosquito population dynamics and fitness (Claessen *et al*., 2002), their effects on *Anopheles funestus* dynamics are not understood. The use of such predators may limit mosquito larval abundance and reduce adult densities (Debrah *et al*., 2021; Eba *et al*., 2021; Kweka *et al*., 2011; Ong'Wen *et al*., 2020).

Although malaria is transmitted by several *Anopheles* species in Tanzania, *Anopheles funestus* contributes to the residual malaria transmission in rural southern eastern Tanzania (Kaindoa *et al*., 2017; Lwetoijera *et al*., 2014; Mapua *et al*., 2022). This species is also highly resistant to common insecticides used for the control of adults, in particular the pyrethroids used in insecticide treated nets (ITNs) (Coetzee & Koekemoer, 2013; Fillinger *et al*., 2008; Matowo *et al*., 2021; Pinda *et al*., 2020). Despite the number of studies on the bionomics of these mosquitoes (Charlwood, 2019; Kaindoa *et al*., 2019; Ngowo *et al*., 2021), and their aquatic habitats (Nambunga *et al*., 2020), the relationship between aquatic predators and *Anopheles funestus* larval population remains unknown.

Therefore, the study aims to assess the predation efficacy of common aquatic predators found in of *Anopheles funestus* larvae aquatic habitats in rural Tanzania. Specifically, we aimed to (a) investigate common predators which co-exist with *Anopheles funestus* group larvae in a rural part of Tanzania (a) assess the impact of aquatic predators on *Anopheles funestus* larval density and adult density in the semi-field system (c) assess the impact of aquatic predators on fitness traits of *Anopheles funestus* (wing size, fecundity, larval and adult survival) in the semi-field system. The efficacy of larval predator in controlling *Anopheles funestus* population, a major vector of malaria transmission in rural southeastern Tanzania, is a critical factor to consider in the design of biological control interventions. Understanding the potential of these predators as a means of reducing vector population can inform the development of effective control strategies.

1.2 Statement of the Problem

Biological control of mosquitoes is recognized as one of the best approaches for controlling malaria vectors in malaria endemic countries (Benelli *et al*., 2016; Eba *et al*., 2021; Kamareddine, 2012). Despite the number of studies on larval predation and mosquito densities

(Kweka *et al*., 2011; Roux & Robert, 2019; Wang *et al*., 2021), there is inadequate information on the common predators and impacts of predation on *Anopheles funestus* mosquitoes in rural Tanzania. Such information would be very important for tackling challenges associated with chemical based interventions including resistance (Benelli *et al*., 2016). Therefore, this study was conducted to assess the predation efficacy on *Anopheles funestus* larvae by invertebrate predators in semi-field environments. The study also aimed to characterize the aquatic habitats which harboured the invertebrate predators and *Anopheles funestus* mosquito larvae and measured the physicochemical parameters of water in all of the aforementioned aquatic habitats.

1.3 Rationale of the Study

Control of *Anopheles* mosquitoes larvae by using predators is very cost effective, environmental friendly and sustainable compared to other methods (Kamareddine, 2012; Moirangthem & Singh, 2018). For example the study conducted by Fillinger highlights controlling mosquitoes larvae from their breeding sites using aquatic predators is cost effective than controlling adult mosquitoes (Fillinger *et al*., 2008). On the other hand, controlling of adult mosquitoes is faced with multiple challenges including the wide spread of insecticide resistance and the changing in mosquito biting/resting behaviours (Coetzee & Koekemoer, 2013; Fillinger *et al*., 2008; Matowo *et al*., 2021; Pinda *et al*., 2020). Utilizing aquatic predators as a complimentary measure for reducing the emergence of adult mosquitoes could be an effective way to prevent and reduce the transmission of malaria in Tanzania and other parts of the world.

Tanzania National Malaria Control Program (NMCP) has suggested the use of bio-control methods to control mosquito larvae as part of their integrated malaria control programs (Malaria & Programme, 2013). The initiatives focus on targeting all larval sources as it has been proved to be very effective (Fillinger & Lindsay, 2011; Imbahale *et al*., 2012; Ingabire *et al*., 2017). However, the use of larviciding techniques which involve application of chemicals poses a major environmental concern (Derua *et al*., 2019). To tackle these challenges, vector control experts came up with bio-insecticides such as *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) which are recommended by World Health Organization (WHO), as environmental friendly pesticides for larviciding (Fillinger & Lindsay, 2011; Zhou *et al*., 2020).

According to a study by Mapua *et al.* (2021), current evidence shows that community members are able to identify *Anopheles funestus* aquatic habitats. It is therefore important to further

characterize these habitats in terms of the presence of predators and assess the impact of these predators on larval and adult density. In addition, using predators that co-exist with mosquitoes in their aquatic habitats provide an opportunity for controlling disease-transmitting mosquitoes.

The findings of this study offer important insight into the interaction between *Anopheles funestus* larvae and predators, which can be used to develop/design a straightforward, simple to use, cost effective, scalable and environmentally friendly vector control method.

1.4 Research Objectives

1.4.1 Main Objective

To assess the predation efficacy on *Anopheles funestus* larvae by aquatic predators in rural, south eastern, Tanzania.

1.4.2 Specific Objectives

(i) Investigating the common invertebrate predators present in *Anopheles funestus* aquatic habitats in rural, south-eastern Tanzania.

(ii) Assessing the impact of aquatic invertebrate predators on *Anopheles funestus* larval and adult density in the semi-field system.

(iii) Assessing the impact of aquatic predation on fitness traits of *Anopheles funestus* mosquitoes (wing size, fecundity, larval and adult survival) in the semi-field system.

1.5 Research Questions

(i) What are the common invertebrate predators present in *Anopheles funestus* aquatic habitat in rural, south-eastern Tanzania?

(ii) What is the impact of aquatic invertebrate predators on *Anopheles funestus* larval and adult density in a semi-field system?

(iii) How does aquatic predation influence the fitness of *Anopheles funestus* (wing size, fecundity, larval and adult survival)?

1.6 Significance of the Study

This study provides useful information for understanding the interactions between aquatic predators and *Anopheles funestus* mosquitoes. These findings lay the groundwork for evaluating the effects of predators on both larval and adult mosquito densities, as well as their

impact on the fitness parameters of *Anopheles funestus* within a semi-field system. Understanding these predator-mosquito interactions is very important for designing effective biological vector control tools to address the challenges associated with chemical-based interventions in rural south-eastern Tanzania. By utilizing the information obtained from this study as a baseline, future research can focus on developing strategies within the framework of Integrated Vector Management (IVM). These strategies may involve the targeted use of predators to control *Anopheles funestus* populations, reducing reliance on chemical insecticides and promoting sustainable vector control methods.

1.7 Delineation of the Study

Previous study in the area have mainly focused only on identifying and characterizing the *Anopheles funestus* aquatic habitats, however, the ecology of *Anopheles funestus especially on* how they interact in with common predators in their aquatic habitats is poorly understood. As it is known, there are many methods for controlling disease transmitting mosquitoes, one effective strategy is to target their aquatic life stages (larval stage). In this stage, mosquitoes are vulnerable to a variety of natural predators that can be used as a form of biological control. Therefore, the current study focused on the understanding the interaction between *Anopheles funestus* and the aquatic predators. This study demonstrates that by utilizing these natural predators, it is possible to effectively control mosquito populations and reduce the risk of disease transmission.

CHAPTER TWO

LITERATURE REVIEW

2.1 Mosquito Life Cycle

The lifecycle of a mosquito includes four main stages egg, larvae, pupae and adult. The first three stages occur in water, while the final stage, the adult, is a highly active flying insect. The first stage of a mosquito's life is the egg stage. Female adult mosquitoes are known to prefer breeding in stagnant or slow moving water bodies, where they lay about 100-200 eggs at a time (Carlström & Renstål, 2020). The second stage starts with the eclosion of larvae from mosquito eggs. Some mosquitoes' larvae (non-anopheline) breathe by using a "siphon" which is located at the end of their tails and penetrates the surface of water. They need stagnant water to survive for 7-14 days depending on the food availability, water temperature and other environmental factor (Bukhari, 2011; California, 2010). In contrast, Anopheline larvae do not have a respiratory siphon but instead, they lie parallel to the surface of water for breathing purposes (William & Pinto, 2012). Mosquito larvae usually feed on organic contents and microorganisms found in water and undergo four aquatic larval stages and growing at each moult (Souza *et al*., 2019) before going into the next stage. During this stage, water can be treated by different larvicides to prevent larvae development into the next stage of the mosquito life cycle (Mbare *et al*., 2014; Ramirez *et al*., 2009). Third stage is aquatic pupae stage where larvae turn into pupae for 1-2 days; mosquitoes' pupae breath by using 'trumpets' which penetrates the surface of water (California, 2010). This is the resting stage and non-feeding which is focusing on shifting to next stage (Ramirez *et al*., 2009). In the pupal stage, water can be treated with specific products that can inhibit growth or cause mortality (Mbare *et al*., 2014). The adult stage is the last one in mosquito life cycle, once mosquitoes successfully reached the adult stage, they will feed on nectar and other sugar sources (Ramirez *et al*., 2009). Female adult mosquitoes will seek blood meals to be able to lay eggs. Adult mosquitoes live much longer under controlled condition and can live up to 14 days in the field (Ngowo *et al*., 2021; Ramirez *et al*., 2009).

2.2 Global Distribution of Anopheles Mosquitoes

Mosquitoes from the genus *Anopheles* are the major vectors of malaria parasites. The genus consists of more than 400 species (Pimenta *et al*., 2015; Sinka *et al*., 2012). The major ones include *Anopheles gambiae*, *Anopheles coluzzii*, *Anopheles arabiensis*, *Anopheles funestus*, *Anopheles moucheti* and *Anopheles nili*, which are responsible for 95% of the total malaria

transmission on the continent (Coetzee *et al*., 2013; Mouchet *et al*., 2004). Other vectors known as secondary vector includes *Anopheles squamosus* (DeMeillon, 1947)*, Anopheles coustani* (Lobo *et al*., 2015)*, Anopheles Anopheles rivulorum* (Gillies & Smith, 1960), *Anopheles ziemanni* Grtünberg (Vincke & Jadin, 1946), *Anopheles pharoensis* (Gillies, 1964) are responsible for other 5% in malaria transmission.

Anopheles funestus group is widely distributed across the African continent and thought to have a significant impact of malaria transmission (Hargreaves *et al*., 2000; Kaindoa *et al*., 2017; Matowo *et al*., 2017; Killeen, 2014; Lwetoijera *et al*., 2014; Gillies & Coetzee, 1987; Mendis *et al*., 2000; Okumu & Finda, 2021). *Anopheles funestus* is a complex group and consists of 13 morphologically identical species; *Anopheles rivulorum* (Mouatcho *et al*., 2018)*, Anopheles funestus*-like (Vezenegho *et al*., 2013), *Anopheles aruni, Anopheles vaneedeni, Anopheles brucei, Anopheles confusus, Anopheles funestus* sensu stricto (*s.s*.), *Anopheles fuscivenosus*, *Anopheles longipalpis* type A, *Anopheles longipalpis* type C, *Anopheles parensis*, *Anopheles rivulorum*-like and *Anopheles leesoni* (Dia *et al*., 2013; Gillies & Meillon, 1968)**.** Only *Anopheles funestu*s s.s. observed to be a dominant malaria vector in rural south-eastern Tanzania where by it contributes for more than 85% of the malaria transmission (Kaindoa *et al*., 2017; Matowo *et al*., 2017; Kaindoa *et al*., 2019). *Anopheles funestus* mosquitoes breed in semi-permanent water with aquatic vegetation and algae (Minakawa *et al*., 1999; Nambunga *et al*., 2020) and are absent in deep permanent water bodies, even if it had a lot of aquatic vegetation (Adoka *et al*., 2016).

Most of *Anopheles funestus* prefer to stay in aquatic habitats that are not exposed to sunlight, with emergent vegetations, large permanent ponds in low and high altitude areas (Nambunga *et al*., 2020). Also, in small ponds and spring fed wells in lower altitude areas, slow-moving water along the riverside and streams at higher altitude above 300 m with emergent vegetation (Nambunga *et al*., 2020).

Targeting *Anopheles funestus* is highly challenging due to the development of insecticide resistance (Kaindoa *et al* 2017; Matowo *et al*., 2017; Lwetoijera *et al*., 2014; Pinda *et al*., 2020, 2022) and changing of biting behaviour (Mathania *et al*., 2016; Moiroux *et al*., 2014; Sougoufara *et al*., 2014). This creates an opportunity for using other bio-control methods for malaria vector. Global distributions of *Anopheles* mosquitoes are influenced by climatic factors, in which they have an impact in the developmental stages. It is clearly shown that *Anopheles* species are highly affected by temperature and humidity (Christiansen-Jucht *et al*., 2014). Temperature is in favour of all the three species which are the major vectors of malaria

transmission in sub-Saharan Africa (Lyons *et al*., 2013). As it shown in a study conducted by (Sinka *et al*., 2012) (Fig. 1), Africa is mainly dominated by three vectors of malaria transmissions as compared to Asian-Pacific region which has more species.

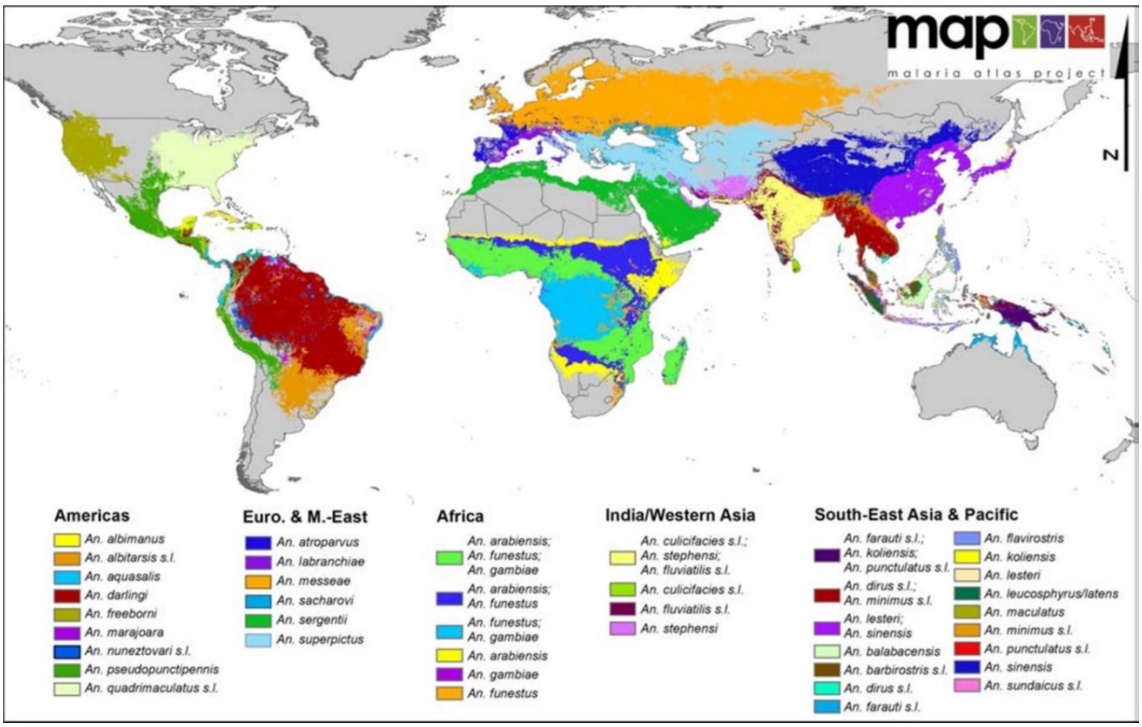

Figure 1: Distribution of malaria vectors globally (Sinka *et al*., 2012)

2.3 Bio-Control of Mosquitoes

Bio-control of mosquitoes involves the use of natural organisms that kill mosquitoes, exploitation of mosquito behavior to increase mosquito mortality, and releasing mosquitoes that are either sterile or unable to transmit the infection (Benelli *et al*., 2016). Bio-control is very essential and effective means for controlling transmission of malaria especially in areas where insecticide resistance is growing (WHO, 2018). There are several biological control approaches which includes the use of bacterial agents like (*Bacillus Thuringiensis and Bacillus sphaericus),* aquatic predators like Notonectidae (backswimmers), Aeshnidae (dragonflies) Dytiscidae (diving beetles) and *Larvivorous Fish* (Benelli *et al*., 2016) (Fig. 2). Pathogens like *entomopathogenic* fungi shown to have a significant impact on anopheles mosquitoes larva (Bukhari *et al*., 2010; Scholte *et al*., 2006). These methods are host specific, environmental friendly, sustainable, cost-effective, lack infectivity and simple application in field (Benelli *et al*., 2016). These methods have a high potential use because they target all different stages of mosquitos' life cycle (Ndava *et al*., 2018).

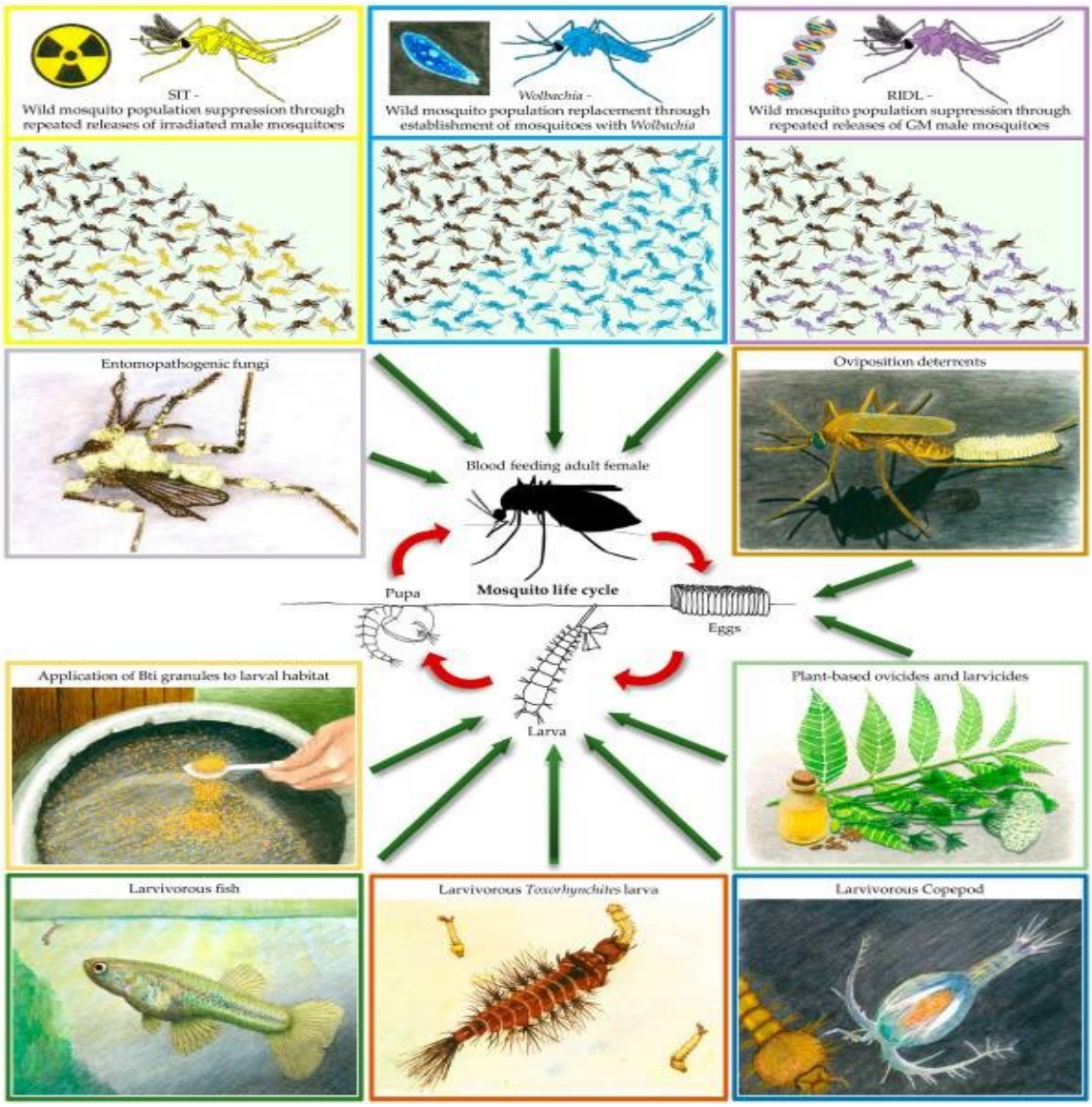

Figure 2: **Bio-control methods used to target different stages of a mosquito lifecycle (Benelli *et al*., 2016)**

2.4 Larval Predators

Different studies shows different kinds of larvae predators which includes; Odonata (dragonfly and damselflies) (Mandal *et al*., 2008), Notonectids (backswimmers) (Ohba *et al*., 2010) Dytiscidae beetles (diving beetles) (Chandra *et al*., 2008; Ohba *et al*., 2010), Belostomatids (giant water bug) (Ohba *et al*., 2010), Crustaceans (Huang *et al*., 2017) and Amphibians (Ohba *et al*., 2010). The use of predators have a significant impact on fitness characteristics of the mosquitoes larvae because it stresses larval development and fecundity rate (Roux & Robert, 2019). Larvae have poor ability to spread and this makes them at high risk of being eaten by predators as compared to adult mosquitoes (Roux & Robert, 2019). Mosquitoes' larvae are able to detect the presence of predators and exhibit phenotypic plasticity, reduce their activity to avoid being easily recognized and reduce the risk of predation (Roberts, 2014). The use of some chemicals for controlling mosquito larvae can have unintended consequence on the local

ecosystem. According to a study by Antwi and Reddy, the use of these chemicals can lead to high mortalities of aquatic mosquito predators, making the intervention ineffective (Antwi & Reddy, 2015).

Several environmental factors have been shown to affect the presence of predators and their prey including temperature, pH and oxygen (Abai *et al*., 2016; Adebote *et al*., 2008; Akeju *et al*., 2022; Anderson *et al*., 2001; Chaiphongpachara *et al*., 2018; El-naggar *et al*., 2013; Musonda & Sichilima, 2019; Spieles & Mitsch, 1999), turbidity and salinity (Dida *et al*., 2015), while under the favourable condition the survival and abundance of predator and prey will be high (Smith-owen, 2008).

2.5 Mosquito Fitness Parameters and their Importance in Malaria Transmission

The pattern and malaria transmission rate within the population depends on the fitness parameters of the mosquitoes (Vézilier *et al*., 2012), in which these parameters fecundity, body size, and survival (Moller-Jacobs *et al*., 2014; Ngowo *et al*., 2021) can be determined and shaped by the condition experienced during larval growth and developmental stages (Moller-Jacobs *et al*., 2014). Mosquitoes survival have an impact on malaria parasite transmission (Smith & McKenzie, 2004), since the higher survival of mosquitoes allow the parasite to complete its extrinsic incubation period and increases the potential of infective bites (Read *et al*., 2009). It is known that the malaria transmission is strongly correlated with body size (Kingsolver & Huey, 2008). Mosquitoes with large body size carry many parasites (Pulkkinen & Ebert, 2004) and show overall body fitness, high fecundity rate and greater survival, which are important factors in determining the vector capacity and transmission of malaria (Moller-Jacobs *et al*., 2014).

2.6 Latest Advances in Malaria Vector Control

Several studies show that malaria vector control are faced with the challenge of the spread of pyrethroids resistance (Kaindoa *et al*., 2017; Ngowo *et al*., 2017; Kawada *et al*., 2011; Lwetoijera *et al*., 2014; Pinda *et al*., 2020, 2022). This threatens the performance of standard LLINs (Martin *et al*., 2021). Moreover World Health Organization (WHO) responded by encouraging manufactures to develop new form of LLINs that contain active ingredients with new different modes of action to address the problem of insecticides resistance (Martin *et al*., 2021; Pennetier *et al*., 2013). Olyset® Plus is a new form of LLINs which contain pyrethroids permethrin and synergist Piperonyl butoxide (PBO) to counter pyrethroids resistance in mosquitoes. Moreover, another study showed evidence for a benefit of combining a PBO with

pyrethroid insecticide mosquito nets (Gleave *et al*., 2021). Other advanced approach includes auto-dissemination of pyriproxyfen (PPF) which act as a growth inhibitor and is used in larviciding (Lwetoijera *et al*., 2019; Opiyo *et al*., 2021).

Auto-dissemination of PPF incorporates exposing the wild-adult mosquitoes in artificial PPF contaminated places, and later such mosquitoes disseminate the chemical to their aquatic breeding sites, thus preventing the emergence of new adult mosquitoes (Caputo *et al*., 2012; Lwetoijera *et al*., 2019). Attractive Sugar Baits (ATB), this method involves the use of manufactured plant sugars that are essential dietary component for both female and male mosquitoes, with the addition of toxin that kill on ingestion or contact (Marshall *et al*., 2013). This technology is new, effective and very target specific with very few or no effects to non-targeted organism and environmental contamination (Beier *et al*., 2018). It targets outdoor-feeding mosquito species by suppressing mosquito populations and diminishes the number of mosquitoes living long enough to pass on the malaria parasite, since many are killed before completing the extrinsic incubation period (Beier *et al*., 2012; Müller *et al*., 2010).

Another method involves using the sexual behaviour of the mosquito by targeting the swarming mosquitoes (Kaindoa *et al*., 207; Matowo *et al*., 2017; Sawadogo *et al*., 2017). This can be done either by spraying the swarms or by collecting them in large sweep nets as used before in Burkina Faso (Sawadogo *et al*., 2017) and Tanzania (Kaindoa *et al*., 2017).

Genetically-modified (GM) mosquitoes, are mosquitoes that have their genetic material (DNA) changed by using a genetic engineering techniques in a way that does not occur naturally or by recombination (Terenius *et al*., 2008). This approach proposed as part of an integrated malaria vector control in endemic areas. Transgenic strains of malaria vector have been developed for the purpose of supressing the vector population responsible for the transmissions and reducing their ability to transmit the infections (Knols *et al*., 2007). All the latest vector controls method shown in Fig. 3.

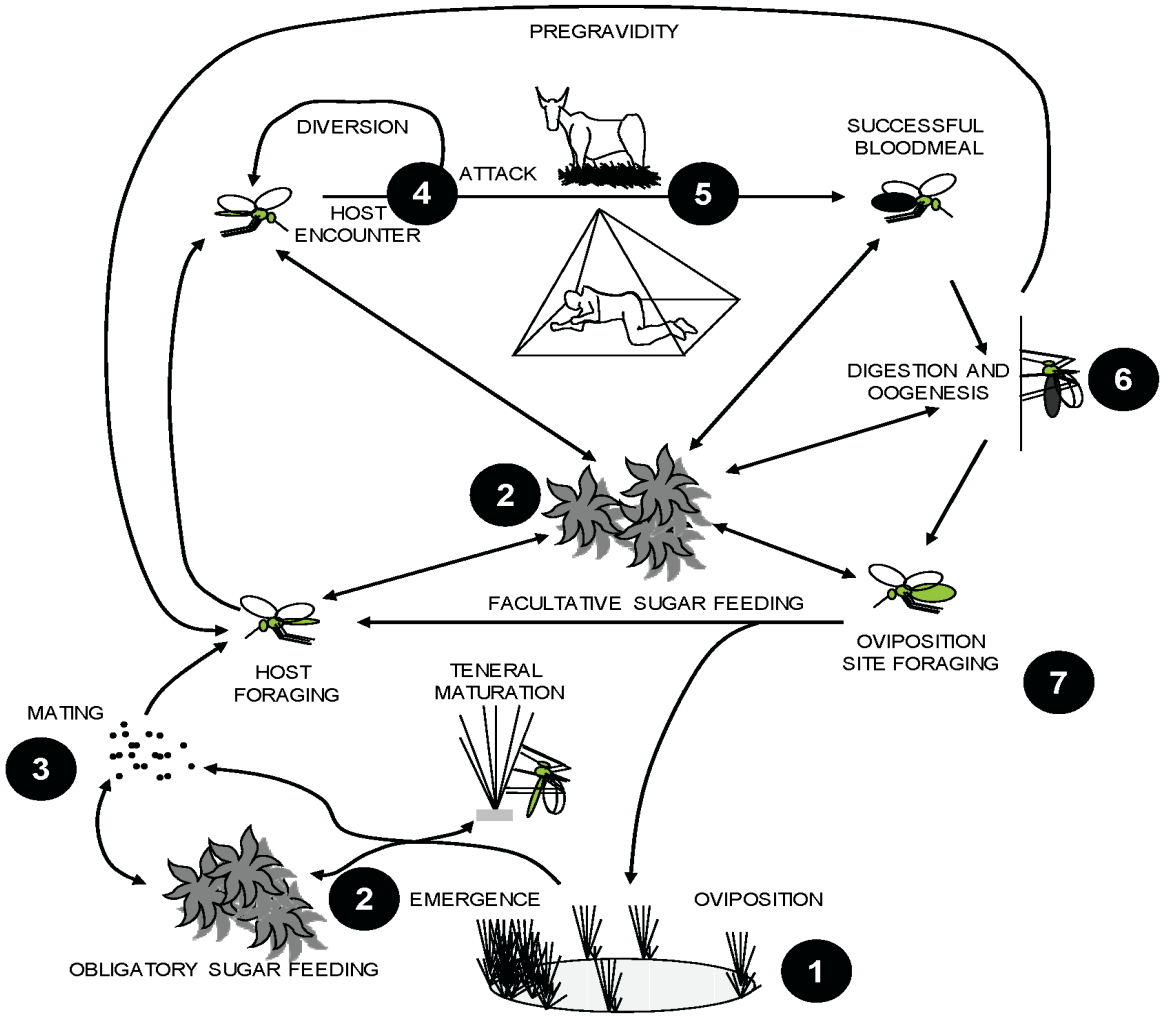

Figure 3: **Life cycle showing latest advanced of malaria vector control and corresponding (Ferguson *et al*., 2010)**

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

A cross-sectional survey was conducted, between March and May 2022, in nine villages in south-eastern Tanzania, namely Chikuti (-8.6028°, 36.7288°), Mzelezi (-8.8934°, 36.7343°), Chirombola (-8.93041°, 36.75753°), Ebuyu (-8.9719°, 36.7608°), Mwaya (-8.91022°, 36.823139°) and Tulizamoyo (-8.35447°, 36.70546°) in Ulanga district and Ikwambi (-7.97927°, 36.81630°), Kisawasawa (-7.89657°,36.88058°) and Mpofu (-8.17220°, 36.21651°) in Kilombero district (Fig. 4). In this area, *Anopheles funestus* is responsible for more than 85% of overall malaria transmission (Kaindoa *et al*., 2017). The residents in some of these villages practise extensive rice farming, which creates suitable habitat for mosquito breeding. Common aquatic habitats for *Anopheles funestus* in the villages are well known and have been previously characterized (Nambunga *et al*., 2020). Eighty-five known habitats from the nine villages were sampled for both mosquito larvae and potential predators.

Controlled experiment was conducted between July and November 2022, in a semi-field system facility (Ferguson *et al*., 2008) located at Ifakara branch, in Kilombero district, South eastern Tanzania. The semi-field system conDsists of three-chambered large screened-enclosures, measuring 28.8 m by 21m, with walls made of UV-resistant shade netting, and a polyethylene roof mounted on a raised concrete platform (Ferguson *et al*., 2008). This study used two chambers of this facility, each measuring 28.8 m by 7 m (Plate 1). In addition, this large semi-field system has self-sustaining colonies of malaria vectors, experimental huts and vegetation to mimic natural environments.

The area (Kilombero and Ulanga) has received LLINs since inception of the distribution campaigns: Tanzania National Voucher Scheme (TNVS) in 2004, mass distribution campaigns, Universal Coverage Campaigns (UCC) and School Net Program (SNP) (Pamungkas *et al*., 2019). The characteristics of *Anopheles funestus* aquatic habitats in this area is well studied (Nambunga *et al*., 2020), however the knowledge on larval ecology and interaction with its aquatic predation is limited. The residents in this area also practice intensive rice and maize farming as well as livestock keeping which have the impacts on the modifications of the habitats including providing conducive environment for mosquito breeding.

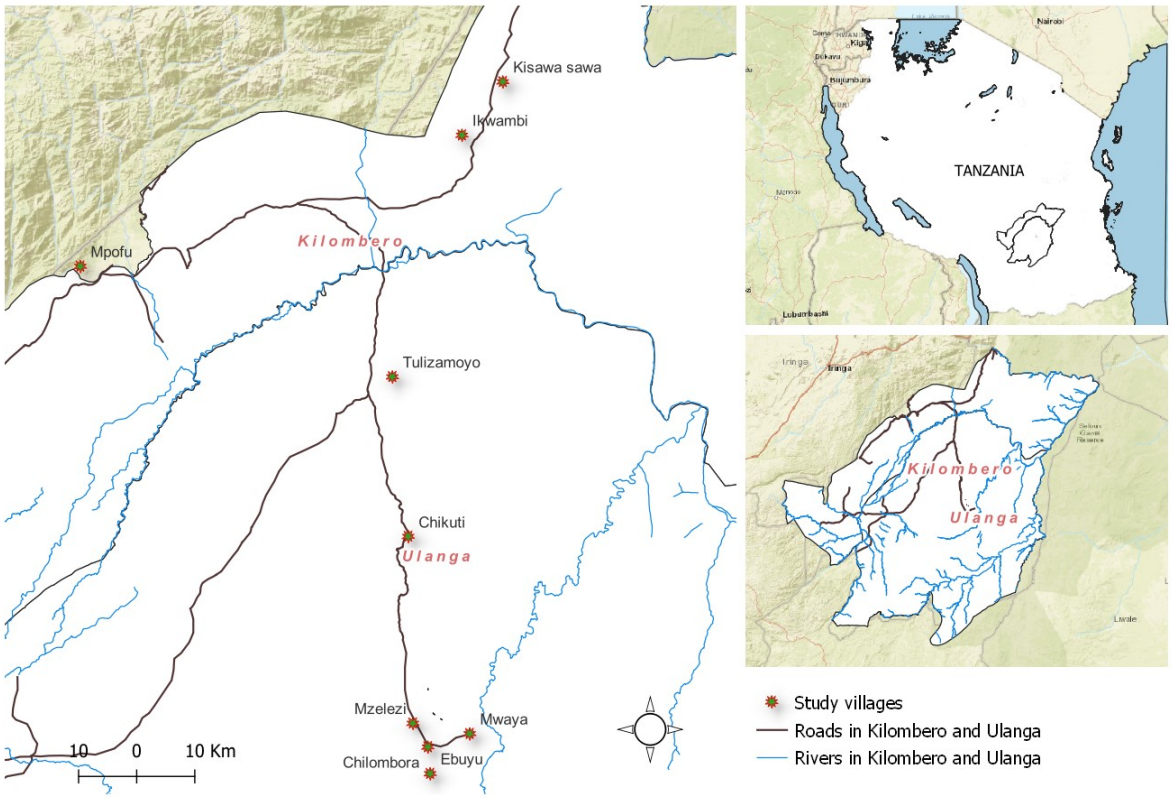

Figure 4: Map of Kilombero and Ulanga districts showing the nine study villages

Plate 1: A large semi-field system at Ifakara Health Institute "Mosquito City"

3.2 Objective one: Identifying the Common Aquatic Predators that Co-Exist with Anopheles Funestus Larvae in the Aquatic Habitats

3.2.1 Sampling and Morphological Identification of Mosquito Larvae and Aquatic Predators

Mosquito larvae and predators were sampled using standard dippers (350 ml) or 10 L buckets, as previously described (Debrah *et al*., 2021; Kweka *et al*., 2011; Nambunga *et al*., 2020). A minimum of three dips and a maximum of twenty dips were taken depending on the size and depth of the habitat. In the previous study, mosquito larvae from the same villages were taken to the laboratory in Ifakara and allowed to emerge and eventually identified to species level by PCR. Of those identified 53% were *Anopheles funestus* s.s. whilst 28% were *Anopheles rivulorum* and 12% were *Anopheles leesoni* (Nambunga *et al*., 2020). All three species were found to occupy the same habitats. During the present study, we followed a similar approach with samples of fourth instar larvae, but we did not identify the species. Earlier stage larvae were identified based on their predominant characteristics and separated into *Anopheles funestus* group, *Anopheles gambiae* s. l. or *Anopheles* sp. Culicines were identified to genera only. Predators were morphologically identified to family level using the keys by (Stroud Water Research Center, 2013) and (Gerber & Gabriel, 2002). Mosquito larvae and predators that were sampled by each dipper or bucket were counted and recorded. Additionally, geographical locations of the surveyed habitats were recorded at access points using a hand-held GPS device (Garmin eTrex 20x Handheld GPS Receiver).

Although many individual dips were negative, all aquatic habitats sampled contained mosquito larvae. Their overall physical characteristics were recorded and physicochemical parameters of the water (pH, temperature, electrical conductivity (EC), total dissolved solids (TDS) were measured using a portable water quality meter (ZJ practical 4 in 1 Water Tester). A Trans Instruments Dissolved Oxygen Meter (HD3030) was used to measure dissolved oxygen (DO), using standard recording procedures. Habitats were classified as being either: swamp, stream, river, rice-field, stream-pool, ground-pool, ditch, spring-fed pool, puddle, hoof-print, man-made wells, brick or sand pit. Water colour was categorized as being clear (transparent and odourless) or coloured (cloudy, not transparent, turbid or with a film of oil). The source of water was also classified as rainwater or others (non-rainwater). Algal quantities in the habitats were classified as none, moderate, or abundant. Algal type was classified as filamentous, green, blue-green or brown. Water was also classified as being stagnant, slow or fast moving. The land use surrounding the aquatic habitats was classified as scrub, cattle grazing or cultivated

field. Shade over the habitats was classified as none, partial or heavy. Habitat size was measured using tape and classified as being less than 100 m^2 or more than 100 m^2. Vegetation quantity and vegetation type were also classified as (none, moderate or abundant) and (emergent, or submerged) respectively. Water bodies known to have existed for three months or more were considered to be permanent whilst other collections of water were considered to be 'temporary'. Water depth was classified as being less than 50 cm or more than 50 cm deep. The distance from aquatic habitats to the nearest houses was estimated visually and classified as being less than 100 m or more than 100 m.

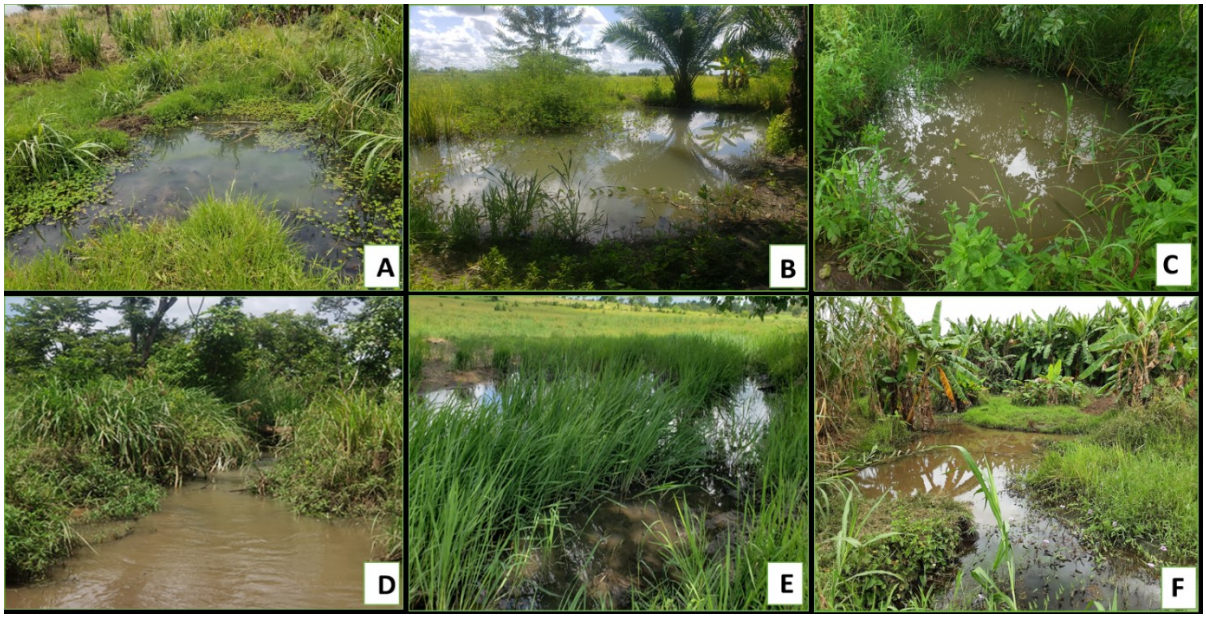

Plate 2: Different aquatic habitats characteristics for *Anopheles funestus* mosquito and their predators: A-Grounded pool, B-Brick/Sand pit, C-Man made well, D-River stream, E-Swamp, F-Grounded fed pool

3.3 Objective two: Assessing the Impact of Aquatic Predators on *Anopheles funestus* Adult and Larval Density in the Semi-Field System

3.3.1 Predators and Mosquitoes Larvae Collection

Predator collections were made from natural *Anopheles funestus* aquatic habitats in which predators were collected using aquatic net (Plate 3A) and 10 L bucket while 10 L bucket and pipette were used to collect *Anopheles funestus* larvae from their habitats (Plate 3C), both predators and *Anopheles funestus* were transferred to buckets with water from their natural habitats and transported to the semi-field (Mosquito City). In this objective we specifically collected only three predator families Aeshnidae, Coenagriondae and Notonectidae and placed them in individual buckets to avoid cannibalism (Plate 3B). *Anopheles funestus* larvae were also placed in a separate basin to prevent early predation. At the semi-field settings *Anopheles*

funestus larvae were given fish food while predators were starved for 12 hours and then introduced in the artificial habitats. Then an evaluation was taken after 24 hours (everyday).

Plate 3: A, B, C-Collection and identification of *Anopheles funestus* larvae and aquatic predators

3.3.2 Experimental Design in the Semi-Field and 24 Hours' Evaluation

Experiments were conducted in one habitat type which contained stone, two Kilogram of sand and grasses (Plate 4a) which mimicked hiding structures found in natural habitats for *Anopheles funestus* larvae against predators (Kweka *et al*., 2011), 5 L of water from the natural breeding habitats, 2 kilograms of soil, aquatic predators and *Anopheles funestus* larvae. The third instar larval was used in the 24-hour evaluation in semi-field experiments.

In this experiment three different predators were evaluated against control. Four groups with different densities of predators were created to evaluate the effect of predatory density on different fitness traits of *Anopheles funestus*. Different number of each predator type were placed in each group (i.e., 20, 15, 10 and 5). In each group a constant number of 100 *Anopheles funestus* larvae were placed and another 100 *Anopheles funestus* larvae placed in the control arm (Fig. 5). Each experimental group had three replicates. In all group the number of surviving larvae were counted and recorded after every 24 hours.

Mosquito larvae that survived were kept until they became pupae. Then, an emergency trap was placed at the top of each habitat to capture emerged mosquito (Plate 4b). The number of emerged mosquitoes in each group was recorded.

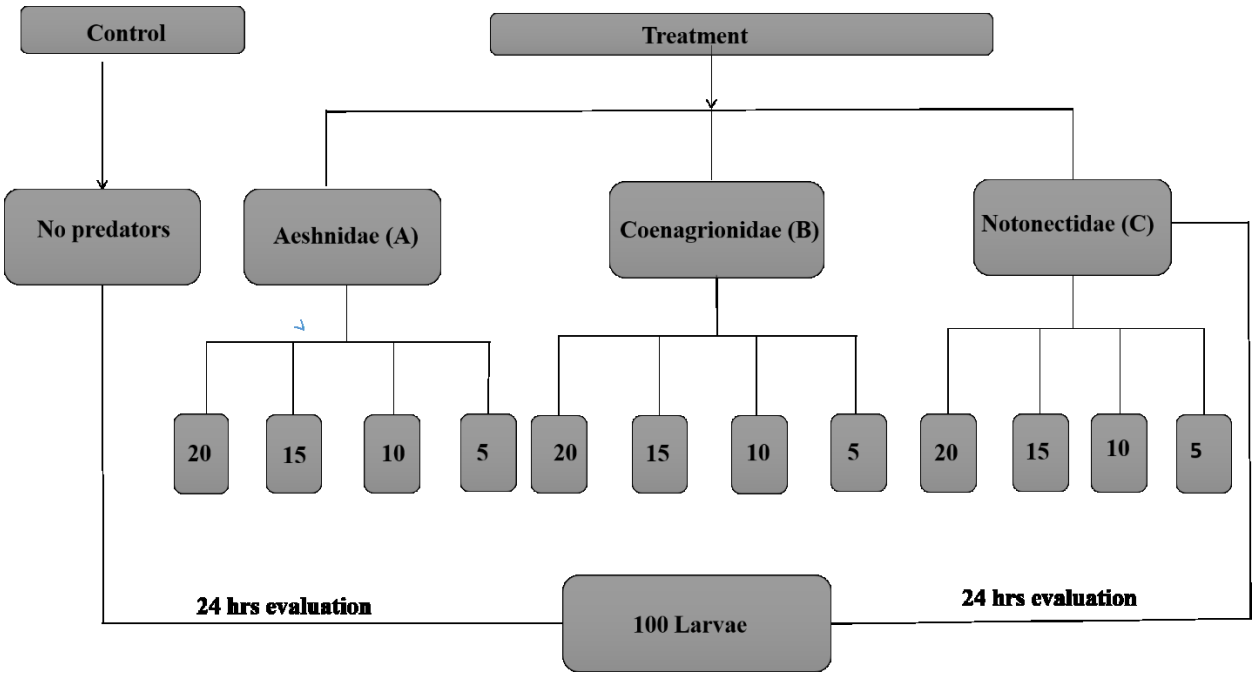

Figure 5: An experimental study design in the semi-field environment

Plate 4: Artificial habitats and 4b - An emergency trap on the top of artificial habitats for capturing the emerged mosquitos in a semi-field system

3.4 Objective 3: Assessing the Impact of Aquatic Predation on Fitness Traits of Anopheles Funestus mosquitoes (Wing Size, Fecundity, Larval and Adult Survivals) in the Semi-Field System

3.4.1 Assessing the Survival of Adult Mosquitoes

Each emerged mosquito from both treatment and control group were transferred into a 15 x 15 cm cage and mosquitoes were provided with 10% glucose solution-soaked cotton wool and their 24 hrs mortality were recorded.

3.4.2 Measurements of Mosquito Wing Lengths

Dead females were removed from the cages, placed in cups, and later transferred to the Ifakara insectary ("Vector Sphere") for wings measurement. A single wing of dead female mosquito was placed in the microscope slide and a drop of distilled water was used for sticking the wing on the slide. Wing length was measured using micrometre ruler under a microscope from alula notch to wing tip following procedures by Lyimo *et al.* (1992). Wing length measurements were used as a proxy for mosquito body size.

3.5 Data Analysis

3.5.1 For the 1st Objective

Analysis was done using open source software R version 4.2.2 (R Development Core Team *et al*., 2022). Generalised linear mixed effects models (GLMM) using template model builder (TMB) with zero-inflated negative binomial implemented under the *glmmTMB* package (Brien, 2022) were used to: (a) assess the associations between water physicochemical parameters and the abundance of aquatic predators (b) assess the associations between water physicochemical parameters and the abundance of *Anopheles funestus* group larvae (c) assess which habitat, characteristics contributed to the abundance of predators and *Anopheles funestus* group larvae and (d) assess the impact of each predator family on the abundance of *Anopheles funestus* group larvae.

Due to a large number of dips with zero larvae the negative binomial with zero inflated models were used. In all models, the study villages in which the aquatic habitats were identified and habitat ID were included as random terms to capture unexplained variations between villages and habitats. The best fitting models were selected using Akaike Information Criterion (AIC) and results presented as risk ratios (RR) at 95% CI and statistical significance was considered when the *P*-value < 0.05.

3.5.2 For the Second Objective

Generalized linear mixed model (GLMM) (Bates, 2010) with binomial variates was used to: (a) estimates the Odds ratio and the absolute proportion of the number of larvae alive in each predator type (b) asses the emergence rate of mosquitoes. The number of larvae alive and emerged mosquitoes in the artificial habitats was first assed in a group (all predators were combined) and later assessed individually. Results were presented as Odds Ratio (OR) with their corresponding at 95% CI and statistical significance was considered when P-value < 0.05.

Marginal prediction on the number of larvae alive were obtained from the *ggeffect* package and the all the plots were produced using *ggplot2* package (Wickham, 2016). The efficacy of each predator family was calculated as:

$$Efficacy = \frac{Control - Treatment}{Control} * 100$$

Where "control" number of larvae alive in a habitat without predators, "treatment" where the number of larvae alive in habitats with predators.

3.5.3 For third Objective

Survival analysis of the emerged mosquitoes were done using Cox proportional hazard model using the *survival* package (Therneau, 2022) to assess odds of mortality for emerged mosquitoes (both males and females) for each predators type in term of hazard ratio (HR). The response variable was the observed time. Graphs were produced by *suveminer* package from a Kaplan Meir survival model (Kassambara *et al*., 2017). Post Hoc Test using the Tukey Honest Significance Difference (THSD) test were used assess wing size differences on mosquitoes emerged in different predators and the control. Figures were produced using the *ggplot2* package (Wickham, 2016).

3.5 Ethical Clearance

Approval for this study was obtained from the institutional review board of Ifakara Health Institute (Ref: IHI/IRB/No: 13-2022) and from the Medical Research Coordinating Committee (MRCC) at the National Institutes of Medical Research (NIMR) (Ref: NIMR/HQ/R.8a/Vol. IX/3353).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Common Predators Present in *Anopheles funestus* Aquatic Habitat Distribution of *Anopheles Funestus* Larvae, other Mosquito Larvae and Aquatic Predators

Eighty-five aquatic habitats that contained *Anopheles funestus* group larvae were identified and characterized. In these habitats, 8295 predators were sampled. Among all sampled predators, only 7906 were identified to eight families. Of these, Coenagrionidae accounted for 57.7% (n=4785), Corixidae 12.8% (n=1060), Notonectidae 9.9% (n=822), Aeshnidae 4.9% (n=405), Amphibian 4.5% (n=370), Dytiscidae 3.8% (n=313), Belostomatidae 1.2% (n=103) and Nepidae 0.6% (n=48) (Table 2). Three hundred and eighty-nine (4.6% of the total invertebrates) were not identified due to lack of an appropriate key (Table 2). A total of 5260 larvae were collected, with *Anopheles funestus* group larvae accounting for 60.3% (n=3170) of the total, *Culex* spp 24.3 (n=1279), *Anopheles gambiae s.l.* 8.3% (n= 438), and other anopheline larvae 7.1% (n= 373) (Table 1).

Overall, *Anopheles funestus* group larvae and predators were samples from different aquatic habitats both man made and natural habitats: includes grounded pool, Brick/ sand pit, man-made wells, river stream, swamp and spring fed pool (Plate 2). However, river stream, rice fields and brick or sand pit found to have higher mean number of *Anopheles funestus* group larvae compared to all other habitats types (Table 1). River stream, spring fed pool and swamps found to have higher mean number of predators compared to all other habitats (Table 2).

(i) Characteristics of Aquatic Habitats and their Influence on the Abundance of *Anopheles Funestus* Larval Group and Predators

Anopheles funestus group larvae and predators were found in high abundance in habitats larger than 100 m^2 and at the edges of streams and rivers (habitats with fast moving water) (P<0.05, Table 5 and 6), whilst low abundance of larvae was associated with habitats with submerged vegetation (P<0.05, Table 5). Predators were positively associated with the permanence of the aquatic habitats *(*P<0.005, Table 6). Other aquatic habitat characteristics including algae quantity and type, shade over the habitats, water depth, vegetation quantity, environment surrounding the aquatic habitats and the distance from the nearest houses were found to have no impact on *Anopheles funestus* group larval abundance and predator abundance (Table 5 and 6).

(ii) Water Physicochemical Parameters and their influence on the Abundance of Predators and Anopheles Funestus Larvae Group

There was no apparent association between physicochemical parameters (pH, temperature, TDS, EC and DO) and predator abundance (P>0.05, Table 9). Temperature, pH, TDS, EC also had no impact on the abundance of *Anopheles funestus* group larvae but DO was positively associated with the abundance of *Anopheles funestus* group larvae (P<0.05, Table 8).

(iii) Association of Different Predators with the Abundance of Anopheles Funestus Group Larvae

Coenagrionidae and Dytiscidae were positively associated with *Anopheles funestus* group larval abundance (P<0.05, Table 10) whilst Notonectidae and Corixidae were negatively associated with *Anopheles funestus* abundance (P<0.05, Table 10). No strong association between abundance of *Anopheles funestus* group larval abundance and some predator families including Aeshnidae and Belomastidae were found (P>0.05, Table 10).

Table 1: Mean number and standard error (se) of different mosquito larvae species sampled from different aquatic habitats

Habitat information		Mean number and standard error (2se) of different mosquitoe larvae				Total
Habitat type	**Total habitats**	***Anopheles funestus group***	***Anopheles gambiae s.l***	**Other anophelines**	***Culex* spp**	
Brick or sand pit	12	37.8 (19.64)	2.7(2.5)	3.2 (6.0)	15.8 (11.9)	713
Ditch	8	16.5 (8.4)	1.5 (1.1)	5.0 (7.4)	20.2 (22.3)	346
Grounded pool	1	19.0 (NA)	3.0 (NA)	0	12.0 (NA)	36
Man- made wells	20	11.2 (3.4)	3.8 (5.3)	0.6 (0.6)	5.6 (3. 6)	422
Rice field	2	40.5 (51.9)	3.0(3.9)	10.5 (20.6)	24.5 (34.3)	157
River stream	34	61.7 (29.8)	9.0(8.0)	5.4 (4.7)	13.3 (10.2)	3039
Spring- fed pool	2	25.0 (17.6)	0	0	29.5 (38.2)	109
Swamp	6	18.8 (4.6)	0.7 (1.0)	13.2 (10.8)	40.7 (19.4)	440
Total	85	3170	438	373	1279	5260

Table 2: **Mean number and standard error (se) of different predators sampled from different aquatic habitats**

Habitat information		Mean number and standard error (2se) of different predators									
Habitat type	**Total habitats (N)**	**Aeshnidae**	**Coenagrionidae**	**Dytiscidae**	**Notonectidae**	**Corixidae**	**Nepidae**	**Belomastidae**	**Amphibians**	**Unidentified species**	**Total**
Brick or sand pit	12	8.25 (6.07)	33.0 (19.42)	3.83 (2.61)	10.33 (6.0)	19.42 (22.95)	0.25 (0.35)	1.42(1.59)	2.08 (1.35)	2.0 (2.84)	967
Ditch	8	1.0 (1.11)	10.3 (6.7)	7.63 (7.27)	6.75 (9.3)	1.13 (0.94)	0.25 (0.49)	3.25 (5.58)	23.5 (45.78)	2.25 (1.92)	448
Grounded pool	1	0.0 (0.0)	46.0 (NA)	0.0 (0.0)	0.0 (0.0)	4.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1 (NA)	0.0 (0.0)	51
Man-made wells	20	7.05 (6.38)	32.2 (33.35)	2.45 (2.53)	23.75 (18.18)	5.95 (6.89)	0.55 (0.78)	0.05 (0.10)	1.45 (1.49)	0.90 (0.98)	1487
Rice field	2	2.50 (2.94)	37.5 (42.14)	9.0(17.64)	5.0 (9.8)	1.0 (9.8)	1.00 (1.96)	0.0 (0.0)	1.00 (0.00)	16.0 (31.36)	129
River stream	34	1.50 (0.98)	97.18 (24.1)	2.0 (1.5)	1.21 (0.89)	17.4 (11.0)	0.76 (0.63)	1.65 (1.33)	3.44 (2.94)	6.76 (4.06)	4485
Spring-fed pool	2	17.5 (22.54)	46.5 (46.07)	0.5 (1.0)	36.5 (2.94)	2.0 (1.96)	1.00 (0.0)	0.0 (0.0)	2.0 (1.96)	1.0 (NA)	214
Swamp	6	11.0 (7.62)	24.17 (13.71)	11.7 (12.0)	7.5 (7.4)	16.17 (15.48)	0.33 (0.41)	0.50 (0.98)	0.67 (0.65)	10.83 (4.54)	497
Total	85	405	4785	296	822	1060	48	103	370	389	8278

Table 3: Number of each aquatic habitats type showing the co-existence of different mosquito larvae group

Study site	Habitat information		Number of habitats with different mosquito species			
	Habitat type	**Total habitats**	***Anopheles funestus* s.l**	***Anopheles gambiae s*.l**	**Other anopheline**	***Culex* spp**
Ulanga and Kilombero	Brick or sand pit	12	12	7	2	10
	Ditch	8	8	5	2	5
	Grounded pool	1	1	1	0	1
	Man-made wells	20	20	10	4	13
	Rice field	2	2	2	1	2
	River stream	34	33	16	9	21
	Spring-fed pool	2	2	0	0	2
	Swamp	6	6	2	5	6
	Total	85	85	46	23	60

Table 4: Number of each aquatic habitats type showing the co-existence of different predators

Study site	Habitat information		Number of habitats with different predators								
	Habitat type	Number of habitat	Aeshnidae	Coenagrionidae-	Dytiscidae	Notonectidae	Corixidae	Nepidae	Belostomatidae	Amphibians	Unidentified group
Ulanga and Kilombero	Brick or sand pit	12	10	12	8	8	7	2	4	6	3
	Ditch	8	3	5	5	4	5	1	2	2	4
	Grounded pool	1	0	1	0	0	1	0	0	1	0
	Man-made wells	20	12	14	6	14	7	4	1	6	5
	Rice field	2	2	2	1	1	2	1	0	2	1
	River stream	34	13	34	11	10	21	9	10	11	21
	Spring-fed pool	2	2	2	1	2	2	2	0	2	2
	Swamp	6	6	6	5	5	4	2	1	3	6
	Total	85	48	75	37	44	49	21	18	33	42

Table 5: **Univariate and multivariate regression analysis of different aquatic habitat characteristics and their association with the abundance of *Anopheles funestus* larvae**

Aquatic habitat	Univariate analysis		Multivariate analysis	
	RR (95% LC, UC)	P-values	RR (95% LC, UC)	P-values
Algae quantity				
None	1		1	
Moderate	1.21 [0.66, 2.20]	0.535	1.44 [0.76, 2.73]	0.260
Abundant	1.94 [0.89, 4.20]	0.094	1.72 [0.72, 4.11]	0.224
Habitat size				
Less than 100 M	1		1	
Greater than 100M	2.54 [1.65, 3.89]	< 0.05	2.56 [1.58, 4.14]	< 0.05
Vegetation type				
None	1			
Emergent	0.73 [0.32, 1.63]	0.435	0.64 [0.35, 1.18]	0.151
Sub-merged	0.21 [0.04, 1.08]	0.062	0.10 [0.02, 0.57]	< 0.05
Water Movement				
Stagnant	1		1	
Slow	1.45 [0.90, 2.35]	0.127	1.29 [0.76, 2.18]	0.343
Fast	1.82 [0.96, 3.45]	0.064	2.79 [1.39, 5.63]	< 0.05
Shade over habitat				
None	1		1	
Partial	0.94 [0.55, 1.61]	0.818	1.13 [0.64, 2.00]	0.675
Water depth				
Less than 50 cm	1		1	
More than 50 cm	1.59[1.07, 2.38]	0.02	0.94 [0.56, 1.59]	0.824
Water type				
Temporary	1		1	
Permanent	1.18 [0.76, 1.82]	0.456	0.77 [0.44, 1.33]	0.343
Environment around habitat				
Cultivated field	1		1	
Scrub	1.48 [0.94, 2.31]	0.089	1.30 [0.80, 2.11]	0. 282
Water colour				
Turbid	1		1	
Clear	0.99 [0.58, 1.71]	0.975	0.77 [0.46, 1.31]	0. 338
Distance from houses				
Less than 100	1			
More than 100 M	1.28 [0.79, 2.06]	0.32	1.02 [0.60, 1.75]	0.937

Table 6: Univariate and multivariate regression analysis of different aquatic habitat characteristics and their association with the abundance of predators

Aquatic habitat	Univariate analysis analysis		Multivariate	
	RR (95% LC, UC)	P-values	RR (95% LC, UC)	P-values
Habitat size				
Less than 100 M	1		1	
Greater than 100M	2.92 [1.72, 4.96]	<0.05	3.52 [1.90, 6.53]	< 0.05
Vegetation type				
None	1			
Emergent	0.75 [0.37, 1.50]	0.409	1.17 [0.53, 2.56]	0.798
Submerged	0.42 [0.07, 2.54]	0.346	0.43 [0.08, 2.27]	0.323
Water Movement				
Stagnant	1		1	
Slow	2.09 [1.22, 3.58]	<0.05	0.98 [0.52, 1.87]	0.961
Fast	2.91 [1.39, 6.13]	<0.05	2.25 [0.94, 5.39]	< 0.05
Shade over habitat				
None	1		1	
Partial	1.25 [0.64, 2.43]	0.508	1.66 [0.77, 3.56]	0.192
Water depth				
Less than 50 cm	1		1	
More than 50 cm	2.03 [1.17, 3.51]	<0.05	1.12 [0.58, 2.18]	0.727
Water type				
Temporary	1		1	
Permanent	2.64 [1.48, 4.69]	<0.05	2.89 [0.99, 4.41]	<0.05
Environment around habitat				
Cultivated field	1		1	
scrub	1.21 [0.71, 2.06]	0.477	1.06 [0.57, 1.98]	0.851
Water colour				
Coloured	1		1	
Clear	1.44 [0.56, 3.67]	0.447	0.86 [0.44, 1.68]	0.651
Distance from home				
Less than 100 M	1			
More than 100 M	1.164[0.64, 2.12]	0.620	0.72[0.35, 1.46]	0.364

Table 7: Mean and range values of water physicochemical parameters in the aquatic habitats

Water characteristics	Mean (Range)
pH	6.3 [5.70-7.82]
Temperature (^{0}C)	28.0[23.3-36.]
TDS (ppm)	126.8[23.0-395.0]
EC (µS/cm)	253.7[40.1-619.0]
DO (mg/L)	6.2[1.12-16.56]

Table 8: **Univariate and multivariate analysis of associations between water physicochemical parameters and the abundance of *Anopheles funestus* larvae**

Water characteristics	Univariate analysis		Multivariate analysis	
	RR (95% LC, UC)	P-values	RR (95% LC, UC)	P-value
pH	0.92 [0.67,1.26]	0.620	0.91 [0.68,1.23]	0.542
Temperature (^{0}C)	0.89 [0.70, 1.14]	0.372	0.86 [0.68, 1.10]	0.231
TDS (ppm)	1.01 [0.74, 1.36]	0.910	1.01 [0.72, 1.42]	0.956
EC (µS/cm)	1.05 [0.77, 1.43]	0.770	0.96 [0.66, 1.40]	0.811
DO (mg/L)	1.43 [1.08, 1.88]	0.010	1.47 [1.11, 1.94]	< 0.05

Table 9: **Univariate and multivariate analysis of associations between water physicochemical parameters and the abundance of predators in *Anopheles funestus* aquatic habitats**

Water characteristics	Univariate analysis		Multivariate analysis	
	RR (95% LC, UC)	P-values	RR (95% LC, UC)	P-value
pH	1.00 [0.74, 1.39]	0.981	1.17 [0.84, 1.64]	0.356
Temperature (^{0}C)	0.85 [0.59, 1.23]	0.390	0.84 [0.57, 1.24]	0.381
TDS (ppm)	1.31 [0.99, 1.74]	0.056	1.44 [0.98, 2.13]	0.064
EC (µS/cm)	1.17 [0.86, 1.58]	0.312	0.83 [0.53, 1.31]	0.432
DO (mg/L)	1.25 [0.93, 1.68]	0.142	1.31 [0.93, 1.85]	1.118

Table 10: **Univariate and multivariate regression analysis of different aquatic predators and their association with the abundance of *Anopheles funestus* larvae**

Aquatic predators	Univariate analysis		Multivariate analysis	
	RR (95% LC, UC)	P-values	RR (95% LC, UC)	P-value
Aeshnidae	1.007 [0.984, 1.031]	0.524	1.014 [0.989,1.039]	0.268
Coenagrionidae	1.008 [1.005, 1.011]	<0.05	1.008 [1.006,1.011]	<0.05
Dytiscidae	1.043 [1.007, 1.078]	<0.05	1.035 [1.006,1.065]	<0.05
Notonectidae	0.998 [0.990, 1.006]	0.685	0.988 [0.978,0.998]	<0.05
Corixidae	0.994 [0.984, 1.005]	0.274	0.990 [0,984,0.997]	<0.05
Belomastidae	0.99 [0.921, 1.069]	0.794	0.973 [0.920,1.028]	0.327

4.1.2 Impact of Predators on the Larval and Adult Density in a Semi-Field System

(i) Preliminary Evaluation within 24 Hours

A preliminary evaluation was performed in order to determine the larval instar stage that would be used in an experiment. Out of the six identified common predators found in the *Anopheles funestus* aquatic habitats during the cross-section study, only three predator types were found to be available during the experiment. As a result, three predators namely, Aeshnidae, Coenagrionidae, and Notonectidae were used in a semi-field experiment to assess their impact on larval and adult density. These three predators were assessed first for their preference and efficacy on all larval instars. In comparison to other larval instars, third larval instars were mostly eaten by all predators after 24 hours of evaluation. Therefore, instar three larvae used in all experiments.

(ii) Impact of Predators on the Larvae Density

The predation impact of the three predator's species varied significant in both treatment and control (P<0.001, Table 11, Fig. 6). Moreover, the absolute proportions of alive mosquito larvae were also varied depending on the concentration/ratio of predators in the artificial habitats. For Notonectidae and Coenagrionidae predator families, proportion of larvae alive was high in habitats with small number of these predators (*P*<0.001, Table 12, Fig. 7). Contrarily, Aeshnidae group, the larvae alive were high in the aquatic habitats with high predators and low in habitats with low predators (*P*<0.001, Table 12, Fig. 7).

Table 11: The efficacy of different predators in reducing the survival of *Anopheles funestus* larvae in the semi-field experiment settings

Predator	Absolute proportion [95%CI] *	OR [95% C]	Relative reduction (%)	P-values
Control	47.85	1		
Notonectidae	11.96	0.15 [0.14, 0.16]	75.01	<0.001
Coenagrionidae	9.29	0.11 [0.10, 0.12]	80.59	<0.001
Aeshnidae	16.26	0.21 [0.20, 0.23]	66.02	<0.001

*Absolute proportions as estimated from Generalized linear mixed effect model

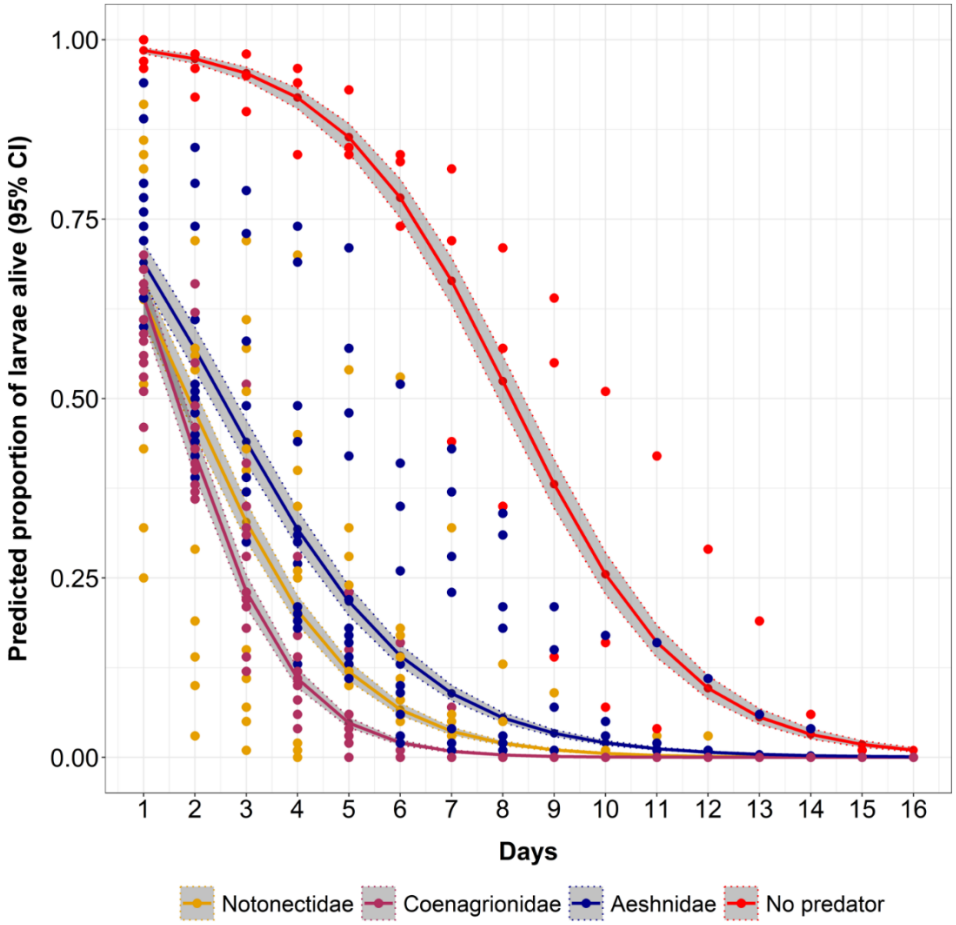

Figure 6: Predicted proportion of *Anopheles funestus* larvae alive as estimated by generalized linear mixed effect model. The coloured lines represent different predators used, dots are observed values, shaded areas are the 95% confidence intervals

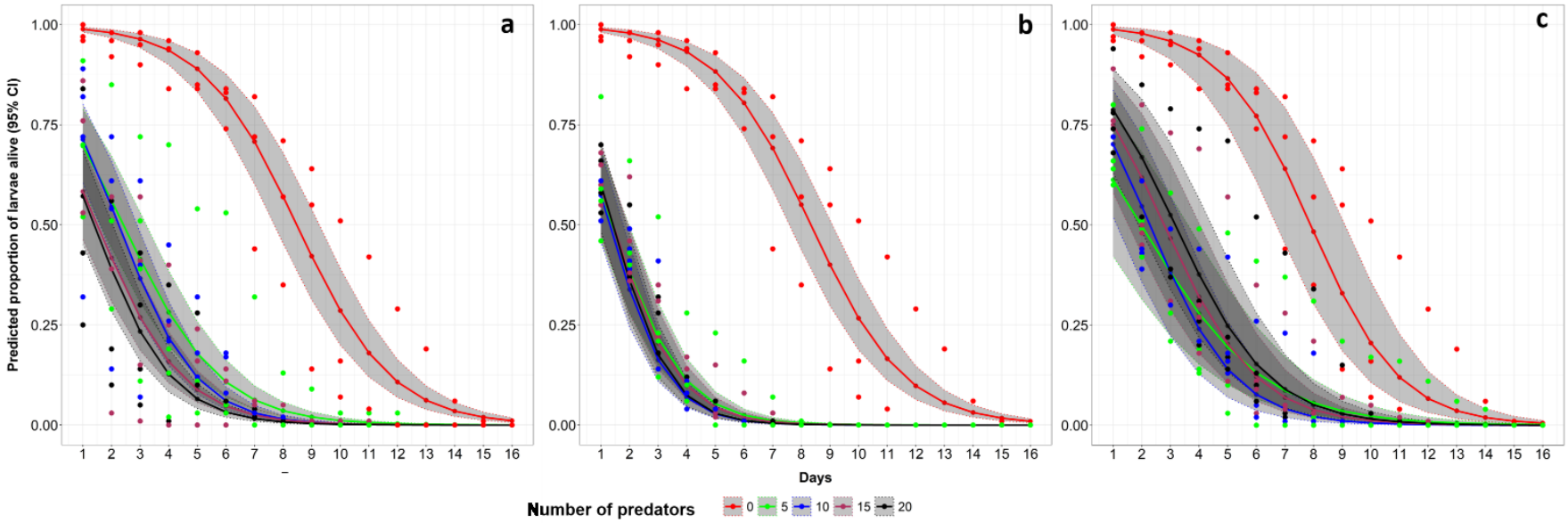

Figure 7: **Predicted proportion of larvae alive in different predator families (a) Notonectidae (b) Coenagrionidae and (c) Aeshnidae predators. The coloured lines represent different number of predators used, dots are the observed values and shaded areas are the 95% confidence intervals**

Table 12: Odds ratio, absolute proportion and their corresponding 95% confidence intervals showing the proportion of larvae alive when exposed to different number of predators

Predator	Number of predators	Absolute proportion [95% CI] *	OR [95% CI]	P-values
	0	50.66 [43.75, 57.53]	1	
	5	14.80 [11.61, 18.67]	0.17 [0.15, 0.19]	<0.001
Notonectidae	10	12.86 [10.03, 16.35]	0.14 [0.13, 0.16]	<0.001
	15	10.06 [7.78, 12.92]	0.11 [0.10, 0.12]	<0.001
	20	9.03 [6.96, 11.65]	0.10 [0.09, 0.11]	<0.001
Coenagrionidae	0	48.87 [44.25, 53.5]	1	
	5	9.22 [7.70, 11.0]	0.11 [0.09, 0.12]	<0.001
	10	7.86 [6.53, 9.43]	0.09 [0.07, 010]	<0.001
	15	8.91 [7.44, 10.63]	0.10 [0.09, 0.11]	<0.001
	20	8.29 [6.90, 9.94]	0.09 [0.08, 0.11]	<0.001
Aeshnidae	0	45.18 [35.20, 55.55]	1	
	5	14.57 [10.10, 20.58]	0.21 [0.19, 0.23]	<0.001
	10	13.22 [9.11, 18.80]	0.18 [0.17, 0.20]	<0.001
	15	16.04 [11.18, 22.48]	0.23 [0.21, 0.25]	<0.001
	20	18.04 [12.67, 25.04]	0.27 [0.24, 0.29]	<0.001

*Absolute proportions as estimated from Generalized linear mixed effect mode

(iii) Impact of Predators on the Adult's Density

There were significant different on the emerged mosquitoes between the treatment and the control group (P<0.001, Table 13, Fig. 8). Moreover, the proportion of mosquitoes emerged in Coenagrionidae predator family were significantly very low compared to those emerged from the other predators (P<0.001, Table 13, Fig. 8). Additionally, the effect of number predators on the adult density showed in (Fig. 9 & 10).

Table 13: The efficacy of different predators in reducing the emergence of *Anopheles funestus* adults in the semi-field experiment settings

Predator	Absolute proportion [95%CI] *	OR [95% CI]	P-values
Control	4.20	1	
Notonectidae	0.27	0.06 [0.05, 0.08]	<0.001
Coenagrionidae	0.04	0.01 [0.0, 0.02]	<0.001
Aeshnidae	0.27	0.06 [0.05, 0.08]	<0.001

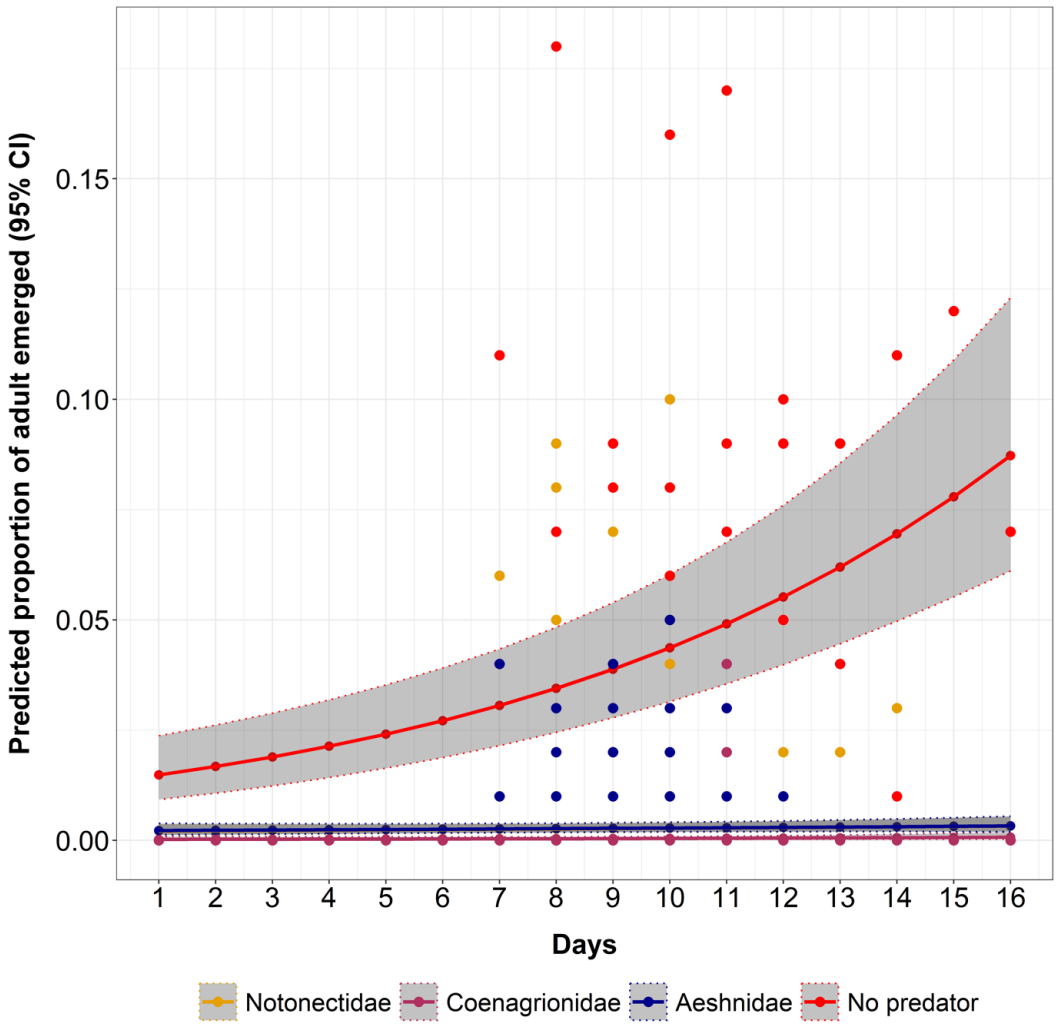

Figure 8: **Predicted proportion of mosquito larvae emerged in all in different predator families: Dots are observed values, shaded areas are the 95% confidence intervals**

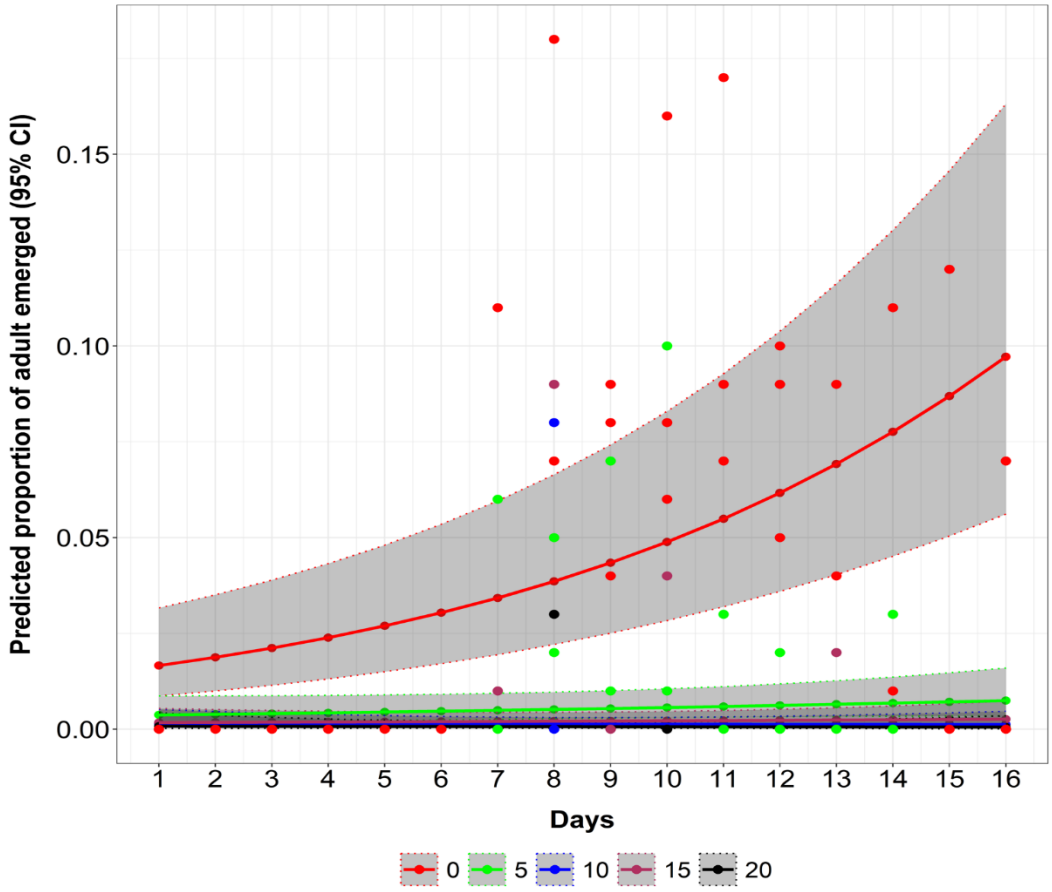

Figure 9: **Predicted proportion of mosquito larvae emerged in different number of Notonectidae predators. The coloured lines represent different number of predators used, dots are the observed values and shaded areas are the 95% confidence intervals**

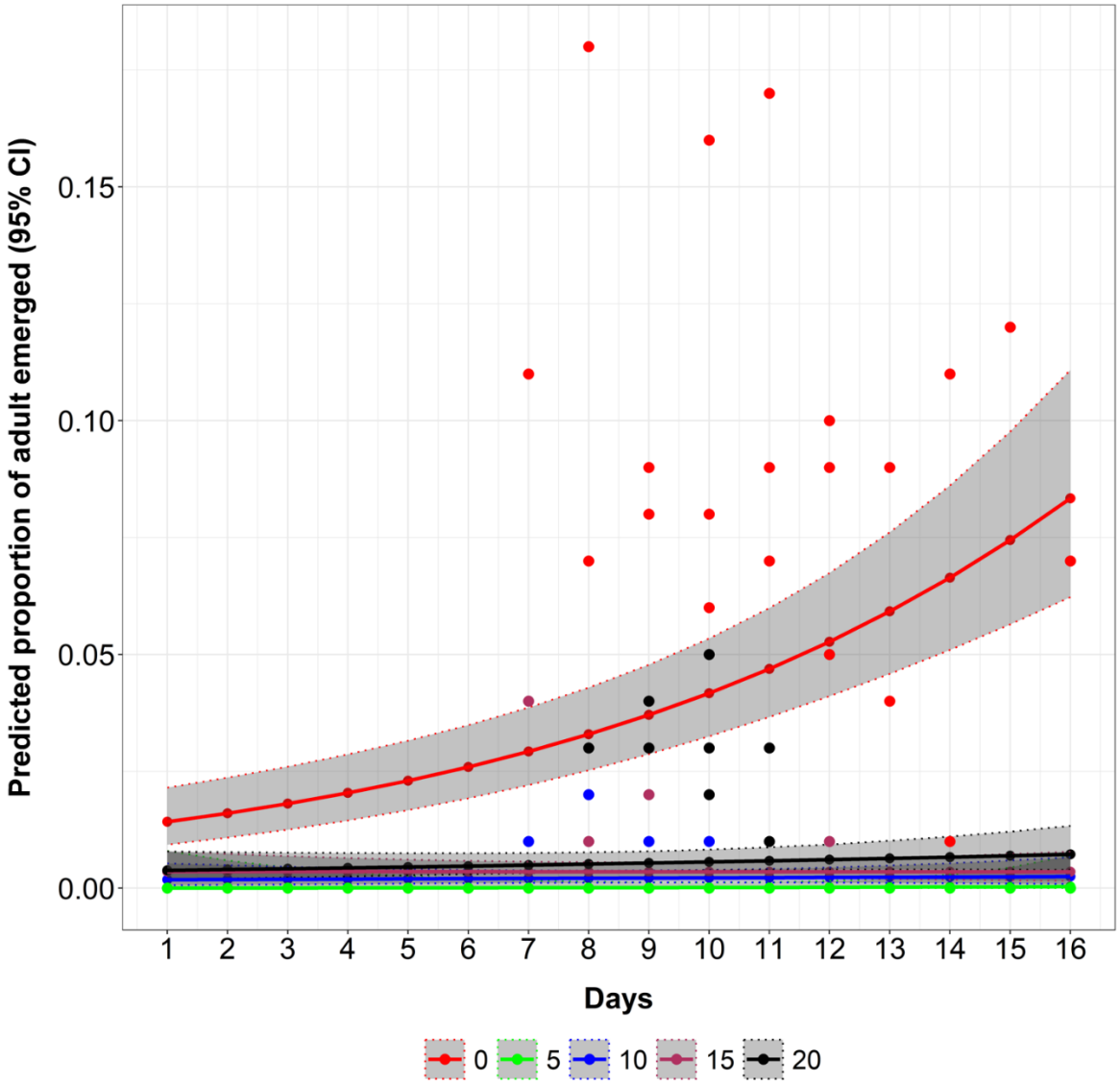

Figure 10: **Predicted proportion of mosquito larvae emerged in different number of Aeshnidae predators. The coloured lines represent different number of predators used, dots are the observed values and shaded areas are the 95% confidence intervals**

4.1.3 Impact of Predators on the Fitness Parameters of the Mosquitoes (Wing Size, Survival and the Fecundity)

(i) Survival Time of Emerged Mosquitoes

Cox regression analysis showed a significant difference between the survival of mosquitoes emerged in the control group and all that emerged in the treatment groups (i.e., their larvae were exposed to Coenagrionidae, Notonectidae and Aeshnidae).

The analysis showed that *Anopheles funestus* mosquitoes emerged from Coenagrionidae were seventeen as likely to die earlier [HR=17.31 (95%: 6.24, 48.02), $P<0.001$] comparing to the control group. Those exposed to Notonectidae and Aeshnidae were 2.82 [HR=2.82(95%CI: 1.70, 4.66), $P<0.001$] and 1.88 [HR=1.88 (1.19, 2.97), $P<0.001$] more likely to die compared to the control group respectively (Table 14, Fig. 11). In addition, there was no significant different between the survival of male and female mosquitoes emerged from different predator type ($P>0.05$, Table 15, Fig. 12 & 13). Overall, surviving mosquitoes in the control group took the longest period, median of 12 (95% CI: 11-15) days while those exposed to predators took the shortest period below the median of 10 days (P-value <0.001, Table 14).

Table 14: Hazard ratios (HR) and median survival days of adult *Anopheles funestus* emerged from different predator types and their corresponding 95% CI and P-values

Predator	Median [IQR]	HR [95% CI]	P-values
Control	12 [11, 15]	1	
Notonectidae	7 [5, 10]	2.82 [1.70, 4.66]	<0.001
Coenagrionidae	2 [1, 2]	17.31 [6.24,48.02]	<0.001
Aeshinidae	8 [5, 12]	1.88 [1.19, 2.97]	<0.001

Table 15: Hazard ratios (HR) and median survival days of adult male and female *Anopheles funestus* emerged from different predator types and their corresponding 95% CI and P-values

Predator	Sex	Median [IQR]	HR [95% CI]	P-values
Notonectidae	Females	7 [4, 10]	1	0.683
	Males	7 [4, 11]	1.18 [0.53,2.64]	
Coenagrionidae	Females	2 [1, 2]	1	0.657
	Males	2 [1, 2]	0.67 [0.11,3.99]	
Aeshnidae	Females	8 [5, 13]	1	0.353
	Males	7.5 [4, 13]	1.42 [0.68,2.97]	

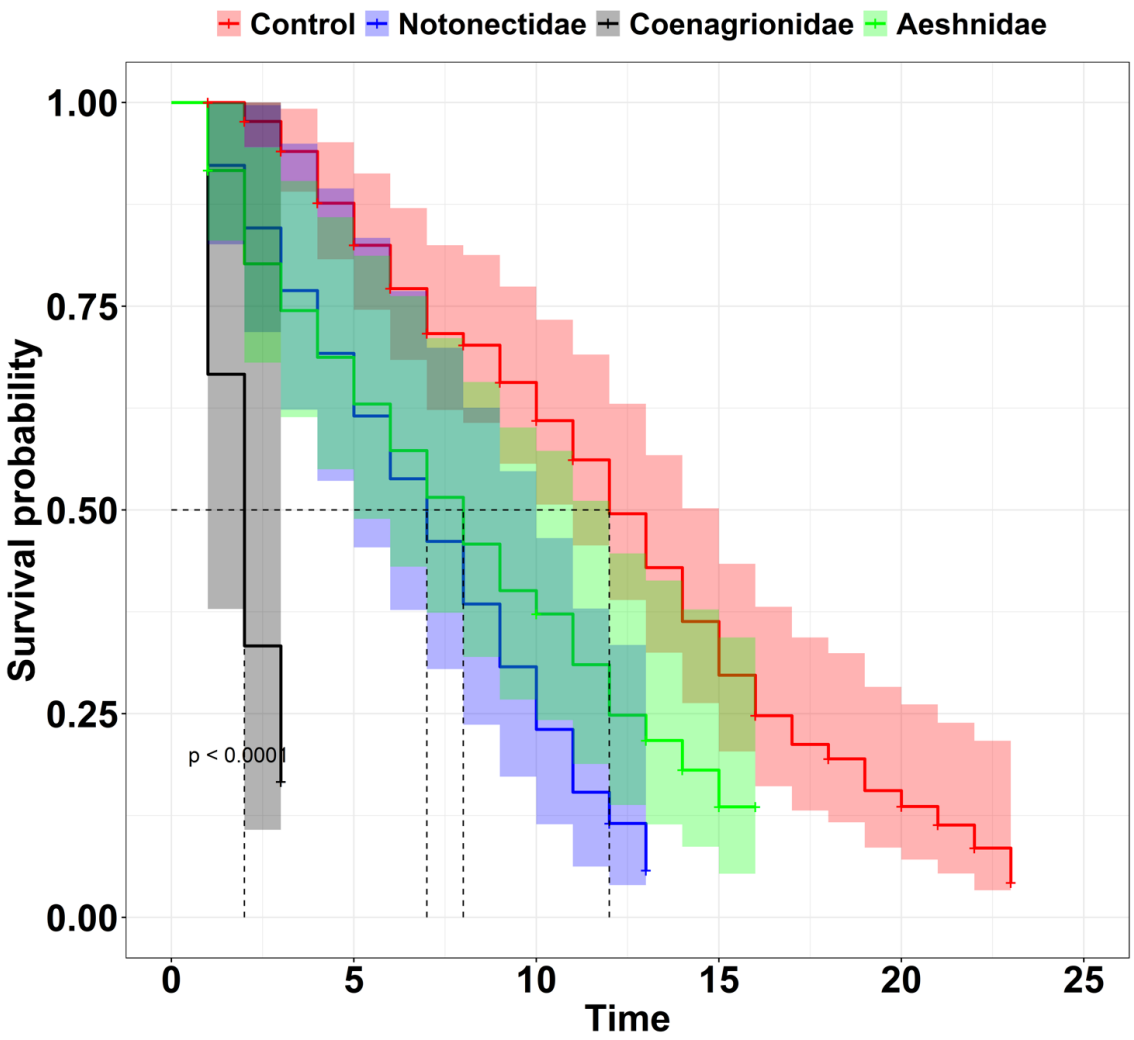

Figure 11: **Survival of *Anopheles funestus* mosquitoes from the different predator families. Full lines represent the survival function as estimated from fitting the Kaplan Meir survival model and shaded area with different colour express 95% CI. Dotted grey horizontal and vertical lines show the median survival days of mosquitoes at 50% probability**

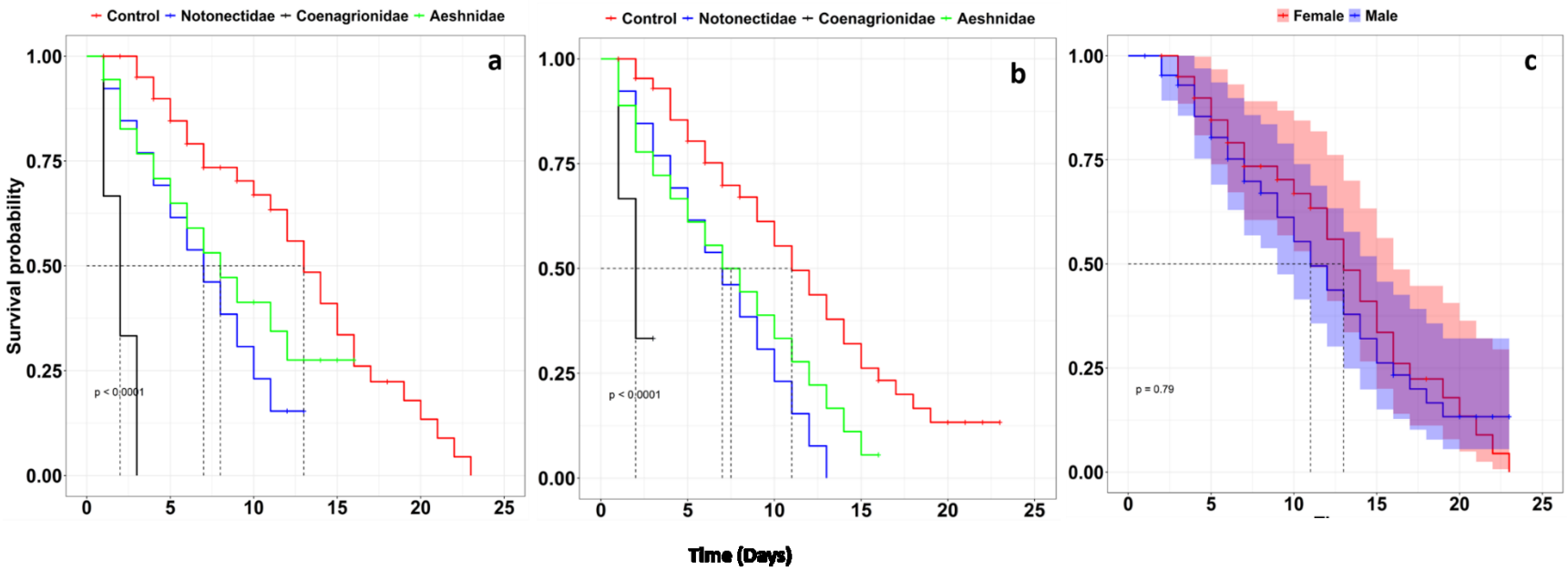

Figure 12: **Survival of (a) Female *Anopheles funestus* (b) Male *Anopheles funestus* and (c) Both female and Male mosquitoes emerged on control group. Full lines represent the survival function as estimated from fitting the Kaplan Meir survival model and shaded area express 95% CI. Dotted grey horizontal and vertical lines show the median survival days of mosquitoes at 50% survival probability**

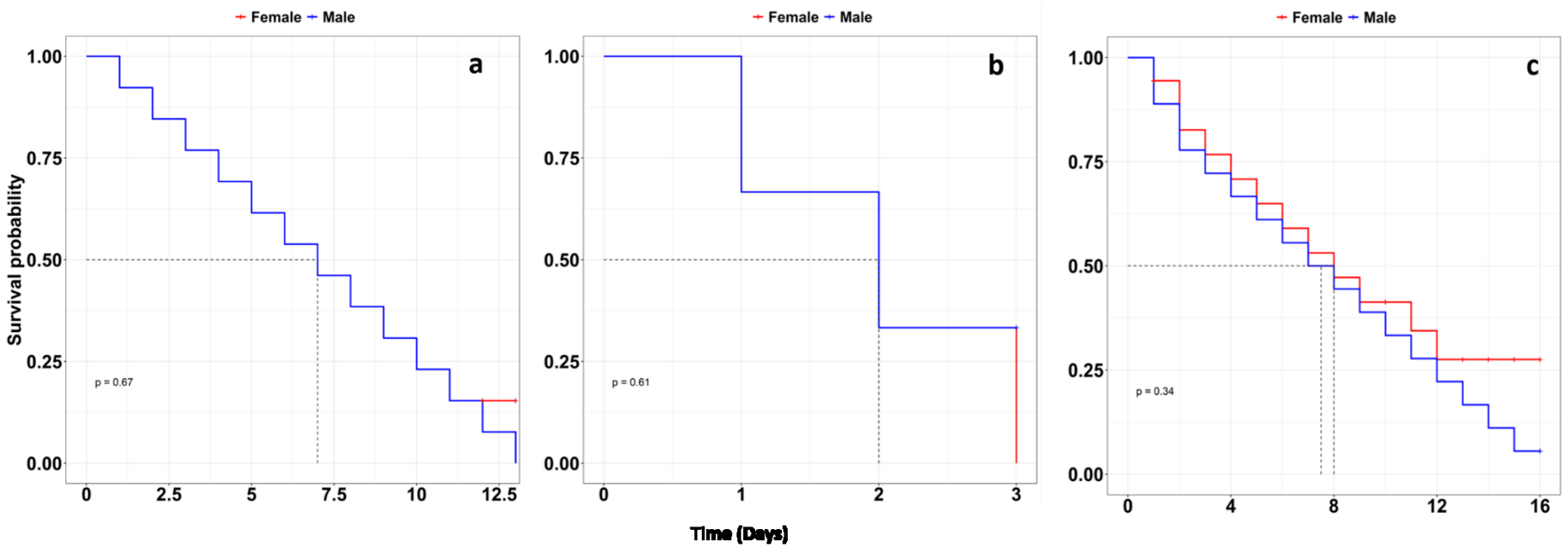

Figure 13: **Survival of male and female *Anopheles funestus* adults emerged from (a) Notonectidae (b) Coenagrionidae and (c) Aeshnidae. Full lines represent the survival function as estimated from fitting the Kaplan Meir survival model and shaded area express 95% CI. Dotted grey horizontal and vertical lines shows the median survival days of 50% survival probability**

(ii) Mosquito Wing Lengths (Size)

A Tukey's post hoc test showed that the *Anopheles funestus* mean wings size varied significantly between the groups i.e., treatment (with different predators' families) and the control ($P< 0.05$, Table 16 & Fig. 14, 15), while there was no difference on wing size in mosquitoes emerged from Notonectidae, Coenagrionidae and Aeshnidae ($P>0.05$, Fig. 14,15).

Table 16: **Mean wing sizes of females *Anopheles funestus* after being exposed to different predators during larvae stages**

Predator type	Predicted Mean [2se] *
Control	2.779 [0.0127]
Notonectidae	2.433 [0.0214]
Coenagrionidae	2.394 [0.0313]
Aeshinidae	2.421 [0.0202]

*Mean values estimated from Generalized linear mixed models

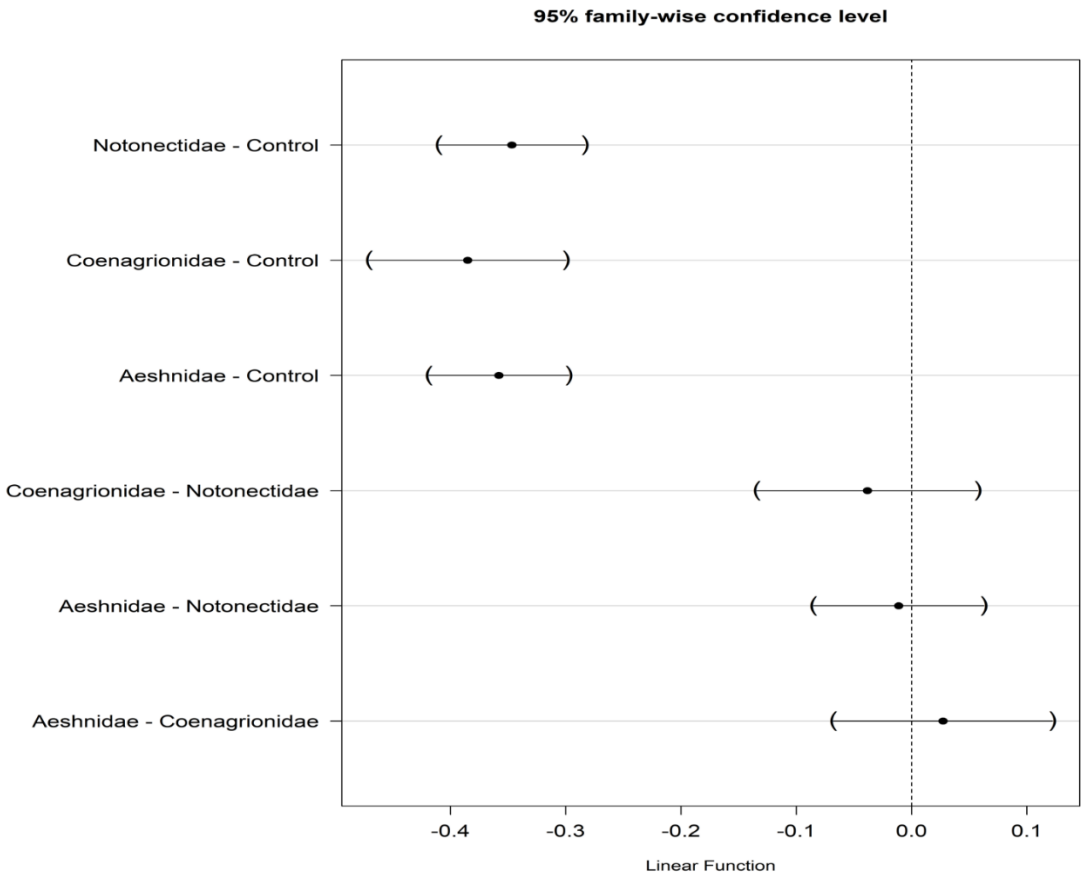

Figure 14: A Tukey's post hoc test showing the difference in mean wing size of *Anopheles funestus* adults mosquitoes emerged from different predator type and the control group

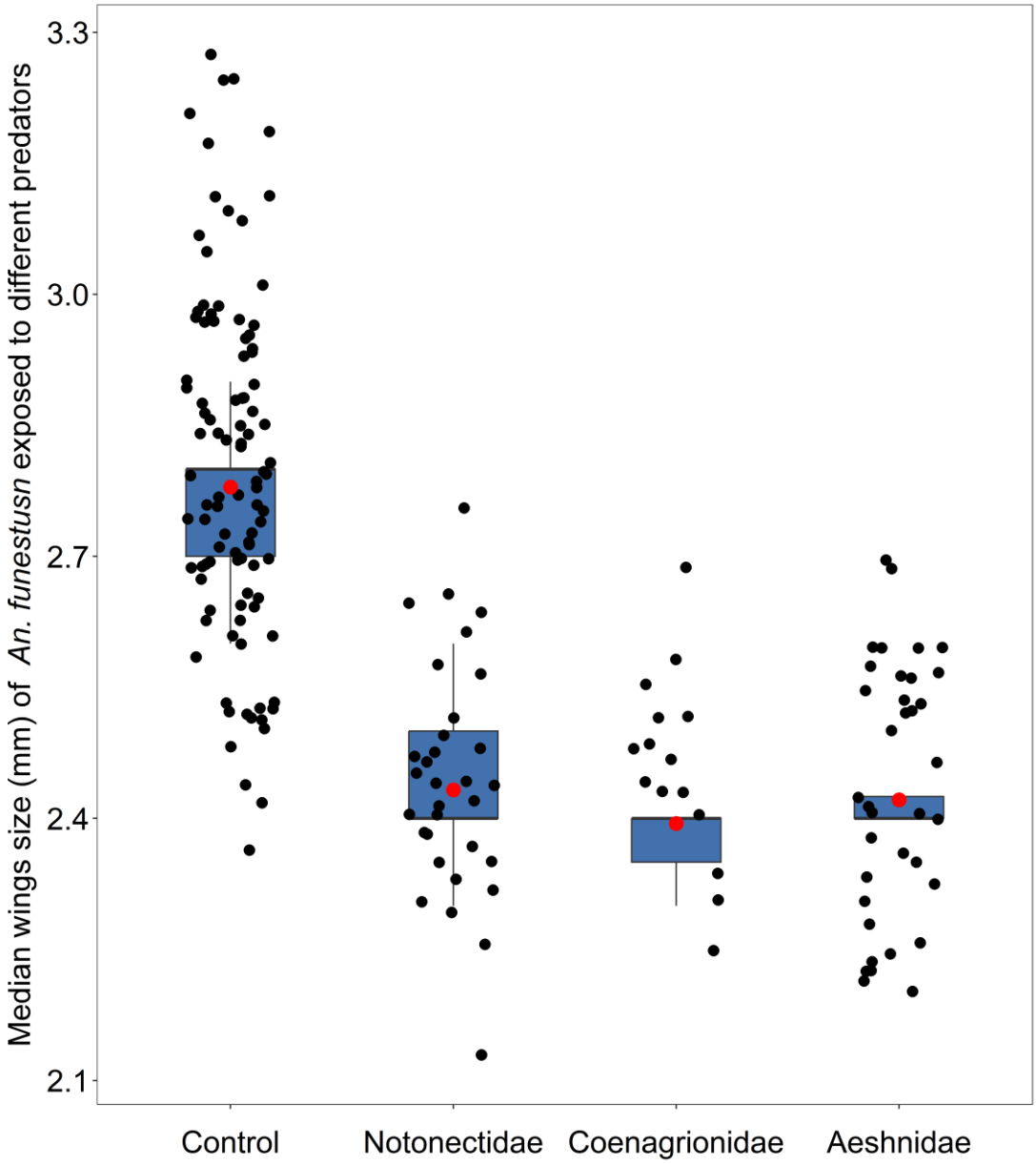

Figure 15 : Median wing size of adult female *Anopheles funestus* emerged from different predator type

4.2 Discussion

Ecological interactions such as predation and competition are key drivers of population size of numerous organisms (Arribas *et al*., 2018). In the context of mosquito borne diseases, predators play an important role in regulating the diseases transmitting mosquitoes directly through feeding on mosquito larvae or indirectly through compromising mosquito fecundity, growth rate and growth trajectories (Arribas *et al*., 2018; Claessen *et al*., 2002). Also, they regulate *Anopheles* populations naturally through predation, parasitism and competition (Walker & Lynch, 2007), the use of aquatic predators represents a potentially simple and practical biological technology for the control of disease transmitting mosquitoes (Kweka *et al*., 2011). Biological control methods,

including the use of naturally occurring predators, have been utilised for vector control in many parts of the world (Benelli *et al*., 2016; Eba *et al*., 2021; Kamareddine, 2012).

The present study was undertaken to assess the predation efficacy of *Anopheles funestus* larvae by aquatic predators in rural southern eastern Tanzania. In selected villages, eight different families of predators co-existing with *Anopheles funestus* group were identified, six of which, namely; Coenagrionidae, Corixidae, Notonectidae, Aeshnidae, Amphibians, and Dytiscidae were common in all habitat types (Table 2). Similarly, previous studies confirmed different predominant family Coenagrionidae (Dida *et al*., 2015), Dytiscidae (Dida *et al*., 2015), and Notonectidae (Chesson, 1984) in mosquitoes larval habitats.

In addition, Notonectidae shown to have direct or indirect effects on mosquito larvae population (Gilbert & Burns, 1999). Current study found consistently, high mean number of Coenagrionidae family in all habitats type as compare to other predator families (Table 2). This implies that the characteristics of the surveyed aquatic habitats were favouring the survival and growth of these predators, hence high abundance. Variation and abundance of different predators across different aquatic habitats were strongly associated with some physical characteristics of the habitat. High abundance of predators was generally observed in permanent habitats with fast moving water and larger than $100m^2$, e.g. brick or sand pits, man-made wells, river streams and swamps, similar to observations from other settings (Banerjee *et al*., 2010; Collinson *et al*., 1995; Diabaté *et al*., 2008; Onen *et al*., 2021; Ong'Wen *et al*., 2020; Williams *et al*., 2004). Such permanent aquatic habitats contain favourable amounts of both decomposed organic and inorganic matter which serve as food for predators, and these habitats allow colonization of the predators than temporal and simple structural habitats (Carlson *et al*., 2004).

Interestingly, aquatic habitats larger than 100 m^2 with fast moving water were positively associated with the abundance of *Anopheles funestus* group larvae. Aquatic habitats with submerged vegetation were negatively associated with the abundance of *Anopheles funestus* group larvae (Table 5). Previous studies have described a positive association between aquatic habitats with emergent vegetation and abundance of *Anopheles funestus* group larvae (Mwangangi *et al*., 2007; Nambunga *et al*., 2020), but not a negative association between abundance and habitats with submerged vegetation. This may be due to the season in which sampling was conducted might have an influence on the nature of the vegetation in the aquatic habitats and movement of water.

With the exception of dissolved oxygen, there was no association between other water physicochemical parameters and *Anopheles funestus* larvae abundance. On the other hand,

predator abundance was not impacted by any of the measured physicochemical parameters. Previous study shows that dissolved oxygen is positively associated with the abundance of *Anopheles funestus* group larvae (Kenawy *et al*., 2013). This is may be due to the preference of *Anopheles funestus* larvae to breed in fresh and clear water, which contains high levels of dissolved oxygen.

The current results are in line with the findings reported by Bashar *et al.* (2016), which indicated that dissolved oxygen is the preeminent predictor for the abundance of *Anopheles* mosquito larvae in aquatic habitat. Several factors, such as physical, chemical, biological and microbiological processes influence the levels of dissolved oxygen concentration in water, such that low dissolved oxygen concentrations, < 3 mg/L in fresh water indicate high level of pollution (Dida *et al*., 2015). In this study the mean of dissolved oxygen was 6.2 mg/L and ranges from 1.12-16.56 mg/L (Table 7), this indicates that most of these aquatic habitats contained the highest amount of dissolved oxygen and aeration which favoured the abundance of the *Anopheles funestus* group larvae and predators.

Water pH is one of the important factors for aquatic organisms (Clark *et al*., 2007). It can limit the abundance and distribution of aquatic organisms because it is directly related to their cellular functions and growth and development as well as their survival (Clark *et al*., 2004; Clark *et al*., 2007). Mosquitoes can tolerate extremely high levels of water pH and the current study shows that pH was not apparently associated with the abundance of either *Anopheles funestus* group larvae or predators in the aquatic habitats (Table 8 & 9). This correlates with Akeju *et al.* (2022), Obi *et al.* (2019), Chaiphongpachara *et al.* (2018) and Dida *et al.* (2015) which suggests that *Anopheles funestus* group larvae and predators are able to tolerate a wide range of pH in different environments. In addition, the current study shows that, the range of pH in the aquatic habitats was 5.70 to 7.82 (Table 7). These results are in line with the previous findings which shows the association of *Anopheles* larvae and aquatic insects including predators in a wide range of pH concentration (Dida *et al*., 2015; Garba & Olayemi, 2015). Both mosquito larvae and aquatic insects including predators have the mechanisms that enable them to inhabit such environments (Clark *et al*., 2007).

Temperature is an important factor mediating predators and mosquito larvae interactions (Johansson & Brodin, 2003). For example, it affects the ecology, physiology, metabolic processes and overall fitness of organisms (Gillooly *et al*., 2001). The interaction between predators and mosquitoes as well as their behaviour performance in the aquatic habitats is mediated by temperature, because temperature plays an essential role as a regulatory mechanism that drives

both physiological and biochemical activities (Teoh *et al*., 2010). Both *Anopheles funestus* group larvae and predators share the same aquatic habitats with temperature ranged from 23.3-36.4ºC (Table 7). This shows that *Anopheles funestus* and predators preferred warm conditions for their survival, development and colonization. The findings of the current study are in line with findings by Dida *et al.* (2015) which reported that both predators and prey preferred temperatures above 18°C while above 25°C had the highest number of predators and prey.

Temperature was not significantly associated with the abundance of *Anopheles funestus* group larvae and predators in the aquatic habitats which correlates with previous findings Nambunga *et al.* (2020), however, some studies reported contrary findings showing positive association between *Anopheles funestus* larvae and by temperature (Akeju *et al*., 2022; Bashar *et al*., 2016; Getachew *et al*., 2020; Kenawy *et al*., 2013). Most studies have mainly focused on the impacts of terrestrial temperature on mosquitoes but a limited number of studies focussed on aquatic habitats in the context of thermal tolerance, particularly for vector mosquitoes and their predators. This necessitates further investigations across seasons.

While studies done elsewhere yielded evidence that electrical conductivity is positively associated with the abundance of *Anopheles funestus* larvae (Akeju *et al*., 2022; Getachew *et al*., 2020). Another study found that higher levels of electrical conductivity was due to the application of agricultural fertilisers, pesticides and herbicides (Musonda & Sichilima, 2019). However, this study did not find any significant association between electrical conductivity and abundance of both, larvae of *Anopheles funestus* group and predators in the aquatic habitats. Electrical conductivity that ranged between 40.1- 619.0 µS/cm (Table 7), shows that *Anopheles funestus* group larvae and predators can survive in a wide range of electrical conductivity. These findings corroborate the report by Dida *et al.* (2015), which suggested mosquito larvae and predators were most abundant in the aquatic habitats with electrical conductivity ranges between 162.9 µS/cm and 166 µS/cm.

Higher total dissolved solids have harmful impacts on aquatic organisms. It changes the mineral water contents, which is important for the survival of predators and mosquito larvae. Furthermore, it determines the flow of water out of an organism's cell. In this study, there was a wide range of total dissolved solids in the aquatic habitats in which it ranges between 23.0-395.0 ppm (Table 7). This variation might be the same as previously reported by another study that total dissolved solids in the aquatic habitat is highly dependent on different factors such as the use of several chemicals in the environments (like agriculture pesticides) (Amini *et al*., 2020). Also, these results correlates with Oyewole *et al.* (2009) findings, but contrary to Abai *et al.* (2016) and (Dida *et al.* (2015)

which suggested that presence of *Anopheles* mosquitoes is associated with very high total dissolved solids (1261.40 ± 1214.31 ppm) or very low (8–87 ppm), respectively.

This study revealed that various predator families share similar aquatic habitats, whereby Coenagrionidae were found in 75 habitats, Aeshnidae, in 48 habitats, Corixidae in 49 habitats, Notonectidae in 44 habitats, Dytiscidae in 37 habitats, Nepidae in 21 habitats, Amphibian in 33 habitats, Belostomatidae in 18 habitats and unidentified group in 42 habitats (Table 4). Furthermore, these results show that among 85 *Anopheles funestus* habitats, 46 were co-inhabited by *Anopheles gambiae s.l,* 23 habitats had other anopheline larvae and 60 habitats had *Culex* spp (Table 3). These findings suggest it is more likely to have other different mosquito species and organisms in the *Anopheles funestus* aquatic habitats similar to the previously studies by Nambunga *et al.* (2020) and Dida *et al.* (2015).

The association of *Anopheles funestus* group larvae and predators was varying. In particular, this study noted some predator families such as Notonectidae and Corixidae were observed in places with low abundance of *Anopheles funestus* group larvae. However, the highest number of predators and low number of mosquito larvae could also reflect the direct predation in the aquatic habitats. Coenagrionidae and Dytiscidae were observed in the area with higher abundance of *Anopheles funestus* group larvae, showing the positive association between these predators and *Anopheles funestus* group larvae. These could be due to variation in feeding preferences among each predator family. More important, further studies should be done to confirm this, because another study has shown that Coenagrionidae are not only significant predators for *Anopheles* larvae but also for *Aedes aegypti* larvae. However, the current study did not find a clear and significant association between different predator families like Aeshnidae and Belostomatidae with *Anopheles funestus* larvae group.

This study further assessed the impacts of three predators on *Anopheles funestus* life history. It was noted that all three predators have an effect on the *Anopheles funestus* larval, adult density and their fitness parameters in south-eastern Tanzania. To my knowledge this is the first documentation that assesses the impact of predators on larval density and adult density as well as on the fitness characteristics of *Anopheles funestus* in Tanzania. Among all the three evaluated predators Coenagrionidae and Notonectidae were the most efficient in predation of *Anopheles funestus* larvae than Aeshnidae predators. Similarly, other studies reported the predatory efficiency of Notonectidae on other mosquitoes species (Kweka *et al*., 2011; Ohba *et al*., 2010). Also, other study reported the direct impact of aquatic predators on the *Anopheles gambiae* larvae and the number of eggs laid by adult *Anopheles gambiae* mosquitoes (Munga *et al*., 2006).

In recent years, Aeshnidae has been evaluated in other parts of the word toward *Anopheles arabiensis* (Gouagna *et al.,* 2012) and *Aedes aegypti* (Samanmali *et al.,* 2018), and has been shown to be efficient. The current study evaluated their predatory efficacy on *Anopheles funestus* larvae and shown to be less efficient as compared to the predators evaluated with (Coenagrionidae and Notonectidae). Similar to Kweka *et al.* (2011), experiment on *Anopheles gambiae* which shows that *Aeshnidae* was inefficient compared to the Notonectidae and other predators evaluated together. This may be due to variation on eating preference and hunting mode of each predator species, though it is very difficult to know what species prefers *Anopheles funestus* over *Anopheles gambiae* because the current study did not morphologically identify these predators to species level. During the preliminary assessment, all predators have shown to consume an intermediate size of *Anopheles funestus* larvae (instar 3) than small and large size of larvae (Instar 1, 2 and 4). Similar result reported on different setting (Kesavaraju *et al*., 2007; Kesavaraju & Juliano, 2008; Kweka *et al*., 2011; Ong'Wen *et al*., 2020). This may be due to difficultness in handling this different larval instar.

Daily and overall predation rate (consumption, pupation rate, emergence and adult survival rate) in the semi field experiment varied depending on which predator families and predator densities in the artificial habitats. All three predators were evaluated based on densities. The rates of consumption varied among predators during the 24 hours of evaluation. Consumptions were higher in the habitats with higher number of predators on Coenagrionidae and Notonectidae. On the other hand, Aeshnidae consumption rate was higher in habitats with small number of these predators. This may be due to the high inter competition on habitats with higher number of Aeshnidae as compared to habitats with small number. Therefore, this may increase the rate of consumption in small number of Aeshnidae.

Among the evaluated predators, Coenagrionidae shown to have higher impact on *Anopheles funestus* larvae in term of consumption rate, emergence, survival of adult mosquitoes and wing size. The number of mosquitoes emerged were lower in Coenagrionidae as compared to the Notonectidae and Aeshnidae predators. Generally, there is a significant difference between the number of mosquitos emerged in the predators and control.

Overall, when mosquito larvae are exposed to aquatic predators their adult survival is diminished. Among all emerged mosquitoes, those that emerged from Coenagrionidae died earlier than those emerged from Notonectidae and Aeshnidae. This may be due to the reason that each predator has its own mode of action toward mosquito larvae and differences in feeding behaviour. Nevertheless, when comparing the effects of all three predators on survival of adult male and female mosquitoes

there was no significant (Table 15). Both males and females of adult *Anopheles funestus* have equal chance of being affected by predators and hence positive impact on malaria vector reduction.

One benefit of utilising biological control is that it may target mosquito species at low densities, and thus it has no impact on non-target organisms and it is simple to use in the field (Walker & Lynch, 2007). Therefore, the study suggest different human intervention should be done to favour colonization and preservation of natural predators through preservation of the natural aquatic habitats in the environments.

Despite the successfulness of this project, a number limitations were observed which includes: (a) this study did not focus on understanding the anthropogenic factors and how they might influence the abundance of predators, (b) the impact of predator on fecundity was not assessed due to small number of female mosquito emergence hence no/too few eggs laid, (c) this study also did not assess the contribution of anthropogenic activities on predation.

Further studies should morphologically identify the aquatic predators to species level using an appropriate identification key and assess the impact of the predators on the fecundity of *Anopheles funestus* mosquitoes. This could help to understand how aquatic predators are distributed in different aquatic habitats and how these predators can play a role in reducing the malaria transmission rate.

Although this does not affect our interpretation of results, a cross sectional study does not represent the variation among habitat characteristics over time including the changes in temperature and water physicochemical parameters. A longitudinal study would help capture seasonal variations between predator and prey abundance. Such study may help in the design of novel interventions focussed on this relationship.

Lastly, it is acknowledged that one of the standard methods adapted from the previous studies, which is intentionally subjecting predators to a period of starvation for the aim of avoiding early predations, may influence the observed outcome because in the natural habitats the experiment introduces an artificial condition that may influence the observed outcomes. This is because, starvation can affect the behaviour, motivation, and hunting efficiency of predators, potentially altering predation rates and outcomes compared to natural conditions. Therefore, this provides a need for another study to be conducted in a real environment to understand the effectiveness of these predators as well as community acceptability on the use of these predators.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study demonstrated the efficacy of three among six common predators on *Anopheles funestus* group larvae. All the three evaluated predators in the semi field experiment were able to consume a significant density of *Anopheles funestus* larvae and affect their fitness parameters (wing size and adult survival). These results suggest that these evaluated predators may play an important role as complementary tool in reducing *Anopheles funestus* larval population and hence contribute to the reduction of the malaria vectors in Southern eastern Tanzania.

Overall, it is clear that aquatic predators play an important role in controlling the population of *Anopheles funestus* mosquitoes, the major vector of malaria transmission in rural Tanzania. By understanding the role of these predators, effective strategies for controlling malaria vector populations could be developed and eventually accelerate malaria elimination. Additionally, for effective malaria vector control, strategies should focus on both permanent aquatic habitats and temporary/seasonal as well as micro-habitats such as ditches and some man-made wells, because these temporary and micro habitats can significantly produce high numbers of disease transmitting mosquitoes at the time and, they limit predator's colonization abilities.

5.2 Recommendations

Further research is needed to fully understand the role of predators in controlling mosquito populations in the wild, including the specific mechanisms by which they prey on mosquito larvae and the relative importance of different predator species. Such research could inform the development of more targeted, sustainable, environmentally friendly and effective mosquito control strategies. Specifically, we strongly recommend the following:

(i) A longitudinal should be done to capture seasonal variations between predator and prey abundance.

(ii) Another study should be done to assess the interaction between other common predators and the mosquito larvae as well as the impacts of all predators on the fitness parameters of adult mosquitoes including the fecundity.

(iii) Additional survey should be done to focus on understanding how the anthropogenic factors and how they might influence the abundance of predators on *Anopheles funestus* aquatic habitats.

(iv) Further studies should also, morphologically identify the aquatic predators to species level using an appropriate identification key and assess the impact of the predators on the fecundity of *Anopheles funestus* mosquitoes.

(v) Studies should be done to assess whether the choice of oviposition site by *Anopheles funestus* mosquitoes could be influenced by the presence of predators and how such information could be used to disrupt mosquito ovipositional behavior.

(vi) Another study should be done to assess the feasibility and effectiveness of these predators on the real environment as well as understanding the community perception on the use of theses predators.

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APPENDICES

Appendix1: Characterization of *Anopheles funestus* aquatic habitats

BREEDING SITE SURVEY FIELD FORM (To be filled in full at the breeding site)

Photo number: ……... Name of field worker……..……

Date:…..…….……. Time:…….……..…….…….

District:…….…….……..

Village name:…….…… Sub-village name……...……

Breeding site ID……...……

S:……E:……Elevation:…… ……..

1. Habitat Type

a) Dry (kavu) ((
b) Swamp/ Marsh (Bwawa) ((
c) Stream/ River (mkondo/mto) ((
d) Rice field (shamba la mpunga) ((
e) Stream pool (bwawa la mto) ((
f) Ground pool (bwawa) ((
g) Ditch (mfereji) ((
h) Spring-fed pool (bwawa la chemchem) ((
i) Puddle (dimbwi) ((
j) Hoof print (kwato) ((
k) Man-made construction (kisima cha kutengenezwa) ((
l) Brick or sand pit (shimo la tofali au mchanga)

2. Water colour (rangiyamaji) ((

a) Clear (masafi) ((
b) Coloured (yenyerangi) ((
c) Polluted (Machafu) ((

3. Water source (chanzo cha maji) ((

a) Rainwater (yatokanayonamvua) ((
b) Non rainy water (sioyamvua)
c) Man created (iliyotokananawatu)

4. Algalquantity(inwaterhabitat)/ (kiasi cha algae)

a) None (hakuna) ((
b) Scarce (kwauchache) ((
c) Moderate (kadirifu) ((
d) Abundant (nyingi) ((

5. Algaetype (aina ya algae) ((

a) Filamentous (asiliyakambakamba) ((
b) Green (kijani) ((
c) Blue Green (kijani blue) ((
d) Brown (brown)

6. Water movement/ mwendowamaji

a) Stagnant (isiyosogea) ((
b) Slow (taratibu) ((
c) Fast (kasi) ((

7. Shadeoverhabitat ((
(Kivulikwenyezalia) ((

a) None (hakuna) ((
b) Partial (Kiasi) ((
c) Heavy (zaidi) ((
((
((

8. Habitatsize:perimeter (Ukubwawazalia)

a) Less than 100 m (chiniya 100m)
b) More than 100 m

9. Vegetation type (in water habitat) (aina ya mimea katika zalia)

a) None (hakuna) ((
b) Submerged (ndaniyamaji) ((
c) Floating (yanaelea) ((
d) Emergent (yaliyochomozajuuyamaji) ((

10. Vegetationquantity(inwaterhabitat) ((
(kiasi cha mimeakatikazalia) ((

a) None (hakuna) ((
b) Scarce (kwauchache) ((
c) Moderate (kadirifu)
d) Abundant (nyingi)

11. Watertype (ainayamaji)

a) Permanent (mudawote) ((
b) Temporary (yamsimu) ((
((

12. Season (msimu) ((

a) Dry season (kiangazi) ((
b) Rain season (masika)

13. Environment(aroundwaterhabitat) (mazingirayaliyozungukazalia)

a) Scrub/Bush (vichaka) ((
b) Cattle grazing (malishoyamifugo) ((
c) Cultivated fields (mashambayamazao) ((

14. Waterdepth (kina cha zalia)

a) Less than 50 cm (chiniya 50 cm) ((
b) More than 50 cm (zaidiya 50 cm) ((
((

15. Distancefrom homes (umbali kutoka makazi ya watu)

a) Less than 100 m (chiniya 100m) ((
b) More than 100 m (zaidiya 100m) ((
((

Observer's comments/ maoniyamtazamaji

_____

_____

_____

PHYSIO-CHEMICAL PARAMETERS

Variable	Value	Unit
pH		
Temperature		^{0}C
Electrical Conductivity (EC)		µS/cm
Total Dissolved Solids (TDS)		ppm
Dissolved oxygen		(mg/L)/ ppm

LARVAL SAMPLING

Larvae present? …….

Anopheles funestuss.l	Y/ N
Anopheles gambiaes.l	Y/ N
Other Anopheline	Y/ N
Culex	Y/ N

Number of dips …….

Number of buckets …….

Larval and pupal density

No of dips/Buckets	Species	No of L1/2	No of L3/4	No of Pupae
1	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
2	***Anopheles funestuss.l***			

	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
3	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
4	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
5	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
6	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
7	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
8	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
9	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
10	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
11	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
12	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			

13	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
14	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
15	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
16	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
17	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
18	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
19	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
20	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			
Total	***Anopheles funestuss.l***			
	Anopheles gambiaes.l			
	Other Anopheline			
	Culex			

PREDATORS SAMPLING

Predators present?.....

Aeshnidae	Y / N
Coenagrionidae	Y / N
Dytiscidae	Y / N

Notonectidae	Y / N
Corixidae	Y / N
Nepidae	Y / N
Belostomatidae	Y / N
Amphibians	Y / N
Unidentified	Y / N

Number of dips / buckets	**Type of predator present**	**Number of predators**
1.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
2.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
3.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	

Number of dips / buckets	Type of predator present	Number of predators
	Amphibians	
	Unidentified	
4.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
5.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
6	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
7	Aeshnidae	
	Coenagrionidae	

Number of dips / buckets	Type of predator present	Number of predators
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
8.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
9.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
10.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	

Number of dips / buckets	Type of predator present	Number of predators
	Belostomatidae	
	Amphibians	
	Unidentified	
11.	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
12	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
13	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
	Aeshnidae	

Number of dips / buckets	Type of predator present	Number of predators
14	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
15	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
16	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
17	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	

Number of dips / buckets	Type of predator present	Number of predators
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
	Aeshnidae	
18	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
19	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	
20	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	

Number of dips / buckets	Type of predator present	Number of predators
	Unidentified	
TOTAL	Aeshnidae	
	Coenagrionidae	
	Dytiscidae	
	Notonectidae	
	Corixidae	
	Nepidae	
	Belostomatidae	
	Amphibians	
	Unidentified	

Appendix 2: Form for recording larval density

Name of investigators……………………………Replicate no…………..Experiment Day/Date………………

Habitat ID	Initial larvae	Larvae remained	Pupae	Adult

Appendix 3: Form for recording wing length habitats

Name of investigators……Replicate no……Experiment Day/Date……

Date	Habitat no	Predator species	Wing Size

Appendix 4: Form for recording survival of mosquitoes

Emerged-Adult mosquito survival evaluation

Name of investigators……Replicate no……Experiment Day/Date……

Habitat no	**Day of death after setup**	**No of mosquito died after-emerged**	
		Female	**Male**
	Day1		
	Day2		
	Day3		
	Day4		
	Day5		
	Day6		
	Day7		
	Day8		
	Day 9		
	Day10		
	Day11		
	Day12		
	Day13		
	Day13		
	Day14		
	Day15		

(i) Publication

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(ii) Poster Presentation