EFFICACY OF CLARIAS ALLUAUDI AS A BIOCONTROL AGENT FOR ANOPHELES MOSQUITOES

BY

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DECLARATION

I declare that this thesis is my original work and has not been presented for the award of a degree in any other University (see originality report in Appendix 1)

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DEDICATION

I dedicate this work to my dear parents Emmanuel Simiyu Wamukota and Jane Nanjala Wamalwa for every effort they have put in to see me grow academically.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACT   : Artemisinin-based combination therapy

cm   : centimeters

EIP   `   : Extrinsic Incubation Period

IRS   : Indoor Residual Spraying

ITNs   : Insecticide treated nets

KMFRI   : Kenya Marine and Fisheries Research Institute

LLINs   : Long Lasting Insecticidal Nets

M   : meters

NMCP   : National Malaria Control Programme

s.l   : sensu lato

s.s   : sensu stricto

US   : United States

WHO   : World Health Organization

ABSTRACT

More research is being tailored towards the biological control of malaria mosquitoes due to resistance of the mosquitoes to the insecticides in use. Larvivorous fishes that are recommended for mosquito control are introduced species, and pose an extinction danger to native flora and fauna. This study sought to evaluate the efficacy of a native catfish (*Clarias alluaudi*) in controlling malaria mosquitoes. The larvivorous efficacy was evaluated on basis of the speed at which a fish took to apprehend a mosquito larva/pupa (exposure time), selectivity index and the predatory index of the fish. *Gambusia affinis* and *Poecilia reticulata* were used as positive controls. Exposure time was measured as the time taken for a single fish to consume a mosquito larva or pupa. Selective feeding of fish was determined by offering paired combinations of *An. gambiae* and *Ae. aegypti* larvae/pupae at intraspecific and interspecific levels. The predatory index of a fish was determined by calculating the mean number of mosquito larvae/ pupae consumed per unit weight of a fish per day. The generalized linear models were used to document differences in the exposure times, feeding preferences and predatory indices among the three fish species. *Clarias alluaudi* took a significantly longer time to consume larval stages of *Anopheles gambiae* as compared to *G. affinis* and *P. reticulata* (P= 0.001). However, the fish took a significantly shorter time to apprehend pupae of *Anopheles gambiae* among the other fish species (P= 0.001). There was no significant difference in apprehension times of late instar larvae of *Aedes aegypti* among the three fish species. *Clarias alluaudi* took a significantly shorter time to consume pupae of *Ae. aegypti* (P= 0.001). In selective feeding experiments, late instar larvae of *An. gambiae* and *Ae. aegypti* were preferred over pupae irrespective of the fish species. Pupae of *An. gambiae* were also preferred over those for *Ae. aegypti*. Although *P. reticulata* had the highest predatory index among the three fish species, the fish ate the least number of prey items offered. *Clarias alluaudi* had the least predatory index despite the fish consuming the highest amount of prey items among the three fish species. Results obtained indicate that *C. alluaudi* is a potentially good larvivorous fish. Field trials using *C. alluaudi* should be carried out to affirm the impact that this fish species has on mosquito larval densities in their breeding habitats.

CHAPTER ONE: INTRODUCTION

1.1 Background

Many countries in sub-Saharan Africa continue to bear a huge burden of malaria despite rigorous efforts in controlling the disease (Drake & Lubell, 2017). Malaria is a devastating vector-borne disease transmitted by female mosquitoes of the genus *Anopheles*. A report by the World Health Organization reveals that malaria cases were approximately 228 million in 2018, with approximately 405,000 deaths occurring in the same year globally. Dejectedly, 93% of the malaria cases and 94% of the malaria deaths occurred in the sub-Saharan Africa (WHO, 2019).

The main mosquito vectors responsible for the transmission of malaria in sub-Saharan Africa belong to the *Anopheles gambiae* complex and the *Anopheles funestus* group (Gillies & Coetzee, 1987). Of the various malaria control methods used (insecticides and antimalarial drugs); vector control involving indoor residual spraying (IRS) and the use of long lasting insecticidal nets (LLINs) has reduced malaria prevalence in the malaria-endemic regions significantly (Bhatt *et al*., 2015). However, the effectiveness of the control measures in place has reduced, mainly because of the evolution of mosquitoes that are restistant to the insecticides used (Hemingway *et al*., 2016; WHO 2018; Ranson & Lissenden, 2016; Strode *et al*., 2014). As a consequence, residual malaria transmission by the resistant mosquitoes together with outdoor biting contribute to high malaria incidence and prevelence in malaria-endemic regions. World Health Organization recommends that integrated vector management (IVM) is the powerful strategy that can lead to successful control of malaria (WHO, 2009).

Natural enemies that feed on mosquito aquatic stages can play a major role in reducing transmission of mosquito-borne diseases. A number of aquatic organisms including fish (Chandra *et al*., 2008), water bugs (Shaalan *et al*., 2007), amphibians (Brodman & Dorton, 2006) and copepods (Schaper, 1999), among others, can feed on mosquito larvae. The use of aquatic vertebrates as control tools for mosquito larvae has mainly focused on the use of larvivorous fish (Griffin & Knight, 2012). Previous studies on the use of larvivorous fishes for mosquito control demonstrate that fish can significantly reduce larval densities in a wide variety of habitats in many parts of the world (Connor, 1922; Van *et al*., 2007; Walshe *et al*., 2017). More than 200 fish species have been evaluated for mosquito control in different parts of the world. However, the most widely recommended fishes for controlling mosquitoes include *Gambusia affinis* and *Poecilia reticulata* (Ghosh & Dash, 2007). These two fish

species are voracious feeders and are reported to be capable of consuming hundreds of mosquito larvae in a single day (Ghosh & Dash, 2007).

Most of the recommended larvivorous fishes have been widely introduced beyond their native regions for mosquito control (Kathleen, 2002). The main challenge with the introduction of voracious fish beyond their native scope is the extinction danger that the introduced fish pose to native biota, inclusive of native fishes (Komak & Crossland, 2000). It is therefore important to carry out rigorous comparisons of native fishes with other introduced larvivorous fishes to establish their control success. This can help in identifying appropriate local biological control agents for Afrotropical malaria mosquitoes. This study sought to evaluate the larvivorous potential of a native catfish called *Clarias alluaudi*, for the control of Afrotropical malaria mosquitoes.

1.2 Problem statement

The continuously high epidemiologic status of malaria infections in sub-Saharan Africa in the phase of increased drug resistance and absence of an effective malaria vaccine calls for a more integrated approach in controlling the disease. A vector control approach requires solid knowledge of the vector ecology and biology. The use of larvivorous fish as part of vector control management systems in Kenya has hardly been exploited. The frontline interventions for malaria control that are currently in place involve the use of insecticides against mosquito vectors and chemotherapy against *Plasmodium* parasites. The insecticides used are in the form of long lasting insecticidal nets (LLINs), which aim at preventing human populations from mosquito bites, and indoor residual spraying (IRS) which are used to kill the mosquitoes (Okumu & Moore, 2011; WHO, 2018). This strategy has reduced malaria transmission significantly in the affected regions (Fullman *et al.,* 2013; WHO, 2018; Huho *et al*., 2013). However, the heavy dependence on insecticide-based malaria control has increased the selection pressure exerted by the pesticides on malaria vectors, hence undermining their effectiveness (Ranson & Lissenden, 2016). The mosquitoes have gained the capacity to resist the action of the insecticides, hence posing severe challenges in controlling the disease. The chemicals used are also implicated in environmental pollution.

Another challenge with the use of insecticides for mosquito control lies in the misuse of bed nets as seen in many malaria endemic communities for instance in the Lake Victoria basin (Minakawa *et al*., 2008). The LLINs are used for drying small fish as well as for fishing (Minakawa *et al*., 2008). The use of IRS and LLINs is also inefficient in curbing outdoor

malaria transmission. This is because the methods only confer protection to people at risk from bites by endophagic and endophilic mosquitoes, leaving room for existence of residual pockets of malaria transmission occasioned by outdoor biting (Olanga *et al*., 2015; Pulford *et al*., 2011; Russell *et al*., 2011). High malaria prevalent areas in Kenya include those inhabited by people whose main socioeconomic activities revolve around fishing (Noor *et al*., 2009). A report by the National Malaria Control Programme (NMCP) (2016) indicated that areas bordering Lake Victoria recorded a high malaria transmission during the same year. Fishing is an activity that is done outdoor, mainly during the night. This means that malaria control strategies that target outdoor biting mosquito populations need to be developed in order to achieve a malaria free world.

The use of alternative control strategies that are ecofriendly include control using larvivorous fishes. A conceptual framework (figure 1) explaining how larvivorous fishes can affect mosquito larval densities in an aquatic environment is provided below. It illustrates the various parameters of the fish that could define the larvivorous potential of the fish. Despite the use of other fish species to control aquatic stages of mosquitoes, malaria transmission still remains high. The fish types widely used have proven to be invasive hence pose an extinction threat to native aquatic biota. Biocontrol using larvivorous fish can therefore contribute to outdoor malaria control as well as provide economic gains to local communities. Therefore, research into testing the native readily available fish for combating malaria-transmitting mosquitoes is paramount.
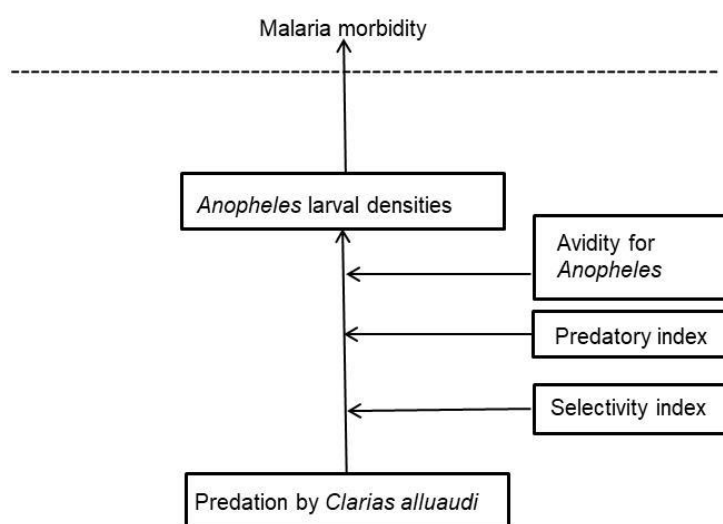
Figure 1: Conceptual framework illustrating the perceived relationship between the insectivorous catfish *Clarias alluaudi* and the population densities of *Anopheles* larvae in an aquatic environment. Boxes indicate the variables of interest, and arrows depict cause-effect relationships.

1.3 Justification and significance of the research

Malaria control strategies currently adopted target indoor biting mosquitoes yet outdoor transmission of the disease significantly contributes to malaria incidence (Githure *et al.,* 2004; Killeen *et al.,* 2016). Alternative control tools targeting outdoor biting mosquitoes therefore need to be developed. One such method is the use of larvivorous fish to control *Anopheles* larvae in breeding habitats. The catfish *Clarias alluaudi* is a good candidate for controlling mosquito larvae because it possesses several potentially desirable characteristics for this purpose. Several authors indicate that this fish species is highly insectivorous (Teugels, 1986; Froese & Pauly, 2019), *albeit* with no information about if it feeds on malaria vectors. The fish is also hardy, being able to withstand harsh environmental conditions and can stay for several hours outside water, hence the name air-breathing/ the walking catfish (Julian, 2018). Like other fishes, *C. alluaudi* are able to reproduce naturally without the need for culturing interventions, hence able to control mosquito larvae for a sustained period of time.

Clarias alluaudi is used for baited hook fishery around the Lake Victoria region (Mkumbo & Mlaponi, 2007). Iterative discussions with community health volunteers on a fishing Island called Mageta inside Lake Victoria disclosed *C. alluaudi* as one of the most efficient baits used for Nile perch fishing on the island. According to focus group discussions, the baits are customarily gathered by women who sell them to fishermen. The fishermen usually stock the baits inside fishing boats before setting out into the lake. Apparently, the boats are suitable breeding habitats for malaria vectors when stationed ashore between fishing rounds or when the boats are not in use (Mukabana *et al.,* 2019). Introduction of *C. alluaudi* into boats prior to fishing will simply turn the breeding habitats into shopping baskets, should the fish be found to be larvivorous. This in turn, can reduce the malaria incidences.

1.4 Research objectives

1.4.1 General objective

To assess the efficacy of *Clarias alluaudi* for controlling Afrotropical malaria mosquitoes

1.4.2 Specific objectives

1. To evaluate the avidity of *Clarias alluaudi* for aquatic stages of *Anopheles gambiae*

2. To determine the selective feeding patterns of *Clarias alluaudi* on aquatic stages of *Anopheles gambiae*

3. To assess the predatory efficacy of *Clarias alluaudi* on *Anopheles gambiae*

1.5 Research hypothesis

1.5.1 Null Hypotheses

Clarias alluaudi does not significantly reduce mosquito larval densities

1.5.2 Alternative Hypotheses

Clarias alluaudi significantly contributes to the reduction in mosquito larval densities.

1.5.3 Assumption made in this study

This study assumed that *Clarias alluaudi* eats up mosquito larvae, and that the fish can effectively control Afrotropical malaria mosquitoes.

CHAPTER TWO: LITERATURE REVIEW

Malaria is a major public health problem globally. Sub-Saharan Africa bears the highest burden of the disease. According to the world malaria report 2019, an estimated 228 million cases of malaria occurred worldwide in 2018. Two hundred and thirteen million (insert %) of the cases occurred in the WHO African Region (WHO, 2019). In Kenya, the disease is a major health concern, accounting for approximately 18% of all outpatient visits to health facilities (NMCP, 2016). A report by the Ministry of health in 2014 revealed that the disease is still a major cause of morbidity and mortality, with more than 70% of the Kenyan population being at risk of infection by the disease (NMCP, 2015). The risk diversity of the disease in Kenya is not homogenous. Four epidemiological zones of malaria namely low risk malaria areas, seasonal transmission malaria areas, endemic areas and highland epidemic prone areas (figure 2) are acknowledged. The risk diversity is mainly determined by malaria prevelence, temperature, rainfall patterns, and altitude (NMCP, 2016). The coastal region and the western parts of Kenya, particularly the areas within the Lake Victoria basin, represent the main malaria endemic regions in Kenya (Okara *et al*., 2010).

Vector control represents one of the major preventative interventions in malaria control. Many documented eradication successes for malaria have been linked to vector control (Patterson, 1979, Karunamoorthi, 2011). The current vector control strategy relies on the use of insecticides in form of LLINs and IRS, which has seen big gains in reducing the incidence of the disease (WHO, 2014; Steketee & Campbell, 2010). Despite the considerable successes in decimating malaria vectors, the disease still remains a major public health concern in many countries. The continued malaria incidences and deaths is attributed to insecticide resistance (Ranson & Lissenden, 2016), changes in biting behavior of malaria vectors (Muirhead-Thomson, 1960; Yohannes & Boelee, 2012; Sougoufara *et al*., 2017), human land use (Lindblade *et al.,* 2000), climate change (Martens *et al.,* 1995), and continuous resistance of the *Plasmodium* parasites towards chemotherapeutic drugs (Blasco *et al.,* 2017; Haldar *et al*., 2018), among others. These control methods mainly aim at curbing indoor transmission of the disease. The development of alternatives to LLINs and IRS, which will lead to the abatement of the outdoor populations of malaria vectors is critical. Biological control of the developmental stages of the malaria vectors in aquatic environments using larvivorous fishes is a strategy that is regaining interest. In the context of this work, larvivorous fishes are construed as those that feed on immature stages of insects found in aquatic environments. The use of larvivorous fishes for mosquito control is an old practice that has existed since the pre-DDT era (Walker & Lynch, 2007).
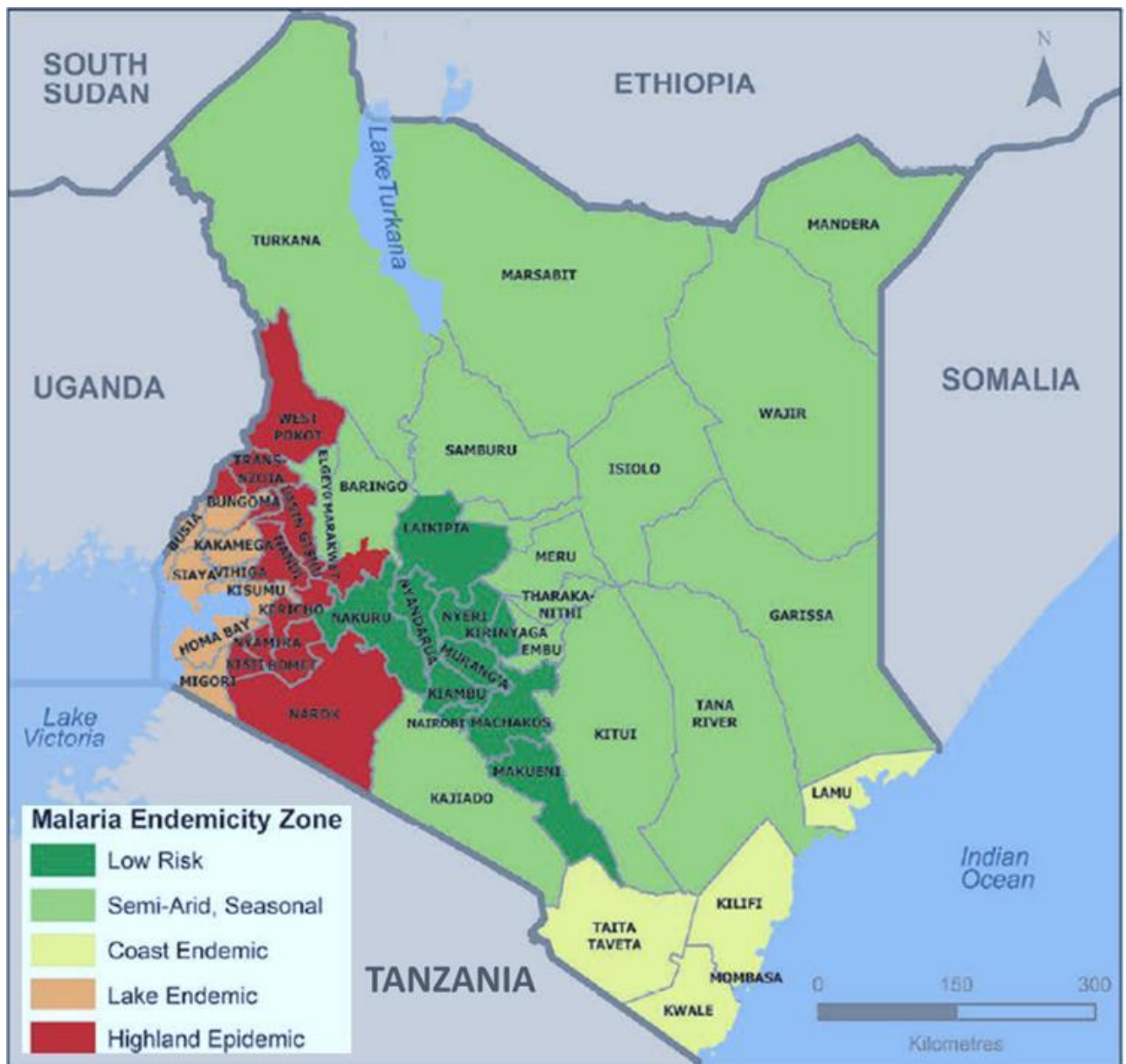
Figure 2: Malaria endemicity zones in Kenya (NMCP, 2016). The low risk areas (central highlands of Kenya) have very low temperatures that hinder growth of malaria vectors. Semi-Arid areas (the Northern and South-eastern parts of Kenya) experience epidemic malaria outbreaks. Coast and lake endemic regions have stable malaria transmission due to suitable climatic conditions that favor mosquito breeding. Highland epidemic regions (Western Kneyan highlands) have seasonal Malaria transmission.

2.1. Malaria transmission

Malaria is a vector-borne disease caused by parasites that belong to the genus *Plasmodium*. For a malaria infection to occur, the *Plasmodium* parasite, a female *Anopheles* mosquito and a susceptible human host must interact. Malaria in man is caused by at least five major species of *Plasmodium* parasites. These include *P. falciparum, P. ovale, P. malariae, P. vivax* and *P. knowlesi*. *Plasmodium falciparum* is the most virulent species amongst the five (WHO, 2018). Female *Anopheles* mosquitoes are the main transmitters of malaria parasites. Transmission of the disease is varied across regions (Beier, 1998). A world malaria risk map (figure 3) shows variations in malaria transmission risk which is mainly attributed to the presence of malaria vectors. Distribution of malaria vectors is majorly influenced by a combination of factors including environmental, socio-economic as well as behavioral factors (Qayum *et al*., 2015). Geographical and seasonal patterns, as well as land use affect the abundance and distribution of malaria vectors (Bashar & Tuno, 2014; Minakawa *et al.,* 2002; Himeidan & Kweka, 2012; Govella & Ferguson, 2012; Klinkenberg *et al*., 2004). High levels of malaria transmission occur in regions that border lakes and water reservoirs in Africa (Root, 1999; Keiser *et al*., 2005).
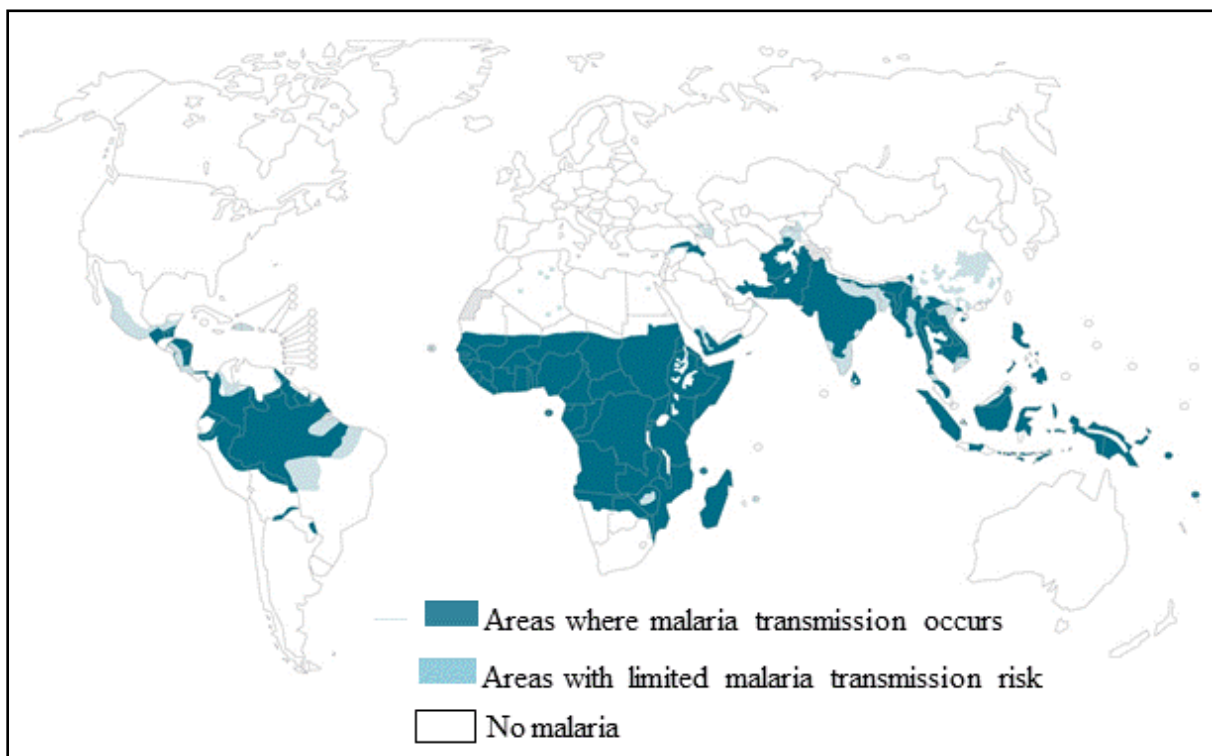
Figure 3: World malaria transmission risk map (WHO 2010).

2.1.1. Distribution of malaria vectors in Kenya

Knowledge on the distribution of the main vectors of malaria in Kenya can be a vital tool for strategic control of the vectors. The distribution of malaria vectors in Kenya is a consequence of complex interactions of both abiotic factors like climate and biotic factors like competition and dispersal of the vectors. Whereas over 140 species of *Anopheles* have been recorded in Africa, at least 8 of them have been found to have the capacity of transmitting *Plasmodium* parasites (Gillies *et al.,* 1968, Gillies & Coetzee, 1987). Of these 8 species, members of the *Anopheles gambiae* s.l complex and *Anopheles funestus* group are the most efficient transmitters of human malaria parasites (White, 1974; Aikpon *et al.,* 2017). Two important species of the *An. gambiae* s.l complex widely known to transmit malaria in Kenya are *An. gambiae sensu stricto* and *An. arabiensis.* Other known species of the complex include *Anopheles merus*, *Anopheles melas*, both of which are localized to coastal regions and breed in salty water, and *Anopheles bwambae*, which are associated with mineral springs (Coluzzi, 1984). A study done by Okara *et al.*, in 2010 revealed that *Anopheles gambiae* were mainly distributed in regions borderring Lake Victoria in western Kenya, and in the Coastal regions of Kenya, with few occurences of the vectors in central parts of Kenya (figure 4). *Anopheles arabiensis* on the other hand was more ubiquitous, having been found distributed along the Kenyan coast, across central regions, into western Kenya, besides occuring in the arid North Western parts of Kenya. *Anopheles merus* were found to be associated with coast regions, signifying the salt-water conditions required for their breeding.

Anopheles funestus was also widely distributed, having been found to occur in the coastal and central regions in Kenya (Keating *et al.*, 2005) and in highland areas near the Lake Victoria basin in western Kenya (Okara *et al.*, 2010). At a glance, the abundance of *An. gambiae, An. arabiensis* and *An. funestus* is favored by warm and moist climates (Minakawa *et al.*, 2002; Ryan *et al.*, 2015).
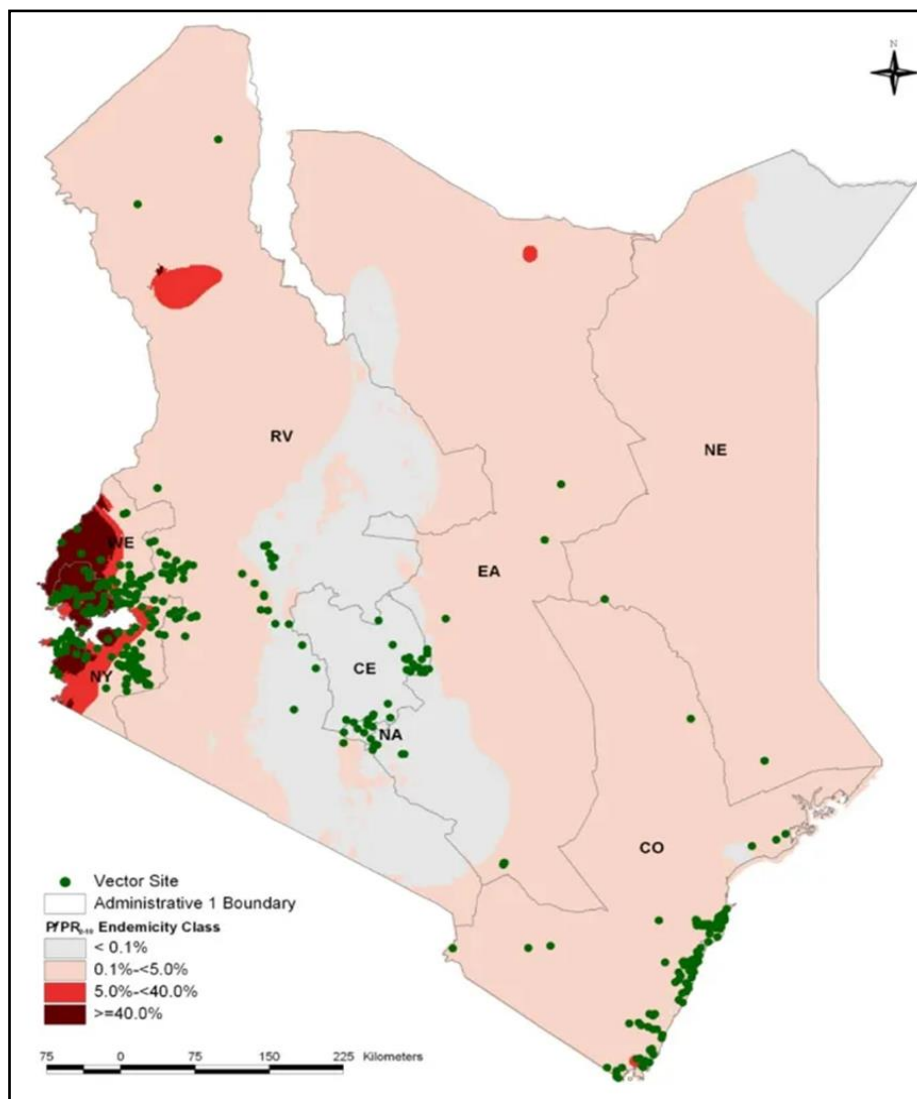
Figure 4: Distribution of malaria vectors in Kenya. *Plasmodium falciparum* endemicity classes as formed by $PfPR_{2-10}$ are indicated with different color intensities in the map (Okara *et al.*, 2010). Endemicity classes were predicted on basis of parasite prevalence rates in children aged between 2-10 years.

2.1.2. Life cycle of *Plasmodium* species

Malaria-causing parasites exhibit a complex life cycle, requiring two host species (Florens *et al.,* 2002). The two host species include an insect vector (in this case, a female *Anopheles* mosquito, which serves as the definitive host) and a vertebrate intermediate host (in this case a human being). *Plasmodium* parasites go through various developmental stages. These include infective sporozoites, merozoites, ring form trophozoites and sexual stages referred to as gametocytes (Figure 5).

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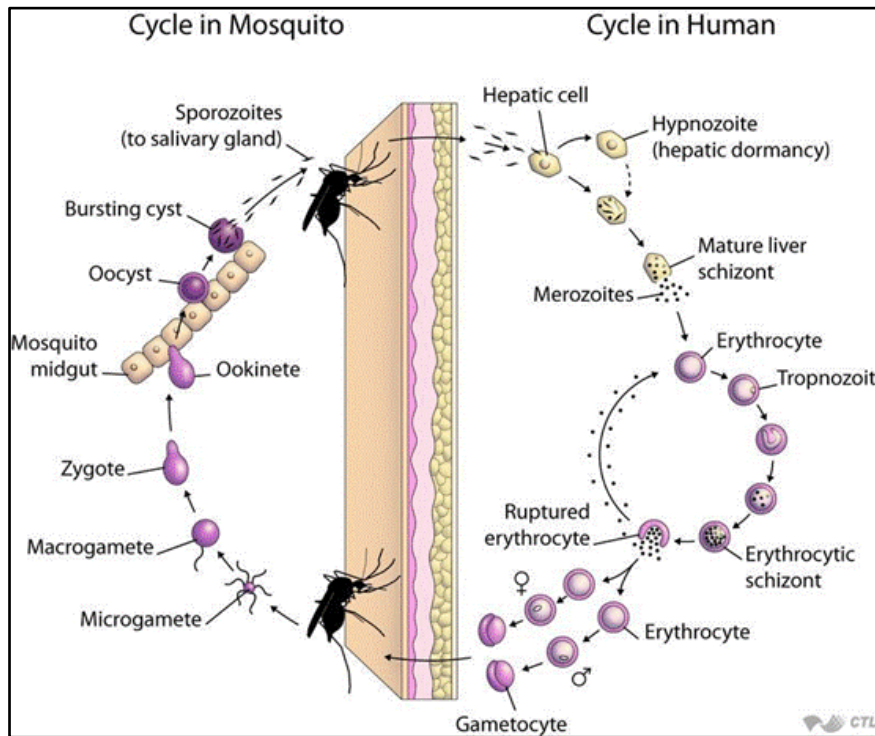
Figure 5: The life cycle of malaria parasite (*Plasmodium*) in the definitive host (*Anopheles* mosquito on the left half of the figure) and intermediate host (cycle in a human on the right half of the figure) (malariasite.com, 2019).

All the developmental stages have distinct characteristics in terms of shape and surface protein structures. The surface proteins are responsible for the difficulties in clearing the parasites by the body's immune system. The proteins keep changing in structure, thereby evading attack by immune cells. Humans become infected when an infected female *Anopheles* mosquito vector bites them while searching for a blood meal to develop their eggs. During this process the mosquito injects infective sporozoites into the human host. The parasites then undergo a series of asexual development inside the human liver cells and erythrocytes.

Once inside liver cells, each sporozoite is capable of developing into thousands of merozoites which once released from the liver, each can invade red blood cells (Miller *et al.,* 2002). In *P. vivax* and *P. malariae* infections, the parasites may remain inside the liver as a dormant stage called hypnozoites. This is what is responsible for relapses and recrudescence in patients. Merozoites released into the bloodstream initiate the erythrocytic schizogony phase, which is responsible for clinical manifestations of malaria. Inside an infected red blood cell, each

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merozoite undergoes growth and division during every erythrocytic cycle, yielding 8-32 new merozoites that develop through ring form trophozoites to schizonts. When the cycle ends, the red blood cell ruptures, releasing the newly formed merozoites into the bloodstream. Released merozoites infect other red blood cells thereon (Greenwood *et al*., 2008). A section of the asexual stages do not undergo schizogony. They differentiate into gametocytes, the sexual parasite forms, instead. The gametocytes are infective to female *Anopheles* mosquito vectors.

The sexual phase (sporogony) of the malaria parasites is initiated when the female *Anopheles* mosquito vector ingests gametocytes during a blood meal from an infected intermediate host. Once in the mosquito gut, the male gametocytes exflagellate under suitable conditions, each producing 8 haploid motile gametes, and the female gametocytes develop to form macrogametes (Billker *et al*., 2004). The male and female gametes fuse to form a zygote which develops within a few hours into actively moving ookinetes (Pradel, 2007). The ookinetes penetrate the walls of the mosquito midgut and develop into oocysts. Sporozoites are produced inside the oocysts. Upon maturation of sporozoites (8-15 days post infection), the oocyst bursts, releasing thousands of the sporozoites into the mosquito's haemolymph. The sporozoites travel through the hemolymph to the mosquito's salivary glands which they invade. They are then transferred to a susceptible human host when the infected mosquito takes a blood meal. This initiates another developmental cycle of the parasite. Mosquitoes infected with malaria parasites exhibit better survival and enhanced search for a blood meal (Heather & Read, 2004; Carolina & Sanjeev, 2005).

2.1.3. Life cycle of Afrotropical malaria mosquitoes

Malaria parasites are transferred to susceptible human hosts during the process of blood feeding by adult female *Anopheles* mosquitoes. The ingested blood is used in egg development. *Anopheles* mosquitoes go through four developmental stages in their life cycle namely the eggs, larvae, pupae and adults (figure 6).
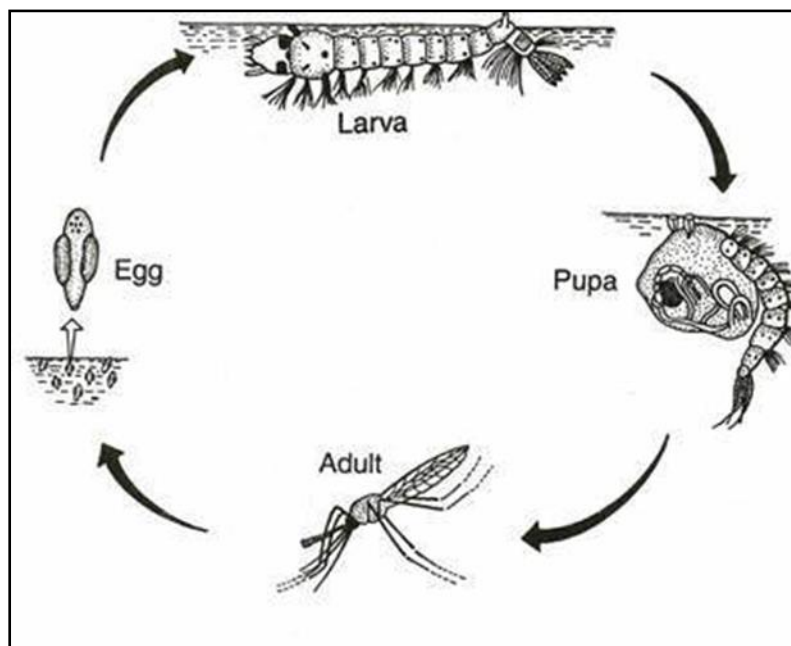
Figure 6: Generalized life cycle of an anopheline mosquito from egg, larva, pupa to adult (Samikhsa, 2019)

The eggs, larvae and pupae are aquatic. The adult stage is terrestrial and is the one responsible for malaria transmission. Maturation of eggs in the female mosquito is dependent on temperature, and occurs in approximately three days after a blood meal in tropical conditions. Oviposition takes place at night (McCrae, 1983) and *Anopheles* mosquitoes prefer laying eggs in cleaner waters with few impurities (Munga *et al*., 2005). One female *Anopheles* mosquito can lay up to 200 eggs per oviposition. The eggs are laid singly on the water surface and have distinct floats on either side, giving them a boat shape. They hatch within 2-3 days with temperatures at 25-30^{0}C, but can take up to two weeks with lower temperatures. Mosquito larvae develop through four instars. *Anopheles* larvae lack a respiratory syphon. It is for this reason that they rest with their bodies parallel to the water surface. They do so in order to breathe through spiracles, which are positioned on the sides of their abdomens. The larvae also spend most of their time feeding on microbes like bacteria and algae on the water surface microlayer, and they dive below the water surface in the event of a slight disturbance. These larval stages are good to target for controlling malaria transmission using larvivorous fish as both species occur in a common environment. Full development of larvae can take approximately 1-2 weeks in tropical regions, but is entirely dependent on density and water temperatures (Gimnig *et al*., 2002). The mature larvae then metamorphosize into pupae, which are comma-shaped and short in size compared to larvae. The pupae do not eat, and mostly float on the water surface, quickly diving in the water when

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they detect any movement or shadow. This stage lasts for two days on average in tropical regions. Adults emerging from the pupae feed on sugar to gain energy, then mate a few days afterwards. Female mosquitoes mate once in their lifetime. Mated females store sperms in a sac known as the spermatheca (Rogers *et al*., 2009). Adult culicine mosquitoes rest at an angle to the surface unlike *Anopheles* mosquitoes whose bodies lie parallel to the surface when resting.

2.2. Ecology of *Anopheles* mosquitoes

Anopheles mosquitoes are the main vectors of human malaria parasites. The most significant malaria vectors in Africa belong to two species complexes i.e. *Anopheles gambiae* complex and the *Anopheles funestus* group (Coetzee *et al*., 2000; Coetzee *et al*., 2013). Sibling species of the *An. gambiae* s.l complex known to transmit malaria in Africa include the *An. gambiae* s.s, *An. merus*, *An. arabiensis*, *An. coluzzii*, *An. bwabae, An. melas* and *An. amharicus* (Coetzee *et al*., 2013). Of the mentioned sibling species of the *Anopheles gambiae* complex, *Anopheles gambiae* together with *An. funestus* of the *An. funestus* group are the most effective and efficient malaria vectors due to their high degrees of anthropophagy (Sinka, 2013; Dadzie *et al*., 2013; Sinka *et al*., 2010; Mwangangi *et al*., 2003; Antonio-Nkondjio *et al.,* 2002; Awolola *et al*., 2003), vectorial capacity and the indoor resting behaviour (Wanji *et al*., 2003; Sinka *et al*., 2010; Cano *et al*., 2004). *An. arabiensis* is a primary malaria transmitter in many regions but is a less efficient malaria vector when compared to *An. gambiae* and *An. funestus*. This is due to the higher plasticity of *An. arabiensis* with regard to its bloodmeal host range (Takken & Verhulst, 2013). *An. gambiae* s.s and *An. funestus* are highly endophilic although some reports on exophily exist (Faye *et al*., 1997; Githeko *et al*., 1996; Mahande *et al.,* 2007; Fontenille *et al*., 1990). *An. arabiensis* on the other hand is highly exophilic (Tirados *et al*., 2006; Fontenille *et al*., 1990).

Different species of mosquitoes are known to occur in the same area (Kirby *et al*., 2008, Minakawa *et al*., 2002), *albeit* oviposition site selection is species specific. *Anopheles* larval habitats are diverse, ranging from natural to man made. The temporal and spatial distribution patterns of the *Anopheles* larval habitats also vary seasonally and annually (Li *et al*., 2009).

2.2.1 Diversity of *Anopheles* breeding habitats

Anopheles breeding habitats can be characterized on the basis of various factors including the size of the habitat (Gimnig *et al*., 2001, Minakawa *et al*., 2004), habitat environment (Gimnig

et al., 2001), and habitat stability (Ndenga *et al*., 2011). Environmental factors that influence the choice of a habit by mosquitoes for oviposition and larval development include turbidity, temperature, availability of competitors, and presence of algae as well as emergent plants (Fillinger *et al*., 2004; Minakawa *et al*., 2004; Dida *et al*., 2018). The characteristics of a habitat to some degree dictate the species type of *Anopheles* present in them. *Anopheles gambiae s.s* prefer breeding in small, temporary non-organically polluted habitats with algae and little or no overhanging vegetation (Gimnig *et al*., 2001, Kudom, 2015). The species avoid ovipositing in water with competitors (Munga *et al*., 2014). In western Kenya, *An. arabiensis* have been associated with non-parmanent habitats which are relatively small, with minimum vegetation, for instance rain pools (Gimnig *et al*., 2001). However, this particular species is regularly common in rice fields, which are large and semi-parmanent with vegetation cover (Mwangangi *et al*., 2007). The occurrence is implicated to be associated with the low turbidity, water depth and dissolved oxygen (Mwangangi *et al*., 2007). *Anopheles arabiensis* is also reported to be colonizing ephemeral natural habitats (Shililu *et al*., 2007). *Anopheles funestus* have been reported to be abundant in large, semi-parmanent habitats that have algae and vegetation cover (Gimnig *et al*., 2001), although a study done by Howard and Omlin in 2008 found an insignificant relationship with vegetation cover (Howard & Omlin, 2008).

Despite the widely documented species seggregation known to be exhibited by *Anopheles* species, niche overlap by the different species has often been recorded (Gimnig *et al*., 2001, Howard *et al*, 2007). Several studies in Kenya indicate that *An. gambiae* s.s and *An. arabiensis* breed in both temporary and parmanent water pools (Gimnig *et al*., 2001, Fillinger *et al*., 2004, Imbahale *et al*., 2011). Studies are increasingly linking *Anopheles gambiae s.l* to man-made habitats. A study by Carlson *et al* (2004) conducted in western Kenya revealed that 87.5% of habitats that contained *Anopheles* mosquitoes were man made. Other man-made habitats colonized by *Anopheles* mosquitoes include swimming pools (Impoinvil *et al*., 2008), burrow pits (Mutuku *et al*., 2009), and those created through agricultural land use (Mutuku *et al*., 2009). Anopheline mosquitoes are also abundant in areas closest to human habitations. It is dimmed that this is to maximize chances of getting a blood meal source. In general, human activities greatly influence the distribution of Anopheline mosquitoes (Mutuku *et al.,* 2006).

2.3. Rationale behind vector control

Malaria transmission efficiency can be quantified in terms of vectorial capacity. Vectorial capacity (C) is termed as the rate at which secondary cases of malaria infections arise from a single infective case per day in a susceptible population (Brady *et al*., 2016). The term vectorial capacity equation as coined by MacDonald (1957) can estimate the ability of a population of *Anopheles* vectors to transmit malarial infections. The equation is expressed as shown below:

$$C = ma^2 p^n / -lnp.$$

where m is the ratio of vectors to humans, a is the total number of blood meals each vector takes per day per human, p is the survival rate of the vectors through a day, and n is the parasite development rate/extrinsic rate in the vectors (Extrinsic incubation period). Whereas the parameters mentioned in the equation are of significant importance, the component of daily human biting rates and vector survival are considered more important, as the human biting rates are squared, and the survival rates for the vectors are raised to the power of the EIP rates. This therefore means that even a slight reduction in the two parameters can impact on the vectorial capacity of local vector populations (Garrett-Jones, 1964). Malaria transmission can therefore be interrupted in the event of reducing the longevity of the malaria vector to levels below the least time for the parasite's EIP in the mosquito.

The mainstay of major current malaria control programs are shown to aim at the two parameters; reducing human contact by the vectors using LLINs, mosquito repellents and screening, and killing the malaria vectors using IRS (Rozendaal, 1997; WHO, 2017). Although the main focus of malaria vector control is decimating adult female *Anopheles* populations by the control programs, the effectiveness of the control strategies is compromised by a number of factors including avoidance of contact by the mosquitoes to insecticides (Killeen *et al*., 2002), development of mosquito populations that are resistant to insecticides (Ranson *et al*., 2011, Ranson & Lissenden, 2016), the foraging and resting behaviors of the malaria vectors (Bekele *et al*., 2012, Fullman *et al*., 2013), among other factors. This implies that reducing mosquito populations by targeting larval stages of the mosquitoes in their breeding habitats is equally important. Larval control however, is considered to be labor intensive, owing to the fact that breeding habitats of the chief malaria transmitters are widely diversified. It is for such reasons that larval control has not been well adopted. Recent works however indicate that larval control can be very effective with

thorough knowledge of the vector's ecology. This can consequently have a direct impact on the vectorial capacity.

2.3.1. Current vector control strategies

The current malaria intervention strategies mainly target the *Anopheles* vectors and the *Plasmodium* parasites. Parasites are controlled using anti-malarial drugs such as artemisinin-based combination therapy (ACT) (Winstanley & Ward, 2006, Banek *et al*., 2014, WHO, 2017). Vectors are majorly controlled using LLINs and IRSs, and to a lesser extend, by use of biological means like *Bti* and larvivorous fishes.

2.3.1.1. Long lasting insecticidal nets

Insecticide-treated bed nets are used to act as a physical barrier to prevent human-vector contact and to compromise the efficiency of mosquitoes in transmitting malaria parasites by killing them and reducing their life-span (Magesa *et al*., 1991, Bayoh *et al*., 2010, and Lindblade *et al.,* 2014). The use of LLINs has greatly reduced malaria morbidity and mortality in many areas in the afrotropical region (Muller *et al*., 2006, Lengeler, 2004, Bhatt *et al*., 2015). The bed nets are impregnated with pyrethroids and do not need to be re-treated for a period of five years, unlike the traditional insecticide-treated nets.

2.3.1.*2*. Indoor residual spraying (IRS)

Indoor resting mosquitoes are the key targets of IRS. The purpose of IRS is to leave residual insecticides on the surface of walls and ceilings inside the house (preferred resting places for mosquitoes that enter houses to seek for a blood meal) in order to increase the chances of killing mosquitoes once they enter inside the house. The encounter of an infected mosquito with the insecticide reduces their chance to live long enough to support *Plasmodium* parasites to maturity. Currently, vector control by IRS is the primary strategy to curb malaria transmission in the Afrotropical region (WHO, 2009).

2.3.2. Challenges in malaria control

Efforts by health agencies to cut down the transmission of malaria mainly involve the use of ITNs and IRSs to prevent bites from mosquitoes. The interventions are insecticide-based, and the continued use of the interventions has led to the emergence of mosquito populations that are resistant to the insecticides in use. Insecticide resistance refers to the ability of an insect, in this case female *Anopheles* mosquitoes, to tolerate insecticide doses that are otherwise lethal to normal populations of the same insect species. Over 50 *Anopheles* species have been reported to develop resistance to pyrethroid insecticides in use (Hemingway & Ranson, 2000). This resistance leads to a reduction in the capacity of the insecticides to kill or repel the mosquitoes. The resistance has also been shown to select for some behaviorally resistant mosquitoes, for instance, those that feed early and exit early from houses containing the insecticide treated nets (Mathenge *et al*., 2001, Pates & Curtis, 2005). This further exacerbates the malaria situation in malaria endemic regions.

Another challenge with the use of pyrethroids for mosquito control is the accumulation of the insecticides in the ecosystem and subsequent harmful effects to non-target organisms, humans included (Eskenazi *et al*., 2009). Many insecticides used were banned following the 2001 Stockholm Convention, although DDT was not banned at this moment (Sadasivaiah *et al*., 2007). However, the insecticide are linked to some detrimetal human conditions like breast cancers, neurodevelopmental disorders, diabetes and reproductive complications (Eskenazi *et al*., 2009, van den Berg, 2009). Reports indicate that DDT has the potential of accumulating in breast milk in humans (Sedera *et al*., 2009). Pyrethroids also have the ability to accumulate in the environment (Yanez *et al*., 2002).

Other challenges in the control of malaria vectors include misuse of bed nets as seen in a study done in some fishing communities around Lake Victoria in Kenya. The study found out that the nets were being used for fishing and for drying small fish among the communities (Minakawa, *et al*., 2008). Inefficiency in relying on control by the nets also occurs due to misconceptions in some communities, political instabilities that render the nets less usable, and the socio-economic statuses of people in malaria endemic regions (Majori *et al*., 1987, Medlock *et al*., 2007, Baume *et al*., 2009). On the flipside the use of IRS has proved to be very costly and it is also logistically demanding (Lengeler *et al*., 2008).

2.3.3. The use of larvivorous fish for mosquito control

Breeding of mosquitoes mainly occurs in temporary and permanent water bodies (Ndenga *et al.*, 2011). The vast availability of mosquito breeding sites hinders successful control of the mosquitoes. Targeting these habitats can lead to sustained control of the vector populations. Controlling the larval stages of mosquitoes can be more efficient as compared to the control of adult stages because the larval stages are normally concentrated in minimal habitats and are immobile (Killeen *et al.*, 2002). This is in contrast to adult mosquitoes, which are rapidly dispersed over a wide area. Source reduction, which is the permanent elimination of mosquito breeding sites**,** is usually an efficient and long-term mosquito control strategy (Floore, 2006). However, some breeding habitats are impossible to eliminate due to the nature and socioeconomic activities of the target region. Biological control tools that can be introduced in the breeding habitats can therefore act as best candidates in controlling mosquitoes in the habitats.

Before the introduction of insecticides, control of mosquitoes was mainly achieved through environmental management such as draining ponds, pyrethrum space spraying, laying oil on breeding habitats and the use of larvivorous fish (WHO, 1982). The deployment of fish that can eat mosquito larvae and pupae in appropriate habitats has been implicated in reduction of malaria incidence for over a hundred years (Bay, 1967). A gradual decline in usage of environmental management strategies was observed in the 1940s after introduction, adoption of DDT for vector Control of mosquitoes using DDT was widely adopted. This happened until the 1970s when malaria resurgence was experienced. During this period, the use of fish and other resource reduction control methods declined. The malaria resurgence, together with the development of insecticide resistance in mosquitoes, accumulation of the insecticide toxicities in the environment, huge cost and logistical complications in use of insecticides is slowly shifting interest to the use of more environmental-friendly control methods. The use of biological control methods like larvivorous fishes was recommended by WHO to be part of an integrated approach to controlling mosquito vectors (WHO, 2003).

2.4. Characteristics of fish suitable for biocontrol of mosquitoes

Larvivorous fishes are the fishes that feed on immature stages of mosquitoes. According to Job (1940) a fish that should efficiently control mosquito larvae should possess a number of characteristics. First, the fish should be hardy. This is because larvivorous fish need to be used in diverse settings, ranging from large to small water bodies, and in different

environmental conditions. It therefore means that the fish should be able to withstand transportation as well as adaptation to new environments. Second, the fish should be small in size, swift and agile in their movement. These traits are to help the fish to be able to easily maneuver in waters and be able to easily escape large predators like other fish predator species. The fish should prefer mosquito larvae over alternative food items, and should be able to survive in absence of the mosquito larvae. Since mosquito larvae are surface feeders, the fish should also prefer feeding from the water surface, so as to easily catch the mosquito larvae. The fish should also have high fecundity both in natural conditions and in captivity (WHO, 2003).

2.4.1. Larvivorous fish that have been used for mosquito control

Over the last three decades, a lot of work has been done to examine the larvivorous potential of both indigenous and exotic fishes against different mosquito species in many parts of the world. The evaluation has been done under laboratory, semi-field and field conditions. Most of the works have reported that the fish used, either solely or as part of an integrated vector management approach, have proved to be effective in reducing mosquito densities. Fish families that contain the most effective species for mosquito control include Cyprinodontidae, Cyprinidae, Hemirhamphidae, Chilidae and Poeciliidae (Ghosh & Dash, 2007). Control of mosquitoes using appropriate native fish is safe, cost-effective, and environmentally friendly, and can be implemented in both natural and artificial breeding habitats (Cote *et al.*, 2010).

Gambusia affinis, commonly known as the mosquitofish or top minnow, is one of the first fishes that were recognized to be having a high larvivorous potential. The fish species is native to Texas, southern United States of America. *Gambusia affinis* is a fresh to saline water fish that was widely introduced beyond its native scope for mosquito control purposes. The fish are small in size, reaching a maximum length of 6.8 cm for female fish, with a life span of approximately four years. The fish are surface feeders, and breed easily throughout the year in a wide range of habitat types. In a study conducted by Chartterjee and Chandra (1997), *G. affinis* was capable of consuming 48, 51 and 31 larvae of *Anopheles subpitcus, Culex quinquefasciatus* and *Armigeres subalbatus*, respectively per day under laboratory conditions. It is reported that feeding rates reduced with an increase in patch size, and increased with an increase in prey and predator densities. The fish has been widely documented to effectively control mosquito populations under field conditions in many parts of the world where the fish were introduced (Hackett, 1937, Menon & Rajagopalan, 1978, Singaravelu *et al.*, 1997, Tabibzadeh *et al.*, 1970). Similarly, studies using the fish to control

mosquito larvae in wells have reported a significant reduction in the number of mosquito larvae in the habitats (Sitaraman *et al*., 1975*). Gambusia affinis*, when used in rice fields to control malaria mosquitoes, produced a significant reduction in the larval and pupal densities of malaria vectors (Rafatjah & Arata, 1975; Tabibzadeh *et al*., 1971; Das & Prasad, 1991; Prasad *et al*., 1993; Bellini *et al*., 1994). This fish species has proved to be capable of significantly reducing *Anopheles* mosquitoes in casuarina pits as well as in overhead tanks (Bheema *et al*., 1982; Pandey *et al*., 1996).

Poecilia reticulata, commonly called the guppy or rainbowfish, originates from tropical America, but is widely distributed beyond its native boundaries. The fish is intolerant to low temperatures, but can tolerate pollution more than the mosquitofish. Reports on use of this fish species for mosquito control indicate that the fish is effective for the purpose of control. In 1908, the fish was deployed in British India and later introduced into many other countries for the control of malaria vectors (WHO, 2003). The fish have been tested both in the laboratory and field. A study done by Elias *et al.,* (1995) in Dhaka on the use of this fish species in controlling *Culex quinquefasciatus* under laboratory conditions indicated that the fish had a high larvivorous potential towards the mosquitoes. Similarly, the fish reduced *Cx. quinquefasciatus* in a wide range of habitats in Cuba (Koldenkova *et al*., 1989; Garcia *et al*., 1991), India (Joshi *et al*., 1989), and Sri Lanka (Ekanayake *et al*., 2007). A study conducted by Martinez-Ibarra *et al.,* (2002) on the indigenous fish species that can control *Aedes aegypti* mosquitoes in water storage tanks in Mexico reported that *P. reticulata* effectively decimated the mosquito populations. In Cambodia, a community-based study on control of *Ae. aegypti* by the fish in water storage containers reported a reduction in the mosquito numbers (Seng *et al*., 2008). In Delhi-India, a study was done to evaluate the role of the fish in controlling *Anopheles* species that breed in wells. It was established that the fish had eradicated the mosquitoes from the wells after 5 years (Ghosh *et al*., 2005). In India, *P. reticulata*, together with *G. affinis*, are produced commercially by fish farmerrs for purposes of use as part of an integrated vector management for malaria control program (USAID, 2007).

Aphianus dispar, commonly called the Dispar topminnow, is a tested larvivorous fish that is native to Ethiopia, India and Pakistan (Frenkel & Goren, 1828). The fish species breeds freely in fresh to brackish water and is able to withstand polluted waters. It is good for controlling mosquitoes in stagnant water bodies. In a study done by Louis and Albert (1988) to test the efficacy of mosquito control by the fish in some urban parts of Djibouti, *An. arabiensis* and *An. gambiae* populations found to be breeding in cisterns were successfully suppressed. Likewise, the fish species successfully controlled *An. culicifacies adanensis*

populations by 97% in barrels and containers in an urban area in Ethiopia (Fletcher *et al*., 1992). The fish was tested for its suitability of being used as a biocontrol agent by Goren (1995) in Israel, and was found to be a suitable biocontrol agent.

Aplocheilus blockii (dwarf panchax) is a small fish native to India. The fish grows to a maximum length of about 9cm, and is implicated to possess a very high larvivorous capacity. The fish typically breeds in freshwaters and has been found to be suitable in controlling malaria mosquitoes in ornamental pools, sheltered waters in tanks, ponds, abounded water bodies, reservoirs and wells among others. A 75% reduction in *An. stephensi* mosquitoes in the coastal belt of Goa was achieved by the fish in a study conducted by Kumar *et al* (1998). When tested against *Aedes albopictus*, the fish was able to effectively control the mosquitoes in manmade habitats like barrels and big cisterns.

Aplocheilus panchax, commonly called the panchax minnow, is a small larvivorous fish native to India, Sri Lanka, Thailand, Burma and Indonesia (Manna *et al*., 2011). The fish species is hardy, and inhabits fresh to brackish shallow waters. Studies with the fish have reported successful control of *An. culicifacies*, *An. sundaicus* , *Cx. quinquefasciatus* and *Cx. vishnui* in irrigation channels, rice paddies, polluted waterways, burrow pits, marshes, septic tanks, swimming pools and ditches among other habitat types (NICD, 1988).

Oryzias melastigma, the estuarine rice fish, is a small larvivorous fish that is widely distributed in India. It grows to a length of about 4 cm. The carnivorous fish is a surface feeder, found breeding both in still and running waters. It typically breeds in estuarine and brackish waters, but has been found to also breed in fresh waters like lakes and rivers. The predatory potential of the fish was tested against forth instar larvae of *Anopheles spp* under laboratory conditions and was found to consume 98 larvae per fish in one day (Sharma & Ghosh, 1994). Under field conditions, the fish was able to reduce third and fourth instar larvae of *Anopheles spp.* and *Culex sp*. in rice fields by 100% (Sharma & Ghosh, 1994).

Carassius auratus, the goldfish, is a widely distributed larvivorous fish whose origin is China, Taiwan, Korea, East Asia, Japan, Siberia and Campuchia (Gupta & Banerjee, 2009). The fish species inhabits and breeds in aquaria and ornamental ponds. It can grow to a length of about 46 cm. Experiments conducted on the fish to assess its biocontrol efficacy indicate that the fish is effective for mosquito control. Vhatterjee *et al* (1997) tested the larvivorous capacity of the fish against forth instar larvae of three mosquito species i.e. *Anopheles subpictus*, *Culex quinquefasciatus* and *Armigeres subalbatus* under laboratory conditions.

The fish was found to consume on average 193, 188 and 132 larvae in 24 hours, respectively. In the field, the fish reduced *An. subpictus* mean larval densities from 34.5 to 0.02 (Chatterjee *et al*., 1997).

Cichlids have also been reported to control mosquitoes. *Oreochromis spirulus spirulus*, commonly called the Sabaki tilapia, is a fresh water fish native to African waters. The fish was tested under field conditions in a study done by Alio *et al* (1985). In every water storage container that the fish was introduced, there was a 100% reduction in mosquito numbers. A similar study done in reservoirs in a village in Somalia found out that the fish reduced mosquito larvae by 52.8% (Mohamed, 2003). Following the latter trial, the local community accepted the use of *O. spirulus spirulus* in their water storage tanks for controlling mosquitoes that bred in them (Mohamed, 2003). Similar finding are reported in a study done by Fletcher *et al* (1993) which sought to assess efficacy of *O. spirulus spirulus* among other indigenous fishes against *An. gambiae* and *Cx. andersoni* under laboratory conditions in Ethiopia.

The Nile tilapia, *Oreochromis niloticus*, is native to African waters. The fish has been tested both in the laboratory and under field conditions and confirmed to be a potential biocontrol agent against mosquito larvae. A study conducted by Asimeng and Mutinga (1993) in Mwea irrigation scheme in Kenya identified *Oreochromis niloticus* together with *Clarias, Barbus* and *Gambusia* in the schemes and tested their affinities for mosquito immatures. The fishes demonstrated strong affinities for mosquito immatures. *O. niloticus* also demonstrated to possess high larvivorous potential when tested against malaria vectors under laboratory conditions in Sri Lanka (Kusumawathie *et al*., 2006). In a field study done in the western Islands of Kenya, *O. niloticus* led to a 94% reduction in the number of *An. gambiae* and *An. funestus* in treated ponds. A 75% reduction was achieved for culicine mosquitoes. Conversely, untreated ponds that were used as controls experienced a drastic increase in the number of mosquito immatures (Howard *et al*., 2007).

Oreochromis mossambicus (the Mozambique tilapia) is another larvivorous fish native to Southern Africa. The fish was found to successfully control *Ae. aegypti* and *Ae. albopictus* populations in Sri Lanka (Ekanayake *et al*., 2007). A pilot study conducted in the laboratory and under field conditions to test the larvivorous potential of the fish reported a high affinity by the fish for *Aedes* and *Anopheles* larvae in Sri Lanka (Surendran *et al*., 2008).

Tilapia guineensis, commonly called the Guinean tilapia, is a fresh water fish that commonly occurs in floodplains. The fish has been tested for use to control mosquito larvae. In a study conducted by Louca et al (2009) to evaluate the potential of controlling mosquitoes in Gambia River floodplains using native fish, *T. guineensis* resulted to a 96% reduction in mosquito larval densities in the floodplains. Another fish species, *Epiplatys spilargyreius*, resulted to a 69% reduction in the same study.

2.5. Impact of introducing non-native larvivorous fish for mosquito control

The mosquito fish *Gambusia affinis* that is a native to Texas, USA, was found to be highly effective in controlling mosquitoes and is the most widely adopted fish that is used in many parts of the world for mosquito control (Kathleen, 2002). However, this fish has been found to have detrimental effects on native aquatic biota when introduced in regions beyond their native geographic ranges owing to their voracious feeding behavior and wide environmental tolerances. *Gambusia affinis* is implicated in the decline in native fish species and other aquatic biota, displacement of other organisms that feed on the same prey as well as interfering with the ecosystem structure and function when they prey on producers.

2.6. Bio-ecology of *Clarias alluaudi* and its potential for use as a mosquito control agent

Clarias alluaudi, commonly called the Alluaud's catfish, is a small freshwater catfish that is native to Africa. The fish belongs to the class Actinopterygi (the ray-finned fishes) of the order Siluriformes (catfish) and family Clariidae (the airbreathing catfishes) (Julian, 2018). According to the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species, *C. alluaudi* is classified as of Least Concern (figure 7). This means that the species is not a focus of conservation.
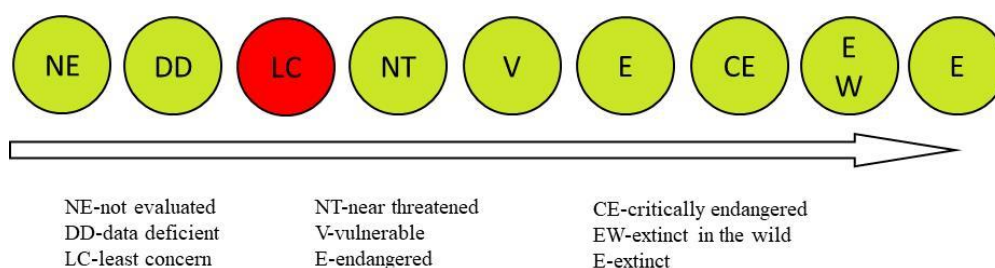
Figure 7: The IUCN red list of threatened species. *Clarias alluaudi* is classified as being of least concern (red fill). Extinction risk rises to the right of the figure as indicated by the arrow (FishBase team RMCA & Geelhand, 2016).

2.6.1. Distribution and ecology of *Clarias alluaudi*

Clarias alluaudi is a small catfish widely distributed in Eastern Africa. They are known to occur in Kenya, Uganda, Tanzania, Congo, and Burundi (figure 8). They occur in the lakes Victoria (Masai *et al.,* 2004), Tanganyika, Kyoga, Rukwa and Edward and their associated rivers (FishBase team RMCA & Geelhand, 2016; Decru *et al.*, 2019). The fish commonly breed in the shallow muddy lake shores, and in waters with papyrus swamps, marginal macrophytes and water lilies in drainage regions of the lakes. The fish also thrive well in rice paddies and in mountainous streams, as well as in side-arms of large water bodies. *Clarias alluaudi* are benthic fish. The fish are construed to be insectivorous, although there is no information on the range of insects eaten. The fish can withstand hypoxic environments as well as long distance transportation as observed when collecting experimental fish for this current study.
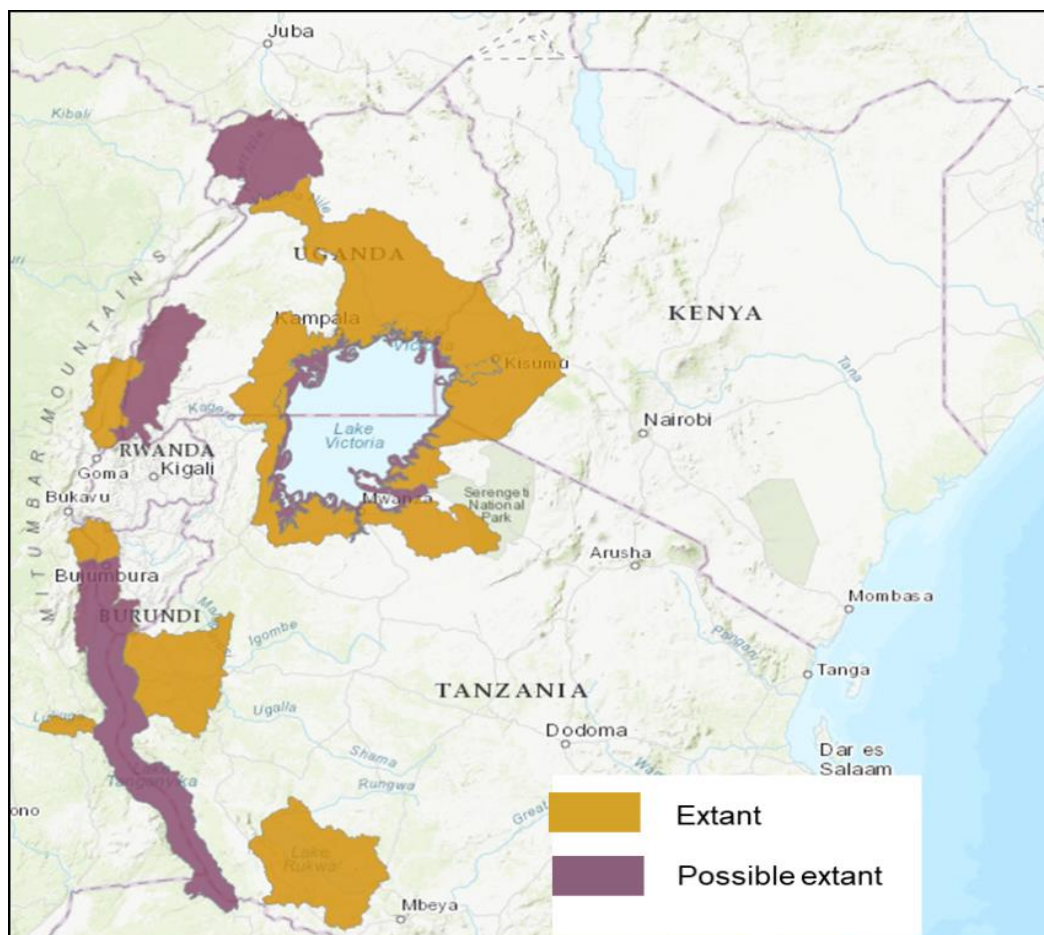
Figure 8: A map showing the distribution of *Clarias alluaudi* . The fish are mainly occur around Lakes Victoria, Tanganyika, Rukwa and Kyoga. (FishBase team RMCA & Geelhand, 2016).

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2.6.2. Morphological characteristics of *Clarias alluaudi*

Clarias alluaudi is a small catfish that reaches up to a length of 23cm total length (Teugels, 1986). The fish is characterized by an elongated body and a depressed head covered with a bony plate. The fish has a long head and a snout that is short and slightly rounded, and dorsally positioned eyes. Tooth plates are relatively small. The dorsal and anal fins are very long and separated from the caudal fin, and the pelvic fin is located forward. The pectoral fin spine is serrated on both the inner and outer sides (Wanja, 2013;Witte & Wim, 1996). Dorsal fins are made up of soft rays (65-79 in total), and they extend to the base of the caudal fins. The anal fin is soft-rayed and extends from behind the anus to the base of the caudal fin, and is made of approximately 57-64 soft rays. The pectoral fins have a bony spine which is serrated on both the inner and outer part. The fish have four pairs of barbels each; the inner and outer mandibulars, nasals and maxillaries. The frontal fontanelle is narrow and elongated, resembling the shape of a knife. The whole first arch of the fish is made up of 12-16 gill rakers. The length measured from the snout of the fish to the anal fin is less than 50% of the standard length (length from snout to start of the caudal fin). The fish is dark khaki to brownish in color, with a lighter belly color (Dignall, 2018).

Several reports indicate that *C. alluaudi* is indistinguishable from *C. werneri*, a type of small catfish sharing the same ecological characteristics with *C. alluaudi*. However, a study done by Mwita in 2015 established shape differences between the two fishes using principal component analysis of morphometric measurements. The two can be distinguished on the basis of the shape and position of the anterior fontanelle in reference to the location of the eyes. *Clarias alluaudi* bears a sharp fontanelle which originates from the front of the eyes, while *C. werneri* bears a blunt one that has its origin between the eyes. The two fishes are also genetically distinct (Mwita & Nkwengulila, 2008). The authors carried out molecular identification of *C. alluaudi* using mitochondrial DNA. The cytochrome *b* gene contained in the mtDNA was amplified using L15267, 59-AAT GAC TTG AAG AAC CAC CGT-39 and H15891, 59-GTT TGA TCC CGT TTC GTG TA-39 primers. The DNA was then sequenced, analyzed and compared to reserved sequences in the GenBank to establish the identity of the fish.

The characteristics described above, in addition to the economic use of the fish in a local setting give the fish the trump card for their augmentative introduction into mosquito breeding habitats for control of aquatic stages of mosquitoes.

2.7. Foraging decisions by fish

Among all living organisms, natural selection will always select for individuals with the most economical foraging patterns. However, for this hypothesis to hold, several factors have to be considered, including the foraging costs, benefits and constraints in a particular situation. The analysis of foraging decisions by predators is mainly based on predator feeding behavior and prey responses (Godin, 1990). Normally, under a typical predator-prey system, predators attempt to maximize their net energy gain, while preys try to minimize predation. To evaluate the effectiveness of an organism as a biocontrol agent, the interaction between the organism and its designated prey should be understood. Several models have been coined to explain the relationship between a predator and a prey.

Predator-prey relationships can be evaluated on basis of the net energy gains and losses. On this basis, an organism has to ensure that optimal conditions are put into place in order to enhance its ecological fitness. This concept of maximizing the energy budgets is referred to as the optimal foraging theory (Kamil, 1983). The basic prey model is an example of the optimal foraging theory which tries to predict the type of prey item that a predator will choose while considering the potential energy gain per unit handling time (Stephens & Krebs, 1986). The basic prey model is applied under circumstances where the available preys differ in terms of profitability or quantity available in the predator's environment. The expected prediction would be that a predator will go for the most profitable prey item if it is available in abundance, or that the predator should forage unselectively on more prey items available. The model has been successful in predicting the choice of some prey types by certain fish, for instance in bluegills (Werner & Hall, 1974).

The size relationship of a predator and prey affects foraging decisions by the predator. Most predatory fish tend to swallow their prey items in whole. This therefore means that prey size is an important constraint when it comes to predation. Depending on the size of the predator, prey items can range from too small organisms that can get trapped in gill rakers to those that are too large for the fish's mouth. Finstad *et al.,* (2006) discloses that the foraging ability of a fish is dependent on its size. This is due to the fact that as fish grows, several aspects like developed visual acuity and motility give them a competitive advantage over the younger fish. Also, as the fish grows so does the metabolic requirements.

Due to the fact that many predator fish consume prey items as a whole, other factors that limit this foraging type come into place. One of the factors is the handling time. Irrespective of the prey type, an increase in the size of prey increases the handling time of the prey by a predator (Gill & Hart, 1994). A fish can therefore decide to swallow low size prey with a lower energy reward, or swallow big sized prey to increase the energy intake but at the same time incur a greater cost in terms of handling time.

Another factor affecting foraging of fish is the probability of prey capture by the fish. As much as a predator fish is supposed to select prey with the maximum energy payoff, the probability of capturing the prey should also strike a balance against the possible energy gain. Literature reveals that predatory fish tend to choose prey with an increased probability of capture (Gill, 2003).

The work contained in this thesis forms part of a broader project whose main aim is to turn mosquito breeding habitats into a wealth source. The results of this work are meant to be used for decimating malaria vectors in breeding habitats on Mageta Island, where *Clarias alluaudi* is one of the main fishing baits for the Nile perch. *Lates niloticus* (the Nile perch), the silver cyprinid (*Rastrineobola argentea*) and tilapia (*Oreochromis niloticus*) are the main fish species caught by the artisanal capture fishers of Mageta Island.

CHAPTER THREE: MATERIALS AND METHODS

The work contained in this thesis sought to assess the efficacy of the catfish *Clarias alluaudi* as a biocontrol agent for *Anopheles* mosquitoes. Two fish species *Gambusia affinis* and *Poecilia reticulata* were used as positive controls. *Aedes aegypti* mosquitoes were used as an alternative prey items. Experiments were carried out in the fish culture house of the School of Biological Sciences at the University of Nairobi.

3.1. Collection of experimental fishes

The three fish species used in this study were collected from their respective natural breeding habitats as described below.

3.1.1. *Clarias alluaudi*

This was the test fish species. *Clarias alluaudi* is a freshwater catfish native to Eastern Africa. It breeds in papyrus swamps and in rivers draining into major lakes where they are known to occur. The fish species also thrive well in rice paddies and in mountainous streams, as well as in side-arms of large water bodies. *Clarias alluaudi* is mainly used as a fishing bait for catching Nile perch by fishermen in the Lake Victoria region (Prof. Mukabana, personal communication). The fish used in this study were collected in western Kenya from the muddy vegetated margins of Lake Victoria in Ahero, Kisumu County, and from Marenga area inside Bunyala swamps, Busia County. The fish were collected using hand held fishing nets which were immersed in the swampy waters and swept towards dry land for fish to collect inside. A total of 500 fish, both male and female, of different sizes were collected and placed in six 20-litre plastic containers perforated on the top side. The fish were then transported by bus to Nairobi where subsequent experiments were carried out. The fish were acclimatized in two dug out ponds. The fish were fed on commercial fish flakes (Raanan Catfish starter feed). After morphometric measurements of the fish were taken, the fish were divided into $60 \times 30 \times 45$ cm glass aquaria according to size groups. The fish were maintained in the glass aquaria until onset of laboratory experiments. The tanks were cleaned and water changed on a daily basis.

3.1.2. *Gambusia affinis*

This fish species was used as a positive control. It is a freshwater fish, commonly called the mosquitofish due to its widely documented ability to consume a large number of mosquito larvae. The fish are native to the Southeastern United States of America, but have been widely introduced in many ecosystems for purposes of biocontrol of mosquitoes. They are widely distributed, inhabiting almost all freshwater bodies in Kenya. Individuals of *G. affinis* used in the present study were caught from the wild, and were supplied by personnel working for the Kenya Marine and Fisheries Research Institute (KEMFRI) at Sagana, Kirinyanga County, Kenya. The fish were transported to Nairobi from Sagana in plastic bags filled with water and oxygen. A colony was established from the wild-caught population, which was later used in subsequent experiments. The colony was maintained in the fish culture house at the School of Biological Sciences, University of Nairobi, Kenya. Fish rearing was done in colorless glass aquaria measuring $60 \times 30 \times 45$cm. The fish were fed on commercial fish flakes (Unga feed) sourced from the KMFRI, Sagana.

3.1.3. *Poecilia reticulata*

This fish is commonly called the guppy or rainbow fish. It is widely distributed beyond its native land of native of South America from where it spread worldwide for mosquito control purposes. This fish was also used as a positive control in the reported work. *Poecilia reticulata* constitute the most widely distributed freshwater fishes and are highly adaptable to many ecological conditions. These fish were sourced from river Kirichwa, which flows by the College of Biological and Physical Sciences, University of Nairobi, Nairobi County, Kenya. Sweep nets were used to scoop the fish from their habitats into plastic containers. The fish were then transferred to the fish culture house at the School of Biological Sciences, University of Nairobi, Kenya. They were reared in glass aquaria measuring $60 \times 30 \times 45$cm. and fed on commercial fish food sourced from KMFRI, Sagana.

3.2. Fish identification

Samples of the three fish species used in this study were taken to the National Museums of Kenya where they were identified morphologically.

3.2.1. Characteristic features of *Clarias alluaudi*

Clarias alluaudi was identified on the basis of phenotypic characteristics including the shape of the anterior fontanelle and its position in reference to the position of the eyes, as described in section 2.6.2 of this thesis. The frontal fontanelle is narrow and elongated. The whole first arch of the fish is made up of 12-16 gill rakers. The fish is dark khaki to brownish in color, with a lighter belly color (Dignall, 2018) (figure 9).
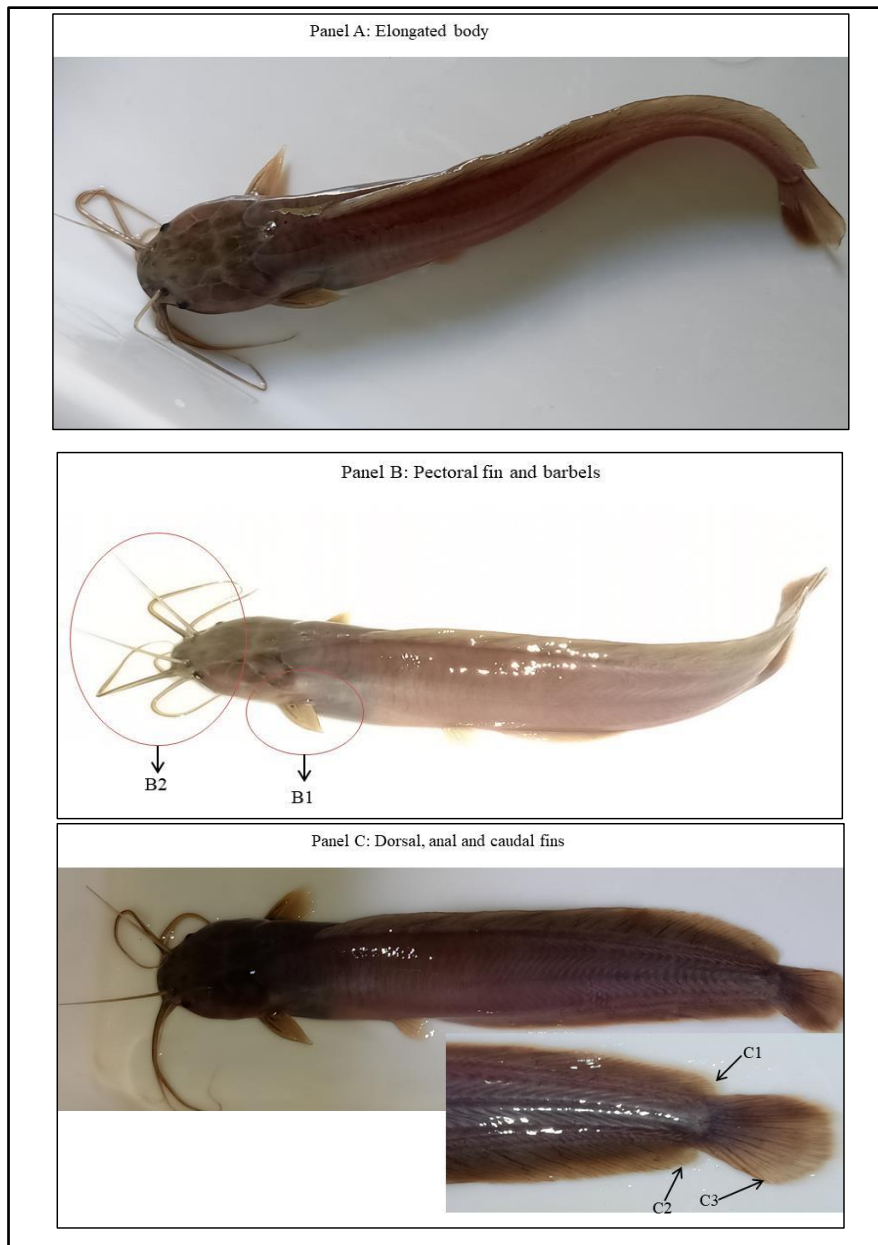
Figure 9: Features of *Clarias alluaudi.* Panel A- slender, elongated body of *Clarias alluaudi*; Panel B1- pectoral fin serrated on both inner and outer margin, B2- four pairs of barbels used for sensitivity; Panel C1- dorsal fin with 65-79 soft rays and extends to base of caudal fin, C2- anal fin with 57-64 soft rays and extends to base of caudal fin, C3- caudal fin.

3.2.2. Characteristic features of *Gambusia affinis*

The fish have an olive green to brown color on the upper body surface, a grey color on the sides, and a silvery white belly (figure 10). They have a relatively large head which is covered with large scales. They have fairly large eyes with a characteristic dark spot below them. The fish have a single dorsal fin that is typically made of 7-9 soft rays, and is short-based and rounded. The caudal fin is rounded, and the peduncle attached to it is long and compressed. The fish are relatively small in size, with males reaching upto a total length of 4cm, and females 7cm (FishBase, 2003). They have a characteristic black spot on the upper rear abdomen which is sorrounded by a golden patch. Small dark spots appear on the dorsal fin, and the caudal fin is characterized by indistinct small black spots that form cross row patterns. The pectoral, anal and pelvc fins have a translucent brownish to yellow color (Vondracek *et al*., 1988). Females that are sexually mature have a distinct gravid spot on the posterior abdomen. Males have a characteristic gonopodium which is basically a metamorphosized anal fin (Turner, 1941).
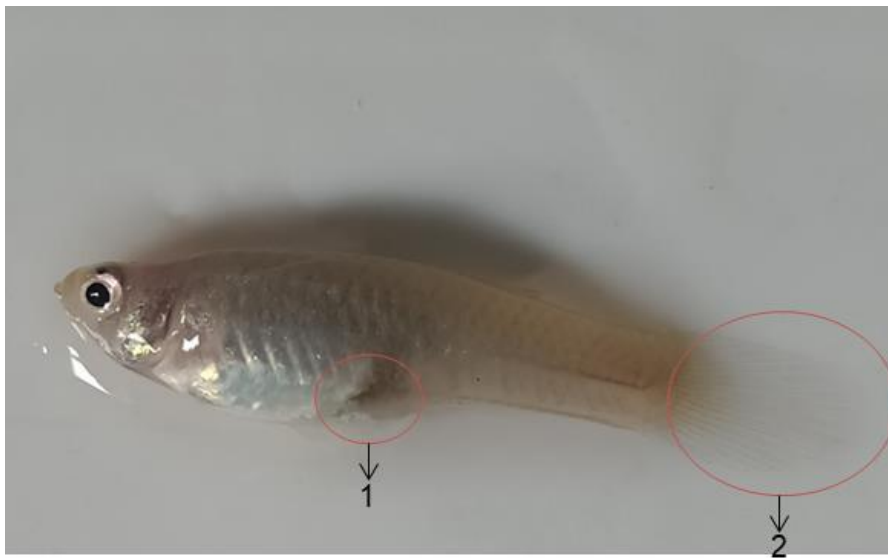
Figure 10: Features of *Gambusia affinis*. A distinct gravid spot (1) on the abdomen and a rounded caudal fin (2) are characteristics of the fish.

3.2.3. Characteristic features of *Poecilia reticulata*

The fish are small, with larger females which can reach a standard length of 5cm long, as compared to shorter males which can reach 3.5cm. The males bear distinct color patterns comprising of pink, purple, black, orange or green spots and speckles (figure 11). The anal

fin in males is modified into a gonopodium. The females are live bearers, giving birth to 20-40 young ones which resemble the females. The females have a uniform silver grey color. Gravid females can be distinguished by a characteristic black triangle that occurs between the pelvic and anal fins. The fish are voracious, preying on their young ones (Peters, 1859).

Figure 11: Features of *Poecilia reticulata.* The fish has a small body characterized by orange, purple, yellow and dark patches.

3.3. Maintenance of mosquito colonies

Colonies of *Anopheles gambiae* s.s and *Aedes aegypti* mosquitoes used in this experiment were established from existing colonies at the insectary of the School of Biological Sciences, University of Nairobi, Nairobi, Kenya. Each mosquito species was reared separately. A batch of eggs for each species was placed inside white larval rearing trays measuring 20×12cm×8cm, with 0.5L of dechlorinated water (figure 12). Environmental conditions in the insectary were set and maintained at a temperature of 32ºC and a relative humidity of 70%. The room was set at a 12h: 12h light: dark photoperiod. Upon hatching, larvae were distributed into rearing trays, each tray holding approximately 100 larvae. The larvae were fed daily on factory prepared fish food (Ranaan catfish starter feed), each tray receiving approximately 10mg of the feed three times in a day. Water was added daily to the trays to freshen up the water and replenish water lost through evaporation. Pupae were harvested using an eye dropper pipette and placed into small plastic containers. The containers were transferred into standard mosquito holding cages measuring $30 \times 30 \times 30$cm. Each cage held a maximum of 1200 adult mosquitoes. A 10% sucrose solution was then placed inside the

cages as food for the emerging mosquitoes. The sugar water was changed daily. Female mosquitoes were blood-fed on human blood on the fourth day onwards after emergence. Filter papers placed in an egg cup and immersed in water were placed inside the cages after blood-feeding for collection of eggs.
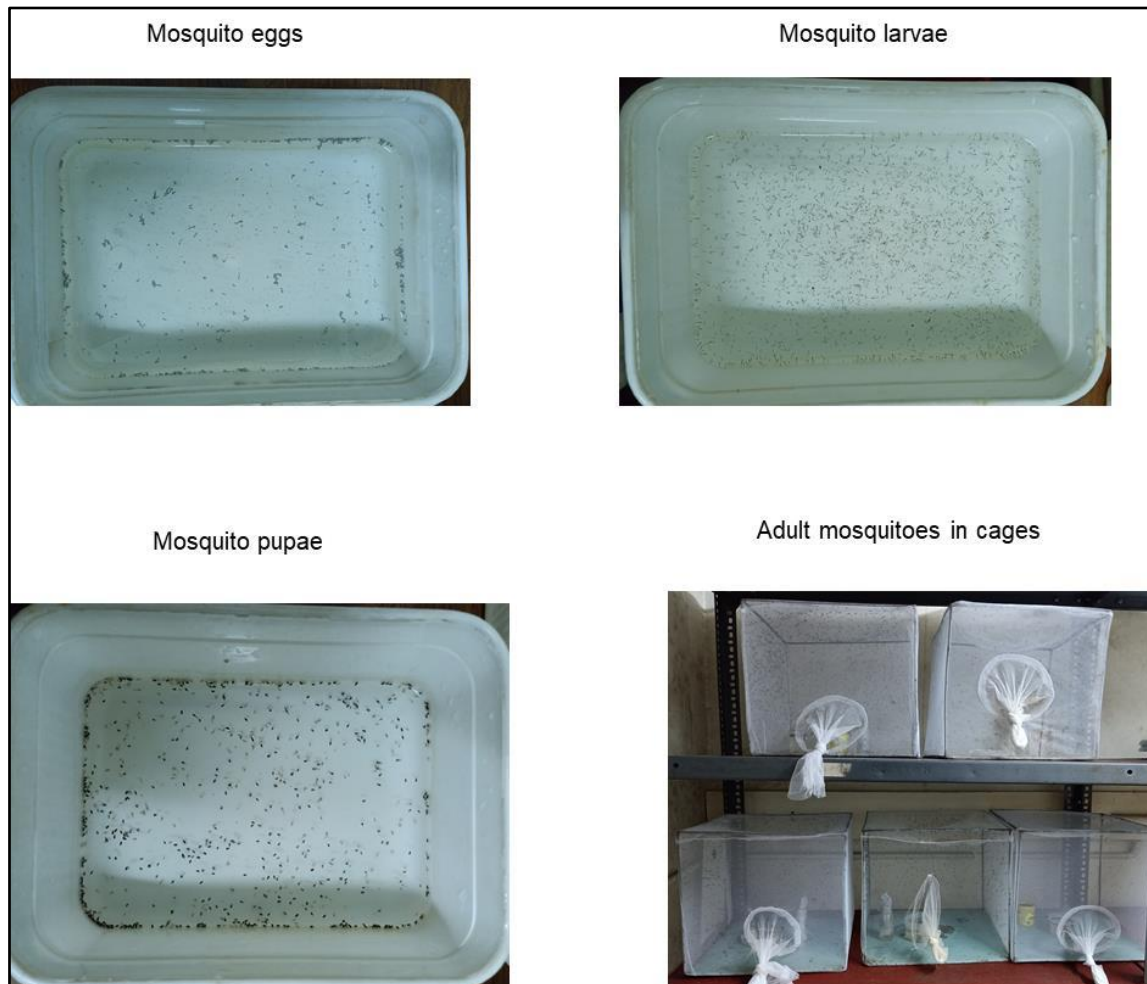
Figure 12: Developmental stages of mosquitoes from eggs, larvae, pupae to adults.

3.4. Morphometric characterization of *Clarias alluaudi*

The main focus of this study was to evaluate the larvivorous potential of *Clarias alluaudi* against *Anopheles* mosquitoes. Besides phenotypic identification of *C. alluaudi*, the fish were characterized in terms of their basic morphology in the laboratory in order to arrange them for consecutive experiments. Morphological characteristics including the total length, standard length, girth and the total weight of all *C. alluaudi* collected from the field were measured and recorded. The fish were weighed using an electronic weighing balance to the

nearest 0.1 g. The total lengths, standard lengths and girth were measured using a meter rule. Fish sexes were also identified.

3.5. Consumption of mosquito immatures by *Clarias alluaudi*

These experiments were carried out under laboratory conditions. Few reports indicate that *Clarias alluaudi* is highly insectivorous (Dignall, 2018), so the main aim of preliminary investigations was to find out and confirm if *C. alluaudi* feed on *Anopheles* mosquito larvae and pupae. This was also done for *Ae. aegypti*. The time taken by individual fish to capture and consume individual prey items was also determined. This length of time, referred to as the 'pre-consumption exposure time' within the context of this thesis, was calculated to find out the avidity of *C. alluaudi* on mosquito larvae. Thereafter the selectivity index of the fish was evaluated through feeding experiments in order to determine preference of *C. alluaudi* for *An. gambiae* larvae over alternative prey items. The same experiments were performed using *G. affinis* and *P. reticulata* as positive controls. Prior to all experiments, the experimental fish were held for an hour in the experimental containers.

3.5.1. Avidity of *Clarias alluaudi* for mosquito immatures

In the context of this work, avidity of fish was construed as the speed at which individual fish located, captured and consumed individual mosquito immatures offered singly. This was measured as the time taken between availing a prey item to a fish and consumption of the prey item by the fish. This test also informed on whether or not *C. alluaudi* consumed *Anopheles* larvae. A completely randomized experimental design was adopted. Individual experimental fishes were selected randomly, weighed and placed separately in plastic experimental containers holding two liters of tap water. A prey item was then availed to each fish using an eye dropper pipette. The time taken for each fish to locate, capture and consume an availed prey item was recorded using a stopwatch. Prey items used in this experiment were late instar larvae and pupae of *An. gambiae* and *Ae. aegypti*. The avidity of *C. alluaudi* on mosquito immatures was compared to that of *G. affinis* and *P. reticulata*.

3.6. Feeding preference of *Clarias alluaudi* on *Anopheles* in presence of alternative prey

The experiments reported in this section sought to test the preference by *C. alluaudi* for *Anopheles* mosquito immatures in presence of alternative prey items, availed at different developmental stages. Late instar larval and pupal stages of *Anopheles gambiae* and *Aedes*

aegypti mosquitoes were simultaneously availed to the fish in different paired combinations. Intraspecific combinations involved availing to a fish the late instar larvae and pupae of a single mosquito species, whilst interspecific combinations involved availing the developmental stages of the two mosquito species at ago. In each experimental session, a total of 200 prey items were availed to a fish (each mosquito species/stage contributing 100 prey items). These experiments were carried out in plastic containers containing 2L of dechlorinated tap water. Each experimental set up lasted for 3hours. The numbers of individual prey categories consumed were then recorded. *Gambusia affinis* and *P. reticulata* were used as comparators. Data on prey selectivity was recorded for each predator fish species.

3.7. Predatory index of *Clarias alluaudi* on aquatic stages of *Anopheles* mosquitoes

The purpose of conducting this experiment was to document the potential of controlling *Anopheles* and *Aedes* mosquitoes using *C. alluaudi* as a larvivorous fish. The predatory index of a fish refers to the number of prey items, i.e. mosquito larvae or pupae in this context, consumed per unit weight of the fish within 24 hours. This is a standard measurement of the predatory capacity of a fish (WHO, 2003). In order to achieve this objective, feeding experiments to determine the average number of prey items consumed per unit weight of experimental fishes in a single day was assessed. The ability of *C. alluaudi* to predate on *Anopheles gambiae* late instar larvae under laboratory conditions was evaluated as previously described (Jaysree & Panicker, 1992; Mohamed, 2003; and Jianguo & Dashu, 1995), *albeit* with slight modifications. A completely randomized experimental design was used for this exercise. The experiments were carried out in plastic containers containing 2 liters of tap water. The weights of each fish were measured before the start of the experiment. Each test fish was placed in a separate container. Each experimental container was then provided with a total of 1000 *An. gambiae* larvae. After 24 hours, the number of mosquito immatures consumed was recorded, and the predatory index of each fish computed using the formula below. The predatory index of *C. alluaudi* was compared with that of *G. affinis* and *P. reticulata*. Similar experiments were repeated using *Aedes aegypti* larvae.

Predatory index = no. of prey items eaten/body weight (g) /day

3.8. Data analysis

Data collected for the standard lengths, total lengths, girth and total weights for *C. alluaudi* was entered into Microsoft Excel spreadsheets and explored using box plots to assess their distribution. The avidity of the three fish species for *Anopheles gambiae* and *Aedes aegypti* was calculated as the mean amount of time taken for each fish to apprehend the availed prey item. Pairwise comparisons were performed using the Generalized Linear Models with a fitted Poisson distribution to document any significant difference in the times taken among the three fishes to apprehend prey items. To compare the feeding preferences among the three fishes for the different prey items offered, the generalized linear models with count data fitted to a Poisson distribution with a log linear function was applied. Pairwise comparisons were performed. Data on predatory indices of fish was analyzed using the GLM to document differences in the feeding rates of the three fish species.

CHAPTER FOUR: RESULTS

Results obtained from all experiments in this study indicate that *C. alluaudi* is a potentially good larvivorous fish as reported in this chapter.

4.1. Morphometric characterization of *Clarias alluaudi*

A total of 500 fish were assessed for their total length, standard length, girth and total weight. The mean total length of the fish was 9.08±0.12, mean standard length was 8.17±0.02, mean girth length was 1.40±0.02, and the mean total weight was 6.51±0.32. The total weight of fish was found to be a function of the fish's total length, standard length, and girth (figure 13).
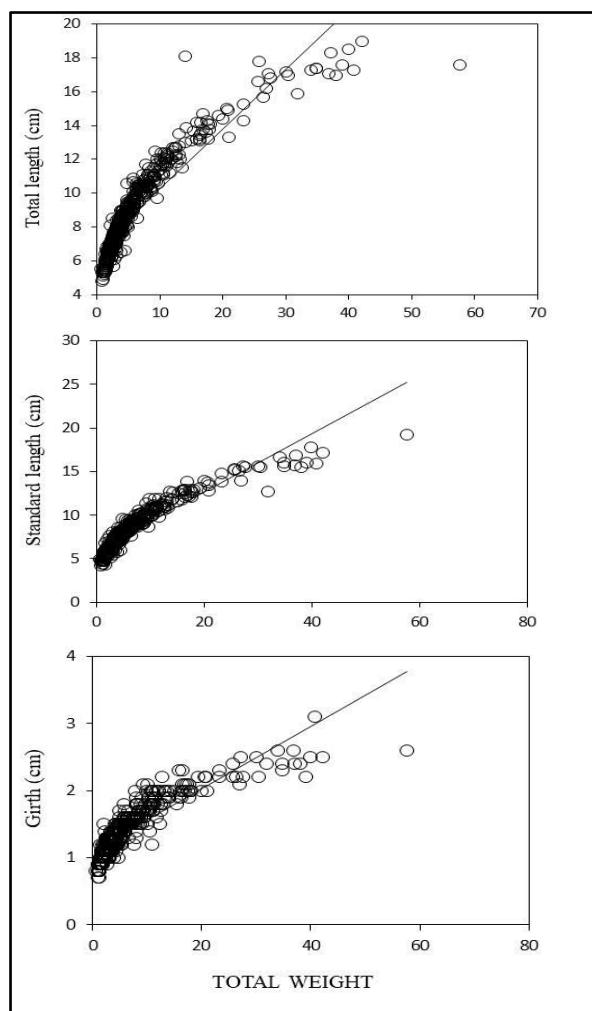
Figure 13: Relationship between the total weight and the total length, standard length, and girth of *Clarias alluaudi*. An increase in the total length, standard length and girth leads to an increase in total weight of fish.

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4.2. Consumption of mosquito immatures by *Clarias alluaudi*

All the three fish species used in these experiments (i.e. 125 *Clarias alluaudi*, 125 *Gambusia affinis* and 125 *Poecilia reticulata*) consumed the prey items that were offered to them, *albeit* at different apprehension speeds and rates. The detailed findings are reported subsequently.

4.2.1. Determining avidity of *Clarias alluaudi* for aquatic stages of *Anopheles gambiae*

Clarias alluaudi took a significantly longer time (34.16±4.19 min) to apprehend and consume individual late instar larvae of *Anopheles gambiae* when compared to *Gambusia affinis* (17.32±2.58 min; P=0.001) and *Poecilia reticulata* (18.65±3.11min; P=0.001). However, there was no significant difference in the time taken to apprehend and consume late instar *An. gambiae* larvae by *G. affinis* and *P. reticulata* (P = 0.220). On the contrary *Clarias alluaudi* took the shortest time to apprehend and consume *An. gambiae* pupae (18.75±3.11 min) when compared to the other two fish species (P = 0.001). *P. reticulata* took the longest time amongst the three fishes to apprehend and consume *An. gambiae* pupae (91.06±6.78 min) and this length of time was significantly longer than that taken by *G. affinis* (48.03±6.16 min) to apprehend and consume *An. gambiae* pupae (P = 0.001). For all the three fish species, there was a statistically significant difference in the time taken to apprehend *Anopheles* larvae when compared to pupae; P=0.001(Figure 14).

Figure 14: Mean (±SE) amount of time (minutes) taken by individuals of three fish species (*Clarias alluaudi, Gambusia affinis* and *Poecilia reticulata*) to consume larvae (Blue) and pupae (Red) of *Anopheles gambiae* mosquitoes.

4.2.2. Determining the avidity of *Clarias alluaudi* for aquatic stages of *Aedes aegypti*

Clarias alluaudi took the shortest time in apprehension of *Aedes aegypti* larvae amongst the three fishes (16.44±4.09). However, the difference in apprehension time was not statistically significant to that for *P. reticulata* (16.88±2.76 min; P = 0.668). The time taken by *C. alluaudi* to apprehend late instar larvae of *Ae. aegypti* was however statistically significant when compared to that for *G. affinis* (18.5±3.55 min; P = 0.048). When compared to *Poecilia reticulata*, *G. affinis* did not differ significantly in the time taken to consume late instar larvae of *Ae. aegypti* (P = 0.122). However, there was a statistically significant difference when the times taken by the three fishes to consume *Ae. aegypti* pupae were compared, with *C. alluaudi* taking the shortest time to consume the pupae (17.83±3.33 min), followed by *G. affinis* (23.17±3.44 min), then *P. reticulata* (41.83±4.51 min; P=0.001 in all comparisons).

There was no difference in the time taken by *C. alluaudi* to consume the late instar larvae of *Ae. aegypti* compared to pupae of the same mosquito species, (P = 0.185). When the time taken by *G. affinis* to consume late instar larvae and pupae of *Ae. aegypti* was compared, there was a statistically significant difference in the consumption times; P=0.001. This was also the case for *P. reticulata*; P=0.001 (Figure 15).
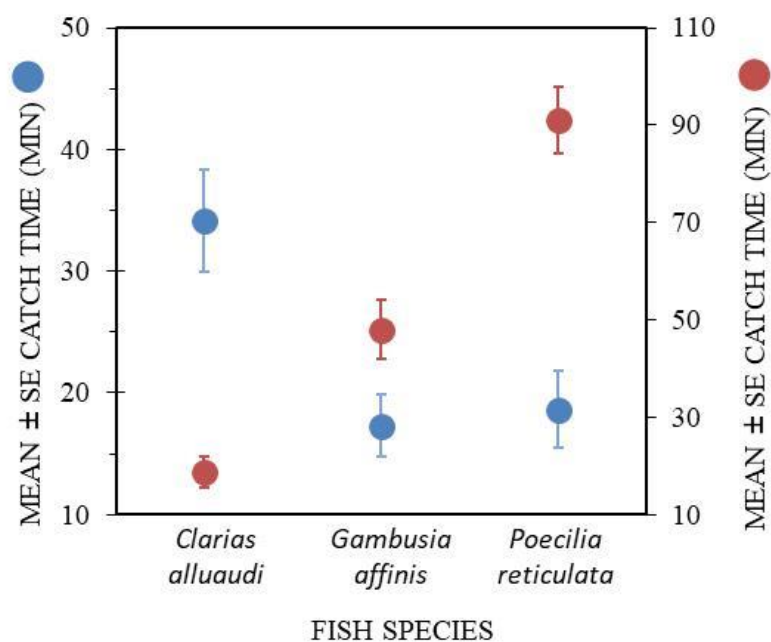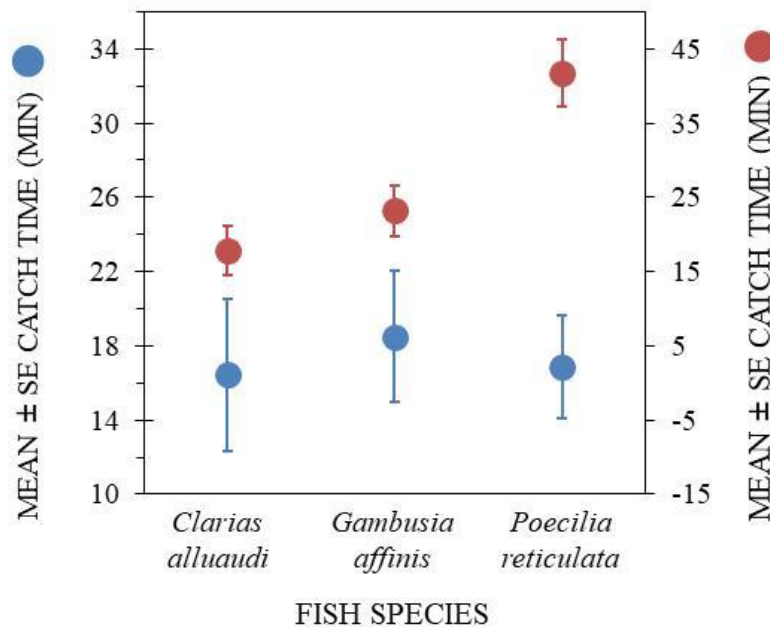

Figure 15: Mean (±SE) amount of time (minutes) taken by individuals of three different fish species (*Clarias alluaudi, Gambusia affinis* and *Poecilia reticulata*) to consume larvae (Blue) and pupae (Red) of *Aedes aegypti* mosquitoes.

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4.3. Selective feeding patterns of *Clarias alluaudi*

For each fish species, a total of 186 fish were used for this experiment. Feeding preferences for the three species of fish were assessed at both intraspecific and interspecific levels.

4.3.1. Selective feeding of *Clarias alluaudi* at an intraspecific level

All the three species of fish used preferred to feed on late instar larvae over pupae, irrespective of the mosquito species (figure 16). *Clarias alluaudi* consumed significantly more late instar larvae of *An. gambiae* (60.83±5.77) than *An. gambiae* pupae (43.67±5.33; P = 0.001). *Gambusia affinis* ate more late instar larvae for *An. gambiae* (19.83±2.03) than *An. gambiae* pupae (10.83±1.62; P = 0.001). Similarly, *P. reticulata* ate more late instar larvae of *An. gambiae* (11.75±1.38) than *An. gambiae* pupae (6.75±1.16), P = 0.001. A similar trend was observed when *Ae. aegypti* mosquitoes were presented to the fish. *Clarias alluaudi* consumed more *Ae. aegypti* late instar larvae (52.67±4.40) than *Ae. aegypti* pupae (24.58±3.52; P = 0.001). *Gambusia affinis* preferred late instar larvae of *Ae. aegypti* (14.67±1.82) over *Ae. aegypti* pupae (4.50±0.89), P = 0.001. The feeding preference was similar with *P. reticulata*, which consumed significantly more late instar larvae of *Ae. aegypti* (9.75±1.74) than *Ae. aegypti* pupae (3.33±0.92), P = 0.001 as shown in figure 16.
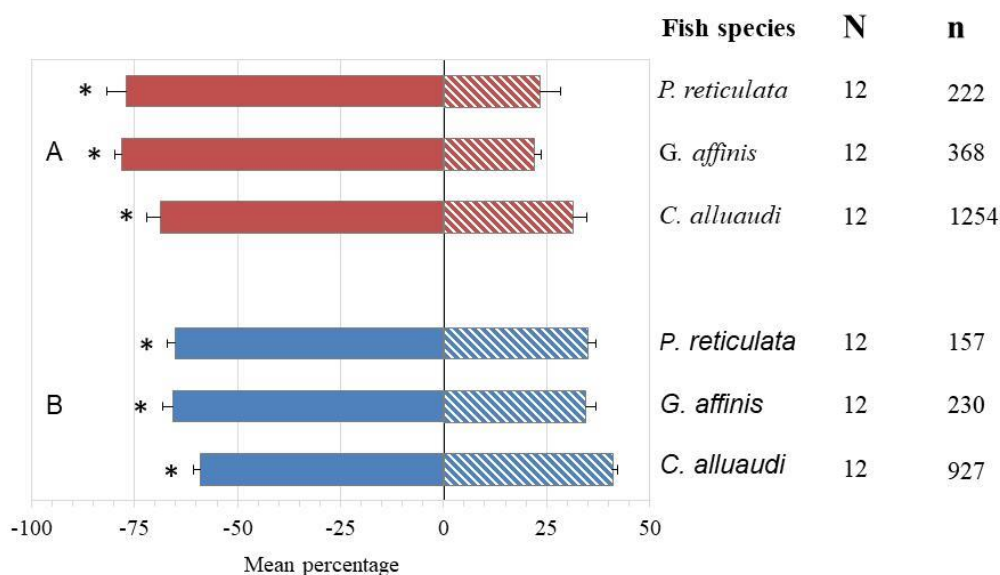
Figure 16: Mean (±SE) percentages of late instar larvae and pupae of *Aedes aegypti* (A) and *Anopheles gambiae* (B) consumed by three fish species offered in different combinations. Solid fills represent larvae while hatched fills represent pupae. N is the number of replicates conducted, while n represents the total number of prey items consumed. * denotes a statistical significance.

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4.3.2. Selective feeding of *Clarias alluaudi* at interspecific level

Late instar larvae of *An. gambiae* were preferred over pupae of *Ae. aegypti* regardless of the fish species. Similarly, the late instar larvae of *Ae. aegypti* were preferred over pupae of *An. gambiae* by the three fish species. All the three fish species preferred *Aedes* larvae over *Anopheles* larvae. On the contrary, all the three fish species preferred eating *Anopheles* pupae over *Aedes* pupae (figure 17).

Clarias alluaudi consumed significantly more late instar *Ae. aegypti* mosquito larvae (56.54±4.93) than late instar larvae of *An. gambiae* (24.97±2.68; P = 0.001), and more *An. gambiae* pupae (42.08±4.65) than *Ae. aegypti* pupae (30.92±5.01; P = 0.001). However, although *C. alluaudi* consumed more late instar *An. gambiae* larvae (39.67±4.70) than *Aedes* pupae (35.83±2.77), the difference was not statistically significant (P = 0.126). There was also no significant difference in consumption of *Ae. aegypti* late instar larvae (31.67±3.89) and *An. gambiae* pupae (29.33±4.60), P = 0.301 by *C. alluaudi* (figure 17).

A similar selective feeding trend applied for *Gambusia affinis*. However, the overall number of prey items consumed by *G. affinis* (n=1850) was significantly lower than that for *C. alluaudi* (n=5204). More late instar *Ae. aegypti* mosquito larvae (20.15±1.69) were consumed compared to the late instar larvae of *An. gambiae* (13.00±1.16), P = 0.001; more late instar *An. gambiae* larvae (15.92±2.07) over *Ae. aegypti* pupae (8.33±1.27), P = 0.001; and more late instar larvae for *Ae. aegypti* (15.00±1.68) over *An. gambiae* pupae (7.58±1.29), P = 0.001. There was however no significant difference between the numbers of *An. gambiae* pupae eaten by this fish (8.33±1.46) compared to the number of *Ae. aegypti* pupae eaten (7.83±1.63), P = 0.667, all shown in figure 17.

Poecilia reticulata consumed the least number of prey items (n=1307) compared to the three fish species used in this experiment. Nonetheless, the trend for selection of prey items was similar to the other two fishes. *P. reticulata* consumed significantly more late instar larvae of *Ae. aegypti* (14.94±1.56) than late instar larvae of *An. gambiae* (9.33±0.88), P = 0.001; more *An. gambiae* late instar larvae (12.17±1.49) than *Ae. aegypti* pupae (5.75±0.78), P = 0.001; more late instar larvae for *Ae. aegypti* (8.33±1.61) than *An. gambiae* pupae (3.67±1.18), P = 0.001; and more *An. gambiae* pupae (7.42±1.63) than *Ae. aegypti* pupae (4.83±0.85), P = 0.011 (figure 17).
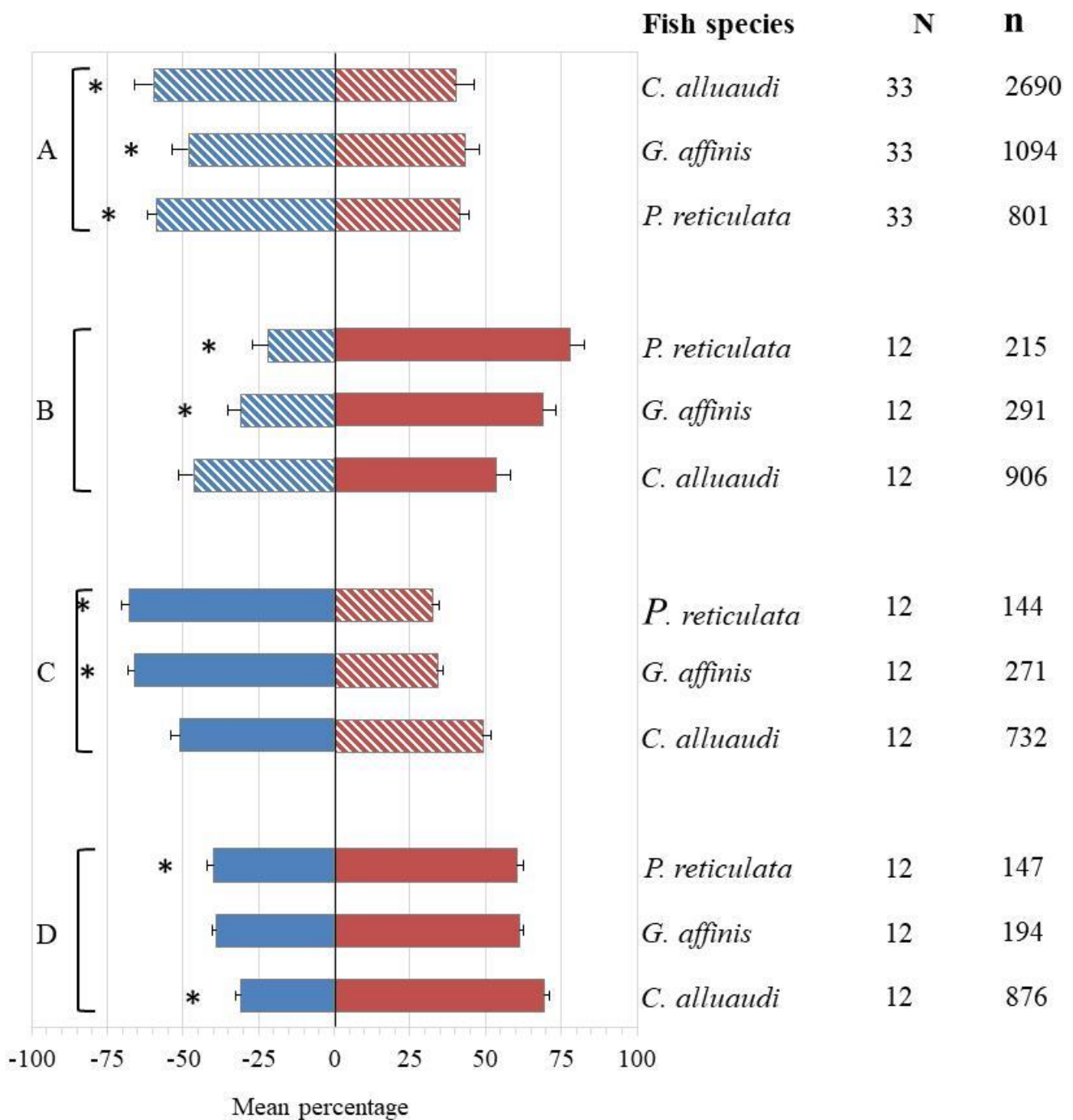
Figure 17: Mean percentages of *Aedes aegypti* and *Anopheles gambiae* late instar larvae /pupae of three fish species offered in different combinations (A, B, C & D). Late instar larvae and pupae of *Anopheles gambiae* is represented by the blue solid fills and blue hatched fills respectively, while late instar larvae and pupae of *Aedes aegypti* is represented by the red solid fills and the red hatch fills, respectively. N represents the number of replicates for each experimental setup, and n represents the total number of mosquito immatures consumed in a particular experimental setup. * denotes a statistical significance.

4.4. Predatory index of *Clarias alluaudi* on aquatic stages of *Anopheles* mosquitoes

Initial ranking of the three fish species for their predatory capacity was based on the total number of late instar larvae of mosquitoes consumed in 24hours. The overall number of both *Anopheles gambiae* and *Aedes aegypti* consumed by the three species of fish reduced in the following pattern: *Clarias alluaudi→Gambusia affinis→Poecilia reticulata,* as documented subsequently.

Clarias alluaudi consumed a significantly higher number of late instar larvae of *An. gambiae* (676.33±72.05) as compared to *G. affinis* (117.33±14.65) and *P. reticulata* (54.0±10.62) as shown in figure 18. A similar trend was observed when the fishes were offered with pupae of *An. gambiae*, with *C. alluaudi* consuming 628±81.03, *G. affinis* consuming 66.00±13.86, and *P. reticulata* consuming 42.4±6.96 pupae. When the fishes were presented with *Ae. aegypti* as prey, *C. alluaudi* consumed the highest number. The fish consumed the highest number of *Ae. aegypti* larvae (301.5±45.27), followed by *G. affinis* (99.73±13.72) then *P. reticulata* (71.67±11.40). Similarly, *C. alluaudi* took the lead in terms of the number of *Ae. aegypti* pupae consumed (427.4±49.90), followed by *G. affinis* (36.27±5.04), then *P. reticulata* (27.67±3.30).

Ranking of the three fish species basing on their predatory potential, that is, the number of mosquito larvae consumed per unit body weight of fish in 24 hours revealed a converse trend in results as compared to ranking using the actual numbers of late instar mosquito larvae consumed. Based on predatory index, *C. alluaudi* was found to have the least index of consumption of *An. gambiae* larvae (88.50±12.90/g body wt/day) as compared to *G. affinis* (376.31±63.03/g body wt/day) and *P. reticulata* (270.0±53.10/g body wt/day). This was a similar case for *Ae. aegypti* larvae where *C. alluaudi* had a predatory index of 50.93±8.39/g/body wt/day, *G. affinis* had 219.98±27.01/g body wt/day and *P. reticulata* had the highest index (344.56±42.75/g body wt/day).

Predatory indices of the three fish species foraging on pupal stages of *Aedes aegypti* go in tandem with those obtained for pupal stages of *An. gambiae* (figure 18). *Clarias alluaudi* had the least predatory index (74.36±5.49), and then followed by *G. affinis* (95.58±12.24), and *P. reticulata* which had the highest predatory index among the three fishes (143.67±16.02).
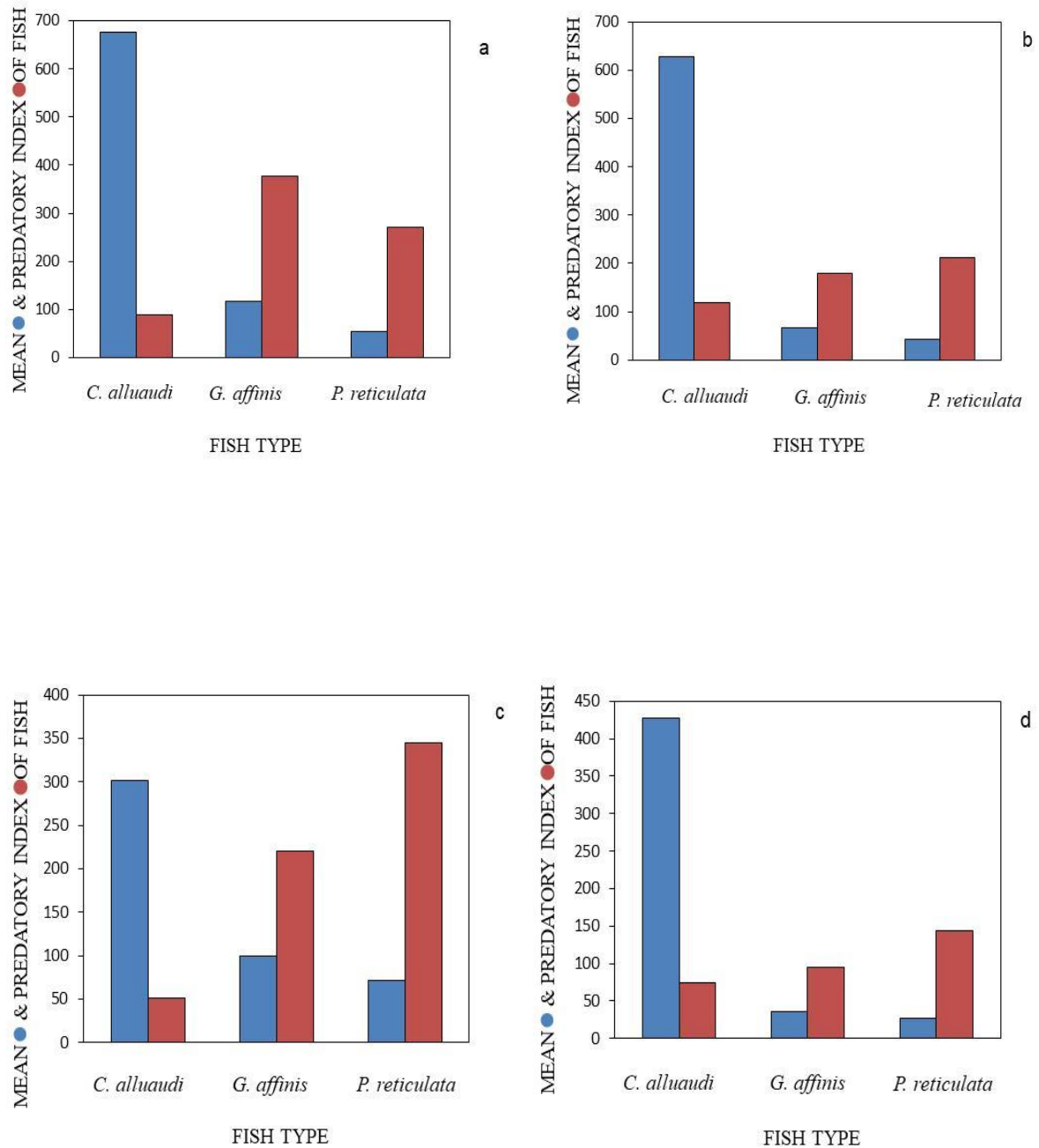
Figure 18: Mean (represented by blue bars) number of *Anopheles gambiae* larvae (**a**), *Anopheles gambiae* pupae (**b**), *Aedes aegypti* larvae (**c**), and *Aedes aegypti* pupae (**d**) with their respective predatory indices (Red bars) for three fish species. In all cases, the mean number of prey items consumed by *Clarias alluaudi* exceeded its predatory index. The inverse is true for *Gambusia affinis* and *Poecilia reticulata*.

CHAPTER FIVE: DISCUSSION

5.1. Discussion

The data acquired demonstrates that *Clarias alluaudi* can consume larval and pupal stages of *Anopheles gambiae* mosquitoes. The time taken for *C. alluaudi* to apprehend late instar larvae of *Anopheles gambiae* was longer compared to that taken by *G. affinis* and *P. reticulata*. However, *C. alluaudi* took the shortest time to apprehend *Anopheles* pupae when the three fishes were compared. Similarly, *C. alluaudi* took the shortest time amongst the three fishes to apprehend both late instar larvae and pupae of *Ae. aegypti*. All the three species of fish preferred late instar larvae over pupae. The three species also preferred late instar larvae of *Ae. aegypti* over *An. gambiae*. On the contrary, the three species of fish preferred *An. gambiae* pupae over *Ae. aegypti* pupae. Ranking of the fish on basis of actual numbers of prey items consumed placed *C. alluaudi* on the lead. However, the inverse was true when predatory indices of the fishes were used to rank them.

Biological control methods involving the use of fish are feasible for controlling immature stages of mosquitoes, including malaria vectors. *Clarias alluaudi* demonstrated good potential in eating up late instar larvae and pupae of both *An. gambiae* and *Ae. aegypti*. Results on exposure time of fish indicated that *C. alluaudi* took a significantly longer time to apprehend *An. gambiae* larvae compared to the *G. affinis* and *P. reticulata*. This result could be explained by physiological and behavioral differences in the fishes and prey which consequently influence foraging decisions by the fish. According to the optimal foraging theory, an organism chooses to feed on a prey item which it will invest less energy in catching, with the aim of maximizing energy gain (Parker & Hawkes, 2018) . *Clarias alluaudi* are demersal in nature (Ssanyu *et al.,* 2014), while *G. affinis* and *P. reticulata* are surface feeders (Pyke, 2005). Larvae of *An. gambiae* feed and rest on the water surface. The foraging and resting behavior of both *C. alluaudi* and *An. gambiae* possibly reduced prey encounter by *C. alluaudi*, hence the longer time of consuming the availed *An. gambiae* larva. This concept substantiates the results obtained on exposure time of *C. alluaudi* on *Ae. aegypti* larvae, considering that *Ae. aegypti* larvae are subsurface feeders. This could have in turn increased the encounter between *Ae. aegypti* larvae with *C. allauaudi*, hence a reduced effort in prey search by the fish.

In this report, we find that *C. alluaudi* choses to consume more larval stages of *Ae. aegypti* as compared to those of *An. gambiae* when presented with a combination of the two prey items. This result can be explained by the concept discussed in the previous paragraph.

The concept of optimization of energy budgets is seen to be displayed by *Clarias alluaudi* when the fish decides to consume *An. gambiae* pupae over *An. gambiae* larvae. According to optimal foraging theories of organisms, predators forage on a prey item from which it gains a high energy payoff (Gill 2003). Aquatic stages of mosquitoes tend to increase the protein, carbohydrate and lipid reserves as they develop (Timmerman & Briegel, 1999). *Clarias alluaudi* consumed pupae which were presumed to contain higher energy reserves than larvae. A report on the prey choices and feeding behavior of the Three-spine stickleback fish corroborate these findings (Gill & Hart, 1994). In this study, larger prey that yielded the best energy returns was preferred by the threespine stickleback, *Gasterosteus aculeatus*.

Results on selective feeding of fish reveal that *C. alluaudi* consumed both prey items that were offered per single experiment. According to the basic prey model which suggests that an organism will only forage on a particular pray item which can yield high energy profits, we expect that *C. alluaudi* choses to forage on one prey item only. However, this was not the case, as the alternative prey item was also consumed. This result possibly points out that *C. alluaudi* could possibly be generalists.

Irrespective of the fish species, larval stages of mosquitoes were preferred over pupae in selective feeding experiments. The concept of foraging decisions exhibited by fish was borrowed to explain this scenario. Comparing the mobility of mosquito larvae with that of pupae, pupae are very fast in their movement, while larvae move at a slower rate. According to Gill, 2003, foraging decisions of a fish are affected by the probability of prey capture. A predatory fish will normally choose to forage on a prey item with the highest probability of capture. *Clarias alluaudi*, together with *G. affinis* and *P. reticulata*, displayed this particular decision, as pupae are less likely to be captured by the fish with regard to their speed of movement as compared to larval stages of mosquitoes. Similar studies done on different fishes indicate that the various fish types chose to forage on prey items that were easier to catch (Dunbrack & Dill, 1983; Hart & Hamrin, 1988; Wankowski, 1979).

Results on the number of prey items consumed per fish indicate that *C. alluaudi* is a potentially good mosquito control agent. The fish, compared to *G. affinis* and *P. reticulata*, which are established mosquito larvivorous species, ate up significantly more *Anopheles* larvae and pupae. The fish also generally ate more *Anopheles gambiae* larvae and pupae as compared to *Aedes aegypti* larvae and pupae. The results also indicated that larger *C. alluaudi* ate more prey items as compared to smaller sized fish. This can best be explained by the concept of size as a function of foraging in fish. The length of fish increases with increase in

its size. The rate of food consumption increases with an increase in the length of intestines in fish (Elliott & Persson, 1978).

Generally, prey consumption rates of *G. affinis* and *P. reticulata* were significantly lower when compared to those of *C. alluaudi*. This similarity in consumption rates of the two, former fish species can be attributed to the closeness in taxonomic relationships between the two fish species, which in turn result in analogous energetic requirements (Harris, 1995).

The mean number of larvae consumed by fish was also expressed per unit weight of fish per unit time (Kusumawathie *et al*., 2006, Ekanayake *et al*., 2007, Phukon & Biswas, 2011). This work assessed the mean mosquito larval consumption by fish in 24 hours as recommended by WHO (2003). *Clarias alluaudi,* which consumed the highest number of prey items, had the least predatory index. *Poecilia reticulata* had the highest predatory index. The observed pattern of predatory indices is a consequence of the total body weight of the fishes. For instance, *P. reticulata* normally weighs approximately 0.2g, while *C. alluaudi* weighed approximately 6.5g according to the morphometric results obtained in this study. This however implies that to achieve a significant reduction in the number of mosquito larvae in a particular habitat, less of *C. alluaudi* fish or more of *P. reticulata* and *G. affinis* fish are required.

The fact that mosquitoes cannot develop physiological resistance towards fish is a major advantage of using larvivorous fish to control mosquitoes. The fish are also self-sustaining, besides being able to survive in absence of larvae. A study by Bond *et al* (2005) revealed prolonged development time of *Anopheles* mosquitoes in presence of fish, yielding smaller females that were less sufficient for malaria transmission. This means that introduction of *C. alluaudi* in the breeding habitats of malaria vectors can possibly lead to production of unfit females that are unable to transmit malaria infection.

5.2. Conclusions

From this study, the following conclusions were made:

1. *Clarias alluaudi* are native larvivorous fish with a very high potential of controlling *Anopheles* breeding. This is explained by the high number of *Anopheles* immatures consumed by the fish as revealed in this thesis.

5.3. Recommendations

1.   Field trials should be conducted to test the impact that *Clarias alluaudi* has on larval densities in habitats that support mosquito breeding.

2.   The control of malaria vectors using larvivorous fish should be practiced alongside other malaria control strategies in order to achieve total elimination of transmission.

3.   The prevalence of malaria in communities where mosquito control is achieved by larvivorous fish should be evaluated in order to establish a direct link between use of fish and reduction in malaria transmission or adult anopheline densities.

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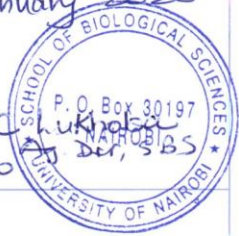
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APPENDIX 2: A table showing results of selective feeding by *C. alluaudi*, *G. affinis* and *P. reticulata*

Prey combination	*C. alluaudi*		*G. affinis*		*P. reticulata*	
	Mean counts	Mean percentage	Mean counts	Mean percentage	Mean counts	Mean percentages
An late instar	24.97±2.68	30.73±1.82	13.00±1.16	38.97±1.49	9.33±0.88	39.81±2.14
Ae late instar	56.54±4.93	69.26±1.82	20.15±1.69	61.02±1.49	14.94±1.56	60.19±2.14
Ae late instar	52.67±4.40	68.62±3.51	14.67±1.82	78.00±1.73	9.75±1.74	76.63±4.95
Ae pupa	24.58±3.52	31.37±3.51	4.50±0.89	22.00±1.73	3.33±0.92	23.3±4.95
An late instar	39.67±4.70	51.03±2.93	15.92±2.07	66.11±2.05	12.17±1.49	67.86±2.42
Ae pupa	35.83±2.77	48.97±2.93	8.33±1.27	33.89±2.05	5.75±0.78	32.14±2.42
Ae late instar	31.67±3.89	53.41±4.80	15.00±1.68	68.88±4.16	8.33±1.61	77.92±4.78
An pupa	29.33±4.60	46.58±4.80	7.58±1.29	31.12±4.16	3.67±1.18	22.08±4.78
An late instar	60.83±5.77	59.14±1.49	19.83±2.03	65.64±2.60	11.75±1.38	65.10±1.99
An pupa	43.67±5.33	40.86±1.49	10.83±1.62	34.36±2.60	6.75±1.16	34.90±1.99
An pupa	42.08±4.65	58.8±3.11	8.33±1.46	48.47±5.32	7.42±1.63	60.03±6.25
Ae pupa	30.92±5.01	41.18±3.11	7.83±1.63	43.19±4.94	4.83±0.85	39.97±6.25