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Effects of Biological Control on Survival and Development of Immature *Anopheles* Mosquitoes

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ABSTRACT: Malaria, a global public health concern, is transmitted through the bite of infected mosquitoes, resulting in significant morbidity and mortality. The overuse of chemical insecticides for malaria vector control has led to the development of resistance and raised environmental and non-target species concerns. As a result, there is a renewed focus on establishing locally sustainable and cost-effective alternative methods for malaria vector control. Predation has emerged as a significant regulatory mechanism for malaria vectors, offering the potential to prevent female anopheles from completing their life stages. This approach holds promise for addressing the challenges associated with traditional insecticide-based control methods. The objective of this study was to assess the predation efficacy of specific predators in an experimental mesocosm, as well as to evaluate the impact of these predators on the development of mosquito larvae, pupae, and adult emergence. The larvae and predators were sourced from various water bodies within the Gilgel Gibe watershed in Southwest Ethiopia. Our semi-field study utilized mesocosms constructed from plastic containers to replicate the natural aquatic habitat of immature Anopheles mosquitoes. The predation efficacy of selected predators, including Belostomatidae, Notonectidae, and Corixidae, on Anopheles mosquito larvae was monitored through daily larval counts until they were consumed, perished, or reached the adult stage within the mesocosms. The study conducted from June to July 2021 involved the collection and rearing of adult mosquitoes that emerged from mesocosms in laboratory conditions. Female adults were monitored to assess the impact of predators on larval development, pupae development, and adult mosquito emergence. The predator to prey ratio in each replicate was maintained at 1:10, and the data collected was analyzed using R software with a risk of species 1 alpha set at 5% for all analyses. The findings revealed that Belostomatidae exhibited the highest efficiency as a predator of Anopheles mosquito larvae, followed by Notonectidae and Corixidae under semi-field conditions. It was observed that these predators exerted sub-lethal effects and indirect non-consumptive effects in addition to direct consumption. The presence of predators significantly affected both the number of adults that emerged and the developmental stage of those that survived predation.

Keywords: Biological control, predators, survival, development, immature, *anopheles* mosquito

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INTRODUCTION

Mosquitoes play a crucial role as arthropod vectors of human disease on a global scale, particularly in the transmission of diseases such as malaria, lymphatic filariasis, yellow fever, and dengue (Eba et al., 2021). According to the World Malaria Report (2020), there were an estimated 229 million malaria cases in 2019 across 87 endemic countries, marking a decline from 238 million cases reported in 2000. The Global technical strategy for malaria 2016–2030 (GTS) baseline of 2015 indicated an estimated 218 million malaria cases at that time. These statistics underscore the significant impact of mosquitoes as disease vectors and the ongoing challenge of addressing mosquito-borne illnesses. The prevalence of malaria cases and related fatalities is notably high in African countries, accounting for 93% of reported cases, followed by the South-East Asia Region at 3.4% and the Eastern Mediterranean Region at 2.1% (Nureye and Assefa, 2020).

Within Africa, there exists a diverse population of Anopheles species, with 140 identified in total, of which only twenty are recognized as transmitters of malaria to humans. In Ethiopia specifically, over 42 species of Anopheles have been identified, with Anopheles arabiensis serving as the primary malaria vector, while An. pharoensis, An. funestus, and An. nili are considered secondary vectors (Adugna et al., 2020). The life cycle of a mosquito consists of four stages: egg, larva, pupa, and adult. Throughout this cycle, the mosquito undergoes two metamorphoses, transitioning from larva to pupa and from pupa to adult (Buxton et al., 2020). The transmission of malaria by these mosquitoes remains a significant global public health concern, contributing to substantial levels of morbidity and mortality, and imposing a considerable burden of disease (Girum et al., 2019).

In Ethiopia, malaria has emerged as a leading cause of hospitalization and mortality, particularly affecting approximately 60% of the population residing in malaria-endemic regions situated below 2000 meters above sea level (Esayas et al., 2020). Given the limited availability of effective preventive treatments against most vector-borne pathogens, the implementation of vector control measures stands out as a crucial strategy in combating mosquito-borne diseases (Id et al., 2020). Since 2000, the control of these vectors has been instrumental in reducing malaria prevalence, necessitating a comprehensive understanding of their ecology, behaviors, and transmission dynamics (Ngowo et al., 2021). However, the excessive use of chemical insecticides in recent years has led to the development of resistance in mosquito populations against various compounds, while also posing detrimental effects on the environment and non-target species (Roux and Robert, 2019a).

In recent years, there has been a resurgence of interest in developing locally sustainable alternative methods for controlling vectors, utilizing non-insecticide-based approaches to complement existing vector control tools. This approach is grounded in a comprehensive understanding of the factors influencing pathogen transmission (Id et al., 2020). Environmental stresses such as competition, predation, and food scarcity during larval development can significantly impact the life-history traits of vectors, including growth rate, fecundity, and longevity. These traits play a crucial role in pathogen transmission by affecting the adult vectorial capacity, which measures the transmission potential of an infectious agent within a vector population (Shapiro et al., 2016). Currently, Environmental Management (EM) has expanded the scope of Aquatic Habitat Management (AHM) for mosquito control, introducing new cost-effective tools and approaches (Layie et al., 2021).

Biological and/or environmental management methods have proven to be effective in reducing mosquito vector populations without causing harm to the environment. In fact, the utilization of biological organisms for controlling mosquito larvae not only aligns with eco-friendly practices, but also offers a more sustainable and efficient approach to population control (Eba et al., 2021). Studies have demonstrated that naturally occurring predators play a significant role in regulating the population of An. *gambiae* larvae, with a wide range of prey and the potential to control the abundance of larval mosquitoes within shared habitats (Kweka et al., 2011; Kweka et al., 2015; Kiszewski et al., 2014). This highlights the importance of considering ecological factors and natural predators in the development of effective mosquito population management strategies.

Predator-induced phenotypic changes can have a significant impact on prey with discrete larval and adult stages, as stress during larval development can carry over to affect adult phenotypes (Roux and Robert, 2019b). Research in insects has identified the Hemiptera (including Notonectidae, Corixidae, and Nepidae), the Coleoptera (including Hydrophilidae and Dytiscidae), and the Odonata (including dragonflies and damselflies) as the three main predator orders associated with Anopheles mosquitoes (Munga et al., 2007). While the possibility of malaria elimination is within reach, doubts have been raised regarding our ability to completely eradicate the disease due to various factors.

In spite of the efforts to maintain zero malaria transmission and to monitor the import of malaria in declared malaria-free countries, it is imperative to expedite interventions aimed at halting transmission in countries striving for elimination (Dhiman, 2019). Recent studies on predator-prey interactions in mosquitoes have revealed that reductions in prey density and changes in crucial aspects of prey, such as development time, size, fecundity, and pathogen infection, can significantly impact the risk of disease transmission (Ecology, 2021). The effectiveness of current malaria vector control methods in various parts of Africa has been compromised by the selection pressure for insecticide resistance across different classes used for indoor residual spray and bed net treatments. This has led to adverse effects on non-target insect species and raised concerns about the long-term impact on the environment. In addition, the characteristics of parasite reproduction, the development of resistance, and shifts in host preference have emphasized the need for alternative approaches to controlling malaria vectors (Yewhalaw et al., 2017). The investigation into alternative control strategies for aquatic mosquito stages continues, with a focus on identifying sustainable and cost-effective methods (Degarege and Erko, 2016). While current strategies are being explored, they are still not meeting efficiency standards. As a result, there has been a renewed interest in utilizing locally available biological resources for controlling aquatic mosquito stages. This study differs from previous research, such as that conducted by Olkeba et al. (2021), in that it specifically examines the impact of Hemipterans predation on emerging adult mosquitoes, as well as the presence of Anopheles mosquito DNA in the predators' gut. Our study, however, focuses on monitoring the development of immature Anopheles mosquitoes in the presence of predators, specifically observing larval and pupal development as well as adult emergence (sub-lethal effects). The overarching objective of this study is to assess the influence of predators on the survival and development of immature Anopheles mosquitoes within controlled experimental microcosms.

MATERIALS AND METHODS

Predators collection and identification

Predators collection

Hemipterans were collected from a pond situated in the Gilgel Gibe watershed, Location coordinates are: Latitude = 7.83487, Longitude = 37.323 of southwest Ethiopia using a scoop net with a mesh size of 300 µm supported by a metal frame within (June – July 2021). Fine sediment were rinsed from the net by forcefully swishing the net through the water a few times, being careful not to lose the organisms captured. Collected hemipterans were identified to family level namely; belostomatids, notonectids, and corixids morphologically. Family-level identified predators were put in a labeled plastic containers containing water from the natural breeding habitat and covered with a net and transported to a space prepared for the study in Jimma University's compound. A few twigs of aquatic plants collected from the same habitat were placed in the container as food and resting sites for the predators. Predators were introduced in semi- field experiments after a 12 hour starvation period (Olkeba et al., 2021).

Anopheles mosquito larvae collection

Anopheles larvae were collected from natural mosquito habitats located in the Gilgel Gibe watershed, Location coordinates are: Latitude= 7.83487, Longitude= 37.323 of southwest Ethiopia by the standard dipping technique (Chapman and Hall, 1993). Water from same habitat was collected in a mosquito-rearing enamel tray and carefully observed for the presence of *Anopheles* larvae. All larvae were sorted to genus *Anopheles* and *Culex*. The *Anopheles* larvae were gently picked up based on their morphological characteristics using a pipette , put in a bowl containing water from the same habitat, covered with a net (mesh size of 1.2 mm), and then transported to the mesocosm prepared for the study. The first-instar *Anopheles* larvae were sorted based on their size and larvae were provided with dog biscuits to forage until the study was started (Olkeba et al., 2021).

Semi-field study deign

Semi-natural habitats (mesocosms) were made-up in plastic containers (volume = 36 L, surface area = 750 cm^2) imitating the natural habitats of immature mosquitoes. The habitat preference of immature *An. gambiae* spp. that includes the most important vector Ethiopia were considered as they prefer habitats that are open-water pools with minimal vegetation/algae, and a lack of emergent plants. Resources that were used to make-up the mesocosms were obtained from the aquatic habitats where *Anopheles* larvae were collected. The bottom of each plastic container was covered with soil (2.5 kg). A few twigs of grasses (sedges) rooted in the soil were used because aquatic habitats with short plants harbor anophelines mosquitoes (Kiszewski et al., 2014, Minakawa et al., 2004; Wamae et al., 2010). Subsequently, the water (12 ltr) used for the study, was checked for the absence of mosquito larvae and were added into the container. The water used in this study was stored for four days to ensure the absence of larval emergence from eggs. The mesocosms were randomly assigned in triplicate to four groups: the three treatment groups (with Belostomatidae, Notonectidae, or Corixidae predators) and the control (no predator). In a recent experiment conducted by Olkeba et al. (2021), fifty first-instar *Anopheles* larvae were introduced into a designated environment along with five predator individuals. The ratio of predators to larvae was carefully maintained at 1:10, as determined by the size of the experimental container. This ratio was established to ensure the free movement of both predators and prey within the environment. This approach allowed for a controlled and balanced setting, enabling the observation of predator-prey interactions in a natural and unobstructed manner. The experiment was carried out using 12 mesocosms in 3 blocks at a time, that is, 4 mesocosms with 3 replicates bearing each predator's name like (C1, C2, and C3) and a control (Cc), Both predators and larvae were introduced into all mesocosms in three blocks at a time. The assumption was that there were no substantial differences between the different blocks in which each predator was tested. In this study, each mesocosm of the treatment groups and control group was placed inside a conically shaped mosquito trap-net made of a metal frame and covered with a net (mesh size of 1.2 mm) to prevent invasion of the mesocosms by other species and any escape of mosquito vectors. The predation efficacy of selected predators namely; Belostomatidae, Notonectidae and Corixidae on anopheles mosquito larvae was monitored by counting larvae once daily until they were either consumed, died or had developed to the adult stage in the mesocosms. Adult mosquitoes that emerged from treatment and control groups were collected using a mechanical aspirator and were given sugar solution with cotton batting**.** Then, the emerged female adults were selected, put in small cages of 30 × 30 × 30 cm, fed with cattle blood and monitored for number of days for effect of predator on larval development, pupae development and adult mosquito emergence (sub-lethal effect of presence of predators) under laboratory condition (Poda et al., 2018).

Larvae production and developmental stages

The female mosquitoes were provided with animal blood to facilitate egg laying, and were housed in cages for the duration of the study. Oviposition substrates consisting of Petri dishes lined with cotton batting, filter paper, and moistened with 25ml sugar solution were placed in each cage (Lyimo et al., 2017). The egg papers were replaced daily, beginning 3 to 5 days after blood feeding (Kafy et al., 2017), (Chaccour et al., 2010). The eggs were transferred to plastic pans filled with distilled water at low density, and the ambient temperature was maintained at 30°C. Larvae that hatched within 2-4 days were counted and recorded (Dreyer et al., 2018). The hatched larvae were provided with instant dry yeast daily in larval pans, and their development into pupae was observed for 7 days. Subsequently, emerged pupae were collected in cups and placed in 30 × 30 × 30 cm cages. The emergence of adults from the pupae was observed for a period of 3 days (Poda et al., 2018).

Data analysis

The data was entered into an electronic database (Microsoft Office Excel Package) and analyzed using R software in version 3.1. The quantitative variables were described using the usual position and dispersion statistics, namely the mean and the standard deviation and Fisher's exact test was performed. For the comparison of means between groups, an independent t-test was used, always according to the assumptions of use of each of these tests. The risk of species 1 alpha was set at 5% for all analyzes.

Data quality assurance

12 liter used for the study, was checked for the absence of mosquito larvae, Family-level predators were identified by a well-trained laboratory technicians and put in a labeled plastic containers and, preliminary test were employed before the main study and also control group were used.

Ethical consideration

Formal consent was taken from the owner of the cattle. The purpose of the study was informed to concern body (Jimma University community and cattle breeder in Jimma, Ethiopia)..

Dissemination plan

After analysis and interpretation of the data, the information was disseminated to concerned bodies, research, and publication office and to Jimma University, Department of Environmental Health Science and Technology whom it needs of findings as a baseline data.

RESULTS

Mosquito emergence rate

Mosquito emergence rate was lower in the Notonectidae, Corixidae and in Belostomatidae groups compare to their control groups with a statistically significant difference ($p < 0.001$). The comparison of the three treatment groups shows a statistically significant difference ($p < 0.001$) (Table 1).

Table 1: Mosquito emergence rate

Predators	Emergence rate (%)	Mean Difference	Independent sample t test	p-value
Notonectidae	10.67	77.33	8.39	< 0.001
Control	88.00	0.0	0.0	0.0
Corixidae	20.00	72.00	7.40	< 0.001
Control	92.00	0.0	0.0	0.0
Belostomatidae	0.00	90.00	9.51	< 0.001
Control	90.00	0.0	0.0	0.0
Notonectidae	10.67	0.0	0.0	< 0.001
Corixidae	20.00	0.0	0.0	0.0
Belostomatidae	0.00	0.0	0.0	0.0

Larvae hatched from eggs means per female mosquito

The means of larvae hatching from the egg per female mosquito is lower in the notonectidae group (24.83 ± 2.40) and corixidae (37.33 ± 3.01) treatment groups compared to their control groups (52.25 **±** 2.63), (52.25 **±**

Table 2: Larvae hatched from eggs means per female *anopheles* mosquito

Groups	Means (± SD)	Mean difference	Independent sample t test	p-value
Notonectidae	24.83 ± 2.40	27.42	7.94	< 0.001
Control	52.25 ± 2.63	0.0	0.0	0.0
Corixidae	37.33 ± 3.01	15.17	4.35	< 0.001
Control	52.50 ± 11.12	0.0	0.0	0.0
Notonectidae	24.83 ± 2.40	12.50	8.445	< 0.001
Corixidae	37.33 ± 3.01	0.0	0.0	0.0

*Note:*SD, Standard Deviation

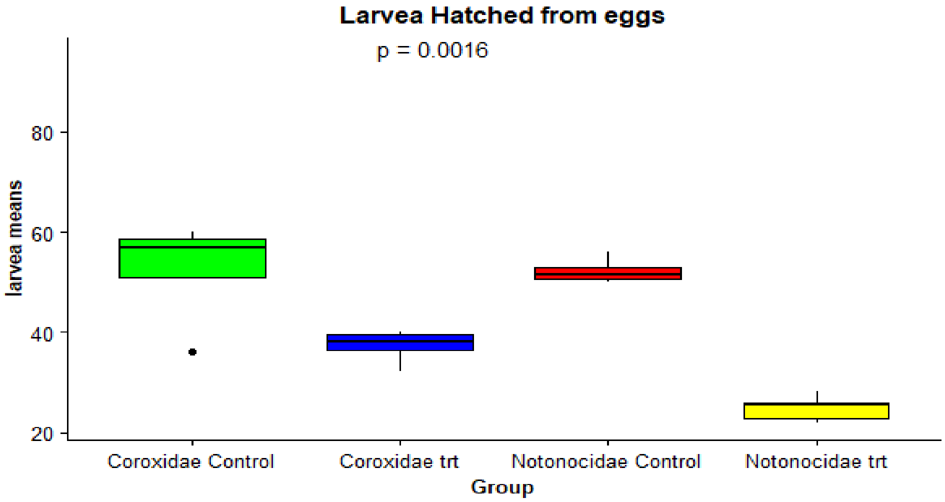

Figure 1: Larvae hatched from eggs means per female *anopheles* mosquito

11.12) with statistically significant difference ($p < 0.001$). When compared with corixidae group (37.33 ± 3.01), the mean of larvae hatching from the egg per female mosquito is lower in notonectidae group (24.83 **±** 2.40), with statistically significant difference ($p < 0.001$) (Table 2 and Figure 1).

Pupae development mean from larvae

The means of pupae developed from larvae is lower in the notonectidae group (7.67**±** 1.03) and corixidae group (20 ± 1.79) compared to their control groups (16 **±** 1.63), (32.5 ± 4.2) with statistically significant difference respectively ($p< 0.001$). When compared with corixidae group (20 ± 1.79), the mean of pupae developed from larvae is lower in notonectidae group (7.67**±** 1.03) with statistically significant difference ($p < 0.001$) (Table 3 and Figure 2).

Mosquito emergence means from pupae (sub-lethal effect of Predators)

The means of adult mosquito emerged from pupae is lower in the notonectidae group (3.83 **±** 0.75) and corixidae group (9.33 ± 1.03) compared to their control groups (7.50 ± 1.00), (15.5 ± 5.25) with statistically significant difference ($p < 0.05$). When compare with corixidae group (9.33 ± 1.03), the mean of pupae developed from larvae is lower in notonectidae group (3.83 **±** 0.75) with statistically significant difference ($p< 0.001$) as shown in (Table 4 and Figure 3).

DISCUSSION

The results of this study have demonstrated ability of locally available aquatic macro invertebrates for controlling aquatic stages of anopheles mosquito. In the semi-field experiments, all evaluated predators were shown to be efficient predators that reduced adult mosquito emergence. Comparing treatment groups with their control groups, the number of adult mosquitoes that emerged from the treatment groups was low compared to the control groups for each predator family evaluated. Comparing treatment groups among themselves, Belostomatidae was most efficient with no adult emergence, followed by Notonectidae while Corixidae were the least efficient predators among all.Predation pressure attributed to predation strategies for prey had a significant effect in the semi- field experiments, which

Table 3: Pupae development mean from larvae

Groups	Means (± SD)	Mean difference	Independent sample t test	p-value
Notonocidae	7.67 ± 1.03	8.33	5.83	< 0.001
Control	16 ± 1.63	0.0	0.0	0.0
Coroxidae	20 ± 1.79	12.50	4.84	< 0.001
Control	32.5 ± 4.2	0.0	0.0	0.0
Notonocidae	7.67 ± 1.03	13.30	9.61	< 0.001
Coroxidae	20 ± 1.79	0.0	0.0	0.0

*Note:*SD, Standard Deviation

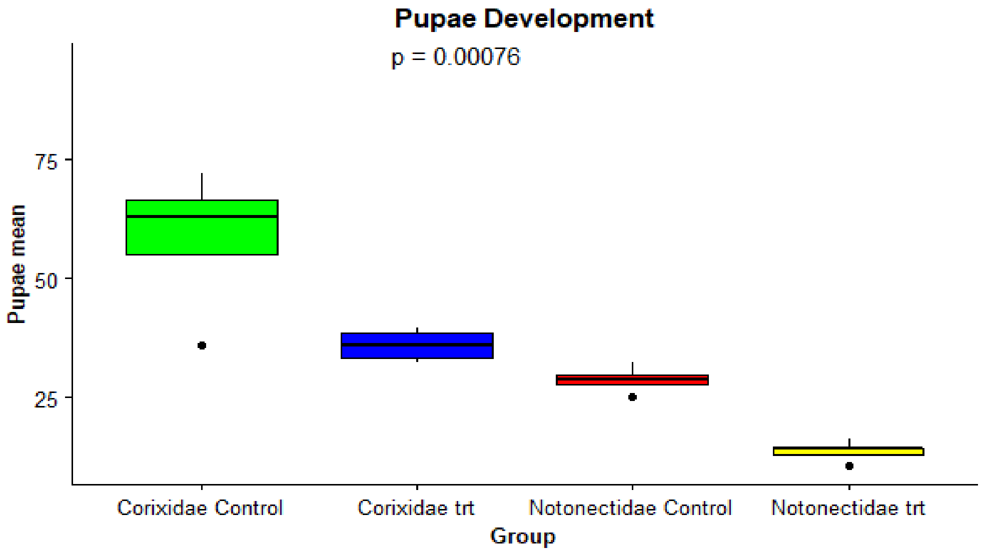

Figure 2: Pupae development mean from larvae.

Table 4: Mosquito emergence means from pupae for (sub-lethal effect of Predators)

Groups	Means (± SD)	Mean difference	Independent sample t test	p-value
Notonectidae	3.83 ± 0.75	3.67	4.66	< 0.001
Control	7.50 ± 1.00	0.0	0.0	0.0
Corixidae	9.33 ± 1.03	6.17	3.98	< 0.01
Control	15.5 ± 5.25	0.0	0.0	0.0
Notonectidae	7.5 ± 1.00	8.00	7.45	< 0.001
Corixidae	9.33 ± 1.03	0.0	0.0	0.0

*Note:*SD, Standard Deviation

suggests that predation pressure may be a significant factor in decreased adult emergence from larva. Other studies has shown that Belostomatids have the capacity to turn and chase prey even at the bottom of water bodies (Olkeba et al., 2021) , while, notonectids demonstrates vertical and horizontal movements (frequently diving swiftly under water, often coming to the surface for breathing) when reversing the swimming direction. This makes them efficient predators for *Anopheles* larvae, and makes the *Anopheles* larvae a preferred prey. This is in line with the result of studies carried out by (Olkeba et al., 2021). However, under laboratory condition, comparing the means larvae hatched from eggs means per female emerged *Anopheles* mosquito between treatments and their control groups each showed a statistically significant difference. Also, comparing the means of larvae development from each emerged female *Anopheles* between treatments only; means of larvae development is lower in notonectidae than in corixidae treatment group with a statistically significant difference. These significant differences between treatments means, could be attributed to strength effects of exposure of prey to indirect non consumptive predation risk carried over through its stages which usually have negative effect on survival and reproduction and thus can have important consequences for population and community dynamics (Sniegula et al., 2020).

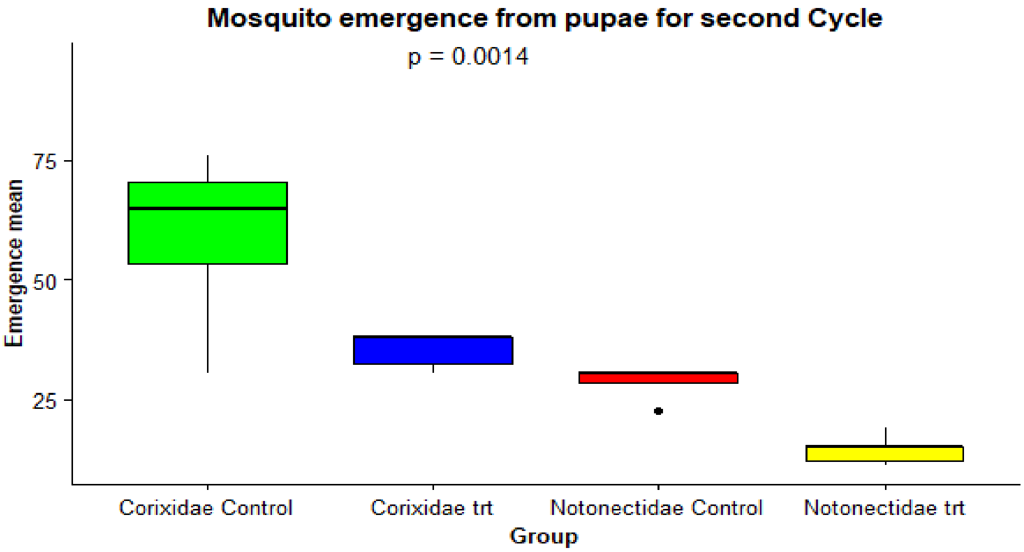

Figure 3: Mosquito emergence means from pupae for (sub-lethal effect of Predators).

The impact of indirect non-consumptive effects can be as strong as or stronger than that of direct consumptive effects. Non consumptive predation risk do not kill the prey but incur costs, such as reduced feeding, a change in diet, increased physiological stress, avoidance behaviors or the production of defenses (Pessarrodona et al.,2019).

Furthermore, the means of pupae developed from larvae was lower in the notonectidae group and corixidae group compared to their control groups and showed a statistically significant difference respectively. Also, when compared with corixidae group, the mean of pupae developed from larvae is lower in notonectidae group with statistically significant difference. These significant differences between treatments means, could be attributed to higher strength effects of indirect non-consumptive effects of Notonectidae on prey. Past studies have found strong carry-over effects from non-consumptive predation between the larval stage and the adult stage in amphibians and insects when exposed to risk organisms usually elongate development times, size and metamorphosis. Pupation is the most energy demanding process in the life cycle of holometabolous insects. The stress and energetic investment of a larva that is unable to naturally bury itself to pupate may be high which could potentially disrupt pupation. In fact, the metabolic heat of wandering larvae while moving around in search of an adequate pupation site can reduce the quality of pupae (Pascacio-Villafan, et. al., 2021).Moreover, the means of adult mosquito emerged from pupae is lower in the notonectidae group and corixidae group compared to their control groups with statistically significant difference. Also, when compared with corixidae group, the mean of pupae developed from larvae is lower in notonectidae group with statistically significant difference. This could be attributed to reduction in number of available immature stages (either larva or pupa) which drastically reduces adult emergence. However, even though the selective environment can change dramatically between each life stage, an individual's fitness (like reproductive success and ability to survive until and beyond a given reproductive stage) is the sum of conditions experienced during the previous life stages (Lindstedt et al., 2019). This trade-off has a cost on both larval development and survival as well as a non-intuitive carry-over effect on adult life-history traits (Roux and Robert, 2019b).

Conclusion

The predators utilized in the study exhibited sub-lethal and indirect non-consumptive effects on the developmental stages of mosquitoes, in addition to direct consumption. The presence of these predators impacted both the number of emerged adults and the developmental stage of those that survived predation. This suggests that these macro-invertebrates could serve as a viable option for controlling the aquatic stages of mosquitoes, potentially influencing the size of anopheles mosquito populations through effects on fitness and migration. The study's findings also indicate that Belostomatidae is the most effective predator of anopheles mosquito larvae, followed by Notonectidae and Corixidae under semi-field conditions. As such, the predation efficiency of these selected predators strengthens their potential use as biocontrol agents for immature anopheles stages. Furthermore, given the abundance of Hemipterans in Ethiopia, mass rearing and introduction into various anopheles mosquito habitats during dry and short blustery seasons could potentially prolong the development of the gonotrophic cycle and contribute to the control of malaria spread.

Recommendation

We recommend increasing the temporal scale of non-consumptive studies to accommodate reproduction, which can provide insight into how non-consumptive effects may influence population cycles since our understanding of how non-consumptive effects influence predator-prey population dynamics is largely based on studies that are limited to mesocosm experiment or small scale laboratory in a short time scale.

Limitation of study

This investigation had a few limitations. The effect of predator on egg production was not analyzed because we were unable to get a microscope to count the eggs laid. Also experiment was done under semi-field conditions and may not show the impacts of predation under normal conditions.

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