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Diversity and Abundance of Controphic Species Associated with Mosquito Oviposition Site in Mbale Municipal Council, Mbale, Uganda

Alhassan, Musa Mohammed

Department of Sciences Laboratory Technology
Umaru Ali Shinkafi Polytechnic Sokoto
+234 (0) 7066946620, +234 (0) 8088580233
musaalhassan33@gmail.com or alhassanmusa44@gmail.com

ABSTRACT

Controphic species diversity and abundance in relation to mosquito larval population within five categories of habitat in Mbale Municipality were estimated using the Shannon index (H'), with the goal of establishing their potential as biological control agents for mosquito larvae. The mosquito Oviposition sites ware sampled between the months of August to November in the year 2014, with field collection using 350ml standard dipper. Controphic species present in all the sites were also collected, counted and identified to the order level of classification. A total of 992 species distributed across 11 orders were recorded. Controphic species diversity was lowest in Pond (H = 1.39) and highest in Ditch (H = 3.47) and Puddle (H = 2.48), whereas Mosquito larval diversity was lowest in rice field (H = 0.58) and Pond (H = 0.60), and highest in Ditch (H = 2.13). Significant differences (p>0.05) were observed in the abundance of both controphic species and mosquito larvae across habitat types. Ponds supported the highest number (51.60 ±7.814) with a range of 31 to74 of controphic species while swamps supported the least (16.71 ±3.490) with a range of 3 to 27 individuals. Pond supported the highest number of mosquito larvae, (28.33 ±8.090) with a range of 14 to 42 larvae while rice field supported the least (16.00 ±6.807) with a range of 3 to 26 larvae. Spearman's correlation results showed that there was a strong positive correlation between the diversity of mosquito larvae and that of controphic species (*r = .900**) and this was highly significant at (*p = .037*).The ecological conditions related to biological interactions between mosquito larvae and associated species could helping the implementation of appropriate control measures of mosquitoes.

Keywords: Controphic species, Diversity, Mosquito larvae, Mbale

INTRODUCTION

Controphic species are the aquatic organisms that shared the same trophic level with mosquito's larvae, though they interact in a complex ways. Those species sharing the same food resources should compete when resources are limiting. Mosquitoes serve as vectors for the transmission of dangerous medical pathogens and parasites such as viruses, protozoan's, and nematodes to humans and animals (Becker *et al* 2003). These parasites and pathogens are etiological agents of serious diseases (e.g. malaria, dengue fever, yellow fever, encephalitis, Filariasis) that are responsible for the disability and the death of millions of people in the world every year (WHO 2005; Reiter 2010). Controphic species of the mosquito larvae have been explored as a possible opportunity for mosquito control with more studies concentrating on predation (Marten and Reid, 2007) and intra-specific competition between mosquito larvae (Costanzo *et al.*, 2011). Competition is often important in structuring many communities (chase *et al.* 2002).

The principal pathogens transmitted by these vectors include viruses (dengue, yellow fever, equine encephalitis, etc.), protozoa (e.g., those causing malaria), and nematodes (e.g. those causing Filariasis) (WHO, 2010). Overall, fewer than 150 species of the genera Anopheles, Aedes and Culex, are the indirect causes of morbidity and mortality among humans, more than any other group of organisms (Hartbach, 2007). However, only the subfamilies Anopheline and Culicinae include vector species of medical or veterinary importance, especially those from the genera: Aedes, Anopheles, Culex, Haemagogus, Mansonia, Sabethes, Psorophora, and Coquillettidia (Bond *et al*., 2014, and Service, 2004). These species Oviposit in a wide range of aquatic habitats that also harbor numerous species of aquatic insects and plant species with which they interact. Species interactions are central to the ecology of any habitat, including those of mosquitoes (Juliano, 2009). Identification of the habitats selected as Oviposition sites is of clear relevance to mosquito surveillance programs as these habitats are also targeted by mosquito control measures involving habitat elimination or larviciding activities (Bond *et al*., 2014).

A wide variety of aquatic environments (e.g. marshes, ponds, wells, drainage channels,. lakes, and rivers) serve as breeding sites for mosquito larvae (Becker *et al.,* 2003). However, many other invertebrate taxa (e.g. *Crustacea, Acaria,* insect larvae) share the same habitats (Campo *et al.*, 2004, Bambaradeniya *et al.*, 2004) and interact with mosquito larvae through competition and predation, i.e. they are controphic in nature. Examples of controphic species of the mosquito larvae is mostly aquatic organism generally found. in the phylum *Arthropoda* with exceptions such as the Anuran larvae and the *Mollusca*. The *Arthropods* include the larvae of *Diptera*, (other than mosquito) *Coleoptera*, *Hemiptera*, *Zygoptera*, *Hydrachnidiae, Anisoptera* and *Crustaceans* among others. The *Coleoptera* adult and larvae, *Anisoptera* and the Chironomidae larvae they act as predator while zooplankton such as *Cyclopoid* and *Ostracoid* act as competitor on mosquito larvae (Kwamboka, 2014). The roles of controphic species in affecting mosquito larval populations has received little attention which is also scarcely published. The published empirical evidence suggests effects on the mosquito larvae by the zooplankton and anuran larvae (Kwamboka, 2014).

The study accompanied with the objective:

To determine the diversity and abundance of controphic species present in selected mosquito larval habitat of Mbale Municipality,

Statement of the Problems

Mosquitoes are not only a nuisance, but also vector of many important human and animal diseases. Despite the support given for the control and prevention of mosquitoes borne-disease, malaria mortality were becoming of serious concern. In Mbale it has been the most important case of illness and death (Namabiwa *et al*, 2011). This is because the Community areas, particularly residential developments, have been located in close proximity to major mosquito or biting midges major breeding sites. Some construction practices in areas which are being developed have also created new mosquito or biting midge breeding habitats Considering the fact that, attempt by government and other donor agencies to eradicate mosquitoes in the Municipality couple with weak control program, used of chemical insecticide for example, ITNs and IRS which is been utilize for a decade causing a negative impact on the environment, non-target organisms, human health and emergence of resistance in vectors.

Research Objectives

To assess the influence of the diversity and abundance of controphic species, on mosquito larval population in selected habitats in Mbale Municipality, so as to improve the knowledge to provide recommendations for the efficient mosquito control practices

Research Hypothesis

The diversity and abundance of controphic species has no effects on the mosquito's larval population

MATERIAL AND METHODS

Study area

The research was conducted at Mbale Municipal Local Government, Mbale District in Eastern Uganda. It is located at 34°10’30.0" East of the prime meridian and 1.04'50.0" North of the Equator. It covers a geographical area of 2,435 hectares (24.35 km2). It is situated 45 kilometers north of Tororo Town, 56 kilometers southeast of Kumi Town, 57 kilometers east of Pallisa Town and 55 kilometers south west of Kapchorwa Town. Mbale is 256 kilometers and 220 kilometers via Tororo and Tirinyi respectively from

Kampala. The study area has a population of approximately 98,189, according to the Uganda population and Housing census 2014, the main agricultural activities mostly cultivation of maize, coffee, vegetable, banana and cocoyam in small scale farming. Cattle, sheep, goats and poultry are owned by member of the community.

Sampling site selection

Thorough search for mosquito larvae positive habitats was done in different water bodies in which five habitat types were: swamps, ditches, ponds, rice field and puddle all within the three division of Mbale municipal council. Namely, Industrial division comprise of pond and ditch, Wanale division with ditch and puddle and Northern division with swamp, puddle and rice field.

Aquatic Organism Sampling and Identification

The samplings was done using a constructed 350ml dipper (diameter, 11.5cm; height 5.5cm) with wooden hand (2 m length) (BioQuip Products, Inc. California, USA) once in a week. Water collected by larval dippers was emptied into a white basin and checked for Mosquito Larvae and controphic species. The organism were identified in the laboratory morphologically using keys developed by Becker *et al.* (2003); Tachet *et al.* (2003); and Streble and Krauter (2006)

After identification, each sample was returned to its preservation bottle using a pipette/forceps. Between the examination of one sample and the next on the counting glass slides were cleaned with distilled water from a wash bottle.

Data analysis

Shannon-weaver indices of species diversity (Longino *et al.*, 2002), for both **mosquito larva and that of controphic species** were calculated for each habitat type. This index is based on the relative composition of species in an area and how equally the individuals are distributed among the species groups or taxa. The Shannon-weaver diversity index, H' was calculated for each count as:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

H' *= the Shannon-Weaver Diversity Index*

Pi *= the proportion of the i^{th} species,*

Loge *= natural log,*

R *= number of species in the community*

Analysis of Variance (One-way ANOVA *followed by post hoc test*) was performed to find out if the abundance of controphic species and mosquitoes larvae varied significantly across habitat types. And also the relationship between the controphic species and mosquito larvae were investigated using Spearman's correlation.

RESULTS AND DISCUSSION

Abundance of controphic species present in mosquito larval habitats

A total of 992 individuals of controphic species were collected, from the sampled mosquito larval habitat. These species belonged to 10 Orders (Table 1). The order Hemiptera had the highest abundance of controphic species with 210 individual and 6 species, followed by the group anural with 201 of individual and 8 species, order Odonata, Diptera (not including mosquito), and Trichoptera, with 135, 135, and 100 individuals, and 10, 3, and 3 species, respectively. Ephemeroptera, Coleoptera, and Cladocera, presented an intermediate abundance with 65, 54, and 39, individuals, 3, 3, and 4, species respectively. The least represented groups in the collections were Plecoptera with only 23 individual and 1 species, Harpacticoida with 22 individual and 5 species (Table 1). The five orders of controphic species were the most encountered which included the Hemiptera, Anural, Diptera, Trichoptera and Odonata dominated the area surveyed amounting to 78.8% of the abundance of all controphic species collected. The remaining order of controphic species accounted for the total of 22.2% of the abundance.

Table 1: Abundances and frequencies of controphic species collected from the different categories of larval habitats in Mbale Municipality.

Order	Total abundance	Mean abundance (Individuals/liter)	Proportion (%)
Hemiptera	218	21.8	22
Anura (Tadpoles)	201	18.3	20.3
Diptera	135	12.3	13.6
Odonata	135	12.3	13.6
Trichoptera	100	9.1	10.1
Ephemeroptera	65	5.9	6.6
Coleoptera	54	4.9	5.4
Cladocera	39	3.5	3.9
Plecoptera	23	2.1	2.3
Harpacticoida	22	2	2.2
Total	**992**		**100**

Distribution of controphic species and mosquito larvae per habitat

In the present study 42 controphic species were recorded and identified based on morphological features in the laboratory. This belonging to 10 orders: belongs to the order Hemiptera, Coleoptera, Plecoptera, Harpacticoida, Diptera, Odonata, Cladocera, Anural, Trichoptera, Ephemeroptera and Nepidae (Table 1). The controphic species and mosquito larvae were collected and recorded in all the five category of larval habitat studied. Ditch habitat had the highest distribution of both controphic species and mosquito larvae, while the swamp recorded least in controphic species distribution and rice field recorded the least distribution in mosquito larvae Table 2.

Table 2: Distribution of controphic species and mosquito larvae per habitat

Habitat type	Controphic species	Mosquito larvae
Ditch	269	191
Pond	258	85
Puddle	212	134
Rice field	136	48
Swamp	117	63
Total	**992**	**521**

Diversity of controphic species and mosquitoes larvae across habitat types

The findings show that ditches had the highest diversity of controphic species (H'= 3.47), while ponds had the lowest (H'= 1.39). Habitat types such as puddle (H'= 2.476), Swamp (H'= 1.45) and Rice field (H'= 1.40), had a moderate diversity of controphic species, (Figure 2). Moreover, based on the diversity of mosquito's larvae the ditch had the highest diversity (H'= 2.13) followed by puddle (H'= 1.44) while the least diverse was rice field which (H'= 0.58) Figure 1.

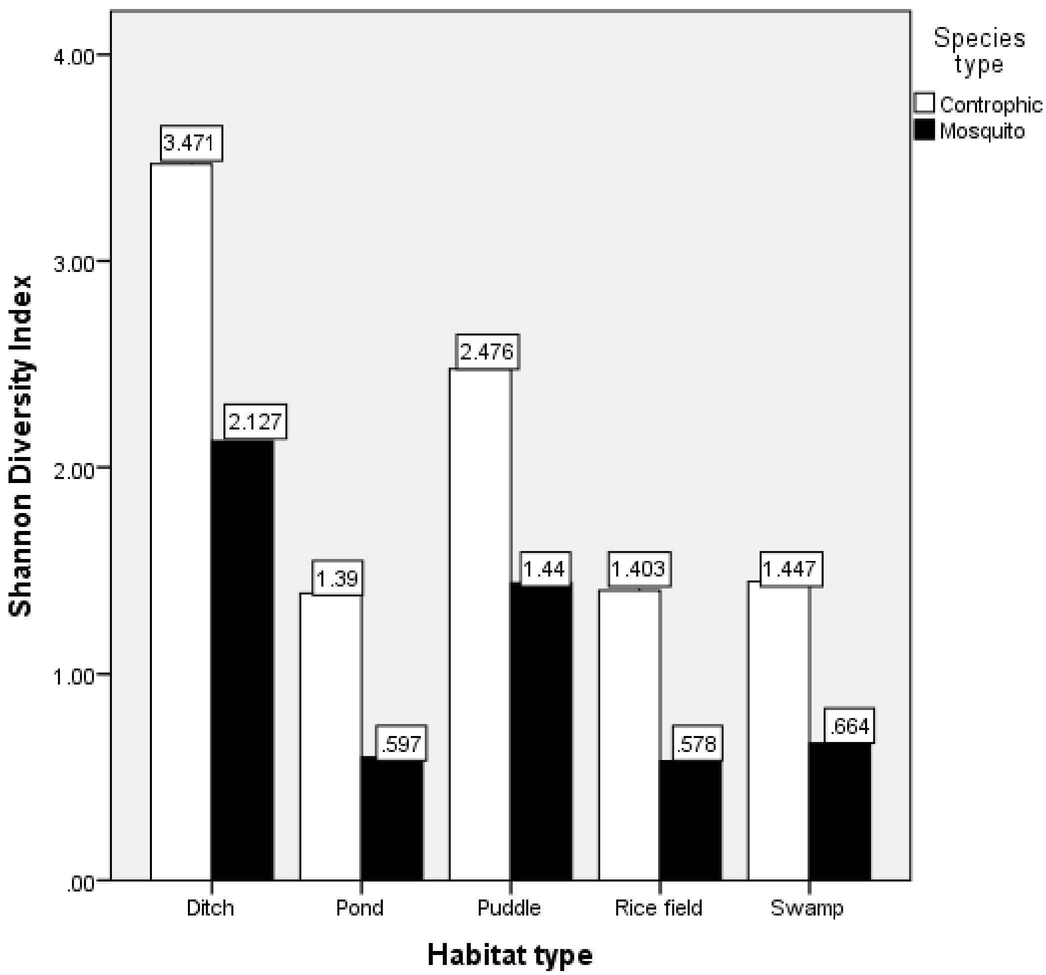

Figure 2: Shannon Diversity index of both Controphic species and mosquito larvae across five category of habitat

Variation in the abundance of Controphic species and mosquitoes larva across habitat types

Results from the one-way ANOVA show that the abundance of controphic species varied across habitats (*ANOVA post hoc test: F= 7.542: p-value = .000*). Ponds supported the highest number (51.60 ± 7.814) with a range of 31 to74 of controphic species while swamps supported the least (16.71 ± 3.490) with a range of 3 to 27 individuals (Table 3). Mosquito larvae were present in all the habitat types but in low abundances as compared to controphic species. The mosquito's larvae abundances ranged from 14 to 42 in Pond with highest mean abundance of 28.33 ± 8.090 larvae, while in the Puddle ranged from 5 to 38 with mean abundance of 22.33 ± 5.875 larvae. Therefore, the Ditch ranged from 2 to 40 with mean abundance of 21.22 ± 5.0747 larvae were not significantly different from Swamp range from 3 to 33 with mean abundant of 21.00 ± 9.165 larvae as observed (*ANOVA post hoc test: F= .276: p-value = .890*) . The rice field ranged from 3 to 26 had the lowest mean abundance (16.00 ± 6.807 larvae) Table 3.

Table 3: The Mean abundances (±SE) of controphic species and Mosquito's larvae across five categories of habitats types namely swamps, rice field pond, ditch and puddle selected for the study

Habitat type	Controphic species	Mosquitoes larvae species
	Mean ± SE (range) S	Mean ± SE (range) S
Ditch	16.81 ± 3.050[a] (4-46) 16	21.22 ± 5.046[b] (2-40) 9
Pond	51.60 ± 7.841[c] (31-74) 5	28.33 ± 8.090[c] (14-42) 3
Puddle	19.27 ± 3.618[ab] (31-74) 11	22.33 ± 5.875[b] (5-38) 6
Rice field	22.67 ± 6.697[b] (7-57)3	16.00 ± 6.807[a] (3-26) 3
Swamp	16.71 ± 3.490[a] (3-27)7	21.600 ± 9.165[b] (3-33) 3

The Means with the same later are not significantly different at 0.05 levels
***S** = absolute number of species,*
***H'** = the Shannon-Weaver Diversity Index*

Relationship between controphic species and mosquito larvae
The relationship between the diversity of controphic species and that of mosquito's larvae was investigated using Spearman's correlation. The results showed that there was a strong positive correlation between the diversity of mosquito larvae and that of controphic species (*r = .900**) and this was highly significant at (*p = .037*) (table 3).

Table 4: Spearman's correlation to examine the relationship between diversity of controphic species and mosquito larvae

			Controphic species diversity	Mosquito larvae diversity
Spearman's rho	Controphic species diversity	Correlation Coefficient	1.000	.900*
		Sig. (2-tailed)	.	.037
		N	5	5
	Mosquito larvae diversity	Correlation Coefficient	.900*	1.000
		Sig. (2-tailed)	.037	.
		N	5	5

*. Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION OF THE FINDINGS

Biological control of mosquitoes using aquatic insect predators and competitors is a feasible alternative to avoid the ill-effects of chemicals hampering the ecosystem functions. The aim of the study was to determine the diversity and abundance of controphic species known to be associated with mosquito larval Oviposition site of Mbale Municipality, with the goal of establishing their potential as biological control agents for mosquito larvae.

Despite its potential for the control of mosquito populations, this strategy has received little attention, and previous work was mostly focused either on the use of insecticide treated bed net (ITNs) or indoor residual spread (IRS) on the mosquito which cause serious environmental problem. The experimental conditions used in this study closely simulated the natural dynamic of an aquatic community dominated by mosquito larvae and the controphic species. A large and taxonomically diverse group of controphic species associated with mosquito Oviposition sites was identified.

The selection of sampling sites within the study area was based on established information available on the type of ecosystem and habitat used by mosquito as likely Oviposition sites. This result indicates that, there is an abundance of controphic species which are known to be associated with mosquito larval habitats of Mbale Municipality. The richness of controphic species collected from the study area is within the range of records of controphic species in mosquito larvae habitats on Rusinga Island and other parts of Kenya as shown in studies by Ohba *et al.,* (2010) and Kweka *et al.,* (2011). They collected similar controphic species from Rusinga Island and a nearby area respectively, to carry out laboratory experiments. Malini, K. *et al.,* (2014) report that numerical abundance and diversity of the constituent species vary in the aquatic habitats thereby influencing the species interactions and shape of the community structure in space and time. This agrees with the results that Ponds supported the highest number of controphic species while swamps supported the least (Table 3). The prey species (i.e. Mosquito larvae) were numerically abundant with low biomass in contrast to the predator species, which is in accordance with the observations for size of prey and predators. Carver *et al.,* (2010) collected similar controphic species which act as predators and competitors in analogous field studies.

Delgado, *et a*l., (1994) also reported on 36 genera, 7 orders of the controphic species were in association with Oviposition sites of mosquitoes. Similarly, 52 genera, 19 families, and 3 orders of controphic species were reported in association with mosquitoes Oviposition sites of which Coleoptera was the most abundant and diverse order (Danis-lozano, *et al.,* 1997).

Bond *et al.* (2014), report on the taxa richness and diversity of aquatic insects associated with breeding sites of mosquitoes in the study he recorded a total of 10 orders, 57 families, 166genera and 247 species, with Shannon index values between3.22 and 3.75.The orders of greatest abundance and species richness were Coleoptera, Odonata and Hemiptera that represented87% of the total species sampled. The diversity of controphic species associated with breeding site of mosquitoes in this study was relatively high. In comparison, with another study done by Bond, *et al.,* (2006) reported 90 genera and 10 Orders of controphic species associated with Oviposition sites of mosquitoes, In this case Shannon index values fluctuated between 2.4 and 3.2.

Furthermore, Kwamboka (2014) collected similar controphic species from the malaria mosquito larval habitats and a further extrapolation by the Jackknife and Shannon index also predicted a richness of 27 species. The presence and abundance of mosquito larvae and controphic species was evident and significantly varied throughout the temporal frame of the study. The abundance of mosquito larvae was higher in Pond and Puddle. Studies by Gimnig *at el.* (2001), found that mosquitoes larvae (e.g. especially *Anopheles spp.*) preferred small aquatic habitats with little or no vegetation. The results showed that the controphic species were the most abundant in the Pond habitats which are temporary in nature. This could be attributed to the abundance of order Hemiptera (which were describe as predators in some literature) were counted in large abundances (unlike the order Harpacticoida that act as competitors in some literature) in these habitats. This demonstrates that, controphic species whose act as predators (data not shown) was of important in reducing mosquito larvae which also abounded in these habitats and therefore potential *Anopheles* vector control tools. This conforms to studies by Chase and Knight (2003), who found predators were of more important in reducing the mosquito larvae in temporary habitats.

The results showed that the diversity of Mosquitoes was mainly positively correlated with the diversity of controphic species. Therefore, when present in the same habitat, species of the order Harpacticoida, such as Assassin bug larvae, Ameridae and larvae of water scorpion compete efficiently for food resources and affect the abundance of mosquitoes Kwamboka, (2014). Similarly, Chase and Knight (2003) demonstrated that non-mosquito competitors (e.g. larvae of Chironomidae, Cladocera) limited the abundance of *Anopheles quadrimaculatus* and *Culex pipiens* to a great extent in temporary ponds.

In addition, the results showed that the controphic species (in some literature report to have act as predators) also influenced, although to a lesser extent than those (report to be competitors in some literature) in the larval populations of mosquitoes in ponds, ditches and other identified mosquito's larval habitat. Cladocera, which are intrigued predators (which were reported by some literature) of mosquito larvae, were found to act as positively in the study. This is in agreement with the results of a number of laboratory and field studies around the world that have shown that Cladocera prey upon mosquitoes (Marten and Reid 2007). Strict controphic species of mosquitoes such as Odonata and Dytiscidae exerted the smallest influence in this study, which might have been due to the relatively low abundances of these taxa in some habitat studied.

CONCLUSION

The study represents the systematic update to the inventory and distribution of controphic species in Mbale Municipal council of Uganda. Believing that this present a valuable contribution to recording the diversity and distribution of the controphic species in this region that is affected by major vector borne diseases, particularly malarial mosquito, Considerably greater sampling effort would be required to yield realistic estimates of total controphic species richness of the region and country, particularly for the Mbale has no comprehensive controphic species inventory, given the great diversity of ecosystems present in this area. This required more of studies on both diversity and abundance of controphic species which might have potential as biological agents for use in the control of the abundance of Mosquito larvae. Such findings with ecological conditions related to biological interactions between mosquito larvae and associated species could help the implementation of appropriate control measures of mosquitoes.

RECOMMENDATIONS

Based on the research findings, the following recommendation were made

1. There is scare information available on the diversity and abundance of aquatic insects (controphic species) in the water bodies of Mbale Municipal council in particular; therefore it is necessary to make continuous studies so that they can be accessible for scientists who are interested in finding proper management plans to protect and as a means to used them for control of mosquito and malaria as well.
2. The controphic species may also be tested for their efficacy while integrating them with various Larval Source Management (LSM) practices such as the addition of *Bti* as well as during short and long hydro period length
3. There is need to collect data in many similar habitat types with proper coordination by the used of global positioning system (GPS) to mapped out the distance covered between one

habitat to another, over a period of time. This will allow better comparison since the sample size will be bigger and similar techniques will have been used.

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