

# Effect of predation on *Anopheles* larvae by five sympatric insect families in coastal Kenya

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## ABSTRACT

**Background & objectives:** The use of insecticides to eliminate mosquito larvae from ground pools may disrupt natural predator-induced control of mosquito larvae. Detrimental effects on predators may be directly from toxicity or by eliminating prey organisms. Identifying the principal predators responsible for mosquito suppression is needed to select non-target indicator species for insecticide studies. In this study, we sought to determine trophic level interactions between predators and immature stages of *Anopheles gambiae* Giles mosquitoes under experimental conditions in the coastal region of Kenya.

**Methods:** To identify effective predation pattern, a series of prey choice experiments was conducted. The relative abilities of five common species of aquatic insects found in the malaria-endemic coastal region of Kenya were assessed in a series of experiments. Experiments were conducted in semi-field conditions at Jaribuni, near the sites of insect collection.

**Results:** In single predator experiments, notonectids consumed most of the mosquito larvae; hydrometrids consumed about half of the mosquito larvae in treatments. Veliids and gerrids had significant, but small effects on larval survivorship. Dytiscids did not have a significant effect on mosquito larvae survivorship. In a two-predator experiment, notonectids significantly decreased survivorship of dytiscids without a change in suppressive effects on mosquito larvae. Of the five common predators evaluated, notonectids were clearly the most voracious consumers of mosquito larvae. The predation pressure on mosquito larvae was not affected by the addition of additional prey items, consisting of small dytiscid beetles. The importance of this notonectid species in coastal Kenya suggests that it would be a valuable non-target indicator species for insecticide studies. Hydrometrids were also efficient at consuming mosquito larvae.

**Interpretation & conclusion:** Of the five common predators from the Kenyan coast evaluated in this study, notonectids were the most voracious consumers of immature mosquitoes. Their predation pressure on mosquito larvae was not affected by the addition of additional prey items, consisting of small dytiscid beetles.

**Key words** *Anopheles gambiae*; insecticides; non-target insects; predation; semi-field conditions

## INTRODUCTION

Natural regulation of mosquito larvae is an important factor in determining the survivorship of mosquito immatures. Predation is recognized as an important factor in the organization of many ecological communities including aquatic communities<sup>1, 2</sup>. Together with insect pathogens, predation can significantly limit numbers of mosquitoes depending on the species and type of habitat<sup>3</sup>. Mosquito larvae and their predators co-exist in a variety of aquatic habitats ranging from large and permanent sites to small and temporary collections of water.

Predation is reported as one of the most limiting factors causing a high level of mortality to immature stages

of mosquitoes<sup>4, 5</sup>. In a study carried out by Service<sup>5</sup> on natural mortalities and predation of immature stages of *Anopheles gambiae* Giles, mortality was observed to be higher in the rice pools than in the small pools and ponds. Precipitin tests on smears of gut contents of possible predators showed that Coleoptera larvae, Hemiptera and predacious adult Dipterans were important predators. Although not all Hemipterans tested produced a positive reaction to *An. gambiae* antisera, species of *Laccotrephes*, *Enithares*, and *Anisops* and various *Corixidae* known predators<sup>6</sup>. In Tanzania, Christie<sup>7</sup> observed intense predation pressure by Notonectids on mosquito larvae and pupae. However, in Japan, Toshihiko and colleagues<sup>8</sup> observed that Notonectids and *Chaoborus* species affected

mosquito larvae more than other predators. The impact of other predators such as the Dragonfly nymphs, Dytiscid and Hydrophilid beetles were limited to large deep containers.

A high level of *An. gambiae* infected with larval nematodes and the fungus *Coelomomyces* was reported in the Kisumu area of Kenya, and in certain instances, larval nematodes inhibit ovarian development in *An. funestus* Giles<sup>4</sup>. At the Kenya Coast, Otieno *et al*<sup>9</sup> reported *Coelomomyces* related mortality of 63% among larvae of *An. gambiae* in a two-season observation near Mombasa. Sabwa *et al*<sup>10</sup> also reported maximum *Coelomomyces* infection levels of *An. gambiae* in between 70 and 100% in semi-permanent and temporary pools. Numerous parasites of mosquito larvae have been tested for mosquito control, including Nematode (mermithid worms) and parasitic protozoa (*Microsporidia*, *Tetrahymena*, *Lambornella* and *Helicosporidium*) but none has proven suitable for operational use. However, heavy parasitic infections of mosquito larvae are not common in nature and it is not possible to release a small number of parasites to multiply and spread throughout a mosquito population. Apart from the natural regulation of mosquito numbers, predators and pathogens have raised interest due to their potential for manipulation of biological control as part of Integrated Vector Management (IVM)<sup>11</sup>.

Studying predation pressure on *Anopheles* mosquito larvae is made more complicated by having at least two distinct guilds of predators<sup>12</sup>. The *Anopheles* mosquito larvae, filter feeding at the surface of the water, must contend with potential attacks from both surface and submerged predators. Since predation is reported to play such a critical role in mortality of mosquito larvae, insect pathogens and predation can significantly reduce numbers of immature stages of mosquitoes. Understanding the factors that shape the patterns of larval density fluctuation, therefore, helps to understand similar changes in the adult populations. The reduction of larval populations from instar to instar through predation would be an ideal base for decisions on where to attack and what to use for the destruction of immature stages of mosquitoes. In this study, we sought to determine trophic level interactions between predators and immature stages of *An. gambiae* mosquitoes under experimental conditions in the coastal region of Kenya.

## METHODS

### Study area

The study was carried out in Kilifi district of Coast Province of Kenya which lies approximately 60 km north of Mombasa city. The district has two main rainfall sea-

sons in a year. The long rains from April to June and the short rains from October to December. The average rainfall ranges from 400 mm in the hinterland to between 900 and 1100 mm in the coastal belt. The district is usually hot with temperature of up to 34°C and humid all the year round with humidity of over 60% in the coastal strip. The experiments were carried out during the wet season between April and September 2004. Malaria is the leading cause of morbidity, constituting 42–48% of all illnesses clinically diagnosed at Kilifi District Hospital. Most cases occur in July following the long rainy period<sup>13</sup>. The study was conducted in small pools along the Jaribuni River (39° 44.34'E, 03° 36.64'S) along the Kenyan coast. The study area has been previously described for entomological surveys<sup>14–16</sup>.

### Collection of predators of anopheline larvae and pupae

Four permanent sites in Jaribuni were sampled for the qualitative description of invertebrate biology. Data collected included families of invertebrates present, species of mosquitoes and descriptions of their habitats. Previous investigations in the study area revealed a stable presence of five predator families in small pools along the Jaribuni River in the Kenyan coastal province which were mainly in two orders: Hemiptera (Gerridae, Hydrometridae, Veliidae and Notonectidae) and Coleoptera (Dytiscidae) (Mbogo, personal communication). Predators were collected by the use of aquatic nets around the perimeter of the habitat. Predators once collected were placed in plastic basins with water and then covered with a net to avoid escape. After collection, predators were sorted out and identified in the field by the use of morphological keys<sup>12</sup>. Reference specimens for future identifications were placed in microfuge tubes with 75% ethanol and labeled accordingly. The reference specimen were identified morphologically at the Kenya National Museums in Nairobi as *Micrivelia* sp (Veliidae), *Hydrometra* sp (Hydrometridae), *Anisops debilis*, Gerst (Notonectidae), *Gerris hypolence*, Gerst (Gerridae) and *Hydrovatus cribratus*, Sharp (Dytiscidae).

### Collection of anopheline larvae and pupae

Collection of mosquito larvae and pupae was done by the standard dipping technique<sup>17</sup>. Every scoop from the aquatic habitat was filtered through a fine mesh and was then backwashed into a white tray. The collected larvae and pupae in the bowl were picked by a pipette and put in petri dishes according to their developmental stages (larval I–IV instars). Mosquito larvae were categorized as either culicine or anopheline according to their morphological characteristics<sup>18</sup>. After identification, the im-

mature larvae and pupae were placed in small petri dishes with water according to their genera and instar stage. A representative sample of III and IV instars of *Anopheles* were preserved in 75% ethanol for further identification of species using morphological features<sup>18</sup>. This subset of larvae was identified microscopically as *An. gambiae* s.l. and *An. funestus*.

*Determination of trophic level interactions between predators and immature stages of anopheline mosquitoes under experimental conditions*

To identify effective predation pattern, a series of prey choice experiments were conducted. Experiments were conducted in a semi-field condition at Jaribuni, near the sites of insect collection. In the semi-field conditions, a temporary structure which served as a field insectary was constructed at Jaribuni River banks. Briefly, the field insectary was constructed of wood pillars and the roof made of palm thatch. The sides of this structure were covered about 3 ft all round using palm leaves. The remaining part of the wall section was left open. This allowed free movement of air and provided natural condition for the larval development as experienced in the natural habitats.

For each experimental treatment, five individuals of two different families of the identified predators were put together with five anopheline larvae and pupae from each instar and placed by use of a pipette into a 3.5 L white plastic basin. The basins contained 1L of net-filtered stream water and were covered with netting to prevent escape of study insects or introduction of outside insects. In total, 20 mosquito larvae and 5 pupae were put in each basin. Five individuals of two different families of predators were added to the larvae and pupae in the basin. The control consisted of mosquito larvae and pupae and one single predator family with larva and pupa as the predator control. Five replicates of each treatment set were done. Numbers of the surviving larvae were counted 6 and 24 h after introduction of predators and the difference between the initial and the final number of larvae was recorded.

Experimental treatments were set up in plastic basins containing five liters of river water, as well as five L1, five L2, five L3, five L4, and five pupae of *Anopheles* larvae, and covered with mosquito net to prevent escape or entrance of insects. Control treatments contained no predators while experimental treatments contained five members of a selected predator species which included gerrids, hydrometrids, veliids, notonectids and dytiscids. The number of replicates for each types of predators were: gerrids (12 replicates); hydrometrids (16 replicates); veliids (12 replicates); notonectids (18 replicates); and dytiscids (20 replicates). The treatments were left for 24

h at ambient conditions, then censused.

A series of experiments were conducted to test the effect of an alternate food source on predation using notonectids, dytiscids, and mosquito larvae. For each of 10 replicates, one treatment contained only mosquito larvae as used for the experiments described above, one treatment contained mosquito larvae and five dytiscids, one treatment contained mosquito larvae and five notonectids, and one treatment contained mosquito larvae, five dytiscids, and five notonectids.

*Data analysis*

Data from pre-coded forms were checked for accuracy, logic and range using SPSS version 15.0 (SPSS, Inc., Chicago, IL). To determine how the presence of predators affected mosquito larvae survivorship, single predator treatments of individual families were compared with no predator treatments using a Mann-Whitney U-test for independence. Means and standard deviations were calculated for each predator to compare the magnitude of the impact.

To assess effects of notonectids on dytiscids, dytiscids mortality rates in the treatments were compared, pairing the mortality rate when alone with the mortality rate in the presence of notonectids in concurrently run treatments (paired *t*-test). The mortality rates of *Anopheles* larvae in the presence of notonectids alone and notonectids and dytiscids were compared using a Mann-Whitney U-test for independence.

## RESULTS

More larvae were consumed by notonectids compared to other potential predators. Hydrometrids were the second most effective potential predators after the notonectids. Veliids significantly reduced II and IV larval instars while gerrids reduced mostly the III instars larvae. Dytiscids had low impact on all larval and pupal stages. When single-predator treatments were compared with controls, only dytiscids failed to significantly decrease numbers of immature mosquitoes (Mann-Whitney U = 231;  $p = 0.32$ ). Notonectids consumed the largest number of larvae and pupae (Mann-Whitney U  $\leq 0$ ,  $p < 0.001$ ) with hydrometrids also consuming over half of the mosquitoes (Mann-Whitney U = 13,  $p < 0.001$ ). Although the presence of veliids (Mann-Whitney U = 113;  $p = 0.02$ ) and gerrids (Mann-Whitney U = 69.5;  $p < 0.001$ ) led to a statistically significant reduction in number of mosquitoes, over 70% of mosquitoes survived for 24 h. Mortality events were not different by mosquito instar (Table 1).

To determine whether there were any competition

Table 1. Mean number of immature mosquitoes surviving 24 h in the presence of five predators<sup>1</sup>

Predator family (n=5)	I instar (n=5)	II instar (n=5)	III instar (n=5)	IV instar (n=5)	Pupae (n=5)	Total (n=25)
Notonectidae (n=18)	0.9 ± 1.19*	0.5 ± 0.97*	0.5 ± 0.78*	0.7 ± 1.11*	1.4 ± 2.06*	4 ± 4.78*
Hydrometridae (n=16)	2.4 ± 1.46*	1.6 ± 1.15*	2.9 ± 1.44*	2.8 ± 1.18*	2.7 ± 1.35*	12.4 ± 4.37*
Gerridae (n=12)	3.3 ± 1.44	3.5 ± 1.39	3.9 ± 1.26	4.1 ± 1.19	3.3 ± 1.60*	18.2 ± 3.05*
Veliidae (n=12)	3.4 ± 1.08	3.3 ± 1.23*	4.6 ± 0.79	4.1 ± 1.24	4.8 ± 0.97	20.2 ± 2.08*
Dytiscidae (n=20)	3.6 ± 1.54	3.8 ± 1.44	4.7 ± 1.25	4.1 ± 1.57	4.4 ± 1.41	20.6 ± 5.05

<sup>1</sup>Mean ± standard deviation of the initial 5 mosquitoes of each instar surviving, adjusted for mortality in paired controls; \*Difference between experimental and control treatments significant at  $p < 0.05$ .

Table 2. Difference in mean number of mosquitoes surviving single and two-predator treatments

Predator (n=5)	I instar (n=5)	II instar (n=5)	III instar (n=5)	IV instar (n=5)	Pupae (n=5)	Total (n=25)
G (n=12) G + H (n=4)	-0.8	-1.3	-1.9*	-2.1*	-1.6	-7.7*
G (n=12) G + V (n=3)	-0.6	-0.2	0.4	-0.1	-0.3	-0.8
G (n=12) G + N (n=2)	-1.6	-3.5*	-3.6*	-3.4*	-2.6*	-14.8*
G (n=12) G + D (n=3)	-1	-0.2	-0.6	-1.4	-0.3	-3.5
V (n=12) V + G (n=3)	-0.8	0	-0.3	-0.1	-1.8	-2.8
V (n=12) V + H (n=4)	-0.7	-2.8*	-1.3*	-1.8*	-3.3*	-9.9*
V (n=12) V + D (n=3)	-0.8	0	-0.3	-0.8	-0.4	-2.2
V (n=12) V + N (n=2)	-1.9*	-3.3*	-4.6*	-4.1*	-3.3*	-17.2*
N (n=18) N + G (n=2)	0.7	-0.5	-0.2	0	-0.7	-0.7
N (n=18) N + H (n=4)	-0.9	-0.2	-0.5	-0.7	-0.9	-3.3
N (n=18) N + V (n=2)	0.6	-0.5	-0.5	-0.7	0.1	-1
N (n=18) N + D (n=10)	-0.8	-0.1	-0.5	-0.5	0.1	-1.8
H (n=16) H + G (n=4)	0.1	0.6	-0.9	-0.8	-0.9	-1.9
H (n=16) H + V (n=4)	0.3	-1.1	0.3	-0.5	-1.2	-2.2*
H (n=16) H + N (n=4)	-2.4*	-1.4*	-2.9*	-2.8*	-2.2*	-11.7*
H (n=16) H + D (n=4)	-0.7	0.4	-0.4	-0.3	-0.9	-1.9
D (n=20) D + V (n=3)	-1	-0.4	-0.4	-0.7	-0.1	-2.6
D (n=20) D + N (n=10)	-3.5*	-3.4*	-4.7*	-3.9*	-2.9*	-18.4*
D (n=20) D + G (n=3)	-1.3	-0.4	-1.4	-1.4	-1.4	-5.9*
D (n=20) D + H (n=4)	-1.9	-1.8*	-2.2*	-1.6	-2.7*	-10.1*

H = Hydrometrids, D = Dytiscids, N = Notonectids, V = Veliids, G = Gerrids; \* $p < 0.05$  (Mann-Whitney U-test); Negative numbers refer to a lower survivorship in two predator treatments; positive numbers refer to a higher survivorship in two predator treatments.

effects, larval and pupal mortalities in one and two predator treatments were compared (Table 2). In the multiple prey experiments, the addition of notonectidae to dytiscidae significantly increased the level of consumption of both larvae and pupae ( $p < 0.05$ , Mean change = -18.4). Furthermore, the addition of dytiscidae to notonectidae did not alter the level of consumption of the larvae by dytiscidae ( $p > 0.05$ , Mean change = -1.8). The addition of other predators to notonectids had no effect on the reduction of larvae and pupae ( $p > 0.05$ ). Interestingly, the addition of notonectids to any of the other four predators (veliids, gerrids, hydrometrids and dytiscids) significantly increased the consumption of larvae.

## DISCUSSION

Of the five predators evaluated, notonectids consumed the most mosquitoes of all immature instars. Notonectids

eliminated most of the immature mosquitoes present, more than twice the number of mosquitoes eaten by hydrometrids, the next most efficient mosquito predator. The predation pressure exerted on mosquitoes by notonectids was not diminished by the presence of additional prey organisms consisting of a species of small dytiscids.

In two predator combinations, the addition of notonectids to any other four predators resulted in increased level of larval consumption. These higher predation effects on mosquito larvae with synergistic effect could have been brought about by competition amongst predators. Earlier studies have shown that intra-guild predation can co-exist and the co-existence disrupts the suppression of their prey<sup>19</sup>. Notonectids evaluated in other parts of the world have also found them to be voracious mosquito predators<sup>20</sup>. Notonectids have the ability to swiftly dive under water (submerged predator) and frequently come to the surface for breathing which makes it



efficient predator for *Anopheles* larvae. Studies on feeding habitats have shown that notonectids preferred to feed on mosquitoes<sup>21–23</sup>. Hydrometrids may also be important for regulating populations of mosquito larvae. The importance of hydrometrids may be poorly described by the single-predator experiments in this study. In the natural habitats, where both hydrometrids and notonectids are present, there may be cross-guild, cooperative suppression of prey. Mosquito larvae, feeding and breathing at the water surface, are prime targets for predacious surface skaters (such as Gerrids, hydrometrids and veliids). When diving to escape this predation, the larvae would be highly susceptible to the back swimming notonectids and other submerged predators.

Resource competition is a common phenomenon amongst insects. Notonectids combined with hydrometrids yielded a higher larvae and pupae consumption rates suggesting that there is an interspecific competition on the prey population. The competition effects of notonectids and hydrometrids contributed to the increased consumption of the larvae and pupae which were their shared natural prey. The results of this study further suggest that there was interspecific competition on the prey populations. The interspecific competition effects of notonectids and hydrometrids contributed to increased consumption of the anopheline larvae and pupae. In natural settings, habitats which have the two predators present may impact negatively the *Anopheles* larval population, lowering the habitat's productivity and contribution to adult mosquitoes.

Dytiscids, like the Coleoptera as a whole, are a highly divergent family of insects, with sizes ranging several orders of magnitude. While the small species of dytiscid used in these experiments was a prey organism for notonectids, it is likely that the many larger species would instead predate upon the notonectids. The impacts of other predators such as dragonfly nymphs, adult and larvae of dytiscid and hydrophilid beetles are important predators in some pool habitats<sup>4, 5, 24</sup>.

In light of the strong predation pressure put upon immature mosquitoes by the notonectids in these experiments, future studies on insecticides in the region should include notonectids as a key non-target indicator. Should an insecticide suppress notonectid populations in addition to the populations of mosquitoes, there is a risk of rebound mosquito outbreaks. Such outbreaks occur whenever natural population regulation mechanisms are disrupted, and have followed predator disturbances from a variety of sources, such as hurricanes<sup>25</sup>.

Malaria vector control efforts have been done majorly through the scaling-up of the distribution of long-lasting insecticide-treated bednets (LLINs)<sup>26</sup>. Larval control

strategies such as larval source management (LSM) mainly targeting the immature stages of mosquitoes while in the aquatic habitats when implemented together with LLINs could offer a promising significant reduction in malaria vectors hence resulting in interruption in malaria transmission<sup>27–31</sup>. Appropriate implementation of LSM could be useful in safeguarding and sustaining the gains achieved so far by the use of LLINs and also reduce the outdoor transmission in even key malaria vectors have behavioral shifts<sup>32</sup>.

In conclusion, there has been little research on the interactions among predators across the surface of the water. Defining the trophic interactions of surface, submerged, and amphibian predators will aid efforts to develop biological control strategies for malaria and other mosquito-borne pathogens. Of the five common predators from the Kenyan coast evaluated in this study, notonectids were by far the most voracious consumers of immature mosquitoes. The predation pressure on mosquito larvae was not affected by the addition of additional prey items, consisting of small dytiscid beetles. If insecticides suppress notonectids in addition to target mosquito larvae, there is a risk of a subsequent mosquito outbreak. Hydrometrids were also efficient at consuming mosquito larvae.

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## Conflict of interest

The authors decline no conflicting interest in publishing this paper.

## REFERENCES

1. Sih AP, Crowley M, Mcpeek JP, Strolaneier K. Predation, competition and prey communities: A review of field experiments. *Ann Rev Ecol Syst* 1985; 16: 269–311.

2. Zaret T. *Predation and freshwater communities*. New Haven: Yale University Press 1980; p. 187.
3. Knight TM, Chase JM, Goss CW, Knight JJ. Effects of interspecific competition, predation and their interaction on survival and development time of immature *Anopheles quadrimaculatus*. *J Vector Ecol* 2004; 29(2): 277–84.
4. Service M. Mortalities of the larvae of *Anopheles gambiae* Giles complex and detection of predators by precipitin test. *Bull Entomol Res* 1973; 62: 359–69.
5. Service MW. Mortalities of the immature stages of species B of the *Anopheles gambiae* complex in Kenya: Comparison between rice fields and temporary pools, identification of predators, and effects of insecticidal spraying. *J Med Entomol* 1977; 13 (4–5): 535–45.
6. Jenkins DW. Pathogens, parasites and predators of medically important arthropods. *Bull World Health Org* 1964; 30: 150.
7. Christie M. Predation on larvae of *Anopheles gambiae* Giles. *Am J Trop Med Hyg* 1958; 61: 168.
8. Toshihiko S, Kenji I, Motoyoshi M. Habitat size: A factor determining the opportunity for encounters between mosquito larvae and aquatic predators. *J Vector Ecol* 2002; 27(1): 8–20.
9. Otieno WA, Odindo MO, Sabwa DM. Occurrence of *Coelomomyces indicus*: A fungal pathogen of malaria mosquito vector *Anopheles gambiae* complex along the Kenya coast. *Insect Sci Appl* 1985; 6(2): 199–204.
10. Sabwa DM, Odindo MO, Otieno WA. Seasonal incidence of *Amblyospora* sp. (Thelohaniidae: Microsporidia) in *Culex sitiens* larvae at the Kenya coast. *Insect Sci Appl* 1984; 5(4): 269–72.
11. *Global strategic framework for integrated vector management*. WHO/CDS/CPE/PVC/2004.10. Geneva: World Health Organization 2004.
12. Merritt RW, Cummins KW. *An introduction to the aquatic insects of North America*, III edn. Iowa: Kendall/Hunt Publishing Company 1996; p. 110–484.
13. Mbogo CM, Snow RW, Kabiru E, Ouma JH, Githure JJ, Marsh K, Beier J. Low-level *Plasmodium falciparum* transmission and the incidence of severe malaria infection on the Kenyan coast. *Am J Trop Med Hyg* 1993; 49: 245–53.
14. Mbogo CM, Mwangangi JM, Nzovu J, Gu W, Yan G, Gunter J, et al. Spatial and temporal heterogeneity of *Anopheles* mosquitoes and *Plasmodium falciparum* transmission along the Kenyan coast. *Am J Trop Med Hyg* 2003; 68(6): 734–42.
15. Mbogo CMN, Glass GE, Forster D, Kabiru EW, Githure JJ, Ouma JH, et al. Evaluation of light traps for sampling anopheline mosquitoes in Kilifi, Kenya. *J Am Mosq Control Assoc* 1993; 9(3): 260–3.
16. Mwangangi JM, Mbogo CM, Muturi EJ, Kabiru EW, Githure JJ, Novak RJ, et al. The influence of biological and physicochemical characteristics of larval habitat on the body size of *Anopheles gambiae* (Diptera: Culicidae) mosquitoes. *J Vector Borne Dis* 2007; 44: 121–6.
17. Service M. *Mosquito ecology: Field sampling methods*, II edn. London, U.K.: Elsevier Applied Science 1993.
18. Gillies MT, Coetzee M. *A supplement to anophelinae of Africa south of Sahara (Afro-tropical region)*. Publication of the South Africa Institute of Medical Research 1987; 55: 1–143.
19. Holt RD, Polis GA. A theoretical framework for intraguild predation. *Am Naturalist* 1997; 149: 745–64.
20. Lacey LA, Orr BK. The role of biological control of mosquitoes in integrated vector control. *Am J Trop Med Hyg* 1994; 50(6): 97–115.
21. Ellies RA, Borden JH. Predation by *Notonecta undulata* on larvae of yellow fever mosquitoes. *Ann Entomol Soc Am* 1970; 63: 963–73.
22. Morales ME, Wesson DM, Sutherland IW, Impoinvil DE, Mbogo CM, Githure JJ, et al. Determination of *Anopheles gambiae* larval DNA in the gut of insectivorous dragonfly (Libellulidae) nymphs by polymerase chain reaction. *J Am Mosq Control Assoc* 2003; 19(2): 163–5.
23. Toth RS, Chew RN. Notes on behavior and colonization of *Buenoa scimitra*: A predator of mosquito larvae. *Environ Entomol* 1972; 1: 534–45.
24. Stav G, Blaustein L, Margalit J. Experimental evidence for predation risk sensitive oviposition by a mosquito, *Culiseta longiareolata*. *Ecol Entomol* 1999; 24: 202–7.
25. Hunter MD. Ecological causes of pest outbreaks. In: Pimentel D, editor. *Encyclopedia of Pest Management*. New York: Marcel Dekker Inc. 2002; p. 214–7.
26. Noor AM, Muthu JJ, Tatem AJ, Hay SI, Snow RW. Insecticide treated net coverage in Africa: Mapping progress in 2000–07. *Lancet* 2009; 373(9657): 58–67.
27. Beier JC, Keating J, Githure JG, Macdonald MB, Impoinvil DE, Novak RJ. Integrated vector management for malaria control. *Malar J* 2008; 7(Suppl 1): S4.
28. Gu W, Novak RJ. Habitat-based modeling of impacts of mosquito larval interventions on entomological rates, incidence, and prevalence of malaria. *Am J Trop Med Hyg* 2005; 73: 546–52.
29. Killeen G, Fillinger U, Kiche I, Gouagna L, Knols B. Eradication of *Anopheles gambiae* from Brazil: Lessons for malaria control in Africa. *The Lancet Infect Dis* 2002; 2: 618–27.
30. Protopopoff N, Bortel WV, Marcotty T, Herp MV, Maes P, Baza D, et al. Spatial targeted vector control is able to reduce malaria prevalence in the Highlands of Burundi. *Am J Trop Med Hyg* 2008; 79(1): 12–8.
31. *Handbook for Integrated Vector Management (IVM)*. Geneva: WHO Press 2012; WHO/HTM/NTD/VEM/2012.3.
32. Mwangangi JM, Mbogo CM, Orindi BO, Muturi EJ, Midega JT, Nzovu J, et al. Shifts in malaria vector species composition and transmission dynamics along the Kenyan coast over the past 20 years. *Malar J* 2013; 12: 13.

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