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ORIGINAL ARTICLE

Predator preferences: a key to effective biological control design

Preferências do predador: a chave para um projeto de controle biológico eficaz

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Abstract

Objetctive: This experimental study aimed to assess the preference of *Gambusia affinis* to mosquito larvae of An.gambiae s.s., *Cx. quinquefasciatus* and *Aedes aegypti*. **Method**: Three *Gambusia affinis* were introduced in a glass container with a dimension of 45cm x 25cmx 25cm. Three larvae densities were used, 90 (30 larvae per species), 120 (40 larvae per species), and 180 (60 larvae per species). Each density experiment was set in triplicate and monitored after 1, 2, 3, and 24 hours. No fish food was added to the container for larvae. Results: **Results** have shown that in all times *A. aegypti* has been the most preferred species by *Gambusia affinis*. Among the tested species, *A. aegypti* was most prayed with time and in different densities. In mixed models including density, species, and time there was no significant difference among the species predation. **Conclusion:** Preliminary results have shown that the appropriate choice of predators for each mosquito species can have a great impact on bio-control to substantially complement existing tools.

Keywords: Larvae. Mosquito. Reduction. Predator. Bio-control.

Resumo

Objetivo: avaliar a preferência de *Gambusia affinis* por larvas de mosquito de *An. gambiae* s.s., *Cx. quinquefasciatus* e *Aedes aegypti*. **Método**: Três *Gambusia affinis* foram introduzidos em um recipiente de vidro com dimensões de 45cm x 25cm x 25cm. Foram utilizadas três densidades de larvas, 90 (30 larvas por espécie), 120 (40 larvas por espécie) e 180 (60 larvas por espécie). Cada experimento de densidade foi estabelecido em triplicado e monitorado após 1, 2, 3 e 24 horas. Nenhum alimento de peixe foi adicionado ao recipiente com larvas. **Resultados**: Os resultados mostraram que em todos os tempos o *A. aegypti* foi a espécie mais preferida por *Gambusia affinis*. Entre as espécies testadas, *A. aegypti* foi a mais predada com o tempo e em diferentes densidades. Em modelos mistos incluindo densidade, espécie e tempo, não houve diferença significativa entre a predação por espécies. **Conclusão**: Os resultados preliminares mostraram que a escolha apropriada de predadores para cada espécie de mosquito pode ter um grande impacto no bio-controle para complementar substancialmente as ferramentas existentes.

Palavras-chave: Larvas. Mosquito. Redução. Predator. Bio-controle.

INTRODUCTION

Malaria vector control still a most important target for malaria disease burden reduction complementing with targeting aquatic habitats[1]. Targeting the larvae habitat where the movement of larvae is restricted is of paramount importance[2, 3]. Mosquito-borne diseases remain a public health problem in tropical and subtropical countries, with some progress made towards malaria control and subsequently eradication[4]. The use of synthetic Insecticides remains one of the most reliable strategies for controlling the spread of disease-carrying mosquitoes[1]. However, studies have revealed that the prolonged use of chemicals has adverse effects on the environment and also non-target animals[5-7], which also causes insecticide resistance to malaria vectors population[8]. Biological control of mosquito vector species is an alternative and efficient, sustainable, and environmentally friendly method[9], which is unlikely to cause resistance[10, 11] and efficient to both susceptible and resistant species. Biological control systems have the advantage of controlling mosquito populations at the larval stage, where it is immobile and easily targeted, consequently disrupting the mosquito life cycle and population reduction from breeding sites[12, 13].

Larvivorous fish have been implemented for mosquito control in various parts either in semifield or full field, with their efficacy being well established and documented[14-18]. The use of indigenous fish species has been favoured over other predators (insects), as not only do they reduce mosquito larvae populations, but also contribute towards indirectly increasing the aquaculture economics[12,18,19]. One of the species that has been extensively studied is, *Gambusia affinis*, a small opportunistic fish whose preys include, zooplankton, invertebrates (insects, worms, molluscs, and others and aquatic plants found surfacing the top of the water column1[2, 20]. These fish have a high feeding potential on mosquito larvae, as shown by studies conducted by Shukla and others[12], hence can serve as a good biological control agent against them.

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An understanding of predator-prey relationships is crucial when selecting the appropriate control system to be implemented. Prey is capable of altering their morphological, behavioural, or developmental features as a means to counteract predation[5, 21]. Hence the consequence such as body size change for emerging adults and sex ratio shift has been observed[10, 11].

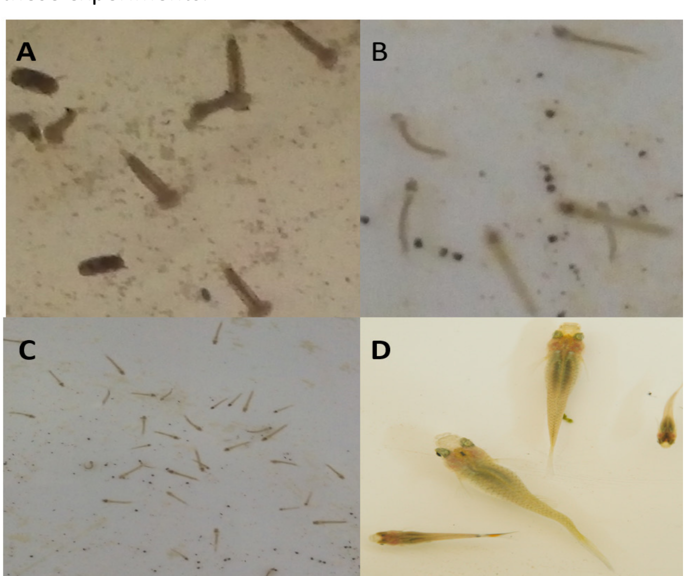
Most of the studies done on the predatory efficacy of *G.affinis* mainly focus on individual mosquito larvae species, this current study aimed to fill this gap. It compared the predatory preferences and efficacy of *Gambusia affinis* on three mosquito larvae species namely: Anopheles gambiae; Culex quinquefasciatus; and *Aedes aegypti* collectively. The mosquito larvae species were each presented at increasing densities. The predatory performance of *G.affinis* will provide a clearer understanding of its prey preferences if there is any selectivity with their dietary choices, and how this knowledge can be exploited when designing appropriate biological control systems that target specific mosquito species.

METHODS

Larvae rearing

Three mosquito larvae species were selected for this study. Third instar larvae of An.gambiae s.s, *Cx. quinquefasciatus* and *Ae.aegypti* (Figure 1) were used. Experiments used three densities containing each species in equal proportions. The densities were 90 larvae (30 larvae for each species), 120 larvae (40 larvae each species), and 180 larvae (60 larvae each species). In each experiment, three predators were placed in each container for 24 hours and monitoring at intervals of 1 hr, 2 hr, 3 hr, and lastly at 24 hr. The predator used was *Gambusia affins* (Figure 1)

Figure 1. Mosquito larvae A) *Cx. quinquefasciatus*, B) Ae. aegypti, C) *An. gambiae* s.s. and D) predator G. affinis used in these experiments.



Data analysis

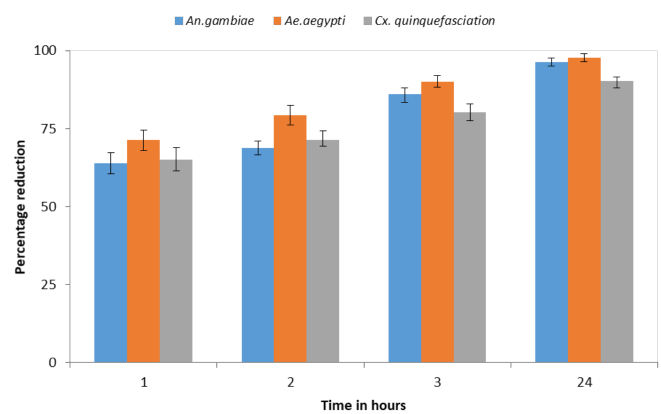
Data were recorded in excel and transferred for analysis in SPSS version 25 (Inc., Chicago, USA). The comparison of the predation rate was done on time, density, and time. The comparison was done with one way ANOVA.

RESULTS

Predation with time

The monitoring of predation time revealed that all three species had significant differences in percentage reduction with time *An. gambiae* ($P<0.001$), *Cx. quinquefasciatus* ($P=0.004$), and Ae. aegypti ($P=0.003$). In all observation time *A. aegypti* was mostly prayed than others (Figure 2).

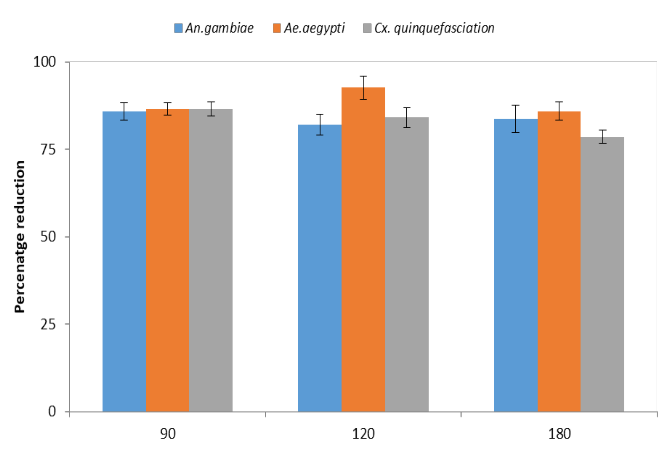
Figure 2. Predation rate for three mosquito species with time



Percentage reduction by density

At the density of 90 larvae, there was no significant difference between the three species ($F=3.022$, $df = 2$, $P = 0.062$), (Figure 3).

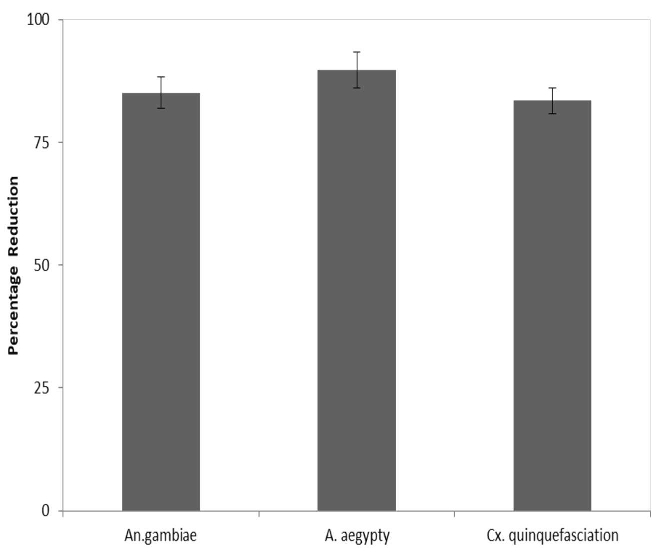
Figure 3. Percentage reduction of mosquito species by density



In the density of 120 larvae, there was a significant difference in larvae reduction *Aedes aegypti* reduced the most (F = 3.468, df = 2, P = 0.043) (Figure 2), while the density of 180 reductions between the species was statistically significant *A. aegypti* reduced most (F = 15.393, df = 2, P<0.001) (Figure 3).

In a mixed factors model, the interaction between density and time in reduction had no significant difference for all three species (F = 0.24, df = 2, P = 0.7880) (Figure 4)

Figure 4. Predation comparison with mixed models including time, density, and predators



DISCUSSION

The findings of this study have shown that exposing larvae predators to the combination of different mosquito species at once, can highly give an appropriate insight of the species to be targeted using the evaluated predator. This study has shown similar results of predator choices against mosquito species when occurring in co-habitation[22-24]. In semi-field studies conducted in western using five predators against An.gambiae larvae alone, showed a great variation on larvae predation[11].

In this study, the density of the predators was fixed to three per artificial habitat but the density of larvae was varying from 90,120 and 180 per artificial habitat with an equal number of each species. The predation rate was species-specific where A.aegypti was most prayed in all densities. In previous studies, predators evaluated showed law rates of preying on *An.gambiae* s.s. larvae which might not be the predator preference[11]. In India, *Gambusia affinis* have shown a high degree of predation on mosquito larvae in semifield experiments and full-field trial choice [25, 26].

In predation monitoring for 24 hr, the predation rate was significant with time for each species, but at the 24 hr of observation, *A.aedes* was mostly preferred. Preference of predators with time has previously been observed for An.gambiae s.s. using different densities and predator species 10, 11. The predation rates for all species with time have shown at 24hr A.aegypti was highly preferred. The availability of different prey at a time in habitat resembles the complex food web of which predator genuinely makes a choice. The ability to be specific in a complex system ascertains the efficiency of semifield and small-scale trials[2,11,23].

CONCLUSION

The findings of this study have shown that predator species specificity is of paramount importance in vector species control. Further experiments should be done with complex settings including refugia for larvae.

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