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#### Acta Tropica

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## Direct and indirect effect of predators on *Anopheles gambiae* sensu stricto



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#### ARTICLE INFO

# Article history: Received 15 July 2014 Received in revised form 7 November 2014 Accepted 21 November 2014 Available online 28 November 2014

Keywords: Anopheles gambiae Carassius auratus Developments Gambusia affinis Predator Kairomones Survivorship

#### ABSTRACT

The increased insecticides resistance by vectors and the ecological harm imposed by insecticides to beneficial organisms drawback mosquitoes chemical control efforts. Biological control would reduce insecticides tolerance and yet biodiversity friend. The predatory and non-predatory effects of Gambusia affinis and Carassius auratus on gravid Anopheles gambiae sensu strict and larvae survivorship were assessed. In determining predation rate, a single starved predator was exposed to third instar larvae of An. gambiae s.s. in different densities 20, 60 and 100. Six replicates in each of the densities for both predators, G. affinis and C. auratus, were set up. The larvae densities were monitored in every 12 and 24 h. In assessing indirect effects: An. gambiae s.s. first instar larvae of three densities 20, 60 and 100 were reared in water from a predator habitat and water from non-predator habitat. Larvae were monitored until they emerged to adults where larval survivorship and sex ratio (Female to total emerged mosquitoes) of the emerged adult from both water habitats were determined. Oviposition preference: twenty gravid females of An. gambiae s.s. were provided with three oviposition choices, one containing water from predator habitat without a predator, the second with water from a predator with a predator and the third with water from non-predatory habitat. The number of eggs laid on each container was counted daily. There were 20 replicates for each predator, G. affinis and C. auratus, Survivorship of An. gambiae s.s., larvae reared in water from non-predator habitat was higher than those reared in water from the predator habitats. Many males emerged in water from non-predatory water habitats while more females emerged from predator's habitats water. More eggs laid in tap water than in water from predator habitat and water from predator habitat with live predator. In 24 h, a starved C. auratus and G. affinis were able to consume 100% of the 3rd instar larvae. The findings from this study suggest that G. affinis and C. auratus may be useful in regulating mosquito populations in favour of beneficial insects. However, a small scale trial shall be needed in complex food chain system to ascertain the observed predation and kairomones effects.

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#### 1. Introduction

Anopheles gambiae sensu stricto is one among eight species of the An. gambiae sensu lato sibling species complex that accounts for the majority of malaria transmission events in sub-Saharan Africa (Coetzee et al., 2000, 2013). According to WHO report, Sub-Saharan Africa accounts for 90% of the 660,000 deaths globally due to malaria and 80% of 219 million malaria cases (WHO, 2013). Currently vector control approach in malaria vectors remains an efficient and mainstay method for reducing malaria transmission in endemic countries. This is because of the slow vaccine development and rapid *Plasmodium* species resistance development against WHO recommended first line anti-malaria drugs (Killeen et al., 2004; Abdul-Ghani et al., 2014a,b). Biological and/or environmental management methods can be used to reduce mosquito vector population without harming environment and therefore biodiversity (Kamareddine, 2012). Currently *An. gambiae* s.l. mosquitoes have developed resistance against pyrethroids used in treated long

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lasting bed nets and indoor residual sprays (IRS) (N'Guessan et al., 2010). Yet chemicals have shown some negative effects on nontargeted organisms particularly beneficial insects and fish (Rao and Kavitha, 2010), and other important aquatic mosquito predators (Service, 1977; Chandra et al., 2008; Ohba et al., 2010). Deployment of bio-control strategies for mosquito in aquatic environment plays significant role in reducing mosquito populations. The methods have little chance of developing resistance, they are cheap, sustainable and environmentally friendly (Yap, 1985; Voyadjoglou et al., 2007). Fish species successfully have been found to control aquatic stages of both Anopheline and Culicine mosquitoes (Louca et al., 2009). However, the technique has not frequently been used in sub Saharan Africa as biological control strategy for mosquitoes (Victor et al., 1994; Blaustein and Chase, 2007; Walker and Lynch, 2007). Among the abundantly and potential aquatic larval predators that naturally coexist in natural mosquito breeding habitats throughout the world include notonectids, belostomatids, amphibians, dytiscid beetles and Gambusia affinis (Ohba et al., 2010; Kweka et al., 2011; Gouagna et al., 2012; Bond et al., 2014).

The predatory effects on prey population may result from direct consumption or the persistence risk to predation (Bolnick and Preisser, 2005). Most of the predator prey relationship studies have shown that, prey responded to reduce the risk of predation by adopting behavioural, morphological or developmental adaptation (Peacor and Werner, 2008; Kweka et al., 2011). In Predator and prey relationship, inducible defences are always associated with costs that are saved when they are not essential (Bolnick and Preisser, 2005), lower mating success (Preisser et al., 2005) and redirecting energy from reproduction to chemical or structural defences (Hay, 2009). An. gambiae s.s. have been reported to lay fewer eggs in rainwater conditioned with a predator backswimmers and tadpoles than in unconditioned rainwater (Munga et al., 2006).

The aim of the present study was to investigate the direct and indirect predatory effects of fish, *G. affinis* and *C. auratus*, on aquatic stages development and oviposition response of gravid *An. gambiae* senso stricto.

#### 2. Methods

#### 2.1. Predators rearing

Predators *G. affinis* and *C. auratus* were obtained from Tropical Pesticides Research Institute (TPRI) laboratory. *G. affinis* originated from lake Duluti, tengeru in Arumeru district, Arusha region since 2009 while *C. auratus* originated from University of Dar es Salaam since 2009; both are maintained at TPRI laboratory. They are reared in aquarier structure provided with dimension of 60 cm length, 30 cm width and 30 cm depth. They are fed once a day with mosquito larvae and chicken layer mash food and kept in a 12L:12D photophase.

#### 2.2. Mosquito rearing

An. gambiae s.s. colony at TPRI insectary originated from Kisumu Kenya and have been reared since 1992. From second instar, An. gambiae s.s. larvae were fed with Tetramine fish food at a rate 0.003gm per larvae. An. gambiae s.s. third instar larvae were used as prey in the predatory effect monitoring experiments. According to Kweka and others, the known predators used consumed the intermediate size prey (third instar) than other sizes (Kweka et al., 2011). This is because movement of larvae at third instar stage produce high amptitude vibrations that increase their risk to predation (Gimonneau et al., 2012). An. gambiae s.s. first instar larvae were used in non-predatory experiment being reared in water which had been used for predator rearing in aquaria. The gravid An. gambiae s.s. mosquitoes used in oviposition experiment were obtained

from female fed on rabbit blood then maintained in the insectary until they became gravid before they were used. They were then fed for 72 h before experimentation.

#### 2.3. Predation rate and feeding time

To determine the predation rate of predators the trial started by starving the predators for 12 h. Then a single starved predator was added in a plate (20 cm diameter and 3 cm depth) containing 1000 ml of water and the 3rd instar larvae in three different densities. The size of the predators ranged from 3.5 cm to 4 cm length. The plates contained 20, 60 and 100 densities of third instar larvae of An. gambiae s.s. The plates were covered with net materials to prevent predators from escaping. The consumed and remained larvae were counted after 12 h and after 24 h which was the end of the experiment. This experiment was also used to determine the feeding time of the predator by initiating the experiment in two different times, some experiments started in the morning at 7:00 h (beginning of light) and others in the evening at 19:00 h (beginning of dark). Each density had 6 replicates on both settings: morning and evening. This procedure was conducted separately for each predator, G. affinis and C. auratus.

#### 2.4. In-direct effect of predator

To determine in-direct effects of the G. affinis and C. auratus on An. gambiae s.s. larvae, two types of rearing water were used (i) water from predator habitat and (ii) water from a non-predatory habitat. Dechlorinated tap water with average pH 9.06  $\pm$  0.81), temperature  $26 \pm 2.3$  °C, conductivity  $1.052 \pm 0.15$   $\mu$ S/cm and turbidity  $0.53 \pm 0.05 \text{NTU}$  was used as non-predatory habitat. Water from predator habitats was taken from G. affinis and C. auratus aquaria. Water from the tap in the laboratory before being used in keeping the predator fish species had an average of pH of 9.67  $\pm$  0.12, temperature of  $23.7 \pm 2.11$  °C, conductivity of  $1.042 \pm 0.4 \,\mu\text{S/cm}$  and turbidity of  $0.52 \pm 0.17$  NTU. The predators, *G. affinis* and *C. auratus*, are kept in different aquaria. Water from G. affinis aquarium had an average pH of  $9.75 \pm 1.13$ , temperature of  $23.8 \pm 1.03$  °C, conductivity of  $1.063 \pm 0.14 \,\mu\text{S/cm}$  and turbidity  $0.53 \pm 0.05 \text{NTU}$  while Water from C. auratus aquarium had an average pH of  $9.9 \pm 0.14$ , temperature of  $23.95 \pm 1.06$  °C, conductivity of  $1.061 \pm 0.17$  µS/cm and turbidity of  $0.54 \pm 0.02$  NTU. These averages were obtained from morning and evening measurements in experimental days. In the insectary where experiments were conducted temperature was maintained at  $27 \pm 2$  °C, relative humidity  $78 \pm 2$ % and 12D:12Lphoto phase. An. gambiae s.s. first instar larvae were reared in different densities of 20, 60 and 100 in the plates filled with water of each category. (20 cm diameter and 3 cm depth). Larvae density from first instar was monitored daily and age structure recorded until when they turned to pupae. Emerged pupae were transferred to transparent vials (5 cm height and 4 cm diameter) by using a plastic pipette. Thereafter the vials were covered with net materials to prevent emerging adult mosquitoes from escaping. Dead larvae and pupae were discarded. The emerged adults were separated by sex and counted every day. The survivorship of the larvae and sex ratio (female emerged/(female + male emerged) of emerged adults were determined and compared between the two water habitat types (Kweka et al., 2012). This procedure was repeated also for C. auratus predator.

#### 2.5. Developmental time

The development time for the mosquito larvae from first instar to pupae stage was assessed. The mean number of days

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