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Laboratory evaluation of the biocontrol potential of *Aphyosemion gularis* against *Anopheles* larvae

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Larvivorous fish have been employed as biocontrol agents of mosquito larvae in several countries across the world including Iran[1], Argentina[2], Australia[3], Turkey[4] and India[5,6]. Despite the fact that this vector control strategy is simple, relatively inexpensive and therefore, particularly suitable for the African situation, information concerning its application in Nigeria is scarcely available. The genus *Aphyosemion* belongs to the family Cyprinodontidae and is commonly called toothed carp, or topminnow. The *Aphyosemion gularis* (Synonyms: *Aphyosemion sjoestedti, Fundulopanchax sjoestedti)* otherwise called blue gularis belongs to the group of fish regarded as killifish and is indigenous to tropical and sub-tropical Africa. Although World Health Organization[7], mentioned *Aphyosemion* as one of the annual killifishes noted to have drought resistant eggs, recent work classified them as semi-annual because they can be water incubated and live longer than the annuals in areas which sometimes dry out to moist mud, but at other times retain water throughout the dry season[8].

Three species, namely *Aphyosemion gardneri, A. arnoldi* and *A. gularis* have been described as part of the freshwater fishes in Nigeria. According to Umeh[9], the three species have similar dietary and ecological characteristics, and are commonly found during the dry season at the surface of small bodies of stagnant water of rain forest pools, ponds and streams where dead leaves are abundant at the bottom. Umeh[9] also classified them as larvivorous, with their diet including mosquito larvae and pupae, detritus and other small terrestrial insects that fall on water surface. *A. gularis* are substrate spawners which could sometimes spawn on aquatic weeds and the eggs can hatch in 16–18 days after they are laid[10]. Having drought resistant eggs that can be water incubated and the ability to survive for longer periods compared to the annuals are features that can make them survive in both temporary and permanent pools where mosquitoes easily breed. However, their larvivorous potential against noxious insects like mosquitoes have not been assessed. In order to complement the existing control measures in the country, this work was undertaken to evaluate the biocontrol potential of this native Nigerian fish species, against *Anopheles* mosquito larvae.

Anopheles mosquito larvae were collected from shallow pools and depressions within Ibadan metropolis with a dipper. Only the III and IV instar larvae were selected for the feeding experiments. *A. gularis* found naturally in some canals and ponds were collected with a mesh aquatic net at Ojoo (3°54'44.23" E and 7°27'38.83" N) and Beere (3°54'00.68" E and 7°22'32.92" N) in Ibadan, Nigeria. The fish identification was based on the taxonomic keys of Umeh[9]. All the fish were acclimatized under laboratory conditions over a three month period before the experiment and were fed with commercially available fish feed and mosquito larvae occasionally. Prior to the experiments, the total length and weight measurements were taken and based on these two measurements, the fish were separated into two main groups, namely large (3.5–4.5 cm, 0.47 ± 0.18 g) and small (1.5–2.5 cm, 0.04 ± 0.02 g). After the three months acclimatization period, the experiments were carried out in glass

aquaria (22×16×16 cm) containing de-chlorinated water (temperature 26–31°C and pH 6.48–6.94). The procedures of Ungureanu *et al*[11] were adopted for the laboratory feeding experiments. Five fish of the same size group were placed in separate glass aquaria containing 2 L of de-chlorinated water, 24 h prior to the start of the experiment. In order to standardize their hunger level, the fish were not fed during this period. Each group of five size-matched fish was offered III and IV instar of *Anopheles* larvae at three separate densities of 50, 150 and 250. Each prey density had four replicates and the number of larvae consumed by each fish group was recorded separately at 3 h interval after which the number of larvae consumed was replenished in each of the aquarium to maintain the prey density for another interval. The observations of prey consumption were categorized into 12 h light and dark periods each comprising of 4 intervals. Student's *t*-test was used to compare the various result obtained.

The total percentage predation of the three different densities of *Anopheles* larvae by the large and small fish groups in the 12 h light and 12 h dark periods are summarized respectively in Figs. 1 and 2. At the larval density of 50, where a total of 200 *Anopheles* larvae were presented to the two fish groups throughout the 12 h light period, 100±0% larval consumption was observed for the large fish group, while the small group consumed 86±3.63% (Fig. 1). However, at the larval density of 150, where a total of 600 *Anopheles* larvae were presented, the large fish group still showed 100 ± 0.57% *Anopheles* larval consumption while the larval consumption by the small fish group decreased to 37 ± 23.29%. At 250 larval density, where a total of 1000 *Anopheles* larvae were presented, the total percentage of larvae consumed by the large and small fish groups was 79±14.03 and 13±8.03% respectively (Fig. 1).

On the other hand, during the 12 h dark period, at the larval density of 50 where a total of 200 *Anopheles* larvae were presented separately to each group, the large fish group consumed 100±0% of the *Anopheles* larvae while the small fish group consumed only 43±15.23% (Fig. 2). At the larval density of 150, where a total of 600 *Anopheles* larvae were presented to each group, the large fish group still consumed 100±0.57% of the *Anopheles* larvae while the small fish group consumed 30±17.12%. Whereas, at 250 larval density, where a total of 1000 *Anopheles* larvae were presented to each group, the total consumption by the large and small fish groups were 74±25.61 and 11±14.34% respectively (Fig. 2).

The results indicate that the number of larvae consumed by the large fish at a larval density of 50 were 1.16 and 2.33 times higher compared to that of the small fish in the light and dark periods respectively. At 150 larval density, larval consumption of the large

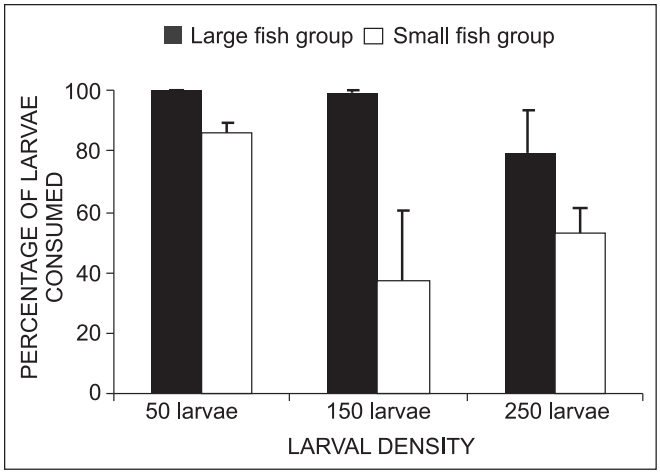

Fig. 1: Percentage predation of *Anopheles* larvae by the large and small fish groups in the 12 h light period.

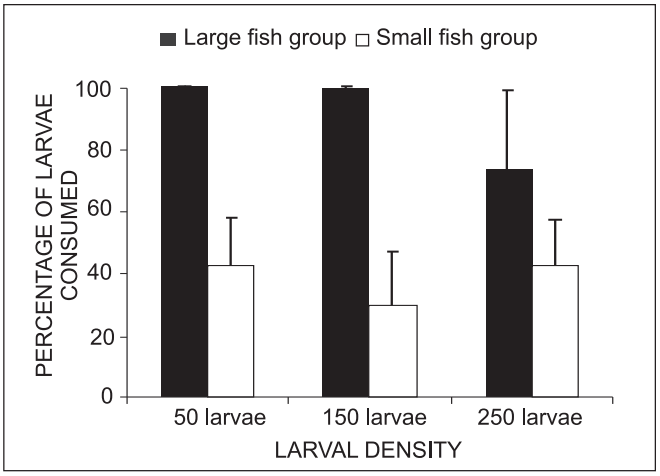

Fig. 2: Percentage predation of *Anopheles* larvae by the large and small fish groups in the 12 h dark period.

fish were respectively 2.68 and 3.31 times higher compared to that of the small fish group in the light and dark periods. Whereas at 250 larval density, the large fish respectively consumed 6.01 and 6.88 times higher than that of small fish group in the light and dark periods. Statistically, at all larval densities, except 50, the difference between the number of larvae consumed by the large and small fish groups were found to be significant ($p<0.05$). This exception could have resulted from the innate predatory ability of the fish species against *Anopheles* mosquito larvae making even the small sized group to feed effectively on a considerable number of the late stages (III and IV instars) of the larvae. These small sized fish group however became overwhelmed at the larval density of 150 and 250 because they could only consume and digest a limited number of larvae per time. At the larval density of 150 where the small fish group were overwhelmed, the large fish group on the other hand, consumed all the larvae presented to them perhaps because they have larger stomach storage chamber and can digest more per unit time. The small fish group therefore tends to get filled easily after consuming relatively less number of larvae. It implies that all adult males which are normally smaller than the mature females will feed on less number of larvae. This suggests that mature adult female fish are more voracious and have higher biocontrol potential compared to the males and other juveniles.

Keeping in mind that a single vector is capable of transmitting the malaria disease, it is necessary to ensure that none of the vector escapes. Therefore, the implication of these results for *Anopheles* mosquito control is that in a situation where only the small fish are present, then a larger population of this group would be required to achieve total control of a considerably high *Anopheles* larval population density in practical field applications.

In accordance with the findings of Yildirim and Karacuha[4] and Manna *et al*[6], the results of this work therefore indicate that the larval consumption increased in relation to the fish size. Moreover, the numbers of *Anopheles* larvae consumed by the fish here is greater when compared with those observed in other laboratory experiments where fishes of the same size range such as *Danio rerio* and *Gambusia affinis* were used as reported respectively by Sharma and Ghosh[12] and Chatterjee and Chandra[13]. Furthermore, in comparison with the adult sizes of other commonly known larvivorous fish species such as *Osphronemus gorami,* which is up to 80 cm in length[14] and *Pseudotropheus tropheops,* which is about 20 cm in length[15], *A. gularis* is smaller in size, making them to have a very reduced food value and hence unattractive to the general public for nutritional and/or economic purposes. In addition, this fish is native to West Africa where malaria is endemic and is therefore not likely to cause any adverse ecological consequences when introduced to the waters in the region. According to WHO[7], the native *Aphyosemion* species also produce drought resistant eggs. These qualities, thus, confer on it an advantage as a biological control agent of mosquito larvae compared to the other ones mentioned above especially in temporary mosquito breeding sites.

The study also revealed that larval consumption increased with increase in larval density until satiation level is reached when the fish became overwhelmed. This finding is in consonance with the work of Willems *et al*[3] who reported that the consumption of *Culex* larvae by *Pseudomugil signifer* and *G. holbrooki* increased with increased density until they became overwhelmed at highest larval density of 200. The implication of this to field application is that in breeding sites of high larval population densities, commensurate number of fish is required to achieve total control. This is because any larva that is allowed to escape into adult cause considerable harm as a disease vector.

The work also revealed that larval consumption was higher in the light than in the dark period suggesting that the fish relies more on visual stimuli in locating their preys. This result agrees with the works of Ghosh *et al*[5] and Pamplona *et al*[16]. The implication of this is that in turbid waters with reduced visibility, the voracity of the fish may be low compared to clean

waters. The results from these experiments indicate that *A. gularis* have mosquito biocontrol potentialities. Considering the limitations of other malaria control measures, there is the need to exploit this relatively less expensive, environmentally compatible alternative in order to complement other efforts in combating the malaria scourge. It could be considered sustainable especially when adopted on a local community basis. In addition, this fish is native to and readily available in Nigeria and therefore would most likely not cause any side-effects as attributed to *G. affinis*[17]. Therefore, we are of the opinion that the potential of this native killifish in reducing malaria burden in endemic zone will not be in doubt if proper and far reaching introductions are made. Malaria endemic sub-Saharan Africa needs relatively inexpensive, efficient and sustainable methods of controlling the mosquito vectors in order to ameliorate the distress of the people especially the rural poor. The field introduction of this fish *vis-à-vis* reduction in malaria transmission in selected areas is currently being assessed.

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