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Research Article

Study the Use of Larvivorous Fish against Larvae of *Anopheles gambiae* (Diptera: Culicidae) Malaria Vector in Laboratory Conditions in Dogbo District in South-western Benin, West Africa

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Abstract: The current study aimed to study the use of larvivorous fish against larvae of *Anopheles gambiae* malaria vector in laboratory conditions in Dogbo district in south-western Benin, West Africa. Larvae of *Anopheles gambiae s.l* mosquitoes were collected from breeding sites using the dipping method in June 2020 during the rainy season in Dogbo district. Alive *Hemichromis fasciatus* fishes were bought immediately once catched by fishers of Ganvié location in So-Ava district and carried by car from Ganvié location to the Laboratory. Laboratory evaluation for larvivorous efficacy was conducted. The results showed that larvivorous fishes ate more larvae of *Anopheles gambiae s.l.* when they were unfed than when they were fed. The results obtained after the introduction of unfed larvivorous fishes in glass jars showed high larva-eating capacity of these fishes.
Keywords: Larvivorous fish, larvae of *Anopheles gambiae s.l,* laboratory conditions, malaria, Benin.

Introduction

According to the World Health Organization (WHO), about half of the world population (3.3 billion) is at risk of malaria. Although the past several years have witnessed tremendous increase in control measures, malaria still remains the number one killer disease especially in sub-Saharan Africa. More than 216 million cases were reported in 2010 alone, with over 660, 000 deaths recorded (World Health Organization, 2013). Malaria is caused by five species of parasite that affect humans, and all of these species belong to the genus *Plasmodium*. Malaria due to *Plasmodium falciparum* is the most deadly form, and it predominates in Africa. This disease is spread from one person to another by female mosquitoes of the genus *Anopheles*.

Malaria is an entirely preventable and treatable disease, provided the currently recommended interventions are properly implemented. These interventions include: vector control through the use of insecticide treated nets (ITNs), indoor residual spraying (IRS) and, in some specific settings, larval control. Insecticides are often deliberately introduced into the mosquito habitat in the fight against the many human diseases they transmit (e.g. malaria, dengue fever, yellow fever and filariasis) (Lounibos, 2002). As a consequence mosquito control programs are now threatened by the selection of mosquito populations resistant to these chemical insecticides (Hemingway *et al*., 2002).

Current researches have to explore several alternative avenues of controlling malaria, and one particular approach that appears to be gaining attention is an environmental management strategy that aims to reduce adult vector population by targeting their aquatic immature stages (i.e., mosquito eggs, larvae and pupae). This strategy is becoming increasingly important in many countries

especially in sub-Saharan Africa and involves different species of mosquitoes including those that transmit malaria. Among the biological control agents of mosquitoes, fishes are the most extensively used species in several countries since the beginning of the twentieth century (Gerberich, 1968). Certain exotic fishes such as *Poecilia reticulata, Gambusia affinis* and *Oreochromis mossambicus* have been used in various ecological conditions in India for mosquito control (Sitaraman *et al.*, 1976; Chand and Yadav, 1994; Prasad *et al*., 1994). Use of these exotic fish has raised environmental concerns in view of their suspected adverse effects on local aquatic fauna (Hulbert and Zedier, 1972). Consequently, fish fauna surveys and evaluation of larvivorous potential of native fishes have been of high research priority in the area of biological control of vectors of disease.

The objective of the current study was to evaluate the eating capacity of the larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions in a context where integrated malaria vector control is necessary.

Materials and methods

Study area

The study area is located in Republic of Benin (West Africa) and includes the department of Couffo. Couffo department is located in the south-western Benin and the study was carried out more precisely in Dogbo district. The southern borders of this district are Lokossa and Bopa districts. The northern border is Djakotomey district. The eastern border is Lalo district and the western border of Dogbo district is Togo republic. Dogbo district covered 475 km^2 and belongs to geographic region of ADJA. The choice of the study site took into account the economic activities of populations, their usual protection practices against mosquito bites, and peasant practices to control farming pests. We took these factors into account to study the use of larvivorous fish against larvae of *Anopheles gambiae* malaria vector in laboratory conditions in Dogbo district in south-western Benin. Couffo has a climate with four seasons, two rainy seasons (March to July and August to November) and two dry seasons (November to March and July to August). The temperature ranges from 25 to 30^{0}C with the annual mean rainfall between 900 and 1100 mm.

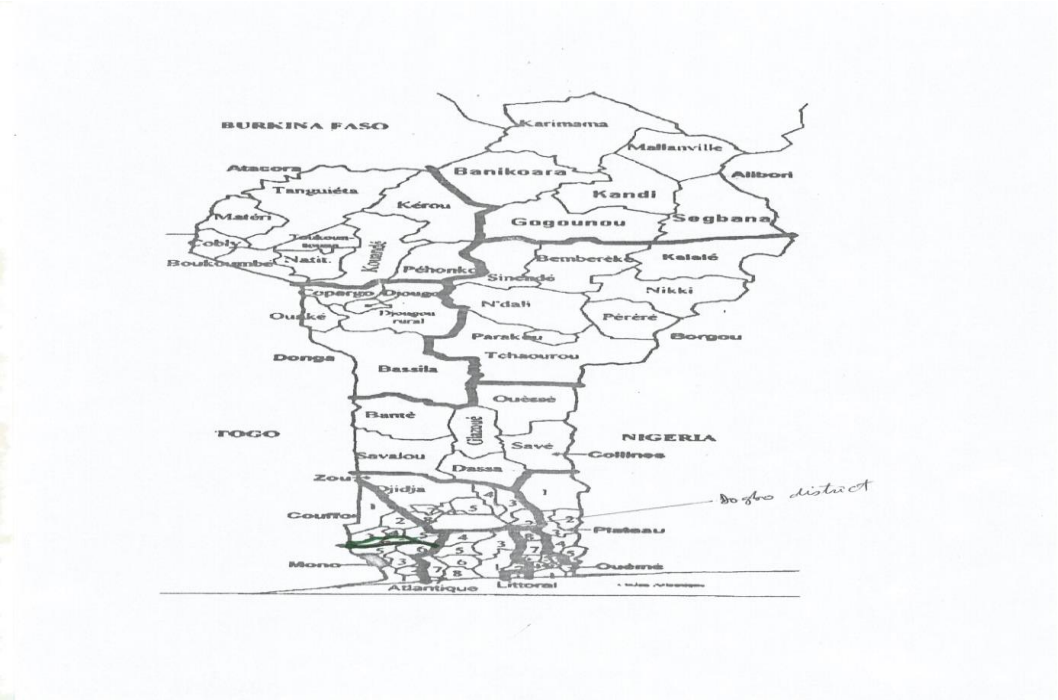

Figure 1. Map of Republic of Benin showing Dogbo District

Mosquito sampling

An. gambiae s.l. mosquitoes were collected in June 2020 during the rainy season in Dogbo district. Larvae were collected from breeding sites using the dipping method and kept in labeled bottles. The

samples were then carried out to the Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques located in Dogbo district.

Hemichromis fasciatus fish collection

Alive *Hemichromis fasciatus* fishes were bought immediately once catched by fishers of Ganvié location in So-Ava district which southern border is Cotonou district. The habitations of Ganvié location are built on water. Fishing is a main activity of people. Then, the *Hemichromis fasciatus* fishes bought were put in some jars contained water and carried by car from Ganvié location to the Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques located in Dogbo district in southern Benin.

Laboratory evaluation for larvivorous efficacy

The sample of fishes (*Hemichromis fasciatus*), indigenous larvivorous fishes were brought from their natural habitats from Ganvié location in So-Ava district in Atlantic department in southern Benin to the Laboratory of Applied Entomology and Vector Control (LAEVC) of the Department of Sciences and Agricultural Techniques located in Dogbo district in south-western Benin. To determine the natural propensity of the sample of *Hemichromis fasciatus* to prey upon mosquito larvae, laboratory evaluation was conducted on larvae of the vector mosquito specie, *Anopheles gambiae* (Diptera: Culicidae), main malaria vector in Benin. Two fishes of the same species of *Hemichromis fasciatus* were released in five glass jars of same dimensions contained each 1 litre of water. A batch of 100 larvae of four instar reared in the insectary of the Laboratory of Applied Entomology and Vector Control (LAEVC) was added in each glass jar for the two fishes in the morning and larval consumption was observed every two hours. Total larval consumption was recorded at the end of 24 h when all remainder larvae removed. A glass jar (without larvae) containing only two fishes of same species of *Hemichromis fasciatus* were used as control for biological tests. The tests were repeated for three consecutive days to establish the maximum devouring capacity of the fishes when they were fed with fish food (without larvae) before tests comparatively to when they were unfed before tests.

Data analysis

Analysis using Fisher's exact test was performed to compare the maximum devouring capacity of the fishes when they were fed with fish food (without larvae of *An. gambiae s.l.*) before tests comparatively to when they were unfed before tests.

Results and Discussions

Larva-eating capacity of fed *Hemichromis fasciatus* fish in the laboratory conditions

The eating capacity of fed larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions was showed in Table 1. The analysis of this table showed that after the introduction of fed larvivorous fishes in each of the five glass jars, the number of larvae of *Anopheles gambiae s.l* was reduced. The maximum reduction was 81% whereas the minimum reduction was 47% and the mean was 64%.

Table1. Reduction in the number of larvae in the glass jars after the introduction of fed larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions

Number of larvae tested			
Number of glass jars	**Before larvivorous fish introduction**	**After larvivorous fish introduction**	**% Reduction**
Control	0	0	0
1	100	53	47
2	100	29	71
3	100	52	48
4	100	47	53
5	100	19	81

Larva-eating capacity of unfed *Hemichromis fasciatus* fish in the laboratory conditions

The eating capacity of unfed larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions was showed in Table 2. The analysis of this table showed that after the introduction of unfed larvivorous fishes in each of the five glass jars, the number of larvae of *Anopheles gambiae s.l* was dramatically reduced. The maximum reduction was 88% whereas the minimum reduction was 59% and the mean was 73.5%.

Table 2. Reduction in the number of larvae in the glass jars after the introduction of unfed larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions

Number of larvae tested			
Number of glass jars	**Before larvivorous fish introduction**	**After larvivorous fish introduction**	**% Reduction**
Control	0	0	0
1	100	22	78
2	100	17	83
3	100	41	59
4	100	36	64
5	100	12	88

Figure 2. *Hemichromis fasciatus* fish

Alternative biological control methods, especially the use of larvivorous fishes, can play a significant role in controlling of mosquito larvae. Some of larvivorous fishes are important predators of mosquito larvae. In fact, in the current study, after the introduction of fed larvivorous fishes in each of the five glass jars, the number of larvae of *Anopheles gambiae s.l* was reduced. In addition, after the introduction of unfed larvivorous fishes in each of the five glass jars, the number of larvae of *Anopheles gambiae s.l* was dramatically reduced. These results showed that larvivorous fishes ate more larvae of *Anopheles gambiae s.l.* when they were unfed than when they were fed. The presence of fish food in glass jars may play a role by limiting the eating capacity of the larvivorous fishes. In fact, because of the presence of fish food in glass jars, larvivorous fishes first tried to eat this food before eating larvae of *Anopheles gambiae s.l.* The results obtained after the introduction of unfed larvivorous fishes in glass jars showed that the larva-eating capacity of these fishes was high.

In Dogbo district as in many other districts of Benin, there is the presence of breeding sites and there is no doubt that introduction of this larvivorous fish in these water sources will dramatically reduce the mosquito population and hence malaria infection. Biological control using larvivorous fishes against larvae of *Anopheles gambiae s.l* has given good results in the current study and may be a

very efficient method of tackling malaria. However, the field conditions are not the same as laboratory conditions. Even if very few studies were published in the country on the use of larvivorous fishes for malaria control, there are many reports elsewhere or in other countries. For example, larvivorous fish were part of an integrated control programme that succeeded in eradicating malaria from the Southwest Pacific (Kaneko *et al.,* 2000). Based on the results of similar studies on the indigenous larvivorous fish *Aphanius dispar* in Ethiopia and Djibouti, fish have been introduced on an operational scale for the control of malaria transmission on those countries (Fletcher *et al*., 1993; Louis and Albert, 1988). It also was reported that under the laboratory conditions *A. dispar* was more successful than *G. affinis* in preying upon the III and IV instars and pupae, and that the two species could complement each other as mosquito control agents in different habitat conditions (Homski *et al*., 1994). The results of the field trial showed that *A. dispar* is capable of controlling mosquito breeding in confined water bodies effectively within a fortnight of its application. An experimental study in Turkey showed high mosquito larval consumption by *A. chantrei* and recommended its use in biological control instead of *Gambusia* spp (Yildirim and Karacuha, 2007).

In the current study, during the laboratory evaluation for larvivorous efficacy, otherwise when the biological tests were in progress in laboratory, all larvae which had pupated and all pupae which had emerged or become adult were taken into account in result recording. The results obtained in the current study in laboratory conditions are encouraging and must permit to envisage the implement of such a strategy to the whole of the territory of the Republic of Benin in order to measure its impact. Most *Anopheles* mosquitoes traditionally breeds in clear, clean and apparently less contaminated surroundings usually around human habitation.

Conclusion

The promoting of integrated control programme is necessary in malaria control in Benin. Despite the use of Indoor Residual Spraying (IRS) and Long-Lasting Insecticidal Nets (LLINs) as cornerstones of malaria prevention more precisely in malaria adult vector control, less effort has been made using strategy to control larvae and pupae in breeding sites such as the use of larvivorous fishes or larvicides in the country. Even if it is difficult to use larvivorous fishes for *Anopheles gambiae* malaria vector control in field conditions, good results were obtained in laboratory conditions in the current study. Biological control against malaria vectors using larvivorous fishes also preserve or respect environment.

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Conflicts of interest

I declare no conflicts of interest.

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