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Larval eating capacity of three indigenous larvivorous fish species under laboratory conditions for malaria control in Dogbo District of South-Western Republic of Benin, West Africa

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Abstract

Because of problems with insecticide resistance, alternative malaria control methods were necessary. These methods include the use of biological control agents, such as larvivorous fish. This study aimed to study the larval eating capacity of three indigenous larvivorous fishes in malaria control in laboratory conditions in Dogbo district in south-western Republic of Benin, West Africa. Larvae of *Anopheles gambiae sensu lato* mosquitoes were collected from breeding sites using the dipping method in July 2020 during the rainy season in Dogbo district. Alive *Hemichromis fasciatus*, *Clarias gariepinus* and *Oreochromis niloticus* fishes were bought immediately once caught 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 obtained in the current study showed that the larval eating capacity of *Hemichromis fasciatus* fish when this fish was fed was the highest among those of the three tested indigenous larvivorous fishes in the laboratory conditions. Similar results were obtained when these different fishes were unfed. In a context of environmental crisis and global changes, environmentally friendly methods should be encouraged. The use of indigenous larvivorous fishes as predators to control malaria mosquitoes is obviously insufficient in the current study.

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Introduction

The World Health Organization (WHO) declared on global malaria elimination programme in 2015 intending to make a malaria-free world by 2030 (WHO, 2015).

Chemical-insecticide-based mosquito control has been successful in reducing the burden of malaria. However, the emergence of insecticide resistance in malaria vectors and concerns about the effect of the chemicals on the environment, human health, and non-target organisms present a need for new or alternative vector control intervention tools.

Environmental management is implemented in the control of mosquito populations along with chemical or microbiological methods in different parts of the world, especially where mosquito-borne diseases are endemic (Amer and Mehlhorn, 2006).

However, mosquito control programs are facing important and timely challenges, including the recent outbreak of arboviral diseases, the development of resistance in several mosquito species and the rapid spreading of highly invasive mosquitoes worldwide (Benelli *et al.*, 2017).

Mosquitoes are becoming increasingly resistant to chemical insecticides and there is growing concern about the potential health and environmental risks of these products (Collins and Blackwell, 2000). The current status of insecticide resistance in mosquitoes (Strode *et al.*, 2014; Liu, 2015), the effects of insecticides on non target insect species (Jacob *et al.*, 2017; Jeffries and Walker, 2015) and the fact that they remain in the environment for decades (Li *et al.*, 2017; Wei *et al.*, 2017; Moschet *et al.*, 2014) are major concerns. Some chemical insecticides also kill non target species including mosquito predators, thereby increasing the occurrence of mosquito vectors as mosquitoes could re-establish their population faster than predators after the application of insecticides (Antwi and Reddy, 2015), whereas predators usually have longer life cycles than their prey (Service, 1977). In addition, predators are late colonizers of a given habitat after certain disturbances including insecticide application. The natural regulation of mosquito larvae is an important factor in determining the survivorship of mosquito immature stages (Eba *et al.*, 2021). The biocontrol methods, especially those involving the use

of macroinvertebrate predators as natural enemies, are recognized as environmentally friendly and are the focus of current research and control of mosquito populations (Naik, 2018; Shaalan and Canyon, 2009). Some fish species eat mosquito larvae and pupae. In disease control policy documents, the World Health Organization (WHO) includes biological control of malaria vectors by stocking ponds, rivers, and water collections near where people live with larvivorous fish to reduce Plasmodium parasite transmission. In the past, the Global Fund has financed larvivorous fish programmes in some countries and with increasing efforts in eradication of malaria, policymakers may return to this option. The goal of the current study was to study the eating capacity of three indigenous larvivorous fishes against larvae of *Anopheles gambiae sensu lato* in laboratory conditions in Republic of Benin, West Africa in a context where integrated 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 (Fig.1). 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 km² 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 eating capacity of three indigenous larvivorous fishes against larvae of *Anopheles gambiae s.l.* in laboratory conditions in Republic of 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°C with the annual mean rainfall between 900 and 1100 mm.

Mosquito sampling

Anopheles gambiae s.l. mosquitoes were collected in July 2020 during the rainy season in Dogbo district.

Larvae and pupae 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.

Fish collection

Alive *Hemichromis fasciatus*, *Clarias gariepinus* and *Oreochromis niloticus* fishes were bought immediately once caught 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, 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 larvivoracious efficacy

The samples of fishes *Hemichromis fasciatus*, *Clarias gariepinus* and *Oreochromis niloticus*, indigenous larvivoracious fishes were brought from their natural habitats from Ganvié location in So-Ava district in Atlantic department in southern Republic of 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 Republic of Benin. To determine the natural propensity of the samples of *Hemichromis fasciatus*, *Clarias gariepinus* and *Oreochromis niloticus* to prey upon mosquito larvae, laboratory evaluation was conducted on larvae of the vector mosquito specie, *Anopheles gambiae* (Diptera: Culicidae), main malaria vector in Republic of Benin. Two fishes of the same species of each type 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 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 hours when all remainder larvae removed. A glass jar (without larvae) containing only two fishes of same species of each type were used as control for biological tests. The test was done 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 three different 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 Discussion

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

The eating capacity of fed *Hemichromis fasciatus* larvivoracious 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 larvivoracious 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%.

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

The eating capacity of unfed *Hemichromis fasciatus* larvivoracious 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 larvivoracious 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%.

Larval eating capacity of fed *Clarias gariepinus* fish in the laboratory conditions

The eating capacity of fed *Clarias gariepinus* larvivoracious fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions was showed in Table 3. The analysis of this table showed that after the introduction of fed larvivoracious fishes in each of the five glass jars, the number of larvae of *Anopheles gambiae s.l.* was reduced. The maximum reduction was 78% whereas the minimum reduction was 43 % and the mean was 60.50%.

Table 1. Reduction in the number of larvae in the glass jars after the introduction of fed *Hemichromis fasciatus* larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions.

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

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

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

Table 3. Reduction in the number of larvae in the glass jars after the introduction of fed *Clarias gariepinus* larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions.

Number of glass jars	Number of larvae tested		%Reduction
	Before larvivorous fish introduction	After larvivorous fish introduction	
Control	0	0	0
1	100	37	63
2	100	28	72
3	100	57	43
4	100	44	56
5	100	22	78

Table 4. Reduction in the number of larvae in the glass jars after the introduction of unfed *Clarias gariepinus* larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions.

Number of glass jars	Number of larvae tested		% Reduction
	Before larvivorous fish introduction	After larvivorous fish introduction	
Control	0	0	0
1	100	32	68
2	100	21	79
3	100	54	46
4	100	36	64
5	100	18	82

Table 5. Reduction in the number of larvae in the glass jars after the introduction of fed *Oreochromis niloticus* larvivorous fish against larvae of *Anopheles gambiae s.l.* in the laboratory conditions.

Number of glass jars	Number of larvae tested		% Reduction
	Before larvivorous fish introduction	After larvivorous fish introduction	
Control	0	0	0
1	100	26	74
2	100	46	54
3	100	61	39
4	100	29	71
5	100	36	64

Table 6. Reduction in the number of larvae in the glass jars after the introduction of unfed *Oreochromis niloticus* larvivorous fish against larvae of *Anopheles gambiae* s.l. in the laboratory conditions.

Number of glass jars	Number of larvae tested		% Reduction
	Before larvivorous fish introduction	After larvivorous fish introduction	
Control	0	0	0
1	100	21	79
2	100	34	66
3	100	59	41
4	100	26	74
5	100	31	69

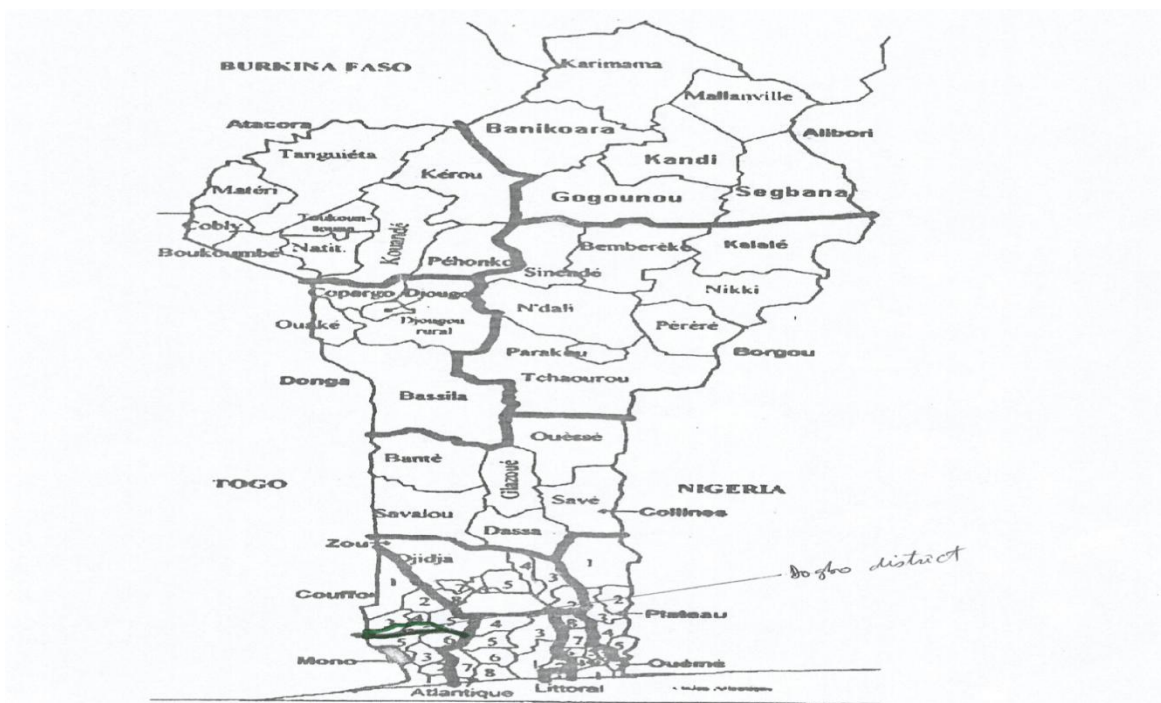


Fig. 1: Map of Republic of Benin showing Dogbo District.



Fig. 2: A breeding site of *Anopheles gambiae* s.l. larvae surveyed in Dogbo district.



Fig. 3: *Hemichromis fasciatus* fish.



Fig. 4: *Clarias gariepinus* fish.



Fig. 5: *Oreochromis niloticus* fish.

Larval eating capacity of unfed *Clarias gariepinus* fish in the laboratory conditions

The eating capacity of unfed *Clarias gariepinus* larvivorous fish against larvae of *Anopheles gambiae* s.l. in the laboratory conditions was showed in Table 4. 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 82% whereas the minimum reduction was 46% and the mean was 64 %.

Larval eating capacity of fed *Oreochromis niloticus* fish in the laboratory conditions

The eating capacity of fed *Oreochromis niloticus* larvivorous fish against larvae of *Anopheles gambiae* s.l. in the laboratory conditions was showed in table 5. 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 74% whereas the minimum reduction was 39% and the mean was 56.5%.

Larval eating capacity of unfed *Oreochromis niloticus* fish in the laboratory conditions

The eating capacity of unfed *Oreochromis niloticus* larvivorous fish against larvae of *Anopheles gambiae* s.l. in the laboratory conditions was showed in Table 6. 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 79% whereas the minimum reduction was 41% and the mean was 60%.

The current study was carried out to identify indigenous larvivorous fish species which could be potential candidates for use as biological control agents. We have evaluated whether introducing larvivorous fish reduce the density and presence of *Anopheles* larvae in water sources in laboratory conditions. That can help in the evidence base for larvivorous fish programmes in malaria control in Republic of Benin particularly and in some countries of Africa in general where the same species of fishes as those used in the current study are present as indigenous or local larvivorous fish species.

The results obtained in the current study showed that the larval eating capacity of *Hemichromis fasciatus* fish when this fish was fed in the laboratory conditions were higher than the larval eating capacity of *Clarias gariepinus* fish in the same condition. In the same way, the larval eating capacity of *Clarias gariepinus* was higher than the larval eating capacity of *Oreochromis niloticus* fish when this fish was fed in the laboratory conditions. Otherwise, the larval eating capacity of *Hemichromis fasciatus* fish was the highest among those of the three tested indigenous larvivorous fishes. Similar results were obtained when these different fishes were unfed. The results obtained in the current study also showed that 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. Even if very few studies were carried out until now in Republic of Benin in the use of larvivorous fishes against larvae of *Anopheles gambiae* s.l. in malaria vector control, good results were found in the current study and corroborated with many results obtained elsewhere, in other countries.

In fact, a study was carried out by Louca *et al.*, (2009) to examine the potential of using native fish species in regulating mosquitoes in the floodplain of the Gambia River, the major source of mosquitoes in rural parts of the Gambia. In this study, in the field, there was less chance of finding culicine larvae where *Tilapia guineensis*, the most common floodplain fish, were present; however, the presence of anophelines was not related to the presence or absence of any fish species. In semifield trials, both *T. guineensis* and *Epiplatys spilargyreus* were effective predators, removing all late-stage culicine and anopheline larvae within one day. Fewer culicines oviposited in sites with fish, suggesting that ovipositing culicine females avoid water with fish. In contrast, oviposition by anophelines was unaffected by fish. This study showed that *T. guineensis* is a potential candidate for controlling mosquitoes in the Gambia (Louca *et al.*, 2009).

Fishes, however, have been extensively studied both in the laboratory and in the field for their ability to eat mosquito larvae and their use as mosquito biological control agents. Among the large range of larvivorous fishes some are particularly efficient at controlling *Anopheles* larvae in many types of reservoirs. The most well-known fishes which are predators of *Anopheles*

larvae, are *Gambusia affinis* (the mosquito fish) and *Poecilia reticulata* (guppies) which have been extensively used all over the world in anti-malarial programs to control mosquito populations in different kinds of reservoirs. *Gambusia affinis* polluted urban water and in low dissolved oxygen concentration.

They are sensitive to pesticides but are able to develop resistances (Pyke, 2005; Pyke, 2008). These large reaction norms to different abiotic conditions make *G. affinis* ideal for introduction in many parts of the world making this species the most widespread freshwater fish in the world (Pyke, 2008; Walshe *et al.*, 2013). *Poecilia reticulata* is less flexible but has a tropical origin and consequently is adequate for use in malaria endemic areas. Both have shown high capacities to reduce the number of *Anopheles* larvae in laboratory and diverse aquatic habitats in the field, sometimes virtually clearing some villages from malaria cases for several years.

However, the voracity of these two species, their ability to prey on almost any animal smaller than them without a real preference for mosquito larvae in the wild and their invasive nature gave rise to some concerns regarding negative environmental impacts (Pyke, 2008; Walshe *et al.*, 2013). Finally, it should be noted that the efficiency of fishes for really reducing malarial transmission, as predators of larval anophelines, is established in some cases but unproven in others (Walshe *et al.*, 2013; El-Sabaawi *et al.*, 2016).

The control of adult mosquitoes is difficult because of widespread insecticide resistance. Hence, the use of potential aquatic predators could be an alternative or complementary control measure for reduction in the adult mosquito population to reduce malaria transmission. In the current study, all species tested were efficient larvivores in the laboratory conditions. However, the larvivorous capacity of *Hemichromis fasciatus* fish was the highest among those of the three tested indigenous larvivorous fishes in the laboratory conditions. In a context of environmental crisis and global changes, environmentally friendly methods should be encouraged. The use of indigenous larvivorous fishes as predators to control malaria mosquitoes is obviously insufficient in the current study.

Conflict of interest statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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