International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 12-2024

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.12.005

Larval eating capacity of indigenous larvivorous fishes against larvae of *Culex quinquefasciatus*, main vector in the transmission of mosquito-borne disease in south-western Republic of Benin, West Africa

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Abstract

Because of problems with insecticide resistance, alternative vector control methods are necessary. These methods include the use of biological control agents, such as larvivorous fishes. This study aimed to study the larval eating capacity of indigenous larvivorous fishes against larvae of *Culex quinquefasciatus* in south-western Republic of Benin, West Africa. Larvae of *Culex quinquefasciatus* mosquitoes were collected from breeding sites using the dipping method from September to November 2023 during the small rainy season and from the March to July 2024 during the great rainy season. Alive *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus* fishes were bought immediately once catched and carried by car from Agricultural Technical Lyceum of Adjahonmè to the Laboratory. Laboratory evaluation for larvivorous efficacy was conducted with fishes weighed 30g, 40g and 50g respectively. The results obtained in the current study showed that all these indigenous

fishes had larval eating capacity but the larval eating capacity of *Heterobranchus longifilis* and *Clarias gariepinus* was higher than that of *Oreochromis mossambicus* and *Oreochromis niloticus* when these fishes weighed 30g and 40g. Also, the larval eating capacity of *Oreochromis mossambicus, Heterobranchus longifilis* and *Clarias gariepinus* was higher than that of *Oreochromis niloticus* when these fishes weighed 50g. The use of indigenous larvivorous fishes as predators to control mosquitoes in the transmission of mosquito-borne disease is obviously insufficient in the current study.

Keywords: Indigenous larvivorous fishes, *Culex quinquefasciatus* larvae, biological control, laboratory conditions, Republic of Benin.

Introduction

Dispite *Culex quinquefasciatus* is not implicated in malaria transmission; it is a mosquito of medical importance. *Culex quinquefasciatus* Say, a member of the *Culex pipiens* group, is a medically important mosquito and major pest species with a worldwide distribution (WHO, 1989). *Culex quinquefasciatus* is known to be a major vector of filariasis (WHO, 1989), St. Louis encephalitis virus (SLEV) (Savage et al., 1993), West Nile virus (WNV) (Kwan et al., 2010) and Rift Valley Fever virus (RVFV) (Sang et al., 2010; Turell et al., 2008). It is considered to be an opportunistic feeder, and while host choice is regionally variable, it feeds on many species of birds, mammals, and occasionally reptiles and amphibians (Mackay et al., 2010; Unlu et al., 2010).

There is an accelerated and disorganized process of urbanization in the last decades, especially in the tropical, low income countries. Additionally, in well-developed regions, the density of the mosquitoes may be positively correlated with seasonal high temperatures (Sang et al., 2010; Turell et al., 2008).

Despite a growing interest in the promotion of integrated vector control strategies co-targeting different vector species, control efforts and relative entomological, epidemiological and insecticide resistance studies primarily focus on anophelines resulting in important knowledge gaps regarding Culex species and their control.

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. So, it is important that biological control also focuses on *Culex quinquefasciatus*, a mosquito of medical importance.

Very few researches were published on the larval eating capacity of indigenous larvivorous fishes against larvae *of Culex quinquefasciatus*, main vector in the transmission of mosquito-borne disease in south-western Republic of Benin. Therefore, there is a need to carry out new researches for this purpose.

The goal of the current study was to study the larval eating capacity of indigenous larvivorous fishes on larvae of *Culex quinquefasciatus*, main vector in the transmission of mosquito-borne disease in south-western 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 departments of Mono and Couffo. Mono department is located in the south-western Benin and the study was carried out more precisely in Lokossa and Comè districts. Regarding Couffo department, it is also located in

the south-western Benin and the study was carried out more precisely in Dogbo and Djakotomey (Fig.1). 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 larval eating capacity of indigenous larvivorous fishes on larvae of *Culex quinquefasciatus,* main vector in the transmission of mosquito-borne disease in south-western Republic of Benin. Mono and Couffo have 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.

Figure 1: Map of Republic of Benin showing the study area

Mosquito sampling

Culex quinquefasciatus mosquitoes were collected from September to November 2023 during the small rainy season and from March to July 2024 during the great rainy season in two districts of Mono department (Lokossa and Comè) and in two districts of Couffo department (Dogbo and Djakotomey). Larvae were collected from breeding sites using the dipping method (O'Malley, 1995) and kept in labeled bottles (Fig.2). The samples were then carried out to the insectary of Laboratory of Pluridisciplinary Researches of Technical Teaching (LaRPET) in Department of Sciences and Agricultural Techniques of Normal High School of Technical Teaching (ENSET) located in Dogbo district (Fig.3).

Figure 2: Mosquito larvae collection in a breeding site

Figure 3: Larvae in labeled plastics in insectary

Fish collection

Alive *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus* fishes were bought immediately once catched in Agricultural Technical Lyceum of Adjahonmè. They were then weighed. For each specie, the different considered weights were: 30g, 40g and 50g.Then, fishes bought were put in some jars contained water with oxygen and carried by car from Agricultural Technical Lyceum of Adjahonmè to the laboratory of the Department of Sciences and Agricultural Techniques located in Dogbo district in south- western Benin.

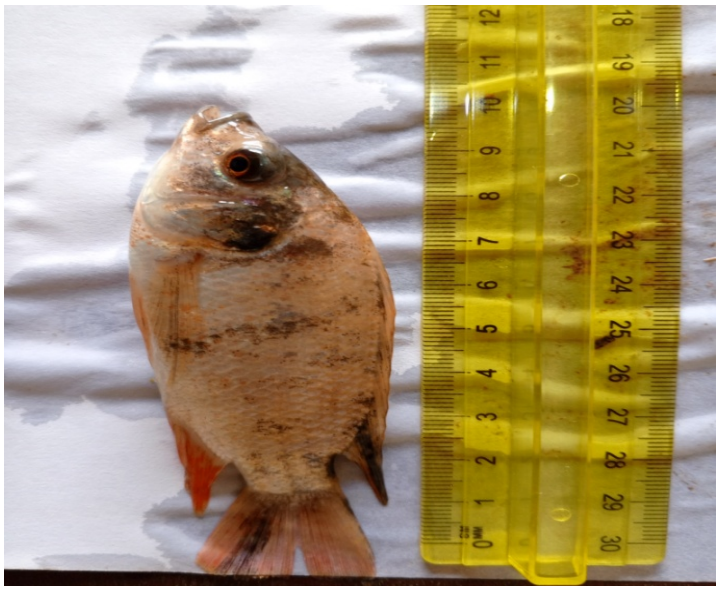

Figure 4: *Oreochromis niloticus*

Figure 5: *Oreochromis mossambicus*

Figure 6: *Heterobranchus longifilis*

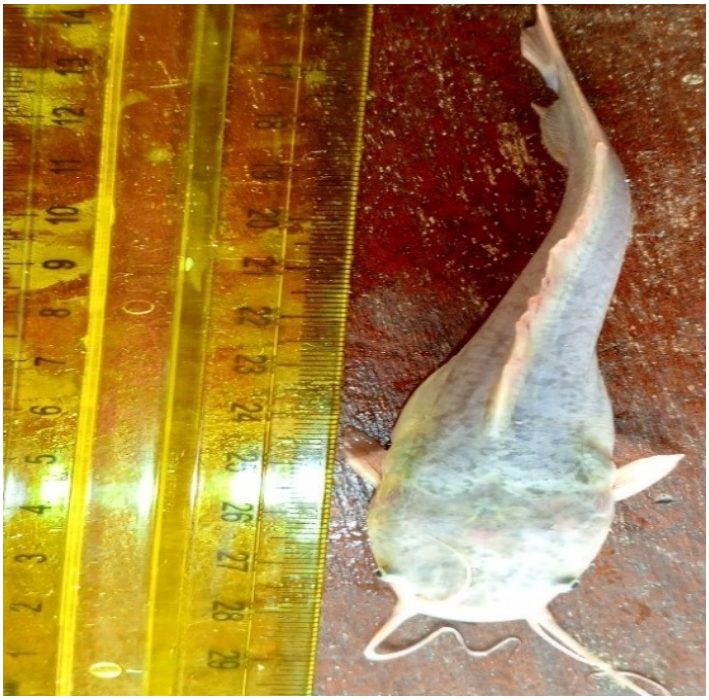

Figure 7: *Clarias gariepinus*

Laboratory evaluation for larvivorous fish efficacy

To determine the natural propensity of the samples of *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus* to prey upon mosquito larvae, laboratory evaluation was conducted on larvae of the vector mosquito specie, *Culex quinquefasciatus*. One fish of the same specie of each type was released in four glass jars of same dimensions contained each 1liter of water. At each range of four test glass jars corresponded a

glass jar (without fish) containing only mosquito larvae as control for biological tests. A batch of twenty (20) larvae of four instars reared in the insectary of the Laboratory was added in each glass jar for the fish in the morning at 8 a.m and larval consumption was observed every two hours. The laboratory evaluation for larvivorous fish efficacy was done for each specie of fish and for the different weights of 30g, 40g and 50g. Total larval consumption was recorded at the end of 24 hours when all remainder larvae removed. The test was done to establish the maximum devouring capacity of the fishes when they were fed with fish food comparatively to when they were unfed.

Figure 8: Laboratory evaluation for larvivorous fish efficacy in progress

Data analysis

Analysis using Fisher's exact test was performed to compare the maximum devouring capacity of the four different fishes when they were fed with fish food to when they were unfed.

Results and Discussion

Larval eating capacity of unfed indigenous larvivorous fishes of 30g on larvae of *Culex quinquefasciatus*

The analysis of figure 9 showed that the number of *Culex quinquefasciatus* larvae introduced in the control plastic glass jar was intact during all the duration of the test. After the introduction of unfed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 30g in each of the four plastic test glass jars, the number of larvae of *Culex quinquefasciatus* was intact at initial time (t=0hour). The number of larvae of *Culex quinquefasciatus* was reduced at middle time (t=12hours) in all plastic test glass jars until became Zero in test glass jars which contained *Oreochromis mossambicus.* At the end of the test (t = 24hours), there was no larvae in the all test glass jars which contained *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* ($P > 0,05$), but there were a few larvae in test glass jars which contained *Oreochromis niloticus.*

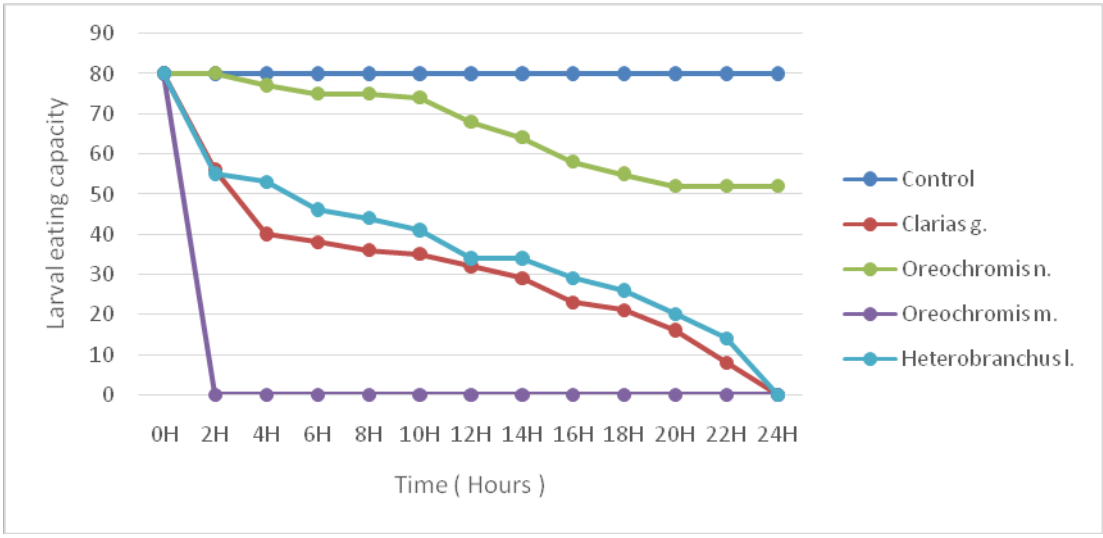

Figure 9: Larval eating capacity of unfed indigenous larvivorous fishes of 30g on larvae of *Culex quinquefasciatus*

Larval eating capacity of fed indigenous larvivorous fishes of 30g on larvae of *Culex quinquefasciatus*

The analysis of figure 10 showed that the number of *Culex quinquefasciatus* larvae introduced in the control plastic glass jar was intact during all the duration of the test. After the introduction of fed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 30g in each of the four plastic test glass jars, the number of larvae of *Culex quinquefasciatus* was intact at initial time (t=0hour). The number of larvae of *Culex quinquefasciatus* was reduced at middle time (t=12hours) in all plastic test glass jars until became Zero in test glass jars which contained *Heterobranchus longifilis.* There was only one larva in the test glass jars which contained *Clarias gariepinus.* At the end of the test (t = 24hours), there was no larva in the all test glass jars which contained *Heterobranchus longifilis, Clarias gariepinus* (P > 0,05), but there were a few larvae in test glass jars which contained *Oreochromis niloticus* and *Oreochromis mossambicus.*

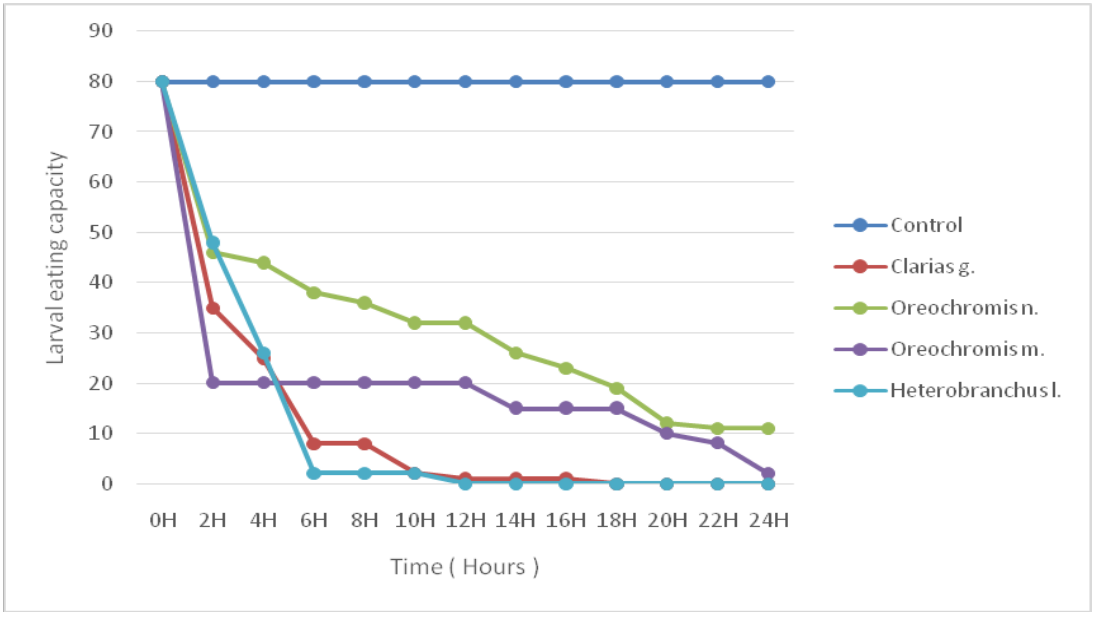

Figure 10: Larval eating capacity of fed indigenous larvivorous fishes of 30g on larvae of *Culex quinquefasciatus*

Larval eating capacity of unfed indigenous larvivorous fishes of 40g on larvae of *Culex quinquefasciatus*

The analysis of figure 11 showed that the number of *Culex quinquefasciatus* larvae introduced in the control plastic glass jar was intact during all the duration of the test. After the introduction of unfed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 40g in each of the four plastic test glass jars, the number of larvae of *Culex quinquefasciatus* was intact at initial time (t=0hour). The number of larvae of *Culex quinquefasciatus* was reduced at middle time (t=12hours) in all plastic test glass jars until became one larva in test glass jars which contained *Oreochromis mossambicus.* At the end of the test (t = 24hours), there was no larvae in the all test glass jars which contained *Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis mossambicus* (P > 0,05), but there was a few larvae in test glass jars which contained *Oreochromis niloticus.*

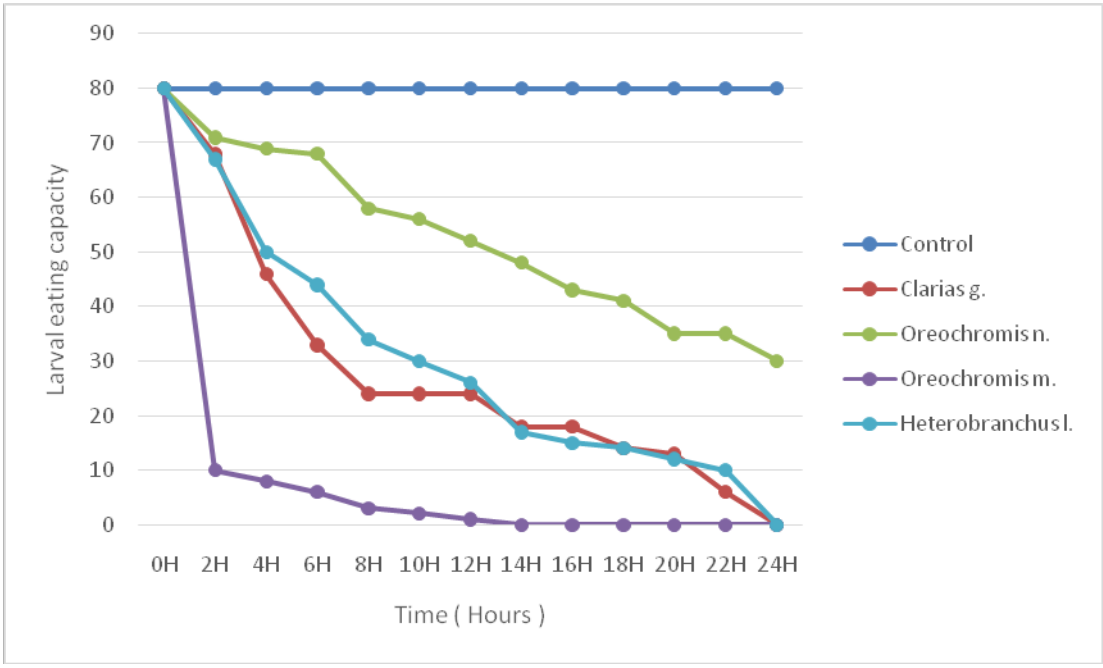

Figure 11: Larval eating capacity of unfed indigenous larvivorous fishes of 40g on larvae of Culex quinquefasciatus

Larval eating capacity of fed indigenous larvivorous fishes of 40g on larvae of *Culex quinquefasciatus*

The analysis of figure 12 showed that the number of *Culex quinquefasciatus* larvae introduced in the control plastic glass jar was intact during all the duration of the test. After the introduction of fed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 40g in each of the four plastic test glass jars, the number of larvae of *Culex quinquefasciatus* was intact at initial time (t=0hour). The number of larvae of *Culex quinquefasciatus* was reduced at middle time (t=12hours) in all plastic test glass jars until became zero larva in test glass jars which contained *Heterobranchus longifilis* and *Clarias gariepinus.* At the end of the test (t = 24hours), there was no larvae in the all test glass jars which contained *Heterobranchus longifilis* and *Clarias gariepinus* (P > 0,05), but there were a few larvae in test glass jars which contained *Oreochromis mossambicus* and *Oreochromis niloticus.*

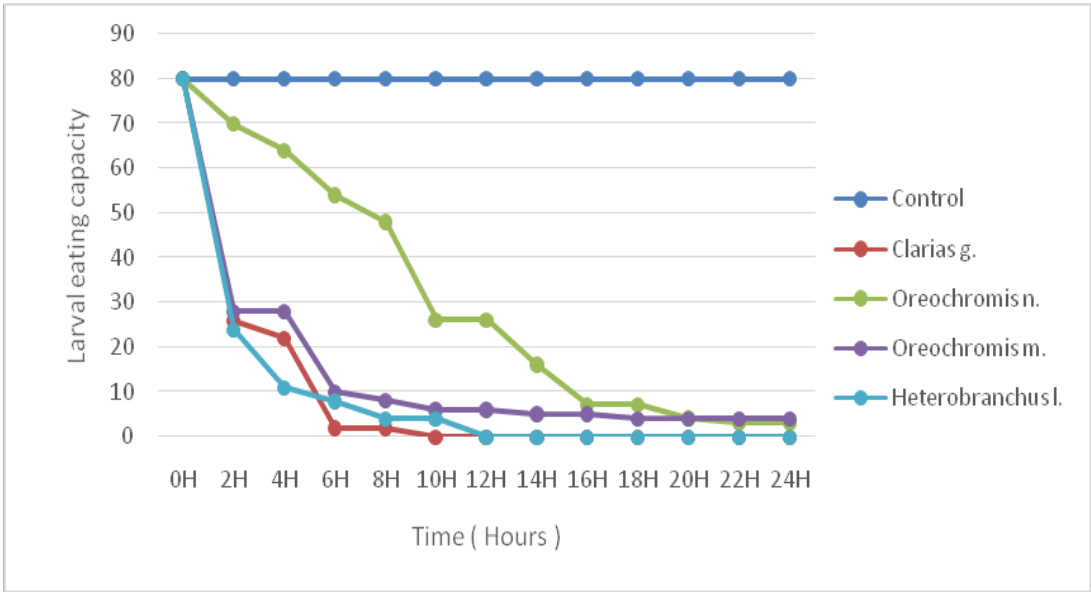

Figure 12: Larval eating capacity of fed indigenous larvivorous fishes of 40g on larvae of *Culex quinquefasciatus*

Larval eating capacity of unfed indigenous larvivorous fishes of 50g on larvae of *Culex quinquefasciatus*

The analysis of figure 13 showed that the number of *Culex quinquefasciatus* larvae introduced in the control plastic glass jar was intact during all the duration of the test. After the introduction of unfed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 50g in each of the four plastic test glass jars, the number of larvae of *Culex quinquefasciatus* was intact at initial time (t=0hour). The number of larvae of *Culex quinquefasciatus* was reduced at middle time (t=12hours) in all plastic test glass jars until became one larva in test glass jars which contained *Oreochromis mossambicus*. At the end of the test (t = 24hours), there was no larvae in the all test glass jars which contained *Oreochromis mossambicus, Heterobranchus longifilis* and *Clarias gariepinus* ($P > 0,05$), but there were a few larvae in test glass jars which contained *Oreochromis niloticus*

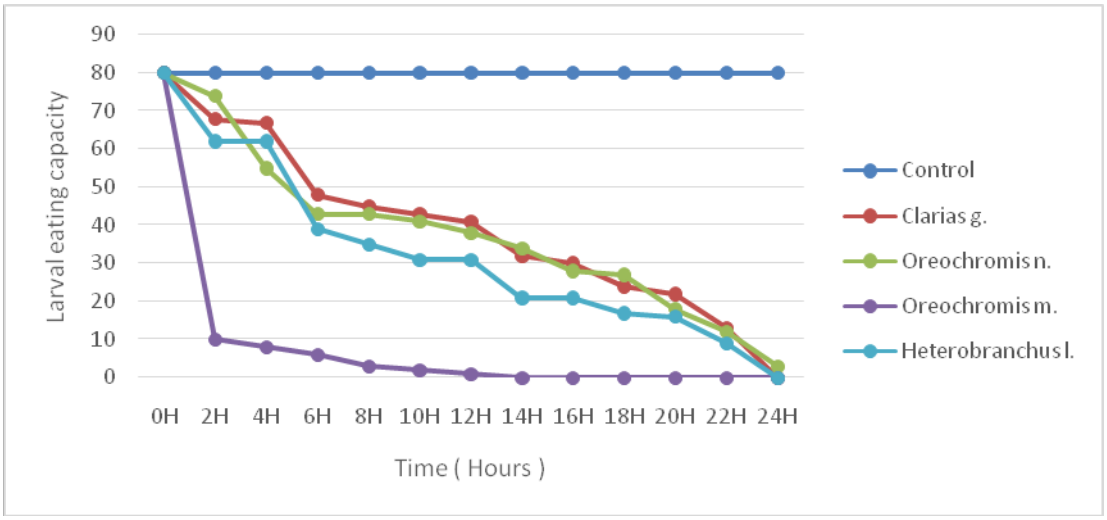

Figure 13: Larval eating capacity of unfed indigenous larvivorous fishes of 50g on larvae of *Culex quinquefasciatus*

Larval eating capacity of fed indigenous larvivorous fishes of 50g on larvae of *Culex quinquefasciatus*

The analysis of figure 14 showed that the number of *Culex quinquefasciatus* larvae introduced in the control plastic glass jar was intact during all the duration of the test. After the introduction of fed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 50g in each of the four plastic test glass jars, the number of larvae of *Culex quinquefasciatus* was intact at initial time (t=0hour). The number of larvae of *Culex quinquefasciatus* was reduced at middle time (t=12hours) in all plastic test glass jars until became zero larva in test glass jars which contained *Oreochromis mossambicus, Heterobranchus longifilis* and *Clarias gariepinus.* At the end of the test (t = 24hours), there was no larvae in the all test glass jars which contained *Oreochromis mossambicus, Heterobranchus longifilis* and *Clarias gariepinus* (P > 0,05), but there were a few larvae in test glass jars which contained *Oreochromis niloticus.*

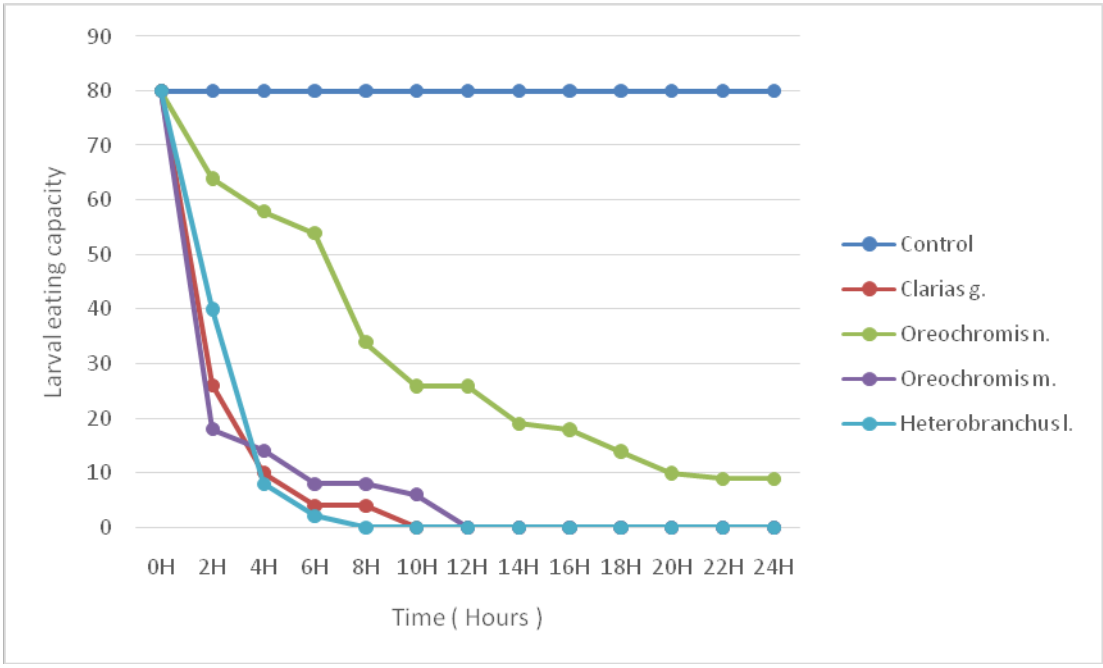

Figure 14: Larval eating capacity of fed indigenous larvivorous fishes of 50g on larvae of *Culex quinquefasciatus*

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 Culex larvae in water sources in laboratory conditions. That can help in the evidence base for larvivorous fish programmes in the control of mosquito-borne disease 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 after the introduction of unfed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 30g in the plastic test glass jars comparatively to those obtained after

the introduction of these fed larvivorous fishes which weighed 30g in the plastic test glass jars, showed that all these fishes had larval eating capacity. But, the larval eating capacity of *Heterobranchus longifilis* and *Clarias gariepinus* was higher than that of *Oreochromis mossambicus* and *Oreochromis niloticus* when these fishes weighed 30g. The role of fish food was not clearly showed in the current study.

In similar way, the results obtained in the current study after the introduction of unfed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 40g in the plastic test glass jars comparatively to those obtained after the introduction of these fed larvivorous fishes which weighed 40g in the plastic test glass jars, showed that all these fishes had larval eating capacity. But, the larval eating capacity of *Heterobranchus longifilis* and *Clarias gariepinus* was higher than that of *Oreochromis mossambicus* and *Oreochromis niloticus* when these fishes weighed 40g. The role of fish food was not also clearly showed in the current study. Our results corroborated with those obtained by Gautam *et al* (2012) who had shown the efficacy of indigenous larvivorous fishes against larvae of *Culex quinquefasciatus.* The larval eating capacity of indigenous larvivorous fishes such as: *Clarias gariepinus* and *Oreochromis niloticus* was recently studied by Aïzoun *et al*. (2022) against larvae of *Anopheles gambiae* sensu lato in malaria vector control in Dogbo district in south-western Republic of Benin with good results.

The results obtained in the current study after the introduction of unfed larvivorous fishes such as: *Oreochromis mossambicus, Heterobranchus longifilis, Clarias gariepinus* and *Oreochromis niloticus*) which weighed 50g in the plastic test glass jars comparatively to those obtained after the introduction of these fed larvivorous fishes which weighed 50g in the plastic test glass jars, showed that all these fishes had larval eating capacity. But, the larval eating capacity of *Oreochromis mossambicus, Heterobranchus longifilis* and *Clarias gariepinus* was higher than that of *Oreochromis niloticus* when these fishes weighed 50g. The role of fish food once again was not clearly showed in the current study. Our results corroborated with those obtained by Parthasarathi *et al* (2016) who had showed the efficacy of indigenous larvivorous fishes such as : *Oreochromis mossambicus* Peters against larvae of *Culex quinquefasciatus.* Another study carried out by Abebe *et al* (2018) also showed the efficacy of tilapia, *Oreochromis niloticus* and *Tilapia zilli* for the control of mosquito larvae around Fincha Valley, Oromia region in Ethiopia.

Conclusion

The use of potential aquatic predators could be an alternative or complementary control measure for reduction in the adult mosquito population in order to reduce the transmission of mosquito-borne disease. In the current study, all species tested were efficient 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 *Culex quinquefasciatus*, which transmits mosquito-borne diseases, is obviously insufficient in the current study.

Acknowledgments

The authors would like to thank people from districts surveyed who had helped us in mosquito collection. The data used in the current study concerned those of the doctoral training of Habib Tamègnon.

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DOI:10.22192/ijarbs.2024.11.12.005

How to cite this article:
Habib Tamègnon, Nazaire Aïzoun, Arlette Adjatin, Thierry Agblonon and Daniel Chougourou. (2024). Larval eating capacity of indigenous larvivorous fishes against larvae of *Culex quinquefasciatus*, main vector in the transmission of mosquito-borne disease in south-western Republic of Benin, West Africa. Int. J. Adv. Res. Biol. Sci. 11(12): 64-75.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.12.005