Feeding rates of Aphyosemion geryi *(Cyprinodontidae) on mosquito larvae in the laboratory and in the field*

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ABSTRACT

Analysis of the gut contents of three populations of Aphyosemion geryi *from Guinea (Conakry) reveals that they feed mainly on small crustaceans, formicidae and chironomid larvae. The predatory capacity of this fish on two species of mosquitos* Aedes aegypti *and* Culex thalassius *was examined in laboratory experiments. The size of prey, which may be related to the motion of larvae, is an important factor determining the predatory capacity.* A. geryi *feeds both during day and night, but it presents a peak of feeding between 10-19h00. Experiments in natural habitats show an important impact of this fish on the younger stages of Culicidae larvae. A 90% reduction of the Culicidae larvae population was reached during the peak feeding period. This species might be considered for possible use in the biological control of mosquito larvae in West Africa.*

KEY WORDS : Fish — Cyprinodontidae — *Aphyosemion geryi* — Culicidae, *Aedes* — *Culex* — Diet — Predatory capacity — Biological control — West Africa.

RÉSUMÉ

TESTS EN LABORATOIRE ET EN MILIEU NATUREL SUR LA CAPACITÉ PRÉDATRICE D'*APHYOSEMION GERYI* (CYPRINODONTIDAE) ENVERS LES LARVES DE MOUSTIQUES

*L'analyse du contenu stomacal de trois populations d'*Aphyosemion geryi *originaires de la Guinée (Conakry) a montré que cette espèce se nourrit principalement de petits crustacés, de formicidés et de larves de chironomes. La capacité prédatrice de ce poisson envers deux espèces de moustiques* Aedes aegypti *et* Culex thalassius *a été examinée par des expériences en laboratoire. La taille des proies, laquelle peut être en relation avec la mobilité des larves, est un facteur important pour déterminer la capacité prédatrice.* A. geryi, *qui se nourrit aussi bien la nuit que le jour, présente cependant un maximum entre 10-19h00. Des expériences réalisées dans des habitats naturels montrent que ce poisson a un impact important sur les plus jeunes stades larvaires. Une réduction de 90% de la population de larves de culicidés a été obtenue pendant la période maximale de prise de nourriture. Au vu de ces résultats, cette espèce de Cyprinodontidae pourrait être considérée pour une utilisation possible dans le contrôle biologique des larves de moustiques en Afrique de l'Ouest.*

MOTS-CLÉS : Poissons — Cyprinodontidae — *Aphyosemion geryi* — Culicidés, *Aedes* — *Culex* — Régime alimentaire — Capacité prédatrice — Lutte biologique — Afrique de l'Ouest.

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INTRODUCTION

There has been a significant increase of malaria since 1979 (WHO, 1983). One reason for this increase is due to the fact that some insecticides have in part become ineffective in controlling the mosquito population, through insecticide resistance parasites. Because mosquito populations have developed resistance to chemical control methods (1983), the World Health Organization is encouraging the biological control of mosquito larvae and several methods have been proposed such as the bacterial control with *Bacillus thuringiensis* H-14 and the use of larvivorous fish (WHO, 1982).

We have been engaged in the search for mosquito feeding fish which are endemic to West Africa. So far two species have been tested and proposed for further studies, *Aplocheilichthys normani* (ROMAND, 1985) and *Epiplatys spilargyreius* (PANDARE and ROMAND, 1986). The present paper reports on the feeding of another Cyprinodontidae, *Aphyosemion geryi* on mosquito larvae. Tests were performed progressively in order to assess the predatory capacity of this species on mosquito larvae : first in small tanks in the laboratory, and later in the field where conditions were close to those of natural habitats.

Aphyosemion geryi is a lowland *Aphyosemion* mainly restricted to the coastal area from The Gambia to Sierra Leone (WILDECAMP *et al.* 1986). However, recently, we caught some populations in the middle of Gambia river and the Upper Casamance river in Senegal. This species can feed on a variety of items such as Cladocera, Hydrocaria, Formicidae and Chironomidae (ROMAND and MORGALET, 1983), and also on mosquito larvae (ROLOFF, 1967). Populations of this species have been observed in a variety of habitats in Guinea (Conakry), Guinea Bissau, Senegal and Gambia. *A. geryi* is found mainly along the banks of small rivers and brooks, and very often in rice fields associated with other Cyprinodontidae species such as *Aplocheilichthys normani, Epiplatys bifasciatus* and *Epiplatys spilargyreius*. The common characteristics of these habitats is the very low level of water and the presence of plants where the fish hide during the day, except for *A. normani*, which prefers the open waters.

MATERIALS AND METHODS

Gut content analyses were made from representatives of three populations of *A. geryi* from Guinea (Conakry); one population from Coya, a second from Kindia and a third from Farmoreya. The gut content of ten specimens of each population was studied after the fish had been fixed with 10% formalin and preserved in alcohol with glycerine. A determination of the biological content of the anterior part of the intestine was made since the stomach cannot be separated in Rivulinae (LABHART and ZISWILER, 1979). This study is based on numerical analysis. We used three quantitative methods :

1 — The occurrence method, in which the total occurrence (Oi) of prey type "i" is the ratio between the number of digestive tracts (ni) containing prey "i" and the total number (N) of tracts examined, normalized for all occurrence and expressed as a percentage:

$$Oi = [(ni/N)/(\Sigma\, ni/N)].100$$

2 — The frequency index (Fi) of prey type "i" is the ratio between the number (ni) of tracts containing prey "i" and the total number (N) of tracts examined :

$$Fi = ni/N$$

3 — The percentage of prey "i" (Pi) is the ratio between the number (pi) of prey type encountered and the total number (P) of prey encountered :

$$Pi = pi/P$$

Experimental feeding-rate tests were carried out with a population collected from a small swamp associated with rice fields on both sides of the road Ziguinchor-Kolda, 3.5 kms before the village of Simbandi-Brassou in the Casamance province of Senegal. Fish were collected with a trap net made up of an iron frame and 2 mm mesh netting. The fish were caught in weeds around the swampy area, held in plastic bags in an isothermic container and transported to the laboratory in Dakar.

For experiments in tanks, or in the field, wild adult specimens of both sexes were used after several weeks of acclimatization in large outdoors basins located in the campus of the University. In these basins, a large variety of Invertebrates including mosquito larvae developed spontaneously.

Two kinds of tests have been performed in order to assess the predatory capacity of this species and its possible impact on natural populations of mosquito larvae :

1 — Laboratory experiments were carried out in glass tanks of 40 liters. The water was at a constant temperature of 25-26 C. Water pH was around neutrality with a conductivity between 400 and 600 µS. Tests on the feeding rate on mosquito larvae were performed mainly with *Aedes aegypti* excepts when otherwise indicated. We used this species because we were able to raise enough larvae for our experiments. We are aware that it is unlikely that

TABLE I

Mean diet composition
Oi = occurence index, Pi = percent in number, Fi = frequency index
Composition moyenne du régime alimentaire
Oi = indice d'occurrence, Pi = pourcentage en nombre, Fi = indice de fréquence

Population Methods of analysis	Coya Oi	 Pi	 Fi	Kindia Oi	 Pi	 Fi	Farmoreya Oi	 Pi	 Fi
Chydoridae	25.6	20.0	0.40						
Cladocera				40.0	37.5	0.33			
Hydrocaria	14.3	10.0	0.20						
Formicidae	42.6	60.0	0.60	20.0	12.5	0.20	100	100	1
Chironomidae	14.3	10.0	0.20	40.0	50.0	0.33			
Vegetal remains									0.20
Animal remains			0.33			0.33			
Algae			0.20						
Diatom			0.33						

Aphyosemion geryi species and *A. aegypti* can be found in the same habitat, since larvae of the later species are mainly found in small pools of clear water trapped by macrophytes under the forest cover. However, because some species of Cyprinodontidae feeding on other species of Culicidae such as *Culex sp.* and *Anopheles gambiae* show the same trends of feeding rate as for *A. aegypti* (ROMAND, 1985), we believe that the model obtained with *A. aegypti* can give good indications on the predatory capacity of a fish with other Culicidae species. For our experiments, *Culex thalassius* were obtained from lentic water from a park near Dakar. Fish were fed each morning with a known number of larvae. The number of larvae given each day for a test was determined in two trials before the experiment and was always greater than the daily feeding rate. The remaining larvae were counted 24 hours later and the number subtracted from the original number of larvae giving the quantity of larvae eaten one day. Each test corresponded to 10 trials in a row. Since one trial lasted one day, the test duration was 10 days in most cases. The feeding rate corresponded to the mean number of larvae eaten per day. The parameter — free U — test was used to compare mean values.

2 — Field studies were performed in small bodies of water located outside a small agricultural village called Hanene close to the town of Thies situated 100 km from Dakar. These bodies of water are called "ceanes" and they correspond to shallow round wells made by the country men for watering gardens. These ceanes are very interesting because they mimic well the natural habitats. In fact, they are restricted in space (3 to 5 m in diameter), the water is shallow and edges are surrounded by plants, bullfrogs, frogs and birds are also present. Moreover, during the rainy season these ceanes contain a large population of mosquito's larvae of different species. Only one trial lasting several months was performed on four ceanes, two filled with 30 couples of adult fish and two served as control. The density of larvae was determined once a week by sampling the surface of the water with a calibrated container of 250 ml. The identification of mosquito's species was done in the laboratory after letting the larvae hatch. In order to assess the impact of the fish on the Culicidae larvae population in the ceanes, we counted only the various stages of larvae which can be done easily by their size.

RESULTS

1. Diet of three populations of *A. geryi* from Guinea

The diet of the three populations showed significant difference (Table I). The diet of the population from Coya is dominated by Formicidae and Chydoridae while the food of the population from Kindia is dominated by Cladocera and Chironomidae. The third population had a diet composed exclusively of Formicidae. Eventhough this result does not show any mosquito larvae in the diet, we have indirect evidences that this species may be a good predator for such larvae :

— First, the presence of *A. geryi* in several other habitats was correlated with the absence of Culicidae

TABLE II

Mean feeding rate of two groups of *A. geryi* (larvae/day-1)
Rythme alimentaire moyen de deux groupes de A. geryi *(larves par jour)*

Groups	Mean	Range
10 adult specimens (5 males and 5 females)	535.8 ± 131.1	310-679
10 adults specimens (7 males and 3 females)	243.2 ± 70.1	145-335

larvae, and the absence of this fish was related to the presence of a large population of larvae.
— Second, another Cyprinodontidae, *Epiplatys spilargyreius* also without larvae in its diet when introduced in large basins containing various species of Invertebrates including Culicidae larvae, cause the disappearance in a few days of mosquito larvae (PANDARE and ROMAND, 1986).

The following experiments were designated to test the predatory capacity of this fish in laboratory on two species of Culicidae larvae.

2. Laboratory experiments on the feeding rate

TEST 1 : MEAN DAILY FEEDING OF TWO GROUPS OF FISH DURING 10 DAYS

From Table II, one can observe the large variation between the two groups, although the fish had the same size, between 24 to 35 mm in standard length, the proportion of males versus females was different.

TEST 2 : FEEDING RATE ON FOUR DIFFERENT STAGES OF *AEDES*

Trials were performed on an adult group with the same size as in test 1 (7 males and 3 females). Each stage was tested successively by 10 repetitions of daily trial. The same number of larvae of each stage was given. Table III shows that the stage of prey is

TABLE III

Mean feeding rate in relation to stage of larvae of pupae given successively (larvae/day-1)
Rythme alimentaire moyen en fonction des stades successifs de développement des proies (larves par jour)

	Stage II	Stage III	Stage IV	Pupae
Total number of larvae eaten by one fish per day (± one SD)	75.2 ± 16.4	53.5 ± 13.1	32.4 ± 8.3	18.5 ± 9.7

TABLE IV

Feeding rate on four different stages of *Aedes* presented simultaneously (larvae/day-1)
Rythme de prédation sur quatre stades différents de Aedes *présentés simultanément (larves par jour)*

	Stage II	Stage III	Stage IV	Pupae
Mean daily feeding rate by 10 fish ± one SD	134.3 ± 43.8	87.4 ± 30.2	71.4 ± 39.2	63.8 ± 30.7
Mean percentage of feeding	37.8 %	25.4 %	20.0 %	17.9 %

an important factor while considering the predatory capacity of *A. geryi*. In fact, the younger stages are eaten in greater quantity than the older ones. The number of stage II larvae eaten is significantly different from that of stage III ($p < 0.05$) and from all other stages ($p < 0.01$). However, there is no significant difference between stages IV and pupae. There is a regular decrease in the number of prey eaten in relation to stages. However, this test cannot give the feeding rate against various stages of larvae when they are presented simultaneously. The following experiment tends to answer this problem.

TEST 3 : FEEDING RATE OF *A. GERYI* FOR DIFFERENT STAGES OF LARVAE AND PUPAE OF *AEDES* GIVEN AT THE SAME TIME

Trials were performed on a daily basis repeated ten times. Fish received each morning at the same time an equal number of four different stages of larvae. Table IV shows that stage II larvae are eaten in greater quantity than older ones and especially pupae which represent only 17.9 % of the

TABLE V

Mean daily feeding rate of three groups of fish on four different stages of *Culex* (larvea/day-1)
Prédation de trois groupes de poissons sur quatre stades différents de développement de Culex *(larves par jour)*

	Stage II	Stage III	Stage IV	Pupae
I daily mean ± one SD	381.8 ± 121.5	168.1 ± 60.6	112.4 ± 32.9	92.4 ± 30.7
II daily mean ± one SD	433.1 ± 106.9	223.8 ± 81.6	137.0 ± 42.6	82.0 ± 17.7
III daily mean ± one SD	286.7 ± 85.5	124.5 ± 48.8	90.5 ± 39.9	59.7 ± 18.8

TABLE VI

Feeding rate for 5 periods of the day
Rythme de prédation au cours de cinq périodes de la journée

	07-10H	10-13H	13-16H	16-19H	19-07H
Number of larvae eaten during 10 days (larvae/period-1)	156	249	314	464	1262
Mean hourly number of larvae eaten (larvae/hour-1)	52	83	104.6	154.6	105
Percentage of hourly feeding rate	10.4 %	16.6 %	20.9 %	31.0 %	21.1 %

total feeding. The number of stage II larvae eaten are significantly different from all other stages ($p < 0.01$).

TEST 4 : MEAN DAILY FEEDING RATE OF *A. GERYI* ON DIFFERENT STAGES OF *CULEX* LARVAE AND PUPAE

Three groups of 5 specimens each were used for this experiment. They were fed of stage II, III, IV and pupae successively by repetitions of 7 daily trial. Table V shows that in all three cases the fish ate more stage II than any other stages ($p < 0.01$). The difference of feeding rate is also significant between stage III and pupae ($p < 0.05$). On the other hand, there is no significant difference in feeding rate between stage IV and pupae.

TEST 5 : COMPARATIVE FEEDING RATES FOR VARIOUS PERIODS OF THE DAY

In order to test the possible variability of feeding during one day, the day was divided into two halves : the first half corresponding to the daylight period (7h00 to 19h00) that was subsequently divided into 4 periods of 3 hours each. The second half corresponded to the night from 19h00 to 7h00. The same number of stage III of *Aedes* larvae were offered to an adult group of 5 males and 5 females at the beginning of each period. The remaining larvae were counted at the end of each period of 3 hours for the daylight and at the end of the night (7h00) for the night period. The computed results for 10 trials are presented in Table VI. It is obvious that the period of 19h00 to 7h00 represents the longest period of the days and also where the largest number of larvae were eaten. In order to get a more meaningful idea of feeding rate during the daylight period and night, it is necessary to compare the same lenght of time. When comparing the hourly feeding rate, one can observe a slight increase of the mean feeding rate from 7h00-10h00 to 16h00-19h00 periods, where feeding reaches a maximum.

TABLE VII

Feeding rate of a group of 10 fishes on 2 species of Culicidae
Prédation par un groupe de 10 poissons sur deux espèces de Culicidae

	Aedes	*Culex*
Average number of larvae eaten 10 fish during one day (larvae/day-1)	243.2	223.9
± one SD	± 70.1	± 26.3
Percentage of feeding	54.7 %	45.3 %

From this experiment it is possible to compare the feeding rate of the daylight period versus the night period. In fact, the beginning of daylight started approximately at 7h00 and the sunset was at 19h00, then we have two equal periods of 12 hours. The comparison of daily feeding rate between these two periods give approximately the same percentage : 51.1 % for the night and 48.9 for the daylight.

TEST 6 : FEEDING PREFERENCE OF *A. GERYI* BETWEEN *AEDES* AND *CULEX*

One group of 10 adult fishes and three other groups of 5 adult fishes each were fed by 10 repetitions of a daily trial with the same number of stage III larvae of both Culicidae species. The *Aedes* were eaten on average more than the *Culex* (Table VII). The *Aedes* represents, 54.7 % of the total feeding, which is not significantly different. A similar result was seen on the three other groups of 5 fishes, each showing a feeding preference for *Aedes* that is significantly different in only one case.

3. Field experiments

The identification of Culicidae larvae in the ceanes shows the presence of several species such as : *Culex decens, C. quinquefasciatus, C. tigripes, C. poicilipes, C. annulioris, Anopheles gambiae s. lato* and *A. arabiensis*. The experiment was performed during the rainy season and in part during the dry season, between July and December. The first four months correspond to an important proliferation of all stages of mosquito larvae as can be observed in the control ceanes (Fig. 1). It is interesting to note that in September the number of stage I and stage II are greater than stage IV, indicating may be, a growing population. Conversely, in November the proportion of pupae is greater than stage I and may indicate a declining population.

In the ceanes filled with 60 fishes the dynamics of the mosquito's larvae population is different from the control ceanes as this can be observed in

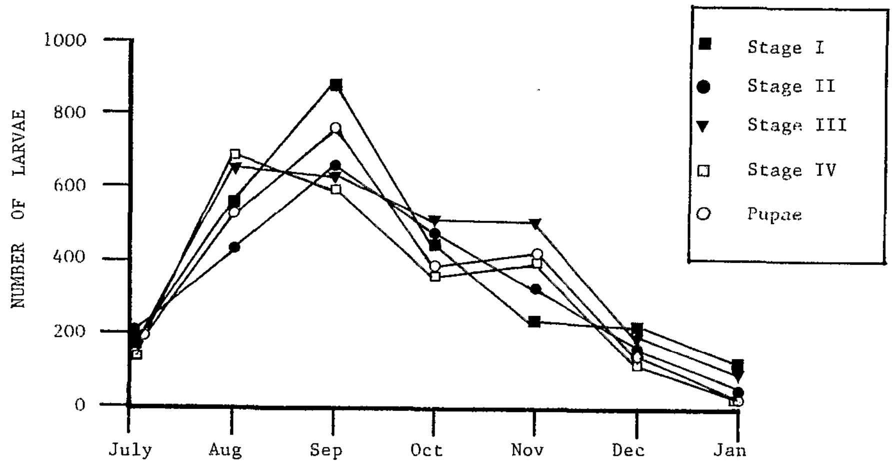

FIG. 1. — Modifications of mosquito larvae populations from a control ceane between July and January
Évolution des populations de larves de moustiques dans une enceinte de contrôle de juillet à janvier

figures 1 and 2. Three very interesting observations can be made from figure 2 :

— first the smaller stage (stage I) do not seem to be affected much by the presence of the fish;
— second, the predatory impact is mainly visible on older stages and more particularly on stage II and stage III;
— third, the number of pupae that will eventually hatch to give adult mosquitos is very much reduced compared with the potential number of mosquitos given by the number of stage I larvae. The reduction of the initial stage I population may reach 90 % in August and between 70 % to 50 % in later months.

DISCUSSION

The absence of mosquito larvae in the diet can not rule out the predatory capacity of the fish for mosquito larvae since we do not know if they are available in the habitat. We know from some other

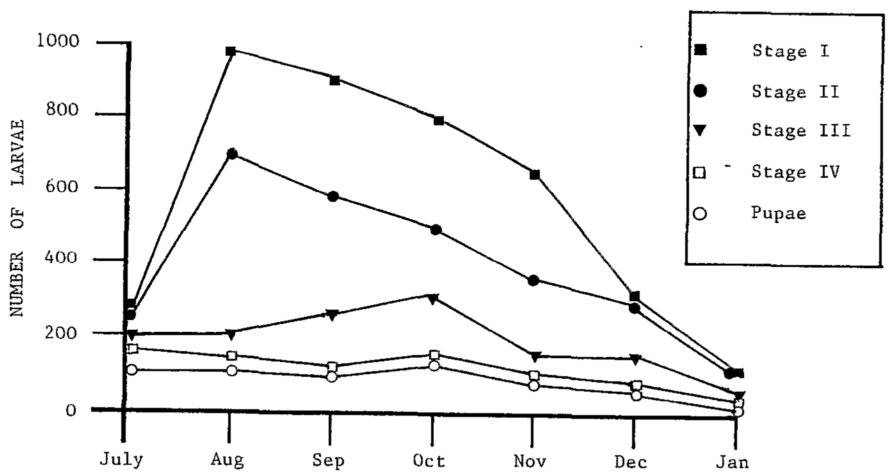

FIG. 2. — Modifications of mosquito larvae populations in a ceane filled with 60 adults of *A. geryi*. One can observe the impact of the fish in comparing with figure 1 from a control ceane
*Évolution des populations de larves de moustiques dans une enceinte peuplée de 60 adultes d'*A. geryi. *L'impact des poissons est donné par comparaison avec la figure 1*

qualitative observations that mosquito larvae were found in the diet when they were present in the habitat. The diet of another *Aphyosemion* species, *A. roloffi* from Sierra Leone, with the same ecology as *A. geryi* was found to be 90 % mosquito larvae (ROLOFF, 1967). Of course, these observations do not give us any information on the prey preference of these fishes. But observations from another Cyprinodontidae (*Aplocheilichthys normani)* gave us the same diet without mosquito larvae (ROMAND, 1985). However, when placed in outdoors basins where mosquito populations developed naturally with some other Invertebrates such as Ostracods and chironomid larvae, the mosquito larvae disappeared in a few days without affecting the other Invertebrate populations.

Aphyosemion geryi represents the third species of Cyprinodontidae tested for its possible predatory capacity on Culicidae larvae. The two previous ones belong to two other genera that exhibit some differences in their biology, eventhough they may be found in the same habitat. *A. geryi* is intermediate in size between *Epiplatys spilargyreius* and *Aplocheilichthys normani*, but its predatory capacity is close to the smaller later species. In aquariums, *A. geryi* shows a greater preference for smaller larvae of stage II than older prey for both mosquito species tested. The same preference was also observed with *A. normani* (ROMAND, 1985) and *E. spilargyreius* (PANDARE and ROMAND, 1986). This may be due to differential mobility of larvae. We observed that older larvae tend to be less mobile in tanks than younger ones.

A. geryi shows little evidence of feeding preference between *Aedes* and *Culex*. However, the two previous species of fish tested in laboratory presented a more obvious preference for *Aedes* versus *Culex* (ROMAND, 1985; PANDARE and ROMAND, 1986). *A. normani* showed a preference with a decreasing order for *Aedes aegypti, Anopheles gambiae* and *Culex quinquefasciatus*. This preference for *A. aegypti* seems to be related to motion of larvae as postulated for this species (ROMAND, 1985) and for *Aplocheilichthis katangae* by THERON and PLESSIS (1985). It seems then, that the determining factor for a larvae to be attractive is its mobility which increases its overall visibility.

Experiments in the laboratory on the feeding capacity of *A. geryi* present serious limitations regarding the biological control of mosquito in natural conditions. In fact, in these experiments, fish have no choice, and they can only feed on available mosquito larvae. Tests undertaken in the ceanes approching natural conditions present a better understanding of the impact of this fish on the Culicidae fauna. *A. geryi* has a very important impact on the various populations of mosquito larvae. This impact corresponds quite well to what we have observed in tanks where younger stages larvae were preferred over older ones and can be related in the ceanes to the more important predatory pressures of *A. geryi* on younger stages, especially stages II. The interesting outcome of this experiment, even if there is not a complete eradication of the Culicidae fauna, is the very important reduction of older larvae, especially during the period when there is a proliferation of mosquitos which corresponds to the rainy season.

This paper presents the first study on the predatory capacity carried out in laboratory on a *Aphyosemion* species and moreover, shows an important impact on the Culicidae fauna in natural conditions. Several authors have stressed the interest of african Cyprinodontidae for the biological control of mosquito larvae such as representatives of the genus *Nothobranchius* (VANDERPLANK, 1941; HAAS, 1965), *Epiplatys* and *Aphyosemion* (ROLOFF, 1967; BAY, 1972) and *Aplocheilichthys* (ROMAND, 1985; THERON and DU PLESSIS, 1985) as recommended by the World Health Organization (WHO, 1982). More studies are underway in order to have a better appreciation of the impact on the Culicidae fauna of *A. geryi* and other related species of Cyprinodontidae in West Africa.

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