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Efficacy of fish as predators of *Aedes aegypti* larvae, under laboratory conditions

ABSTRACT

OBJECTIVE: To evaluate the efficacy of fish as predators of the *Aedes aegypti* larvae in laboratory conditions.

METHODS: The male and female of five different fish were included in the experiment. The tests to measure their consumption ability lasted five weeks for each species. Each trial involved four test tanks and four control tanks. Two control tanks contained just one fish, and the other two just larvae. Each of the test tanks contained one fish and the larvae. During the first week, 100 larvae were placed in the tank, with an additional 100 added every week, up to a maximum daily amount of 500 larvae. The length and weight of the fish were measured at the beginning and end of every week.

RESULTS: A total of 369,000 larvae were used. The *Trichogaster trichopteros* was the only species in which both sexes ate 100% of the available larvae. The *Betta splendens* failed to eat only 15 larvae. The male *Poecilia reticulata* showed a strong capacity for larvae eating, compared with the female of the same species. In terms of weight and size, the *Betta splendens* proved capable of eating 523 larvae per gram of weight per day.

CONCLUSIONS: The female and male *Trichogaster trichopteros* and *Astyanax fasciatus*, and the female *Betta splendens* and *Poecilia sphenops* proved to be the most effective predators of the *Aedes aegypti* larvae. And although the male *Poecilia sphenops* and female *Poecilia reticulata* were less effective, they were also capable of eradicating the total number of *Aedes aegypti* larvae that could appear over 24 hours in a breeding site under natural conditions. The male *Poecilia reticulata*, however, proved incapable of doing so.

KEY WORDS: *Aedes aegypti*. Larva. Fishes. Mosquito control. Pest control, biological. Laboratory experiment. *Betta splendens*. *Trichogaster sp.*. *Poecilia sp.*. *Astyanax sp.*.

INTRODUCTION

Currently dengue has the highest incidence rate of all vector-transmitted diseases both globally and in Brazil. *Aedes aegypti*, which is the main vector of this arbovirus, breeds in any kind of storage container or receptacle that is found in the home and that can hold water. During the last 40 years, the control of dengue has been based on the reduction of potential breeding sites in homes and the chemical control of young and adult forms of *Aedes aegypti*.¹⁵

The control of *Aedes* using chemicals has declined because of the resistance to insecticides that the mosquito has developed¹⁰ and because of the consequences of large-scale use of larvicides and insecticides for the environment.⁵ In Brazil, the Ministry of Health, through the Dengue Control Program, has made major

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efforts to reduce breeding sites and to control the larval and adult forms of *Aedes aegypti*. In spite of this, it has not managed to prevent the cyclical occurrence of dengue epidemics.^{19,21} This justifies a search for alternative ways of controlling the dengue vector, such as the biological control of immature forms through the use of larvae-eating fish.

For several decades, different species of fish have been used in biological control of mosquito larvae, especially in natural breeding sites.^{3,8,9,13} Fish bred in artificial containers, such as large domestic tanks^{14,17,18,20} and rain-water vessels, have already been used as an alternative to biological control in various parts of the world, including Nicaragua and Mexico.^{11,12}

In the State of Ceará, Northeastern Brazil, various species of fish are being used as an alternative form of biological control of *Aedes aegypti* in domestic tanks. In the year 2000, larvivorous fish were being used for this purpose in ten municipalities in Ceará and in the city of Fortaleza alone, there were approximately 419,000 domestic tanks with fish in 2004. Among the various species of fish used, only the male of the *Betta splendens* species is known to have predatory capability, and is able to consume approximately 400 of *Aedes aegypti* larvae per day.

The aim of the present study was to identify the efficacy of different fish species found in Ceará state as predators in the control *Aedes aegypti* larvae in laboratory conditions that replicate field conditions.

METHODS

The species of fish used in the trial were *Poecilia sphenops*, *Trichogaster trichopteros*, *Astyanax fasciatus*, *Poecilia reticulata* and the female *Betta splendens*. All these species, with the exception of the *Trichogaster*, can easily be found in lakes, rivers and lagoons in Ceará. Both the *Trichogaster* and the *Betta* are anabantidae. The poeciliids are from the Poeciliidae family and the *Astyanax* is a Characidae.

The experiments were carried out in the Entomology Laboratory of the Community Health Department at the Universidade Federal do Ceará, in the city of Fortaleza (known by the Portuguese acronym, UFCE). Adult fish that had been collected from natural breeding sites were used, although it was not possible to determine their exact age. Prior to the tests, the fish were quarantined for 15 days in 310 liter fiberglass water tanks, four of which contained fish and larvae (the experiment tanks), and two just fish with no food and two just larvae (the control tanks). The tanks were kept in an open area and were covered with a screen to prevent any insects or predators entering. They were kept at room temperature – an average of 27.5°C, ranging from a high of 32.6°C and a low of 21.9°C.

A total of 369,000 third-stage larvae were used in the experiments, with the tests on each species of fish lasting five weeks. In the first week, 100 larvae were placed daily in each tank (experiment and control tanks), during five consecutive days. In the second week, 200 larvae per day, 300 in week three, 400 in week four and 500 larvae per day in the final week. The experiment was halted before the fifth week, when the proportion of larvae eaten was less than 70%. This process was carried out for each of the five species, and with male and female separately, with the exception of the *Betta splendens*. For each species of fish, two additional tanks with the same amount of larvae were used in parallel, to act as a control for mortality.

The same fish specimens were used throughout the experiment. The fish were measured and weighed before and after each week of the experiment, with the aim of calculating the average and maximum number of larvae consumed for each gram weighed and centimeter of length for each fish. A pachymeter and a digital scale (Gehaka, model BG 200VP) were used, with a maximum capacity of 200g, a minimum of 0.025g and increments of 0.001g. In order to calculate the number of larvae eaten per gram and centimeter of fish, the average and maximum numbers of larvae eaten by each species was divided by the size and weight of each specimen on the final day of the experiments.

RESULTS

A total of 369,000 *Aedes aegypti* larvae were used. The female of the *Betta splendens* species displayed almost the maximum capability possible in this experiment, namely consumption of up to 500 larvae daily. Just one of the females did not eat 15 larvae during the first week and the control group had a mortality rate of less than 3% during the whole period of the experiment. The two specimens used as controls survived without adding larvae during the five weeks of the tests (Table 1).

Both the female and male *Trichogaster trichopteros* showed a high capacity to consume *Aedes aegypti* larvae during the five weeks of the experiment, with both sexes achieving 100% consumption. The average mortality rate of the control larvae was 1.1%. The fish that acted as a control also survived during the five week period (Table 1).

The females of the *Astyanax fasciatus* species consumed 100% of the larvae available in the experiment. The average mortality of the control was around 0.92%. Nonetheless, one specimen among the males of this species left uneaten 300 larvae daily during the final week (Table 1).

The female *Poecilia reticulata* only ate 100% of the larvae during the first week of the experiment, when

Table 1. Percentage of *Aedes aegypti* larvae eaten by the five species of fish, during five consecutive weeks, in 310 litre water tanks. Fortaleza, Northeastern Brazil, 2005.

Weeks	N° of larvae deposited*	<i>Betta splendens</i> Female**	<i>Trichogaster trichopteros</i>		<i>Astyanax fasciatus</i>		<i>Poecilia reticulata</i>		<i>Poecilia sphenops</i>		% of larvae dead in the control	
			Male	Female	Male	Female	Male	Female	Male	Female	Min	Max
			Percentage of larvae consumed (%)									
First	2,000	99.3	100.0	100.0	100.0	100.0	89.3	100.0	100.0	100.0	0.5	2.6
Second	4,000	100.0	100.0	100.0	100.0	100.0	39.0	92.3	100.0	100.0	0.5	1.7
Third	6,000	100.0	100.0	100.0	100.0	100.0	–	85.7	97.6	100.0	0.0	0.3
Fourth	8,000	100.0	100.0	100.0	100.0	100.0	–	91.5	79.7	100.0	0.0	2.3
Fifth	10,000	100.0	100.0	100.0	90.7	100.0	–	67.7	79.4	100.0	0.2	1.5

* Four tanks with 100 larvae, for five consecutive days. Every week, a further 100 larvae were added up to a maximum of 500 larvae per tank per day.

** Only females of the *Betta splendens* fish were used.

Table 2. Minimum and maximum amounts of larvae consumed per day by weight of fish (larvae/day/gram weighed), and per day by length (larvae/day/centimeter length), for the five species of fish, by sex. Fortaleza, Northeastern Brazil, 2005.

Species of fish/sex	Larvae/day/g weight		Larvae/day/cm length	
	Minimum	Maximum	Minimum	Maximum
<i>Betta splendens</i>				
Male	-	-	-	-
Female	406	523	119	125
<i>Trichogaster trichopteros</i>				
Male	117	200	69	81
Female	116	188	69	79
<i>Astyanax fasciatus</i>				
Male	267	349	96	102
Female	281	452	98	111
<i>Poecilia sphenops</i>				
Male	380	523	116	156
Female	301	405	109	125
<i>Poecilia reticulata</i>				
Male	230	307	21	43
Female	438	456	91	102

100 larvae were made available daily. This percentage decreased by the week until less than 70% (of the 500 daily larvae) was consumed. The male *Poecilia reticulata*, meanwhile, showed low capacity to consume larvae, compared with other species in the experiment. Its consumption capacity was less than 90% in the first week when 100 larvae were offered daily, and during the second week, they proved able to eat less than 40% of the 200 larvae offered daily. Therefore the male *Poecilia reticulata* were only included in the experiment for a two-week period (Table 1).

A difference between the male and female *Poecilia sphenops* was also observed in terms of their capability of consuming larvae. The females were able to eat 100% of the larvae provided daily during the tests, while some

male specimens began to eat less from the third week onwards. During the final two weeks, more than 20% of the available larvae remained. The larval controls displayed a mortality of less than 4% (Table 1).

Among the different species, the larvae-eating capacity in relation to weight and size was greatest for the *Betta splendens* and the female *Poecilia sphenops*, with 523 larvae consumed daily, for every gram weighed. In terms of size, the larvae-eating capacity was also greatest for these two species, eating 125 and 156 larvae per centimeter, respectively (Table 2).

The tests on both sexes of the five species lasted for 25 days, with the exception of the male *Poecilia reticulata* which was terminated after ten days. Thus, with the

exception of the male *Poecilia reticulata*, the number of larvae consumed daily varied from 180 larvae on the 25th day, by the female *Poecilia reticulata* to 500 larvae on the 25th day by the female *Betta splendens* and the male and female *Trichogaster trichopteros*, *Astyanax fasciatus* and *Poecilia sphenops*.

DISCUSSION

Very little research into the use of fish to control mosquito larvae has been published in the recent Brazilian literature.¹⁶ In Ceará state, various fish species have been used to control the immature forms of *Aedes aegypti*. In the first instance, under laboratory conditions, a male specimen of the *Betta splendens* was found to be capable of removing 500 *Aedes aegypti* larvae in less than 24 hours. In subsequent field work, male specimens of the *Betta splendens* were placed in various kinds of domestic containers. The results of this pilot study were particularly promising since at no stage were *Aedes aegypti* larvae found in the containers that housed the *Betta splendens*. As a result of high levels of interest among the local and national press, uptake by the population followed almost immediately and was accompanied by a massive rise in demand for larvae-eating fish. In the city of Fortaleza, demand for the *Betta splendens* fish outstripped the supply capacity of the natural breeding sites (large lakes) in the city. This failure in supply gave rise to the search for other species of larvae-eating fish, mainly from the countryside of Ceará state. Although these fish can be found in natural breeding sites in various locations in the state, the majority of them are exotic species to the region.

A fish's efficacy as a predator depends on its weight and sex, among other variables. The present study evaluated the preference or consumption capacity of five species of fish that are being used to control *Aedes aegypti* larvae in the State, against these two variables. With the exception of the male *Betta splendens*, all other species were introduced as part of the Dengue Control Program, with no prior knowledge of the larvae-eating capacity of the fish, whether using natural or laboratory conditions.

Estimates of the number of larvae consumed per gram weighed by each fish make it possible to compare the larvae-eating capacity of the fish species. Of the species involved in the present study, the number of larvae removed varied from 188 larvae/gram/day (female *Trichogaster trichopteros*) to 523 larvae/gram/day (male *Poecilia sphenops* and female *Betta splendens*). Jayasree & Panicker⁶ reported that the *Trichogaster trichopteros* is capable of consuming up to 47 *Culex quinquefasciatus* larvae/gram/day. Molloy¹² found that the *Poecilia sphenops* kills up to 200 larvae in 24 hours or up to 405 larvae/gram/day. Considering that all the species of fish involved in this research are relatively

small, comparisons of larvae-eating capacities can be made by measuring the number of larvae consumed during a specific time period. Thus, the smallest and largest number of larvae removed during 24 hours were 180 and 500. Vargas²² studied the behavior of the fish from the Poeciliidae family and found that the *Poecilia gilli* consumed ten larvae in 67 seconds or 75 larvae in 15 minutes. Work by Gene et al⁴ showed that the *Astyanax bimaculatus* has a very high consumption capacity, managing to consume between 342 and a thousand larvae in 24 hours (at an average of 655 larvae).

The results shown here are comparable to those found in the literature and lead to the conclusion the fish from the *Astyanax*, *Trichogaster* and *Poecilia* genera are proficient larvae-eaters. The laboratory conditions used in this study are very similar to those found in the field, since these kinds of water containers most commonly found in houses were used.

The total number of larvae killed in a day, irrespective of fish weight or size represents an important parameter in evaluating the larvae-eating potential of fish as a strategy for controlling insect larvae. The control can be considered effective when a specific number of fish prove capable of consuming all the larvae present in a specific breeding container. The number of larvae present in a container is a complex matter and depends on several factors including the material of the container, its size and location.⁷ Independently of the type of container, the number of larvae also depends indirectly on the choice of location for positing *Aedes aegypti* eggs. Research by Zahiri & Rau²³ shows that *Aedes aegypti* pregnant females have a preference for choosing breeding sites with lower density of larvae and ones that do not contain undernourished larvae or larvae infected by certain pathogens. In another study located in what the authors call "a city with low levels of infestation", an average of 66 culicid larvae (of *Aedes* and *Culex* genera) per domestic container, counting only those containers that housed larvae.¹ In one area of New Orleans in the United States with high infestation levels of *Aedes aegypti*, a total of 24,609 *Aedes aegypti* and *Culex quinquefasciatus* larvae were collected in 107 domestic containers¹⁰ or an average of 158 larvae per container.

Nonetheless, the individual daily consumption capacity of the fish in the present study is higher than the number of larvae present in the breeding containers referenced in these three studies. In addition, the results obtained suggest that the number of fish needed to guarantee the effective control in artificial domestic containers of *Aedes* larvae, or of Culicidae in general, would be less than that proposed by Martinez-Ibara et al.¹¹ These authors suggest that ten *Poecilia sphenops* or *Astyanax fasciatus* specimen be used in each container. However, an analysis of the number of larvae that a container

can host suggests that only one specimen of the fish is capable of removing, in a short space of time, the *Aedes* larvae that can possibly exist in domestic containers. Furthermore, in natural conditions, it is probable that the fish will eat the larvae at the rate that the latter are born, and that the number of larvae that appear per day is much less than the number found in a breeding container at any given time.

With regard to the sustainability in the use of fish in the biological control of larvae, the most important variable is the duration of the fish in the container. This depends on the fish's survival capacity and the relative difficulty of leaving the container. In the case of the *Betta splendens* fish, according to incomplete results from a prospective study that is currently being carried out, after between 35 and 90 days, the fish was still present in just 41.3% of containers (findings not published). The reasons for this disappearance of fish have not yet been studied, but empirical observations made at field sites suggest that the *Betta* cannot tolerate water with high concentrations of chlorine. The exit from a container can be prevented by making small hydraulic modifications. In the containers in Fortaleza, PVC adapters were placed on the tubes that pumped water from the containers, to prevent the fish from leaving. Another aspect that needs consideration is the survival rate of the species used as a control during the five weeks of the experiment, where no additional food was provided. All, with the exception of the *Poecilia reticulata*, survived during the experiments, without the daily addition of larvae, which suggests that they could survive in large domestic containers, even during periods with low levels of larvae.

The use of fish in containers in the home raises questions about the biosafety of this method. The larvae-eating fish species are obtained from natural water sources, meaning that it is possible that they may carry with them

micro-organisms from the original breeding sites that are potentially pathogenic for human beings.

A study carried out in Trinidad by Chadee² showed that the individual *Poecilia reticulata* fish sourced from either natural or laboratory conditions, were infected with *Escherichia coli*, *Citrobacter freudi* and *Pseudomonas aeruginosa*. These important findings are the only of their kind in the literature, meaning it will be necessary to investigate the level of susceptibility of the *Poecilia reticulata* in Brazil to these species of bacteria. On the other hand, it is possible that the contamination is only an indicator of the presence of the bacteria in the water source, and if this is indeed the case, the implications are less serious. Chadee's² results cannot be generalized and new studies will be needed that reflect the particular region and ecology of the breeding sites from which the fish are sourced.

In conclusion, the fish included in this experiment, with the exception of the male *Poecilia reticulada*, showed an excellent capacity to remove *Aedes aegypti* in laboratory conditions. Further studies are needed to measure the appropriateness of this biological control method in field conditions. In other words, there is a need to know the kind of water and the kinds of containers that facilitate maximum larvae consumption and longest survival of the fish. Among other factors, studies are needed on the kind of material, the volume of water and the location of the container (whether inside or outside the house), the physio-chemical levels of the water, primarily, and also of the chlorine and organic material.

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