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Control of Dengue Carrier Aedes Mosquitoes (Diptera: Culicidae) Larvae by Larvivorous Fishes and Putting It into Practice Within Water Bodies

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Abstract

There is growing awareness on the effects of insecticides used for controlling the vectors spreading human diseases. Manipulating or introducing of a self reproducing predator into the ecosystem may present continual biological control of vector populations. In order to achieve an acceptable control of the primary *Aedes aegypti* (Linnaeus) and secondary *Aedes albopictus* (Skuse) mosquitoes (Diptera: Culicidae) as vectors of dengue fever, this study puts side by side a range of control methods and found that keeping fishes is the most effective strategy. Larval control of vector mosquitoes appears to be promising in rural and urban areas, given that the density of humans needing protection against dengue is higher than the limited number of breeding sites. One of the most successful and widely used biological control agents against mosquito is the top water minnow or mosquitofish *Gambusia affinis* (Baird & Girard) that can consume 100 to 300 larvae per day. Fish other than Gambusia which has received the most attention as a mosquito control agent is guppy *Poecilia reticulate* (Peters), and single fish can eat about 80 to 100 mosquito larvae per day. Both these larvivorous fishes such as Gambusia and Poecilia are small in size and inedible, highly tolerant and carnivorous, prefer shallow water and frequent to the margins of the water bodies where mosquito larvae also breed. The use of biologically managed larviciding fishes for the control of dengue disease carrier mosquitoes is feasible and effective only when breeding sites of vectors are relatively identified and treated. These fish can be placed in ornamental ponds, stock watering tanks, ponds without game fish, and a variety of other locations as biological control agents for mosquito larvae. When these predators are placed in container habitats, decorative ponds and pools, they prey on mosquito larvae for effectively preventing mosquito's development. For self perpetuating control of dengue vectors, the implementation of larvivorous fish should be accompanied by an adequate participatory education of peoples to make it more acceptable and potentially sustainable for communities. When biological tools are not always feasible in certain container habitats and all water storage containers cannot be removed, cleaned or covered, then a combination of mosquito control devices can be more effective. One of the benefits of integrated vector management (IVM) is that it overcomes the disadvantages of using individual methods, proves popular in certain trials and shows great promise for controlling disease carrying mosquitoes.

Keywords

Mosquito Control, Larvivorous Fish, Biocontrol Agent, Dengue, Biological Control, Predator

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1. Introduction

Some of the most prevailing mosquito-borne (Diptera: Culicidae) human diseases, for instance dengue and malaria, symbolize a most important load on public health worldwide. Other than sickness causing pathogens, mosquito vectors are furthermore constantly bared to a wide diversity of microbes

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within their environment. A number of other microbes are likely to be obtained by mosquito vectors from the honey dew, nectars or breeding water used for their persistence. For transmission of vector-borne diseases, victorious contacts between insect vector, human host and the pathogen are required. The greater part of investigation intended at control of vector-borne sickness has primarily targeted on the contacts among human and pathogen (development of drug and vaccine) otherwise involving links between human and vector (development of insecticide). Alternative approaches for control of insect, pathogen and disease are desirable to decrease the risk from arthropod-borne sicknesses. Owing to the complicacy to build up a dengue vaccine and the mosquitoes have also developed resistance to insecticides, alternative approaches are needed for control of insect, arbo-pathogen and disease. However, in order to fight invading insect, interest is growing for the manipulating or introducing of biological tools to control the vectors of human diseases to provide persistent control of dengue or malaria for reducing global public health trouble (Sarwar, 2014 a; 2014 b). Since long times, fishes have been employed for controlling of mosquito larvae breeding in water containers, ponds or other ground water collections, where their usage has proved popular in certain trials and shown a great promise.

Fishes have been widely used in public health due to an environment friendly method of control for mosquitoes larvae, are self-perpetuating after their establishment, continuous to reduce vector density for long time, and the cost of their introducing is relatively lower than that of chemical control. Furthermore, a study found that in rural areas 43.7% of containers without fish have *A. aegypti* larvae, compared to just 7.0% of containers that have fish; this effect is also seen in an urban area (40.6% vs 8.3%). This study compared a range of control methods and found that keeping fish is the most effective (Phuanukoonnon et al., 2005). Larvivorous fishes have further advantages, unlike some of the invertebrate predators, peoples feel familiar with fish (Martinez-Ibarra et al., 2002; Sarwar, 2014 c) and this means that they are able to apply this control tool themselves. This also happened in another locality where the successful use of *Betta splendens* fish has been broadcasted in the media, resulting in the peoples to place this fish in their water storage containers of their own accord (Lima et al., 2010). In addition, the success of a trial in other region has been attributed in part to the adoption of the larvivorous fish as pets by the local children (Martinez-Ibarra et al., 2002). Thus, a sound knowledge of various attributes of interactions between the vector population and the fish predator to be introduced is desirable.

2. Criteria to Quantify the Efficacy of Larvivorous Fish

The success in measuring the efficacy of the candidate agents depends on a multitude of factors: - (i) characterization of natural enemy candidates including ecological, morphological, taxonomical or genetic markers; (ii) selection of climatically matching candidates; (iii) evaluation of semi-field or field cage conditions following quarantine evaluations prior to proceeding with natural release; (iv) assessment of unintended impacts; and (*v*) the potential efficacy of existing indigenous agents against larval populations (Chandra et al., 2008). The two main factors determining the efficacy of the fish are the suitability of the fish species to the water bodies where the vector species breeds and the ability of the fish to eat enough larvae of vector species to reduce the number of infective bites. The first factor is best addressed by finding a native fish species that thrives under the conditions prevalent in breeding sites rather than to change breeding sites to accustom the fish, although Wu et al., (1991) have recommended a ditch ridge system for rice fields to better accommodate the fish. It has to be kept in mind that the use of pesticides and fertilizers can negatively influence fish stocked in irrigated fields (Lacey and Lacey, 1990). The second factor may be strongly influenced by aquatic vegetation, which in turn, can interfere with fish feeding and can also provide refuge for the mosquito larvae. The effectiveness of larvivorous fish to control mosquitoes may vary due to environmental complexity. It needs to evaluate the efficacy of several indigenous larvivorous fish in seasonal wetlands and larger water bodies and their role in the trophic flow from the community view point (Blaustein and Chase, 2007).

3. Common Species of Larvivorous Fish

One of the most successful and widely used biological control agents against mosquito larvae is the top water minnow or mosquitofish *Gambusia affinis*. Fish other than Gambusia which has received the bulk concentration as a mosquito control agent is *Poecilia reticulata*, the common guppy. Both these larvivorous fishes such as Gambusia and Poecilia prefer shallow water where mosquito larvae also breed.

3.1. Western Mosquitofish *Gambusia affinis* (Baird & Girard)

The eastern mosquitofish *Gambusia affinis* (Baird & Girard) is a member of the family Poeciliidae of order Cyprinodontiformes and a species of freshwater. The common name mosquitofish is derived from its diet, which under some circumstances consists of large numbers of mosquito larvae relative to body size. Based on diet, mosquitofish is classified as larvivorous fish and its diet consists of zooplankton, small insects and insect larvae (beetles, mayflies, caddisflies), other invertebrates (mites), and detritus material. Mosquitofish feeds on mosquito larvae at all stages of life and adult females can consume hundreds of larvae in one day (100 to 300 larvae per day). Maximum consumption rate in a day by one mosquitofish has been observed to be from 42-167% of its own body weight. Mosquitofish is small, of a dull grey colouring, have rounded dorsal and caudal fins and an upturned mouth in comparison to many other freshwater fishes, and females reaching an overall length of 7 cm and males at a length of 4 cm. The female can be distinguished from the male by its larger size and a gravid spot at the posterior of its abdomen. Fish breeds throughout the year, fertilization is internal, the male secretes milt into the genital aperture of the female through its gonopodium and within 16 to 28 days after mating, unlike to many native fishes the female gives birth to about 60 young ones that are about 8 to 9 mm in length. The females reach sexual maturities within 21 to 28 days while the males within 43 to 62 days. Because of their notable adaptability to harsh conditions such as low oxygen concentrations, high salt concentrations (up to twice that of sea water) and temperature up to 42°C (108°F) for short period, surface feeder for feeding on both anophelines and culicines, small in size and inedible, withstand for transportation, and global introduction into many habitats for mosquito control, this has been described as the most widespread freshwater fish in the world. These are now considered just slightly better at eating mosquitoes than at destroying other aquatic species (Dionne, 1985; Campton and Gall, 1988; 1988; Koya and Kamiya, 2000).

Classification of the other species western mosquitofish *Gambusia holbrooki* has been difficult due to its similarity to the eastern mosquitofish *G. affinis*. This has a dorsally flattened head, rounded tail and a single dorsal fin, back is green to brown, becoming grey with a bluish tinge down the sides, silver on the belly and also has an upturned mouth. Adult females mosquitofish can be identified by a gravid spot (carrying eggs) these possess on the posterior of their abdomens, and reaching a much larger size of up to 6 cm compared to males. Males grow to around 3 cm in length, lack the prominent stomach bulge as present in females and possess a longer anal fin which is used as a breeding tool. Western *G. holbrooki* more commonly known as mosquitofish, as a biological control agent for mosquitoes has been recommended by World Health Organization to use for malaria control programs (Arthington and Marshall, 1999; Willems et al., 2005).

3.2. Guppy *Poecilia (Lebistes) reticulata* (Peters)

Similar to Gambusia, guppy *Poecilia* (*Lebistes*) *reticulata* is also easy to care, is hardy fish, survives in all types of water bodies and reproduces quickly and prolifically. Females reach up to 6 cm in length, compared to males, which is only around 3 cm in length, and both can live for 4 to 5 years. The females breed throughout the year at about four weeks interval after maturity and take about 90 days to mature. The each ovary of guppy female can bear from 100 to 160 eggs, gives birth to young ones averaging 5 to 7 young per brood at a time and about 50 to 200 young ones are released after every four weeks. The larvivorous effectiveness of Poecilia is comparatively less than Gambusia, and as a result, a single fish can eat about 80 to 100 mosquito larvae per day. It can survive and reproduce when introduced into new water bodies, is a surface feeder as well as highly carnivorous and older brood may eat up their own young ones. The larvivorous efficiency of guppy is due to its more tolerance than Gambusia to salinity and high degree of pollution with organic matter, frequents to the margins of the water bodies, no any specialized equipments are required for its transportation, and once established well can be found in the habitat even after many years (Sabatinelli et al., 1991; Chatterjee and Chandra, 1997). The researchers Nalim and Tribuwono (1987) studied the rice field breeding mosquito *Anopheles aconitus* and its effective control using *P. reticulata* through community participation. They noticed a sharp decline in the number of malarial cases after introduction of effective biocontrol procedures with larvivorous fishes.

Earlier attempts to introduce the so-called mosquito fish, *G. affinis* or *G. holbrooki* to tropical areas may have or not been successful in most places as the species does not breed well in tropical environments. For that problem, examples of other common larvivorous fishes include in the genera Poecilia (Lebistes), Apochelius, Panchax and Macropodus, which are common and widespread in many tropical and subtropical areas where dengue is endemic to exclude *A. aegypti* mosquito from breeding in containers of drinking water. The small fishes such as gold fish *Carassius auratus* (Linnaeus), *Claris fuscus*, *Tilapia nilotica* and *Macropodus* spp., have been used in many regions to eliminate the larvae in the domestic water containers with considerable success. The use of catfish appears to be particularly effective, such fish may have potential elsewhere in the world where its introduction can be maintained and where the human population does not have objection to the presence of these animals (Neng et al., 1987).

4. Guidelines on the Use of Larvivorous Fish for Vector Control

Of course, the experience on the development of larvivorous fish network for mosquito control can demonstrate the feasibility of using such natural enemies in urban and rural areas provided if a systematic and planned approach is practiced.

4.1. Hatcheries for Larvivorous Fish

The hatchery for larvivorous fish can be established in the following two ways:-

4.1.1. Natural Water Body Hatchery

The criteria for selecting a water body for a fish hatchery are, it should be a permanent, confined and without big natural outlet, free from other carnivorous fish, not be contaminated by any chemical or other harmful substances, easily accessible for daily or periodic inspection and for collection, and periodically weeds cleaning in and around of water. The depth of water should be at least 1.5 m or more and the minimum size of water body may be at least 5 m × 4 m, however, water body of 10 m × 5 m can support 50000 fish.

4.1.2. Special Hatchery

The subsequent points might be kept in view, while constructing the special hatcheries for the rapid reproduction of larvivorous fish, there should be a constant supply of fresh water so that the required level of water in the tank does not drop, submerged vegetation such as Hydrilla or Vallisneria should be available in the tanks, and hatchery should not be subjected to strong water current and be protected from heavy rains and floods. Entire tanks should be brick made, lined with good quality of cement plaster with thickness of wall about 0.5 m. The tank should be divided into two portions of equal size of 5 m × 4 m (one for laying young ones and other for holding mature full grown fish) with central separator of 0.5 m thick, and tank should be allowed to mature for 10-15 days. The depth of water in the hatchery should be 1.5 m and floor of tank 0.5 m thick with slope from the partition towards sides. Proper outlet at the bottom of tank should be provided; overflow outlet about 5 cm below, inlet protected with proper wire mesh to prevent escape of fish and inlet should be at 1.25 m height. Bottom of tank should be covered with uniform thickness of sand for about 10 cm and the bottom seeded with organic matter about 2 kg/ m^2. At least 25% amount of water should be replaced once a week and the fish should be transferred from the tank to avoid over population. Chlorination of water beyond the tolerance levels or presence of insecticides can be lethal to the fish, and salinity of water should not exceed 20 gm per liter. In case of scarcity of natural food, artificial food such as waste flour may be given.

4.2. Collection of Larvivorous Fishes

Fishes are collected with the help of netting fitted on a circular iron ring of 60 to 90 cm diameter with a wooden handle; sufficient quantity can be collected by repeated dips and put collections in bucket or drums till these are packed for transportation. Preferably the fishes should not be given any food for 10-12 hours period prior to packing for transportation.

4.3. Transportation of Larvivorous Fish

The fishes are best transported in small containers of up to 40 liters, such as plastic buckets, cans or in strong plastic bags, half filled with water from the rearing pond, containers should have sufficient openings to allow flow of air and should not be exposed to direct sunlight. Another way is, take polythene bag of 3-5 liter capacity, fill it with 1.5 liter of water, and introduce the fish in the bag till the total volume of water plus fish is two liters. Bubble the oxygen in bag from O_2 cylinder or from air pump, close the mouth of bag with a string leaving sufficient space at the top, put the bag in a thermocol container and close the mouth of container. The container can be transported for a period of 24 hours without further filling of oxygen, but, if the period of transport is more than 24 hours then arrange for change of water and oxygenate.

4.4. Release of Larvivorous Fish

For release of the fish in a water body, measure the boundary of water body, release the fish at the rate of 5-10 per linear meter and if the larval density is high more fish up to 20 can be released. Fishes should be released in the morning hours or in the evening and before releasing ensure that the temperature of water both in container and in larval habitat is more or less similar. Further, ensure that water body is clean and free from dense vegetation and predators of larvivorous fishes.

4.5. Use of Larvivorous Fish

Fish should be preferably introduced in all unused wells in the rural and urban areas before the high mosquito breeding season to maximize their impact. However, the fresh water bodies in rural areas such as stagnant ponds, slow moving streams quarry pits, large borrow pits and margins of ponds should be targeted apart from wells. In open mosquito breeding sites or when used in rice fields, the fishes need to be protected from pesticides applied to crops or rice fields.

4.6. Monitoring of Larvivorous Fish

The care taker of the operation should check the fish hatcheries at least once a month during the high transmission season, and at least 10% of the sites where fishes have been introduced should be checked for whether fishes have been introduced or not, these are surviving or not and identify possible reasons in case the introduced fishes are not surviving.

Mosquitofish is introduced directly into ecosystems in many parts of the world as a biocontrol agent to lower mosquito populations. Mosquitofish can be placed in decorative ponds and other large container habitats to prey on mosquito larvae and effectively prevent mosquito development. Larvivorous fish (top feeding minnows) can be used as a complementary means of Aedes immature's control especially in large and permanent water storage containers. However, a rule in larvivorous fish usage is that only easily found indigenous species might be used. Importation of exotic fish species for mosquito control is not recommended due to the potential adverse environmental impact on the local ecosystem.

In short, amongst vertebrates mostly fishes have been highlighted in biological control of mosquito larvae. But fish, especially when introduced, could cause ecological damage by becoming a threat to native organisms including amphibians whose populations are often in decline. Furthermore, fish needs interconnected waterways to spread and are often not found in isolated pools, tree holes, rock pools, ponds and most ephemeral water bodies, which are ideal breeding grounds for mosquitoes. However, biological tools are not always feasible in certain small container habitats, and all water storage containers cannot be treated with natural enemies. One of the benefits of IVM (Integrated Vector Management) is that it overcomes on mosquito control tools and can be more effective than any tool used in isolation. Authors of certain studies have concluded that integrated vector control is the best and most advantageous over using of individual methods, and is a combination of effective methods for dengue control (Chen et al., 1994; Sarwar, 2014 d).

5. Conclusion

Chemicals are capable to contaminate the atmosphere, are pricey and insecticide resistance has been developed in Aedes mosquitoes. There is no silver bullet for dengue vector control, and each of the intervention categories has its own advantages and disadvantages. Different types of fishes have been used so far in the operational technique against dengue vectors, and many varieties of fish, including mosquitofish, guppyfish and goldfish, eat mosquito larvae. However, use of fishes of indigenous origin is found to be more appropriate in the vectors control operation, so, the use of native larvivorous fishes is advocated. Also somehow the use of larvivorous fishes is practical because most of *A. aegypti* and *A. albopictus* populations are produced in small man-made containers such as tires, buckets, cans, bottles and so on, located at the backyards of houses. Nevertheless in few tropical countries, it is common to have large cement-built deposits to store water in houses; hence the use of fishes in those structures, water containers, ponds or other ground water collections is effective. As a result, the selection of a biological agent should be based on its self replicating capacity, preference for the target vector's population in the presence of alternate natural prey, adaptability to the introduced environment, and overall interaction with indigenous organisms. Consequently, the broad-spectrum properties of fishes with their ability to destroy mosquito larvae can make these motivating candidates for the control of vector-borne diseases. However, adoption of Integrated Vector Management (IVM) is an inclusive tactic to accomplish a good collapse of dengue like vector borne diseases.

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