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Comparative efficiency of Larvivorous fishes against *Culex* mosquitoes: Implications for biological control

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Abstract

Biological control of mosquitoes using larvivorous fishes is a sustainable practice when compared to the traditionally used chemical methods. The present study made an attempt to evaluate the comparative larvivorous potential of three indigenous fish namely *Danio rerio*, *Danio malabaricus* and *Aplocheilus panchax* with two exotic species *Gambusia affinis* and *Poecilia reticulata* against *Culex* larvae. Predatory potential of fishes was assessed at different prey densities for a period of 24 hour. The study revealed that all the fishes showed larvivorous potential with differences in their feeding efficiencies. The sequence of predation efficacy noted in the present study was *Danio rerio*>*Aplocheilus panchax*>*Gambusia affinis*>*Danio malabaricus*>*Poecilia reticulata*. The study also shown that the foraging behavior of all the fishes showed a significant increase ($P<0.05$) with increase in prey densities. Hence, the strategy of biological control through indigenous larvivorous fishes can be practical on a large scale which is economically viable and pollution free.

Keywords: Larvivorous fish, *Danio rerio*, *Culex* larvae, indigenous fish, biological control

1. Introduction

Mosquitoes, the blood sucking dipteran insects are well known vectors for the transmission of several life-threatening diseases all over the world and all these mosquito-borne diseases continue to be a key problem in almost all tropical and subtropical countries [1]. Different species of mosquitoes are estimated to transmit diseases more than 700 million people annually in Africa, Mexico, South America, Central America, and most of the Asian countries with more than one million deaths every year globally [2]. More than 3, 000 species of mosquitoes have been identified all over the world that comes under the family culicidae. Mosquitoes are holometabolous insects experiencing a total transformation in their life history including egg, larva, pupa and adult. The initial three stages are aquatic and last for 5 to 13 days, depending upon species and ambient temperature. Generally, mosquitoes flourish in stagnant water and have distinctive rearing surroundings based on species. The most common genera of mosquitoes are *Aedes*, *Culex*, *Anopheles* and *Mansonia*, each having hundreds of species with its own unique characteristics and potential to transmit various diseases [3]. *Aedes* mosquitoes are flood water mosquitoes that prefer damp soil or small man-made or domestic water collections containers like opened vessels or bottles, discarded tyres, discarded coolers or even if coconut shell etc. to lay eggs [4]. *Culex* is the very common type of mosquito prevalent throughout year, laying eggs in rafts seen on the surface of any type of standing water [5]. *Anopheles* mosquitoes are marsh mosquitoes that prefer ground pools, irrigated areas, small streams, freshwater marshes, forest pools or any place with clean, slow-moving water, are all considered prime breeding grounds for egg-laying [6]. The *Mansonia* mosquitoes breed in ponds and lakes containing aquatic plants, especially floating types and eggs are laid in star-shaped clusters on the under surface of leaves of these plants [7].

Both male and female mosquitoes are nectar feeders, while females of several species feed upon blood of humans, other mammals and livestock especially during breeding season, since female requires certain proteins from the host's blood for the development of eggs [8]. Thus, female mosquitoes act as vector that carry disease causing viruses, bacteria, protozoans and nematodes from person to person without causing diseases themselves [9].

Common viral diseases transmitted by mosquitoes are dengue fever, yellow fever, chikungunya, epidemic polyarthritis, West Nile fever, Rift Valley fever, Japanese encephalitis, La Crosse encephalitis and several other encephalitis type diseases. Malaria is the protozoan disease and filariasis is the nematode disease transmitted by different species of mosquitoes^[10].

Members of the genus *Aedes* are responsible for the transmission of several viral infections like dengue fever, chikungunya, yellow fever, West Nile fever, eastern equine encephalitis, and Zika virus. The two most prominent species such as *Aedes aegypti* and *Aedes albopictus* act as vectors for the above said viral diseases^[11]. The deadly disease malaria is transmitted from man to man by different species of *Anopheles* mosquitoes like *Anopheles culicifacies*, *Anopheles stephensi*, *Anopheles gambiae*, etc. Apart from malaria, anopheline mosquitoes are also known to transmit the filarial worm *Wuchereria bancrofti*, the Timorese filarial worm *Brugia timori*, several arboviruses including eastern and western equine encephalitis, Venezuelan equine encephalitis etc.^[12]. Similarly, different species of *Culex* mosquitoes like *Culex pipiens*, *Culex quinquefasciatus*, *Culex vishnui* etc. are act as vectors for the transmission of several arbovirus infections such as West Nile fever, Japanese encephalitis, St. Louis encephalitis. *Culex* mosquitoes are also responsible for the transmission of avian malaria and lymphatic filariasis in different parts of the world^[13].

Thus, in order to reduce the increased incidence of mosquito-borne diseases and to raise the quality of public health, effective vector control is essential. Moreover, in recent years, mosquito borne diseases are spreading to newer territories and its vulnerability is constantly increasing to global environmental changes, that creates newer varieties of breeding places for mosquitoes. In addition, anthropogenic accomplishments including industrialization, urbanization, movement of industrial and agricultural labours, rapid demographic and climatic changes, human population growth, improper water storage and irrigation, trade, travel, inadequate waste management and disposal, environmental pollution and development of resistance in vector species to most of the available public health insecticides also contribute increased risk for the recurrent transmission of mosquito borne diseases^[14]. In this serious scenario, proper eradication of mosquitoes is becoming a difficult task due to the global environmental changes that favours amplified population of mosquitoes and concomitant threat of vector borne diseases^[15]. The most extensively used control methods are catching, usage of bed nets and indoor residual sprays, application of mosquito repellent sprays and insecticides as well as biocontrol agents^[16]. Out of these, chemical insecticides have represented the most widely used methods to control both larval and adult mosquito populations. Yet, the practicality of many synthetic insecticides in mosquito control programmes has become restricted because of the lack of novel insecticides, the high cost of such insecticides, concern for environmental sustainability as well as harmful side effects on human health and other non-target species. Moreover, the effects of such chemical insecticides on mosquito populations are usually transitory because those vectors can rapidly develop resistance against them^[17]. And also, most of these insecticides are carcinogenic and have the potential of bio-concentration and bio-magnification, so as to transfer through food chain, which in turn cause adverse health issues in non-

target organisms^[18, 19].

Therefore, the need for alternative strategies has driven researchers to look for environment friendly, cheap, non-toxic and highly effective mosquito control solutions. In this scenario, various organisms referred to as natural biological management agents, are being used to regulate vector populations, so as to evade the application of chemicals and also for the drop of harmful effects induced by such insecticides on non-target species as well as to the environment. In contrast to commercially available synthetic insecticides, bio-control agents can be host specific, target-specific and are stringently safer to the environment due to their biodegradability. Moreover, have little evidence of resistance development in target mosquito species and predators of mosquito larvae and pupae are taxonomically diverse in nature^[20].

Biological control involves introduction of biocontrol agents like predator species against immature stages of mosquitoes like larvae and pupae in the aquatic habitat to reduce their population size which in turn ultimately decrease the overall disease transmission rate^[21]. Several species of aquatic insect and some invertebrates and vertebrates have been proved to be effective predators against mosquito larvae. This include larvivorous fishes, frogs and tadpoles, turtles, larvae of dragon fly and damselfly, predatory mosquitoes in the genus *Toxorhynchites*, copepods, some nematodes and aquatic beetles^[22]. Furthermore, the bacteria *Bacillus thuringiensis* and the intracellular parasite *microsporidia* are also employed as an agent for mosquito control^[23, 24]. Of these, a major boost in this direction was given by the use of larvivorous fishes for mosquito control under the patronage of World Health Organization.

The use of larvivorous fish is one of the oldest practices in vector control, but it was replaced by chemical insecticides after its discovery. However, due to the problems of environmental contamination and insecticide resistance, there has been renewed concern in biological methods^[25]. Use of fish in vector control is an environment friendly method without having any significant detrimental effects on non-target populations and the fishes are self-perpetuating after its establishment and continues to reduce mosquito larvae for long time. Besides, the cost of introducing larvivorous fish is comparatively lower than that of chemical control^[26]. And also, fish live in the same aquatic habitat as larval mosquitoes and have been able to adapt in varied mosquito larval habitats so that there is no need to create an artificial directing habitat^[27]. Larvivorous fishes have numerous promising characteristics for mosquito control, including its small in size with low cost, robustness, survive in shallow waters where mosquito larvae breed, surface feeder and carnivorous habit having predilection for mosquito larvae even in the presence of other food materials. Besides, these larvicidal fishes should be easy to rear, able to withstand a wide range of temperature, pH and light intensity, drought resistant and a prolific breeder in confined water. Above all, they should have no food value, so that the fish-eating people discard them.^[28]

Studies have reported that there are more than 253 fishes, including exotic and indigenous species have been considered for mosquito biocontrol programme throughout the world^[29]. Among them most commonly used larvivorous fish species are *Gambusia affinis* (mosquito fish), *Poecilia reticulata* (Guppy), *Rasbora daniconius*, *Trichogaster* species, *Puntius* species, *Oreochromis* species, *Carassius auratus* (Gold fish),

Nothobranchius guentheri (Killi fish), *Danio rerio* (Zebra fish), *Danio malabaricus* (Malabar danio), *Colisa* species (Gourami), *Aplocheilus* species etc. [28]. But generally exotic species have traditionally been used for controlling mosquito larvae rather than indigenous ones. One of the most important concerns when introducing exotic fish for mosquito control is their impact on native species. Several studies have reported that introduction of exotic species lead to decline and complete absence of endemic species in different parts of the world [30, 31]. The teething troubles with introducing exotic species have incited interest in the use of native species for controlling mosquitoes all over the world [32, 33]. Thus, the present investigation has been undertaken to find out the comparative predation potential of some selected exotic and endemic fish species for the fourth instar larvae of *Culex* mosquito in Kozhikode, Kerala, India under laboratory conditions.

In view of the potential of mosquito control by different exotic and native larvivorous fishes, the present study was aimed to evaluate the comparative predation potential of five different fishes including both exotic and endemic species using *Culex* larvae. The fish species considered are *Gambusia affinis* and *Poecilia reticulata* as exotic varieties and *Danio rerio*, *Danio malabaricus* and *Aplocheilus panchax* as endemic ones to India. The common mosquito fish, *Gambusia affinis* is an exotic species and has been distributed throughout the tropical and some temperate parts of the world. It is a very resilient fish and can tolerate a wide range of temperature as well as to chemical and organic content of the water but does not tolerate very high organic pollution [34]. *Poecilia reticulata*, the guppy fish is also an exotic fish introduced in India in 1910. It is easy to care for and reproduces quickly and prolifically. It is now widely distributed in India and is an important larvivorous fish. Once well established, it can be found in the habitat even after many years [35]. *Danio rerio*, the zebra fish is endemic to India, which is distributed all over northern India, Bangladesh and Myanmar with a standard length of 5cm. They are surface feeders found in stagnant and slow-moving streams, rice fields, ditches, rivers and ponds. Zebrafish is omnivorous, mainly eating zooplankton, phytoplankton, insects and insect larvae. It is suitable for feeding mosquito larvae and also acts as a model organism globally [28]. *Danio malabaricus*, the malabar danio is a tropical fish, widely distributed in India and Sri Lanka which grows to a maximum length of 10-12cm. It is surface feeder with omnivorous food habit and usually found in a variety of habitats like ponds, streams, rivers and ditches. Spawns in shallow water and around 200 eggs are laid each time [36]. The 'whitespot', *Aplocheilus panchax* is widely distributed in peninsular India and Sri Lanka having a maximum body length of 9cm. It is commonly found in paddy fields, ponds, rivers, ditches, lowland wetlands, estuaries and canals. It is a surface feeder, mainly feeds on small insects and larvae so that it acts as a potential larvivorous fish in controlling several vector species in different types of natural and man-made habitat [37].

2. Materials and Methods

2.1. Collection and acclimatization of fish

The indigenous fish species namely *Danio malabaricus*, and *Aplocheilus panchax* were collected using drag nets from rice fields, nearby rivers and irrigation canals in and around Kozhikode, Kerala, India and two exotic fishes *Gambusia*

affinis and *Poecilia reticulata* as well as the endemic one *Danio rerio* were brought from local aquarium shops. The fish specimens weighing 2 ± 0.5 g and length 3.5 ± 0.5 cm were brought to the laboratory with least disturbances, segregated, and maintained separately species-wise in glass bottles with 10L capacity. The fishes were acclimatized to the laboratory conditions for 15 days prior to experiments. All fishes were fed with artificial food pellets and then starved for 24 h prior to experiments, as this period would increase the motivation to feed [38].

The glass bottles contained well aerated and dechlorinated tap water and were placed in an open area, under natural light. The physico-chemical features of the tap water were estimated as per APHA [39]. Water temperature ranged from 28 ± 2 °C during the experiment; oxygen saturation of water ranged between 70 and 100%; pH was from 6.5 to 7.5, which were monitored using standardized procedures. Moreover, the biological filtration in the bottles was obtained through sand sediments and pebbles. For each experimental trial a single fish was used only once. The collection of the fishes was continued throughout the experimental period (September 2019 to February 2020).

2.2. Collection and maintenance of *Culex* mosquito larvae

Larvae of *Culex* species (Diptera: Culicidae) were collected by fine netting from bogs, sewage drains, cesspits and also from rice fields in and around Kozhikode, Kerala, India and were transported to the laboratory. In the laboratory, the collected larvae were emptied in glass bowls to segregate larger one (IV instar) based on body length. The selected IV instars fed with crushed fish food and kept in bowls with good aeration, were immediately used for the experiments.

2.3. Experimental methods

2.3.1. Evaluation of predation potential of fishes on *Culex* larvae

The predatory potential of fishes was evaluated under varying prey densities for one day. To assess the predatory potential and to compare the consumption of *Culex* larvae, a single individual of each fish species was allowed to consume independently, 25, 50, 75 and 100 IV instar larvae in different glass bottles with 5l capacity. The number of larvae consumed were noted at the end of 24-hour duration. The experiment was repeated using ten different fish individuals for each species. The same experiment was repeated per prey density for all selected indigenous and exotic species, and the variations in prey consumption were analyzed using one-way analysis of variance (ANOVA).

3. Results

3.1. Effect of prey density on the predation potential of fishes on *Culex* larvae

In the present study, all the species of fishes consumed considerable numbers of fourth instar *Culex* larvae, although variation in feeding rate was noted depending upon fish species and prey density. The average prey consumption ranged from 10 to 40 for *Gambusia affinis*, 5 to 21 for *Poecilia reticulata*, 6 to 30 for *Danio malabaricus*, 16 to 62 for *Danio rerio* and 12 to 58 for *Aplocheilus panchax* at different prey densities such as 25, 50, 75 and 100 under laboratory condition against fourth instar *Culex* mosquito larvae for one day. (Table 1-4)). The predation potential is maximum for the indigenous fish *Danio rerio* and minimum

for the exotic fish *Poecilia reticulata*, and the others lie in between these two in the order *Aplocheilus panchax*, *Gambusia affinis* and *Danio malabaricus* from higher to lower. Moreover, the foraging behavior of all the fishes showed a significant increase ($P < 0.05$) with increase in prey densities, with a maximum predation at maximum density of *Culex* larvae, that is 100 in number (Figure 1).

Table 1: Feeding efficiency of larvivorous fish for 24 hour duration

Name of fish	Number of larvae supplied	Number of larvae consumed (Average)
<i>Gambusia affinis</i>	25	10
<i>Poecilia reticulata</i>	25	5
<i>Danio malabaricus</i>	25	6
<i>Danio rerio</i>	25	16
<i>Aplocheilus panchax</i>	25	12

Prey density 25 numbers

Table 2: Feeding efficiency of larvivorous fish for 24 hour duration

Name of fish	Number of larvae supplied	Number of larvae consumed (Average)
<i>Gambusia affinis</i>	50	21
<i>Poecilia reticulata</i>	50	10
<i>Danio malabaricus</i>	50	14
<i>Danio rerio</i>	50	32
<i>Aplocheilus panchax</i>	50	25

Prey density 50 numbers

Table 3: Feeding efficiency of larvivorous fish for 24 hour duration

Name of fish	Number of larvae supplied	Number of larvae consumed (Average)
<i>Gambusia affinis</i>	75	30
<i>Poecilia reticulata</i>	75	17
<i>Danio malabaricus</i>	75	21
<i>Danio rerio</i>	75	45
<i>Aplocheilus panchax</i>	75	39

Prey density 75 numbers

Table 4: Feeding efficiency of larvivorous fish for 24 hour duration

Name of fish	Number of larvae supplied	Number of larvae consumed (Average)
<i>Gambusia affinis</i>	100	40
<i>Poecilia reticulata</i>	100	21
<i>Danio malabaricus</i>	100	30
<i>Danio rerio</i>	100	62
<i>Aplocheilus panchax</i>	100	58

Prey density 100 numbers

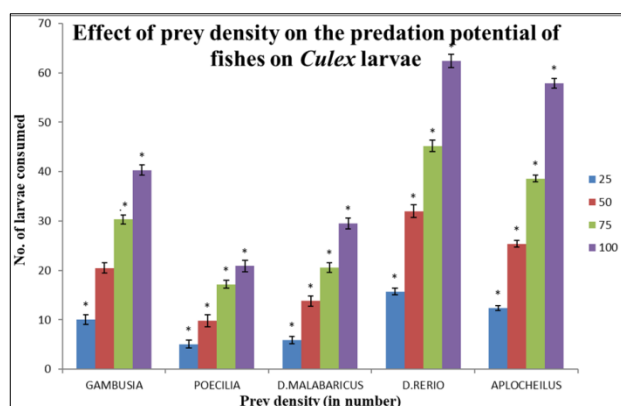


Fig 1: Effect of prey density on the predation potential of fishes on *Culex* larvae

4. Discussion

4.1. Effect of prey density on the predation potential of fishes on *Culex* larvae

Different types of larvivorous fishes have been used so far in the biological control of mosquitoes all over the world. However, use of fish of indigenous origin is found to be more appropriate in this operational technique. Moreover, the predation potential of larvivorous fish is regarded as an important criteria for the biological control of mosquitoes. The present study reveals a density dependent increase in the predation potential of five fishes *Gambusia affinis*, *Poecilia reticulata*, *Danio rerio*, *Danio malabaricus* and *Aplocheilus panchax* against *Culex* larvae for one day. The feeding efficiency of all the fishes were positively correlated with the increasing density of *Culex* mosquitoes and the indigenous varieties showed maximum predation potentials when comparing with exotic ones under laboratory conditions. Thus, from the results it is clearly evident that native larvivorous fishes *Danio rerio*, *Danio malabaricus* and *Aplocheilus panchax* can consume considerably more number of IV instar *Culex* larvae in contrast to the exotic varieties *Gambusia affinis* and *Poecilia reticulata*.

Nowadays, there is an increasing trend of ecological and environmental modifications by anthropogenic activities, creating diverse forms of breeding sites for mosquitoes, that have countless impact on the epidemiology of mosquito-borne diseases. Especially in urban areas, it creates suitable habitats for vector mosquitoes in which there are a reduced number of predators, and human hosts are widely available [40]. The population of mosquito larvae, both quantitatively and qualitatively depends on the competitors and predators present in the concerned habitat. Some decades before, the most widely used biological control agents against mosquito larvae are the exotic varieties *Gambusia affinis* and *Poecilia reticulata* in several countries including India. But a number of studies have reported that use of larvivorous fish, especially when introduced, could cause serious ecological damages by becoming a threat to native organisms including amphibians whose populations are often in decline. Thus, larvivorous fishes of indigenous origin is found to be more appropriate in vector control operations, advocating its widespread applications [41].

Thus, various researches have been going on worldwide in order to assess the predation potential of different indigenous fish species for the effective suppression of mosquito population. One of the native voracious feeder, *Aplocheilus panchax* is one of the frequently used fish for mosquito control all over India due to its increased larvivorous efficiency [42]. Different species of *Aplocheilus* has been used for the control of *Anopheles* mosquitoes in various breeding habitats like rain water pools, irrigation channels, sluggish streams with sandy margins with little vegetation, cemented tanks, cleared mangroves and lagoons, lakes and pits in coastal areas and brackish water habitats of Goa, India, when severe outbreaks of malaria occurred [43]. Similarly, the same fish is also used for the control of various species of *Culex* larvae in regions with severe outbreak of filariasis and avian malaria [37]. Field trial studies have stated that introduction of *Gambusia* and *Aplocheilus* in the wells of Pondicherry resulted in the reduction of *Anopheles* mosquitoes by 32.8%. The study also reported that *Aplocheilus* was more tolerant to varying salinity and pH than *Gambusia*, which is evidenced by the death of *Gambusia* after

few months of introduction^[44].

Thus, the present study confirms that our own indigenous fishes like *Danio rerio*, *Danio malabaricus* and *Aplocheilichthys panchax* can be rightly employed for the control of mosquitoes in different aquatic resources singly or integrated with other vector control programmes instead of commonly used exotic varieties, since these indigenous fishes have higher efficiency of predation than the exotic ones. The results of the present study will help to evaluate the efficacy of these indigenous fishes in regulating vector mosquitoes, apart from promoting the significance of conservation of these indigenous species.

5. Conclusion

Considering the biocontrol potential of indigenous fishes like *Danio rerio*, *Danio malabaricus* and *Aplocheilichthys panchax* as observed in the current study, these fish species could be used as effective biological agents for the control of mosquitoes, especially *Culex* mosquitoes in our surroundings. Nevertheless, further studies are needed on their effects on aquatic communities by the application of these fishes in concerned fields, for the broad understanding of actual potentialities and the overall impact on biodiversity and benefits in the control of mosquito populations. So, field trial experiments will be done in future for the promotion of indigenous varieties in mosquito control programmes along with supporting the significance of conservation of these native species.

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