International Journal of Current Science Research
www.drbgrpublications.in

Volume: 2; Issue: 2; February-2016; pp 333-345 . ISSN: 2454-5422

Feeding preference and efficiency of mosquito fish, *Gambusia affinis* and its density in the natural water resources of higher altitude areas of The Nilgiris, Western Ghats, India

R. Ravikumar[1]*, K. Sendesh Kannan[2] L.J. Kanheker[1], R. Sanil[4] N. Yasothkumar[3] P.Periyakaruppiah[3] and Saritakumar[4]

[1]NCDC, M/o Health and FW, Govt. of India, 22-Sham Nath Marg, Delhi, India
[2]Department of Microbiology, Yadava College, Madurai, India
[3]Department of Botany, Yadava College, Madurai, India
[4]Department of Zoology & Wild Life Biology, Govt. Arts College, Ooty, India
*Corresponding Author E-mail:karsar43@gmail.com

Abstract

The mosquito fish, *Gambusia affinis* is the best alternative to insecticides and a good biological control agent. The mosquito fish collected from natural water resources, is about 2000 meter above mean sea level areas viz. Avalanche, T.R.Bazaar, Gori, Murlimund, Katteri and Ralia Dam are located in the Ooty and Coonoor Taluks of Nilagiri district of Tamil Nadu state. In order to assess the food preference of *G.affinis* in the natural water resource i.e. affinity towards mosquitoes immature, individual mosquito fish 50 numbers from every area were dissected the gut content and examined for the remnants of mosquito larvae, zooplanktons and other food and further estimated in terms of percentage. The result indicates that except at T.R.Bazaar and Murlimund the presence of mosquito larvae in the gut content is almost zero. The mosquito larvae forms food share 12.0% and 9.0 % in T.R.Bazaar and Murlimund, respectively. Gut content analysis shows that the other prey forms the major share in all the natural water than mosquito immature. Due to cold climate prevailing in the higher altitude areas, density of mosquitoes immature was recorded very low and it may be the limiting factor.

Keywords: *Gambusiaaffinis*; *adlibitum*; *Anopheles stephensi, Culex quinquefasciatus* and *Aedes aegypti.*

Introduction

The indigenous mosquitocidal fishes like *Danio rerio, Esomus danricus, Badis badis, Chanda nama, Puntius ticto, Rasbora daniconius, Colisa fasciata,* etc. are commonly encountered in Indian fresh waters and were not found to be effective in controlling mosquitoes. They cannot be mass produced or they are not hard enough to withstand transportation, variation of water quality, turbidity and temperature. So the exotic species *Gambusia affini*s gains significance as a biological control agent and is not a contributory factor for bio magnification. *G.affini*s is remarkably successful in controlling mosquitoes (Nelson *et al* 1992; Boklund 1997). The ecological and biological properties of mosquito fish populations in India, such as distribution, biological properties, success in malaria control and interactions with other living things in the ecosystem, have not been studied sufficiently. The mosquito fish is an important alternative to pesticides, which cause environmental problems. The structure of the gonopodium was a significant criterion in the distinction of the mosquito fish species (Page and Burr 1991). The mosquito fish dwells in small lakes, ditches, ponds and slow flowing warm waters and in rich vegetation. This species, which survives and reproduces easily, is resistant to poor environmental conditions such as low oxygen levels, high temperature, high salinity etc. (Peterson and Mark 1990). Yousef S. Al-Hafedh (2007), the mosquito fish found to be well adapted to some extreme conditions such as high turbidity, high salinity, high ammonia, low oxygen, shallow water, waste water contaminated with oil droplets or dairy by-products and hard water. The mosquito fish is carnivorous, feeding on the floating larvae of mosquitoes, small crustaceans and insects. Mosquito larvae were the second most dominant food item for the *G.affinis* instead of other preys (Yousef S.Al-Hafedh, June 2007). Cannibalism is also observed in the feeding of the mosquito fish (Dionne 1985).

The Nilgiri hills located in Tamil Nadu of South India is highly diverse in terms of ecology and culture. The terrain features and climatic conditions like temperature, humidity, rainfall, the forest cover and availability of breeding habitats render the area suitable for the existence of mosquitoes (Balakrishnan *et al* 1995). Mosquitoes breed in confined water instead of running /disturbed water source. Mosquitoes are important single groups of insects in terms of public health significance and they are remarkably adapted to co-exist with man and domestic animals. Increasing population, unplanned organization, rapid transportation, unreliable water supplies and

water storage practices of peoples are leads to rapid spreading of mosquito species (Ravikumar *et al* 2000). The bionomics of mosquitoes essentially refers to the various events associated with the life of a mosquito species, some of which may inherent and species specific e.g. selection of a breeding place by a mosquito species, the act of egg laying, mating behaviour, preference for feeding on man or the act of animal, time of biting human host, and the choice of a resting place. A sound knowledge on bionomics of a vector species is a prerequisite for epidemiologically effective planning and implementation of a cost effective control strategy. Studies on the mosquito fish were carried out in its natural range and several areas (Krumholz 1948) of introduction. However, detailed studies on *G. affinis* population density, feeding and efficiency in the higher altitude areas have not been performed. Same way, the prevalence of mosquitoes immature in the natural water has also not been studied on epidemiological aspect. Under these circumstances the present study was undertaken to understand the feeding preference and feeding efficiency of this fish under natural and artificial conditions.

Materials and Methods

The study was conducted in Ooty and Coonoor taluks of the different areas of Nilgiris of Western Ghats during April 2012-August, 2013. The major study sites include Avalanche, T.R. Bazar, Gori, Murlimund, Katteri and Raliah Dam and located in the height of 2000 meters above the sea level. The fish *G. affinis* collected mainly in the shallow areas where small grass and plants are growing and were transported to the laboratory and acclimatized with artificial feed for further experiments.

Plate-1 Study area map, The Nilgiris District, Tamil Nadu, India

Sites of study Raliah Dam T R Bazaar Gori Murlimund

Preparation of standard fish meal

Feed pellets were prepared by taking equal quantity of rice bran and well grinded groundnut cake were mixed with the sufficient water and squeezed into pellets. Then it was dried up under sunlight and used as feed for fish.

Plate-2 Preparation of artificial-feed

1. Mixing of ingredients

2. Squeezing the mixture in a tray **3. Mixture kept under sunlight to dry**

Mosquito larvae feeding rate of *Gambusia affinis*

The feeding efficiency of the mosquito fish was studied in experimental conditions. Different mosquito vectors viz., *Anophelesstephensi, Culexquinquefasciatus* and *Aedesaegypti* were obtained from National Centre for Disease Control, South India Branch, M/o Health & FW, Govt. of India, Brooklands, Coonoor, The Nilgiris, India. The experimental group is divided into two major groups. One group contains mosquito larvae alone and the second group contains both mosquito larva and artificial feed. Each of these groups is subdivided into four sub groups. In every sub group, one healthy female *G.affinis* was introduced to see the feeding preference and efficiency. The first sub-group is fed with *Cx.quinquefasciatus*, the second sub-group with *An.stephensi*, the third sub-group with *Ae.aegypti* and the fourth contain all the three mosquito larvae in equal proportion. 2nd and 3rd instarlarvae were used for this experiment. In the second group and its entire sub group the artificial feed was fed *adlibitum*. The stocking density of the mosquito larvae at initial stage in each of these groups was estimated using the plankton counting chamber. The density of larvae after 24 hour exposure was estimated using the same procedure. This experiment was repeated for 7 times on various days and the results were noted.

Body Length to Gut Length Ratio of *G. affinis*

Fishes were collected from the water resources irrespective of sex are used to estimate the ratio. The total body length of fishes collected from the natural sources was measured. Further the

fishes were dissected out and the gut length was measured and the ratio was estimated (Plates-2 & 3). Further data have been correlated and the regression equation was developed.

Gut content analysis of *G.affinis*

In order to assess the total mosquito larval consumption by this fish, volumetric method of estimation of gut content is used as per the method of Lima-Junior and Roberto Goitein (2001). Individual mosquito fish (50 fish per sample) were examined, dissected and their gut content was removed. The gut content was searched for the presence of remnants of mosquito larvae. This was further estimated in terms of percentage and expressed.

Density estimation in the natural water

Density of planktons in the natural environment was estimated using plankton net and counted using plankton counting chamber. Similar method was adopted to estimate the density of mosquito larvae in surface water. To assess the density of mosquito larvae a known volume of water is collected from the shallow areas where *G.affinis* fish normally seen in the study areas. Also, the number of *G.affinis* trapped in known volume is counted and expressed in number of fish collected per 100 liters of water.

Plate-2 Alimentary system of mosquito fish, *Gambusia affinis*

Plate-3: Body and Gut length *G. affinis* **Plate-4: Analyzing the Gut Content**

Results

The experimental analysis of the feeding efficiency of the *G. affini*s (Table-1). Group I experiment shows the larval intake by a fish in 24 hour duration. When the fish was given with different types of mosquito larvae and it was observed that the feeding rate alters. The statistical analysis shows that the fish is significantly reducing the mosquito larval density irrespective of the species of mosquito. Further, it was observed that the fish prefers *Anopheles stephensi* more than *Culex quinquefasciatus* and *Aedes aegypti*. It is also observed that when a mixed mosquito larvae were supplied the reduction rate is averagely fare.

In group II the fish was fed with mosquito larvae and standard fish meal in order to assess the larval preference by the fish. It was observed that the intake of the mosquito larvae was reduced significantly than the artificial food. The statistical analysis shows that the fish is significantly reducing the mosquito larval density. When comparing the species wise preference when artificial food is available, it showed that the preference pattern was same as that of when supplied independently. The unpaired `t` between the groups after the 24 hours in Group I and II shows that the availability of food is a governing factor controlling the larval intake.

Table-2 shows the density of the mosquito fish, different mosquito immatures and planktons in various natural bodies. The study areas surveyed were the catchments areas for various dams like, Avalanche, T.R. Bazaar, Gori, Murlimund, Katteri and RaliahDam. Many of these reservoirs supply drinking water or hydroelectric project. Catchment areas like Ghori,

Murlimund and the T. R. bazaar are very close to human habituations and others are away from the human habituations. Data shows that the mosquito fish *G.affini*s is present in all the water bodies, but the density of distribution differs. At Ghori and the Murlimind the fish density was more than the other water resources. Least density is observed in the Ralia Dam and the Katteri. However the analysis of mosquito larval density is less in all the study areas except at T.R. Bazaar and Murlimund, which recorded the maximum density of the mosquito larvae. Regarding planktons, comparatively more numbers were collected from T.R. Bazaar and Gori than Avalanche and Katteri and very less numbers were collected from Murlimund and Ralia Dam.

Table-3 shows the fish length and its gut length in various water bodies in the Nilgiris. It was observed that the gut length is slightly more than the fish length in an average. The one way analysis of the variance showed that there is no significant variation with respect to the sample sites. This helps to prepare a prediction equation for the gut length based on the fish length. Table also shows the details of the gut content analysis of fish collected from various water resources. This indicates that except at T.R. Bazaar and Murlimund the presence of mosquito larvae in the gut is almost zero. However in T. R. Bazaar it was observed that the mosquito larvae forms 12% of food share and in Murlimund it was 9.0%.

Fig.-1 shows the distributional relation between mosquito larvae and fish. However the picture clearly depicts the presence of mosquito larvae is not a limiting factor for the distribution and abundance of the *G.affinis*. Maximum mosquito larval density was observed in the T.R. Bazaar where the *G.affinis* density was comparatively less. Maximum *G.affini*s density was observed at Ghori and Murlimund. However in Ghori the mosquito larval density is almost zero. Fig.-2 shows the correlation graph between the fish gut and fish length; the values are perfectly positively correlated.

Table: 1 Data shows the mosquito larval feeding rate of G*ambusia affini*s

Live mosquito larvae feed	Group I (Mosquito larvae alone)		Paired"t"	Group II (Mosquito larva + feed)		Paired"t"	Unpaired "t" between after 24 hr groups
	Larval Denisity Initial	Larval Density after 24 hr		Larval Denisity Initial	Larval Density after 24 hr		
Aedesaegypti	400± 40	240 ± 35	P<0.05	400± 30	310 ± 25	P<0.05	P<0.05
Anopheles stephensi	400± 35	160 ± 40	P<0.05	400± 25	240 ± 35	P<0.05	P<0.05
Culexquinquefasciatus	400± 64	196 ± 28	P<0.05	400± 40	270 ± 40	P<0.05	P<0.05
Mixed mosquito larvae	400± 45	180 ± 30	P<0.05	400± 35	290 ± 25	P<0.05	P<0.05
Anova	NS	P<0.05	--	NS	P<0.05	--	--

NS: Not Significant

Table: 2 Data shows the area-wise distribution of fish, *Gambusiaaffinis*, mosquito larvae and planktons in the natural water in the Nilgiris areas.

Water Source	*Gambusiaaffinis* Density (/100L)	Plankton (~No/100L)	Mosquito Larvae (No/100L)
Avalanche	04.56 ± 0.54	470 ± 34	00.00 ± 00.00
T.R. Bazar	06.86 ± 1.23	690 ± 25	42.86 ± 12.12
Gori	16.42 ± 3.89	555 ± 45	00.00 ± 00.00
Murlimund	14.34 ± 6.22	345 ± 30	24.00 ± 06.88
Katteri	03.65 ± 2.10	455 ± 20	00.00 ± 00.00
Ralia Dam	02.86 ± 1.89	345 ± 40	00.00 ± 00.00
Anova	P<0.05	P<0.05	P<0.05

Table: 3 Data of area-wise distribution, bodylengths to gut length ratio and food preference of the *Gambusia affinis* in the natural water sources

Water Source	Fish length (cm)	Gut Length (cm)	Food share of mosquito larvae (%)	Food share of other food (%)
Avalanche	4.01 ± 0.24	4.75 ± 0.36	0	100
T.R. Bazar	3.67 ± 0.89	4.10 ± 0.84	12	88
Gori	4.12 ± 0.36	4.80 ± 0.62	0	100
Murlimund	3.87 ± 0.88	4.50 ± 0.74	9	91
Katteri	3.68 ± 0.94	4.25 ± 086	0	100
Ralia Dam	4.26 ± 0.40	4.98 ± 056	0	100
Anova	NS	NS	----	----

Note: NS-Not Significant

Fig.:1 Distribution of *Gambusia affinis* and mosquito larvae present in natural water sources at different altitude areas

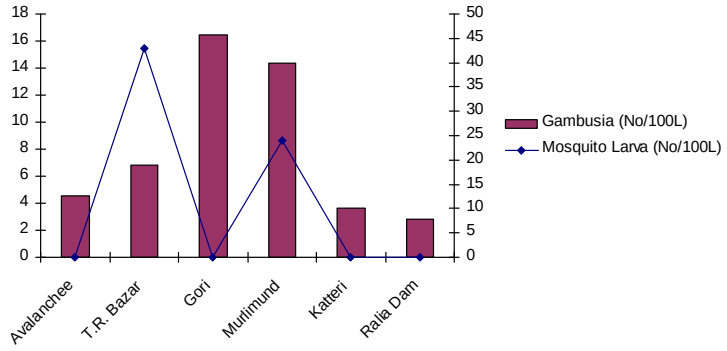
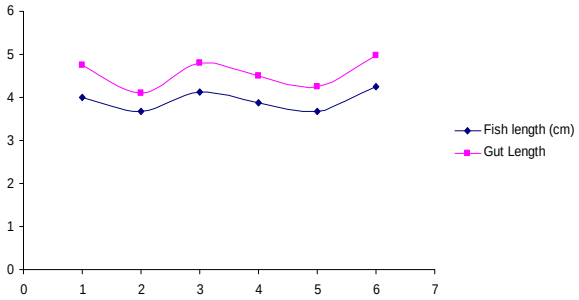


Fig.: 2 Correlation of fish`s whole body length and gut length of *G.affinis*



Discussion

The indirect effects of predators on two or more prey species have recently received increasing recognition as being important in structuring communities. Mosquito fish (*G.affnis*) feed on mosquito immatures and have been used as biological control agents. In addition, mosquito fish may have beneficial effects on reduction of mosquito populations (Washino and Hokama 1967). Zooplankton may initially decrease predation intensity of fish on mosquito larvae. But, feeding on zooplankton probably increases the population growth rate of mosquitofish which in time probably increases predation intensity on mosquito larvae (i.e. apparent competition occurs). Another plausible effect of mosquito fish is a reduction in competition between mosquito larvae and zooplankton (Mogi 1978). Mosquito larvae and cladocerans have a high overlap in particle size and in diet, consuming algae, protozoans, bacteria, and organic detritus (Clements 1963; Pennak 1978). YousefS.Al-Hafedh (2007) analyzed the gut content of mosquito fish and found that the green algae dominated among the ingested food items. Mosquito fish, prefer cladocerans to other prey (Bence 1985) may increase food available to mosquito larvae by decreasing cladoceranpopulations. Mosquito fish have been associated with a decrease in zooplankton abundance (Hurlbert *et al* 1972; Hurlbert and Mulla 1981). Comparative studies on feeding preference of least chub and mosquito fish in the laboratory conditions indicated that least chub could be potential replacement for mosquito fish for the mosquito control (Eric J.Billman *et al* 2007). The above observation is in concordance with the current studies, where the gut content analysis shows that the other prey forms the major share in all the natural water than the mosquito larvae. However, it should also to be noted that the density of mosquito larvae in the natural water is less and have a good plankton population. It also has to be taken into account that the cold condition prevailing in the Nilgiris may act a limiting factor in this case. The in vitro studies using the artificial feed (Table-1) shows that the fish does not prefer the feed on mosquito larvae completely when the other food sources are available. If the fish was forced to feed on the mosquito larvae, the fish was seen to be a voracious feeder of the larvae. The fig.-1 is also supporting the same data that the density of fish in natural water is not correlated with mosquito larval distribution.

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