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**Research Article**

# Biological control of *Culex quinquefasciatus* Say (Diptera: Culicidae) larvae

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**ABSTRACT:** Local larvivorous fish *Aphanius dispar* has potential for regulating vector mosquitoes. The consumption of larvae of the mosquito *Culex quinquefasciatus* Say (Diptera: Culicidae) by *Rasbora daniconius*, *Puntius ticto* and *Puntius conchonius* was evaluated in the presence of different prey densities under simple habitat conditions. The number of mosquito larvae consumed in fresh and ditch water at three seasons was observed. Only third and fourth instars of *Culex* larvae were used on a particular prey density of 1 to 10 (increase 100). The maximum and minimum value of larvae eating per day in ditch and fresh water were observed to 267 to 876 and 277 to 880 respectively. The comparisons for the pair using Tukey-Kramer HSD indicated the fact that there was non-significant difference. The respective mean values in fresh and ditch water were observed 525 and 493 respectively. The analysis of water (ditch and fresh) was non-significant (F = 1.0116). *Rasbora daniconius* species of fish played a dominating role in eating the *Culex* larvae in ditch and fresh water.

**KEY WORDS:** Biological control, mosquito larvae, vector management, local larvicidal fish

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## INTRODUCTION

*Culex quinquefasciatus* Say mosquito is a vector transmitting of filaria disease in India. Broad scale applications of synthetic insecticides are often associated with high levels of insecticide resistance and environment and health concerns. The biological vector control plays an increasingly important role in integrated management (Ghosh and Dash, 2007; Aditya *et al.,* 2012; Haq and Srivastava, 2013). Vector-borne diseases associated with water resources like artificial storage system, natural water storage system, unused water storage system and polluted water storage system. All these water storage systems are suitable for mosquitoes breeding, egg laying, larvae hatching, pupal formation, moulting and development of adult mosquito (Martinez-Ibarra *et al*., 2002; Bhattacharjee *et al*., 2009; Fang, 2010; Quintans *et al*., 2010). Larvivorous fish are biological control agents of mosquitoes. Prior to the implementation of a biological control program, detailed studies of local fish species and testing under pretend or natural field conditions are required to evaluate both their efficacy as control agents and their impact to the aquatic ecosystem (Chandra *et al*., 2008). The guppy, *Poecilia reticulata* Peters and mosquito fish, *Gambusia affinis* (Baird and Girard) have long been promoted for biological control of mosquitoes and their success has been documented (Aditya *et al*., 2010). The exotic larvicidal fishes are inefficient, considering their invasive nature and failure to provide desired results in certain instances, the indigenous larvivorous fishes are being promoted as an alternative option (Chandra *et al*., 2008). In view of the potential for mosquito control by native larvivorous fish, the present study was aimed at evaluating the prey preference of *Puntius ticto, Puntius conchonius* and *Rasbora daniconius,* using *Culex quinquefasciatus* as target prey.

## MATERIALS AND METHODS

The experiments were carried out in cemented tanks in Chitrakoot villages during 2010 to 2013. The experiment setup was located at Rajaula ($25^0$ 08’ 39.6” N & $80^0$ 50’ 39.9”), Khohi ($25^0$ 08’ 58.6” N & $80^0$ 50’ 48.5”), Mokamgharh ($25^0$ 08’ 07.4” N & $80^0$ 51’ 20.1”), Nayagoan M.P. Harigan Basti Ward ($25^0$ 10’ 33.2” N & $80^0$ 52’ 44.2”) and Nayagoan Sharma Harigan Basti ($25^0$ 10’ 29.8” N & $80^0$ 52’ 40.8”). To assess the effective suppression of larvae of *C. quinquefasciatus* by the use of three larvivorous fish es, *Puntius ticto, Puntius conchonius* and *Rasbora danico-*

*nius* and to evaluate the efficiency of feeding rate in fresh and ditch water in three session of a year, the experiment was conducted in five villages, cemented tanks of a size (10 x 10 x 5 meter) with a control tank having no fishes were also set in this trials. Only III and IV instars of *C. quinquefasciatus* larvae were used on a particular prey density of 1 to 10 (increase 100). Three replicates were set to assess the feeding trend of three fish species of same size (2cm long and 1.5g weight) and control replicate was also set for each treatment. The experiments were carried out on five weeks for each season and Random Block Design was applied for field trial (Ungureanu *et al*., 1981). Number of prey consumed by three fish species was noted at the end of each week. This procedure was followed for the reminder of testing period, so as to obtain the average number of larvae consumed per week per fish over a period of five weeks. The survival rate was estimated as follows:

$$Survival\,rate\,(SR\%) = \frac{(No.of\ \ fish\ harvested)}{Initial\,no\,of\ \ fish} \times 100$$

To assess the effectiveness of larvivorous fish under different ecological conditions, potentiality was calculated using SAS-JMP (USA) Software 9.03 version. Statistical technique such as analysis of variance (ANOVA) includes one-way and two-way classification, test of significance such as t test, F test using graphic user interpreter on SAS.

## RESULTS AND DISCUSSION

### Response of fish on prey consumption

The feeding response of *P. ticto*, *P. conchonius* and *R. daniconius* have been presented in Table 1 where in *P. conchonius* the range of eating *C. quinquefasciatus* larvae was observed be between 267 and 480, in *P. ticto* 272 and 560 and the maximum consumption of *C. quinquefasciatus* larvae was observed in case of *R. daniconius* which ranged between 560 to 880, it is evident from the Fig.1 that *R. daniconius* performance remained superior to *P. conchonius* and *P. ticto.*

**Table 1. Number (statistical mean) of prey consumed by the larvivorous fishes, whenthe preys were present in same proportions**

| Treatment | Mean |
|---|---|
| *Puntius conchonius* | 321.027b |
| *Puntius ticto* | 526.474b |
| *Rasbora daniconius* | 879.415a |
| CD (*P*= 0.05) | 235.560 |
| CV | 018.055 |

**Table 2. The results of two-way ANOVA and Tukey test on the consumption of IV instar larvae of *Culex quinquefasciatus* by three larvivorous fishes**

| Source | DF | SS | MS | F ratio | F |
|---|---|---|---|---|---|
| Fish | 2 | 3337751.0 | 1668875 | 320.4123 | 0.0001* |
| Error | 123 | 0640648.5 | 5209 | | |
| C. Total | 125 | 3978399.5 | | | |

In order to compare all pairs using Tukey-Kramer HSD was applied. Statistically there was significant difference among *R. daniconius*, *P. ticto* and *P. conchonius*. The preference of *P. conchonius* remained least (369.26), *P. ticto* (420.33) and the highest for the fish *R. daniconius* (737.21). F-values in bold indicate significance at p <0.001 level effect of fish predators on mosquito prey.

### Field evaluation of predatory efficiency

Ecology and environment plays an important role in the process of consuming of mosquito larvae. The environmental parameters namely pH, temperature, humidity and rainfall were dealt separately in model building and ecological factors, fresh and ditches water. Feeding capacity of each fish was observed at the rate 1000 larvae. The maximum and minimum number of larvae eating per day in ditches and fresh water were observed to 267 to 876 and 277 to 880 respectively. The comparisons for the pair using Tukey-Kramer HSD indicated the fact there is non-significant difference while this fish eat the *C. quinquefasciatus* larvae. The respective mean values in fresh and ditch water were observed 525 and 493 respectively. The analysis of water (ditch and fresh) was non-significant (F = 1.0116)

**Table 3. The results of two-way ANOVA and Tukey test on the consumption of IV instar larvae of *Culex quinquefasciatus* by the larvivorous fish**

| Source | DF | SS | MS | F ratio | F |
|---|---|---|---|---|---|
| Water | 1 | 0032192.0 | 32192.0 | 1.0116 | 0.3165 |
| Error | 124 | 3946207.5 | 31824.3 | | |
| C. total | 125 | 3978399.5 | | | |

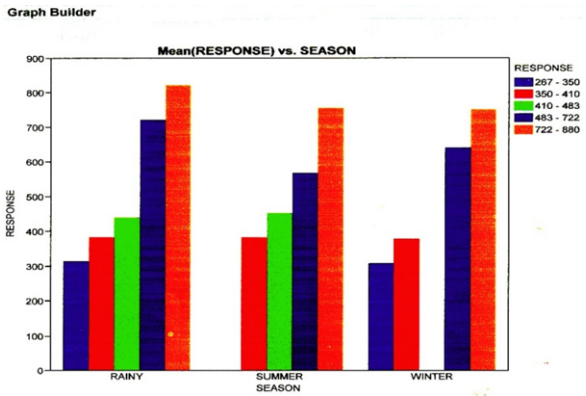


**Fig. 1. Response of fish within water in three seasons.**

*Rasbora daniconius* species of fish played a dominating role in eating *C. quinquefasciatus* larvae in ditch and fresh water fallowed by *P. ticto* & *P. conchonius.* F-values in bold indicate significance at $P < 0.001$ level effect of water type on consumption of prey

**Response of fish against mosquito larvae in summer, rainy and winter season**

Fish, *P. ticto, P. conshonius* and *R. daniconius* and three seasons along with two types of water it is concluded that the fish, especially *R. daniconius* and rainy / summer season played a significant role in the consumption of *C. quinquefasciatus* larvae. The data was analyzed using prediction profiler and visualized. This revealed that fresh water is more congenial than the ditch water for the fish to eat *C. quinquefasciatus* larvae because the presence of alternative food likes chironomous larva & other insect larvae. *Rasbora daniconius* played a dominating role in eating the *C. quinquefasciatus* larvae in ditch and fresh water.

In water tanks, *R. danoconius* reduced 93% larval counts by Day 7 and 98% by Day 21($P<0.01$), showing high larval propensity. Consolidated information on bionomics, tolerance and physicochemical properties of water taken from natural habitats of *R. danoconius* indicate its high larvivorous potential. Large-scale evaluation is warranted to evaluate its potential in vector-borne disease control. After *R. danoconius* introduction, mosquito densities immediately dropped in the treated tanks but increased in the control tanks. This increase was apparently due to climatic factors. The results showed that after 15 weeks the fish caused a more than 94% reduction of mosquito and more than 75% reduction in culicine mosquitoes. There was a highly significantly reduction in *Culex quiquefasciatus* larvae numbers when compared to pre-treatment levels.

The present investigation is clear that these fish species are very specific for the control of *C. quinquefasciatus* mosquitoes in Chitrakoot weather. From the results it is evident that local larvivorous fishes *P. conchonius, P. ticto & R. daniconius* can consume considerable numbers of IV instar larvae of *C. quinquefasciatus*. The efficacy of these fish species as strong biocontrol agents have been proved under field conditions. Larval population was significantly decreased at 30 and 45 days after introduction of these fishes. Similar the present finding if concord to the result of observations were found by some scientist i.e. Al-Akel and Suliman 2011; Ghosh *et al*., 2011; Aditya *et al*., 2012; Haq and Srivastav 2013. The sound increase in the larval abundance after the removal of fishes which proved that the reduction was due to the larvivorous potentiality of fish. In mosquito control programmes, especially those using biocontrol agents, it is highly desirable to have materials and agents that will yield long lasting control with one or few treatments or introductions so as to be cost-effective (Haq and Yadav 2011). Under the alternative strategy of vector control by means of bio-environmental improvement techniques, primary importance is given to anti larval operations. As all the fish species studied here are very active, hardy, prolific breeders in both fresh water and stagnant water and attain high growth rates under field conditions, they can effectively be used as strong biocontrol agent against immature mosquitoes similar finding were reported by Ungureanu, *et al*., 1981; Howard and Omlin 2008; Manna *et al*., 2008; Imbahale *et al*., 2011.

These characteristics provide an advantage for their augmentative release in sites where mosquitoes occur and make these fish useful for aqua cultural practices in rice field and other wetlands. Further studies in respect to the effects of these fish on the aquatic community food web can decipher their overall impact on the biodiversity and benefits in reducing mosquitoes (Haq and Srivastava 2013).

Global climate change can potentially increase the transmission of mosquito vector-borne diseases such as Japanese encephalitis, malaria, lymphatic filariasis, Chikungunya and dengue in many parts of the world. These changes are based on the affect of changing temperature, rainfall, and humidity which ultimately effect on mosquito breeding and survival, the more rapid development of ingested pathogens in mosquitoes and the more frequent blood feeds at moderately higher ambient temperatures. Larvicidal fish play an important in biological control by consuming mosquito larva; help in reducing the population of vectors, minimize the occurrence of mosquito borne diseases.

Our results particularly suggested that the local larvicidal fish is not required additional energy for culture and easily adaptable in resource water where of breeding dangerous mosquitoes takes place. The farmer and panchayat could help in maintain mosquito vector disease free village. Indigenous and local larvivorous fish provide dual benefits i.e. reducing the mosquito populations, nutritional benefits and agriculture economics.

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