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Biological Control of Vector Mosquitoes by Some Common Exotic Fish Predators

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Abstract: Predation experiments using *Cyprinus carpio* Linnaeus (1758), *Ctenopharyngodon idella* (Valenciennes 1844), *Oreochromis niloticus niloticus* (Linnaeus 1758) and *Clarias gariepinus* (Burchell 1822) were conducted against fourth instar *Anopheles stephensi* Liston (1901) larvae and pupae at varying prey and predator densities. The relative prey consumption rates of the four fish species for *An. stephensi* larvae and pupae during 24-hour experiments under laboratory conditions were *C. gariepinus* > *C. idella* > *C. carpio* > *O. n. niloticus.* Predatory efficacy was positively related with prey density and inversely related with water volume (search area). A significant decrease in larval abundance in dipper samples was observed at 30 and 45 days after introduction of fishes (30 individuals each) in field conditions. The efficacy of the fish under field conditions was also indicated by significant increases of larval mosquito abundance at 30 and 45 days after removal of fishes.

Key Words: Biological control, larvivorous fish, *Anopheles stephensi*, immature mosquitoes

Introduction

Biological control is defined as the action of predators, parasites (parasitoids) or pathogens in maintaining the density of another organism at a lower average than would occur in their absence. Biological control of vectors is an essential and effective means for controlling transmission of several mosquito-borne diseases such as malaria, filariasis, JE, dengue fever, etc. Modern researchers are inclined to use biocontrol agents rather than chemical insecticides due to the residual effects of chemical insecticides, widespread resistance in target insects, high refusal rate for indoor spray, soaring price of chemical insecticides and other operational difficulties (1-6). Among the numerous mosquito predators, fish have been used since the early 1900's for mosquito control and are the most commonly used biological control agent for the vectors of malaria in many countries. Use of larvivorous fishes in the field of mosquito control is well documented (7-11). The present study was conducted to assess the biocontrol potential of four exotic fishes, *Cyprinus carpio* Linnaeus (1758), *Ctenopharyngodon idella* (Valenciennes 1844), *Oreochromis niloticus niloticus* (Linnaeus 1758) and *Clarias gariepinus* (Burchell 1822), which are well adapted in the native environmental conditions of India and are widely cultured in most of the fresh water biomes throughout the country. Our investigation was also aimed at analyzing the efficacy of co-existing bioagents of Anopheline mosquitoes, the relationship of predatory efficacy with reference to prey density and search area, and the efficacy of the four fish species under field conditions.

Material and Methods

The first instar larvae of *Anopheles stephensi* Liston (1901) were collected from shallow ponds at Burdwan (23°16′N, 87°54′E), and reared until the fourth instar with supplementary food consisting mixture of protein biscuit (60%) and dried yeast powder (40%). Some of the larvae were reared through pupation for the predation experiment. *Cyprinus carpio, C. idella, O. n. niloticus* and *C. gariepinus* were collected from Industrial Fish and Fishery Division, Burdwan (23°16′N, 87°54′E) and were maintained with mixed plankton diet for 3 days. For the experiments, 8 exotic fishes (two *C. carpio* weighing 2.0 g, 2.5 g and total length of 3 cm, 4.1 cm respectively; two *C. idella* weighing 2.1 g, 2.3 g and total length of 3.2 cm, 3.9 cm respectively; two *O. n. niloticus* weighing 1.9 g, 2.3 g and total length of 2.6 cm, 3.3 cm respectively and two *C. gariepinus* weighing 3.3 g, 3.8 g and total length of 4.2 cm, 5.1 cm respectively) were used individually as predators. Two thousand fourth instars and one thousand five hundred pupae of *An.*

stephensi were separately given as prey to each species of fish to determine the larval and pupal consumption rate respectively. Predation experiments were conducted in 2 litre of pond water in a glass aquarium [measuring 45 cm (length) x 20 cm (breadth) x 20 cm (width)]. Pond water used was almost neutral and sieved through a net (500 holes per square inch) to exclude any larvae of other insects, phytoplankton and zooplankton. The water fluctuated between a temperature from 26 to 31˚C, pH from 6.34 to 6.61 and dissolved oxygen content from 5.28 to 6.47 mg/L, during the experimentation. Each experiment had three replicates (n = 3) and was conducted on three separate days. The number of larvae consumed by individual fish species in two litres of water was noted throughout a day at an interval of 3 hours. After counting the number of consumed larvae and pupae at every 3-hour interval, the same number of larvae and pupae were replenished within the aquarium to maintain the same prey density. The experiments were conducted during light on (05.00 – 17.00h, IST-Indian Standard Time) and light off (17.00 – 05.00h, IST) phases to examine the efficacy of the fishes. The length of the light on and light off phases were maintained by the application of artificial lights (Tube lights), set on the walls of the laboratory (6 x 40 Watt). The light on phase in the laboratory synchronized with the photophase and the light off phase with the dark phase of the nature. Although, the entry of some dim scattered sunlight through two windows and a door of the laboratory was allowed during photophase experiment.

Variation in the predatory potential of the four fish species was assessed across changes in the number of predators and water volume (search area). Two or four fishes of each species were allowed to feed on two thousand fourth instars and three thousand *An. stephensi* pupae, separately, in variable amount of pond water (2 litre and 4 litre respectively) over 24 h period. Statistical significance of predation was analyzed using two sided *t* tests. The relationship of feeding rate (Y) with changes in the number of predators (X_1), and water volume (X_2) was examined by least squares regression using Microsoft Excel (Office 2000). This multiple regression of Y, X_1 and X_2 gives first order relationship of the variables.

In the next phase of experiment, variations in per dip density of larval mosquitoes (comprising different species) were recorded in the field conditions (in a trench measuring about 9.11 m in length, 1.52 m in breadth and 0.61 m in depth). The field study was conducted in Kalna, a small rural village in the district of Burdwan. The trench was free of any larvae or nymphs of larvivorous insects or fishes before the introduction of fishes as confirmed by fine netting (about 130 holes/inch^2), which allowed the passage of mosquito larvae. Larval densities (per dip density) in the experimental trench were assessed with a 250-ml dipper (12). Each time, 30 dips (n = 30) were taken at different spots of the trench and the mean density was calculated. Exotic fishes (30 individuals, each weighing about 2-2.5 g) of each species were introduced into the same trench, one after another, in a sequence of *C. carpio*, followed by *C. idella, O.n.niloticus* and *C. gariepinus.* Changes in the larval frequency were recorded at an interval of 30 days and 45 days after the introduction of each species of exotic fishes, as well as after the removal of the fish species (after 30 days and 45 days). The individuals of each of the exotic fishes were introduced after the complete removal of the previous fish species from the trench by fine netting, which allowed the passage of mosquito larvae. All the data were statistically analyzed by the application of *t* test. The temperature of the water ranged from 21 - 28˚C, pH from 6.77 – 7.11 and dissolved oxygen content from 5.31 – 6.11 mg/L during the study.

The experiments (both in laboratory and in the field) were conducted in 2004. Morbid larvae and pupae were not considered as consumed.

Results and Discussions

Three hourly and daily consumption rates (mean ± SE, of three experiments) of *C. carpio, C. idella, O. n. niloticus and C. gariepinus* on *An. stephensi* larvae and pupae are presented in Table 1. When a 24-hour period was divided into 2 phases, it was found that during dark phase *C. carpio*, *C. idella*, *O. n. niloticus* and *C. gariepinus* consumed 135, 213, 126 and 813 larvae (mean value of three experiments) and 106, 154, 98 and 706 pupae respectively. The corresponding consumption rates during the light phase were 160, 158, 130 and 705 larvae and 121, 109, 113, 543 pupae respectively (Table 1). The change in the biocontrol potentiality in dark versus light phases probably exhibited some behavioural response with no practical significance in biocontrol strategy.

Variations in daily larval and pupal feeding rates of individual fish species with variation in water volume and predator density are depicted in Table 2. Water volume was inversely related whereas number of predators was positively related to the larval and pupal feeding rates of each fish species (Table 2). The two independent variables were strong predictors of feeding rates because for each fish species the multiple correlation (R) is fairly close to 1.

Regression equations obtained from experimental data are given below:

Predators	Equations for larval feeding rate	Values of R	Equations for pupal feeding rate	Values of R
Cyprinus carpio	$Y = 492.82 + 20.7\ X_1 - 21X_2$	0.88	$Y = 104.5 + 0.96\ X_1 - 27X_2$	0.99
Ctenopharyngodon idella	$Y = 654.09 + 18.14\ X_1 - 19X_2$	0.84	$Y = 124.75 + 0.97X_1 - 0.24X_2$	1.00
Oreochromis niloticus niloticus	$Y = 103.00 + 0.96X_1 - 0.27X_2$	0.99	$Y = 858.75 + 0.94X_1 - 0.32X_2$	0.99
Clarias gariepinus	$Y = 525.25 + 0.99X_1 - 0.12X_2$	0.99	$Y = 858.75 + 316.25\ X_1 - 107.25X_2$	0.99

Where, Y = Feeding rate of larvae/pupae; X_1 = Number of predators; X_2 = Amount of water (search area); R = Multiple correlation coefficient.

Table 1. Three hourly and daily consumption rates (average of 3 experiments) of different exotic fishes on larvae and pupae of *Anopheles stephensi*.

Name of the Fish species	Life stage of *An. stephensi*	Amount of Water (litres)	Average consumption at an interval of three hours								Mean value
			5 a.m. to 8 a.m.	8 a.m. to 11 a.m.	11 a.m. to 2 p.m.	2 p.m. to 5 p.m.	5 p.m. to 8 p.m.	8 p.m. to 11p.m	11 p.m. to 2 a.m.	2 p.m. to 5 a.m.	
Cyprinus carpio	Larva	2	31±2.52	42±2.08	27±3.21	35±1.15	55±3.05	48±3.51	21± 3.00	36±5.51	295
	Pupa	2	22±3.05	28±1.53	25± 3.00	31±3.21	21±3.21	44±3.05	30±4.58	26±3.21	227
Ctenopharyng odon idella	Larva	2	59±2.31	57±5.03	67±2.52	30±4.16	40±4.21	20±2.08	52±3.79	46±2.65	371
	Pupa	2	44±3.00	41±5.69	47±2.31	22±2.08	20±1.73	14±2.08	33±1.53	34±4.04	255
Oreochromis niloticus niloticus	Larva	2	16±2.52	12±1.51	55±4.00	43±5.60	45±3.84	32±2.65	27±4.51	26±3.06	256
	Pupa	2	21±2.08	31±2.08	22±2.52	24±2.08	17±2.52	39±3.05	15±2.52	16±2.08	185
Clarias gariepinus	Larva	2	141±4.16	194±11.06	163±7.84	315±15.87	225±13.3	194±5.20	140±5.20	146±9.61	1518
	Pupa	2	150±10.01	189±6.90	173±10.58	194±6.66	162±8.51	150±9.61	137±3.79	94±6.25	1249

Table 2. Variations in daily larval and pupal feeding rates of different fishes with variations in water volume and predator density.

Name of the species	Number of fishes (X1)	Amount of water (in litre) (X2)	Mean larval consumption per day (Y)	Mean pupal consumption per day (Y)
Cyprinus carpio	2	2	295 ± 1.73	227 ± 6.12
		4	205 ± 3.79	181 ± 8.08
	4	2	525 ± 7.81	403 ± 3.22
		4	423 ± 8.39	351± 7.43
Ctenopharyngodon idella	2	2	371 ± 3.51	255 ± 4.36
		4	245 ± 3.79	212 ± 6.03
	4	2	62 ± 16.39	427 ± 5.04
		4	621 ± 2.00	385 ± 4.16
Oreochromis niloticus niloticus	2	2	256 ± 4.51	185 ± 4.16
		4	192 ± 4.85	137± 3.06
	4	2	463 ± 2.09	343 ± 2.00
		4	407 ± 2.11	292 ± 2.00
Clarias gariepinus	2	2	1518 ± 4.76	1249 ± 9.46
		4	1325 ± 7.51	1090 ± 1.16
	4	2	2550 ± 5.87	1937 ± 6.11
		4	2480 ± 5.69	1667 ± 6.11

The change in the per dip density of a mixed population of mosquito larvae [mainly comprising *An. stephensi, An. subpictus* Grassi (1899), *Armegeres subalbatus* Coquillett (1898), *Culex quinquefasciatus* Say (1823) and *Cx. vishnui* Theobold (1901)] at a fixed time interval (after 30 and 45 days) after the introduction of fish species as well as after the removal of fish species is presented in Figure 1. After 30 days from the introduction of fishes (30 individuals) in the fish-free trench, average density (mean value of 30 dips; n = 30) of mosquito larvae per dip was reduced to 22.54 from 30.85 (in case of *C. carpio*), 22.23 from 29.31 (for *C. idella*), 19.83 from 26.87 (for *O. n. niloticus*) and 10.31 from 25.59 (for *C. gariepinus*), which are statistically significant (t = 13.73, 6.68, 15.60 and 28.97 respectively) in comparison to the tabulated value (t = 2.04 at 29 degrees of freedom) at 0.05 level of probability. The corresponding values were 17.69 (for *C. carpio*), 17.46 (for *C. idella*) 12.93 (for *O. n. niloticus*) and 7.69 (for *C. gariepinus*) after 45 days from the introduction of fish species with t = 8.17, 9.19, 18.28 and 5.82 respectively, which were statistically significant in comparison to the mosquito density after 30 days from the introduction of fishes.

To know whether this reduction was due to the introduction of exotic fishes, per dip larval density was assessed after 30 days from the removal of fish species and the density was increased to 22.31 from 17.70 (for *C. carpio gariepinus*), 22.54 from 17.46 (for *C. idella*), 21.2 from 12.93 (for *O. n. niloticus*) and 13.38 from 7.69 (for *C. gariepinus*) which was statistically significant (t = 8.84, 9.91, 23.20 and 12.57 respectively) at 0.05 level of probability. The corresponding figures were 29.31 (for *C. carpio*), 26.87 (for *C. idella*) 25.59 (for *O. n. niloticus*) and 22.31 (for *C. gariepinus*) respectively after 45 days from the removal of fishes which are statistically significant against the per dip larval density after 30 days of removal (t = 14.12, 7.11, 10.77 and 18.42 respectively) at 0.05 level of probability against table value of t = 2.04 (when number of observations = 30).

The results obtained from our experiments indicated clearly that *C. carpio*, *C. idella*, *O. n. niloticus* and *C. gariepinus* had potential as biocontrol agents for mosquito larvae and pupae. It was also found that consumption rates of immature mosquitoes were similar in light on and light off phase but was inversely related with the amount of holding water. Average daily feeding rate on fourth instar *An. stephensi* larvae in laboratory experiments was highest for *C. gariepinus* and it was higher than for other larvivorous fishes, such as *Gambusia affinis* Baird and Girard 1853, *Poecilia reticulata* Peters 1859 and *Carassius auratus* Linnaeus 1758 (13-15). Therefore *C. gariepinus* should be very effective controlling mosquitoes in shallow ponds and marshy areas.

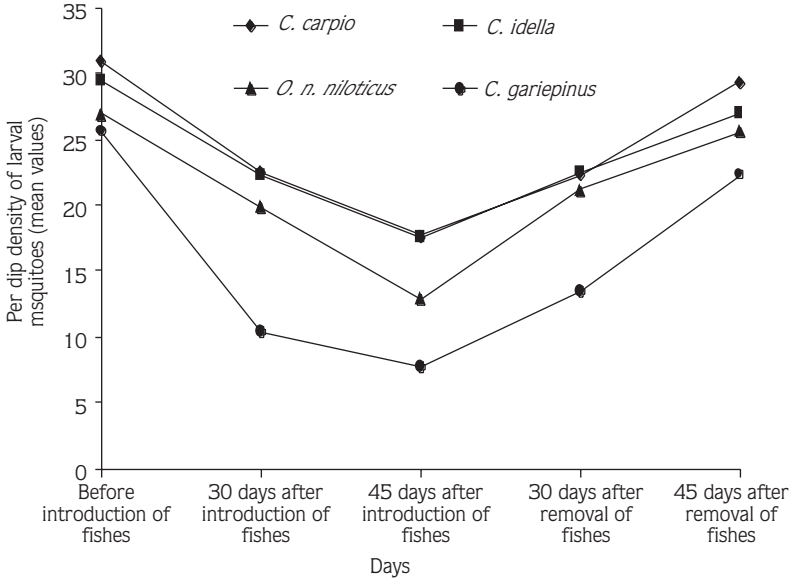

Figure 1. Change in the per dip larval density after introduction as well as after removal of exotic fishes in field condition.

The efficacy of four fish species as strong biocontrol agents was also proved under field conditions where larval mosquito abundance decreased significantly at 30 and 45 days after the introduction of fishes in the natural habitat of larval mosquitoes. There was also a steady increase in the larval abundance after the removal of fishes which suggested that the reduction was due to the larvivorous potentiality of fish species.

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. Under the alternative strategy of malaria control by means of bio-environmental improvement techniques, primary importance is given to antilarval 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.

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References

1. Sharma MID, Krishnamurthy BS. Note on the development of resistance to D.D.T in *Anopheles subpictus* Grassi, 1899 in a Delhi State Village. Bull Nat Soc Ind Mal Mosq Dis 5: 78-81, 1957.
2. WHO. Technical Report; Resistance of vetors and reservoirs of diseases to pesticides TRS/*585*. Geneva. 1975.
3. WHO. Report of the WHO Expert Committee on vector biology, control and chemistry and specification of pesticides. Tech. Rep. Ser. No.699. 1984.
4. Rathor HR, Taqir G, Reisen WK. Status of insecticide resistance in Anopheline mosquitoes in Punjab Province,Pakistan. South East Asian J Trop Med Pub Hlth 11: 332-340, 1980.
5. Vittal M, Deobhankar RB, Hasan MM. Experiments on feeding behaviour of some common larvivorous fish. Pestology. 8: 28-31, 1984.
6. Sankaralingam A, Venkatesan P. Larvicidal properties of water bug *Diplonychus indicus* (Venk and Rao)and its use in mosquito control. Ind. J Exp Biol 27: 174-176, 1989.
7. Hoy JB, O' Berg AG, Kauffman EE . The mosquito fish as a biological control agent against *Culex tarsalis* and *Anopheles freeborni* in Sacramento Valley rice fields. Mosq News 32: 146-52, 1971.
8. Menon PKB, Rajagopalan PR. Mosquito Control Potential of some species of indigenous fishes in Pondicherry. *Ind J Med Res* 66: 765-767,1977.
9. Menon PKB, Rajagopalan PK. Control of mosquito breeding in wells by using *Gambusia affinis* and *Aplocheilus blochii* in Pondicherry town. Ind J Med Res 68: 927-33,1978.
10. Tabibzadeh I, Behbehani G, Nakhari R. Use of *Gambusia affinis* in the Malaria Eradication Programme of Iran Bull WHO 43: 623-26, 1977.
11. Wee N, Liao GH, Li DF et al. The advantage mosquito biocontrol by stocking edible fish in rice paddies. Southeast Asian J Trop Med Pub Hlth 22: 436-42, 1991.
12. WHO. Manual on Practical Entomology in Malaria: Part II (Methods and Terminology). Geneva, 1975.
13. Chatterjee SN, Chandra G. Laboratory trials on the feeding pattern of *Anopheles subpictus*, *Culex quinquefasciatus* and *Armigeres subalbatus* larvae by *Gambusia affinis*. Sc Cult 63: 51-52,1997.
14. Chatterjee SN, Chandra G. Feeding pattern of *Gambusia affinis* and *Lebistes reticulatus* on *Anopheles subpictus* larvae in the laboratory and field conditions. J Appl Zool Res 8: 152-153,1997.
15. Chatterjee SN, Das. S, Chandra G. Gold fish (*Carrassius auratus*) as a strong larval predator of Mosquito. Trans Zool Soc India 1: 112- 114, 1997.