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Comparative Study of Gender based Larvicidal Potency of *Puntius ambassis, Ompak bimaculatus, Channa marulius* and *Heteropneustes fossilis* Native Fish Species of Gwalior (M.P.) India: A Laboratory examination

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ABSTRACT

Present study is focused on using some common native fish species of Gwalior (M.P.) as predator of mosquito larvae. All Predation experiments were conducted against mosquito larvae, pupae and alternative food (aquatic insects) at varying pray densities in separate aquariums containing analysed pond waters of Jal-vihar and fish farm of Gwalior (M.P.). The time duration of each predation experiments was three days and in each day, fishes were fed with mosquito larvae, pupae and alternative food at varying densities at different time intervals of a day. The study revealed that, Larvae consummation was positively related with the small fish species having female gender and prefered mostly small pray as food. Thus, small sized female fish species possessed greater predation efficacy on mosquito larvae and the sequence of larvicidal potency of female fish species was noted in the present study is *Puntius ambassis>Ompak bimaculatus> Channa marulius> Heteropneustes fossilis*. As per the results of our study, it is predicted that females have greater value of larvicidal potency than that of males. Thus, female gender plays an important and positive role of mosquito biocontrol at source level.

Figures : 02 References : 04 Tables : 04
KEY WORDS : Biocontrol, Eco-friendly. Gender, Potency, Zooplankton.

Introduction

From 1900 century, mosquito control has become a point of focus in scientific communities specilly health providing units. All tropical and sub-tropical countries have to face mosquito borne diseases every year like dengue fever, malaria, filariasis, Japanese encephalitis *etc*. In order to overcome the vectors of these dreadful diseases at source level various chemical methods have been employed so for but have certain environmental issues. Biological control is the best alternative, cost effective, and eco-friendly method for mosquito control at source level.

Mosquitoes are blood sucking dipteran insects that act as vector of several important life-threatening diseases, including Malaria, Lymphatic Filariasis, Chikunguniya, Dengue fever and Japanese encephalitis[1]. While feeding on blood, they may become carriers for many animal diseases, transmitting harmful zoonotic and other important diseases. Oogenesis female mosquitoes feed on host blood and find their way to swamps, ponds and marshy areas where females lay eggs. Ephemeral aquatic ecosystems (pools, puddles, ponds, floodplains *etc.*) become ideal breeding sites for mosquitoes. Thus, mosquitoes originate from such water bodies, hence we can say these water bodies are the actual sources of mosquitoes. Among the live feeds that fish consumes, mosquito larvae are the most favourite food items for the larvivorous fish. Fish that are predators of the immature stages of mosquitoes are referred to as larvivorous fish. Biological control refers to the introduction or manipulation of animals to suppress the population of vector. A wide range of organisms helps to regulate mosquito populations naturally through predation, parasitism and competition.

Among all the biological control agents, larvivorous fish are the most common and widely used in vector control. In India an exotic larvicidal fish (*Gambusia affinis*) is used as a predator of mosquitoes which is currently running in many rivers and other water bodies. This exotic mosquito fish is quite effective in minimising mosquito

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TABLE-1 : Larvae consumption by males and females in different intervals of time on the separate days.

Species name and body size	Gender	Experi-mental days	No. of larvae supplied at 9 am	No. of larvae supplied at 2 pm	Total no. of larvae supplied in a day	Total no. of larvae consumed in a day	Total no. of unconsumed larvae in a day
Puntius		1	30	40	70	70	0
ambassis	**Female**	2	30	40	70	68	2
(8.5cm)		3	30	40	70	70	0
Puntius		1	30	40	70	65	5
ambassis	**Male**	2	30	40	70	67	3
(8.2cm)		3	30	40	70	63	7
Ompak		1	30	40	70	65	5
bimaculatus	**Female**	2	30	40	70	62	8
(12.3 cm)		3	30	40	70	58	12
Ompak		1	30	40	70	63	8
bimaculatus	**Male**	2	30	40	70	60	10
(12.cm)		3	30	40	70	55	15
Channa		1	30	40	70	52	18
marulius	**Female**	2	30	40	70	50	20
(16.3 cm)		3	30	40	70	53	17
Channa		1	30	40	70	42	28
marulius	**Male**	2	30	40	70	50	20
(16 cm)		3	30	40	70	45	25
Heteropneustes		1	30	40	70	42	27
fossilis	**Female**	2	30	40	70	40	30
(18.5 cm)		3	30	40	70	39	31
Heteropneustes		1	30	40	70	40	30
fossilis	**Male**	2	30	40	70	35	35
(18cm)		3	30	40	70	30	40

TABLE-2 : Egg consumption by males and female fish species in different intervals of time on the separate days.

Species name and body size	Gender	Experi-mental days	No. of eggs supplied at 9 am	No. of eggs supplied at 2 pm	Total no. of eggs supplied in a day	Total no. of eggs consumed in a day	Total no. of unconsumed eggs in a day
Puntius		1	25	35	60	58	2
ambassis	**Female**	2	25	35	60	60	0
(8.7cm)		3	25	35	60	59	1
Puntius		1	25	35	60	55	5
ambassis	**Male**	2	25	35	60	56	4
(8.4cm)		3	25	35	60	52	8
Ompak		1	25	35	60	54	6
bimaculatus	**Female**	2	25	35	60	51	9
(12.5 cm)		3	25	35	60	51	9
Ompak		1	25	35	60	50	10
bimaculatus	**Male**	2	25	35	60	51	9
(13.cm)		3	25	35	60	48	12
Channa		1	25	35	60	50	10
marulius	**Female**	2	25	35	60	43	17
(16.5cm)		3	25	35	60	42	18
Channa		1	25	35	60	41	19
marulius	**Male**	2	25	35	60	41	19
(16.2 cm)		3	25	35	60	38	22

larvae, but it has also certain environmental issues related to ecological imbalance, possible impacts on native fish species and other effects on biodiversity of the water bodies.Since mosquitoes are considered dangerous and are responsible for direct and indirect damages, their control has always been focused in community scientific studies. Mosquito-borne diseases have been major problem in almost all tropical and subtropical countries and currently there are no successful vaccines against most such diseases. These diseases not only become burden in our daily life but are also responsible for more than 3 million deaths per year. To overcome these diseases, the reduction in the population size of mosquitoes is essential. Many synthetic insecticides are widely used for controlling adult and larval mosquito populations, which is harmful for human health and nontarget animals and arthropod populations and an important cause of environmental pollution. Chemical

control methods were used to eradicate them but due to the intense use of these chemicals, mosquitoes become less effective because of development of resistance[4].

Scientists have negated serious ecological consequences upon complete eradication of mosquitoes, creating a room for developing a safest and environment friendly control methods. Among them biological control is the deliberate use of natural enemies to reduce the number of pest organisms, which has gained acceptance for controlling nuisance. Biological larvicides are the safest and attracting methods in mosquito control and this is by use of biological agents (*e.g.,* mosquito fish) that eat or destroy the mosquito larvae.

This study will emphasise on the use of native larvivorous fish species of India with special reference to Gwalior (M.P.) in order to evaluate their larvivorous potential property, so that we can use them for malaria biocontrol programmes. Thus, the study envisages assaying and providing an inventory biological control. It is considered to be not only best low price, eco-friendly method but highly effective in vector management as compared to the other chemical methods.

Materials and Methods

During this study, attempts were made to evaluate the comparative larvicidal potency on gender basis of some common native fish species against mosquito pupae, larvae and aquatic insects as alternative food, under laboratory conditions.

2.1 Collection, maintenance and identification of fishes

Twelve fishes of four native fish species namely *Puntius ambassis, Ompak bimaculatus, Channa marulius, Heteropneustes fossilis* of Gwalior region have been brought alive in fish shipping boxes from the Tigra Dam which is located about 23kms away from Gwalior (M.P), India. Before experiments in the laboratory the fish species were segregated and were maintained separately species wise, acclimatized them for seven days under laboratory conditions and were kept in glass containers of aerated tap water and were fed with commercial fish feed (tubifex worms) and pieces of earthworms. Fishes were identified with the help of standard taxonomic keys.

2.2 Collection of mosquito eggs

The mosquito eggs have been collected from local (in an around Gwalior city (26.22p N 78.18p E) shady pond, water stored containers and stagnant drains and ditches with the help of mosquito egg collecting net and O type brush. The eggs have been brought to the laboratory and have transferred to 18 cm × 13 cm × 4 cm size enamel trays containing 500 ml of pond water.

2.3 Collection and maintenance of mosquito larvae

The mosquito larvae have been collected in the month of September in order to get maximum larvae by dipping, netting and pipetting methods from shallow ponds, water stored containers, ditches, drains and other stagnant water bodies of the Gwalior city and have been brought to the laboratory in plastic buckets and they were sieved to remove the phytoplankton's, zooplanktons and other dried leaves and then transferred to glass containers and were reared with supplementary food consisting of protein biscuit (60%) and dried yeast powder (40%).

2.4 Collection of aquatic insects

Water insects were simply collected from localwater bodies adjacent to the Jiwaji University Gwalior (M.P.) India.

2.5 Predation experiments

As this work is laboratory based, the intent of the experimental design was to stimulate natural conditions in fish glass aquariums (25cm×20cm×20cm)by filling the laboratory aquariums with the analysed pond water. Acclimatized fish species were weighed and allowed to release into separate aquariums in the morning hours. All the predation experiments carried in 10 L glass aquariums (25cm×20cm×20cm) half filled with analysed pond waters. Each experiment was carried out separately in triplicates. The duration of each predation experiment was one week, and all the four species namely *Puntius ambassis, Ompak bimaculatus, Channa marulius* and *Heteropneustes fossilis* were fed with equal number of eggs, larvae and alternative food in different experiments at different intervals of time in each day of a week. After the feeding duration of each experiment, the number of foods left in the experimental aquariums were counted and removed on daily basis at the end of the experiment and the native larvivorous fish species were removed from experimental aquariums to plastic buckets (5L) and were fed with commercial feed (Tubifex worms)and small pieces of Earth worm (*Eisenia fetida*) during night. The data obtained from each of the experiment were analysed statistically.

Statistical analysis

MS excel 2007 (Average ± Standard Error) was the tool that was used to analyse the data.

Result and Discussion

Reduction of mosquito has been tried by various methods, however, due to certain drawbacks, related with environmental issues; there is a shift in approach to control the mosquito population. Due to ban or restrictions by environmental protection agencies, there are now fewer chemicals available than there have been for the last 20

TABLE-3 : Aquatic insects' consumption by males and female fish species in different intervals of time on the separate days.

Species name and body size	Gender	Experi-mental days	No. of aquatic insects supplied at 9 am	No. of aquatic insects supplied at 2 pm	Total no. of aquatic insects supplied in a day	Total no. of aquatic insects consumed in a day	Total no. of unconsumed aquatic insects in a day
Puntius		1	10	5	15	2	13
ambassis	Female	2	10	5	15	3	12
(8.5cm)		3	10	5	15	1	14
Puntius		1	10	5	15	2	13
ambassis	Male	2	10	5	15	2	13
(8.2cm)		3	10	5	15	1	14
Ompak		1	10	5	15	10	5
bimaculatus	Female	2	10	5	15	10	5
(12.3 cm)		3	10	5	15	11	4
Ompak		1	10	5	15	11	4
bimaculatus	Male	2	10	5	15	12	3
(12.cm)		3	10	5	15	10	5
Channa		1	10	5	15	15	5
marulius	Female	2	10	5	15	13	7
(16.3 cm)		3	10	5	15	13	7
Channa		1	10	5	15	11	4
marulius	Male	2	10	5	15	13	2
(16 cm)		3	10	5	15	12	3
Heteropneustes		1	10	5	15	15	0
fossilis	Female	2	10	5	15	15	0
(18.5 cm)		3	10	5	15	15	0
Heteropneustes		1	10	5	15	14	1
fossilis	Male	2	10	5	15	14	1
(18cm)		3	10	5	15	13	2

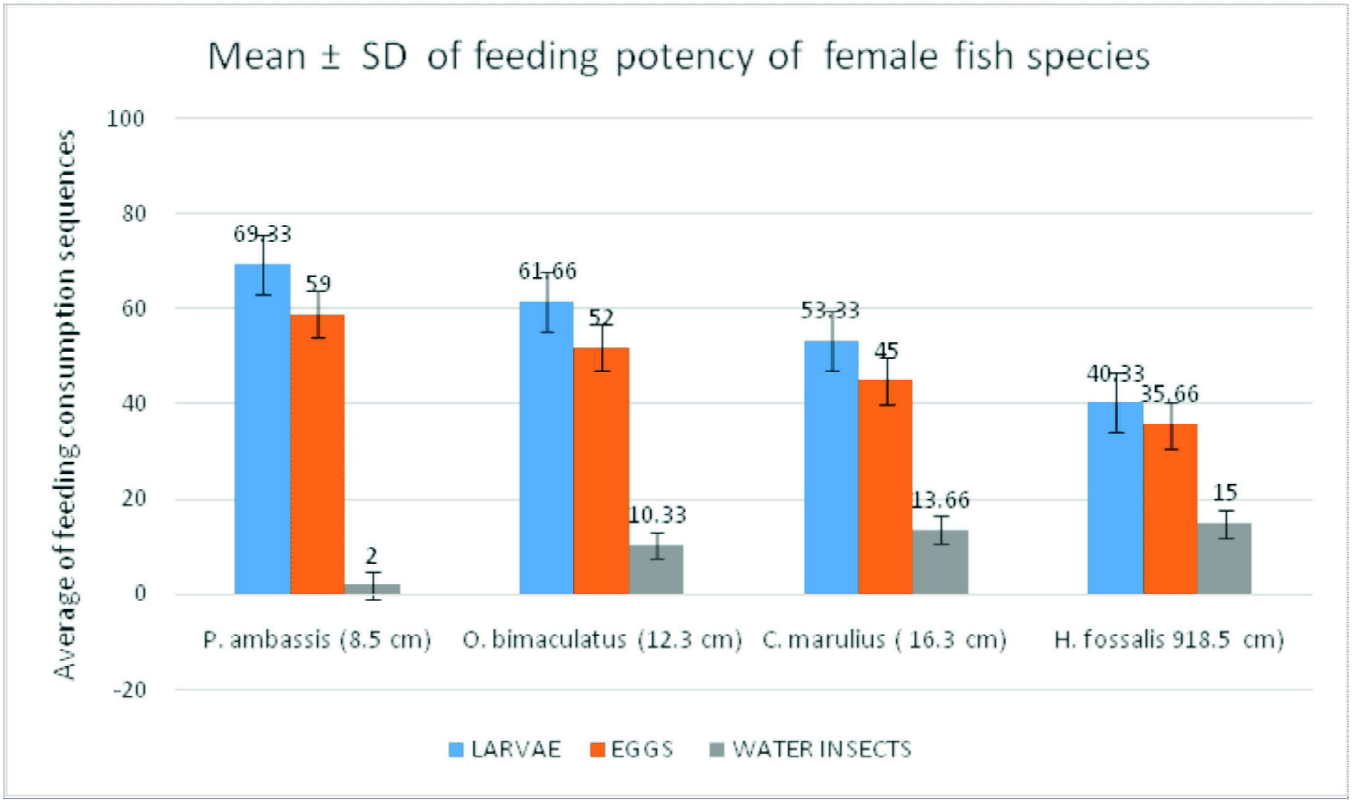

Fig. 1: Comparative feeding potency of female genders of native fish species upon larvae, eggs and aquatic insects.

years[2]. Thus, the biological control of mosquitoes has become more practical. In this regard, larvivorous fishes have been used worldwide for controlling mosquito larvae. Researchers all around the world have evaluated indigenous fish species to identify appropriate local biological control agents[3]. Several studies have suggested introduction of fish mosquito fish species, *Gambusia affinis and Poecilia reticulata* and indigenous species to be effective in minimising mosquito populations breeding places. Concern has also been felt about introducing exotic fish for mosquito control due to their possible impacts on native fish species. The introduction *of Gambusia affinis* in Greece (Europe) led to decline of the endemic fish species *Valencia letourneuxi*[2] and similar findings were reported in United States, Spain and Australia. Keeping above mentioned concerns in mind, in this study, native fishes were tested for their larvicidal potential and predatory potential.

Our three predation experiments under laboratory conditions, convinced that the native fishes have also greater potential to be used as biological control agents for mosquito larvae and other pests. The first experiment was performed on mosquito larvae as a predation experiment in three separate days. Equal number of larvae were fed to both genders of different species of native fishes on daily basis at different intervals of time in a day. Larvae consumption data of both females and male fishes are given in (Table-1).

The second experiment was conducted on mosquito eggs as a predation experiment on three separate days. Equal number of eggs were fed to both genders of different native fish species on daily basis at different intervals of time in a day. Egg consumption data of both females and male fishes are given in (Table-2).

The third experiment was performed on aquatic insects as a predation experiment on three separate days to determine the carnivorous nature of the native fish species. Equal number of aquatic insects were fed to both genders of different native fish species on daily basis at different intervals of time in a day. Aquatic insect's consumption data of both females and male fishes are given in (Table-3) and the average value of all consumptions are given in (Table-4).

All the three predation experiments were preformed to analyse the comparative larvicidal potency and carnivorous nature of the native fish species on the basis of gender. The total number of unconsumed food material were counted every day at the end, in order to get the appropriate food consumption rates on each day of the experiment.

TABLE - 4 : Average no. of eggs, larvae and aquatic insect consumptions by males and female fishes at different intervals of time entire experimental days.

S. No	Species name	Mean $\pm SD$ of eggs, larvae and aquatic consumption by fishes					
		Males			Females		
		Eggs	Larvae	Water insects	Eggs	Larvae	Water insects
1	*Puntius ambassis*	54.33±0.98	65±0.94	1.66±0.27	59±0.47	69.33±0.54	2±0.47
2	*Ompak bimaculatus*	49.66±0.72	59.33±1.90	11±0.47	52±0.81	61.66±1.65	10.33±0.27
3	*Channa marulius*	40±0.81	45.66±1.90	12±0.47	45±2.05	53.33±0.42	13.66±0.54
4	*Hetreopneustes fossilis*	30±0.94	35±2.35	13.66±0.27	35.66±1.90	40.33±0.72	15±0

Abbreviations

PA.*Puntius ambassis*

OP. *Ompak bimaculatus*

CM.*Channa marulius*

HF.*Heteropneustes fossilis*

The data on feeding consumptions averages with standard deviations (SD) of both the genders are given in (Table-4).Three feeding consumption sequences of female genders are larvae consumption sequence P.A 69.33 ± 0.54 > O.B 61.66 ±1.65 > C.M 53.33 ±0.72 > H.F 40.33 ± 0.72, egg consumption sequence P.A 59 ± 0.47> O.B 52 ± 0.81 > C.M 45 ± 2.05 > H.F 35.66 ± 1.90, and aquatic insect consumption sequence P.A 2 ± 0.47< O.B 10.33 ± 0.27< C.M 13.66 ± 0.54 < H.F15 ± 0and three male gender feeding consumption sequences are larvae consumption sequence P.A 65 ± 0.94> O.B 59.33 ± 1.90> C.M 45.66 ± 1.90> H.F35 ±2.35, egg consumption sequence P.A 54.33 ± 0.98> O.B 49.66 ± 0.72> C.M 40 ± 0.81> H.F 30 ± 0.94 and aquatic insect consumption sequence P.A 1.66 ± 0.67 <O.B 11 ± 0.47< C.M 12 ± 0.47< H.F 13.66 ±0.27.

On the basis of the averages of feeding consumptions sequences of males and females, we plotted two graphs (Fig.1 and Fig.2) separately for males and females. Females possess greater larvicidal potency as compared to males. The body size of the fish species also affects their larvivorous potency. The small fish species have greater value of larvicidal potency than that of large fishes. Small fishes usually select small pray as food like mosquito larvae and large fishes usually select large prays as food like aquatic insects. Out of the four native species *Puntius ambassis* possess greater power of larvivorous property followed by *Ompak bimaculatus,* because of their small body sizes.While the other two species namely *Channa marulius* and *Heteropneustes fossilis* show less interest towards larvae because their body size is larger and hence usually prefer large food like aquatic insects for their survival.Thus, the carnivore's sequence of the native fishes is PA<OM<CM <HF. This sequence shows that carnivorous nature of these four native fish species increases as the fish size increases.

Conclusion

The comparative study of larvivorous native fish species revealed that, Larvae consummation was positively related with the small fish species having female gender and prefers mostly small pray as food. Thus, small sized female fish species possess greater predation efficacy on mosquito larvae and the sequence of predation potency of female fish species was noted in the present study is *Puntius ambassis>Ompak bimaculatus> Channa marulius> Heteropneustes fossilis*. As per the results of our study, it is predicted that females have greater value of larvicidal potency than that of males. Thus, female gender plays an important and positive role of mosquito biocontrol at source level. The indigenous larvivorous fish species showed excellent results with high predation efficiency and good survival ability in small volumes of water containers. Besides this they don't cause any harm to other native species of fishes and also breed naturally. As the size of the mosquito larvae and eggs are minute, are easily predated by the small native larvivorous fish species espically with feminine character.The carnivorous nature of the female native larvivorous fish species increases as the body size of the

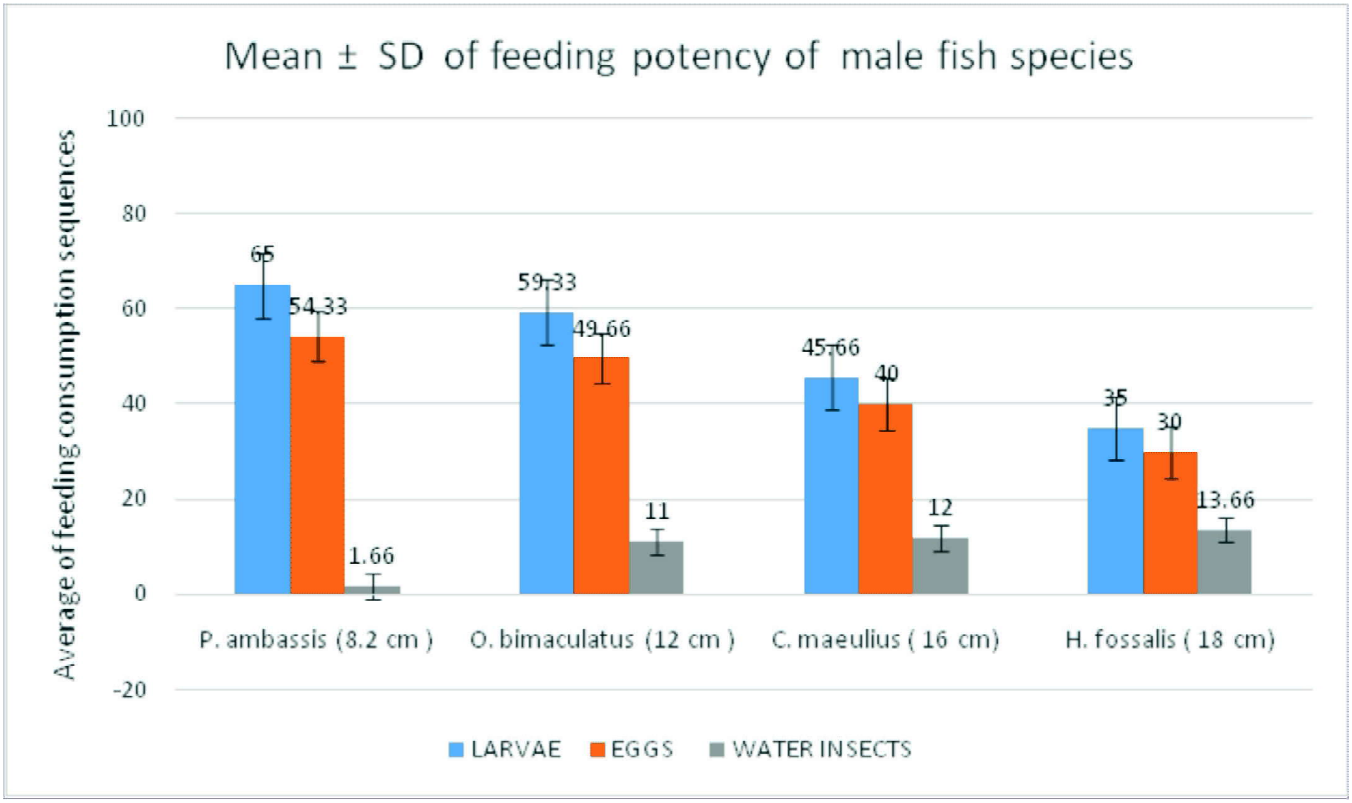

Fig. 2: Comparative feeding potency of male genders of native fish species upon larvae, eggs and aquatic insects.

fish species increases. Large sized native larvivorous fish species feeds voraciously on the large pray like aquatic insects *etc.* Out of all the four native larvivorous species of Gwalior (M.P) selected for larvivorous activity *Puntius ambassis* with female gender seems the most effective and powerful in larvicidal potency followed by *Ompak bimaculatus.* Female genders of native larvivorous fish species are more powerful than that of male genders in mosquito-biocontrol.Biological control by fishes is considered effective, eco-friendly, low-cost and safe to human and other non- target populations. It is suggested that, only native fish species should be used as biological control of mosquitoes in order to avoid the invasive nature of exotic species such as *Gambusia* and *Poecilia.*

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