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BIOLOGICAL CONTROL OF *Aedes* MOSQUITO LARVAE (DIPTERA: CULICIDAE) USING THREE LARVIVOROUS FISH SPECIES IN LABORATORY CONDITION

RAJAMANICKAM DHIVYA & MUTHULADCHUMY VINOBABA

Department of Zoology, Faculty of Science, Eastern University of Sri Lanka, Vantharumoolai, Sri Lanka

ABSTRACT

The mosquitoes *Aedes aegypti* and *Aedes albopictus* are the main vectors of Dengue virus in Sri Lanka. The larvivorous fish used as biological control agents to diminishing mosquito larval populations. This study was carried out to investigate the larvivorous potential of *Aplochelius parvus, Gambusia affinis,* and *Poecilia reticulata*, against *Aedes* mosquito larvae in laboratory condition. Solitary female, solitary male and female and male companion of all three fish species were fed by each three hundred larvae mixture of 3rd and 4th instar of *Aedes aegypti* and *Aedes albopictus* mosquito species under laboratory conditions. The mean number of larvae consumed in every 10 min, 20 min, 30 min, 1 hour, 2 hours, 5 hours, 8 hours and 24 hours were counted for every five replications. The mean larval consumption patterns showed statistical significance at $p<0.05$ level with different time intervals. Solitary female, solitary male and female and male companion of *Aplochelius parvus* had higher feeding potential than *Gambusia affinis* and *Poecilia reticulata*. Therefore these fish species can be utilized as environmental friendly potential biological control agents for dengue mosquitoes.

KEYWORDS*:* *Aplochelius parvus,* Biocontrol, Larvivorous Fish, Larva & Vector

INTRODUCTION

The mosquitoes *Aedes aegypti* (Linnaeus 1762) and *Aedes albopictus* (Skuse 1894), are the main vectors of Dengue virus, Chikungunya virus, and Yellow fever [4] and Zika virus [1] around the world. Both species flourish quickly all over the world mainly in tropical and subtropical areas (Benedict *et al*., 2007). Dengue (Backbone fever) causes severe illness and flu, finally become as hemorrhagic fever and shock syndrome and lead to death. Every year nearly 50-100 million human populations are under the risk of fever dengue. In 2018 500,000 dengue hemorrhagic fever cases and 22,000 deaths are reported from 128 countries [15].

In Sri Lanka, for more than 30 years, all four serotypes of DENV viruses transmitted by the dengue vectors. New changes in genotypes increase the risk of dengue fever around the country [9]. Climatic changes with heavy rain and flooding increase the massive outbreak of dengue vectors. According to the Ministry of Health in 2017, 186,101 dengue fever cases and 320 deaths reported in Sri Lanka. Spraying of the vast amount of insecticide and larvicide developed resistance population of *Aedes aegypti* and *Aedes albopictus* and its lead to the outcome of co-evolutionary forms and inspiring the search of more viable pathogens [5]. Therefore an immediate solution is needed to control the dengue vector population in addition to the existing control strategies. Biological control agents are used to diminishing the different developmental stages of larvae and pupae of *Aedes* mosquito in aquatic environments with the focus on maintaining a healthy and sustainable environment [3]. Larvivorous fishes feed on immature stages of mosquitoes and extremely effective at diminishing mosquito larval populations which breeds in household wells, natural ponds, and tanks [14].

The study was performed to analysis the larvivorous feeding potential of *Aplochelius parvus*, *Gambusia affinis* and *Poecilia reticulata* against the *Aedes aegypti* and *Aedes albopictus* in laboratory condition. There were very few studies carried out in Sri Lanka regarding the larvivorous potential of wild *Poecilia reticulata*, *Gambusia affinis* against the *Aedes* mosquito larvae. There is no significant record on the use of *Aplochelius parvus* [7].

MATERIALS AND METHODS

The experiments were carried out from January to July 2018.

Collection of *Aedes* mosquito larvae using ovitraps

Twenty black plastic containers (Height 15 cm, Diameter 15cm) with the capacity of 1 liter was used as artificial ovitraps. The traps were filled with 600 ml of dechlorinated, clear water. A small wooden stick was vertically placed inside each container to facilitate egg-laying of female mosquitoes [10]. Small leaves and petioles were put inside the traps in order to provide food sources for emerging larvae (Figure 1). Finally, traps were placed outside in dark forestry areas. The ovitraps with eggs were allowed to hatch. The 2nd, 3rd and 4th instar larva were collected for laboratory studies.

Figure 1: Outdoor Ovitraps

Collection of Larvivorous Fish Species

All three adult mosquito larvivorous fishes were collected in Batticaloa district, Sri Lanka. *Poecilia reticulata* was collected from the Medical Officer of Health (MOH) department, Batticaloa. *Gambusia affinis* was collected from the water bodies of channels, local ponds and municipal concrete drainages in urban areas and *Aplochelius parvus* was collected from the water bodies of mangrove, swamps, and marshes in suburban areas by using fine fishing nets. All fish species were brought to the laboratory to maintain the culture.

The water quality parameters of fish habitat were measured during the collection of fish. Salinity was measured by Refractor Salinometer (ATAGO S/ Mill-E, Japan) in ppt. The pH, dissolved oxygen level and temperature were measured by using Multiparameter (EUTECH, PCD 650, Singapore) in pH unit, mg/l, and ^{o}C respectively.

Acclimatization of Fish for Bioassay

All three fish species were reared in three separate experimental glass tanks Three-quarter parts of tanks were filled with dechlorinated water and maintained in laboratory conditions (RT 27.9^{o}C± 2^{o}C) for seven days of acclimatization period before they used for bioassay. The fish were fed with 0.1- 0.5 mm artificial feed two times per day (5g for 100 fish). The experimental tanks were fixed with artificial aerators. The water was changed within every two days to prevent the accumulation of wastes. In order to maintain natural conditions, some aquatic plants were introduced into each tank. The fishes were starved before 24 hours to the bioassay.

Bioassay

The separate glass tanks (60cm (L) × 30cm (H) × 30cm (W) contained 28 liters of dechlorinated well water used for experiments. The fish species which ready for bioassay were introduced into the experimental tanks before three hours in order to adapt in the new environment. Daily feeding of all three fish species fed by a mixture of three hundred 3rd and 4th instar larvae stages of *Aedes aegypti* and *Aedes albopictus* were recorded (Figure 2). Three different gender ratios were tested as following in laboratory conditions (27.9^{o}C±2^{o}C),

- **Tank A:** One adult female fish exposed to 300 mosquito larvae for 24 hours
- **Tank B:** One adult male fish exposed to 300 mosquito larvae for 24 hours
- **Tank C:** One adult female and one adult male exposed to 300 mosquito larvae for 24 hours

The number of larvae consumed in every 10 min, 20 min, 30 min, 1 hour, 2 hours, 5 hours, 8 hours and 24 hours were counted. Five replications were conducted for each trial of three fish species.

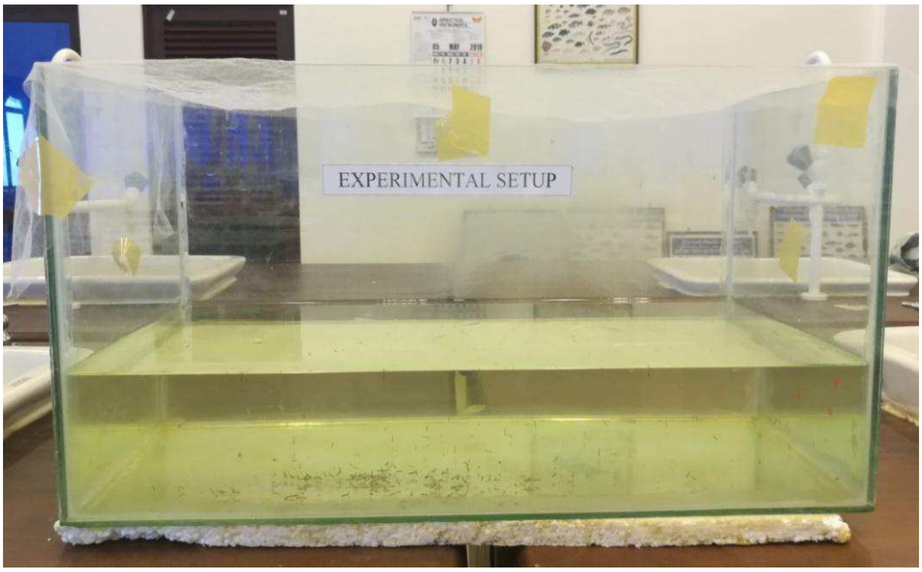

Figure 2: Experimental Tank

Statistical Analysis

All statistical analysis was performed using MINITAB, 2014 (14.0 version). The one way un-stacked ANOVA ($p<0.05$) was used to compare the statistical significance among the mean larval consumption among females, males and female-male companions of all three fish species at different time intervals. Two hypotheses were tested: a) larval consumption was affected by the gender of fish (female and male), and b) larval consumption vary among the different fish species.

RESULTS AND DISCUSSIONS

Table 1: Physio-Chemical Parameters of Field and Laboratory Water Samples

Water Parameters	Levels Detected in Field Water			Tested Water Conditions in the Laboratory Water
	Poecilia reticulata	*Gambusia affinis*	*Aplochelius parvus*	
Salinity ($^0/_{00}$)	1	1.3	12.5	0.4±0.1
pH	7.5	7.7	7.9	7.4±0.3
Dissolved oxygen level (mg/l)	6.85	7.66	5.59	6.98±1
Temperature (^{0}C)	28.8	35.5	33.1	27.5 ±5

Salinity (0.4 ± 0.1ppt) and pH were (7.4 ± 0.3) lower in the laboratory water sample and higher in field water samples (Table 1). The *Aplochelius parvus* had higher adaptability to tolerate the salinity changes [7] than the *Poecilia reticulata* and *Gambusia affinis*.

Mean larval consumptions of females and males of three fish species at eight different time points were presented in Table 2. According to the results of ANOVA, there was significance in mean larval consumption among females and males of three fish species. The larval consumption patterns increased with different time intervals of both female and male fish. The statistical analysis showed that the average larval consumption pattern at different time intervals at $p<0.05$ level.

Mean larval consumption patterns between female male companions of three fish species showed a statistically significant at $p<0.05$ level. The consumption rate was increased with the time of all three fish species (Table 3).

The larvivorous activity of three fish species; *Aplochelius parvus*, *Gambusia affinis*, *Poecilia reticulata* showed a considerable larvivorous potential against the *Aedes* mosquito larvae. The average larval consumption among three fish species with different gender comparison in 24 hours followed as *Aplochelius parvus* > *Gambusia affinis* > *Poecilia reticulata*.

Table 2: The Average (Mean± SE) Larval Consumption (n=5) of Female and Male at Different Time Intervals of all Three Fish Species

Time	Average Number of Larvae Consumed by Female and Male Fish of All Three Fish Species								
	Aplochelius parvus			*Gambusia affinis*			*Poecilia reticulata*		
	Female	Male	*p* Value	Female	Male	*p* Value	Female	Male	*p* Value
10 minutes	9 ±0.24	4± 0.58	0.000	11±0.71	2±0.55	0.000	5±0.86	1±0.32	0.003
20 minutes	6 ±0.97	2± 0.36	0.007	6±0.66	0±0.24	0.000	4±0.58	0±0.24	0.000
30 minutes	7 ±0.51	4± 0.68	0.008	8±1	4±0.71	0.011	3±0.49	2±0.45	0.108
60 minutes	12 ±0.86	7± 0.54	0.003	10±0.37	7±0.8	0.009	6±0.73	3±0.55	0.008
2 hours	10 ±0.89	11±0.66	0.633	14±1.16	8±0.86	0.005	9±0.84	6±0.71	0.026
5 hours	17 ±1.65	13±1.27	0.117	18±0.86	7±1.02	0.000	11±0.70	4±0.86	0.000
8 hours	21 ±0.93	15±1.01	0.006	16±1.02	13±0.89	0.073	13±0.66	13±1.16	1.000
24 hours	93 ±4.03	46±2.43	0.000	88±4.50	36±1.65	0.000	52±1.41	32±1.93	0.000

The females of *Aplochelius parvus* (175±10.32) and *Gambusia affinis* (171±9.62) were very active predators than the *Poecilia reticulata* (103±5.72). The males of *Poecilia reticulata* (61±3.76) and *Gambusia affinis* (77±4.02) were consumed a low number of *Aedes* larvae. But *Aplochelius parvus* (102±5.04) had a higher preference than other males.

In the presence of female-male companion, higher mean numbers of larvae were consumed by *Aplochelius parvus* (283±18.575) than *Gambusia affinis* (208±12.175), and *Poecilia reticulata* (166±10.867) (Table 4).

In the experimentation, body sizes of male and female fish reflected the different capacity in searching and consuming *Aedes* mosquito larvae. The prey consumption capability increases with body size. The feeding efficacy of fish was found to be increased as the size of a group also increased.

Table 3: Average Number of Larvae Consumed by Female and Male Companions of three Fish Species at Different Time Intervals

Time	Average Number of Larvae Consumed by Female and Male Companions			*p*- Value
	Aplochelius parvus	*Gambusia affinis*	*Poecilia reticulata*	
10 minutes	11 ± 0.58	9±0.86	8±1	0.054
20 minutes	7 ± 0.51	6±0.71	4±0.86	0.024
30 minutes	8 ± 0.51	8±0.58	6±1.07	0.150
60 minutes	20 ± 1.36	16±0.86	11±0.63	0.000
2 hours	21 ± 0.73	20±1.26	12±0.95	0.000
5 hours	19 ± 1.21	17±1.05	10±0.8	0.000
8 hours	30 ± 1.88	22±0.73	19±1.30	0.000
24 hours	164 ± 3.17	110±3.94	96±1.95	0.000

Table 4: Average (Mean± SE) Larval Consumption of Three Fish Species in 24 Hours

Different Gender Composition	Three Larvivorous Fish Species		
	Aplochelius parvus	*Gambusia affinis*	*Poecilia reticulata*
Female	175±10.32	171±9.62	103±5.72
Male	102±5.04	77±4.02	61±3.76
Female+Male	283±18.57	208±12.17	166±10.87

In all experiments, the efficient larval consumption was observed at initial 10 minutes that because of fish were starved for 24 hours prior to the experiments.

The larval consumption improved with time was a good suggestion for the larvivorous potential habit of a fish [4]. Because increasing the feeding rate of fish with time is necessary due to the short larval period when compare to pupal and adult stages.

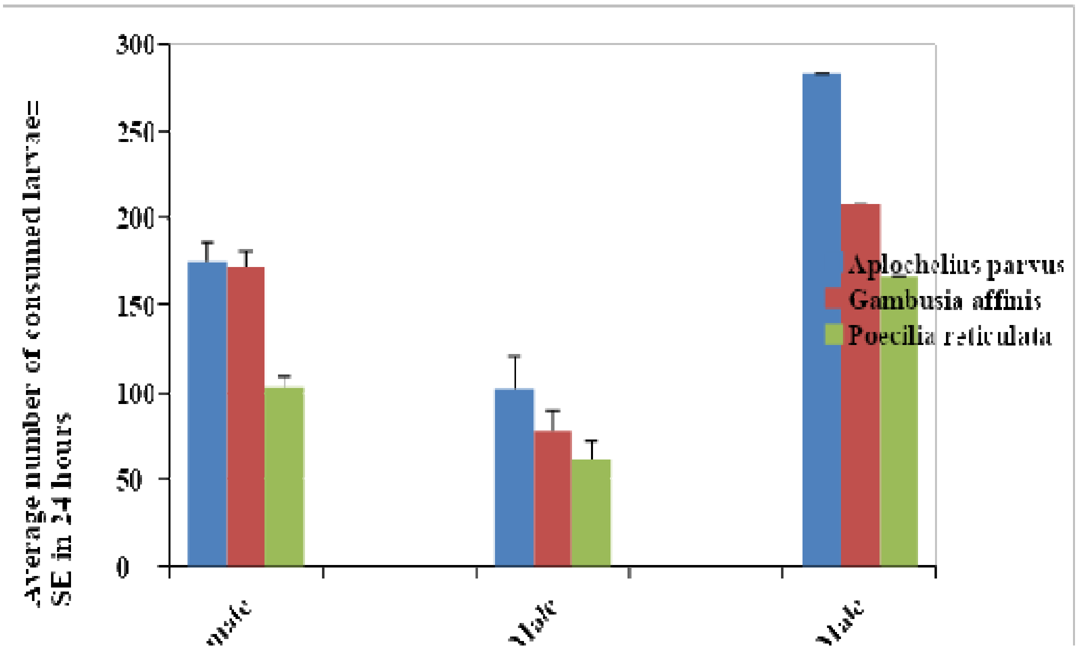

Figure 3: Comparison of Total Average Larval Consumption of three Fish Species in 24 Hours

In many countries, field trials conducted to quantify the reduction in *Aedes* mosquito larval density achieved by using larvivorous fish species of *Aplochielus* sp, *Gambusia affinis* and *Poecilia reticulata* in water storage containers, elevated tanks, ground tanks, cisterns, household wells, drums, open wells, ponds, water holes, jars and tanks [8]. *Aplochelius parvus* is our native species and diurnal feeder, both females and males are active predators [7]. *Gambusia affinis* and *Poecilia reticulata* are exotic fish species to our country. Utilization of our native fish species, as opposed to exotics, should be encouraged.

Further studies are needed to measure the appropriateness of this biological integrated control method in field environments to assess its field impact for all three fish species.

CONCLUSIONS

In conclusion, the average larval consumption of Solitary female, solitary male and female and male companion of *Aplochelius parvus* had higher feeding potential than *Gambusia affinis* and *Poecilia reticulata*. The females had a higher feeding rate on *Aedes* larvae compared to the males.

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