

Assessment Of Guppy Fish *Poecilia Reticulata* And *Poecilia Sphenops* As Biological Control Agent Of Mosquito Larvae Under Aquarium Conditions

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Abstract

There are various mosquitos borne diseases playing major role of transmission as vector; these include dengue, malaria, filariasis and yellow fever. Various places around us everywhere act as breeding sites for different developmental stages of mosquitoes. Variety of chemicals are being used to control breeding and spread of mosquitoes which include mercuric chloride, phenols, Paris green cresols, naphthalene, Bordeaux mixture, calcium arsenate, rosin-fish oil soap, and nicotine sulfate which are prone to various risks on environment and biodiversity. In present study an alternative approach of control through insectivore fish *Poecilia reticulata* and *Poecilia sphenops* was used against mosquito's larvae as an alternative approach. The predation effect of insectivore fish on mosquito larvae (200) of *Culex* and *Aedes* was observed at different temperatures including 20°C, 25°C and 30°C for one hour duration and observation was recorded at 5 minutes interval. At 20°C the maximum consumption of larvae was observed at 35 minutes while for 25°C the maximum consumption was observed at 55 minutes similar results are observed at 30°C with increase of time for *Poecilia reticulata*. *Poecilia sphenops* also showed significant results at recorded temperatures. Also the morphometric parameters of mosquito's larvae are shown in results which gave significant results. This study gave a useful alternate for control of breeding sites of mosquitoes which can save mankind from various serious mosquitoes' related diseases.

INTRODUCTION

Mosquitoes are deliberately parasitic and responsible for transmission of one of the world's mainly terrible life threatening and viral infections including malarial infection, dengue viral infection, filariasis, yellow fever, chikungunya, encephalitis and Zika viral infection etc rendering it the deadliest animal in the world. The main mosquito vector, *Culex*, and *Aedes aegypti* breeds in stagnant, clear bodies of water and thrives in human-made receptacle such as unnecessary pots, tires, cans and water storage containers [1]. Life cycle of mosquitoes go through four stages which are Egg, Larva, Pupa, and adult. These life stages normally last 5 to 14 days, depending on temperature and species. Females lay 100-200 eggs during their life cycle [2].

Geographically Mosquitoes are frequently distributed all over the world commonly found in Tropical and sub-tropical regions. More than 3,000 diverse species has been reported as a possible vectors caught up in disseminations of life threatening out breaks all over the earth [3]. About 50 million of dengue infections are reported every year and 500,000 people are hospitalized with dengue viral infection, typically in the Pacific, Southeast Asia and the Americas [4].

In order to control and prevent the mosquito borne diseases different actions have been developed aiming to destroy every developmental stages of mosquito's larvae [3]. From two decades, the use of insecticides in controlling vector borne infections was the most reliable method. Also several compounds like mercuric chloride, phenols, Paris green cresols, naphthalene, Bordeaux mixture, calcium arsenate, rosin-fish oil soap, and nicotine sulfate, were usually applied as habitual pesticide [5]. These strategies consume millions of dollars. Such chemical insecticides are to be avoided due to their ill effects on the water community and biodiversity [6]. So, there is need to design a comprehensive integrated management strategy to overcome the harmful effects of insecticides.

In order to defend the environment as well as peoples from mosquito born disease an alternative approach is needed which is through control of mosquito by biological means particularly using larvivorous fishes (that feed on mosquitoes larvae) in an eco-friendly manner [7]. In biological control, use of larvivorous fish that feed on mosquito larvae was one of the most recommended methods for controlling vector borne diseases at the larval stage [8]. More than 200 fish species has been reported as larvivorous [9]. Among them one of the most successful and widely used biological control agent is *Poecilia reticulata* (Guppy), can eats about 80 to 100 mosquito larvae in 24 hours. A study conducted in a number of riverbed pools and dams in Sri Lanka as well showed the potential of *Poecilia reticulata* in mosquito control [10].

Present study was designed to help the future workers to utilize fishes as biological control agents in integrated management program as a source of effective bio control agents for mosquito management. Feature work on comparative potential between *Poecilia reticulata* (guppy) and *Poecilia sphenops* (Molly) for mosquito bio control in Pakistan perspective is still lacking. So, the present work was designed to compare the mosquito bio control efficiency of guppy and molly, two popular fishes which so far have been used for mosquito bio control.

MATERIAL AND METHODS

Collection of Fishes

Poecilia reticulata and *Poecilia sphenops* was collected from local market of Rawalpindi, brought to the Bio Control Laboratory and were kept in four batches of glass aquaria having size 15×15×12" each containing 25 L of water at a temperature 27 to 30°C. The fishes were maintained with fish feed for a week earlier to the experiment [11].

A 5 gallon bucket was placed in summer and allowed it to fill with rainwater than place bucket in sun. Algae were grown in warm water. Mosquitoes lay tiny rafts of dark brown eggs on the surface of the water. Within 48 hours the eggs hatched into larvae. Mosquito's larvae were also collected from Rawalpindi and its adjacent areas with the help of plastic dipper.

Experimental Design

The trial was carried out in glass beakers. Five fishes were placed in glass beakers and were held without food for 24 hours in order to standardize hunger level. Predation experiment was conducted over a period of 5 minutes in 1 hour, after which the fish were removed from beaker and remaining larvae were counted. Each trial was replicated 3 times in parallel. (i.e., n= 12 for a trial with 3 treatments). The treatments were observed and recorded for 1h with an interval of 5min, 15min, 30 min, 45 min and 60 min. treatment, a known number of mosquito larvae were offered and the numbers of larvae consumed by fishes were recorded. Total numbers of larval consumption in 1h duration as well as feeding behavior were noted carefully for each size groups.

Preliminary Comparison of Predation Efficiency

In each experiment, both species *P. reticulata* and *P. sphenops* were tested against 200 larvae of culex and aedes. Total number of larvae consumption their feeding behavior and their comparison were noted.

Morphometric of Mosquitoes Larvae

Observations and measurements of total body length (BL), length of Siphon length (SL), Siphon width (SW), were made cleared in lacto phenol for study in temporary mounts on a microscopic glass slide.

Calibration

Ocular micrometer was used to measure the width and length of internal structure of Mosquitoes larvae. It was calibrated with stage micrometer by using following procedure. In the eye piece of microscope, the ocular micrometer was placed. For calibration, stage micrometer was placed on the stage of micrometer. Microscope was adjusted at 40X in such a way that zero lines of both stage and ocular micrometer were coincided. The lines of stage micrometer were counted from left to right between zero and most super imposed line (20th spaces). Similarly, the lines of ocular micrometer were counted from left to right between zero and most super imposed line (20th spaces). The smallest space on stage micrometer was 0.01mm. So, divisions of stage micrometer on divisions of ocular micrometer were observed by using following formula

20 divisions on ocular micrometer = 20 divisions on stage division

1 divisions on ocular micrometer = $20 \times 0.01\text{mm} / 20 = 0.01\text{mm}$

1 ocular division = 0.01mm

= 0.01×1000

= 10 μm

Thus, the calibration factor used in this study was 10 μm . The width and length which were measured from the ocular micrometer were multiplied

with

10 μm calibration factor to get the actual value of width and length in μm .

Statistical Analysis

Data was analyzed for ANOVA using micro-soft excel program to show descriptive summary. Program SPSS 13 (SPSS Inc.) was employed for one way ANOVA analysis to compare efficiency of male and female. The level of significance for the statistical analysis was set at $p < 0.05$.

RESULTS

The result for 1hour experiment at 20⁰ C, showed maximum average consumption rate by *P. reticulata* of mosquitoes larvae during initial 5 minutes (male=10.1 \pm 0.09 and female= 23.2 \pm 0.19), 10 minutes (male = 5.5 \pm 0.09 and female = 10.4 \pm 0.19) which were statistically significant ($P = 0.02$). While average consumption rate of mosquitoes larvae during 40 minutes (male = 2.5 \pm 0.28 and female = 8.5 \pm 0.19), 60 minutes (male = 8.5 \pm 0.25 and female = 8.33 \pm 0.09) ,50 minutes (male = 6 and female = 8) were significantly different with 60 minutes (male = 8.67 \pm 0.25 and female = 8.33 \pm 0.09). Mean comparison indicated that during starting 5 minutes average consumption rate was found maximum (male = 10.1 \pm 0.09 and female = 23.2 \pm 0.19) followed by 35 minutes and 25 minutes. During 20 minutes minimum consumption rate were found, this may be due to environmental stress (fig 1). The bars on graphs show standard error.

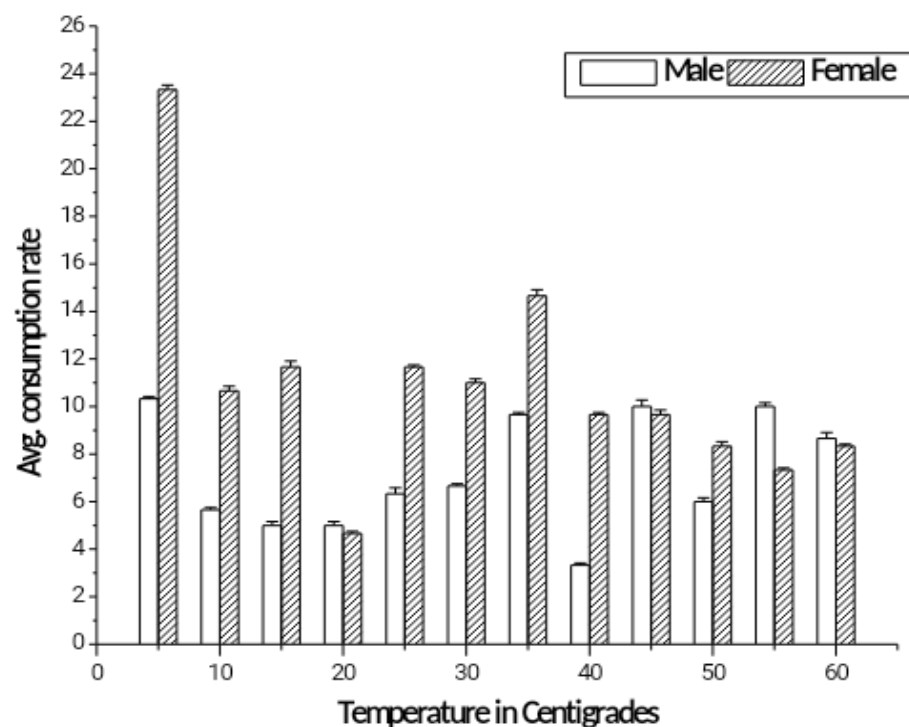


Figure. 1 Number of mosquitoes larvae consumed by *P. reticulata* at temperature 20°C

The maximum average consumption rate at 25° during initial 5 minutes (male = 26.67 ± 0.48 and female = 41.67 ± 0.96) followed by 10 minutes (male = 11 ± 0.44 and female = 12.67 ± 0.59) were found significant ($P= 0.01$). Average consumption rate of mosquito larvae recorded during 20 minutes (male = 7.33 ± 0.69 and female = 8.66 ± 0.69) followed by 30 minutes (male 9.66 ± 0.41 and female 9 ± 0.92) 35 minutes (male = 7.33 ± 0.58 and female = 9.66 ± 0.67) 40 minutes (male = 4.66 ± 0.19 and female = 6.33 ± 0.25) 45 minutes, 50 minutes, 55 minutes and 60 minutes (male = 5 ± 0.5 and female = 7.66 ± 0.53) were statistically significant ($P= 0.02$) Figure 2.

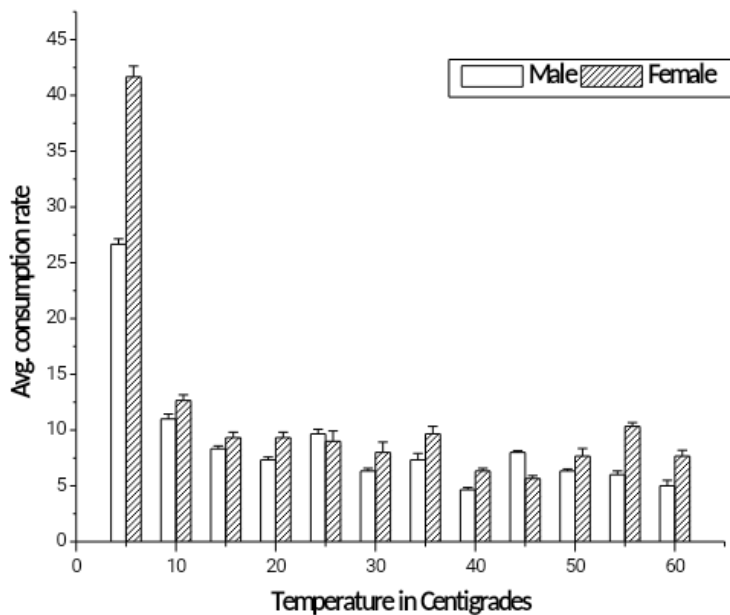


Figure 2. Average consumption of mosquitoes larvae by *P. reticulata* at temperature 25°C

Similarly observations recorded at 30°C showed significant ($P=0.03$) increase in the consumption rate with increase in time which showed 5 minutes consumption rate to be 16.66 ± 0.33 in males and 51.66 ± 0.88 by female followed by 10 minutes (male 15 ± 0.57 and female 14 ± 0.33) 15 minutes (male = 12.33 ± 0.33 and female 0.33) 20 minutes (male 13 ± 0.66 and female 8.66 ± 0.69) 25 minutes (male = 9.66 ± 0.41 and female = 9 ± 0.92) 30 minutes (male 6.33 ± 0.25 and female 8 ± 0.92) 35 minutes. Similar significant results ($P=0.01$) were recorded up to 60 minutes (Fig. 3). The data revealed that average consumption to three different temperatures on mosquito's larvae during summer season at 5% level of probability showed maximum number of their consumption during 30°C (male = 8.34 ± 0.84 and female = 13.63 ± 1.95). The average consumption rate was minimum at lower temperature 20°C. Average consumption rate increased as the temperature increases to 25°C.

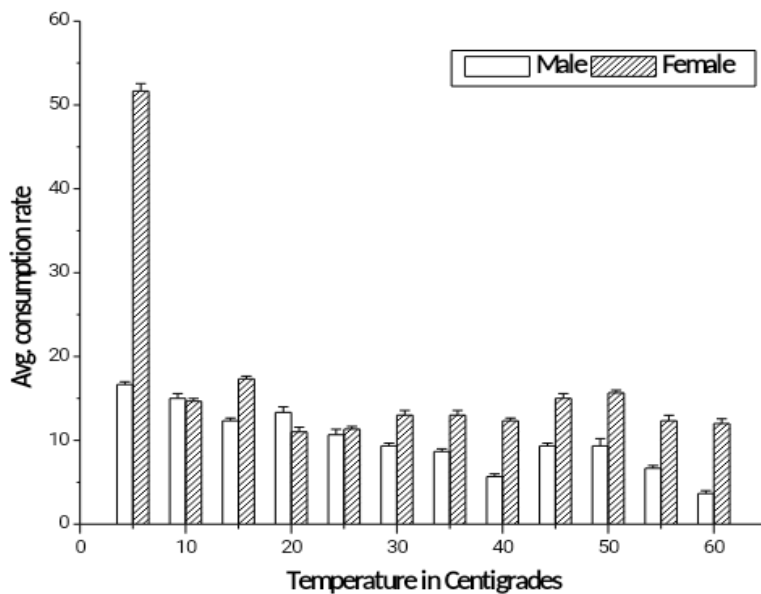


Figure 3. Average consumption of mosquitoes larvae by *P. reticulata* at temperature 30°C

Maximum consumption rate for *Poecilia sphenops* at 20⁰ recorded during initial 5 minutes for male were 8.33 ± 0.88 and for female 18.66 ± 0.88 followed by 35 minutes (male = 6 ± 0.55 and female = 11.66 ± 0.66) which were statistically significant ($p < 0.05$). Average consumption rate of mosquitoes larvae during 5 minutes (male = 8.33 ± 0.88 and female = 18.66 ± 0.88) were found significant with 10 minutes (male = 3.66 ± 0.88 and female = 11.33 ± 1.85) followed by 15 minutes

20 minutes 25 minutes (male = 4 ± 0.57 and female = 10 ± 1.15) 30 minutes (male = 4.66 ± 0.33 and female = 9 ± 0.57) 35 minutes (male = 6 ± 0.57 and female = 11.66 ± 0.88) 40 minutes (male = 3.66 ± 0.33 and female = 8.33 ± 0.33) 45 minutes (male = 6.33 ± 0.66 and female = 7.33 ± 0.33) 50 minutes (male = 3.33 ± 0.88 and female = 5.33 ± 0.33) 55 minutes (male = 5.66 ± 0.33 and female = 4.66 ± 0.33) 60 minutes (5.33 ± 0.33 and female = 6 ± 0.57) were found significant ($P = 0.03$). Maximum consumption per minutes was recorded during 5 minutes followed by 10, 15 and 35 minutes were significantly different (figure 4).

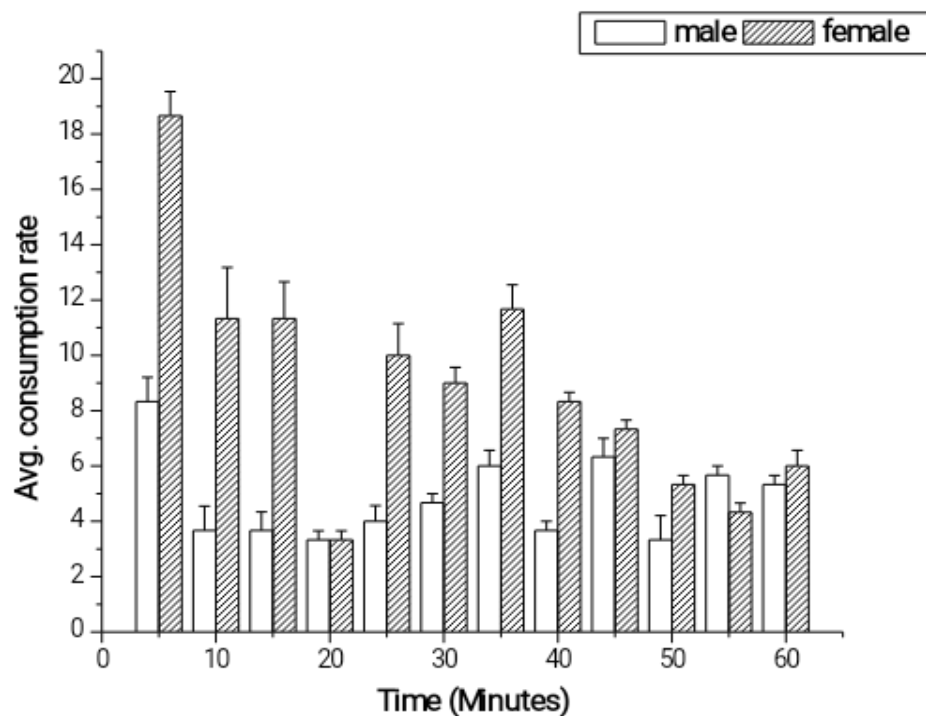


Figure 4. Average consumption of mosquitoes larvae by *P. sphenops* at temperature 20°C

On the other hand maximum consumption rate at 25° was recorded during initial 5 minutes for male was 15 ± 0.57 and for female = 30.33 ± 0.88 followed by 10 minutes (male = 11.33 ± 0.66 and female = 13.33 ± 0.88) 15 minutes (male = 6.66 ± 0.33 and female = 11.33 ± 0.33) 25 minutes (male = 5.66 ± 0.33 and female = 7.66 ± 0.33) 35 minutes (4 ± 0.57 and female = 7.33 ± 1.52) 40 minutes (male = 2.66 ± 0.33 and female = 6.66 ± 0.88) 55 minutes (male = 3.66 ± 0.33 and female = 4.33 ± 0.33). Minimum consumption rate was recorded during 45 minutes (male = 2.66 ± 0.33 and female = 6.66 ± 0.88) Figure 5.

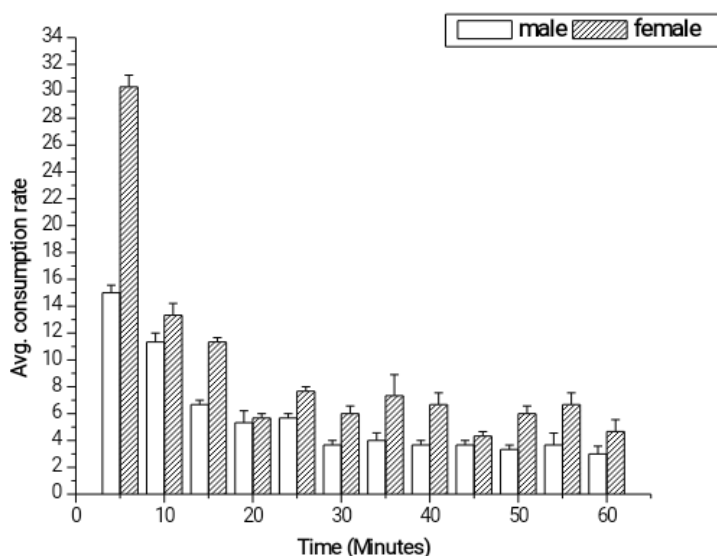


Figure 5. Average consumption of mosquitoes larvae by *P. sphenops* at temperature 25°C

At 30° C during initial five minute consumption rate was found maximum (male = 15.33±0.33 and female = 40±0.33) followed by 10 minutes (male 11.33±0.33 and female 13.66±0.88) 15 minutes (male = 9.66±0.33 and female = 13.66±0.88) were statistically significant. Minimum consumption rate was found during 25 minutes (male = 7.33±0.66 and female = 6.33±0.88). During 20 minutes male *P. sphenops* consumed more larvae than female (male 10.66±0.66 and female 9±0.57). During 40 minutes consumption rate was (male = 3±0.57 and female = 10.66±0.66) followed by 30 minutes (male = 8±0.57 and female = 10.33±0.88) 35 minutes (male = 5.66±0.48 and female = 8.33±0.66) 50 minutes (male = 6.33±0.88 and female = 10.33±0.88) 55 minutes (male = 4±0.57 and female = 10±0.57) 60 minutes (male = 3±0.57 and female = 9.66±0.33) 45 minutes (male = 5.66±0.66 and female = 9.33±0.33) 20 minutes (male = 10.66±0.66 and female = 9±0.57) 25 minutes (male = 7.33±0.66 and female = 6.33±0.88).

The average total body length of the 30 collected mosquito's larvae of both species *Culex quinquefasciatus* and *Aedes aegypti* were 3.110667±0.816984 and 1.975±0.110215 respectively. Average length of siphon tube of both *Culex quinquefasciatus* and *Aedes aegypti* larvae were 0.427333±0.022175 and 0.339667±0.022725 respectively. The larvae *Culex quinquefasciatus* and *Aedes aegypti* siphon tubes mean width were 0.145333±0.022175 and 0.122±0.011481 respectively (Table 1&2)

Table 1: Morphometric measurements of Mosquito Larvae of *Culex*

S.NO	Total body length (mm)	Siphon length (mm)	Width of siphon tube (mm)
1	2.37	0.45	0.01
2	1.92	0.4	0.4
3	1.6	0.39	0.11
4	2.27	0.41	0.15

5	2.74	0.41	0.15
6	2.74	0.48	0.12
7	2.34	0.37	0.73
8	2.79	0.45	0.18
9	2.48	0.44	0.12
10	2.65	0.46	0.14
11	2.8	0.44	0.13
12	2.44	0.44	0.1
13	26.6	0.47	0.11
14	2.46	0.46	0.12
15	2.92	0.56	0.13
16	1.93	0.35	0.1
17	2.96	0.4	0.11
18	0.14	0.22	0.07
19	1.32	0.21	0.08
20	2.34	0.42	0.12
21	2.3	0.96	0.12
22	2.6	0.35	0.08
23	2.57	0.42	0.1
24	2.5	0.41	0.09
25	1.98	0.4	0.11
26	2.7	0.38	0.14
27	2.26	0.4	0.13
28	2.82	0.47	0.19
29	1.37	0.37	0.08
30	2.41	0.43	0.14
Total	93.32	12.82	4.36

Mean	3.110667	0.427333	0.145333
S.D			
S.E	0.816984	0.022175	0.022175

Table 2: Morphometric Measurements of Mosquito Larvae of Aedes

S. No	Total body length (mm)	Length of siphon tube (mm)	Width of siphon tube (mm)
1	2.9	0.43	0.2
2	2.43	0.4	0.12
3	1.4	0.03	0.13
4	2.43	0.44	0.16
5	2.74	0.45	0.12
6	1.46	0.3	0.1
7	2.49	0.43	0.12
8	2.36	0.42	0.12
9	2.51	0.43	0.13
10	2.2	0.42	0.1

DISCUSSION

Present study reveals that at temperature 20°C female *P. reticulata* (23.2±0.19) is more active than male (10.1±0.09) ones in consuming mosquito larvae. Similar findings were in agreements with the findings of [12], [13] concluded that female fish was more active than male fish. Female *P. reticulata* is more active than male *P. reticulata* because of having greater body size and also greater food requirement for giving birth to young one. This result also supports the finding of [14] who reported that the efficacy of fish as a predator of mosquito larvae directly depends on its sex and weight. Therefore, female *P. reticulata* are more capable to eradicate *A. aegypti* larvae than male *P. reticulata*. The results are also in line with findings of [15] and have found that all the fish species consumed greatest number of mosquito larvae during the initial minutes and subsequently the feeding potential was gradually decreases.

Present study reveals that female *P. reticulata* consumed more mosquitoes' larvae than male *P. reticulata*. Similar findings were also reported by [12], [13] concluded that female fish was more active than male fish. The results of study are in agreement with the findings of [15] who has reported that the average consumption rate was increase with increase in time this may be because of acclimatization factor. Also this study revealed that at higher temperature 30°C, both male and female *P. reticulata* consumed higher number of mosquitoes' larvae and their after the feeding intensity decreased. Similar findings were in agreements with that of [13], [15]. The feeding rate was found to be optimum at temperature 30-35°C for *P. reticulata* [16], which indicated that average consumption rate of mosquitoes' larvae by fish generally an increase with increase in temperature till an optimum level is reached.

Also it is evident from present study that female *Poecilia sphenops* consumed more larvae as compared to female because of having larger in size as compared to male which is similar to the findings of [14]. The results are in line with findings of [15] found all the fish species consumed maximum numbers of mosquito larvae during initial minutes and thereafter the feeding amount was gradually slowed down. It was observed that all the fish species consumed maximum numbers of mosquito larvae at first 30 minutes and after that feeding potential decreases with time similar to the findings of [15]. Female *P. reticulata* in present study consumed more mosquitos' larvae than male *P. reticulata*. Similar findings were also reported by [12, 13, 14, 15] concluded that female fish was more active than male fish. Present study revealed that female *Poecilia sphenops* consumed more larvae as compared to female because of having larger in size as compared to male which is similar to the findings of [14]. Female *P. sphenops* was more active than male *P. sphenops*. Similar findings were in agreements with the findings of [12] concluded that female fish was more active than male fish. This was may be because of having greater body size and greater food requirement for giving birth to young one.

The consumption efficiency of *P. sphenops* was comparatively lesser than *P. reticulata*. It was observed that average larval consumption was greater during the earlier minutes of the study and reduced with the progression of the time with several exceptions. Female fish of both sexes consumed more larvae than male as the size of female fish is larger than male fish. In line to our study [17] the size of fish is directly proportional to rate of larval consumption. Present study revealed that female *Poecilia sphenops* consumed more larvae as compared to female because of having larger in size as compared to male which is similar to the findings of [12]. The results are in line with findings of [15] found all the fish species consumed maximum numbers of mosquito larvae during initial minutes and thereafter the feeding amount was gradually slowed down. Present study reveals that female *P. reticulata* consumed more mosquitos' larvae than male *P. reticulata*. Similar findings were also reported by [12] concluded that female fish was more active than male fish. Present study revealed that female *Poecilia sphenops* consumed more larvae as compared to female because of having larger in size as compared to male which is similar to the findings of [14].

Conclusion

It is concluded from above mentioned results that we can use fish larvae for controlling mosquito population and can save spread of related epidemic. Also it is use full alternative approach instead of using chemicals with adverse effects. In future there should be expended research using alternative approaches for controlling mosquito population.

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Data Availability Statement

Data can be provided on demand

Competing Interests

Authors have declared that no competing interests exists

Ethical Approval: The ethical issues is not applicable

Consent of Participate: Not applicable

Consent of Publication: Not applicable

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