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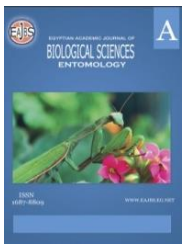

Functioning of Mosquitofish in Different Bionomics as A Biocontrol Agent to Lower the Population of *culex pipiens* L. Larvae

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ABSTRACT

The effect of salinity levels, water source and prey density on the predatory efficiency of mosquitofish, *Gambusia affinis* (Baird and Girard) against the 4th instar larvae of *culex pipiens* was studied. The salinity levels of 5, 10 and 15% significantly affected the rate of predation of *G. affinis*. All fishes have died at a salinity level of 15%. However, the predatory efficiency of both males and females was significantly higher at a 5% salinity level than that at 10%, despite fish sex or size. The number of mosquito larvae consumed by a female was higher than male at a 5% salinity level, but the reverse was observed at a salinity level of 10% despite the fish size. The type of water in which mosquito larvae or fish were bred affected the predatory efficiency of *G. affinis* for both male and female fishes and for all sizes of fish. Distilled water was the highest significant favorable water for predation. The density of mosquito larvae affected the predatory efficiency of *G. affinis*. Results showed that mosquito larvae at a density level of 40 larvae/200 ml water were the most favorable density for predation by *G. affinis* despite sex or size of the fish. In general, females were more efficient in predation than males despite fish size or prey density.

INTRODUCTION

Mosquitoes still constitute a severe nuisance for humans and domestic animals in many rural, suburban and urban areas in Egypt and many countries in the world. Several species of *Culex* act as transmitters of one or more significant diseases of birds, humans and other animals, such as arbovirus infections, West Nile virus, filariasis, Japanese encephalitis, St. Louis encephalitis, and avian malaria. These diseases have been a major problem in almost all tropical and subtropical countries and currently, there are no successful vaccines against most such diseases (Hemingway *et al*., 2004). For instance, *Culex quinquefasciatus* is a transmitter of elephantiasis, which can infect ca. 120 million persons worldwide, and around 400 million persons are under the attack of contracting elephantiasis universal, causing an annual economic loss of about 1.5 billion dollars (WHO, 1997).

Many non-natural insecticides are extensively used for suppressing adult and larval mosquito populations. However, the destructive effects of chemicals on non-target organisms and the growth of resistance to these chemicals in mosquitoes besides the recent

resurrection of different mosquito-borne diseases have provoked to discover alternative, easy and sustainable methods of mosquito control (Milam *et al*., 2000).

Biological control of mosquito larvae with predators and other biocontrol agents would be more effective and environmental-*friendly* methods, besides avoiding the use of chemicals and the coincident damage to the environ. Using or introducing a self-reproducing predator into the ecosystem may deliver continuous biological control of the pest populations (Kweka *et al.,* 2011).

Several predators have proved to be effective against mosquito larvae, among the popular biocontrol agents against mosquito populations are the larvivorous fish, or western mosquitofish, *Gambusia affinis*, which was introduced to more than 60 countries in the last century and remains as a preferred larvivorous fish on mosquitoes. The study was planned to manifest the effect of different bionomics on the predatory efficiency of *G. affinis* against *Culex* larvae.

MATERIALS AND METHODS

Rearing and Maintenance of *Culex spp.:*

Initially, Mosquito eggs, larvae and pupae were collected with a 300 ml dipper from stagnant water bodies in Assiut governorate and transported to the laboratory on tap water in plastic containers and allowed to hatch in metal trays (30 cm long × 15 cm wide × 3 cm deep) containing 2 L of tap water. In the laboratory, the immature mosquitoes were transferred to enamel larval trays until adult emergence. The larvae were fed with dog biscuits and yeast in a 3: 1 ratio. After emergence female adults were fed on their principal feeding diet which is blood from any source (mainly vertebrates) (Apperson *et al*., 2002), and reared at 25 – 27°C and 44 – 50 % R.H for several generations to conduct some biological studies.

Gambusia Collection and Rearing:

The Gambusia fishes were collected with a mesh aquatic net (25 cm x 25 cm) with a wooden handle; enough numbers were collected by repeated dips and were transported in plastic containers of up to 5 liters, half-filled with water from the rearing pond, containers, which had sufficient openings to allow the flow of air. Fishes were transported quickly to the laboratory in dechlorinated water and were acclimatized to its condition for several days and then were fed with Culicine larvae and artificial foods.

Factors Affecting The Predation Efficiency of *Gambusia affinis* on Mosquito Larvae.

Salinity Levels:

Twenty *Culex* spp. 4th instar larvae were added to plastic containers (10 cm x 10 cm x 10 cm) with 500 ml of dechlorinated water, *Gambusia affinis* were measured in three different size groups of males and females (1.5, 2.5 and 3.5 cm length) before the experiment and were allowed to predate on the mosquito larvae under four degrees of salinity (0, 5%, 10% and 15%). The number of *Culex* spp larvae consumed by *G. affinis* was recorded through, at an interval of 2, for 24 hours.

Type of Water:

Three types of water were used (pond water, tap water and distilled water). Twenty *Culex* spp. 4th instar larvae were added to plastic containers (10 cm x 10 cm x 10 cm) with 500 ml of each water type. *G. affinis* at the size of 1.5, 2.5 and 3.5 cm length were allowed to predate on the mosquito larvae under the three types of water (pond water, tap water and distilled water). The number of *Culex* spp. Larvae, which were consumed by *G. affinis* was counted through one day, at an interval of 2 hours.

Prey Density:

For comparison with individual predation rates, schools of 2 fish (males and

females) (measures 1.5, 2.5 and 3.5 cm) consumption at prey density 20,40 and 60 individuals mosquito larvae were tested under the same conditions (25°C) as individual tests using twenty larvae of 4th instars of *Culex* spp.

Each experiment was replicated 3 times using different fishes in each trial. The number of larvae consumed per fish per school was then calculated based on the assumption that individuals within each school consumed equal numbers of larvae.

Statistical Analysis:

Data were subjected to the Analysis of variance (ANOVA) and means were compared by the least significant difference (LSD) test at a 5% probability level (Steel & Torrie, 1984).

RESULTS AND DISCUSSION

Salinity Levels:

Figure 1 represents the effect of three salinity levels (5, 10 and 15%) on the predation efficiency of both males and females of *G. affinis* on the 4th instar larvae of *culex* mosquito under laboratory conditions. Results revealed that salinity at a level of 15% was unfavorable for fish since all individual died. For all fish sizes (1.5, 2.5 and 3.5 cm), the predation efficiency of both males and females was significantly higher at a salinity level of 5% than that at 10% level. The average number of mosquito larvae consumed for one male fish at 5% level was 19.0, 19.0 and 17.33 larvae/fish/day for 1.5, 2.5 and 3.5 cm fish size, respectively, while for females were 18.67, 20.0 and 19.33 larvae/fish/day. The consumption of fish male was higher than female at 10% salinity level and the reverse was observed at 5% level. In general, the salinity level of water significantly affects the predation efficiency of *G. affinis* against mosquito larvae. The optimum level was 5% and increasing salinity level showed a negative effect on the predation efficiency of *G. affinis*. *Gambusia* is tolerant of a wide range of salinity from very low salinity freshwater to fully marine conditions (Arthington and Lloyd, 1989). It occurs in power plant cooling ponds with salinity as high as 15 ppt (Coykendall, 1980 and Meisch, 1985). However, many publications confirmed that a high level of salinity effect *G. affinis*, Al-Daham and Bhattit (1977) found that all *Gambusia* died within 24 h after transfer to 31 ppt saltwater. Also, Nordlie and Mirandi (1996) found that 60% of *Gambusia* fish survived at salinity 10 ppt, and decreased to 37% at 12 ppt. Survival of fish was 0% at salinity levels of 24 and 28 ppt.The survival of *Gambusia* in water with relatively high salinity is increased if the fish are subjected to slow increases in salinity, which allows for gradual adaptation to the new conditions (Chervinski, 2006).

On other species of indigenous larvivorous fish *Macropalus cupanus*, Jacob *et al*. (1983) found that salinity up to 12.83% do not affect predation rate, except that owing to stress conditions. predation efficiency at a salinity of 0.12%, 7.38% and 14.52% were 29.0-6.6, 27.64-6.2 and 27.04-6.3 larvae per individual, respectively. However, predation at 21.83% (26.2-5.9 larvae/fish) was significantly lower than that recorded at 0.1%.

Type of Water.

Figure 2 shows the effect of water used for mosquito breeding on the efficiency of *G. affinis* for predatory mosquito larvae. The predation efficiency of *Gambusia* was significantly high when distilled water was used. The average number of mosquito larvae consumed by one male sized 1.5, 2.5 and 3.5 cm length was 18.00, 15.67 and 17.33 larvae/fish/day, respectively, while for females were 16.00, 17.00 and 18.33 larvae/fish/day. No significant differences in consumption by the male when tap water or pond water was used, but for females, the consumption was significantly higher in pond water than that of tap water. Generally, the distilled water was more favorable for

Gambusia predation. On the other hand, the predation efficiency of females was significantly higher than males for all types of water.

The effect of water source on the survival of *Gambusia* was studied by Strearns and Sago (1980) who found that *G. affinis* grew more slowly in freshwater rather than in brackish (10 ppt) water. However, Zimmerer (1983) obtained the opposite result for *Gambusia holbrooki* in salinity as high as 15 ppt.

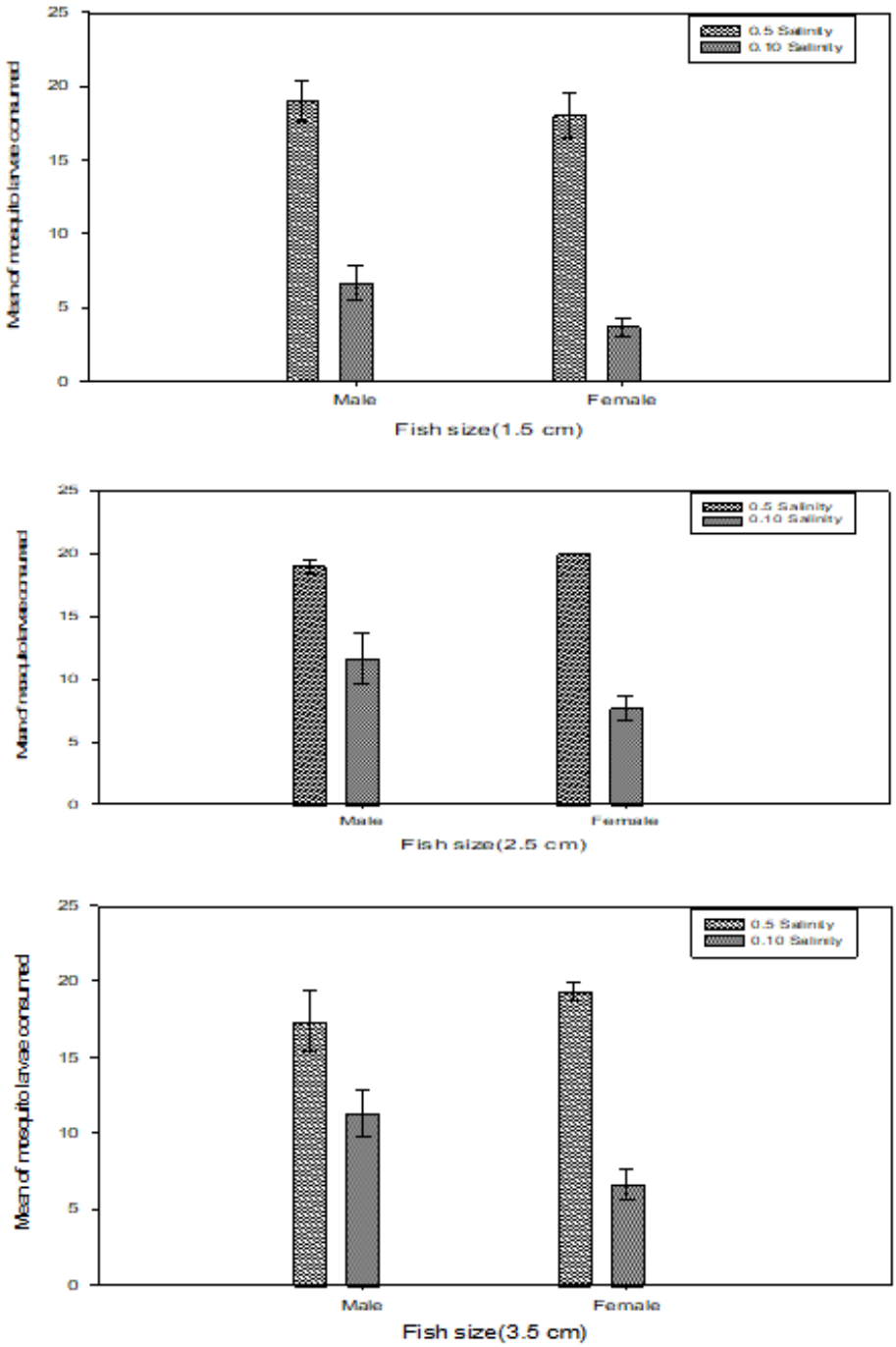

Fig. 1: Effect of salinity levels on the predation efficiency of *G. affinis* on 4th instar larvae of *C. pipiens* under laboratory conditions.

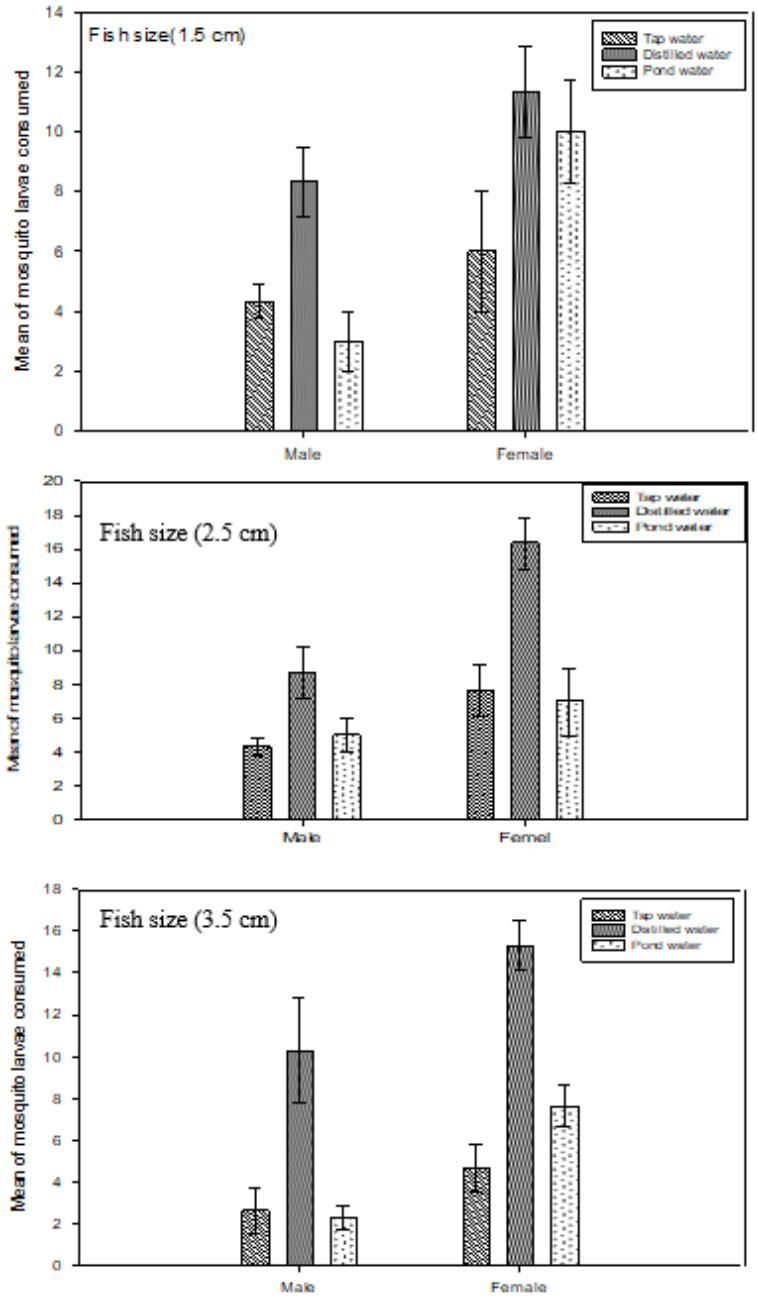

Fig. 2: Effect of water source on the predation efficiency of *G. affinis* on 4th instar larvae of *C. pipiens* under laboratory conditions

Prey Density:

Figure 3 shows the effect of larval density of mosquito on the predation efficiency of *G. affinis*. For males, the average number of mosquito larvae consumed by one fish was significantly higher when 60 mosquito larvae used, than that when 20 or 40 larvae used, regardless of fish sizes. The average number of mosquito larvae consumed was 40.33, 43.67 and 40.00 larvae/fish/day for fish sized 1.5, 2.5, 3.5 cm, respectively. However, the consumption at prey density of 20 and 40 individuals was comparable with no significant differences. Results of fish female took the same trend of male, but the consumption was significantly high. At the prey density of 60 larvae, the average number of mosquito larvae consumed was 49.33, 40.67 and 48.33 larvae/fish/day, for fish sized 1.5, 2.5 and 3.5 cm, respectively. In general, the prey density significantly affected the predation efficiency.

The density of mosquito larvae of 60/200 ml was more favorable for *Gambusia* predation, whereas densities below or above 60 larvae were less favorable. No literature was available on the effect of prey density on the predation efficiency of fish on mosquito larvae. However, the effect of fish density was studied, Stearns (1983) found that female of *Gambusia* subjected to crowding in the first 10 days of life reached reproductive maturity at about 80 days, whereas those kept on their own took 40 days. Also, Zulian *et al*. (1993) found that both age at maturity and body length at maturity were higher for males grown in groups compared with males grown individually. On *Aedes* sp. Larvae, Sanyal and Ghosh (2014) found that in simple habitat and after one hour one male consumed 9 larvae, 2 males consumed 13 larvae, 4 males consumed 25 larvae, while in complex habitat 1 male consumed 35 larvae, 2 males consumed 40 larvae, and 4 males consumed 45 larvae.

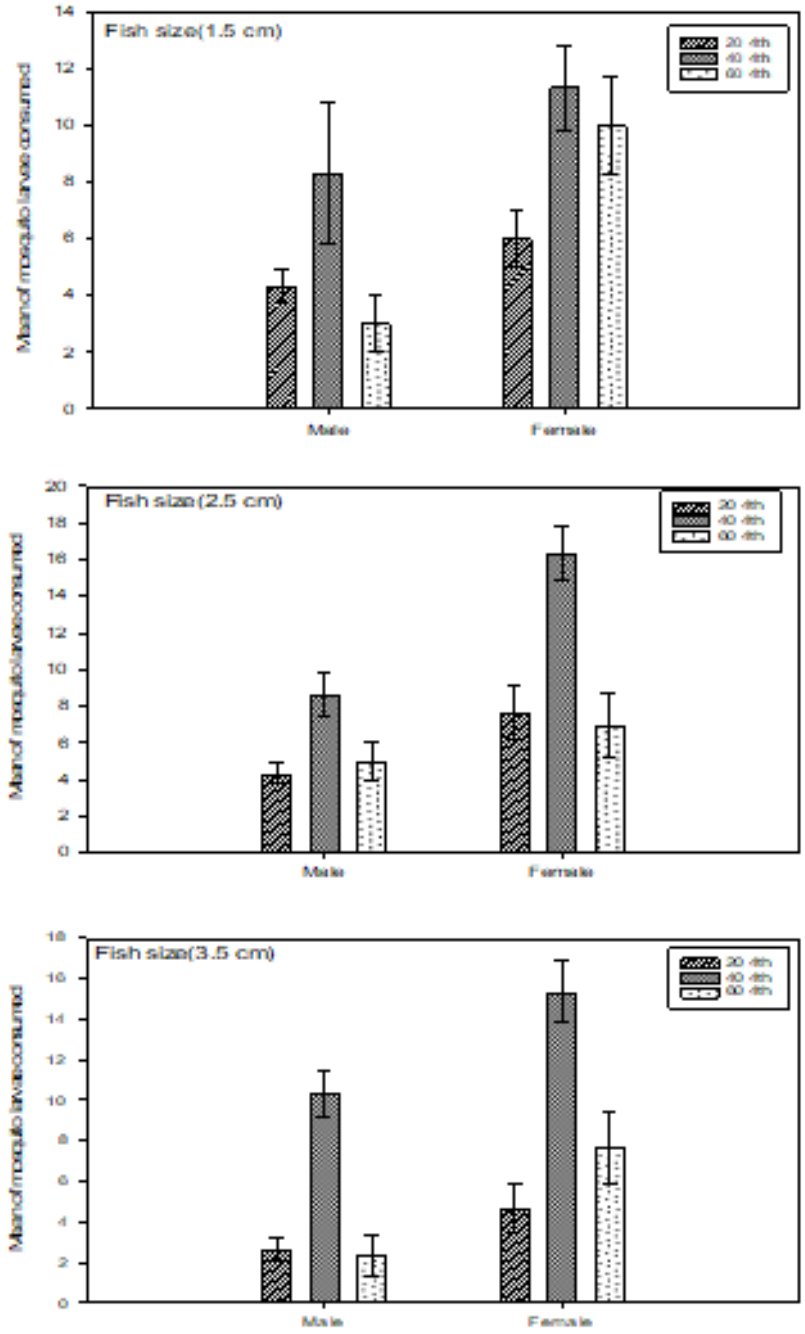

Fig. 3: Effect of mosquito numbers on the predation efficiency of *G. affinis* on 4th instar larvae of *C. pipiens* under laboratory conditions.

In conclusion, the predation efficiency of *G. affinis* is dependent on many measurable variables of the prey-predator ecosystem including salinity level, types of water (pond water, tap water and distilled water) and density of prey (20, 40 and 60 larvae/200 ml). Thus, this study on *G. affinis* proved the efficiency of the fish holds a good promise as an effective biological agent to lower the population of mosquito larvae. These results are recommended to be taken in biological control programs of mosquito larvae.

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