Egyptian Journal of Biological Pest Control, 25(2), 2015, 351-354

Laboratory Evaluation of the Biological Control of the Snail, *Biomphalaria pfeifferi*, the Intermediate Host of *Schistosoma mansoni*, Using the Fish, *Gambusia affinis*

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(*Received: June 22, 2015 and Accepted: August 22, 2015*)

ABSTRACT

The fish *Gambusia affinis* (Baird and Girard, 1853) was used under laboratory conditions to evaluate its efficacy as a predator against different stages of the snail, *Biomphalaria pfeifferi*, the intermediate host of human *Schistosoma mansoni*. Results showed that *G. affinis* was a voracious predator towards egg-masses and neonate snails with a shell diameter length (SDL) of up to 3.5mm, as well as of adult snails with a SDL greater than 3.5mm. The results showed also that *G. affinis* had a significant impact on *B. pfeifferi* snail populations. However, long term studies under natural field conditions are needed to support these results.

Key words: Biological control, *Biomphalaria pfeifferi, Gambusia affinis,* schistosomiasis.

INTRODUCTION

Schistosomiasis (commonly known as bilharzia) is a prevalent disease in developing countries in Africa, Asia and South America, which has a huge impact on public health and socio-economic development (King *et al.*, 2005 and Hodges *et al.*, 2012). Eliminating or decreasing the intermediate host species of schistosomiasis is an appropriate method for controlling infections with the disease and this can be accomplished by using chemical molluscicides, biological control agents and environmental management (WHO, 1993). However, efforts to control snail populations in the past through the use of chemicals, or through alteration of their habitats have resulted in environmental pollution and damage (McCullough, 1992 and Spear *et al.*, 2006). Therefore, utilizing locally available biological control agents seems to be a relatively safe and more economical approach.

Among the potential biological control agents, many invertebrate and vertebrate predatory species have been evaluated, including belostomatid bugs, sciomyzid flies, leeches, crabs, crayfish, and some species of fish (Alexander and Covich, 1991; Ledford and Kelly, 2006; El Bardicy *et al.*, 2009; Khalil and Sleem, 2011 and Sulieman *et al.*, 2013). In addition, a number of fish species such as *Gambusia affinis* (Baird and Girard, 1853), *Tilapia melanopleura* (Dumeril, 1861), *Astronotus ocellatus* (Agassiz, 1831) and *Mylopharyngodon piceus* (Richardson, 1864) have also been used to control snails (Acra *et al.*, 1986; Feitosa and Milward, 1986 and Ben-Ami and Heller, 2001).

G. affinis, commonly known as the mosquitofish, is a small (standard length: 40mm), viviparous freshwater fish native to south-eastern North America that has been widely introduced into fresh and brackish waters around the world (Pyke, 2008). *Gambusia* fishes are opportunistic omnivores, eating mainly small invertebrates, small fishes and algae (Leyse *et al.*, 2004). This fish is adaptable and hardy, inhabit warm, fresh and brackish waters at low elevations and it can withstand environmental conditions, such as high temperatures and low levels of oxygen (Nordlie, 2006).

The objective of the present study was to investigate the efficacy of the mosquitofish, *G. affinis,* as a potential biological control agent against different stages of the snail, *Biomphalaria pfeifferi* (Krauss, 1848), the intermediate host of human *Schistosoma mansoni* (Sambon, 1907), under laboratory conditions.

MATERIALS AND METHODS

Fish and snail samples

The *G. affinis* fish used in this study, with a mean body length of 45.2mm, was collected from the natural farms of the Malaria Control Program, Khartoum State, Sudan. The fishes were transported to the Schistosomiasis Research Laboratory at the University of Khartoum, Sudan and maintained in groups of 10 fishes, in 30 x 20 x 20cm aquaria, each containing six liters of dechlorinated tap water, maintained at 25-27°C and equipped with an aerator. All the fishes were fed daily on goldfish-specific food.

Altogether three stages of development of laboratory bred, *B. pfeifferi* snails were used in the experiments. Two of these were classified into groups, based on their shell diameter length (SDL): group (1), neonates had an SDL of up to 3.5mm, while group (2), adults had an SDL greater than 3.5mm. In addition a third group, group (3) used in the experiments was the egg-mass of the snail.

Experimental design

Four sets of experiments were designed for assessment of the effectiveness of the proposed bio-control agent, *G. affinis*, against the three groups (egg-masses, neonates and adults) of *B. pfeifferi*. The first three sets consisted of eight aquaria (four control aquaria and four experimental for each stage). While the fourth set, made for the combination between all stages and consisting of six aquaria (three controls and three experimental). In the first set, 34 egg-masses (300±2 eggs) of the snail were placed in each aquarium of the set, 50 neonate snails were placed in each aquarium in the second set and in the third set, 40 adult snails were placed in each aquarium. Two *G. affinis* fishes were added to each of the 12 experimental aquaria with no fish being placed in the12 control aquaria. Frequency of the experimental agents used based on their observed field frequency. For the combination set, each aquarium was stocked with 34 egg-masses, 50 neonates and 40 adult snails, thereafter, six *G. affinis* fishes were added to the experimental aquaria with none being added to the control aquaria.

In each experimental set, number of: remaining eggs, or surviving neonates or adult snails were counted every four days, for eight such intervals. The water was changed after each periodical counting. The water was sieved with a small meshed sieve to avoid washing-out the target organisms. Snail food (fresh lettuce plant) was added to each experimental aquarium when needed.

Data analysis

Data analysis was performed using independent sample t-tests. The SPSS 16.0 statistical software, was used to conduct the data analysis and values were considered significant when $P < 0.05$.

RESULTS AND DISCUSSION

Results showed that the egg-masses of the *B. pfeifferi* snail, were completely consumed by the *G. affinis* fish in the experimental aquria by the end of the second period of intervention (Table 1) and the consumption values were significantly different when compared to the corresponding controls ($t = 17.1$, df $= 62$, $P < 0.001$). This finding can be explained by the eggs of the snail being soft since they lack a protective shell and are therefore, easily swallowed and consumed by the fish. This finding revealed the ability of this fish to consume soft prey items. Previously, it has been reported that *G. affinis* preferred to consume the egg-masses and juveniles of the snail, *Bulinus truncatus* which grows up to a size of 2mm (Acra *et al.*, 1986).

Further, the neonate snails with an SDL of up to 3.5mm were completely consumed by the end of the fourth period of intervention (Table 1), with a significant difference when compared to the control groups ($t = 21.4$, df $= 62$, $P < 0.001$). Thus, prey size played a key role in determining their vulnerability, as neonate snails were much more vulnerable to the proposed bio-control agent, *G. affinis*, than were larger ones. This might be due to that the shells of the neonate snails being relatively thin and easier to break. Previously, it has been reported that smaller snails were easier prey and can be attacked and crushed by predators such as species of crayfish (Ibrahim *et al.*, 1995 and Reynolds and Donhoe, 2001).

In the present study, adult snails with an SDL greater than 3.5mm, were reduced to their lowest number by the end of the last period of intervention (Table 1), with a significant difference compared with the control groups ($t = 6.4$, df $= 62$, $P < 0.05$). Previously, it has been reported that, *G. affinis* is able to consume the flesh of the snail *Bulinus truncatus* at size 3 to 6mm in absence of other food material (Acra *et al.*, 1986). However, it has been reported that snail species with thicker shells are often less susceptible to predators and strategies behind the choice of snails as prey by predators may be influenced by the presence or absence of an operculum, prey movement and speed as well as the hunger level of the predators (Preston *et al.*, 1996 and Krist, 2002).

Table (1): Mean number of different stages of *B. pfeifferi* remaining after predation by *G. affinis* in different experimental sets comparing with control

Intervals	*B. pfeifferi* stages remained after predation by *G. affinis*					
	Eggs (n = 300 each)		Neonates (n = 50 each)		Adults (n = 40 each)	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
1st (4 days)	294±3.5	196 ± 5.2	48.3±0.5	24.3 ± 4.0	39.5±0.6	34.0 ± 2.9
2nd (8 days)	282±6.6	118 ± 3.1	45.5±1.3	11.0 ± 4.1	39.0±0.0	31.3 ± 2.2
3rd (12 days)	269±4.2	0.00	42.8±0.5	6.5 ± 4.7	37.5±0.6	29.0 ± 2.7
4th (16 days)	266±2.2	0.00	40.8±0.5	0.8 ± 1.5	37.0±0.8	26.5 ± 6.5
5th (20 days)	262±3.3	0.00	39.5±0.6	0.00	34.3±0.5	15.8 ± 1.1
6th (24 days)	258±2.6	0.00	38.8±0.5	0.00	32.8±1.7	4.8 ± 4.5
7th (28 days)	254±0.8	0.00	38.5±1.0	0.00	30.0±0.8	2.0 ± 2.8
8th (32 days)	251±0.9	0.00	37.0±0.0	0.00	27.0±0.8	0.3±0.5

Table (2): Mean number of different stages of *B. pfeifferi* remaining after predation by *G. affinis* in the combination experimental sets comparing with control

Intervals	*B. pfeifferi* stages remained after predation by *G. affinis*					
	Eggs (n = 300 each)		Neonates (n = 50 each)		Adults (n = 40 each)	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
1st (4 days)	289±4.5	40.3±2.7	48.0±1.0	2.3±0.6	39.3±0.6	32.3±2.1
2nd (8 days)	277±6.1	8.3±7.4	46.0±1.0	0.00	39.0±0.0	30.7±1.5
3rd (12 days)	266±4.9	0.00	42.3±1.2	0.00	37.7±0.6	27.0±1.0
4th (16 days)	265±0.6	0.00	40.7±0.6	0.00	36.7±1.2	23.0±1.0
5th (20 days)	260±2.5	0.00	39.3±1.2	0.00	34.3±0.6	13.0±7.5
6th (24 days)	254±0.6	0.00	38.3±0.6	0.00	33.3±1.5	3.3±4.2
7th (28 days)	252±2.1	0.00	36.3±1.2	0.00	31.7±1.5	2.0±3.5
8th (32 days)	190±1.1	0.00	29.3±2.5	0.00	30.7±0.6	0.3±0.6

In the combination sets, the results confirmed that *G. affinis* preferred to consume the soft eggs ($t = 52.1$, df = 46, $P < 0.001$) and neonate snails ($t = 37.9$, df = 46, $P < 0.001$) first (Table 2) and only when these had all been consumed did they turn to attack the larger snails ($t = 6.8$, df = 46, $P < 0.05$). However based on the present results, it is important to point out that the fish *G. affinis* is small and is able to live successfully even in small waterways with dense vegetation, where the schistosome-bearing snails are able to live and breed.

In conclusion, the present laboratory results showed that the fish *G. affinis* has a significant impact on populations of the snail, *B. pfeifferi*. However, long term studies under natural field conditions are needed to support these results.

ACKNOWLEDGMENTS

The authors thank the staff of the Schistosomiasis Research Laboratory, University of Khartoum, Sudan for their assistance. Also, they thank the authority of the Malaria Control Program, Khartoum State for providing the *Gambusia* fish used in this study.

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