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Sphaerodema urinator Duforas (Hemiptera: Belostomatidae) as a Predator of *Fasciola* Intermediate Host, *Lymnaea natalensis* Krauss

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ABSTRACT

The water bug *Sphaerodema urinator* Duforas (Hemiptera: Belostomatidae), inhabits lakes, ponds, marches and rice fields, was found to predate on a wide range of other aquatic organisms like insects and snails. To evaluate the predatory potential on the *Fasciola* intermediate host, *Lymnaea natalensis* Krauss snail, laboratory studies involved searching; encounter, consumption and prey preference were achieved. Obtained results indicated that the searching time of the predator ranged between 3.4±0.5 to 7.8±0.9 min and handling time ranged between 18.8±1.01 to 25±1.0 min, depending on the snail size and the predator vulnerability. The adult water bug could kill and consume small-, medium- and large-sized snails. Number of snails consumed/ day differed according to their size and density. Preference experiments showed that the adult predator prefers small size snails than medium or large ones. This study suggested that the predator, *S. urinator* may be potentially suitable as a bio-control agent against *Fasciola* intermediate host, *L. natalensis.*

Key words: Predator, *Sphaerodema urinator*, *Fasciola*, intermediate host, snail.

INTRODUCTION

Some of the world's most serious epidemics are caused by liver flukes, whose intermediate hosts are freshwater snails. The liver fluke, *Fasciola gigantica* Cobbold 1855, is an economically important parasite that infects a wide range of livestock species (Charlier *et al.*, 2009). The snail *Lymnaea natalensis* Krauss 1848 functions is an obligatory intermediate host of *Fasciola* infection (Mungube *et al.*, 2006 and Morely, 2007). Liver flukes can cause huge losses to livestock industries and affect the health of humans where fascioliasis is an important human disease (Walker *et al.*, 2011 and Fürst *et al.*, 2012). In their general distribution, freshwater pulmonate snails are benthic animals living in the shallower water of lakes, ponds, marches, rivers, streams and ditches (Rondelaud *et al.*, 2011 and Fu & Meyer-Rochow, 2012).

Snail control strategies are considered a priority for the reduction of transmission. Synthetic molluscicides (Niclosamide) have been widely used as a chemical control method (Jaiswal and Singh, 2008), although they induce only temporary reduction snail density. The biological methods, especially those involving the use of indigenous predators as natural enemies, were traditionally perceived as environmentally friendly and have been the foci of research and management of these freshwater snails (Suhardono *et al.*, 2006 and Younes *et al.*, 2015).

In view of this, the present study aimed to evaluate predation potential of the adult water bug, *Sphaerodema urinator* Duforas 1863 (Hemiptera - Belostomatidae) which lives in the water's surface. As well, searching behavior of the predator towards the snail sizes was also measured.

MATERIALS AND METHODS

Insect collected

S. urinator adults were collected from ponds and lakes at Abou-Rawash area, Giza governorate, Egypt. Sampling was performed with a dip net (1-mm mesh), approximately biweekly. The net was calmly pressed into the water, the plants were shaken and some of them were pulled by the net because water bugs are associated with vegetation in nature. The collected samples were kept separately in plastic jars provided with pond water. In the laboratory, insects were released in glass aquaria (50 × 30 × 20 cm in length, width and height, respectively). The aquarium was filled by pond water up to 15 cm, to provide the resting site for insects where few branches of the collected aquatic plants (*Ceratophyllum demersum* or *Eloden* sp.) were kept in the aquarium. The snails, *L. natalensis* of various sizes were regularly supplied as food for insects. Dead snails and insects were removed from the aquarium regularly and water was changed every 2-3 days to provide a proper living conditions.

Snails collecting and rearing

Individuals of the experimental snail, *L. natalensis,* collected from lakes and ponds in the Abou-Rawash area, Giza Governorate, Egypt were kept in glass aquaria (50 × 30 × 20 cm) filled with pond water up to 15 cm of height (10 snails / liter) for a period of one week prior to the start of the experiment. The snails culture began with the individuals collected from the field. After being washed thoroughly, the snails were confined into the aquaria. Parts of aquatic plants for aeration and small pieces of polyethylene sheets as sites for deposition of snail egg-masses were added to the aquaria. The plants were previously washed thoroughly by tap water and left into aquaria containing water only for one month before introducing to the rearing aquaria to avoid snail egg-masses that may be associated with it. For feeding, the snails were provided by fresh lettuce leaves as a basic food, dried lettuce was provided when the green was not available. For obtaining dried lettuce leaves, the leaves were washed thoroughly under tap water, boiled in water for 2-3 minutes, picked up to be spread on a white card-board to be dried, crushed and then offered to snails in little amount. Fish food (Tetramin®) and blue green algae (*Nostoc muscorum*) were used as an additional food source for newly hatched and juvenile snails. Deposited egg-masses were removed by a smooth brush or by a forceps, washed thoroughly under tap water into a small strainer and then placed into Petri-dishes to be examined by stereomicroscope. Re-washing should be carried out if the microscopic examination showed any other attached organisms. After being completely free of microorganisms, egg masses were placed into small covered plastic aquaria containing pond water and dried lettuce or blue green algae (*N. muscorm*). Additionally, some water plants (*C. demersum*, *Lemna gibba* or *Elodea* sp.) were placed in the aquarium to simulate natural conditions. Small-, medium- and large-sized snails measuring 2-5, 6-9 and 10-13 mm in shell height, respectively, were used in the experiments.

Experimental procedures

Ten glass aquaria, 5 l in total volume, containing 3 l of pond water were used in each experiment. Among these, each containing a predator and experimental snails, the experimental group was comprised of five glass aquaria containing snails only. The remaining five glass aquaria constituted the control with only snails. The aquaria were covered with nylon net to prevent snail escape. Snails that may leave the water and sit on the aquarium wall were not considered and deleted from the count. The snails were allowed to acclimatize for 1 hour before introducing the predator. Snails and predators were used only once in the experiments. All experiments were carried out in the laboratory at temperature of 25±2ºC and 60-70% R.H. Fluorescent tubes (10 cm long, 32 watt) were placed 100 cm above the tanks to provide a photo period of L12: D12.

1. Searching and handling times

Predator and prey behaviors were observed during a continuous 60-min period. Foraging behaviors (searching and handling time) for both starved and satiated predators were quantified. Handling time per prey was calculated as the total time taken to manipulate a single prey item, from encounter to the end of consumption. Encounters between predators and preys and the outcomes of the encounters were also quantified. The encounter rate was calculated as the total number of encounters divided by predator search time. Encounters with prey could result in attacking, avoidance or consumption of prey. Each trial involved introducing an individual predator into the experimental glass aquaria filled with clear pond water (to facilitate observation) and containing 10 live snails of one of the three different snail's sizes.

2. Effect of prey density on consumption and predation rate

For each predator, prey snails (small, medium or large) were supplied at densities of 5, 10, 15, 20 or 25 snails. Predators were allowed to prey for a period of 24 h. Five replicates for each prey density were performed to determine the mean number of preys consumed/day and subsequently the predation rate.

3. Prey preference

Two sets of experiments; in the first set (Dual-choice preference) preference experiments with two-prey sized combination and with all snail sized combination were achieved, each individual of predator was supplied with two different prey sized snails in combination *i.e.* (small & medium), (small & large) and (medium & large). Starting density of each prey sized snails was ten individuals with a total of twenty snails/ predator. In the second set of experiments (Three-choice preference), preference was examined when all the three snail sizes (small-, medium- and large) were offered. In this set, each predator individual was supplied with thirty preys (ten from each snail size) and after 24 h and number of consumed preys was recorded separately.

Prey preference was subjected to the analysis of selectivity, following Rehage *et al.* (2005) as equivalent to Manly's index:

$$S_i = W_i \,/ \sum_{j=1}^{m} W_j$$

Where: S_i = equivalent to Manly's index for prey type (i); W_i = proportion of prey (i) consumed at the end of the experiment relative to the original input. $\sum W_j$ = total proportion of all prey types consumed (i= 1, 2,…m); and m = number of prey type. Manly's index can take value between zero and one, and the values of different prey types always are sum up to one. In case of dual-choice combination, the threshold value is 0.50, while in case of three-choice combination, the threshold value is 0.33 (S_i = $^1/_m$). Values higher than the threshold value are indicated selectivity against it.

Data analysis

Data considering searching, handling times, foraging behavior, prey consumed and predation rate were expressed as mean ± SE. Comparison among three or more different groups was analyzed using one-way ANOVA. Regression analysis was carried to estimate the relation between the prey density and predation rate. Data were analyzed using Graph Pad In Stat software (Graph Pad In Stat, 2009).

RESULTS AND DISCUSSION

Searching and handling times

Adults (♂ or ♀) of *S. urinator* showed clear differences in searching and handling times towards the three-prey sizes (Table 1). Data obtained showed that the adult required more time in searching for the small- and medium-sized snails as compared to the large-sized ones. Maximum searching time (7.8±0.9 min) was observed towards small-sized snail in case of satiated adults, whereas the minimum searching time (3.4±0.5 min) was obtained with the large-sized snails for starved adults. Statistically, insignificant differences (P < 0.05) were obtained considering the searching times of *S. urinator* adult towards small-, medium- and large- sized snails. Significant differences (P < 0.05) were obtained in handling time of the predator adult towards the small size and the two other sizes of the snail. Handling time of the starved predators towards the snails was (18.8±1.01), (23.4±1.1) and (25±1.0) min for the small-, medium- and large-size snail, respectively. A similar relationship was also obtained with the handling time of the satiated predator. Comparing searching time or handling time of starved and satiated predators showed significant difference (P < 0.05) in both searching and handling times. Starved predators required shorter period for attacking (searching) and handling the preys in comparison to the satiated individuals. Searching and handling times are major factors in the determination of the functional response of the predator to its prey and should predict how the predators behave when different prey species are presented (Turner, 2008). Predator searching efficiency is another important determinant of the predation rate, and it should decline with increasing use of refuges by prey, otherwise density dependent predation at low prey density may not occur (Hassell, 1978).

Encounter between the predator, *S. urinator* and the prey, *L. natalensis*

Data in table (2) and figure (1) show the encounter behavior of the predator, *S. urinator* adult toward the prey snails. Most observed encounters with snails ended in avoiding the preys before preying or attacking them. Maximum number of encounters (65±3.53) was found with the large snail, followed by the medium (51.2±2.1), whereas the number of encounters recorded by the predator toward the small snail was the lowest (43.6±2.7). The encounter as well as the encounter rate (no. / min.) significantly differed (P < 0.05) when the large snail was compared to the other two sizes. Also, a significant difference (P < 0.05) was obtained for the prey consumed / encounter. The predator, *S. urinator* adult consumed more small-sized snail than the medium-ones. Average number of prey consumed/ encounter was (0.11±0.01) and (0.02±0.01) considering small- and medium-sizes, respectively. On the other hand, no large-sized snail were consumed, where, the number of preying/ encounter was zero. In contrast, insignificant differences (P > 0.05) were obtained for the number of attacks/ encounters and the number of avoidances/ encounters, considering small-, medium- and large- sized snail. The nature of interactions between predators and their prey are important because they determine: which prey is sought by predators, how prey are captured and processed by the

(a) (b)

Fig. (1): Foraging behavior of *S. urinator* adult towards the freshwater snail, *L. natalensis*; (a) Encounters. (b) Devour.

Table (1): Searching and handling times of the predator, *S. urinator* adult towards *L. natalensis* snail

Behavior	Adult condition	Snail size			
		Small	Medium	Large	P-value
Searching time (min)	Starved	5 ± 0.7^{a}	4 ± 0.7^{a}	3.4 ± 0.5^{a}	0.251
	Satiated	$7.8\pm0.9^{a*}$	$7.4\pm1.2^{a*}$	$6.2\pm0.73^{a*}$	0.477
	p-value	0.0361	0.0374	0.014	
Handling time (min)	Starved	18.8 ± 1.01^{a}	23.4 ± 1.1^{b}	25 ± 1.0^{b}	0.003
	Satiated	$22.2\pm0.92^{a*}$	$28.6\pm1.1^{b*}$	$28.8\pm0.9^{b*}$	0.0007
	p-value	0.0381	0.0092	0.0259	

- Means in the same row, followed by the same letter are not significantly different (P> 0.05).
- * Significant at P <0.05 with starved value.

Table (2): Foraging behavior of *S. urinator* adult towards *L. natalensis* snail

Behavior	Snail size			
	Small	Medium	Large	P-values
No. of encounter	43.6±2.7[a]	51.2±2.1[a]	65±3.53[b]	0.0006
Encounter rate (no. /min)	0.72±0.05[a]	0.85±0.34[a]	1.1±0.06[b]	0.0007
No. of attack/encounter	0.37±0.02[a]	0.42±0.01[a]	0.41±0.05[a]	0.5040
No. of avoided/encounter	0.63±0.02[a]	0.57±0.014[a]	0.58±0.1[a]	0.5508
Preying/encounter	0.11±0.01[a]	0.02±0.01[b]	0±0 [b]	<0.0001

- Means in the same row followed by the same letter are not significantly different (P > 0.05).

Table (3): Effect of snail density on the consumption rate of *S. urinator* adult

Prey size	Daily number of preys consumed (Mean ± SEM) at prey density				
	5	10	15	20	25
Small	3.6±0.4[a]	7.2±0.37[a]	12.2±0.7[a]	16.2±0.8[a]	19.2±1.4[a]
Medium	2.8±0.37[a]	6.4±0.5[a]	10.6±0.5[a]	14.8±0.9[a]	16±1.41[a]
Large	0.6±0.24[b]	1.2±0.37b	1.4±0.5[b]	2.2±0.66[b]	2.4±0.5[b]
P-values	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
F	20.11	58.96	106.17	98	56.84

- Means in the same row followed by the same letter are not significantly different (P > 0.05).

Table (4): Regression analysis of the effect of *L. natalensis* snail density on the predation rate of *S. urinator* adult

Snail size	Slope	(r)	P-value
Small	0.372	0.641	0.244
Medium	0.520	0.591	0.294
Large	-0.116	-0.719	0.170

- Regression analysis based on the mean predation rate (n = 5 sets/prey density).

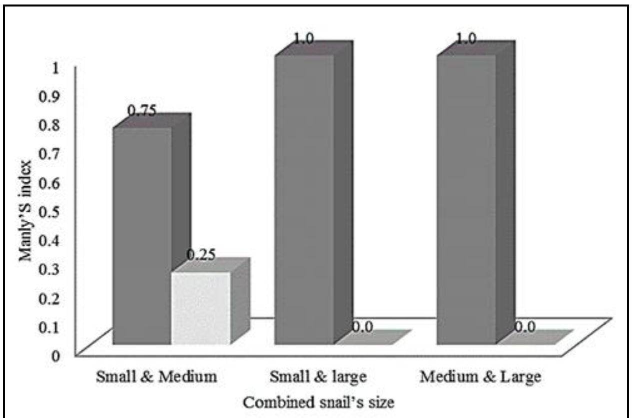

Fig. (2): Mean value of Manly's preference index for *S. urinator*adult towards the freshwater snail, *L. natalensis*(Dual-choice preference).

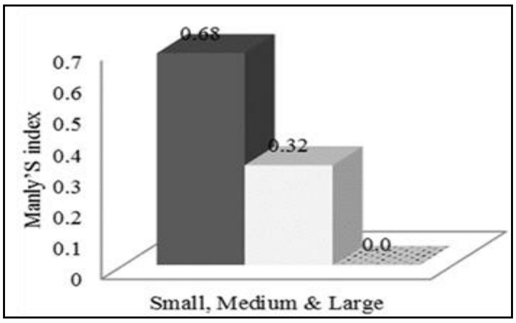

Fig. (3): Mean value of Manly's preference index for *S. urinator*adult towards the freshwater snail, *L. natalensis* (Three-choice preference).

predator, and ultimately, the effect of predation on prey abundance and population dynamics (Taylor, 1984). This study showed that the predator may distinguish between preys of differing profitability and select the more profitable ones. Prey-predator attributes that affect selection involve encounter rate with a particular prey type, probability of attack given in an encounter, probability of capture given an attack and probability of ingestion in a capture (Sih, 1982).

Prey consumption and predation rate

Table (3) shows the effect of prey densities on number of preys consumed considering the prey sizes. Statistically, significant differences (P < 0.05) were obtained between each of the numbers of preys consumed at all densities 5, 10, 15, 20 or 25 when comparing small- and medium-sized snails to the large-sized one. The number of small *L. natalensis* consumed by *S. urinator* adults was always the greatest in comparison to medium and large sizes at all densities. Obtained data showed that at density of 25 preys, one adult could consume a mean of 19.2±1.4, 16.0±1.41 and 2.4±0.5 preys/day considering small-, medium- and large-sized snails, respectively. The predation rate of *S. urinator* adults on *L. natalensis* snails at different densities is shown in table (4). Regression analysis based on the mean predation rate (n=5 sets/ prey density) showed insignificant differences in predation rate of *S. urinator* adults towards small-, medium- and large-sized snail (r = 0.641, 0.591 and -0.719 with P-value = 0.244, 0.294 and 0.170), respectively. Obtained data showed that the predation rate towards large-sized snails was very low and did not exceed 12%, even by increasing the prey density from 5 till 25 preys. On the other hand, the predation rate of the predator towards small and medium sizes was greater. Statistically, insignificant difference was obtained

(P>0.05).The present study reveals that the adult water bug *S. urinator* can consume a quite number of *L. natalensis* snails and thus can significantly reduce the population of them, though the number of small- or medium-sized snails consumed per day was quite higher than that of large-sized ones.

Prey preference

In the choice experiment, *S. urinator* adults consumed more individuals of small-sized than medium- or large-sized *L. natalensis* snails. Obtained preference index showed that it preferred consumption of one prey from the two exposed (Fig. 2). Preference indexes value (Manly's index) were (0.75 & 0.25), (1.0 & 0.0) and (1.0 & 0.0) considering preys offered as (small & medium), (small & large) and (medium & large), respectively. Fig. (3) shows the preference tests of starved *S. urinator* adults when the three snail sizes were offered in combinations. The preference differed clearly among the three-prey sizes. The predator nymph preferred small-sized *L. natalensis* snail than the other sizes. The mean numbers of the prey snails preferred in a descending order were 0.68, 0.32 and 0.0 considering small-, medium- and large-sized *L. natalensis*, respectively, according to Manly's preference index. Preference experiments showed that *S. urinator* adults, in general, preferred small- sized snailscompared to medium- or large- sized ones. Though the number of small- or medium-sized snails consumed per day was quite higher than the large-sized one, it remains a question, about the situation when all prey types will be equally available. Since, from the predator, *S. urinator* view-point, capturing a small snail is an easier job involving less energy to carry from the water bottom to the water surface where the predator begins to feed. Predators in nature often include an array of the prey type; they often select certain prey types over others (Saha *et al.*, 2014). Further, in the presence of predators, small snail individuals tend to crawl above the water more than large individuals and so they become more suitable to predation by *S. urinator*. Successful biological control may depend on use of native predators that share the habitat and are part of the natural food web (Yanoviak, 2001 and Younes, 2008).

In conclusion, obtained results showed that the predator had the ability to search, encounter, attack and devour the examined snails. Determination of the daily prey consumed and the predation rate confirmed the predatory efficacy against *L. natalensis* snail. Further field studies are required to determine the ability of these predators with the other control types in reducing the snail population. The results may provide a primary assessment basis of this predator as biological resource against freshwater snails.

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