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Experimental evaluation of Odonata nymph in the biocontrol of schistosomiasis intermediate hosts

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ABSTRACT

Objective: To evaluate the predatory potential of the Odonata nymph on freshwater snails that serve as intermediate hosts for *Schistosoma* species (*Bulinus truncatus* and *Biomphalaria alexandrina*).

Methods: Observations on the searching, attacking and devouring of the two snail types with series of laboratory-based predation experiments, whose aims were to determine daily predation rate, differential predation, prey preference considering small-, medium- and large-sized snails were conducted.

Results: Laboratory evaluation revealed that, the Odonata nymph could kill and consume the two intermediate hosts. The number of snails consumed differed according to the snail type, size and density. The times taken for searching and handling times were dependent on the snail size, type and satiation of the predator. The predation rate varied also with respect to snail type, size and density. This study also evaluated that Odonata nymphs consumed more *Bulinus truncatus* than *Biomphalaria alexandrina* per unit time, and that there may be a preference for smaller than larger snails.

Conclusions: According to our observation, the predator, *Hemianax ephippiger* nymph may be a suitable biocontrol agent in connection with *Schistosoma* intermediate hosts.

1. Introduction

Schistosomiasis is a parasitic disease estimated to affect more than 200 million people around the world, causing high levels of morbidity and mortality in 74 countries in tropical and subtropical areas [1]. Three main schistosome species infect humans, two of them exist mainly in Africa, *Schistosoma haematobium* and *Schistosoma mansoni*. Freshwater gastropods, *Bulinus truncatus* Audouin 1827 (*B. truncatus*) and *Biomphalaria alexandrina* Ehrenberg 1831 (*B. alexandrina*) are intermediate hosts for *Schistosoma haematobium* and *Schistosoma mansoni*, respectively. Snail control is a major component of any successful schistosomiasis control program [2]. Recently, World Health Organization raised the alarm with a necessary return to snail

control strategies in association with chemotherapy [3]. Snail control strategies are considered as a priority for the reduction of transmission. Synthetic molluscicides (niclosamide) have been widely used [4] although chemical control gives only a temporary reduction. Biological control of snails through use of micropathogens, predators, parasites and competitors has been considered as an alternate method to the use of chemical molluscicides [5]. Biological methods, especially those involving the use of indigenous predators, are traditionally perceived as environmentally friendly and have been the foci of research and management of these hosts [6]. Predators in nature often include an array of prey types in their diet. Further, in the presence of multiple prey types, they often select certain prey types over others [7]. Predation is a major force affecting species abundance, population dynamics and community structure [8].

Dragonflies are ideal predators of many insect pests and have an important role in biological control in various ecosystems. Dragonfly nymphs, in particular, are potential biocontrol agents of mosquitoes and are looked upon as important predators of various macro invertebrates in aquatic systems [9,10]. Potential of the nymph of *Hemianax ephippiger* Burmeister, 1839 (Odonata: Aeshnidae) (*H. ephippiger*) as predator of the snail *Lymnaea*

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natalensis an intermediate host of *Fasciola gigantica* was demonstrated [11]. In view of these facts, the present study was aimed at evaluating the predation potential of the dragonfly *H. ephippiger* nymph towards the freshwater snails which are intermediate host for *Schistosoma* species. The preference behavior of the predator towards the snails was also detected. The results of the present study provide a primary assessment of the potential of this predator to control freshwater snails.

2. Materials and methods

2.1. Collection of Odonata nymphs

H. ephippiger nymphs were collected from ponds and lakes in Giza Governorate, Egypt during summer season. They were kept in glass aquaria (50, 30 and 20 cm in length, width and height, respectively), fed daily to satiation on different sizes of the freshwater snails. Fully grown nymphs (the last two instars) with sizes ranging from 3.2 to 4.5 cm in length were used in the experiments. They were starved for a period of 24 h before the share of the experiments.

2.2. Collection of snails

The experimental snails, *B. truncatus* and *B. alexandrina*, were collected from lakes and ponds in Giza Governorate, Egypt. They were kept in a glass aquarium (50, 30 and 20 cm in length, width and height, respectively), filled with pond water up to 15 cm of height for a period of one week prior to the start of the experiment. The snails were provided with fresh or dried lettuce leaves as basic food. Fish food (Tetramin®) and blue green algae, *Nostoc muscorum* (Nostocales, Nostocaceae) were used as an additional food source for newly hatched and juvenile snails. Only laboratory-bred snails were used in experiments as prey for the nymphs. Additionally, some water plants, *Ceratophyllum demersum* (Ceratophyllales, Ceratophyllaceae) and *Elodea* sp. (Alismatales, Hydrocharitaceae) grows in still or very slow-moving water in lakes, ponds and quiet stream in Giza Governorate, Egypt were collected in summer season. The collected plants were placed in the aquaria to simulate natural conditions. Small-, medium- and large-sized snails measuring 2–5, 6–9 and 10–13 mm in shell height or width for *B. truncatus* and *B. alexandrina*, respectively, were used in the experiments.

2.3. Experimental methods

Ten glass aquaria, each 5 L in volume, containing 3 L of pond water were used in each experiment. Among these, the experimental group was comprised of five glass aquaria, each containing a predator and experimental snails. The remaining five glass aquaria constituted the control with only snails. The aquaria were covered with a nylon mosquito net to prevent possible 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 h before introducing the predator. Only separately experiments (one of the two snail species was offered to the predator) were done. Snails and Odonata nymphs were used only once in the experiments. All experiments were carried out at constant temperature of (25 ± 2) °C and 60%–70% relative humidity. Fluorescent

tubes (10 cm long, 32 W) were placed 100 cm above the tanks to provide a photoperiod of light: dark (12:12).

2.4. Searching and handling time

Predator and prey behaviors were quantified during a continuous 60 min period. Foraging behaviors (searching and handling prey) 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. Encounters with prey could result in attacking, avoidance or consumption of prey. Each trial involved introducing an individual nymph into the experimental glass aquaria filled with clear pond water and containing 10 live snails of one of the two different snail species. Each Odonata nymph was tested only once and the large-sized snails were used in determining the searching and handling times.

2.5. Effect of prey density on the consumption and predation rate

One nymph of *H. ephippiger* was placed in each aquarium with small-, medium- or large-sized snails of *B. truncatus* or *B. alexandrina* at densities of 5, 10, 15, 20 or 25 snails for a period of 24 h. Five replicates for each prey density/prey size/prey type were performed to determine the mean number of prey consumed/day and subsequently the predation rate.

2.6. Prey preference

Two sets of experiments were conducted to determine how prey size influenced prey preference. In the first set of experiments (dual-choice preference), each individual predator was supplied with two different prey sized snails from the same prey type, i.e. small and medium, small and large, and medium and large *B. truncatus* or *B. alexandrina* snail. The starting density of each prey sized snail was ten individuals, with a total of twenty snails per 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 within. In this set of experiments, each predator individual was supplied with thirty preys (ten from each snail size). After 24 h, the numbers of snails consumed were recorded separately. Prey preference was subjected to the analysis of selectivity following Rehage *et al.* [12]; an equivalent to Manly's index.

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

where, S_i is equivalent to Manly's index for prey type (i), W_i is proportion of prey (i) consumed at the end of the experiment relative to the original input. $\sum W_j$ is total proportion of all prey types consumed ($i = 1, 2, \dots, m$); and m is number of prey types. Manly's index can take the value between zero and one, and the values of the different prey types always 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 ($S_j = 1/m$). Values higher than the threshold value indicated selectivity preferred.

2.7. Data analysis

Data considering searching, handling times, foraging behavior, prey consumption and predation rate were expressed as mean ± SE. The comparison between three or more different groups was analyzed using One-way ANOVA. The correlation between prey density and predation rate was determined using regression analysis. Contingency table analysis (*Chi-square test*) to compare frequency of encounter outcomes for each snail type is represented. Data were analyzed using GraphPad InStat software (2009). An alpha value of 0.05 was used to determine the significance.

3. Results

3.1. Searching and handling times

The Odonata *H. ephippiger* nymph showed clear differences in searching and handling times towards the two snail species. The Odonata nymph required more time in searching for the *B. alexandrina* snail than the snail, *B. truncatus*. The maximum searching time [(19.20 ± 1.50) min] was obtained towards *B. alexandrina* snail when predator nymphs were satiated while the minimum searching time [(3.80 ± 0.58) min] was obtained with the snail *B. truncatus* snail when the predator nymphs were starved. Searching times significantly differed in both starved and satiated predator individuals comparing the search time of the predator towards all snails. Statistically, a significant difference appeared in comparing the search time of the predator towards *B. truncatus* snail and those of *B. alexandrina*. As for the handling time, significant differences were obtained in the handling time of the predator nymph towards the two snail species. Handling time of the starved predators towards the snails was (14.00 ± 1.40) and (9.80 ± 1.02) min, concerning, *B. truncatus* and *B. alexandrina* snails, respectively. A similar relationship was also obtained with the handling time of the satiated predator. After comparing searching time or handling time of starved and satiated predator, significant differences were obtained in both searching and handling times of the predators towards the two snail types. The satiated predator takes more time in searching and handling towards the two types of snails. These times were (12.80 ± 0.86) and (19.20 ± 1.50) min considering the searching times and were (20.00 ± 1.14) and (14.40 ± 0.93) min considering the handling times of the predators towards *B. truncatus* and *B. alexandrina*, respectively.

3.2. Encounter behaviors between the predator and the preys

Data in Table 1 and Figures 1 and 2 show the encounter behavior of the predator, *H. ephippiger* nymph towards

Table 1

Contingency table of prey encounters outcomes behavior of *H. ephippiger* nymph towards the freshwater snails, *B. truncatus* and *B. alexandrina* (%).

Snail type	Attack	Avoided	Consumed	Total
<i>B. truncatus</i>	81 (77.9)	14 (21.5)	24 (19.5)	119
<i>B. alexandrina</i>	67 (70.0)	27 (19.0)	13 (17.5)	107
Total	148	41	37	226

Values between brackets are expected value; *Chi square* = 8.102; *df* = 2; *P* = 0.0174.

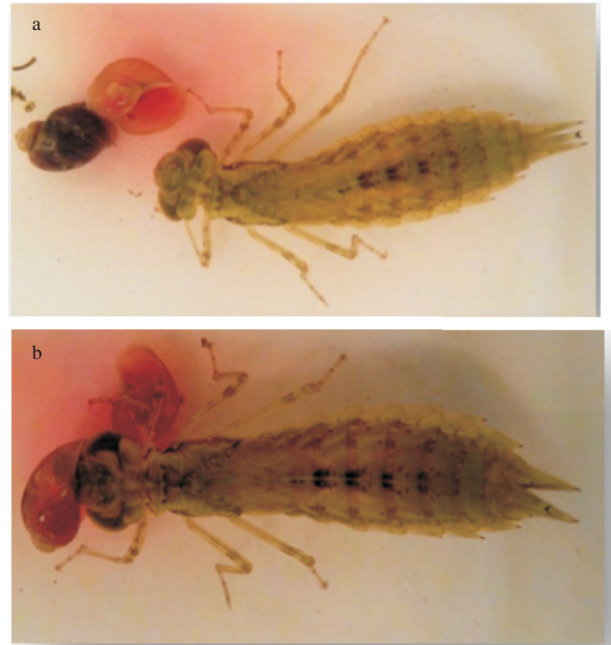


Figure 1. Foraging behavior of the Odonata, *H. ephippiger* nymph towards the freshwater snail, *B. truncatus*. a: Encounters; b: Devour.

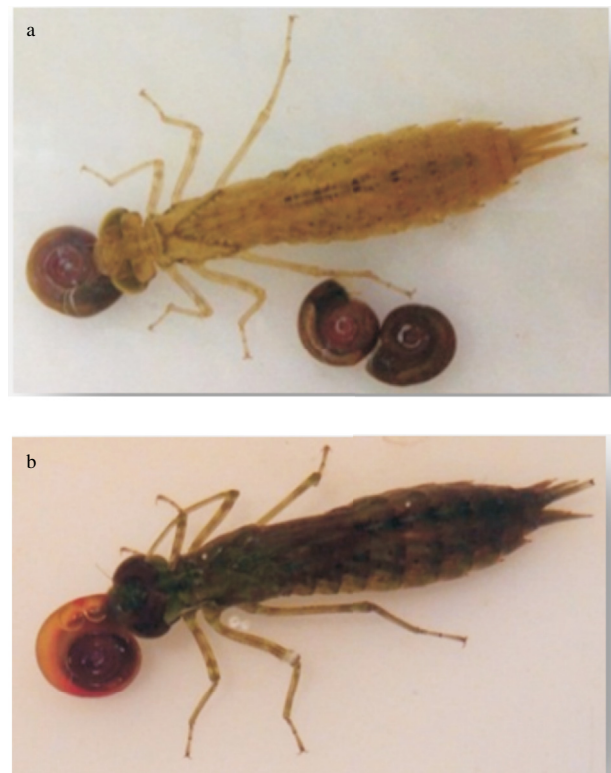


Figure 2. Foraging behavior of the Odonata, *H. ephippiger* nymph towards the freshwater snail, *B. alexandrina*. a: Encounters; b: Devour.

B. truncatus and *B. alexandrina* snails. Most observed encounters began with snails attacks, followed by avoidance or consumption. More encounters occurred with *B. truncatus* snail than with *B. alexandrina* snail. Encounter percentage outcomes in Table 1 indicated that the predator encounters outcomes (attacks, avoidance and consumption) towards *B. truncatus* or

B. alexandrina are not identical. The contingency table showed that the proportions of outcomes in the different columns vary significantly with the two snail types ($\chi^2 = 8.102$; $df = 2$ and $P = 0.0174$). This indicated the relationship between the snail type and the predator encounter outcomes.

3.3. Prey consumption and predation rate

Table 2 shows the effect of *B. truncatus* snail densities on the number consumed. Obtained data show that *H. ephippiger* nymph could consume a mean \pm SE of 17.60 ± 0.50 , 15.00 ± 0.32 and 13.00 ± 0.45 individuals of *B. truncatus* snail per day with the snail density of 25 individuals considering the small-, medium- and large-sized snails, respectively. Statistically, significant difference was obtained with the prey density of 25 individuals per day while no significant differences were obtained between small-, medium-, and large-sized *B. truncatus* snail at density of 5, 10, 15 and 20 preys. On the other hand, Table 3 shows significant differences in the mean numbers of *B. alexandrina* snail consumed per day at a density of 20 and 25 individuals considering small-, medium- and large-sized snail, respectively. Subsequently, *H. ephippiger* nymph consumed a mean \pm SE of 15.00 ± 0.43 , 11.00 ± 0.45 and 8.00 ± 0.71 individuals of *B. alexandrina* snail per day at the same density (25 individuals) considering small-, medium- and large-sized snail, respectively. The predation rate of *H. ephippiger* nymphs towards the two snail species at the different densities were shown in Figures 3 and 4 and Tables 4 and 5. The regression analysis showed that, significant differences in the predation rate of *H. ephippiger* towards *B. truncatus* by comparing the predation rate with increasing the snail densities ($r = -0.921$, -0.933 and -0.974 with P -values = 0.026, 0.021 and 0.005) considering small-, medium- and large-sized *B. truncatus* snails, respectively. Linear regression analysis also showed that by increasing the density of *B. alexandrina* snail from 5 to 25 individuals, significant differences in the predation rate of medium- and large-sized snail could obtain ($r = -0.894$ and -0.948 with P -values = 0.0409 and 0.0143)

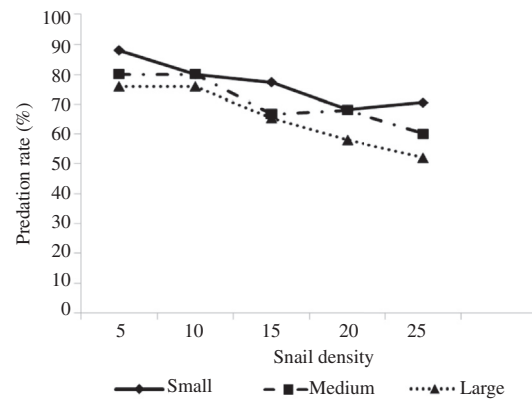


Figure 3. Predation rate of the Odonata, *H. ephippiger* nymph on the freshwater snail, *B. truncatus*.

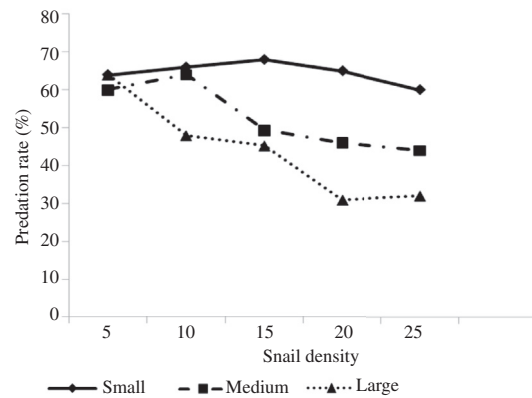


Figure 4. Predation rate of the Odonata, *H. ephippiger* nymph on the freshwater snail, *B. alexandrina*.

considering medium- and large-sized *B. alexandrina*, respectively. While, no significant difference was obtained in the predation rate of small-sized *B. alexandrina* snail with increasing the prey density ($r = -0.479$, P -value = 0.4130).

Table 2

Effect of *B. truncatus* snail density on the consumption rate of the Odonata, *H. ephippiger* nymph.

Prey size	Daily number of preys consumed at prey density				
	5	10	15	20	25
Small	4.40 \pm 0.24 ^a	8.00 \pm 0.32 ^a	11.60 \pm 0.51 ^a	13.60 \pm 0.93 ^a	17.60 \pm 0.50 ^a
Medium	4.00 \pm 0.32 ^a	8.00 \pm 0.32 ^a	10.00 \pm 0.45 ^a	13.60 \pm 0.51 ^a	15.00 \pm 0.32 ^b
Large	3.80 \pm 0.37 ^a	7.60 \pm 0.24 ^a	9.80 \pm 0.37 ^a	11.60 \pm 0.51 ^a	13.00 \pm 0.45 ^c
<i>P</i> -value	0.4200	0.5566	0.0283	0.0940	< 0.0001

Values were expressed as mean \pm SEM. Means followed by the same letter in the same column are not significantly different ($P > 0.05$).

Table 3

Effect of *B. alexandrina* snail density on the consumption rate of the Odonata, *H. ephippiger* nymph.

Prey size	Daily number of preys consumed at prey density				
	5	10	15	20	25
Small	3.20 \pm 0.20 ^a	6.60 \pm 0.24 ^a	10.20 \pm 0.49 ^a	13.00 \pm 0.45 ^a	15.00 \pm 0.43 ^a
Medium	3.00 \pm 0.32 ^a	6.40 \pm 0.24 ^a	7.40 \pm 0.24 ^b	9.20 \pm 0.87 ^b	11.00 \pm 0.45 ^b
Large	3.20 \pm 0.20 ^a	4.80 \pm 0.37 ^b	6.80 \pm 0.37 ^b	6.20 \pm 0.58 ^c	8.00 \pm 0.71 ^c
<i>P</i> -values	0.8	0.0018	< 0.0001	< 0.0001	< 0.0001
F	0.22	11.23	22.25	26.7	41.11

Values were expressed as mean \pm SEM. Means followed by the same letter in the same column are not significantly different ($P > 0.05$).

Table 4

Regression analysis of the effect of *B. truncatus* snail density on the predation rate of the Odonata, *H. ephippiger* nymph.

Snail size	Slope	<i>r</i>	<i>P</i> -value
Small	-0.94	-0.921	0.026
Medium	-1.04	-0.933	0.021
Large	-1.32	-0.974	0.005

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

Table 5

Regression analysis of the effect of *B. alexandrina* snail density on the predation rate of the Odonata, *H. ephippiger* nymph.

Snail size	Slope	<i>r</i>	<i>P</i> -value
Small	-0.18	-0.479	0.4130
Medium	-1.00	-0.894	0.0409
Large	-1.62	-0.948	0.0143

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

3.4. Preference experiments with all-prey combination

When given the choice (dual-choice preference), *H. ephippiger* nymph consumed more individuals of large-sized than small- or medium-sized *B. truncatus* snail. The preference indexes obtained were (0.28 and 0.72), (0.23 and 0.77), and (0.42 and 0.58) considering the dual choice of (small and medium), (small and large) and (medium and large) *B. truncatus* snail, respectively. The same result was obtained with *B. alexandrina* snail. The obtained preference indexes were (0.19 and 0.81), (0.11 and 0.89) and (0.30 and 0.70) considering the dual choice of (small and medium), (small and large) and (medium and large) *B. alexandrina* snail, respectively. Obtained preference index showed that, *H. ephippiger* nymph preferred the consumption of one prey from the two sizes exposed. Preference tests with starved *H. ephippiger* nymph when the three-prey snail sizes offered in combinations (three-choice preference) were showed the ability of the predator to prefer some stages than others. The preference of *H. ephippiger* nymph differed clearly among the three-prey sizes. The predator nymph preferred large-sized *B. truncatus* than the other sizes. According to the preference index (Manly's index), the preferred snails in a descending order were the large-, medium- and small-sized *B. truncatus* snail, respectively. The same results were obtained with *B. alexandrina* snail where the Manly's preference indexes obtained were large-, medium- and small-sized snail, respectively in a descending order.

4. Discussion

From the results, it appears that the Odonata, *H. ephippiger* nymphs are able to consume the medically important snails, substantially through the rate of consumption varying between the species sizes as well as with the prey density. The obtained data showed that the consumption rate of the predators increases with the prey density. Although Odonata predators are important biocontrolling agents against mosquitoes larvae in lakes and ponds [10,13], their use in snail regulation in these habitats requires more attention. Recently, the predatory potential of *H. ephippiger* nymph against *Lymnaea natalensis* snail, the intermediate host of

Fasciola gigantica was evaluated [11]. Laboratory experiments with the Odonata nymph, *H. ephippiger*, showed that the nymph was a voracious predator of *B. truncatus* and *B. alexandrina* snails. Starved predators required significantly shorter time in attacking (searching) and handling time towards the preys in comparison with the satiated predators. This indicated that the starved *H. ephippiger* nymph reacted fast when exposed to the preys. Searching and handling times are major factors in determination of the functional response of the predator to its prey and should predict how the predators behave when different prey species are present [14]. Predation rate of *H. ephippiger* on the freshwater snails varied with snail species and density. In natural situations where the habitat is structured and the temporal and spatial variations of species abundance are more complex, the predation rates of the Odonata nymph are expected to vary as has been noted in other aquatic predators [15]. Preference experiments showed that *H. ephippiger* nymph in general preferred *B. truncatus* than *B. alexandrina*. As a result of most gastropods must also be prepared to execute avoidance or escape behavior since they inhabit the same habitat as their predators, a difference in the snail preference were obtained [16]. Many studies indicated that successful biological control may depend on the use of native predators that share the habitat and are part of the natural food web [17,18]. The present study suggests that *H. ephippiger* nymph may act as a potential biocontrolling candidate sharing the same habitats of snails. Thus, the use of this Odonata nymph alone or in combination with other aquatic insects can be a viable option for regulation of freshwater snails in wet lands as an extension of conservation biological control. This proposition needs to be tested under field conditions to promote regulation of these snails and conservation of useful insects.

Conflict of interest statement

We declare that we have no conflict of interest.

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