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Potential of *Hemianax ephippiger* (Odonata-Aeshnidae) nymph as predator of *Fasciola* intermediate host, *Lymnaea natalensis*

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ABSTRACT

Objective: To evaluate the predatory capacity of the Odonata, *Hemianax ephippiger* nymph as a biocontrol agent for the freshwater snail *Lymnaea natalensis*, intermediate host of *Fasciola gigantica*.

Methods: Observations on the searching, attacking and devouring of the snails with a series of laboratory-based predation experiments, whose aims were to determine daily predation rate, differential predation on small-, medium- and large-sized snails were carried out.

Results: Laboratory evaluation revealed that, the Odonata nymph could kill and consume all three sizes of snails. Searching and handling time of the predator differed depending on snail size and predator vulnerability. The predation rate varied also with respect to snail size and density.

Conclusions: Our observations suggested that the predator *Hemianax ephippiger* may be a suitable bio-control agent of *Lymnaea natalensis* snail population.

1. Introduction

The liver fluke, *Fasciola gigantica*, is an economically important parasite that infects a wide range of livestock species [1]. The snail *Lymnaea natalensis* Krauss 1848 (*L. natalensis*) functions as an obligatory intermediate host for *Fasciola gigantica* in the old world and thus plays an important role in the epidemiology of *Fasciola* infection [2,3]. Liver flukes can cause huge losses to livestock industries and affect the health of humans where fascioliasis is an important human disease [4–6]. In their general distribution, freshwater pulmonate snails are benthic animals living in the shallower water of lakes, ponds, marshes, rivers, streams and ditches [7,8]. Snail control strategies are considered a priority for the reduction of transmission. Synthetic molluscicides (niclosamide) have been widely used for chemical control [9]; although chemical control only gives a temporary reduction in snail density. The biological methods, especially those involving the use of indigenous predators, were traditionally perceived as environmentally friendly and have been the foci of research and management of this pest [10]. Predators in nature often include an array of prey types in their diet. Furthermore, in the presence of multiple prey types, they often select certain prey types over others [11]. Predation is a major force affecting species abundance, population dynamics and community structure [12]. Dragonflies are ideal predators of many insect pests and have an important role in biological pests control in various ecosystems. Dragonflies have proved to be potential bio-control agents of mosquitoes and are considered an important predators of various macro-invertebrates [13,14].

Many studies on biological control of freshwater snails using natural enemies have been reported [15–17]. Although some insect predators of snail-host species have been taken into account but other predatory insects like Odonata and Dytiscidae require further study [18]. In view of this, the present study was aimed at evaluating the predation potential of the dragonfly *Hemianax ephippiger* Burmeister 1839 (*H. ephippiger*) nymph of the *Fasciola* snail intermediate host, *L. natalensis*. The searching behavior of the predator towards the different snail

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sizes was also measured. The results of the present study will provide a primary basis for assessment of this predator as biological resource against freshwater snails.

2. Materials and methods

2.1. Collection of Odonata nymphs

H. ephippiger nymphs were collected from ponds and lakes in the Abou-Roash area, Giza Governorate, Egypt. They were kept in a glass aquarium (50, 30 and 20 cm in length, width and height), respectively. Collected nymphs were reared in an aquarium in the laboratory and fed daily to satiation on different sizes of *L. natalensis*. Nymphs were starved for a period of 24 h before they were used in experiments. Fully grown nymphs (the last two instars) with sizes ranging from 3.2 to 4.5 cm in length were used in the experiments.

2.2. Collection of snails

The experimental snails, *L. natalensis* were collected from lakes and ponds in the Abou-Roash area, Giza Governorate, Egypt. They were kept in glass aquaria (50, 30 and 20 cm), 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 lettuce leaves as a basic food, dried lettuce is provided when the green was not available. Fish food (Tetramin®) and blue green algae (*Nostoc muscorum*) were used as an additional food source for newly hatched and juvenile snails. Only laboratory-bred snails were used as preys for the nymphs used in experiments. Additionally, some water plants (*Ceratophyllum demersum* and *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.

2.3. Experimental methods

Ten glass aquaria, 5 L in total 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 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 h before introducing the predator. Snails and Odonata nymphs were used only once in the experiments. All experiments were carried out at constant temperature of (25 ± 2) °C, 60%–70% relative humidity. Fluorescent tubes (10 cm long, 32 watt, were placed 100 cm above the tanks to provide a photo period of L12: D12.

2.4. Searching and handling time

Predator and prey behaviors were observed 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 prey, 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 (no./min). Encounters with prey could result in attacking, pre-capture, avoidance or consumption of prey. Each trial involved introducing an individual Odonata nymph 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.

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

For each *H. ephippiger* nymph, *L. natalensis* snails (small, medium or large) were supplied at densities of 5, 10, 15, 20 and 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 prey consumed/day and subsequently the predation rate.

2.6. Data analysis

Data considering searching, handling times, foraging behavior, prey consumed and predation rate were expressed as mean ± SE. Comparison between three or more different groups was analyzed using One-way ANOVA followed by Bonferroni multiple comparison test for least significant difference. The correlation between prey density and predation rate was determined. Data were analyzed using GraphPad InStat software (version 3.1, GraphPad InStat., California, USA).

3. Results

3.1. Searching and handling times

The Odonata *H. ephippiger* nymphs showed clear differences in searching and handling times towards the three-prey sizes (Table 1). Data obtained show that, the nymph required more time in searching for the small and medium snails as compared to the large snails. The maximum searching time (21.40 ± 1.90) min was obtained by the predator nymph towards small snail when predator nymphs were satiated, whereas the minimum searching time (6.00 ± 0.71) min was obtained with the large snail when the predator nymphs were starved. Significant differences ($P < 0.05$) were obtained in the handling time of the predator nymph towards the three sizes of snails. Handling time of the starved predators towards the snails was (6.80 ± 0.86),

Table 1

Searching and handling times of the predator, *H. ephippiger* nymph towards *L. natalensis* snails.

Behavior	Adult condition	Prey snail size		
		Small	Medium	Large
Searching time (min)	Starved	15.00 ± 1.73^{a}	15.40 ± 0.93^{a}	6.00 ± 0.71^{b}
	Satiated	$21.40 \pm 1.90^{a*}$	18.80 ± 1.20^{a}	$12.40 \pm 1.20^{b*}$
	P-value	0.037	0.127	0.003
Handling time (min)	Starved	6.80 ± 0.86^{a}	7.80 ± 1.20^{a}	12.00 ± 0.71^{b}
	Satiated	8.80 ± 1.10^{a}	$11.20 \pm 0.60^{a*}$	$15.40 \pm 0.40^{b*}$
	P-value	0.183	0.018	0.005

Means in the same row, followed by different letters are significantly different ($P < 0.05$); *: Significant at $P < 0.05$ compared with starved value.

Table 2

Forage behavior of the Odonata, *H. ephippiger* nymph towards *L. natalensis* snails.

Behavior	Prey snail size		
	Small	Medium	Large
No. of encounter	26.00 ± 1.70[a]	25.60 ± 1.20[a]	19.40 ± 1.50[b]
Encounter rate (no./min)	0.43 ± 0.02[a]	0.42 ± 0.02[a]	0.32 ± 0.03[b]
No. of Attack/ encounter	0.50 ± 0.01[a]	0.65 ± 0.02[b]	0.91 ± 0.03[c]
No. of Avoidance/ encounter	0.49 ± 0.02[a]	0.35 ± 0.02[b]	0.14 ± 0.02[c]
Preying/encounter	0.22 ± 0.01[a]	0.20 ± 0.01[a]	0.21 ± 0.01[a]

Means in the same row followed by different letters are significantly different ($P < 0.05$).

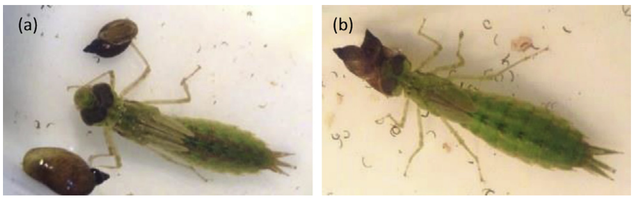

Figure 1. Foraging behavior of the Odonata, *H. ephippiger* nymph towards freshwater snail, *L. natalensis*.
a: Encounters; b: Devour.

Table 4

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

Prey size	Slope	*r*	*P*	*F*
Small	−0.400	−0.995	0.004 0	306.12
Medium	−1.428	−0.991	0.009 0	173.87
Large	−0.902	−0.931	0.021 7	19.41

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

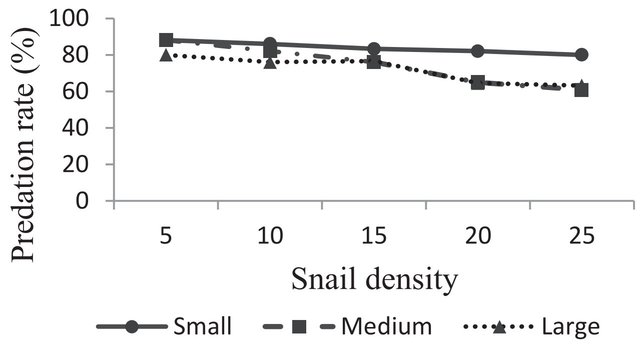

Figure 2. Predation rate of the Odonata, *H. ephippiger* nymph on the freshwater snail, *L. natalensis*.

(7.80 ± 1.20) or (12.00 ± 0.71) min for the small, medium and large snails, 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, significant difference ($P < 0.05$) were obtained in both searching and handling times of the predators towards the three different prey snails (Table 1).

3.2. Encounter between the predator and the preys

Data in Table 2 and Figures 1a and 1b show the encounter behavior of the predator, *H. ephippiger* nymph toward the prey snails. Most observed encounters with snails ended in attacking the preys before preying or avoiding it. The maximum number of encounters (26.00 ± 1.70) was obtained with the small snail followed by the medium snail (25.60 ± 1.20), whereas the number of encounters recorded by the predator toward the large snail was the lowest (19.40 ± 1.50). The encounter and also the encounter rate (no./min) significantly differed ($P < 0.05$) when the large snail was compared with the two other snail sizes. Also a significant difference ($P < 0.05$) was obtained for the number of attacks/encounters and the number of avoidances/encounters. In contrast, no significant differences ($P > 0.05$) were obtained for the prey consumed/encounter. Obtained preying rate was (0.22 ± 0.01), (0.20 ± 0.01) and (0.21 ± 0.01) considering small-, medium- and large-size of the snail.

3.3. Prey consumption and predation rate

The Odonata *H. ephippiger* nymph uses its grasping mouth to catch the prey (snail) in one swift motion and then consume it. Table 3 shows the effect of prey densities on the number of prey consumed considering the prey sizes. Statistically, no significant differences ($P > 0.05$) were obtained between the number of preys consumed at lower density (5, 10 or 15 preys) whereas significant differences ($P < 0.05$) were obtained for the higher densities (20 or 25 preys) in comparison to the snail size consumed. Small *L. natalensis* snails consumed by *H. ephippiger* nymphs were significantly different ($P < 0.05$) from large snails at high density (20 and 25 preys). Obtained data shows that the nymph could consume a mean of (20.00 ± 0.71), (15.20 ± 0.90) and (15.80 ± 1.10) preys/day considering small-, medium- and large-size snail, respectively, at density of 25 preys. The predation rate of *H. ephippiger* nymphs on *L. natalensis* snails at different densities are shown in Table 4 and Figure 2. Regression analysis based on the mean predation rate (n = 5 sets/prey density) showed significant differences in the predation rate of *H. ephippiger* towards small, medium and

Table 3

Effect of 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 ± 0.24[a]	8.60 ± 0.40[a]	12.50 ± 0.80[a]	16.40 ± 0.51[a]	20.00 ± 0.71[a]
Medium	4.40 ± 0.24[a]	8.20 ± 0.58[a]	11.40 ± 0.92[a]	13.00 ± 1.87[b]	15.20 ± 0.900[b]
Large	4.00 ± 0.32[a]	7.60 ± 0.51[a]	11.50 ± 0.40[a]	12.90 ± 1.60[b]	15.80 ± 1.100[b]

Data are represent as mean ± SEM. Means followed by different letters in the same column are significantly different ($P < 0.05$).

large snails (r = −0.995, −0.991 and −0.931 with P value = 0.004 0, 0.009 0 and 0.021 7), respectively. Obtained data showed, increasing the snail density decrease the predation rate, at density of 5 snail individuals, the predation rates were (88.0 ± 4.8), (88.0 ± 4.8) and (80.0 ± 6.4)% while these were differ at density of 25 snail individuals to (80.0 ± 2.8), (61.0 ± 3.6) and (63.0 ± 4.4)% considering small-, medium- and large-size snail, respectively.

4. Discussion

H. ephippiger nymphs are able to devour and consume substantial numbers of the medically important snail *L. natalensis* although the rate of consumption varies with snail size and prey density. The consumption rate increased with increasing the prey density. Odonata predators are cited as important bio-control agents against mosquito larvae in lakes and ponds [19,20]; although their use in snail regulation in these habitats has not been recorded. The nymph encountered and attacked small, medium and large *L. natalensis* snails. Most aquatic insects detect their preys by mechanical, vibratory or visual cues and react to the slightest agitation of the water [21]. The Odonata nymphs are sprawlers and snatch the moving prey in ambush, rarely hunting for the prey [22]. Starved predators required shorter time in attacking (searching) and handling time towards the preys in comparison with the satiated predators. 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 were presented [23]. The predation rate of *H. ephippiger* on the freshwater snails was found to vary with snail size and density of the prey. The predation rate varied significantly against snail sizes at different density. A low predation rate was observed against large snails, likely due to the greater amount of time required to prey those snails. In natural situations where the habitat is structured and the temporal and spatial variations of species abundance is more complex, the predation rates of the Odonata nymph are expected to vary as has been noted in other aquatic predators [24]. The present study suggests that the Odonata *H. ephippiger* nymph is a potential candidate as a bio-control agent sharing the same habitats of snails. Thus the use of the Odonata nymph alone or in combination with other aquatic insects can be 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.

The freshwater snail, *L. natalensis* is the intermediate host of liver fluke, and elimination or reducing density of this snail will reduce the chances of the disease transmission. Studies conducted in lakes show that fish and crayfish predators play an important role in determining the abundance of freshwater snails. In contrast, shallow ponds and marches often lack fish and crayfish but have abundant insect predators. Our laboratory study on one of those insect predators; *H. ephippiger* nymph showed that, this predator had the ability to search, encounter and devour this snail. Determination of the daily prey consumed and predation rate confirm the predatory efficiency of this predator against *L. natalensis* snail. Further field studies are required to determine the ability of this predator with the other control types in reducing the snail population.

Conflict of interest statement

We declare that we have no conflict of interest.

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