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Predation of the Diving Beetle, *Eretes sticticus* (Coleoptera: Dytiscidae) on Mosquito Larvae, *Culex pipiens* L. (Diptera: Culicidae)

Younes, A. A.
Department of Entomology, Faculty of Science, Cairo University, Giza, Egypt
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ABSTRACT

The dytiscid beetle, *Eretes sticticus* Linnaeus, 1767, (Coleoptera: Dytiscidae) was noted to predate on the mosquito larvae in ponds and pools. Laboratory evaluation of predation by *E. sticticus* on *Culex pipiens* L. larvae revealed that a single adult of *E. sticticus* could kill and consume all larval instars of the mosquito. The number of preys consumed differed according to their size and density. The predator searching time ranged between 26 and 96 minutes, and the handling time ranged between 0.36 and 3.4 minutes depending on the size of the prey and the predator. The predation rate varied also with respect to prey size and density. The predation rate obtained varied from 8.0 to 52 %. It was also noticed that the predator, *E. sticticus* prefered in particular oldest instar when they were offered in both or in combinations.

Key Words: Aquatic predator, Dytiscidae, *Eretes sticticus*, Prey, *Culex pipiens* larvae.

INTRODUCTION

Mosquitoes are probably the best known group of insects because of their importance as nuisance pests and vectors of animal and human diseases. They are widely distributed through the world and occur in a variety of habitats (Yan and Zhong, 2005). Larvae of mosquitoes have been traditionally controlled with synthetic pesticides. This type of control can be hazardous to human, result in pesticide resistance in mosquitoes, damaging fauna and flora, contaminating air, water and soil, and being expensive (Hemingway *et al.*, 2004). In spite of the continuous use of synthetic pesticides, mosquito populations have not been reduced significantly (Boyer *et al.*, 2006 and Poupardin *et al.*, 2008). Therefore, it is necessary to evaluate other control methods. Many species of dytiscid beetles normally occur in ponds where they often constitute one of the major components of the biota (Larson, 1985 and Nilsson & Svensson, 1994). According to Campos *et al.*, 2004, dytiscid beetles have been noticed to regulate the population of mosquito species through predation.

E. stictius beetles are distributed through the Africa, Middle east, South America from Peru, the Galapagos islands north to the Virgin islands and to the Southern USA (Miller, 2001 & 2002). During this study, *E. sticticus* was recorded through different regions in Egypt.

Several factors are known to be important in the relationship between predators and their preys. These include, density of the prey as well as that of the predator, predator searching efficiency and prey activity (Hubbard *et al.*, 1988; Sherrat and Tikasingh, 1989; and Yasuda and Mitsui 1992). Prey age structure may also be an important aspect of this relationship (Blaustein and Margalit, 1994).

In view of these facts, the dytiscid beetle, *E. sticticus* predating on the mosquito larvae in nature, an assessment of predatory efficiency of the adult beetle on *Culex pipiens* larvae were evaluated. Also, a prey preference of the predator beetle towards the prey stages was determined.

MATERIALS AND METHODES

Biological material and experiment condition

Laboratory colonies of the predator, *E. sticticus* and the prey, *C. pipiens* larvae were established from adults and egg rafts, respectively, collected from standing fresh water-ponds located in Kom Oshim area in Fayoum Province, Egypt. Egg rafts hatched in the laboratory (in 3-liter plastic containers); every two days 40 mg of a 1:1 mixture of Tetramin® and yeast was added to each container as food for the mosquito larvae. Water of the container was replaced every 6 days. Larvae were transferred to the experimental treatments as a 1 day old.

The dytiscid beetle, *E. sticticus* adults were sampled with a small hand net (frame diameter 150 mm, mesh size 0.6 mm). Collected adults were immediately transferred to a flat bowel with some water, from which the insects were removed. Adults beetle were kept in 1-m^3 tanks filled with tap water, the predators were provided by mosquito larvae as preys and kept for approximately one week before being used in tests.

Searching and handling time

The main intended searching outcomes were to measure the costs implied for starved and satiated predators in pursuing and processing the prey as well as measuring prey vulnerability (latency to capture). Each trial involved introducing an individual beetle into a glass bucket of 10-liter capacity filled with distilled water, after which a 10 live mosquito larvae of one of the four different instars were introduced. Each single beetle was tested only once. Searching time (latency to capture prey), handling time (the time from capture to end of feeding) were recorded. Search time was measured as the time from introduction of prey to successful capture.

Effect of prey density on the consumption and predation rate

For each adult individual of the dytiscid predator beetle, *E. sticticus*, mosquito larvae of a first, second, third or fourth instars were supplied at densities of 5, 10, 15, 20, 25 and 30 larvae. Predators were allowed to predate for a period of 24 hours. Five replicates for each of prey instar as well as prey density was carried out for determination of the mean number of preys consumed and so predation rate.

Prey preference

In the first set of experiments (preference experiments with two-prey combination), each individual of the dytiscid beetle was supplied with two different prey instars in combination. Starting density of each prey instar was five individuals. Mathematical formula of Sherrat and Harvey (1993) was used as following: $(E_1 / E_2) = C\ (N_1/N_2)$; where N_1 and N_2 represent the number of each prey instars available for the predator, and E_1 and E represent the number of consumed preys. The parameter (C) is the preference index. A value of the preference index between zero and one indicates a preference for prey type (N_2), whereas a value of the preference index between one and infinity indicates a preference for prey type (N_1). In the second set of experiments, preference was examined when all prey instars are in combinations (preference experiment with four-prey age combinations). In this set of experiments, each predator individual was supplied with all four prey instars simultaneously. Starting density of each prey instar was 5 individuals. After 24 hours of the start, numbers of consumed prey were recorded separately. Prey preference was measured using the Manly's index (Manly, 1974; Chesson, 1983; and Krebs, 1989), which takes the prey depletion into account:

$$\alpha_i = \log P_i / \sum_{j=1}^{m} \log P_j$$

where α_i = Manly's index for prey type I ; P_i = proportion of prey i remaining at the end of the experiment relative to the original input (i = 1,2,3,......m) p_j = proportion of the experiment relative to the original input (j = 1,2,3,......m); and m = number of prey types. Manly's index can take value between zero and one, and the values of the different prey types always sum up to one. In case of four-prey combination, the threshold value is 0.25 (α = 1/m). Values higher than 0.25 indicate preference for this prey type, smaller values indicate selectivity against it.

All experiments were carried out at a constant temperature of 24 ± 2 °C, 60-70 % RH. Fluorescent tubes (10 cm long, 32 watt) were placed 100 cm above the tanks to provide a photoperiod of 12 L: 12 D. before each test; individuals of *E. sticticus* were starved in a container for 2 days, in order to increase their motivation to forage.

Data analysis

Data considering the number of prey consumed were subjected to ANOVA followed by Fisher's pairwise comparison for least significant difference (LSD). T-test and regression analyses were also represented. Obtained types of data were analyzed using computer program, SAS institute, version 8.0 (2000).

RESULTS AND DISCUSSION

Searching and handling times

Adult *E. sticticus* beetle showed clear differences in search and handling times towards the four-prey instars (table 1). Searching times significantly differed in both starved and satiated predator individuals comparing the search time of all instars ($P<0.05$). Statistically, no significant differences appeared ($P>0.05$) comparing the searching for time of the predator to the third and fourth prey instars. Data obtained show that, the predator beetle required more time in searching for the smaller instars and less time in searching for the bigger ones. The maximum searching time (96 ± 4.3 min.) was obtained by the first prey instar when the beeltes were satiated while the minimum searching time (26 ± 2.4 min.) was obtained by the fourth prey instar when the beetles were starved. Concerning the handling time, significant differences ($P<0.05$) were obtained in the handling time of the beetle towards the four-prey instars. Handling time of the starved predator to fourth instar was 9.1, 2.7 or 1.5 times longer compared with those of the starved predator to first, second or third instars, respectively. A similar relation was also obtained with the handling time of the satiated predator. Comparing

Table (1): Search and handling times of the predator, *E. sticticus* adults towards the mosquito, *C. pipiens* larvae.

Behavior	Adult condition	Mosquito larval instar (means ± SE) 1st	2nd	3rd	4th
Searching time (min.)	Starved	55 ± 5^{a}	44 ± 3.5b	34± 4.3^{c}	26±2.4
	Satiated	96±4.3a*	76±4.4b*	51±3.3^{c*}	50±3.5^{c*}
Handling time (min.)	Starved	0.36±0.05^{a}	1.2±0.06^{b}	2.1±0.2^{c}	3.3±0.3^{d}
	Satiated	0.39±0.02^{a}	1.36±0.05^{b}	1.9±0.07^{c}	3.4±0.15^{d}

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

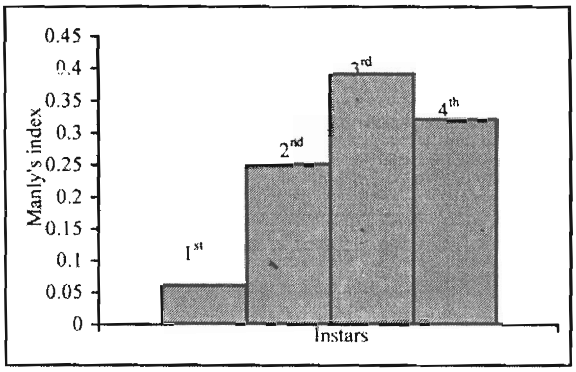

Figure (1): Mean values of Manly's preference index for the adult predator, *E. sticticus* predate on the mosquito, *C. pipiens* larvae for 24 hrs (all instars are offered in combination).

Table (2): Effect of prey density on consumption of *C. pipiens* larvae by the adult predator, *E. sticticus*.

Prey larval instar	Prey consumed (mean ± SE) at prey density 5	10	15	20	25	30	F
1st	0.4±0.24a	0.8±0.37a	3.2±0.58b	5.8±0.4c	6.4±0.37c	8.4±0.5e	52
2nd	0.8±0.37a	2±0.7a	3.8±0.5ab	6±0.7bc	7.4±0.6c	8±0.7c	18.2
3rd	2.4±0.67a	3.2±0.73ab	6±0.71bc	9±0.8c	9.2±0.86c	9.4±0.7c	15.7
4th	2.6±0.5a	3.4±0.5	6.6±0.5b	8.6±0.7b	8.2±0.7b	9±0.8b	15.9

- Means in the same raw followed by the same letter are not significantly different (P>0.05). F- value was estimated at df : 4,29.

Table (3): Predation rate (% ± SE) of *E. sticticus* predator prayed on *C. pipiens* larvae

Prey larval instar	**Predation rate (% ± SE) at prey density** 5	10	15	20	25	30
1st	8.0 ± 2.4	8.0 ± 3.7	21.3 ± 3.8	29 ± 1.9	25.6 ± 2.5	28 ± 1.6
2nd	16 ± 7.4	20 ± 7	25.3 ± 3.3	30 ± 3.5	29.6 ± 2.8	26.6 ± 2.3
3rd	48 ± 13.4	32 ± 7.3	40 ± 4.6	45 ± 4.3	36.8± 3.4	31.3 ± 2.3
4th	52 ± 10	34 ± 5	44 ± 3.3	43 ± 3.5	32.8±2.8	30 ± 3.2

Table (4): Predation rate regression analysis of the adult predator, *E. sticticus* prayed on the mosquito, *C. pipiens* larvae at different prey density.

Mosquito larval instar	Slope	(r)	p- value
1st	1.146	0.899	0.014 (<0.05)
2nd	0.834	0.494	0.039 (<0.05)
3rd	- 0.366	- 0.504	0.51 (>0.05)
4th	- 0.655	- 0.728	0.11 (>0.05)

Table (5): Prey preference of adult predator, *E. sticticus* prayed on the mosquito, *C. pipiens* larvae (the preys were offered in paired instars).

Exposed instars (N_1&N_2)	Prey consumed (mean ± SE) (E_1&E_2)	Prefer index (C)	Pry preference	t-	p-value
1st & 2nd	1.4±0.5 & 3.6±0.5	0.38	N_2 (2nd instar)	3.05	0.015
1st & 3rd	0.8±0.37 & 4.2±0.4	0.19	N_2 (3rd instar)	6.42	0.0002
1st & 4th	0.6±0.2 & 4.6±0.3	0.13	N_2 (4th instar)	11.54	0.0001
2nd & 3rd	2.6±0.3 & 4±0.3	0.65	N_2 (3rd instar)	3.67	0.006
2nd & 4th	1.6±0.24 & 4.2±0.3	0.4	N_2 (4th instar)	5.9	0.0003
3rd & 4th	4.8±0.6 & 3.2±0.4	1.5	N_1 (3rd instar)	2.31	0.04

searching time or handling time of starved and satiated beetle, significant differences (P<0.05) were obtained in the searching time of starved and satiated beetle to the different prey instars. On the other hand, no significant differences (P>0.05) were obtained in the handling time of both starved and satiated beetles to the different prey instars.

Prey consumption and predation rate

The adults of *E. sticticus* were observed to use their forelegs to grasp the prey (mosquito larvae) in one swift motion and then consume it. Table (2) shows the effect of prey density on the number of prey consumed. Obtained data indicated that, increasing prey density led to increasing in the prey consumption. Significant differences (P<0.01) were obtained between the number of preys consumed at low or intermediate density in comparison to those ate at high density. In general, dytiscid beetles given 1^{st} or 2^{nd} instar larvae ate less than those given 3^{rd} or 4^{th} larval instar.

Predation rate

Rate of predation of *E. sticticus* adults on mosquito larvae varied with the mosquito larval age and density (Table 3). The regression equations obtained on the predation rate at different prey densities are presented in table (4). Linear regression test carried out on the rate of predation showed a significant difference in the predation rate with the first (r = 0.8987, P<0.05) and second larval instar (r = 0.8337, P<0.05), with respect to prey density. The rate of predation was found to be increasing with the increasing densities of the first and second prey instars (slope = 1.146 and 0.4943, respectively). On the other hand, no significant differences (P>0.05) in the predation rate of *E. sticticus* adults were obtained with change of prey density of both third and fourth mosquito larval instars. A decreasing value in the predation rate of the beetles was obtained with increasing the prey density of third and fourth prey instar (slope = - 0.3663, - 0.6549, respectively)

Preference experiments with two-prey combinations

Table (5) summarizes six series of pairwise preference tests. When given the choice, *E. sticticus* consumed more individuals of the third mosquito larval instar than the first, second or fourth ones. Exposed first with the second or first with the fourth prey instars, *E. sticticus* consumed much low first instar compared with second or fourth ones. Obtained preference index showed that, *E. sticticus* preferred consumption of one prey from the two exposed. Paired t-test carried out on each two prey instars consumed varied significantly through all pairwise preference tests (P<0.05; table 5).

Preference experiments with all-prey combinations

Figure (1) shows preference tests with starved *E. sticticus* when the four-prey instars were offered in combinations. The preference of the dytiscid beetle differed clearly among the four prey-instars. The beetle preferred the third mosquito larvae than the others. The mean numbers of the prey instars preferred in a descending order were 3.6, 2.8, 2.4 and 0.6 larvae, considering third, fourth, second and first prey instars, respectively. Manly's preference index (Fig. 1) showed a significant preference towards the third prey instar in comparison with the others instars.

The diving beetles often been cited as important predators in lakes and ponds that contribute to the suppression of the mosquito larvae (Larson, 1985 and Lundkvist *et al.*, 2003). Laboratory experiments with the diving beetle, *E. sticticus* showed that, the adult was a voracious predator of the mosquito *C. pipiens* larvae. The adult predator encountered and attacked oldest instars than the youngest ones. According to Le Louran and Cloarec (1997), most aquatic insects detect their preys by mechanical, vibratory or visual cues and react to the slightest agitation of the water and this may explain why *E. sticticus* attacked the oldest instars. 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 *E. sticticus* 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 predator behaves when different prey species are present (Cock, 1978).

Predation rate of *E. sticticus* on *C. pipiens* larvae was found to vary with the size and density of the prey. At high density the predation rate was usually similarly for both youngest and oldest preys (Table 4). On the other hand, the predation rate varied significantly towards youngest and oldest preys at lower density. Low predation rate observed towards youngest preys at lower density are likely due to their low attack rates and longer searching time. This is understandable because the first and second prey instars are small and their detection in water at low density is difficult for the predator. 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 beetle are expected to vary as has been noted in other aquatic predators (Blaustein, 1998; Eitam *et al.* 2002 and Hampton, 2004).

Preference experiments showed that, *E. sticticus* in general prefered oldest preys than youngest ones. Considering the availability of alternate preys along with heterogenity of the habitats in the ponds and pools, deviation from the present finding on the prey preference may be found. Lundkvist *et al.* (2003) stated that, prey preference of dytiscids beetles are known to vary under natural and artificial conditions. In natural conditions, dytiscids beetles of the genus *Hydroporus*, *Ilybius*, *Colymbetes*, and *Rhantus* were found to lower the populations of mosquito larvae while under laboratory conditions, mosquito larvae were less preferred than other prey type like Daphnia by those dytiscids beetles. Also some authors suggested that the nutritional quality of a prey, or the specific nutrient needs of a predator, could explain predator preference for some preys (Krebs and Davies, 1994). It seems that, the choice of prey may differ with the community representatives, as far the dytiscids beetles are concerned. Overall, the present study suggests that, assessment of predation of *C. pipiens* larvae by the dytiscid beetle, *E. sticticus* under such natural circumstances would help to substantiate its role in the aquatic communities.

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