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Description and Factors Affecting Activities of *Gammarus* sp., a Crustacean Predator of *Culex pipiens* Mosquito Larvae in Egypt

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ABSTRACT

A species of a fresh water amphipod, *Gammarus* sp., collected from Wadi El-Rayan, Fayoum governorate, Egypt was described. Predation rate of *Gammarus* sp. on mosquito larvae was calculated. *Gammarus* sp. was found feeding on second instars more than first ones and it didn't feed on 3rd or 4th instars mosquito larvae. Effect of light and aeration on the survival of *Gammarus* sp. was also tested. Light had no significant effect on the survival of *Gammarus*, while aeration significantly increased its life period under laboratory conditions.

Key words: *Gammarus* sp., *Culex pipiens*, Mosquito larvae, Predation, Activities.

INTRODUCTION

Genus *Gammarus* is represented by more than 200 species worldwide (Vainola *et al.*, 2008). *Gammarus* sp. is widespread throughout a diverse range of fresh water habitats and can be the dominant part of many benthic macroinvertebrate assemblages, in terms of both numbers and/or biomass. In general, *Gammarus* is much more active at night than during the day hours. It crawls and walks using its legs in addition to flexing its whole body. When *Gammarus* swims, it often rolls over on its side or back that gives it the name of side-swimmer.

Gammarus sp. exhibited wide variations in physiochemical tolerances, habitat requirements, abilities to invade and susceptibility to replacement (Macneil 1999). The effect of different environmental factors on habitat selection in *Gammarus* sp. is well documented in literature. Henry and Danielopol (1999) proved that *Gammarus roeseli* actively selects habitat based on two important environmental variables; dissolved oxygen concentration and direction of water flow.

All *Gammarus* spp. are omnivores and detritivores. They feed on living plant and animal organisms such as fungus, bacteria and algae. They also feed on decaying matter, or detritus. Although many studies have emphasized fish predation on *Gammarus* spp., they themselves preyed upon juvenile and wounded/trapped fish (Macneil *et al* 1997). Few studies have been carried out on *Gammarus* predation on mosquito larvae. Roberts (1995) examined the predatory potential of *Gammarus duebeni* to investigate its effect as predator of mosquito larvae. Mature gammarids ate 4-8 *Aedes detritus* larvae in 24 hours.

In the current study, the effect of *Gammarus* sp. as a predator of mosquito larvae and its feeding preference on different larval instars was examined. Also, the effect of aeration and light on its survival under laboratory conditions was tested.

MATERIALS AND METHODS

1- Collecting and preparing crustacean samples

Water Samples were collected from the pools of Wadi El-Rayan, Fayoum governorate, Egypt. All samples were taken from a spot on the shore that contains aquatic plants all year round. A small bucket tied from the handle with long rope was used to take the samples. All samples were taken after sunset. Water samples were poured into plastic bottles, transported instantly in an ice box to the laboratory and kept in aluminum dishes. Samples were kept at room temperature (27 °C) and 16:8 light and dark cycle. Aquatic plants which were found in the pool were taken in plastic bags and placed with the samples. *Gammarus* individuals were isolated from these plants before starting any experimental work. Larvae of *Culex pipiens* mosquito were provided as food source for the *Gammarus* individuals during experiments.

Some specimens were cleared in Nesbitt's solution and mounted in Hoyer's medium (Krants, 1978). Different stages of *Gammarus* were examined under light microscopy for morphological details. All measurements were taken with a micrometer slide and a lens. Nomenclature for morphological characters follows that of Hou and li (2002).

2- Predation rate of the *Gammarus* sp. on *Culex pipiens* larvae

This experiment consisted of ten replicates and conducted for eight consecutive days. Rounded plastic containers of 10 cm diameter were used as replicates. Each container was provided with five crustaceans and 100 mosquito larvae (25 mosquito larvae from each larval instar). All containers were filled with natural pool's water from the field, kept at room temperature (27 °C) and were exposed to a 16:8 h light and dark cycle. Fish-tank air pumps were used for aerating the containers. Dead *Gammarus* individuals were replaced by fresh live ones on a daily basis.

3- Effect of aeration on the survival of *Gammarus* sp.

Two treatments, each with ten replicates were used in this experiment. Each replicate consisted of one rounded 10 cm plastic container. Each container had 10 crustaceans and was provided daily with 50, 2nd instar mosquito larvae. All containers were kept at room temperature with a 16:8 h light and dark cycle. Containers of the first treatment were aerated by a fish-tank air pump while the others were kept without aeration. Surviving crustaceans were counted every 24 hours.

4- Effect of light on the survival of *Gammarus* sp.

This experiment was divided into two treatments, one was kept at the dark and the other was kept at a normal 16:8 h light and dark cycle. Ten replicates were used for each treatment. Each replicate consisted of one rounded 10 cm plastic container filled with pool's water from the field, kept at room temperature (27 °C) and contained 10 crustaceans. Every container was provided with 50, 2nd instar mosquito larvae daily. Surviving crustaceans were counted every morning.

RESULTS AND DISCUSSION

Taxonomy of *Gammarus* sp.:
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Amphipoda
Suborder: Gammaridea
Genus: *Gammarus*

1- Description of *Gammarus* sp.
Holotype- male

Collected from Wadi El-Rayan, Fayoum and deposited in the collection of the Faculty of Agriculture, Cairo University, Department of Zoology and agricultural Nematology, Giza, Egypt.

Antenna 1 (Fig. 2 B): Peduncles bearing few setae, length ratio of peduncular articles 1.0: 0.7: 0.5; primary flagellum 12-17 articulate, most articles with aesthetascs; accessory flagellum 3-4 articulate.

Antenna 2 (Fig. 2 A): Peduncle densely setose, article 3 with one group of setae on anterior margin. article 4 and 5 subequal in length, article 4 with two groups of setae on outer margin, one group of setae on the middle of anterior margin, one group of setae on inner margin and one group of setae on the inner face. Article 5 with two groups of setae on the inner margin, one group of setae on the outer margin, one group of setae on the middle of anterior margin and two groups of setae on the inner face. The flagellum 9-10 articulates each article with 2-3 groups of setae.

Gnathopod 1 (Fig. 1 B): Basis with long setae on posterior margin and short setae on anterior margin, bearing stiff setae distally; carpus trapezoid shaped; propodus about 1.3 times of carpus in length, carpus and propodus with some groups of distally serrate setae, palm of propodus weakly oblique bearing 4 spines on posterior corner; dactylus bearing 1 seta on outer margin.

Gnathopod 2 (Fig. 1 A): A little larger than gnathopod 1; basis with long marginal setae, carpus triangular shape with sub-parallel anterior and posterior margin, with 11 groups of setae; propodus 1.2 times as long as carpus with 8 groups of setae along posterior margin and 5 groups of distal setae; and two groups of setae on anterior margin, palm transverse, bearing 4 spines and one little spine and 12 small spines on posterior corner, dactylus with 1 seta on outer margin.

Pereopod 3 (Fig. 3 B): Slender. Article two-three with long marginal seta; article 4 with 4 groups of long setae on posterior margin and one group of setae on anterior margin, article 5 with 3 groups of setae and 3 spines on posterior margin and one group of setae on anterior margin. Article 6 with two groups of setae and one spine on posterior margin and one group of setae on anterior margin, dactylus curved bearing one seta on outer margin

Pereopod 5 (Fig. 3 A): Coxal gills small and kidney shaped. Article 2 with few setae and 6 spins, article 3-6 with some groups of setae and spines. Dactylus with 3 setae.

Urosomites (Fig. 4 A): Without humps dorsally. Urosomite 1 with few spines and few setae, urosomite 2 with two groups of spines and a single spine. Urosomite 3 with 10 groups of spines, 3single spines and 11setae.

Uropod 3 (Fig. 4 B): Cone shape with many simple setae and, peduncle with 2 pairs of spines on outer and inner margin respectively. Inner and outer ramous equally in length. Both two articulates with 3 groups of spines and few setae.

Pleopdes (Fig. 4 C): Subequal in length, peduncle

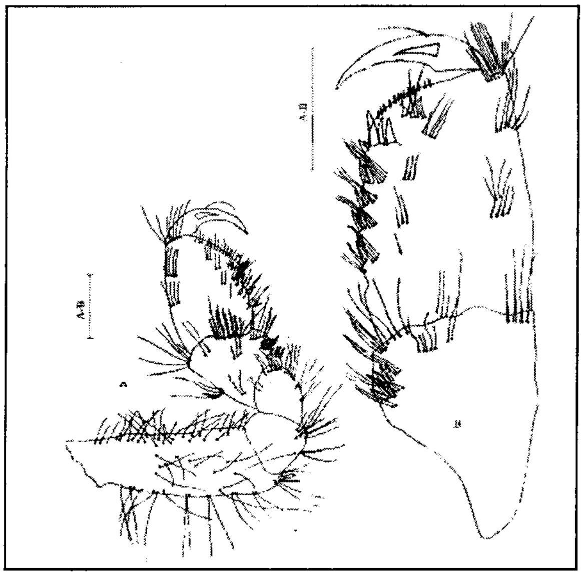

Fig. (1): *Gammarus sp.*, A. gnathopod 2, B. gnathopode 1. Scale: A-B = 0.01 mm.

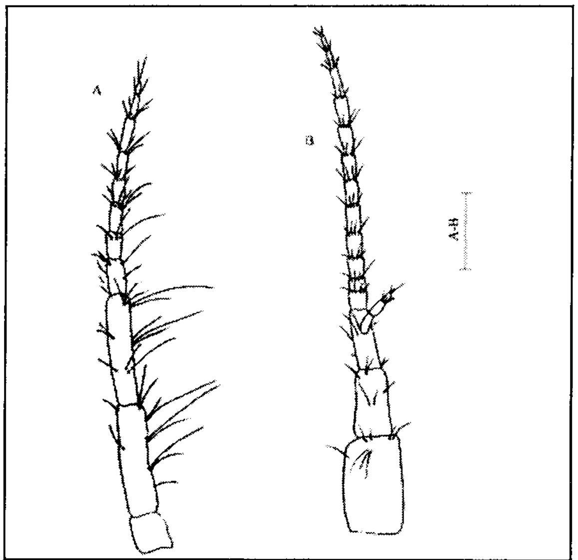

Fig. (2): *Gammarus sp.*, A. antenna 2, B. antenna 1. Scale: A-B = 0.01 mm.

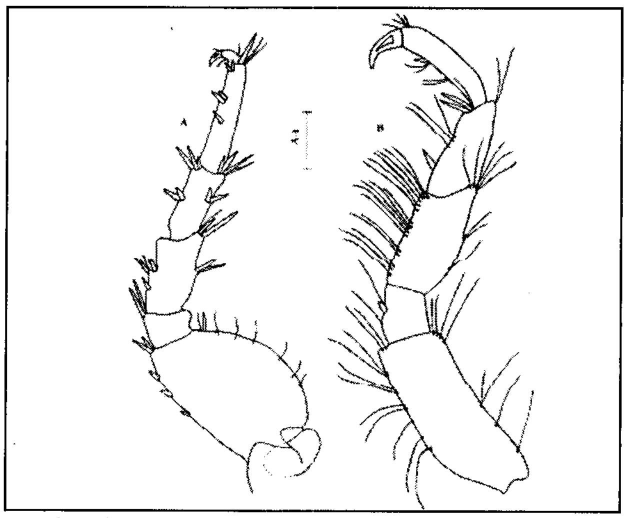

Fig. (3): *Gammarus sp.*, A. pereopod 5, B. pereopod 3. Scale: A-B = 0.01 mm.

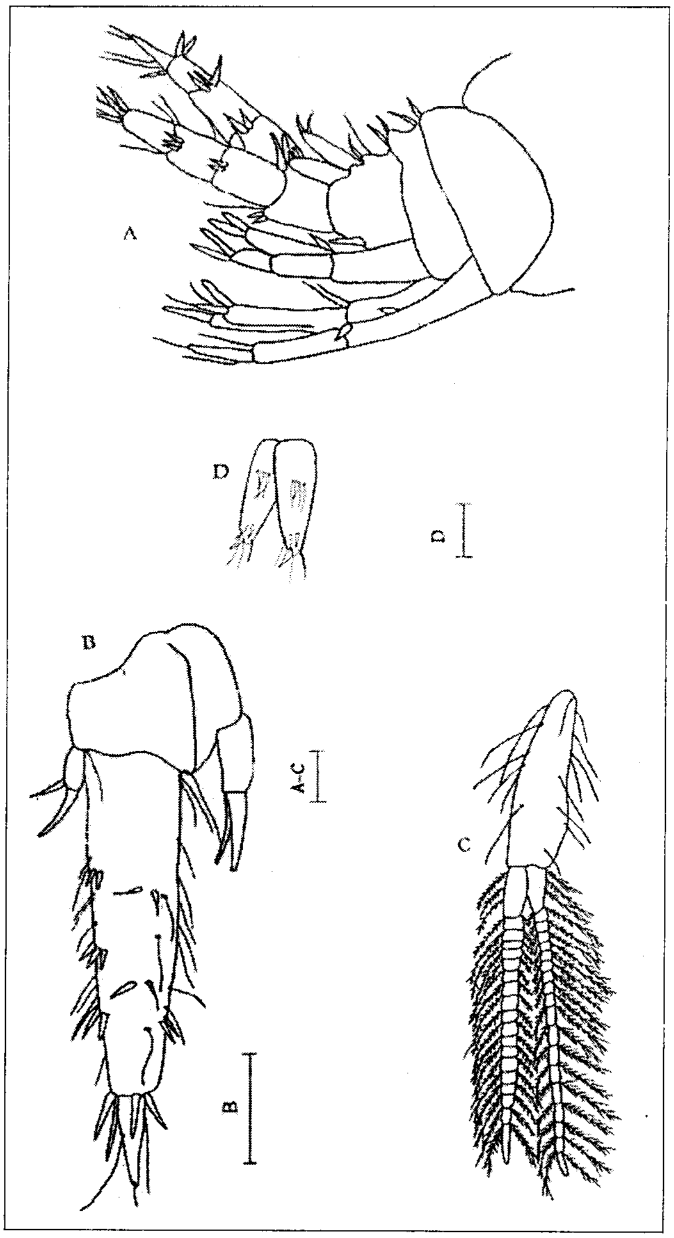

Fig. (4): *Gammarus sp.*, A. urosomites 1-3 (lateral view), B. uropod 3, C. pleopd. Scale: A-B = 0.01 mm.

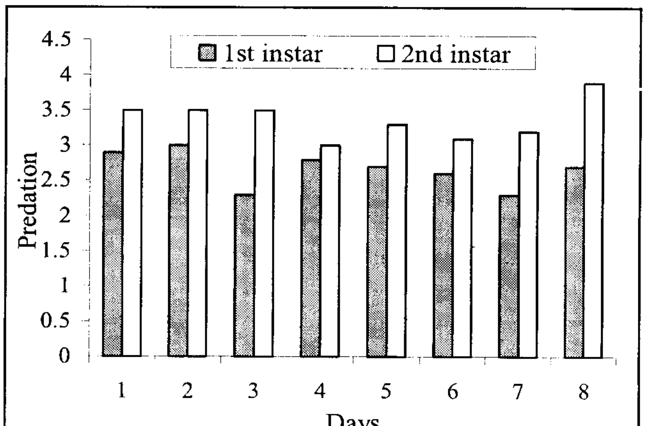

Fig 5: The predation of *Gammarus* sp. on mosquito larvae.

with 15 setae. Rami with 19 articles fringed with plumose setae.

Telson (Fig. 4 D): Cleft deeply, longer than wide. Each lobe bearing one distal spine accompanied by two long setae. Dorsal surface with one spine and three long setae on the upper part and one spine and two long setae on the lower one.

2- Predation rate of the *Gammarus* sp. on *Culex pipiens* larvae

Fig. (5) shows that the highest predation rate (4 larvae/ day) was on the 8th day, with the larvae being 2nd instars, while the lowest (2 larvae/ day) was recorded when it fed on the 1st instars. From Table (1), it can be noticed that there were significant differences between the predation rates in the case of 1st and 2nd instars. The results determined that *Gammarus* individuals preferred to feed on the 2nd instar larvae than the 1st ones. On the other hand, they didn't feed on the 3rd or 4th instars may be due to their large size. There were no significant differences among predation rates all over the days and *Gammarus* individuals fed on almost the same number of larvae every day.

In the study of Roberts (1995), *Gammarus duebeni* fed on 4-8 *Aedes detritus* larvae/ 24 hours. In the present study, *Gammarus* sp. fed only on 2-4 *Culex pipiens* larvae/ 24 hours. Different larval stages were offered to determine the most preferred stage for predation.

Gammarus individuals fed on the 2nd instar larvae of mosquito more than 1st instars while they didn't feed on 3rd or 4th instar larvae. First instars of mosquito larvae are very small and not very active which made them very hard to spot by predators. On the other hand, 2nd instars are bigger and more dynamic which is thought to be the reason why *Gammarus* individuals preferred them and *Gammarus* could spot them easier in the water and the probability to meet them is higher than the 1st instars. Wise de Valdez (2007) showed that mosquito infection with mermithid nematodes reduced the risk of predation by the mosquito *Toxorhynchites rutilus*, a predator of mosquito larvae. The avoidance behavior of mosquito larvae to predation by *Toxorhynchites rutilus* was explained by a decrease in larval activity due to infection with mermithid nematodes. The idea from the late data agree with this study that predators like *Gammarus* sp. would feed more on less active preys such as mosquito larvae.

Gammarus individuals didn't feed on any of the 3rd or 4th instar larvae. These instars are bigger in size and stronger than 1st and 2nd instars, this makes them more vigorous and resistant to infection with pathogens and not easy to be handled by small predators such as *Gammarus* sp. and they usually avoid feeding on them.

In nature, *Gammarus* is omnivorous but it might have a significant predation potential against mosquito larvae in water bodies that are poor in aquatic plantations.

3- Effect of aeration on the survival of *Gammarus* sp.

Fig. (6) shows that aeration was very important for the survival of *Gammarus* individuals. The rate of survival declined from 100% to 0% through a period of 21 days, when using fish-tank air pumps to aerate the water, while the survival rate was zero in the 8th day, when the air pumps were not used. Table (2) shows highly significant differences when testing the effect of aeration on the survival of the *Gammarus* individuals.

Gammarus individuals survived for a longer period in the containers supplied with aeration source. This proved the hypothesis that aeration would increase the life period of the individuals in

Table (1): ANOVA for the predation of *Gammarus* sp. on mosquito larvae

Variables	d.f.	*F*	*P*
Larval instars	1	36.41*	0.000
Days	7	1.51	0.1683
Larval instars × Days	7	1.13	0.3424
Error	144		

* Significant

Table (2): ANOVA for the effect of aeration on the survival of *Gammarus* sp.

Variables	d.f.	*F*	*P*
Aeration	1	3781.890*	0.000
Days	20	629.520*	0.000
Aeration × Days	20	94.7309*	0.000
Error	378		

* Significant

Table (3): ANOVA for the effect of light on the survival of *Gammarus* sp.

Variables	d.f.	*F*	*P*
Light	1	0.48*	NS
Days	7	330.97*	0.000
Larval instars × Days	7	0.22*	Ns
Error	177		

* Significant

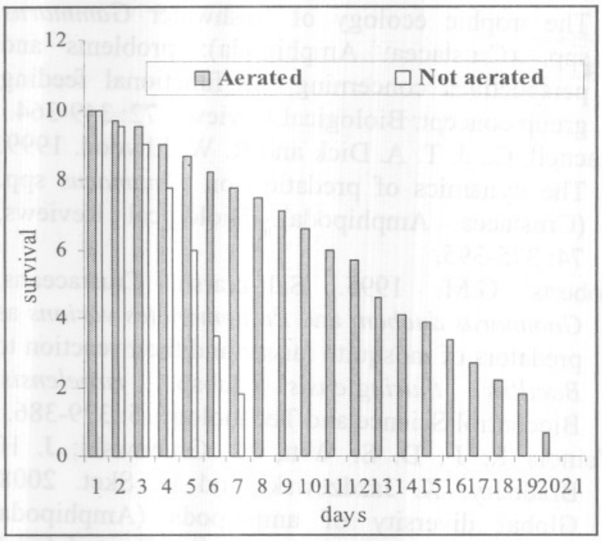

Fig 6: The effect of aeration on the survival of *Gammarus* sp.

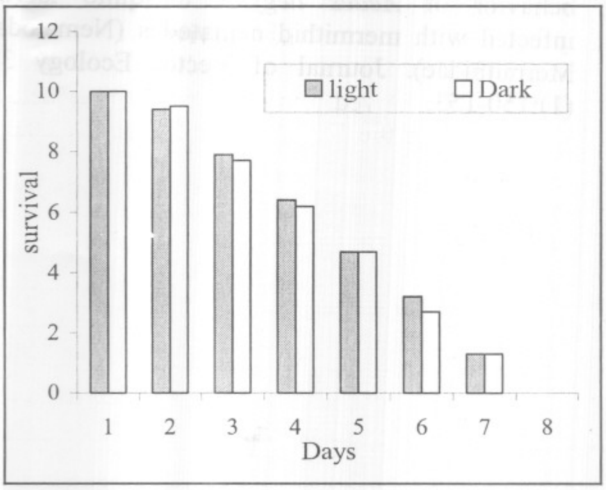

Fig. (7): The effect of light on the survival of *Gammarus* sp.

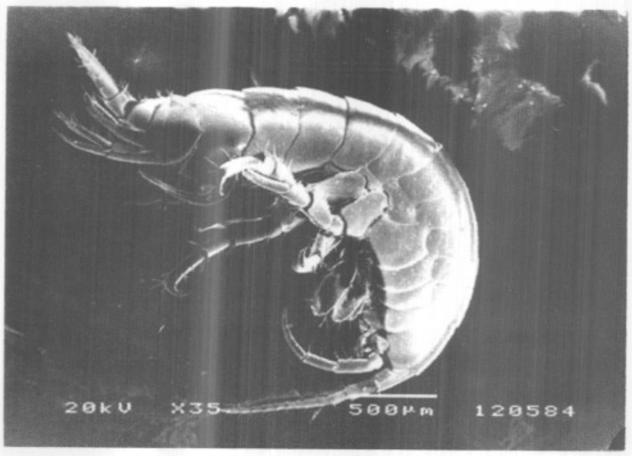

Fig. (8): lateral view for the full body of *Gammarus* sp. using Scanning Electron microscope (SEM).

the laboratory. Aeration continuously provides oxygen in the water which is vital for most living organisms to maintain their life. *Gammarus* which is not equipped with air syphons like the ones mosquito larvae possess, can't reach the air outside the water to acquire its needs of oxygen. In nature, *Gammarus* sp. consumes the dissolved oxygen in the water generated by continues water movement. Henry and Danielopol (1999) proved that dissolved oxygen concentration was one of two important environmental variables that affect habitat selection in *Gammarus roeseli*. In the laboratory, *Gammarus* individuals were maintained in small containers that lack water movement and therefore, they needed artificial aeration to provide them with dissolved oxygen for survival.

4- Effect of light on the survival of *Gammarus* sp.

As shown in Fig. (7), light had no effect on the survival of *Gammarus*. Table (3) shows no significant differences between the survivals rates of individuals kept either in the dark or in a normal light and dark cycle (16:8 h) and both treatments had the same mortality rate. All individuals from the two treatments died after day 7.

In nature, *Gammarus* lives in a balanced dark-light cycle but it hides among aquatic plants and decreases their activities during day light. This made us test the hypothesis that light may have a negative effect on these crustaceans. In the laboratory, *Gammarus* individuals were maintained under 8:16 (L: D) hours photoperiod but unlike nature they were exposed to the light during the whole light period; in the laboratory, they didn't have a shelter such as aquatic plants to hide from the light. The main idea behind this experiment was that dark could have a positive effect on the survival of *Gammarus* in the laboratory but the experiment didn't show any significant differences in survival among individuals kept in the dark and those kept under normal light-dark cycle. Individuals in both treatments had almost the same life period. Data from this experiment proved that artificial light in the laboratory didn't have any negative effect on *Gammarus* survival. Natural day light, however, contains UV which could be the main factor negatively affects *Gammarus* individuals in nature.

Another hypothesis to explain the low activity of *Gammarus* during the day could be that *Gammarus* tries avoiding predators during the day. It reduces its activity level and attaches itself to aquatic plants as an attempt to hide from predators. If this hypothesis was correct, this would mean that light, in both nature and laboratory doesn't have any effect on *Gammarus*.

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