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Certain Bioecological Characteristics of the Predatory Water Bug, Sphaerodema (Diplonychs) Nepoides (Fabricius 1803) (Heteroptera: Belostomatidae) and its Medical Importance

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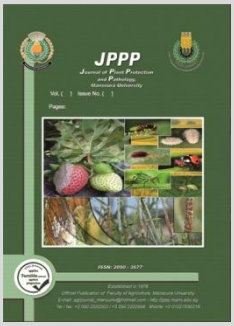

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ABSTRACT

The bioecological research conducted on the belostomatid predatory water bug, *Sphaerodema nepoides* showed that its studied habitat included 31 taxa as 9 different species from 5 families in Heteroptera, 16 species from 2 families in Coleoptera, 3 species from 3 families in Diptera, 2 species from 2 families in Odonata, and one species from Baetidae in Ephemeroptera. The feeding habits, mating behaviors, and egg-laying processes were examined. The egg stage was characterized by an incubation duration of 11 ± 1.8 days. The average lengths of the immature feeding instars were recorded as 3.1, 3.6, 5.1, 6.2, and 15.9 days for the first, second, third, fourth, and fifth instar nymphs, respectively. The nymphal stage was completed within a range of 26 to 39 days, with a mean duration of 33.9 days. The lifespan of males and females was found to be 320 ± 30.2 days and 311 ± 28.8 days, respectively. Fecundity averaged 2120 ± 66.5 eggs per female, distributed across 21 ± 7.5 egg masses, which were laid on the backs of the males. In terms of disease vector potential, microscopic analysis of air-dried smears from the salivary glands of wild-caught adults and nymphs of *S. nepoides*, stained with carbol fuchsin, revealed no presence of the bacterium *Mycobacterium ulcerans*, the pathogen responsible for Buruli ulcer disease. This disease is not endemic to Egypt.

Keywords: *Sphaerodema nepoides*, ecology, biology, vector, Buruli ulcer disease

INTRODUCTION

The belostomatid water bug, *Sphaerodema* (*Diplonychus*) *nepoides* (Fabricius 1803), is a predatory species on certain aquatic fauna members inhabiting various stable freshwater environments globally, particularly in rice fields, marshes, ponds, lakes, and rivers (Mukai *et al*. 2005). Its prey includes mosquito larvae and pupae, small fish, juvenile nymphs from the order Odonata, small snails and tadpoles (Victor and Ugwoke, 1987; Saha and Raut, 1992, Sivagnaname, 2009). Different biological aspects of *S. annulatum* were studied by Saha and Raut (1992). Females of *S. nepoides* lay their egg masses glued to elytra of the males. Thus, eggs are effectively aerated maintaining oxygen. Brood stroking is required for egg development and survival (Smith 1997). Moreover, it protected the eggs against natural enemies by male. Trumbo (2012) mentioned that eggs of water bugs is highly attacked by predators and parasitoids but are protected through parental care phenomenon. Parental care was studied in the giant water bug, *Kirkaldyia deyrolli* by Ohba and Maeda (2017). Males of *Lethocerus deyrollei* copulate several times with the same female before and between egg laying (Ichikawa, 1989). Otherwise, the females stopped oviposition and waited for the next copulation when males were experimentally removed. This result (Ichikawa, 1989) suggests that belostomatid females need to copulate before each oviposition bout. Since eggs fail to hatch without care of males, females have to detain males until end of sequential ovipositions. As in all species of family Belostomatidae, *S. nepoides* has five nymphal stadia. Molting takes place under water while nymphs attach themselves to water grass keeping the respiratory retractile organ penetrating the water surface for respiration. Sibling cannibalism was noticed in *S. nepoides* as in many of the belostomatid water bug as in *L. deyrolli* (Ohba *et al.*, 2006).

Belostomatid adults could fly and attracted to light. In central Ivory Coast, Duvirad (1974) stated that the flights of *S. neoides* present a well-marked lunar rhythmicity where 79% of the catches occur during the fortnight around full moon. In absence of their prays when the water habitat dry, adults fly attracted to lights in nearby housed areas and bite humans and animals seeking for nourishments. We found few adults in August swimming at the corners in the swimming pool at Faculty of Agriculture, Cairo University; may be due to dryness of their nearby habitat. Allai *et al.* (2019) studied the ecology of aquatic heteropterans in two ponds with endemicity different from Buruli ulcer disease also in the south of Cote d'ivoire (West Africa). In the same location, Conan *et al.* (2024) proved that the common Buruli ulcer disease caused by the bacterium *Mycobacterium ulerans* is transmitted to humans by bites of *S. neoides,* where they found the pathogen in aquatic heteropterans in urban rice fields. They added that cases of this disease have been reported in more than 33 countries with the main burden occurring in West and sub-Saharan Africa. If left untreated, infections can result in considerable skin scarring and disability. This skin condition progresses slowly, leading to destructive infections of soft tissues characterized by painless ulcers or plaques, with a marked absence of an inflammatory

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response (Fokou *et al.,* 2015). Buruli ulcer disease is a neglected tropical disease, continues to pose challenges to public health in endemic countries. The disfiguring ulcers may lead to permanent disability, and there may be significant socio-economic, stigma and mental health impact for patients (Phillips and Asiedu 2023). Early diagnosis of infection in humans followed a PCR specific test (Röltgen *et al.,* 2019). This disease is non-endemic in Egypt (WHO, 2023).

MATERIAL AND METHODS

Ecological studies:

The source of the insect material was an agricultural drainage canal containing nearly stagnant water, situated in the village of Abou Rawash, within the Giza governorate. Water was rich in vegetation especially grass and filament green algae. Twice a week, a metal net was used to sieve the water under grown algae blankets for collecting the different water insect adults and their immature feeding stages present underneath during the period from April 2024 till April 2025. Collected material was poured into a white enamel tray and the insects were separated from organic matter, picked out and placed into a plastic buckle half filled with water from the same habitat and transferred to the laboratory. Insect individuals were separated, and each was easily identified in the Biological Control Center at Faculty of Agriculture, Cairo University.

Biological studies:

Adults and nymphs of *S. nepoides* were placed individually in small glass aquaria (15 x 10 x 10 cm) and provided with algae and water grass. Grass upper parts should penetrate water surface to serve molting process of attached immature nymphs. Aquaria were provided daily each with accounted numbers of mosquito larvae and pupae as preys collected daily from the same habitat to calculate prey consumption and recording molt of the confined nymphs. Newly emerged adults were coupled in glass aquaria (30x25x25 cm) filled with water to a depth of 20 cm. Adult aquaria were covered with muslin textile to prevent their escape by flying. Mating takes place in the aquaria; female pre-oviposition period was calculated and eggs laid on elytra of males were counted. Egg incubation period, durations of the five immature nymphs, number of consumed preys were calculated. Predation and molting processes were described under summer and winter laboratory conditions of 25°C±1 and 14-20ºC±2, respectively. Also, a laboratory mosquito colony supported the needed prey individuals especially in winter.

Wild collected adults and nymphs of *S. nepoides* were placed in Saline solution for removing salivary glands. A direct smear from the salivary gland was prepared on glass slide, allowed to air dry, and then stained with carbol fuchsin. The smears were examined by light microscope under the oil lens for the presence of the bacterium *M. ulerans,* the causative agent of Buruli ulcer disease. This is the most used staining technique for detection for detection of acid-fast bacilli (AFBs) and diagnosis of different mycobacterial agents and known as Ziehl-Neelsen (ZN) method based on carbol fuchsin staining (Röltgen *et al.,* 2017).

RESULTS AND DISCUSSION

The Collected Water Insect Fauna

Data presented in Table 1 indicates the presence of 31 taxa, which comprise 9 distinct species from 5 families within Heteroptera, 16 species from 2 families in Coleoptera, 3 species from 3 families in Diptera, 2 species from 2 families in Odonata, and a single species from the Baetidae family in Ephemeroptera.

Table 1. Collected aquatic insects from the research area in Abou Rawash village, Giza

Order	Family	Species
Hemiptera	Belostomatidae	*Lithocerus niloticum* Stal.
		Limnogeton fieberi Mayr.
		Sphaerodema nepoides (Fabr.)
	Nepidae	*Ranatra vicina* Sing.
	Notonectidae	*Anisops sardea* H.S.
	Corixidae	*Coryxa hieroglyphica* Dut.
		Micromecta plicata Costa
	Hydrometridae	*Hydrometra aegyptiaca* Hung.
	Gerridae	*Limnogonus aegyptiacus* Fut.
Coleoptera	Dytiscidae	*Cybister tripunctatus* Cast.
		Eretes sticticus L.
		Hyphoporus sclieri Aubé
		Herophydrus guineemsis Aubé
		Herophydrus musicus Klug.
		Hydatiscus leander Rossi
		Laccophilus umbrinus Motsch
		Rhantus pulverosus Steph.
		Hydrovatus sordidus Sharp
		Bidessus signatellus Klug.
	Hydrophilidae	*Hydrous aculaetus* Sol.
		Ampliopslucida Er.
		Laccobius leucaspis Kies..
		Sternolophus solieri Cast.
		Octhebius seriocus Mols.
		Enchorus bicolor F.
Diptera	Chironomidae	*Chronomus* sp.
	Culicidae	*Culex pipiens* L.
	Syrphidae	*Eristalis* sp.
Odonata	Coenagrionidae	*Ischnura senegalensis* Rambur
	Aeshnidae	*Hemianax ephippiger* Burmeister
Ephemeroptera	Baetidae	Cloeon sp.

Various authors have identified a range of water insect species across different countries. Duvirad (1979) collected five belostomatid species by light traps located 3 km away from a small artificial lake in central Ivory Coast, where only *S. nepoides* was abundant. In the present study, the most abundant species were those of Belostomatidae, Notonectidae, Corixidae, Dytiscidae and Hydrophilidae, with S. *nepoides* was the most abundant. In West Africa, Allali *et al.* (2019) recorded 35 taxa, belonging to 9 families of heteropterans, at two study sites in Cote d'ivoire where the number of recorded taxa was 32 and 25 at pools in Sokrogbo and at Vieil Aklodj sites, respectively.

Feeding Habits

The main function of forelegs in *S. nepoides* is capturing its prey, meanwhile middle and hind legs are adapted to swimming. The raptorial forelegs are characteristic with a claw on the terminal tarsus segment which is crucial for capturing prey. Forelegs of the early three nymphal instars have highly curved claws enabling them to catch proportionally larger prey than older nymphs with less-curved claws. This observation is on line with those of Ohba *et al*. (2008). When young nymphs attack preys larger than themselves, they first insert the claws of their raptorial legs into the prey hooking it, and then use all their legs to fully embracing the prey body. Sometimes, the young nymphs are forced to capture prey larger than themselves when smaller preys are less abundant (Ohba *et al*. 2008). Older nymphs have an overall well-developed body size, but smaller prey are regularly attacked. Ohba and Tatsuta (2016) mentioned that all *Kirkaldyia*

deyrolli belostomatid nymphs can feed on prey of approximately 3cm in length and they alter catching technique according to changes in raptorial characteristics to enhance prey resources at each developmental stage. Victor and Ugwoke (1987) stated that *S. nepoides* is an ambush predator in aquatic habitats with abundant vegetation in Nigeria.

Mating and Egg Laying

Mating usually takes place 13-16 days after adult emergence (pre-mating period) in water. Saha and Raut (1992) in West Bengal, India recorded an average of 31.1± 0.99 days for *S. annulatum* to reach sexual maturity. During displayed courtship, the male of *S. nepoides* performs a pumping movement (push-up) display under the female and to the water surface. This behavior might secure more than one copulation simultaneously with the same female. Similar behavior was recorded by Kraus (1989) in the belostomatid *Abedus indentatus* (Haldeman). More studies are required for more understanding of mating behavior (Smiseth, 2014). The females are somewhat larger in size than the males. Accep;8ting the male courtship, the female let the male climbing onto her back embracing her with all his legs proceeding the mating process while their retractile respiratory organs contacting the air over water surface. The mating couple rest quite motionless during copulation. If, for any reason, they were disturbed, that the male was forced to release its hold, mating may continue on the water surface, or the couple may separate for a while to combine again when properly settled. Generally, the mating couple release themselves and get free drawing their terminalia and combined genitalia to their normal position at abdomen terminal. This suggests that mating in *S. nepoides* is practiced repeatedly after short intervals of 2-4 minutes. These interruptions may be due to lack of oxygen so that the insects need to store air. This view is supported by the action which the insects show in getting up to water surface and diving back under water while retracting their retractile organs and repeating the process several times before finishing copulation. Thereafter, the male repeated the pump-up display carrying the female on his back on which she embraces the male with all her legs. The female takes a sideway position as she fastens her eggs in form of a mass glued with a yellowish secretion onto the male's elytra. The male repeated pump-up display is effectively aerated the egg mass maintaining oxygen and is essential for egg development and survival (Smith 1997).

In the present study, males bearing egg masses are collected from water body of the study location during March, April, June and August 2024. They were not observed in this natural habitat during the period from October to the next spring in April 2025.

Egg Stage and Incubation Period

Eggs are oval in shape with shiny yellowish gray to yellowish brown color, arranged contacting each other in the egg mass by a yellowish secretion from the accessory gland of the female. By the time, they increase in volume by absorbing water through the hydropyle (Venkatesan and Rao 1980). Sometimes, few eggs at margin of the latest masses are seen to be smaller in size than the rest. Generally, the incubation period lasted between 9 – 12 with an average of 11± 1.8 days.

Immature Feeding Nymphs and Durations

The newly hatched nymph (first instar) is yellowish white in color; its integument is quite transparent showing the tracheae and heart, the compound eyes are pink. Picking it out of the water, the lateral edges of the body are bent ventrally; placed it again in water, surface of these lateral edges straighten holding air underneath over the spiracles as reservoir for respiration. Nymphs increase in size after each molting and became pale brownish in color reaching the final size at the fifth nymphal instar. When fed on mosquito larvae and pupae, the first nymphal instar lasted 3-5 days with a mean of 3.1 day as shown in Table 2. Second, third, fourth and fifth nymphal stadia lasted 2-5, 4-6, 5-7, and 12-16 days with means of 3.6, 5.1, 6.2, and 15.6 days, respectively. The total nymphal period accounted 26-39 days with a mean of 33.9 days (Table 2).

Table 2. Durations (/days) and individual numbers (n) of *Sphaerodema nepoides* nymphal instars

Instars	Minimum	Maximum	Mean	n
First	3	5	3.1	80
Second	2	5	3.6	52
Third	4	6	5.1	44
Fourth	5	7	6.2	23
Fifth	12	16	15.9	16
Total duration	26	39	33.9	

Adult Longevity, Fecundity and Generations

The lifetime in *S. nepoides* from egg hatching till death of the male ranged between 290-352 days with an average of 320±30.2 days (Table 3). Life span of the female ranged between 281-320 days with an average of 311± 28.8 days. Saha and Raut (1992) reported a maximum lifespan for male and female of *S. annulatus* by 363 and 351 days, respectively with an average of 310.55± 4.30 days and the female survived for 293±7.63 days for the female.

Table 3. Adult longevity (/days) and female fecundity (number of eggs) in *S. nepoided* reared under laboratory conditions

	Longevity		Fecundity/female	
	Male	Female	Total eggs	No. egg masses
Min.	290	281	2111	19
Max.	352	320	2180	26
Mean	320	311	2120	21
SS	30.2	28.8	66.5	7.5

During its lifetime, female of *S. nepoides* laid 2120± 66.5 eggs distributed among 21±7.5 egg masses opposed to 2279.3±73.09 eggs in 19.0±0.26 egg masses in case of *S. annulatus* reported by Saha and Raut (1992).*S. nepoides* exhibited three generations annually. The adults of the final generation enter a state of quiescence towards the end of October, remaining inactive until the onset of spring (April), when mating occurs, oviposition takes place, and the first generation emerges.Nevertheless, nymphs from natural habitat were collected and reared in the laboratory until they reached the adult stage mostly in June. Males carrying egg masses of the second generation were kept in aquaria till egg hatching. The nymphs were reared on larvae and pupae of mosquitos and completed their life cycle by adults occurred in August. The last third generation was handled similarly in the laboratory among the period between September and the beginning of November where the adults again entered the quiescence period.

Testing for Buruli ulcer Pathogen in *S. nepoides*

The direct smears from the salivary glands of wild collected adults and nymphs of *S. nepoides* were prepared on glass slides, air dried and stained with carbol fuchsin. The smears were examined microscopically under the oil lens for the presence of the bacterium *M. ulerans,* the causative agent of Buruli ulcer disease. All tested 166 individuals showed negative results. This confirms the WHO report (2023) that this disease is non-endemic in Egypt.

CONCLUSION

The present study dealt with investigating the natural habitat of the belostomatid predatory water bug, Sphaerodema nepoides (F.) in awater body in Giza governorate, Egypt. The habitat harbored insect fauna from 31 taxa; 9 species from 5 families in Heteroptera, 16 from 2 families in Coleoptera, 3 from 3 families in Diptera, 2 from 2 families in Odonata, and one from Baetidae in Ephemeroptera. Feeding habits, mating and egg laying wree described. Egg stage was described; and incubation period, duration of the five nymphal instars were determined, as well as lifespan of both sexes, fecundity and number of generations. As vector of the Buruli ulcer disease, caused by the bacterium Mycobacterium ulerans, microscopic examination of direct smears prepared from salivary gland of wild collected nymphs and adults, stained with carbol fuchsin, showed no presence of the pathogen; the disease is not endemic in Egypt.

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بعض الخصائص الحيوية البيئية لحشرة بق الماء المفترسة سفيروديما (Heteroptera: Belostomatidae) وأهميتها الطبية

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الملخص

تناولت الدراسة الحالية فحص بيئة المسكن للبقة البيلوستوماتيدية المفترسة *Sphaerodema nepoides* (F.) وبعض جوانبها الإيكوبيولوجية في منطقة أبو رواش بمحافظة الجيزة، وأهميتها الطبية كناقل للبكتريا *Mycobacterium ulerans* المسببة لمرض التقرح الجلدي Buruli ulcer. وتم تعريف ٣١ نوعا حشريا في البيئة المائية , تضمنت ٩ أنواع من ٥ عائلات لرتبة نصفية الأجنحة، ١٦ من عائلتين لرتبة غمدية الأجنحة، وثلاثة من ثلاث عائلات لرتبة ذات الجناحين، وإثنان من عائلتين لرتبة الرعاشات، ونوع واحد لعائلة من رتبة ذباب مايو. كما تم وصف العادات الغذائية للمفترس، والتزاوج، ووضع البيض. كما تمت دراسة طور البيضة وحساب طول فترة حضانتها حتى الفقس، وطول فترات الأعمار الحورية الخمس، ومدة حياة كل من الذكر والأنثى، وخصوبة الإناث، وعدد أجيال المفترس في السنة. وكناقل لبكتريا التقرح الجلدي، تم اختبار تواجد مسبب المرض في الغدد اللعابية للحوريات والكوامل البرية بالفحص الميكروسكوبي لمسحات مباشرة من الغدد اللعابية مصبوغة بالكريول فوكسين لمشاهدة البكتريا، وجاءت جميع الفحوصات سالبة بعدم وجود مسبب المرض مما يؤيد تقرير منظمة الصحة العالمية (٢٠٢٣) بأن المرض ليس مستوطنا في مصر.