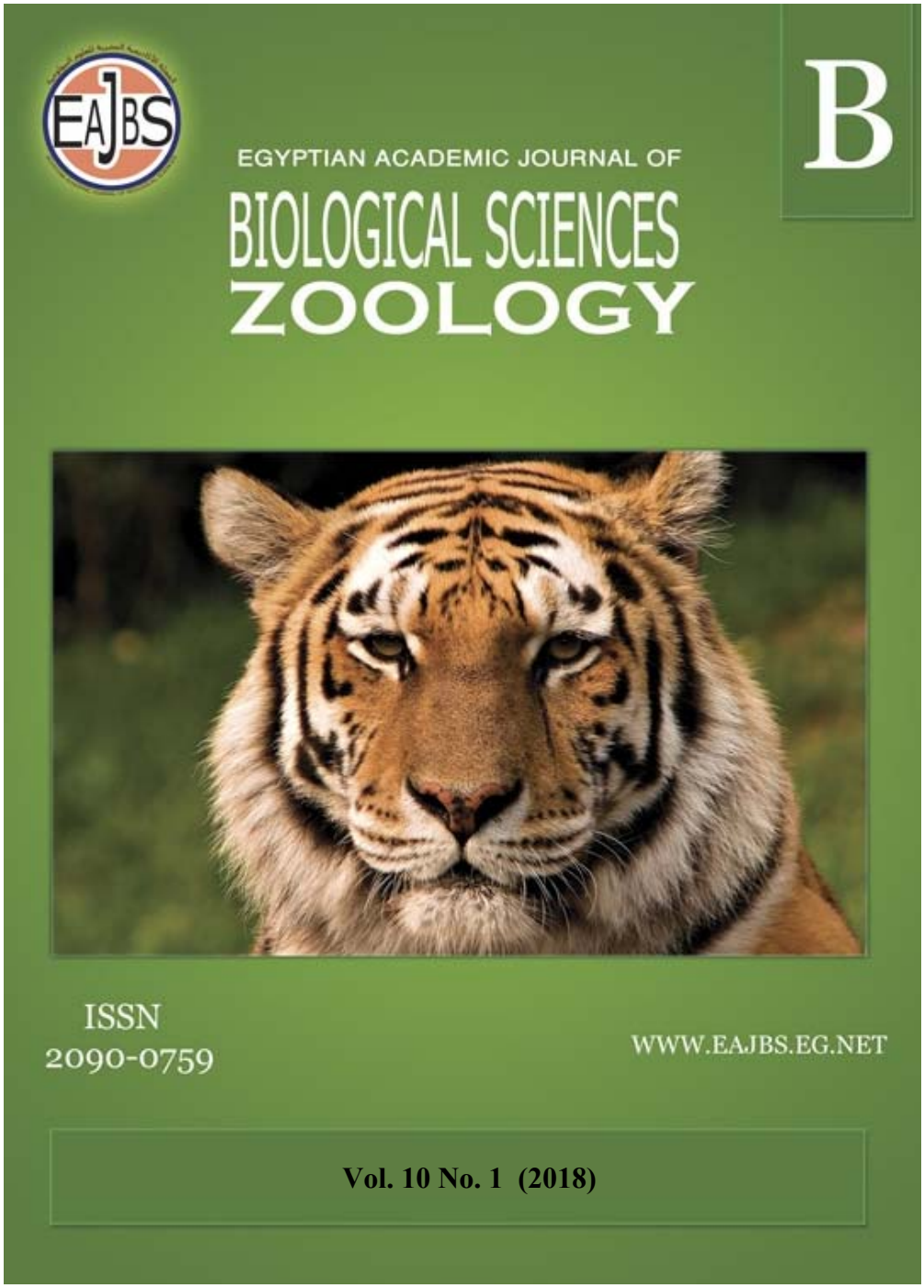

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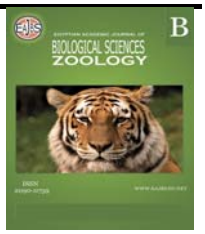

Seasonal variations in abundance and sex ratio of the freshwater crayfish *Procambarus clarkii* from four freshwater localities, River Nile, Egypt

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ABSTRACT

A total of 693 specimens of the freshwater crayfish, *Procambarus clarkii* were collected from four sites chosen along the River Nile, at Helwan (Site #1), El Warraq (site #2), El Rahawy (Site #3) and El Rayah El Mounofy (Site#4) during the period from summer 2014 to the following spring 2015. Most individuals of the collected specimens were characterized by hard-rigid outer exoskeleton, with dark-red, or deep brownish color carapace. But some specimens had old green or grey color carapace with scars and epibiotes. The collected specimens showed spatial and temporal variations, recorded 242 specimens (34.92% of all) from El Rayah El Mounofy, declined gradually into 173 (24.96%), 163 (23.52%) and 115 (16.59%) at El Warraq, El Rahawy and Helwan, respectively. Seasonally, the highest number of individuals was 266 (29.4%), collected in spring, of them 115 were obtained from El Warraq, but declined gradually into 193 (28.9%) in summer, and 183 (24.3%) in autumn and reached the minimum number (51 individuals, 17.3%) in winter. The present results also showed that, no specimens were collected from Helwan during winter, and only 3 individuals were obtained from El Warraq, compared with 17 and 31 specimens collected from El Rahawy and El Rayah El Mounofy, respectively. In contrast, during summer, only 11 specimens were collected from El Rayah El Mounofy. The fluctuations in number of collected individuals of this species were greatly correlated with increasing in water temperature and water level after winter enclosure. The general sex ratio was 1:0.47 males: females and showed significant statistical variations between sites and seasons, recorded 1:0.40, 1:49, 1:0.63 and 1:0.40, males to females at Helwan, El Warraq, El Rayah El Mounofy and El Rahaway, respectively. The present data showed that, the whole populations of this species were varied from 6.6 to 14.3 cm in total length, from 26.84 ±14.42 at Helwan to 34.48±22.5g at El Rahawy.

INTRODUCTION

The freshwater red swap crayfish, *Procambarus clarkii* (Girard, 1852), belongs to family Cambaridae. This species is distributed mainly in USA (Huner, 1995), but had invaded other countries including Europe, Australia and China (Huner *et al*., 1993). Out of over 400 species of the freshwater crayfishes belonging to the families Astacidae, Cambaridae and Parastacidae around the world, the red swap crayfish *P. clarkii* became the most common wide spread species (Huner and Lindqvist, 1995). This species had been introduced into the Egyptian freshwater systems (the River Nile and its drainage canals), during the early 1980's via a private fish farm (Ibrahim *et al*., 1995; Ibrahim and Khalil, 2009). During the last few decades of twentieth century, the introduced freshwater red swap crayfish, *Procambarus clarkii* became very common and widely distributed in main River Nile and its tributaries comprised canals and ditches from the south (Aswan) to the north Delta and even in north Sinai (Ibrahim and Khalil, 2009).

In spite of several attempts had been carried out to remove *P. clarkii* from its new habitats around the world and especially in Egypt because of its harmful effects on natural biodiversity and resident biota particularly native fish species, but all were failed. Consequently, most of these attempts concentrated to maximize its benefits as source of animal protein for human or used it as biological control against vector of intermediate host as snails of *Biomphalria*, *Physa* and *Bulinus* species which consider as vector for schistsomiasis or used as live bait, animal diet, or even as experimental animals in the class room in order to eliminate a considerable number of their population (Lodge *et al.,* 2000; Ibrahim and Khalil, 2009). This species can tolerate a wide range in differences in water qualities (Ibrahim and Khalil, 2009), and about 20-25% of the total body weight is edible meat, even other wastes (carapace, viscera and cephalothorax) have high protein sources, and can be used as fish meal (Agouz and Tonsy, 2003), or as food for egg producing and meat producing poultry (Rafaat, 2006).

In last decades of the twentieth century, *Procambarus clarkii* accounted at least 80% of all wild and cultured crayfish harvest around the world (Huner, 1989), and over 60,000 tons are produced annually in the USA and China (Huner *et al*., 1993). In contrast, only about 4.6 tons represented the annual yield in Egypt (Emam and Khalil, 1995). At the present time, this species became widely distributed in all freshwater systems (Ibrahim and Khalil, 2009), and represents a new natural protein resource with high nutritive value (Hamdi and Abd El Monem, 2006; Hamdi and Zaghloul, 2006). This species can be consumed by the Egyptian people as cheap food, with high protein (Mona *et al*., 1999; Rafaat, 2006; Ibrahim and Khalil, 2009), instead of the other high expensive marine shrimps and lobsters.

Recently, several studies were carried out on *P. clarkii* from the Egyptian freshwaters comprised studies of the effects of eye stalk ablation on molting cycle (Amer *et al*., 2015a), investigation changes in telson setae and uropods during different molt stages and substages (Amer *et al*., 2015b), and changes in the incubated egg of ovigerous females of the freshwater crayfish, *P. clarkii* during early autumn under laboratory conditions (Amer *et al*., 2015c). While physiological changes in organic components of hepatopancreas, muscles and haemolymph during molting stages were treated by Ghanem *et al*. (2016), and studies on the biometric relationships (El Sayed *et al*., 2015) and histological changes in cuticle structure and number of layers of carapace cuticle during different molting stages (Amer *et al*., 2016) as well as gatstroliths formation, biomineralization and X-ray micro analyzer (EDX) during molting cycle of the red swamp crayfish, *Procambarus clarkii* from

Egyptian freshwaters (Amer *et al*., 2017) were also treated. However, no detailed studies are available on the fluctuations in the abundance and sex ratios of this species at different localities subjected to variable human impacts.

Therefore, this study aims to throw light on the biometric relationships of *Procambarus clarkii* indicating the possibility of exploitation as mass stock resource for proteins from this invasive aquatic animal into the Egyptian freshwater.

MATERIALS AND METHODS

A total of 693 specimens of *Procambarus clarkii* were collected seasonally from the four chosen sites extending along the River Nile at Helwan, El Warraq, El Rahawy and El Rayah El Mounofy during the period summer 2015 to spring 2016. These individuals were collected by fishermen using Egyptian trap (Gobia) as demonstrated by Ibrahim and Khalil (2009). They were alive and transported immediately to the Hydrobiology Lab at National Institute of Oceanography and Fisheries, Al Kanater.

At the laboratory, all individuals were sexed and weighed to the nearest 0.1 gm using an electric balance with accuracy of 0.01 g after blotting excess water with absorbent tissues. The total body length, right chela length and abdomen length were measured with a Caliper Vernier with accuracy of 0.01 mm. Most of the collected specimens were sexually mature. They varied from 6.6 to 14.3 cm in total length, and ranged between 3.84 (female without right chela) and 68.275 gm in total body weight.

All notes on carapace case comprised hard or soft, color, texture, and epibiotes and scars occurrence were rerecorded. As well as notes on missing legs, presence of new buds and broken limbs were recorded.

The general morphology for immature and mature individuals was recorded, and sexual dimorphism was determined based on appearance of secondary sexual characters as demonstrated by Ibrahim and Khalil (2009). Based on sex determination, the general sex ratio for the seasonally collected specimens were determined as ratios of males to females according to Frutiger *et al*. (1999) and Ibrahim and Khalil (2009).

RESULTS AND DISCUSSION

A-General Morphology:

The fresh water crayfish, *Procambarus clarkii* (Girard, 1852) belongs to family Cambaridae. It is a freshwater crustacean, has lobster like (Plate 1). It is characterized by an outer hard exoskeleton, classified relatively on the dorso-lateral sides of cephalothoracic segments forming a carapace, which protects the body and makes it rigid. The carapace in most adult collected specimens has dark-red to deep brownish color with prominent whitish granules during mid- and late intermoult stage. In newly molt individuals with soft carapace, a bright brown or brownish color is prominent. However, old carapace had green or grey dull color with several scars and epibiontes.

The abdomen of this species also has the same carapace color but free from granules; while in very few obtained premature individuals, it has grayish, faint brownish or faint green color, with wavy dark lines without spots in few specimens. This species has relatively protruded triangular rostrum, characterized with spike-like protrusion extends over the head with two lateral spines or notches near its tip.

This species has five pairs of walking legs. The first three pairs are chelate, while the last two pairs are subchelate. The first pair of chelipeds is elongate, has

narrower pincers longer in males than females. The cheliped palm has rows of tubercles extend along the mesial margin. Males are sexually differentiated from females by presence of hooks on the ischia of the 3rd and 4th thoracic appendages (walking legs). The general characters of the present specimens are in well agreement with those mentioned by Ibrahim and Khalil (2009).

A B

Plate (1): Dorsal and ventral views of mature female (8.1cm in SL) of the freshwater *Procambarus clarkia* (After Amer, 2012).

B- Sexual Dimorphism:

In males, the first two pairs of abdominal appendages are modified for semen transfer in addition to presence of hooks on the 3rd and 4th pairs of periopods and used for male sexual dimorphism. While in females, the abdominal appendages called as swimmerets and used for egg incubation, in addition to presence of annulus ventralis between the 4th and 5th periopods and used for storage of semen. These characters are in agreement with those mentioned by De La Bretonne and Romaire (1989), Mubarak (2001), Ibrahim and Khalil (2009), Amer (2015), and Amer *et al*. (2016).

In spite of the collected number of *P. clarkii* individuals, no ovigerous females were detected during the present study due to collecting during late spring while ovigerous females appeared two weeks after emerging from burrows and commencement feeding according to Mubarak (1997) or retreating in their burrows according to Sommer and Goldman (1983) at late autumn.

C- Sex Ratio:

The results in Table (1) indicate that out of the collected 693 specimens of *P. clarkii* from the study sites, 471 (67.97%) were significantly characterized males compared with only 222 (32.03 %) distinct females. These data showed that males were overdominated females and was represented by general sex ratio of 1:0.47 males: females. The difference between sexes was very clear and has high statistical significant Chi –square (X^2=89.46, P< 0.01).

The sex ratio had similar pattern at all sites, characterized by low ratios of females than males and represented by: 1:0.40, 1:49, 1:0.63 and 1:0.40, males to females at Helwan, El Warraq, El Rayah El Mounofy and El Rahaway, respectively. These ratios are in well agreement with that estimated by Frutiger *et al*. (1999) on *P. clarkii* from Swiss lake and was 1.33: 1 males : females, but in contrast with that reported by Rafaat (2006) on the same species in Egypt and was 50% males and 50% females. Also, there were sharp seasonal fluctuations with high number for males (except only during summer at El Rayah El Mounofy) with statistical highly significant differences with Chi –square(X^2= 62.93, P <0.01).

The disappearance of females reflects their slow movement or low activity particularly at the main River Nile and its associated tributaries and large canals. The same results were obtained by Amer (2012), where 74 females were collected in El Kanater compared with 92 males, with sex ratio of 1:0.80 males to female. On the other hand, Ibrahim and Khalil (2009) stated that, most individuals of *P. clarii* inhabited shallow and small canals and were retreated to live in burrows 1-2 m deep and tunnels at low temperature below 16ºC throughout late fall winter and early spring, which reflects fluctuations in seasonal sex ratios. Huner and Barr (1991) stated that, the best ratio is 1:1 males: females, and when males become greater than females, the latter are damaged and their mortality is high.

D- Abundance of Collected Crayfish Specimens:

1- The Overall Abundance:

A total of 693 specimens of *P. clarkii* (Table 1) were collected from the four selected sites during this study. The given data show that there were remarkable spatial variations in the number of collected specimens. The highest number of individuals was 242 specimens representing 34.92% collected from El Rayah El Mounofy, followed by 173 (24.96%) from El Warraq, 163 (23.52%) from El Rahawy and only 115 (16.59%) from Helwan (Figure 1). The variations in specimens' number may be attributed to nature of site, water quality and effects of water currents. At Helwan and El Warraq (the main River Nile), the water current is strong in comparison with that at El Rayah El Mounofy (small canal) and water quality is good in comparing with that at El Rahawy Drainage.

2- Seasonal Variations in Abundance:

The numbers of obtained specimens was seasonally fluctuated. The highest number of individuals was 266 (29.4%), obtained during spring, of them 115 specimens (43.23 %) were collected at El Warraq. However, these individuals declined gradually into 193 (28.9%), and 183 (24.3%) during the following summer and autumn, respectively and reached the minimum number of 51 individuals (17.3%) in winter (Table 1 and Fig. 1). During winter, no specimens were detected at Helwan, and only 3 individuals were obtained at El Warraq, but they increased into 17 and 31 specimens at El Rahawy and El Rayah El Mounofy, respectively. On contrast, during summer the number of collected individuals was declined remarkably into 11 at El Rayah El Mounofy, in spite of it was high at other sites.

The fluctuations in number of collected individuals of this species were greatly correlated with water temperature and site's depth (Ibrahim *et al*. 1997; Habashy, 2004). Most of the collected species were collected from El Rayah El Mounofy and El Warraq during spring. This reflects either the availability for occurrence of this species in the former site or to increasing fishing activities particularly during spring at the latter one or increasing activity with increasing water temperature in spring as demonstrated by Ibrahim *et al*. (1997), and Ibrahim and Khalil (2009). However, the complete disappearance and low number of obtained specimens during winter are correlated with low temperature and escaping crayfish specimens in deep burrows which agrees well with data reported by Ibrahim *et al*. (1997) and Ibrahim and Khalil (2009). Ibrahim *et al*. (1997) mentioned that *P. clarkii* begun its activity with increasing water temperature to 22ºC during late March and associated with increasing water levels in different channels and ditches after the winter closure. The individuals of this species are emerged from their burrows and moved to all habitats to feeding on decomposing plant matter, snails and decayed fish.

3- Spatial Variation in Body Weight and Length:

The present data showed that the whole populations of this species were varied from 7.7-13.3, 6.6-14.1, 7.7-14.1 and 6.8-14.3 cm in total length, and averaged 26.84 ±14.42, 27.48±18.6, 34.48±22.5 and 27.52 ±17.22 gm at Helwan, El Warraq, El Rahawy, and El Rayah El Mounofy, respectively. It is obvious that, the highest average biomass was recorded at El Rahawy, but the lowest average was calculated at Helwan. These data are in agreement with those determined by Amer (2012) on *P. clarkii* collected from Al-Kanater which varied from 6.5 to 11.5 cm in total length and ranged between 10.38 and 56.34 g and averaged 30.01 g in total body weight. The increasing body weight at El Rahawy Drainage may be attributed to low density, availability of food resources and other environmental conditions as mentioned by Reynolds (2002).

Table (1): Number of seasonal specimens and sex ratios for *P. clarkii* collected during the present study(F=females, M=males).

Sites & sex Seasons	Helwan			El-Warraq			El-Rahawy			EL-Rayah El-Menoufy			Total
	F	M	Total	F	M	Total	F	M	Total	F	M	Total	
Summer	14	28	42	13	33	46	38	56	94	6	5	11	193
Autumn	13	42	55	3	6	9	7	6	13	14	92	106	183
Winter	0	0	0	1	2	3	4	13	17	10	21	31	51
Spring	6	12	18	40	75	115	14	25	39	39	55	94	266
Total	33	82	115	57	116	173	63	100	163	69	173	242	693
Sex ratio	**1:.0.40**			**1:49**			**1:0.63**			**1:0.40**			**1:0.47**

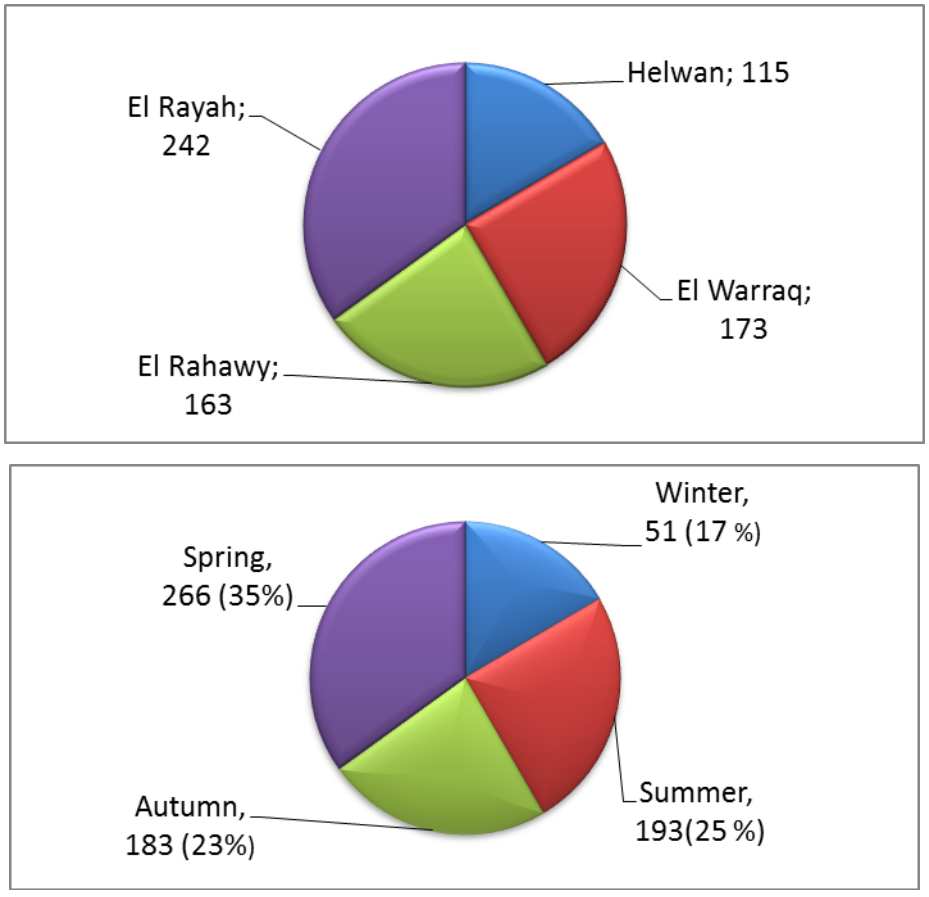

Fig. (1): Overall abundance at sites (upper) and during seasons (lower) of *P. clarkii* collected during the present study.

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ARABIC SUMMERY

التغيرات الموسمية والمكانية في الوفرة العددية والنسب الشقية وتكرارات الحجوم في استاكوزا الماء العذب، بروكامبارس كلاركي، بالمياه المصرية

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2- المعهد القومي لعلوم البحار والمصايد.
3- كلية العلوم للبنات، جامعة الأزهر، مدينة نصر، القاهرة.

تلقي هذه الدراسة الضوء على ديناميكية التغيرات الموسمية والمكانية في تجمعات عشائر استاكوزا الماء العذب " بروكامبارس كلاركي" بالمياه المصرية المجمعة من أربع مناطق مختلفة شملت حلوان، الوراق ، مصرف الرهاوي والرياح المنوفي، أظهرت النتائج تميز الأفراد الناضجة بهيكل خارجي صلب يتلون باللون الأحمر أو البني الداكن ودرقة أكثر صلابة تبدو أدكن قليلا من اللون العام للجسم، كما يتغير اللون إلى الأخضر أو البني حسب حالة الهيكل الخارجي والدرقة قبيل وأثناء وبعد عملية الانسلاخ. وتميزت الأفراد الناضجة بظهور حبيبات ذوات قمم بيضاء باهتة، خاصة في المرحلة البينية قبيل عملية الانسلاخ. وتشير نتائج النسب الشقية عامة إلى زيادة أعداد الذكور في جميع المناطق بنسبة 1: 0.47 ذكور إلى إناث، مع وجود اختلافات إحصائية موسمية ومكانية عالية، غير أنها سجلت نسب متقاربة جدا للنسبة العامة في المناطق المختلفة. كما أوضحت النتائج أيضا تجميع 694 عينة من هذا النوع خلال فترة الدراسة، منها 242 (34.92 %) من الرياح المنوفي، تناقصت تدريجيا إلى 173 (24.96%) ، 163 (23.52%) و 115 (16.59%) في الوراق، والرهاوي وحلوان، على التوالي. كما أختلفت أعداد الأفراد المجمعة موسميا حيث وصلت إلى 266 عينة (29.4 %) خلال الربيع منها 115 عينة من حلوان و11 عينة فقط من الرياح المنوفي، تناقصت تدريجيا إلى 193 (28.9%) في الصيف، 183 (24.3%) في الخريف حتى وصلت إلى أقل عدد (51 عينة) بنسبة 17.3 % تم الحصول عليها في موسم الشتاء، منها 17 من الرهاوي و 31 عينة والرياح المنوفي في نفس الموسم، مقابل عدد 3عينات فقط من الوراق وغيابها تماما في منطقة حلوان. وتشير نتائج الدراسة إلى الارتباط الوثيق بين عدد العينات المجمعة وارتفاع درجة حرارة المياه بدءا من موسم الربيع وخلال الصيف مع اختفاء معظمها في موسم الشتاء باستثناء الرياح المنوفي ومصرف الرهاوي. كما تشير الدراسة إلى تراوح العينات المجمعة في الطول الكلي من 6.6- 14.3 سم ، وتراوح متوسطات الأوزان من 26.84±14.42 في عينات حلوان إلى 34.48± 22.5 جم في عينات الرهاوي.