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DESCRIPTION, REPRODUCTIVE BIOLOGY AND ECOLOGY OF THE *SPHAEROMA WALKERI* (CRUSTACEA: ISOPODA) ALIEN SPECIES FROM THE TUNIS SOUTHERN LAGOON (NORTHERN TUNISIA, CENTRAL MEDITERRANEAN)

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ABSTRACT

Sphaeroma walkeri *Stebbing, 1905 is a non-indigenous species first recorded in the Tunis Southern Lagoon in 2002, where it is at present established as a result of the environmental restoration of this brackish area. Specimens of* S. walkeri *were collected monthly at 10 stations from February 2012 through January 2013. This species occurs in the Tunis Southern Lagoon throughout the year, but only reproduces during the warm period between April and October. From May 2012 onwards, the number of gravid females exceeded 50 % of the total sample, with a peak of 88.59 % in July 2012. Fecundity ranged between 13 and 26 eggs. Among the gravid females, a significant relationship between brood and total body length of specimens was only noted in the smallest (4.8–5.8 mm) and largest (8.8–9.8 mm) ovigerous females. In our sample, the females outnumbered the males. This isopod colonizes empty balanoid barnacle shells, ascidians and especially sponges.*

Keywords: bioinvasion, fecundity, habitat, Tunisian waters

DESCRIZIONE, BIOLOGIA RIPRODUTTIVA ED ECOLOGIA DELLA SPECIE ALIENA *SPHAEROMA WALKERI* (CRUSTACEA: ISOPODA) NELLA LAGUNA TUNISINA MERIDIONALE (TUNISIA SETTENTRIONALE, MEDITERRANEO CENTRALE)

SINTESI

Sphaeroma walkeri *Stebbing, 1905* è una specie *non indigena trovata per la prima volta nella laguna tunisina meridionale nel 2002. La specie vi si è stabilita in seguito al recupero ambientale di quest'area salmastra. Esemplari di* S. walkeri *sono stati raccolti mensilmente in 10 stazioni tra febbraio 2012 e gennaio 2013. Questa specie* è *trovata nella laguna meridionale tunisina durante tutto l'anno, ma vi si riproduce solo durante il periodo più caldo, da aprile a ottobre. Il numero di femmine gravide ha superato il 50 % del campione totale a partire da maggio, con un picco dell'88,59 % nel mese di luglio 2012. La fertilità* è variata tra *13 e 26 uova. Tra le femmine gravide, è stata trovata una relazione significativa tra covata e lunghezza totale del corpo degli esemplari solo per le femmine ovigere più piccole (4,8–5,8 mm) e quelle più grandi (8,8–9,8 mm). Nei campioni analizzati le femmine superavano in numero i maschi. Questo isopode colonizza il carapace vuoto dei cirripedi balani, ascidie e soprattutto spugne.*

Parole chiave: bioinvasione, comportamento, habitat, acque della Tunisia

INTRODUCTION

Sphaeroma walkeri is an isopod species commonly found in the Indian Ocean and the Red Sea among intertidal fouling communities, and reported in warm and warm-temperate waters (Galil, 2008). The species was first recorded in Tunisian waters, and the record took place in the northern brackish area, the Tunis Southern Lagoon (Ben Souissi *et al*., 2003).

While the original description by Stebbing (1905) was rather succinct, Jacobs (1987) represented *S. walkeri* anew and in much more detail. However, discrepancies found in the literature between several authors (Stebbing, 1910; Loyola e Silva, 1960; Mak *et al.*, 1985; Jacobs, 1987; Khalaji-Pirbalouty & Wägele, 2010) offer us an opportunity to provide a thorough description of the species in the present paper, based on the specimens collected from the Tunisian waters.

Isopods are considered as an important component of benthic communities and play a fundamental role in the ecosystems (Guarino *et al.,* 1993). Nevertheless, except for some morphological descriptions and checklists, there are no data concerning the biology and ecology of the species available. This paper represents the first contribution to enhancing the knowledge about the reproductive biology of *S. walkeri* and, consequently, about its behaviour in the Tunis Southern Lagoon. The results are useful to explain the establishment of this non-indigenous isopod in its new environment.

MATERIAL AND METHODS

Specimens of *Sphaeroma walkeri* were collected monthly in the Tunis Southern Lagoon. The lagoon adjoining the city of Tunis is located in the southwestern part of the Gulf of Tunis (36° 47′ N and 10° 17′ E) and divided into northern and southern areas by a navigation channel (Fig. 1). Both areas used to be heavily polluted (Zaouali, 1983; Ben Souissi, 2002), but have recently been rehabilitated with success (Jouini *et al*., 2005). Tunis Southern Lagoon extends over an area of 720 ha with a regular depth of about 2.1 m, except in restricted areas where it reaches a maximum of 4 m. It appears as an ellipse stretching in a SW-NE direction, between 36° 46′ 47″ and 36° 48′ 00″ N and 10° 12′ 22″ and 10° 16′ 41″ E. Its shores have been excavated and protected by large rocky stones.

The sampling conducted between February 2012 and January 2013, took place in ten stations (Fig. 1) situated in shallow, less than 3 m deep waters. In stations 1 through 6, characterized by muddy grounds, samples were obtained by dredging and scuba diving, whereas in stations 7 through 10, located in intertidal rocky shores covered by algae, the samples had to be collected manually from under the stones. Our investigations were conducted regularly between 2012 and 2013, at least twice a month for abiotic parameters, such as temperature, salinity, pH and transparency (measured with a Secchi disk), and monthly or seasonally for benthic communities. Historical data (1995–1997) for abiotic and trophic variables before the ecological restoration of the lagoon are also available (Ben Souissi, 2002).

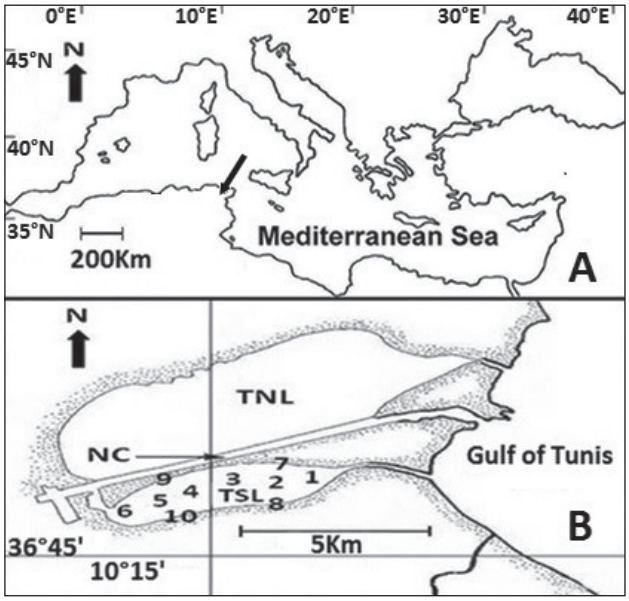

Fig. 1: (A) Map of the Mediterranean Sea, with the arrow pointing at the site of the Tunis Southern Lagoon, located in northern Tunisia. (B) Tunis Northern Lagoon (TNL), separated from the Tunis Southern Lagoon (TSL) by a navigation channel (NC). Numbers 1 to 10 denote sampling stations in TSL.
Sl. 1: (A) Zemljevid Sredozemskega morja z označeno Tuniško južno laguno v severni Tuniziji. (B) Tuniška severna laguna (TNL) je od Južne (TSL) razmejena s plovnim kanalom (NC). Številke od 1 do 10 označujejo vzorčevalne postaje v TSL.

Vegetation and benthic communities were delivered to the laboratory, sorted and rinsed with seawater prior to being filtered through a 0.47 mm sieve. Identification of specimens belonging to *S. walkeri* was confirmed using a binocular microscope. Samples were stored in a 70 % ethanol solution. Of the 1191 specimens examined, 801 were females. Appendages were dissected, mounted on slides in a glycerine solution for observation and drawing under a camera Lucida. The total length of females was measured to the nearest 0.01 mm by means of a micrometre scale. The eggs in the marsupia of ovigerous females were removed and counted. Monthly sexual activity was determined as the value (in %) obtained by dividing the number of gravid females by the total number of females (Guarino *et al.,* 1993). The relationship between the total body length of ovigerous females by size classes and number of eggs was estimated. The size of the smallest gravid female was taken as the definition of the size at the first sexual maturity (Garcia-Guererro & Hendrickx, 2005).

The identification of specimens and the terminology adopted in this paper follow the description and illustrations provided by Jacobs (1987).

The differences in size classes were tested by analysis of variance (ANOVA, $p < 0.05$). To explain correlations between ovigerous females and different environmental variables the method of 'Analyses of the Principal Components' was used. Data analyses and treatment were conducted by means of the Statgraphics Centurion software.

RESULTS

Environmental parameters

Prior to the lagoon's ecological rehabilitation, the average monthly salinity had ranged between 30.9 and 48.9, with a peak value of 51.9 registered in 1995. After the rehabilitation, it has been registered between 28 and 38.8 with a monthly average of 37.15. There have been no significant changes in the monthly and annual temperature values before and after the restoration.

Abiotic parameters recorded simultaneously with *S. walkeri* sampling showed that the average temperature was 20.72 °C with a minimum of 11.75 °C and a maximum of 26.4 °C (observed in February 2012 and July 2012, respectively). The mean salinity value was 37.05 with a minimum of 36.1 measured in January 2013 and a maximum of 38 in August 2012. The average pH was 8.02 (7.74 in December 2012 and 8.29 in July 2012). The mean transparency was 1.7 m (1.1 m in December 2012 and 2.1 m in July 2012).

Description of Tunisian *Sphaeroma walkeri*

Sphaeroma walkeri is easily identified among its congeners by the presence of numerous prominent tubercles running the entire dorsal face of the carapace. Its length may exceed 10 mm. This isopod is characterized by a net sexual dimorphism. Males are larger than females (Fig. 2A); by contrast, females are more convex (Fig. 2B). The surface of pereonites 1 and 2 is smooth, but pereonites 3 and 4 each have two irregular rows of low tubercles. Pereonites 5 and 6 and the pleon show one row of prominent round tubercles, additionally, the posterior edge of the pleon presents a second row of small round tubercles. The pleotelson is long and tapers to a rounded point that is slightly upturned with four rows of 3–6 tubercles along the surface. The tubercles of pleotelson are less prominent in females (Fig. 2B).

Antenna (Fig. 2C). Antenna with a five-segmented peduncle and flagellum of 14 to 16 articles. Each article of the flagellum bears a fringe of smooth setae at the distal interior angle. Adult males with these setae are more abundant, the seta measure up to 2–3 times the length of the articles. In females, these setae are shorter and less dense (Fig. 2E).

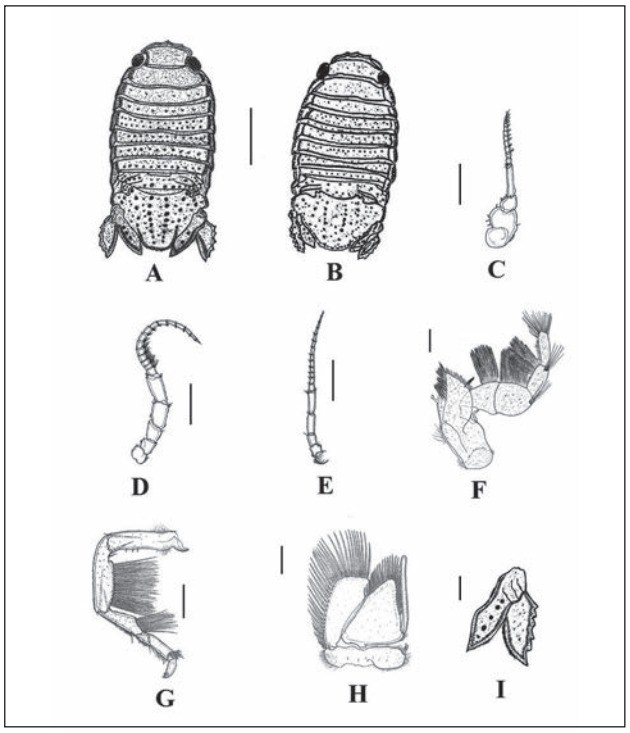

Fig. 2: *Sphaeroma walkeri* ***Stebbing, 1905. (A) Adult male in dorsal view, scale bar = 2 mm; (B) adult female, in dorsal view, scale bar = 2 mm; (C) male antenna, scale bar = 0.5 mm; (D) male antennules, scale bar = 0.5 mm; (E) female antenna, scale bar = 0.5 mm; (F) maxilliped, scale bar = 0.25 mm; (G) pereopod 1, scale bar = 0.5 mm; (H) male pleopod 2, scale bar = 0.5 mm; (I) male uropod, scale bar = 0.5 mm.***
Sl. 2: *Sphaeroma walkeri* ***Stebbing, 1905. (A) Odrasel samec s hrbtne strani, merilo = 2 mm; (B) odrasla samica s hrbtne strani, merilo = 2 mm; (C) samčeva antena, merilo = 0,5 mm; (D) samčeve antenule, merilo = 0,5 mm; € samičja antena, merilo = 0,5 mm; (F) maksiliped, merilo = 0,25 mm; (G) pereopod 1, merilo = 0,5 mm; (H) samčev pleopod 2, merilo = 0,5 mm; (I) samčev uropod, merilo = 0,5 mm.***

Antennule (Fig. 2D). With a three-segmented peduncle; article 3 is elongated, slender and about 3 times the length of article 2; flagellum 13-articled, articles 3-12 each bear aesthetascs extending to the posterior margin of pereonite 1.

Maxilliped (Fig. 2F). Endite wide distally, with some plumose setae set amongst fine simple setae in a semicircular distal margin, mesial margin with a single coupling hook, ventral surface with a row of about 19–20 long robust, plumose setae; palp articles 2–4 bearing dense fringes of long fine, plumose setae on superior margin, articles 3–4 with some slender fine, plumose setae on inferodistal angle.

Pereopods (Fig. 2G). Basis proximal superior margin with dense fine setae; ischium superior margin fringed

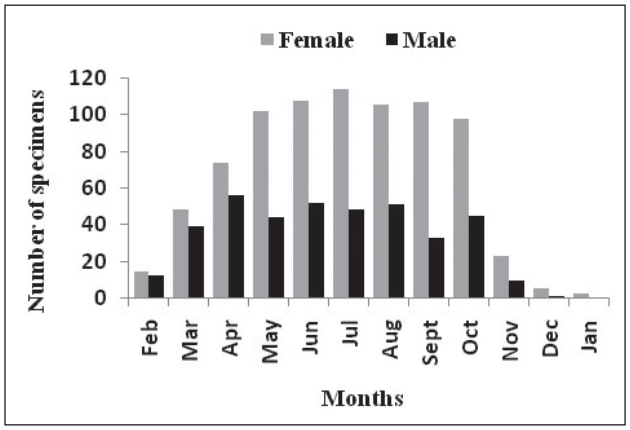

Fig. 3: Monthly distribution of male and female specimens of** S. walkeri **collected from the Tunis Southern Lagoon, February 2012–January 2013.
Sl. 3: Delež samcev in samic vrste** S. walkeri **iz Tuniške južne lagune po posameznih mesecih v obdobju februar 2012–januar 2013

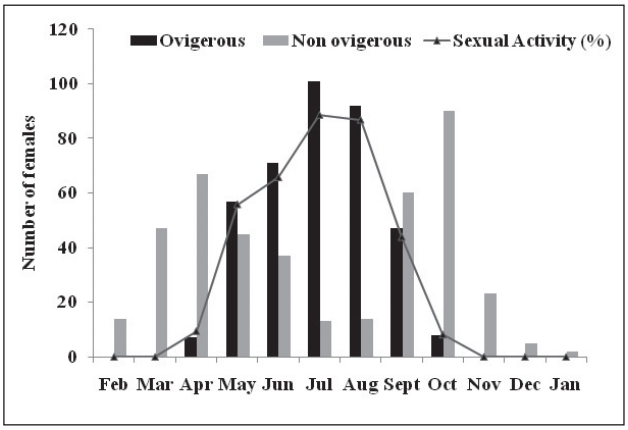

Fig. 4: Monthly distribution of ovigerous and non ovigerous females and sexual activity of** S. walkeri **collected from the Tunis Southern Lagoon, February 2012–January 2013.
Sl. 4: Delež ovigerih samic in samic brez jajc ter spolne aktivnosti vrste** S. walkeri **iz Tuniške južne lagune po posameznih mesecih v obdobju februar 2012–januar 2013

with numerous long, plumose setae, proximal superior corner with a single robust seta; merus, carpus and propodus inferior margins with a dense fringe of short setae; propodus of pereopod 1 with one serrated rostro-distal spine. Near rostro-distal spine transverse row of 10–15 long, plumose setae being inserted.

Pleopod 2 (Fig. 2H). With short sympodite bearing three lateral setae. Endopodite larger than exopodite, with a broad masculine appendix, laterally inserted with an acute tip, much longer than the endopodite itself. This pleopod also features long marginal plumose setae.

Uropods (Figs. 2A, B, I). Flattened and attached to the side of the pleotelson towards the front. Endopod rigidly fused, while exopod movable and larger or equal to the length of endopod. External margin of exopod has 5–6 pronounced teeth, mostly situated in the caudal region. Endopod margins fringed with dense simple setae**,** dorsal surface bearing 2–3 prominent tubercles. Uropods exceed the distal end of the pleotelson and are more developed in males than in females (Fig. 2I).

Based on the schemes presented above, the Tunisian specimens are morphologically similar to those from Africa (Jacobs, 1987) and those from the Persian Gulf (Khalaji-Pirbalouty & Wägele, 2010). These authors describe only 5–6 teeth and an acute apex. Conversely, Stebbing (1905) counted 6 or 7 teeth on the outer margin of the uropod.

Reproductive biology

Males and females were present throughout the year (Fig. 3) and the sex ratio showed that the females outnumbered the males from May 2012 to November 2012 (df = 2, $\chi^2 > 3.84$, $p > 0.05$) (see Table 1). *S. walkeri* presented a discontinuous spawning period throughout the year. However, no monthly sexual activity was observed during two periods: from February to March and from November to January. Ovigerous females were collected from April to October. The number of gravid females exceeded 50 % of the total sample from May to August, and two peaks were recorded in summer, 88.59 % in July 2012 and 86.79 % in August 2012. In autumn, the sexual activity decreased, reaching 43.92 % in September 2012, and showing an even more significant decline in October 2012 (about 8 %) (Fig. 4).

The size at the first sexual maturity observed in *S. walkeri* was 4.8 mm. The fecundity ranged between 13 and 26 eggs. Among the gravid females, a significant

Tab. 1: Monthly sex ratio of** S. walkeri **(Sr) and χ^2 test (significant if $\chi^2 > 3.84$).
Tab. 1: Delež samcev in samic vrste** S. walkeri **(Sr) in χ^2 test (značilen v primerih, ko je $\chi^2 > 3,84$)

Month	Feb-2012	Mar	Apr	May	Jun	July	Aug	Sept	Oct	Nov	Dec	Jan-2013
Sr	0.86	0.81	0.76	0.43	0.48	0.42	0.48	0.31	0.46	0.39	0.20	0.00
χ^2	0.15	0.93	2.49	23.04	19.60	26.89	19.27	39.11	19.64	6.13	2.67	2.00

relationship between the importance of brood and the total body length of specimen was only noted in the extreme size classes, in the smallest (4.85 mm) and largest (8.89 mm) ovigerous females, respectively (ANOVA, $p < 0.05$) (Fig. 5).

Monthly distribution in size classes of females

The largest females were observed during the warmest period of the year, from April to August. From February to April, the size of the specimens sampled ranged between 3 and 6.5 mm. The predominant size classes were 6–7 mm in the May to June period, and 7.5–8.5 mm in July and August. From October onwards, the big size classes were substituted by juveniles (2–3 mm). Figure 6 shows that the autumn and winter seasons coincide with the recruitment of juveniles in the area. To highlight a possible correlation between the ovigerous females and environmental variables we applied the principal component analysis (PCA). Two principal components explaining 86.33 % of the total inertia were retained. The number of ovigerous females was found to be positively correlated with all the studied abiotic parameters, but a highly significant correlation was observed particularly with transparency and temperature (Fig. 7).

DISCUSSION

This study was conducted in the Tunisian Southern Lagoon following the environmental rehabilitation of its ecosystem, as a significant improvement of ecological variables, such as water quality, and invertebrate species previously unknown in the lagoon, in the nearby Gulf of Tunis and along the Tunisian coasts had been recorded (Ben Souissi *et al.,* 2003; Rifi *et al.,* 2011; Rifi *et al.,* 2013).

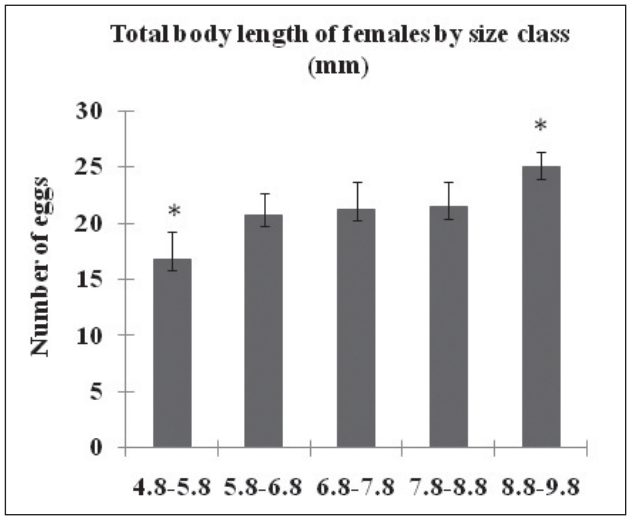

Fig. 5: Relationship between females' size classes and the number of eggs in the brood of** S. walkeri **specimens collected from the Tunis Southern Lagoon.
Sl. 5: Odnos med velikostnimi razredi samic in številom jajc v leglu pri vrsti** S. walkeri **iz Tuniške južne lagune

Sphaeroma walkeri is present in Tunisian brackish waters throughout the year except in December and January. Mak *et al.* (1985) noted that the species was also found in India, but only during the warm period from April to August. This alien species has established in Tunisian waters and reproduces during the period oscillating between April and October, as corroborated by the presence of gravid females. In Hong Kong, *S. walkeri* breeds almost continuously throughout the year with peaks during spring and autumn (Morton, 1987). In our study, fecundity ranged between 13 and 26 eggs per female and the importance of the brood was positively correlated with extreme body size lengths. Similar results were recorded for *Paradella dianae* (Garcia-Guererro & Hendrickx, 2005). The monthly distribution of females by size classes in the Tunis Southern Lagoon showed that except for the spawning period, the largest females disappeared from October to March and were replaced by young specimens (juveniles). This period concerns the recruitment phase, where class sizes ranged between 2 and 6 mm, with the smallest specimen (2.2 mm) collected in November. The virtual disappearance of large specimens of this species in Tunisia during the mentioned period was probably due to intense predation by *Zeus faber* Linnaeus, 1758, which is particularly abundant in the lagoon in winter. A study of the stomach contents of Z. *faber* confirmed this hypothesis (Ben Souissi *et al.*, 2004). Reproductive effort can also induce and explain the mass mortalities observed. This phenomenon was observed in non-indigenous behaviour outside the native habitats of the species (Rifi *et al.,* 2011). According to Shafir & Field (1980) and Kroer (1989), dispersion could also explain the absence of adults. PCA showed that the reproductive biology of *S. walkeri* was positively correlated with environmental variables (salinity and pH) and highly linked to transparency and temperature. Similar patterns were recorded by Radhakrishnan *et al.* (1987) concerning salinity, temperature and suspended solids that affect the species' occurrence. These results corroborated the feeding behaviour of the species, *S. walkeri* being considered a voracious detritus-feeder (Guarino *et al.,* 1993).

S. walkeri was found in the lagoon in very shallow water, generally less than 3 m deep. All records are from intertidal zone except for one from India, where the isopod was collected in waters up to 46 m of depth (Carlton & Iverson, 1981). The species has the flexibility to occupy different habitats related to its distribution. In the studied area, this sphaeromatid species is a typical animal of biofouling found in high densities, mainly among benthic communities, algae, as well as under stones and among dead sea squirts, as in the present study. *S. walkeri* is able to burrow in mangrove roots

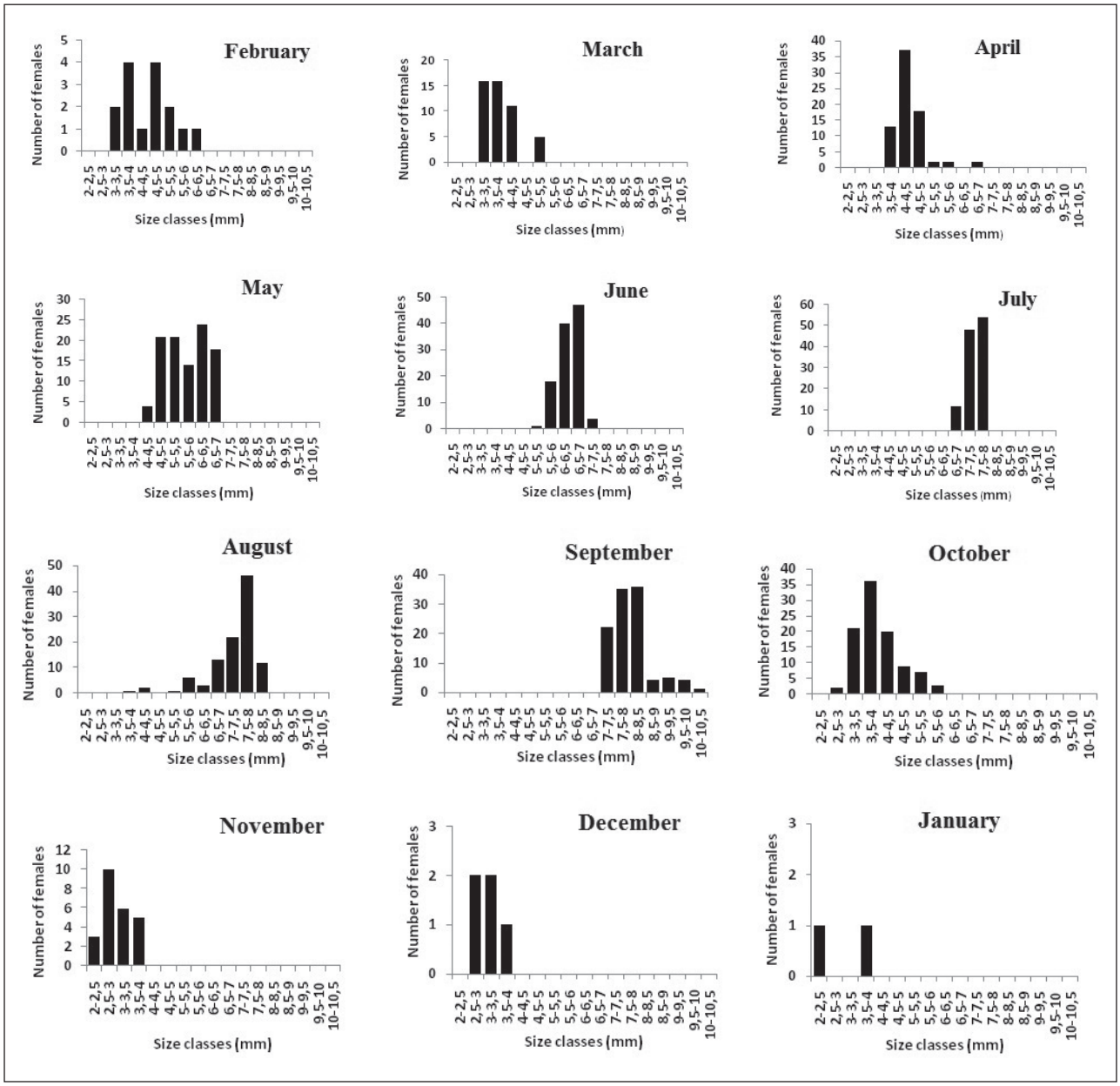

Fig. 6: Monthly size class distribution of** S. walkeri **females collected in the Tunis Southern Lagoon.
Sl. 6: Velikostni razredi samic vrste** S. walkeri **iz Tuniške južne lagune po posameznih mesecih

to live and breed, as described by Khalaji-Pirbalouty & Wägele (2010), who define the species as a wood-borer.

In the Tunis Southern Lagoon, *S. walkeri* inhabits different bottoms and is found among fouling communities. This isopod species colonizes empty barnacle shells of balanoids, such as *Balanus amphitrite amphitrite* Darwin, 1854, spaces between benthic fauna, oscula of sponges, especially *Ircinia* sp., *Chondrosia reniformis* (Nardo, 1847), and ascidians *Phallusia mammillata* (Cuvier, 1815), *Ecteinascidia turbinata* (Herdman, 1880), *Ascidiella aspersa*, (Müller, 1776) and *Ciona intestinalis* (Linnaeus, 1767). In this study, we observed that *S. walkeri* invades via oral and cloacal siphons, burrowing into the thick tunic of dead ascidians of the genus *Microcosmus*, to escape from predators and cope with large environmental variations in the lagoon ecosystem.

This species takes refuge among the dense herbaria of the lagoon constituted by *Gracilaria verrucosa* ((Hudson) Papenfuss, 1950) and *Cystoseira barbata* ((Stackhouse) C. Agardh, 1820) associated with two non-indigenous isopods *Paradella dianae* (Menzies, 1962) and *Paracerceis sculpta* (Holmes, 1904) and the native congeners

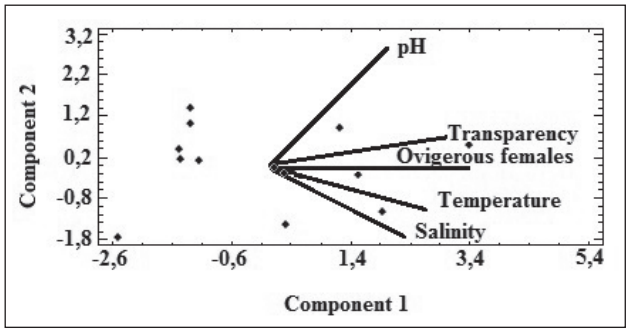

Fig. 7: Principal Component Analysis (PCA): environmental parameters and ovigerous females of *S. walkeri* ***specimens collected from the Tunis Southern Lagoon.***
Sl. 7: Analiza glavnih komponent (PCA): okoljski parametri in ovigere samice vrste *S. walkeri* ***iz Tuniške južne lagune***

S. serratum (Fabricius, 1787) and *Cymodoce truncata* (Leach, 1814). This non-native isopod prefers the habitat of sponges to that of ascidians. Ounifi Ben Amor *et al.* (2010) noted that the highest densities of *S. walkeri* were observed among sponges during spring (18 ind./ m^2) and summer (23 ind./m^2) seasons. Conversely, the lowest densities of *S. walkeri* were among ascidians during autumn (3 ind./ m^2) and winter (2 ind./ m^2) seasons. In the Tunis Southern Lagoon, a significant decrease of the *S. serratum* density was observed subsequent to the environmental restoration. This, however, could also be explained by an interspecific competition pressure between *S. walkeri* and its congeneric species *S. serratum*. The improvement in the water quality in the lagoon after its rehabilitation, in its salinity and pH, for instance, is to the advantage of non-indigenous rather than native species. Indeed, *S. serratum* does not tolerate salinity changes, especially during determinate life stages (Charmantier & Charmantier-Daures, 1994).

CONCLUSION

Sphaeroma walkeri is a thermophilic isopod introduced a decade ago in the Tunis Southern Lagoon, where it rapidly spread throughout the restricted brackish area and colonized both muddy and rocky grounds. Specimens in all developmental stages were observed throughout the year. High densities of *S. walkeri* were recorded especially during spring and summer, showing that the species is substantially established in the Tunis Southern Lagoon. Such settlement is probably due to a successful rehabilitation of the area, which facilitated the introduction of species previously unknown in the region (Mejri *et al*., 2004; Ben Souissi *et al*., 2004, 2005). These results are useful to explain the establishment of this non-indigenous isopod in its new environment.

The establishment of *S. walkeri* in the Tunis Southern Lagoon constitutes one of the best examples of biological change that has affected Tunisian waters for the last four decades at least. New marine species are regularly and continuously recorded in Tunisian waters, incoming from the eastern tropical Atlantic through the Strait of Gibraltar, on the one hand, and from the Indo-Pacific and Red Sea via the Suez Canal, on the other (Spanier & Galil, 1991). Such changes are owed to an intensification of shipping activities (Galil, 2009) and a significant increase in the average water temperature throughout the Mediterranean Sea (Francour *et al*., 1994), which further the introduction and establishment of several species belonging to different zoological groups.

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OPIS, RAZMNOŽEVALNA BIOLOGIJA IN EKOLOGIJA TUJERODNE MOKRICE *SPHAEROMA WALKERI* (CRUSTACEA: ISOPODA) IZ TUNIŠKE JUŽNE LAGUNE (SEVERNA TUNIZIJA, OSREDNJI MEDITERAN)

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POVZETEK

Sphaeroma walkeri *Stebbing, 1905, je tujerodna vrsta, ki je bila leta 2002 prvič potrjena v tuniški južni laguni, kjer je ustaljena vrsta, najverjetneje zaradi okoljske oživitve tega brakičnega okolja. Avtorji so primerke vrste* S. walkeri *zbirali v mesečnih intervalih na 10 postajah od februarja 2012 do januarja 2013. Ta vrsta se v Tuniški južni laguni pojavlja skozi vse leto, razmnožuje pa v toplem delu leta od aprila do oktobra. Število oplojenih samic je v maju bilo več kot 50 % celotnega vzorca, v juliju 2012 pa doseglo višek 88,59 %. Število jajc je bilo od 13 do 26. Pri oplojenih samicah je bil razviden značilni odnos med leglom in celotno dolžino telesa le pri najmanjših (4,8–5,8 mm) in največjih ovigerih samicah (8,8–9,8 mm). V vzorcu je bilo več samic kot samcev. Ta rak enakonožec naseljuje prazne lupine rakov vitičnjakov, kozolnjake in še posebej spužve.*

Ključne besede: bioinvazija, plodnost, habitat, tunizijske vode

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