ISSN: 0001-5113 AADRAY	ACTA ADRIAT., 59 (1): 61 - 70, 2018	ORIGINAL SCIENTIFIC PAPER

Sexual maturity, habitat and ecological aspects of the range expansive Isopod *Sphaeroma venustissimum* in Tunisian waters (Central Mediterranean Sea)

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A total of 4665 specimens of Sphaeroma venustissimum *Monod, 1931 were collected between February 2012 and January 2013, in Tunis Southern Lagoon, brackish area located in northern Tunisia. This species was mainly recorded in the Atlantic Ocean and recently extended its natural range toward southern Mediterranean Sea. Biological and ecological traits were not available to date with special regard to its native and recipient habitat.* S. venustissimum *isopoda inhabits area below stones covered by biofouling and empty nests of barnacles. The species occurs and reproduces in Tunis Southern Lagoon throughout the year with a peak in early spring and late summer. Males significantly outnumbered females during high reproductive activity period (May - July). The first gravid female was observed at 7 mm and the fecundity ranged between 6 and 17 eggs. Among the gravid females, a significant relationship was noted between the importance of brood versus total body length for all ovigerous female cohorts. The recruitment occurred throughout the year with a winter peak.* S. venustissimum *have established a sustainable population in Tunisia and have spread into the wild in its new host area.*

Key words: isopod, *Sphaeroma venustissimum*, fecundity, behaviour, Tunis,

INTRODUCTION

/Following BOCQUET & REZIG, (1972) and REZIG (1974, 1976) two indigenous species of the genus *Sphaeroma* occur in Tunisian waters *Sphaeroma serratum* (Fabricius, 1787) and *S. emarginatum* Grube, 1864. Two more species have recently entered the list, *Sphaeroma walkeri* Stebbing, 1905 originating from Red Sea BEN SOUISSI *et al*., (2003) which is now considered established in northern Tunisia (OUNIFI-BEN AMOR *et al*., 2016), and *Sphaeroma venustissimum* Monod, 1931 from the eastern tropical Atlantic (BEN SOUISSI *et al*., 2005).

Sphaeroma venustissimum was described from specimens collected off the Mauritanian coast (MONOD, 1931), and is distributed northwards, off Morocco, the southwestern Iberian Peninsula (HOESTLANDT, 1959), off Portugal (JACOBS, 1987) and Spain (JUNOY & CASTELLÓ,

2003), The species is assumed to have entered the Mediterranean Sea via the Strait of Gibraltar and was firstly recorded in Tunis Southern Lagoon, brackish area located in northern Tunisia (BEN SOUISSI *et al*., 2005). This species of tropical Atlantic origin, was initially considered as "alien" , but has been removed from earlier alien species lists because its presence in the Mediterranean Sea can be explained by a natural range expansion rather than a human mediated introduction (ZENETOS *et al*., 2012). The aim of this study is to explain the success of the eastward spread and establishment of this thermophilic isopoda through some bio-ecological traits.

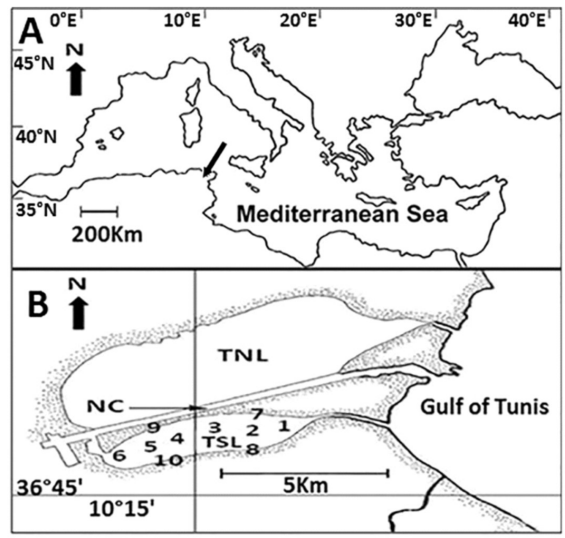

Fig. 1. Sphaeroma venustissimum; *A. Map of the Mediterranean Sea, with arrow pointing out the site of Tunis Southern Lagoon located in northern Tunisia. B. Tunis Northern Lagoon (TNL) separated from Tunis Southern Lagoon (TSL) by a navigation channel (NC). 1-10 sampling stations in TSL*

MATERIAL AND METHODS

Study area

Tunis Southern Lagoon adjoins the city of Tunis and is located in the southwestern region of the Gulf of Tunis (36°47' N and 10°17' E). It constitutes the southern part of Tunis Lagoon divided in two areas by a navigation channel (Fig. 1). Tunis Southern Lagoon extends over an area of 720 ha with a regular depth of about 2.1m, the maximum depth being 4m. 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.

Sampling method

Monthly samplings were carried out in Tunis Southern Lagoon between February 2012 and January 2013 in 10 stations (Figure 1B). Specimens of *S. venustissimum* from soft bottoms were obtained by dredging (stations 1 to 6). In the rocky shores (stations 7 to 10), specimens under stones and among empty barnacle tests, were manually collected and their density was assessed using a 1m quadrate sampler.

In the laboratory, small stones with fouling communities were cleaned with sea water prior to be filtered through a 0.47 mm sieve to collect all stages of *S. venustissimum*. We identified the species following field guides such as REZIG (1976) and JACOBS (1987). Samples were stored in a 70% ethanol solution. The total length of specimens was measured to the nearest 0.1 mm with a micrometer scale for population analysis. The eggs in the marsupia of ovigerous females were removed and counted. Monthly sexual activity was determined as the value (in %) obtained by dividing number of gravid females by total number of females (GUARINO *et al*., 1993).

Table 1. Monthly sex ratio of Sphaeroma venustissimum *(February 2012- January 2013)*

Months	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan
Sr	1.15	2.25	1.74	2.3	2.12	2.75	1.81	1.02	1.06	0.96	1.13	0.69
χ^2 - test	0.15	0.93	2.49	23.04	19.6	26.89	19.27	39.11	19.64	6.13	2.67	2

Sr: Sex ratio (M/F) and the χ^2 test: (significant for > 3.84)

Table 2. Sphaeroma venustissimum; *Analysis of the differences between size classes with a confidence interval of 95% (Tukey (HSD))*

Contrast	Difference	Standardized difference	Significance
> 12 mm vs 7-8 mm	8.672	25.960	*
> 12 mm vs 8.1 - 9 mm	7.206	27.813	*
>12 mm vs 9.1 - 10 mm	2.940	11.595	*
>12 mm vs 10.1- 11 mm	1.054	4.138	*
10.1 - 11 mm vs 7 - 8 mm	7.619	25.123	*
10.1- 11 mm vs 8.1 - 9 mm	6.153	28.233	*
10.1 - 11 mm vs 9.1 - 10 mm	1.887	8.927	*
9.1 - 10mm vs 7-8 mm	5.732	18.955	*
9.1- 10 mm vs 8.1 - 9 mm	4.266	19.682	*
8.1 - 9 mm vs 7- 8 mm	1.466	4.775	*

The relationship between the total body length of ovigerous female *versus* size classes and eggs number was assessed. Size at first sexual maturity was considered as smallest gravid female (GARCÍA-GUERERRO & HENDRICKX, 2005).

The differences of the size classes were tested by analysis of variance (ANOVA) (p <0.05).The method of 'Analyses of the Principal Components' was used to explain correlations between ovigerous females and different environmental variables. Data analysis treatments were performed by the Statgraphics Centurion XV software (2006).

Our investigations were regularly conducted between 2001 and 2014, at least twice a month for abiotic variables (temperature, salinity, pH and turbidity measured with a Secchi disk). The water temperatures were measured using a thermometer with accuracy of 1/10°C and a multitype Lab (WTW) for pH and salinity. In order to highlight a possible correlation between the ovigerous females and environmental variables, we applied a principal components analysis (PCA).

RESULTS

Environmental parameters

The water temperature varied between 11.75°C in February 2012 and 26.4°C in July 2012 with an average of 20.72°C. The salinity oscillated between 36.1 psu measured in January 2013 and 38 psu in August 2012 (average value 37.05 psu). The average pH varied between 7.74 in December 2012 and 8.29 in July 2012 with a mean value of 8.02. The turbidity varied from 0.6 m to 2.5 m with an average of 2.1 m.

Some ecological aspects

Sphaeroma venustissimum is an intertidal species, often found below stones covered by biofouling or inside empty tests of the barnacle *Amphibalanus eburneus* (Gould, 1841). The species rather occupies rough stones with crevices colonized by algae and, is rare or absent on smooth clear stones. In barnacle tests at the intertidal zone, *S. venustissimum* apparently tolerates relatively long emersions. Of the 835 empty barnacle tests collected throughout the year, a maximum of 4 specimens from all stages (subadults and adults) was observed associated with other isopod species such as *Paracerceis sculpta* (Holmes, 1904), *Cymodoce truncata* Leach, 1814 and *Dynamene edwardsi* (Lucas, 1849). Below stones, the highest densities were observed early spring and late summer (April to August) (Table 3).

Reproductive biology

Out of 4665 specimens of *S. venustissimum*, 2346 were sexed including 850 females and 1496 males; while 2319 other specimens were

Table 3. Density of Sphaeroma venustissimum *in the Tunis Southern Lagoon (February 2012- January 2013)*

Months	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan
Density (ind/m²)	272	672	912	985	1168	1728	1776	1056	768	624	432	352

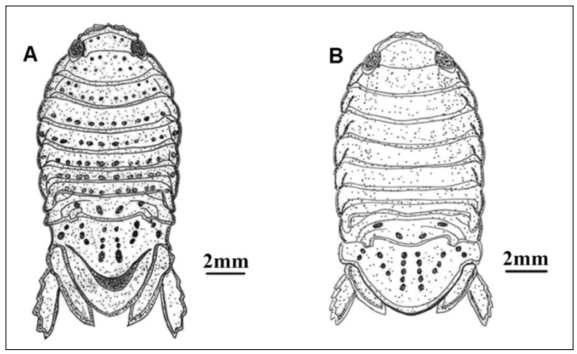

Fig. 2. Sphaeroma venustissimum; *(A) adult male in dorsal view (B) adult female in dorsal view*

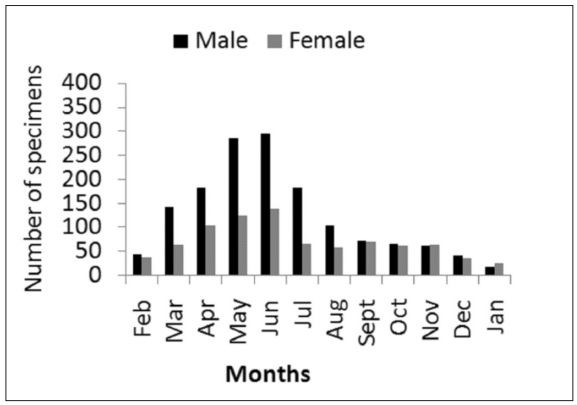

Fig. 3. Sphaeroma venustissimum; *Monthly distribution of male and female specimens* of Sphaeroma venustissimum *collected from Tunis Southern Lagoon. (February 2012- January 2013)*

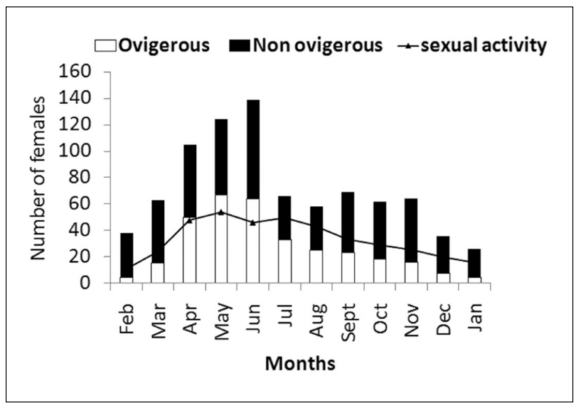

Fig. 4. Sphaeroma venustissimum*; Monthly distribution of ovigerous, non ovigerous females and sexual activity of* Sphaeroma venustissimum *collected from Tunis Southern Lagoon (February 2012- January 2013)*

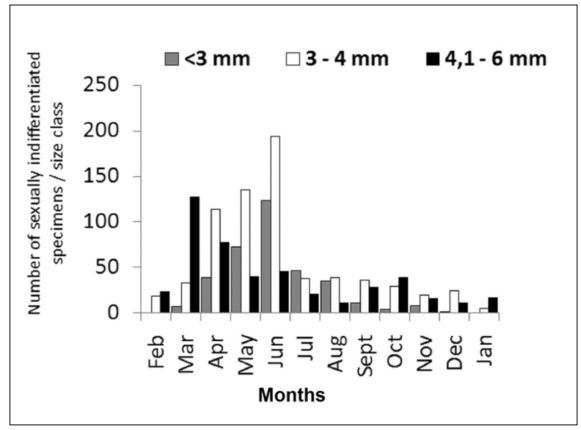

Fig. 5. Sphaeroma venustissimum; *Temporal evolution of the number of sexually undifferentiated specimens. (February 2012- January 2013)*

sexually undifferentiated. The species displays an evident sexual dimorphism with males (7 to 16 mm) larger than females (7 to 14 mm) and represent well developed pleotelson, its length slightly larger than 1/3 of total length (Fig. 2A), while The body of females are more convex (Figure 2B). Males, females and sexually undif-

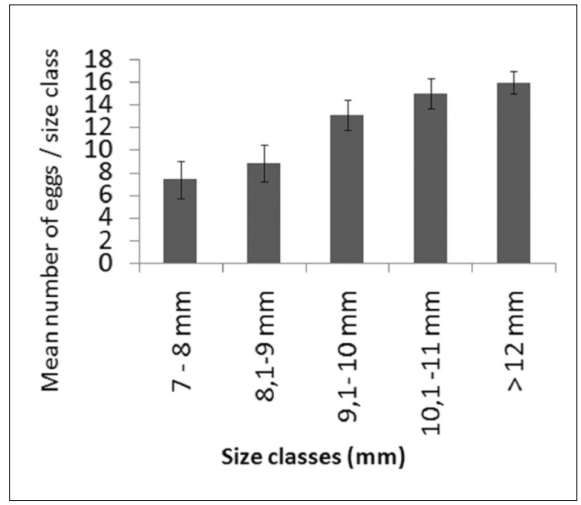

Fig. 6. Sphaeroma venustissimum; *Relationship between female's size classes and the number of eggs in the brood for* Sphaeroma venustissimum *specimens collected from Tunis Southern Lagoon (February 2012- January 2013)*

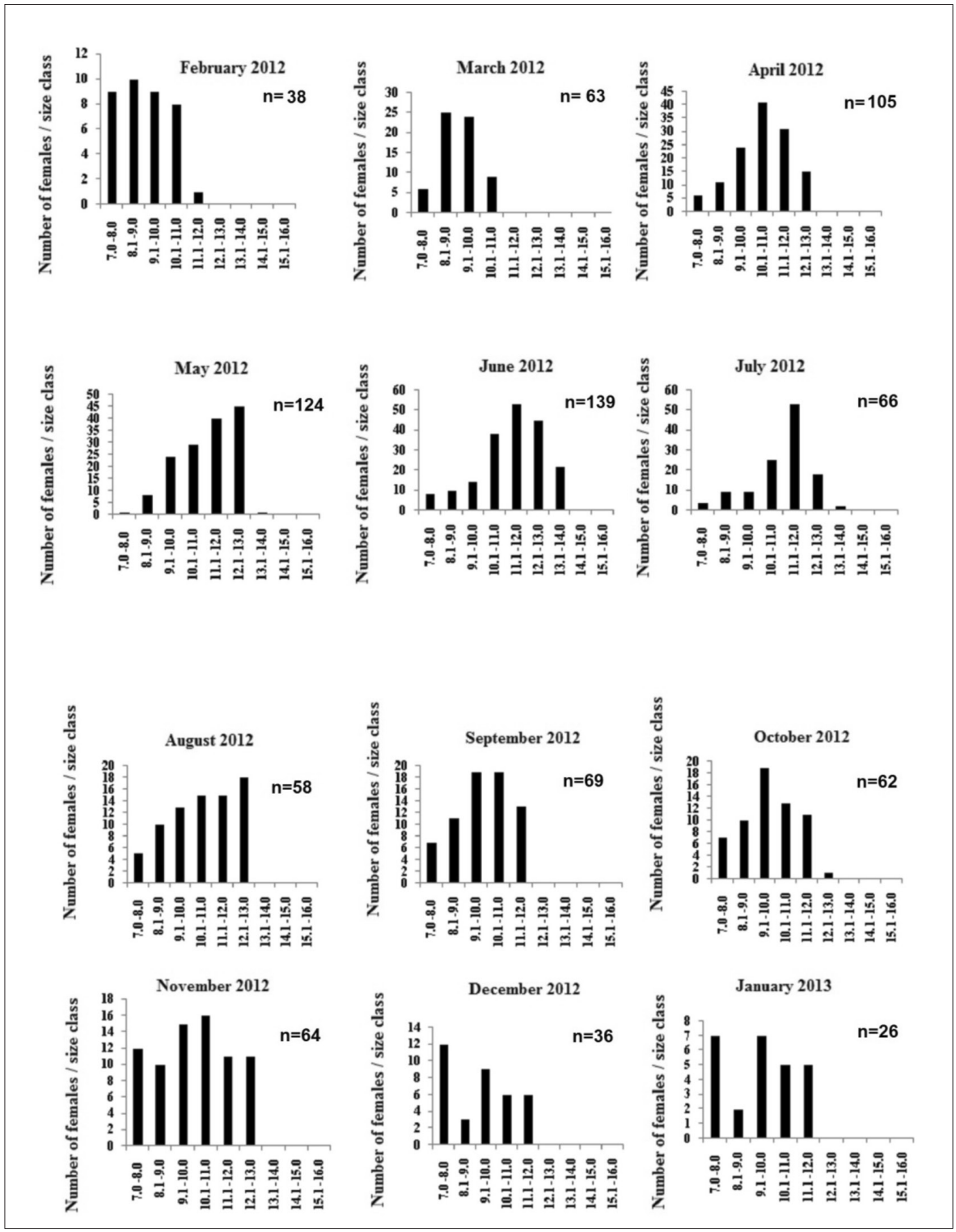

Fig. 7. Sphaeroma venustissimum; *Monthly size classes distribution of Sphaeroma venustissimum females collected in Tunis Southern Lagoon (February 2012- January 2013)*

ferentiated specimens were present throughout the year. Males significantly outnumbered females from May to November (Tab. 1, Fig. 3).

Ovigerous females were collected throughout the year with a spring peak occurring in May (54.03%) and a minimum in February (10.52%) indicating a continuous spawning period. *S. venustissimum* was rare in winter and a similar pattern was observed for gravid females. Off the winter period (November-February), sexual activity showed a slight variation reflecting a spread reproductive effort (Fig. 4).

The juvenile recruitment occurred throughout the year with a spring peak in total concomitance with the massive presence of gravid females. However, very small specimens (<3 mm) showing a recent maternal marsupium release were absent in winter (December - February). During the warm period (July to October), different juvenile sizes coexist in similar proportions (Fig. 5).

First sexual maturity size was observed in females at 7 mm. The fecundity varied from 6 to 17 eggs. The number of eggs was correlated with female body length (Figure 6). Among the mature females, a significant relationship between the importance of brood *versus* total body length of specimens was noticed for all size classes (ANOVA, p <0.0001, Tukey Test) (Table 2 and Fig. 6).

Monthly distribution of size classes of females

The largest sizes (from 11 to 14 mm) were observed during the warmest season of the year (May to July). During the cold period (December to February), the predominant size class was 7 to 8 mm reflecting a recruitment. Furthermore, our data showed the coexistence of all sizes during late spring and early summer, mainly from April to June, highlighting an intensive reproductive activity and a fast growth of juveniles. During this period, the females recruitment appeared to occur continuously with a short break in May (Fig. 7).

Environmental parameters and ovigerous females

Two principal components explaining 75.94 % of the total inertia were retained showing that the number of ovigerous females was positively correlated with all studied abiotic variables (Fig.8).

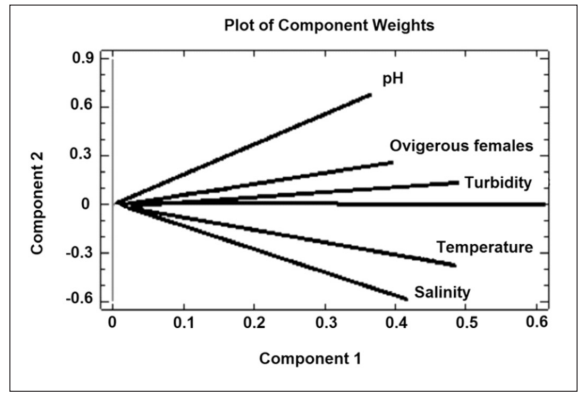

Fig. 8. Sphaeroma venustissimum; *Principal Component Analysis (PCA) - environmental parameters and ovigerous females for* Sphaeroma venustissimum *specimens collected from Tunis Southern Lagoon (February 2012- January 2013)*

DISCUSSION

Before the ecological rehabilitation of the lagoon in 2001 water and sediment qualities and the macrobenthic communities composition were degraded, pointing a high anthropogenic pollution (BEN SOUISSI, 2002). Indeed, the abiotic parameters fluctuated considerably altering the Lagoon becoming eurytherm, euryhaline (between 30.9 and 48.9 psu, with a pick of 51.9 psu), euryoecious, turbid and hypereutrophic. As a consequence of the high level sediment toxic heavy metals contamination, disturbed benthic communities and azoic zones were observed (VANDENBROCK & BEN CHARRADA, 2001; BEN SOUISSI, 2002).

Measurements of abiotic variables concomitantly carried out during *S. venustissimum* samplings showed that the water quality of Tunis Southern Lagoon was considerably improved, compared to its ecological state before restoration (OUNIFI BEN AMOR *et al*., 2017).

Tunis Southern lagoon is at present a prosperous recipient ecosystem for *S. venustissimum,* allowing the settlement of a sustainable population of the species. ENZENROSS & ENZENROSS (2001) noted that most of the first records of alien species in Tunisia stem from ports and lagoons which appeared to be favorable 'transit sites'. *S. venustissimum* occupied different habitats, mainly stones with crevasses and empty tests of barnacles. The empty tests barnacles provide refuge and an incubator of large females. *S. venustissimum* showed other habitat types such as annelid tubes in Morocco (REZIG, 1976) and shelly sand in the Iberian Peninsula (HOESTLAND, 1959).

S. venustissimum occurs throughout the year with highest densities during the warm period (from May to July). The decrease of this crustacean, during the cold season, could be attributed to mortalities related the high sexual activity period as in *S. terebrans* (THIEL, 1999). Furthermore, in winter, decline in salinity and temperature may explain the mortality and dispersion of *Sphaeroma* populations, a phenomenon already observed in other isopod species such as *Paradella dianae* (Menzies, 1962) and *Uromunna sp*, (SHAFIR & FIELD, 1980; KROER, 1989; GARCÍA -GUERERRO & HENDRICKX, 2005).

The juvenile recruitment occurred throughout the year with a peak in spring concomitant to the large abundance of gravid females. Size rapidly increased in juveniles, during the study period, and could be explained by successive moults, and stimulated by higher temperature values. Similar patterns were observed in *S. terebrans* Bate, 1866 by THIEL (1999).

The reproductive biology of *S. venustissimum* was related with the environmental features. Males significantly outnumbered females especially during the period of high reproductive activity. Female first sexual maturity was observed at a size of 7 mm, no gravid females were observed in lower sizes. Moreover, laboratory cultures performed on *Sphaeromatidae* showed that the juveniles leaving the maternal marsupium are sexually undifferentiated, due to the fact that sexual differentiation displays after several months.

Reproductive activity in *S. venustissimum* permanently occurred in Tunis Southern Lagoon, with a peak in early spring and late summer, decreasing during the cold season from December to February. *S. venustissimum* exhibited similar reproductive strategies to its congeneric species *S. terrebrans* (THIEL, 1999). Such pattern was not recorded in *Lekanesphaera rugicauda* (Leach, 1814) which breeds only once during its lifetime (HARVEY, 1969; BETZ, 1979). The number of eggs was significantly correlated with female body length and the same trend was reported in its congener *S. walkeri*, in *Idotea spp* and *Paradella dianae* in which largest females produced more eggs (OUNIFI BEN AMOR *et al*., 2015; KROER, 1989; GUARINO *et al*., 1993; GARCÍA -GUERERRO & HENDRICKX, 2005).

CONCLUSIONS

Sphaeroma venustissimum 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. High densities and occurrence of all developmental stages of this isopod showed that a sustainable population of *Sphaeroma venustissimum* is at present well established in its new environment. Such settlement could be considered as a main consequence of the successful rehabilitation of the area, which enhanced the introduction of the species previously unknown in the region. New marine species are regularly and continuously recorded in Tunisian waters mainly in the lagoon environments, considered as hotspots and nursery areas for the installation of invasive species.

ACKNOWLEDGEMENTS

We wish to acknowledge the suggestions and comments of two anonymous reviewers, which helped to improve the quality of the manuscript.

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Received: 28 October 2015
Accepted: 1 February 2018

Spolna zrelost, stanište i ekološki aspekti rasprostranjenosti jednakonošca *Sphaeroma venustissimum* u tuniskim vodama

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SAŽETAK

Ukupno 4665 primjeraka *Sphaeroma venustissimum* Monod, 1931., prikupljeno je između veljače 2012. i siječnja 2013. u tuniskoj Južnoj laguni, u bočatoj vodi koja se nalazi u sjevernom Tunisu. Ova vrsta je uglavnom zabilježena u Atlantskom oceanu, te je nedavno proširila svoj prirodni raspon prisutnosti prema južnom Sredozemnom moru. Do danas nisu bile dostupne biološke i ekološke osobitosti ovog izopoda s posebnim osvrtom na stanište i primatelje. Izopod *S. venustissimum* nastanjuje se ispod kamenja prekrivenih obraštajem i praznim gnijezdima rakova vitičara ispitivanja stabala kao staništa. Vrste se pojavljuju i reproduciraju u Južnoj laguni u Tunisu tijekom cijele godine s vrhuncem u rano proljeće i kasno ljeto. Mužjaci su brojčano značajno nadmašili ženke tijekom razdoblja visokog reproduktivnog djelovanja (svibanj - srpanj). Prva promatrana gravidna ženka je bila veličine 7 mm, a plodnost je iznosila između 6 i 17 jaja. Među gravidnim ženkama, zabilježena je značajna veza između važnosti legla u odnosu na ukupnu dužinu tijela za sve ženke s prekomjernom veličinom. Novačenje se odvijalo tijekom cijele godine s vrhuncem u zimskom periodu. Uspostavljene su održive populacije izopoda *S. venustissimum* u Tunisu, te su se proširile u divljinu kao svom novom prostoru domaćina.

Ključne riječi: jednakonožac, *Sphaeroma venustissimum*, plodnost, ponašanje, Tunis