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ON THE SUCCESSFUL ESTABLISHMENT OF THE ALIEN ISOPOD *PARACERCEIS SCULPTA* IN TUNISIAN WATERS (CENTRAL MEDITERRANEAN SEA)

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ECOLOGICAL ASPECTS
POPULATION DYNAMICS
REPRODUCTIVE TRAITS
SETTLEMENT

ABSTRACT. – A total of 2103 specimens of an alien species *Paracerceis sculpta* (Holmes, 1904) was collected between November 2016 and July 2017 in Tunis Southern Lagoon, brackish area located in northern eastern Tunisia. The species is a native from southern California and invaded the Mediterranean Sea during the late seventies. *P. sculpta* was found below stones covered by biofouling and empty tests of barnacles. From the three distinct sexually mature male morphs of *P. sculpta*, only the larger (alpha) was found, confirming the absence of the two smaller male morphs (beta and gamma) in an introduced population. Females reached size at first sexual maturity from 4 mm in length, the fecundity ranged from 5 to 14 eggs. The high densities and occurrence of all stages of development of the intertidal isopod *Paracerceis sculpta* in the Tunis southern lagoon, suggests that the species is established in its new environment.

INTRODUCTION

The lagoon of Tunis is located in the northeast of Tunisia. Cutting across the lagoon and connecting the Mediterranean with the old port of Tunis, the Canal of Tunis (NC) divides the lagoon into two basins, commonly known as the southern and the northern lagoon of Tunis. In the last decades, Tunis southern lagoon was polluted by local anthropic activities (domestic and industrial discharge), which induced dystrophic crises together with destruction of benthic communities (Ben Souissi 2002). In order to resolve the pollution problem and to improve the water quality of the Tunis southern lagoon for ecological and economical purposes, an environmental restoration project was conducted from April 1998 to July 2001. The overall aim of the project was to achieve a good ecological status in the lagoon and to realize substantial land reclamation all around.

The Tunis southern lagoon, azoic during the restoration, was progressively invaded by several species, therefore, new activities have been developed such as the collection of mussel on artificial rocky banks of the lagoon and fishery of cephalopod species such as common cuttlefish *Sepia officinalis* Linnaeus, 1758 and musky octopus *Eledone moschata* (Lamarck, 1758) (Ounifi Ben Amor *et al*. 2019a).

Several Indo-Pacific immigrants were established in the area since autumn 2001 (Ben Souissi *et al*. 2005). For crustaceans, thirteen alien species were recorded in the area, among them 5 isopods, of which two spheroma; *Sphaeroma walkeri* and *Sphaeroma venustissimum* are totally established (Ounifi Ben Amor *et al*. 2015, 2018). The alien isopod *Paracerceis sculpta* (Holmes, 1904) was firstly recorded in the Tunis lagoon, in the northern part (TNL) (Rezig 1978) and in its southern part (TSL) (Ounifi Ben Amor *et al*. 2006).

Invasive Alien Species (IAS) have negative ecological and socio-economic impacts and represent a major threat to marine biodiversity by displacing native species, changing community structure and food webs (Streftaris & Zenetos 2006). While in the Tunis southern lagoon, the negative impact of the introduced species is not highlighted, alien and native species survive and coexist together (Ounifi Ben Amor *et al*. 2017).

Paracerceis sculpta, subject of the present study, native to the Northeast Pacific from southern California to Mexico (Menzies 1962, Miller 1968), has been recognized as alien species in several marine environments (Brusca 1980). Several authors pointed that isopods possess typical traits of non-indigenous species (NIS), such as high dispersal ability, tolerance of wide range of environmental conditions, phenotype plasticity… employing ships as vectors and colonizing ports and nearby marine environments worldwide (Carlton & Iverson 1981, Hewitt & Campbell 2001). *Paracerceis sculpta* essentially associated with algae and calcareous sponges are considered as NIS in the Atlantic coast of Europe, also in Southwestern Atlantic (Rodriguez *et al*. 1992), the Mediterranean Sea (Tunisia: Rezig 1978; Italy: Forniz & Maggiore 1985), Hong Kong (Bruce 1990), and Australia (Harrison &

Holdich 1982, Hass & Knott 2000). *Paracerceis sculpta* formed the subject of several studies concerning physiology of moult and growth (Shuster 1989; Shuster & Guthrie 1999), population biology (Munguia & Shuster 2013, Rumbold *et al*. 2018) and reproductive behavior (Shuster 1989, Shuster & Wade 1991, Shuster 1992).

The intertidal isopod *P. sculpta* is a model organism for understanding alternative mating strategies related to its three different male morphs: alpha, beta and gamma males (Shuster 1987, Shuster 1990, Shuster & Wade 1991). Alpha males are the largest specimens with enlarged pleotelsons and elongated uropods, which guard cavities of their common habitat, the calcareous sponge *Leucandra losangelensis* (Laubenfels, 1930). Within these cavities, alpha males maintain a harem composed of one or more females. Beta males are morphologically similar to females and live as females, do to infiltrate harems and mate (Shuster 1987). Finally, gamma males are very small relative to the other two morphs and are able to sneak undetected into harems (Shuster 1987).

Paracerceis sculpta is well known as a component of the biodiversity studies of Tunis Southern Lagoon (Ounifi Ben Amor *et al*. 2017). However, some aspects of their population dynamics, reproductive traits and the distribution / absence of the two male morphs are to date poorly known. The aim of the present study is to analyze its life history strategies, population biology and reproductive traits in order to state if a viable population is at present successfully established in Tunis southern Lagoon.

MATERIALS 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 man made navigation channel (NC) (Fig. 1). Tunis Southern Lagoon extends over an area of 7.2 km² with a regular depth of about 2.1 m, the maximum depth being 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 artificially protected by large rocky stones. Tunis southern lagoon is a coastal lagoon, which maintains a connection to the sea through the navigation Channel.

The lagoon is characterized by a semiarid Mediterranean climate. The annual temperature ranges between 7 and 30° C, the mean annual rainfall is 528 mm with interannual variations. The luminosity is about 6 h per day, with 155 h in December and 359 h in July, evaporation ranges between 62 and 201 mm, respectively, in January and July (Abidi *et al*. 2018).

Sampling & laboratory procedures: *Paracerceis sculpta* were manually collected from November 2016 to July 2017 in the intertidal zone of the lagoon, not exceeding 0.5 m depth, among rocky shores covered by algae. The empty barnacle tests

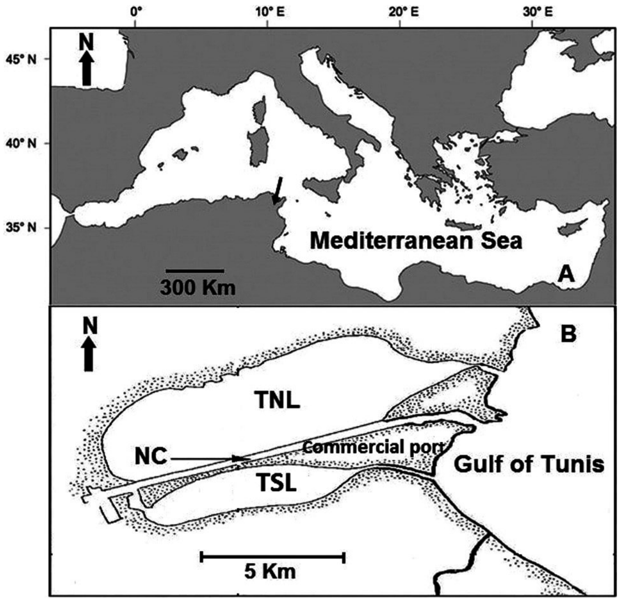

Fig. 1. – **A**: Map of Tunisia pointing out the site of Tunis Southern Lagoon (TSL) located in the north. **B**: Tunis Northern Lagoon (TNL) separated from Tunis Southern Lagoon by a navigation channel (NC).

were removed gently at the base of the barnacle using a mallet. Each test and its occupants were carefully placed inside a polyethylene zip-lock bag. Small stones covered by algae containing isopods were also transported to the laboratory for identification and counting. The densities were assessed using a one 0.1 m² quadrate sampler with 10 replicates per month totalizing an area of 1 m². Simultaneously with the biological sampling, the water temperature were measured using a thermometer with accuracy of 1/10° C and a multi-type Lab (WTW) for other parameters such as pH, salinity and dissolved oxygen.

In the laboratory, specimens were removed from algae by washing and sieving through a 0.47 mm sieve, all occupants of each barnacle test were removed. Samples were preserved in a 70 % ethanol solution and subsequently identified using taxonomic description (Loyola e Silva *et al*. 1999). The total length of specimens was measured from the frontal edge of the cephalon to the posterior end of the pleotelson under a stereoscopic microscope with a micrometric ocular (± 0.01 mm accuracy). Animals were classified into sex and age groups: juveniles (individuals lacking any observable trait of sexual differentiation), alpha males (possessing rugose pleotelsons and elongated uropods), ovigerous females (*i.e*., with eggs in the marsupium) and non-ovigerous females (*i.e*., without marsupium). The eggs in the marsupia of ovigerous females were removed and counted.

Data analysis: Densities and size-frequency distributions were plotted. Sex ratio (Sr) was calculated each month, the heterogeneity hypothesis and the 1: 1 sex ratio hypothesis are tested by the Chi-square χ^2 test (Scherrer 1984). The monthly average data were submitted to the analysis of variance (ANOVA) and, if necessary, to *posthoc* Tukey test, with a significance level at $P < 0.05$ (Zar 2010).

Linear regression and the Pearson's correlation coefficient (Zar 1999) were calculated to assess the relationship between female size and the number of eggs in the marsupium and between the number of ovigerous females and the lagoon water temperature. Monthly reproductive activity was determined as the proportion (%) of ovigerous females (Guarino *et al*. 1993). Size at first sexual maturity was considered as the size of the smallest ovigerous female (Garcia-Guererro & Hendrickx 2005).

RESULTS

Environmental parameters

The water temperature range between 12° C in January 2017 and 27.1° C in July 2017 with an average of 18.77° C. During the study period, the salinity oscillated between 36.1 and 38 with an average value of 37.05. The average pH range between 7.70 in December 2016 and 8.29 in June 2017 with a mean value of 8.02.

Some ecological aspects

Paracerceis sculpta is an intertidal lucifugous species, often found inside empty tests of barnacles of the two species *Amphibalanus eburneus* (Gould, 1841) and *A. amphitrite* (Darwin, 1854) and below stones covered by biofouling. The species is rather found below rough stones with crevasses colonized by algae and rare or absent in the smooth stones without fouling. Males and ovigerous females are more abundant in the empty tests of barnacles, while non-ovigerous females and juveniles were mainly observed among algae (Table I). The species was observed associated, both in algae and in empty barnacle tests, with other crustaceans species essentially its congener *Sphaeroma venustissimum* and the native isopods *Dynamene edwardsi* (Lucas, 1849) and *Cymodoce truncata* Leach, 1814.

Description and population biology

From November 2016 to July 2017, a total of 2103 of *Paracerceis sculpta* were sampled and sorted into three different classes; alpha males (824), females (1136) and juveniles (134). The mean size of males was 6.80 ± 1.21 mm, females (5.3 ± 1.3 mm). Juveniles showed a mean size of 2.8 ± 0.5 mm. Alpha-males were larger than females, and exhibited robust ornamented telsons and elongated uropods (Fig. 2).

Alpha male, 7 mm (Fig. 2A): body relatively slender, about 2.1 times as long as wide. Anterior margin of head with median projection. Dorsal surfaces of head and pereon almost smooth, each bearing 3 or 5 bundles of setae posteriorly. Pleon and anterior part of pleotelson granulose. Posterior margin of pleon and middle part of pleotelson each with 3 setose tubercles. Apex of pleotelson cleft, with 3 pairs of notches, anterior and middle notches deep and posterior shallow. Uropod with endopod reduced; exopod greatly elongate, gradually curved, bearing many bundles of setae, apex acute.

Table I. – Different associations and total number of *Paracerceis sculpta* individuals recorded in Tunis Southern Lagoon. ov: ovigerous females.

Association	Males	Females	Juveniles
Empty tests of barnacles	530	275 (ov: 95)	51
Algae	294	861 (ov: 50)	92

Fig. 2. – *Paracerceis sculpta*. **A**: Adult male in dorsal view; **B**: Adult female in dorsal view. Scale bar: 3 mm.

Adult female (non-ovigerous), 5.5 mm (Fig. 2B): body ovate, about 1.5 times as long as wide. Anterior margin of head rounded. Dorsal surfaces of head and pereon smooth, each bearing a few posterior setae. Pleon also smooth, posterior margin with three bundles of setae. Middle part of pleotelson with 3 setose tubercles. Apex of pleotelson shallowly concave. Endopod and exopod of uropod flattened with marginal setae; exopod lanceolate, apex pointed. Males are light brown and pink. Females are dark red with a dorsal dark or light brown band. Juveniles do not show the reproductive structures that adult isopods have, *e.g*. enlarged penes or oöstegites, and therefore cannot be sexed. Our data showed the coexistence of all cohorts throughout the study period, however during the warm period (April to June) different cohorts coexist in almost similar proportions (Fig. 3).

The highest densities were observed for the largest cohorts (from 5 to 7 mm) during the sampling period; 5.1 to 6 mm for females and 6.1 to 7 mm for males (Fig. 3). No significant difference was found between the mean sizes of males and females ($P = 0.2422$). Juveniles represent 7 % of the entire population sampled. The densities of juveniles decreased from March to July 2017 when the adults appeared in large numbers. Females outnumbered males with significant differences from November to February, while males predominated over females from March to May (Table II).

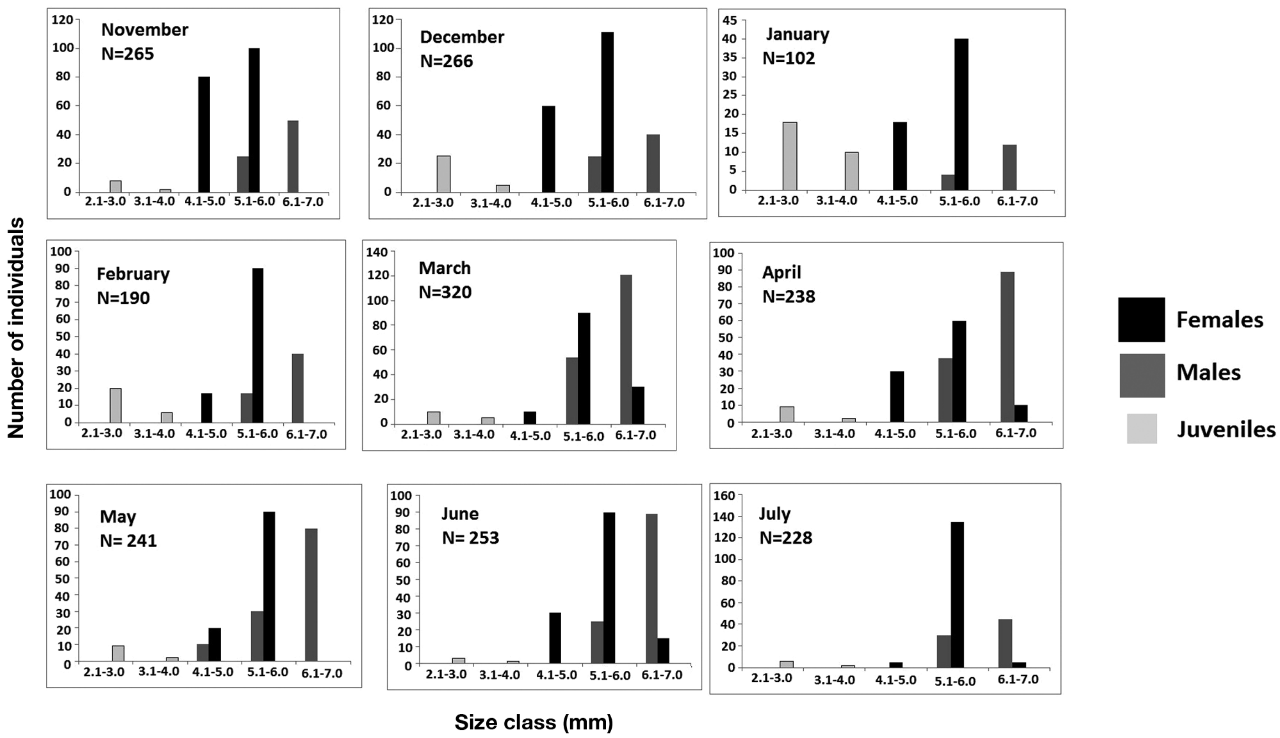

Fig. 3. - *Paracerceis sculpta*. Monthly size class distribution (November 2016-July 2017).

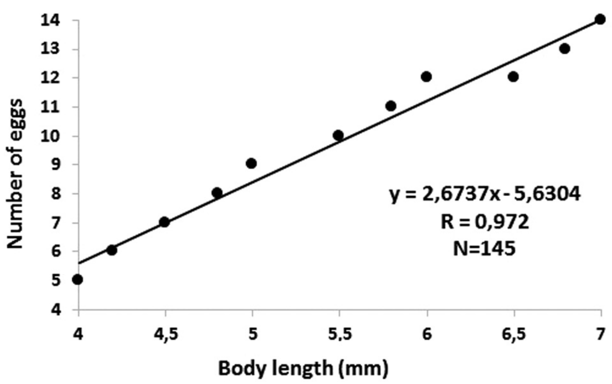

Fig. 4. – *Paracerceis sculpta*. Relationship between the number of eggs and the total body length of ovigerous females.

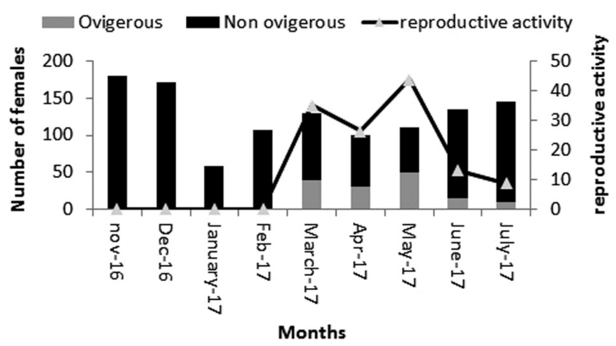

Fig. 5. – Monthly distribution of ovigerous, non-ovigerous females and reproductive activity of *Paracerceis sculpta* collected from Tunis Southern Lagoon (November 2016- July 2017).

Table II. – Monthly sex ratios of *Paracerceis sculpta* in Tunis Southern Lagoon.

Month/year	Females	Males	% F	% M	Sr	χ^2
Nov 2016	180	75	70.58	29.41	0.41	*43.23
Dec 2016	171	65	72.46	27.54	0.38	*47.61
Jan 2017	58	16	78.38	21.62	0.27	*23.84
Feb 2017	107	57	65.24	34.75	0.53	*15.24
Mar 2017	130	175	42.62	57.38	1.34	*6.64
Apr 2017	100	127	44.05	55.95	1.27	3.21
May 2017	110	120	47.83	52.17	1.09	0.43
June 2017	135	114	54.21	45.78	0.84	1.8
July 2017	145	75	65.90	34.09	0.52	*22.27

Size at first sexual maturity occurred in females at 4 mm in length. The fecundity ranged from 5 to 14 eggs. Larger females carried more eggs than smaller ones; the number of eggs was positively correlated with female body length (r = 0.972) (Fig. 4). An important correlation between the number of ovigerous females and the water temperature was highlighted in the Tunis southern lagoon (the Pearson correlation coefficient r = 0.8652, P = 0.002592) (Table III).

Ovigerous females were collected from March to July and reproductive activity showed with a peak in May (44 %) and a minimum in July (8.80 %) (Fig. 5).

Table III. – Number of ovigerous females and water temperatures in the Tunis southern lagoon.

Month/year	Ovigerous females	Water temperature (°C)
Nov-16	0	18.18
Dec-16	0	14.56
Jan-17	0	11.88
Feb-17	6	13.46
Mar-17	17	16.01
Apr-17	19	18.66
May-17	30	22.7
Jun-17	45	26.45
Jul-17	28	27.1

DISCUSSION

Prior to the lagoon's ecological rehabilitation, the abiotic parameters fluctuated considerably altering the water quality of the lagoon; the average monthly salinity ranged between 31 and 48.9 (Abidi *et al*. 2018). Measurements of abiotic variables after the restoration work showed that the water quality of Tunis Southern Lagoon was considerably improved (Ounifi Ben Amor *et al*. 2019b). Improvement of the physico-chemical parameters of the lagoon waters have favored the establishment of new species in the region. The two alien isopods *Sphaeroma walkeri* and *S. venustissimum* are the best instances (Ounifi Ben Amor *et al*. 2018). The sampling of the alien isopod *P. sculpta* in the Tunis southern lagoon reveals its high tolerance for fluctuating salinities, such as the ones encountered in ballast tanks during transoceanic voyages, typically ranging between 4 and 30 ‰ (Ellis & MacIsaac 2009). The influence of several factors as salinity, temperature, turbidity, chlorophyll-*a*, phosphate and nitrate concentrations on crustacean distribution in Tunis southern lagoon, including *P. sculpta,* have been previously determined (Ounifi Ben Amor *et al*. 2017). These results showed that temperature plays an important role in the population changes of other well established alien species in the area by increasing the growth rate (Ounifi Ben Amor *et al*. 2018). Furthermore, in winter, decline in salinity and temperature may explain the mortality and dispersion of *Paracerceis* populations, a phenomenon already observed in the thermophilic isopod species (Ounifi Ben Amor *et al*. 2015, 2018) and in amphipods such as *Monocorophium acherusicum* (Costa, 1853) and *Ericthonius punctatus* (Spence Bate, 1857) (Rumbold *et al*. 2016).

In Tunis southern lagoon, *P. sculpta* is always sampled essentially among algae, which constitute their essential food, and in the empty tests of barnacles unlike its common habitat, calcareous sponge, *Leucandra losangelensis* (Shuster, 1987). Habitat change in this species proves its tolerance to a wide range of environmental conditions such as inhabiting hard substrates and even soft-sediment areas (Espinosa-Perez & Hendrickx 2001, Hewitt & Campbell 2001). *Paracerceis sculpta* share the same habitat as the established alien isopod *Sphaeroma venustissimum* in the lagoon unlike *S. walkeri* which inhabits different bottoms and is found among fouling communities (Ounifi Ben Amor *et al*. 2018). *Sphaeroma walkeri* colonizes empty barnacle shells of balanoids, such as *Balanus amphitrite amphitrite* Darwin, 1854, oscula of sponges, especially *Chondrosia reniformis* (Nardo, 1847), and ascidians *Phallusia mammillata* (Cuvier, 1815), *Ecteinascidia turbinata* (Herdman, 1880), *Ascidiella aspersa* (Müller, 1776) and *Ciona intestinalis* (Linnaeus, 1767) (Ounifi Ben Amor *et al*. 2015).

The sampled population is composed of males, females and juveniles. Only alpha males, possessing the conspicuously elongated uropods, were found, in accordance with observation of invasive populations from Europe, Australia, Asia, North, and South America (Brazil and Argentina) (Loyola e Silva *et al*. 1999, Hewitt & Campbell 2001, Munguia & Shuster 2013, Marchini *et al*. 2017). Beta-males are smaller and do not exceed 4.3 mm and the gamma-males are tiny, not exceeding 3 mm; all three male morphs are sexually mature (Shuster 1992). The long lifespan of alpha males allows them to survive long voyages and ballast water since ships take at least six weeks and could even take up to two or three months to cross the Atlantic Ocean from the American continent. This could explain their invasion compared to beta and gamma males that have a shorter lifespan (Munguia & Shuster 2013, Marchini *et al*. 2017). Transporting individuals across large bodies of water could create genetic bottlenecks, which can result in large populations (Shuster 1989). However, perhaps only alpha males were able to colonize some new sites (Hewitt & Campbell, 2001). The variation in the sex ratio of *P. sculpta* remains without detailed explanation since those studies have showed that the sex ratio varies considerably through time, both within and between populations (Shuster & Wade 1991).

The early maturation at small size of females (4 mm) of the Tunisian population requires detailed genetic study to explain it. Geburzi & McCarthy (2018) attributed this change, among other mechanisms, to a phenotypic plasiticity, a key feature of successful invaders.

Paracerceis sculpta present a discontinuous spawning period throughout the year, however, no reproductive activity was observed during cold months. This suggests that temperature may play an important role in the gonad maturity. This discontinuity in reproductive activity is also explained by the lack of alternative mating morphs, which could have rapid and profound changes in mating strategies and life history traits (Shuster 1989). However, the alien isopod *sphaeroma venustissimum* has continuous spawning period (Ounifi Ben Amor *et al*. 2018).

Larger females carried more eggs than smaller ones; the number of eggs was positively correlated with female body length. The same trend was observed in *S. walkeri and* in *S. venustissimum* (Ounifi Ben Amor *et al*. 2015, 2018). The fact that fecundity is positively related to female body length is a feature common to many marine isopods (Guarino *et al*. 1993, Garcia-Guererro & Hendrickx 2005).

The similarities observed also in the dynamics population of *P. sculpta* in the Tunis southern lagoon with those of Mexico (Munguia & Shuster 2013) and the Southwest Atlantic (Rumbold *et al*. 2018), where the species is declared definitively established, could explain the invasive capacity of this species linked, for example, to their phenotypic plasticity. Broad environmental tolerance is also likely to be a favorable trait for successful establishment, introduced species must be able to cope with a range of contrasting environmental conditions, which are often different from those encountered in native areas (Geburzi & McCarthy 2018).

Among several alien crustaceans introduced into the lagoon, only a few species have successfully established. Several ecological and life-history traits are associated with their success, many of these traits are associated with reproduction, an essential factor determining whether a species successfully establishes and spreads (Geburzi & McCarthy 2018). Therefore, a study of population dynamics and reproductive traits of *P. sculpta* was required.

High densities and occurrence of all developmental stages of the intertidal isopod *Paracerceis sculpta* in Tunis Southern Lagoon suggest that the species is established in its new environment. Similar patterns were reported in the same area for the lessepsian isopods *Sphaeroma walkeri* and *S. venustissimum* (Ounifi Ben Amor *et al*. 2015, 2018). Such settlement could be considered as a main consequence, which displays the successful ecological rehabilitation of the area by introduction of the species previously unknown in the region (Ben Souissi *et al*. 2005). Lagoon ecosystems also provide favorable trophic conditions for alien growth and reproduction and a relatively low biotic resistance due to impoverished native communities (Azzurro *et al*. 2014). Ounifi Ben Amor *et al*. (2016, 2019b) noted that most of the first records of alien species in Tunisia stem from ports and lagoons appeared as favorable 'transit sites'. Tunis Southern Lagoon could be considered at present as a prosperous recipient ecosystem for the settlement of a viable population and a potential spread zone of invasive species of *P. sculpta*.

The location of the Tunis southern lagoon near the ports has always played an important role in the bioinvasion phenomenon (Ounifi Ben Amor *et al*. 2019b).

The present study represents an additional contribution, highlighting how the life history traits can be the key in determining the success or failure of the settlement process of an introduced species. Studies of genetic diversity and evolutionary changes should be useful for understanding the lack of the two morphs in introduced invasive populations, the potential for colonization and establishment, geographic patterns of invasion and range expansion.

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