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## Reproductive features of the invasive crab *Libinia dubia* H. Milne Edwards, 1834 from the Central Mediterranean Sea

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### Abstract

Reproduction is a key feature in the dynamics and establishment of non-indigenous species in new areas. Thus, reproductive biology studies are particularly important for predicting whether alien species can become invasive and to pinpoint the most effective time to implement management options and mitigation actions. Here, we report the reproductive aspects of the invasive crab *Libinia dubia* from the central Mediterranean Sea. The population was sampled monthly for two years (January 2015 to December 2016) on the southern Tunisian coasts (Gulf of Gabès). A total of 4469 specimens were examined, of which 50.88% were males and 49.12% were females (Confidence Interval, CI = 0.015). The overall sex ratio was slightly male-biased (1: 0.97). The first gonadal sexual maturity was earlier in females than in males. Fecundity increased with crab size and weight. *Libinia dubia* has a single annual spawning season (March to July) in the study area. Species reproductive activity was greatest in spring, with a peak in May, while the sexual rest phase occurred during October and November in females and from September to November in males. Reproduction is one of the most influential factors in the ability of invasive species to successfully thrive and establish a population in a new environment. Our results provide important biological inputs for improving the understanding of *L. dubia* reproductive performance and support recommendations for research relevant to the prediction of future *L. dubia* invasions.

**Keywords:** Brachyura; Tunisian coasts; climate change; non-indigenous species; size at first maturity; gonad maturity stages; reproduction indices; Fecundity.

### Introduction

Biological invasions, known as the spread and establishment of Non-Indigenous Species (NIS) into a new ecosystem, are a major concern in terms of changing environmental dynamics globally (Vermeij, 1996). This phenomenon has been increasingly occurring as a consequence of species' accessory transport alongside goods, services, and human travel across long distances (Galil *et al.*, 2007; Ros *et al.*, 2014; Crowley *et al.*, 2017). Bioinvasions have been gaining attention as a significant threat to the marine environment due to their severe multi-level impacts (Piras *et al.*, 2019). In fact, introduced marine species have been documented to displace native species, alter biodiversity, and food web dynamics, disrupt recipient ecosystems, and affect socio-economic factors and human health (Galil, 2011; Galil *et al.*, 2014; Katsanevakis *et al.*, 2014; Ojaveer *et al.*, 2015). Crustaceans are among the most frequently reported invaders in the Mediterranean Sea (Galil, 2011; Galil *et al.*, 2014; Zenetos,

2019; Crocetta *et al.*, 2021). The distribution of exotic marine species introduced to the Mediterranean Sea over the past 30 years (491 taxa) highlighted the dominance of invertebrates, with more than 58% (287 species) represented mainly by decapods (Zenetos, 2019).

Reproduction is the main mechanism that facilitates the species proliferation and continuity. In brachyurans, this process is extremely diverse and ultimately shaped to maximize the number of offspring (Hartnoll & Gould, 1989; López Greco *et al.*, 2000). Several methods were used to determine the reproductive cycle of crabs, including the sex ratio, size at first sexual maturity, fecundity, and gonadal development (O'Brien *et al.*, 1999; González-Gurriarán *et al.*, 2002; Safaie *et al.*, 2013; Gonçalves *et al.*, 2016; Waiho *et al.*, 2017). Gonad macroscopic evaluation is commonly and widely used in fishery resource assessment because of the large number of samples that can be examined (Da Silva Castiglioni & Negreiros-Fransozo, 2006; Sal Moyano *et al.*, 2011; Safaie *et al.*, 2013; Gonçalves *et al.*, 2016; Basilone *et al.*,

2021). In this way, gonadosomatic index (GSI, unit mass of gonad per unit mass of bodyweight) (Nolan *et al.*, 2022) can be used to investigate gonad variations throughout the year (Tapella *et al.*, 2002; Lawal-Are, 2010; Baklouti *et al.*, 2015; Basilone *et al.*, 2021). Moreover, condition factors have been used to understand population health and variability (Lloret *et al.*, 2012; Baklouti *et al.*, 2015; Brosset *et al.*, 2017) and to measure stored body energy (Vila-Gispert & Moreno-Amich, 2001).

For efficient stock management, it is essential to determine the carapace width at which 50% of individuals reach maturity. Size at first sexual maturity is a fundamental parameter for defining the mechanism controlling the balance of parental stock and may contribute to the study of environmental effects on population biology (Mori *et al.*, 1990). The size at onset sexual maturity in brachyurans can be evaluated by comparing physiological, morphological, functional and behavioral criteria (López Greco & Rodríguez, 1998; Pinheiro & Fransozo, 1998; Castiglioni & Santos, 2000; Flores *et al.*, 2002; Castiglioni Da Silva & Negreiros-Fransozo, 2006). This parameter can be evaluated by considering growth allometry, gonad development stages, presence of spermathecae in spermathecae, and fecundity (López Greco & Rodríguez, 1998; Flores & Paula, 2002).

Fecundity estimation has increasingly become the preferred measure of stock reproductive potential and may be incorporated into scientific recommendations that provide biological reference points for sustainable fishery management (Goni *et al.*, 2003; Tallack, 2007; Cooper *et al.*, 2013; Soundarapandian *et al.*, 2013). This may provide important information on replacement rates (Cobo & Okamori, 2008) and the measurement of reproductive performance in natural populations (Mantelatto & Fransozo, 1997; Varisco & Vinuesa, 2011). In brachyurans, the reproductive effort resulting from egg incubation is strongly correlated with body size or weight (Cobo & Okamori, 2008; Carmona-Osalde & Rodríguez-Serna, 2012; Lin *et al.*, 2021). The lack of a relationship between fecundity and body size may have several causes, such as individual variation in egg production, seasonal food availability, and multiple spawning events in addition to natural egg loss (Hines, 1982; Hartnoll, 1985).

Acquisition of morphometric and physiological maturity is crucial for successful mating (Sainte-Marie *et al.*, 1995; Barón *et al.*, 2009). Majoidea crabs present a terminal molt after the prepubertal stage, featuring morphometric maturity and end of their growth (Hartnoll, 1978). This group reveals some reproductive particularities that have frequently been the subject of investigations (Sampedro *et al.*, 1999; Sal Moyano *et al.*, 2010; Sal Moyano *et al.*, 2011; Gonçalves *et al.*, 2016). In particular, Majoidea females' reproductive tracts are composed of spermatheca, which accumulate sperm from males after mating (Sal Moyano *et al.*, 2010).

The Western Atlantic native spider crab *Libinia dubia* H. Milne Edwards, 1834 has been observed in southern Tunisian coasts nearly two decades ago (Enzenross *et al.*, 1997) in the vicinity of an oil terminal; it was likely, introduced via ballast water (Enzenross & Enzenross,

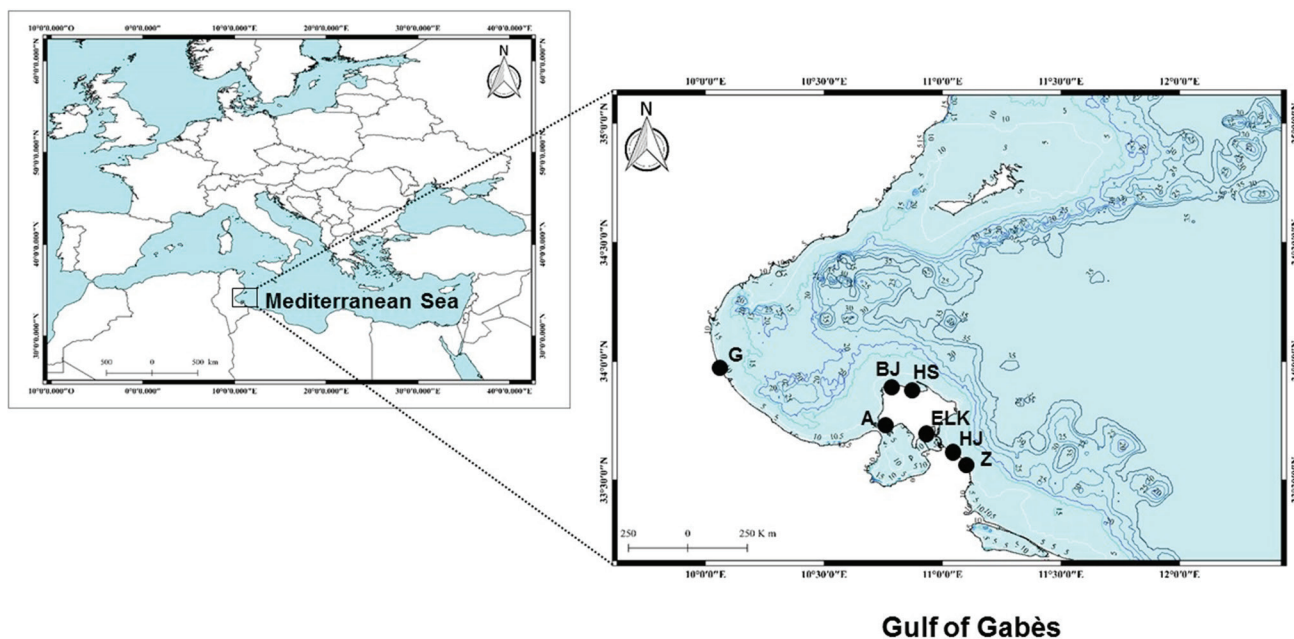
2000). Since its first sighting, this species has spread widely in the Gulf of Gabès, causing negative impacts on local ecosystems (such as competition with native crabs) and coastal artisanal fisheries (by altering catches and net clogging) (Ben Souissi, 2015; Ounifi-Ben Amor *et al.*, 2016; Rjiba-Bahri *et al.*, 2019). Adapted management practices are critical for mitigating the impacts of invasive species. Therefore, new information on *L. dubia* can provide resources that can be used to limit the spread of invasive populations. *L. dubia* is naturally distributed from Cape Cod to southern Texas, including Cuba and Bahamas (Williams *et al.*, 1977). Juveniles of this species are common in seagrass beds, and adults are frequently found in sandy bottoms (Williams, 1984). Immature crabs are often found attached to jellyfish species such as, *Stomolophus meleagris* Agassiz, 1860, which breeds seasonally along the coasts (Tunberg & Reed, 2004), *Chiropsalmus quadrumanus* (F. Muller, 1859) (Phillips *et al.*, 1969), *Aurelia aurita* (Linnaeus, 1758) (Jachowski, 1963) and *Gonionemus* sp., while adults are benthic (Carman *et al.*, 2017). Juveniles have also been associated with other pelagic organisms such as the loggerhead sea turtle *Caretta caretta* (Linnaeus, 1758) (Frick *et al.*, 2004). In Mexico, some aspects of the reproductive biology of *L. dubia* have been studied under laboratory conditions. Females spawned at least twice and were receptive to males after each spawning event (Carmona-Osalde & Rodríguez-Serna, 2012). The congener species *Libinia ferreirae* Brito Capello, 1871, presented a continuous reproductive pattern, as observed for *Libinia spinosa* Guérin, 1832 (Braga *et al.*, 2007; Gonçalves *et al.*, 2017). In *L. ferreirae* males, gonadal maturity was attained before morphometric maturity, whereas females showed synchronic morphometric, gonadal, and functional maturity (Gonçalves *et al.*, 2016).

The longnose spider crab *L. dubia* is the only species of this genus known to occur in the Mediterranean Sea, suggesting its invasive behavior and adaptive potential. In the Mediterranean Sea, studies on this species have examined its morphological and biochemical aspects in addition to its trophic habits (Rjiba-Bahri *et al.*, 2019; Chaffai *et al.*, 2020). This study is the first to investigate the reproductive biology of *L. dubia* outside of its natural range. The aim is to establish new information on the species to help decision makers for designing management plans.

## Materials and Methods

### Study area and sampling design

This study was conducted in the Gulf of Gabès (southern Tunisia). The gulf extends 750 km from Ras Kapoudia at 35 °N, parallel to the Tunisian Libyan border. From January 2015 to December 2016, seven sites were sampled monthly. In total, 4469 crabs (2195 females and 2274 males) were used in this study (Fig. 1, Table 1). All samples, except ovigerous females, were caught using gillnets (22, 23, 25, and 28 mm mesh size) and trammel nets (26/28 and 30/40 mm mesh size). Because of bury-



**Fig. 1:** Study area and sampling sites.

**Table 1.** Sampling details of *L. dubia* from the Gulf of Gabès.

Code	Site name	Geographic coordinates	Habitat	Number of <i>L. dubia</i> caught
G	Ghannouch	33.5633° N, 10.0358° W	Sandy	41
BJ	Borj Jilij	33.5339° N, 10.4621° W	Sandy	134
HS	Houmet Souk	33.5236° N, 10.5128° W	Muddy	177
A	Ajim	33.4353° N, 10.4538° W	Rocky	133
ELK	El Kantara	33.4109° N, 10.5508° W	Sandy	2854
HJ	Hassi Jerbi	33.3716° N, 11.0110° W	Seagrass	693
Z	Zarzis	33.3136° N, 11.0632° W	Muddy	437

ing behavior, ovigerous females were caught in one site (El Kantara, shallow waters) from March to July over the two sampling years. Ovigerous crabs were collected with a dip net (25 mm mesh size) during night foot-fishing surveys (Barton *et al.*, 1999; Mannino & Thomas, 2002; Mannino *et al.*, 2011; Privat *et al.*, 2013).

### Sample processing

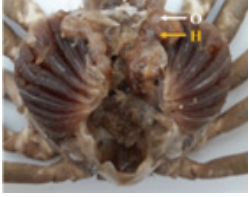



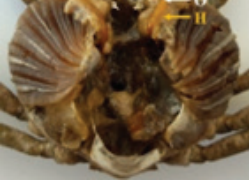
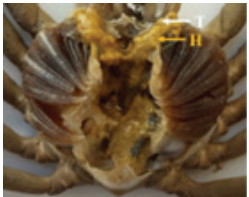
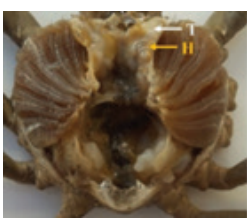

Species identification followed Williams *et al.* (1977). Crabs were sexed based on abdomen shape (thin triangular in males and oval in females) and the number of pleopods (two pairs for males and four pairs for females). Individuals were classified as juveniles or adults based on their secondary sexual characteristics. In both sexes, juveniles presented a sealed abdomen, whereas adults presented an unlocked flexed abdomen (Guinot & Bouchard, 1998; Bolla Jùnior & Negreiros-Fransozo, 2015). Furthermore, juvenile females had flat abdomens, whereas adults had domed abdomens. In males, the distinction be-

tween immature and mature crabs was also made based on chela development, as explained by Le Foll (1993).

For both sexes, gonad macroscopic analysis was conducted, and development stages were characterized according to gonad state (color, shape, consistency, and volume in relation to the hepatopancreatic region) (Table 2). There are five gonad development stages for females: (1) immature, sealed abdomen with filamentous and translucent ovaries; (2) rudimentary, unlocked abdomen with thin and white-yellowish ovaries; (3) developing, unlocked abdomen with relatively voluminous and light orange-colored ovaries; (4) developed, unlocked abdomen with thicker dark orange ovaries; (5) sexual rest, unlocked abdomen with deflated pale orange ovaries. Likewise, three gonadal developmental stages have been characterized for males: (1) immature, with sealed abdomen and filamentous translucent testis; (2) rudimentary, with a thin white testis and unlocked abdomen; (3) developed, with a thicker white-colored testis and unlocked abdomen (Choy, 1988; Abelló, 1989; Zara *et al.*, 2011; Gonçalves *et al.*, 2016).



**Table 2.** Macroscopic sexual maturity scale in *L. dubia* from the Gulf of Gabès. O: Ovary, T: Testis, H: Hepatopancreas.

♀				
Stage	Gonad color	Gonadal development	Life cycle	Illustration
1	Transparent, colourless	Filamentous translucent ovaries, not clearly differentiated	Prematuration	
2	White-yellowish	Thin, very slightly swollen ovaries not exceeding the hepatic area	Early maturation	
3	Light orange	Ovaries clearly visible, relatively voluminous occupying most of the hepatic area, oocytes not visible to the naked eye	Maturation	
4	Dark orange	Ovaries at full development occupying the entire hepatopancreas, visible oocytes	Spawning	
5	Pale orange	Deflated ovaries	Post-spawning	
♂				
1	Transparent, colourless	Filamentous testis, undifferentiated and translucent	Prematuration	
2	White	Thin and differentiated testis that occupy only a small part of the hepatopancreas	Early maturation (subadult)	
3	White	Opaque and swollen testis covering almost the entire hepatic region	Maturation	

For each specimen captured, the carapace width (CW, estimated to 0.01 mm), carapace length (CL, 0.01 mm), and total weight (TW, 0.01 g) were measured. Cheliped metric and mass characteristics were also recorded (length (CHL, 0.01 mm), width (CHW, 0.01 mm), height (CHH, 0.01 mm) and weight (CHWe, 0.01 g).

### Sex ratio

Within a population, the sex ratio is defined as the proportion of males to that of females. According to Kartas & Quignard (1984), this can be expressed as the rate of masculinity or femininity. In this study, we evaluated the sex ratio by calculating the rate of femininity, which was determined by month, season, and size class. The results of the sex ratio were tested using a statistical compliance test, Chi-square ( $\chi^2$ ) at  $\alpha=5\%$  (significance,  $\chi^2_{cal} > \chi^2_{th} = 3.84$ ,  $p < 0.01$ ) among sexes, seasons and size classes.

### Gonadosomatic and hepatosomatic indices, and condition coefficients

The gonads and hepatopancreas of 1036 crabs (546 females and 490 males) were weighed (to 0.001 g). The mean gonadosomatic index (GSI) was analyzed monthly for both sexes between November 2015 and October 2016. In fact, GSI results in changes in gonad weight throughout the year:

$$GSI = \frac{GW}{TW} \times 100$$

GW: Gonad Weight (g); TW: Total Weight (g) (DeVlaming *et al.*, 1982).

The non-parametric Kruskal-Wallis test (H test), was applied to compare the GSI values among gonad maturity stages in both sexes (Katz, 2006).

The hepatic cycle was studied monthly for both sexes by assessing the mean hepatosomatic index (HSI) and analyzing Fulton's condition coefficients K and  $K_{ch}$  (Ricker, 1975). These parameters reflect changes in the hepatopancreas, body, and cheliped weight.

$$HSI = \frac{HW}{TW} \times 100$$

$$K = \frac{TW}{CW^3} \times 100$$

$$K_{ch} = \frac{CHWe}{CW^3} \times 100$$

HW: Hepatopancreas Weight (g), CW: Carapace Width (mm), CHWe: Cheliped Weight (g).

### Size at first sexual maturity

The carapace width at first maturity (at which 50% of crabs are mature),  $CW_{50}$ , was estimated by selecting carapace width data from August to February. Of 2448 samples collected during this period, gonads of 1688 crabs were examined. These samples, comprising 856 males and 832 females, were classified as mature and immature

based on macroscopic examination of the gonads.

The data were allocated to 2 mm CW size classes. Percentages of mature crabs in each size class and for both sexes were calculated. The  $CW_{50}$  was determined and correlated based on the logistic function described by Ghorbel *et al.* (1996):

$$p = \frac{1}{1 + e^{-r(CW - CW_{50})}}$$

p: proportion of mature individuals, CW: Carapace Width,  $CW_{50}$ : Carapace Width at which 50% of the individuals are mature, r: curve inclination.

The XLSTAT software (iterative method) was used to adjust the function. The results were tested using a statistical compliance test, Chi-square ( $\chi^2$ ), at a 5% level of significance ( $\alpha = 5\%$ ).

### Fecundity

In brachyurans, absolute fecundity is estimated by counting the number of eggs stored or carried by ovigerous female abdomens (Kumar *et al.*, 2003). During the breeding season (March to July), oocytes of 72 ovigerous females (with bright orange to orange eggs in early stages of development) were collected, weighed to the nearest 1/100 g, and maintained separately in Gilson's solutions to ensure their total dissociation.

For each female, oocytes were diluted in 200 ml of water. After each solution was homogenized using a magnetic stirrer, five 1 ml samples were taken for each individual. The oocytes were counted in a Dollfus tank under a binocular microscope. The number of oocytes ( $N_o$ ) was estimated using the following power functions:

$$N_o = a CW^b, N_o = a TW^b$$

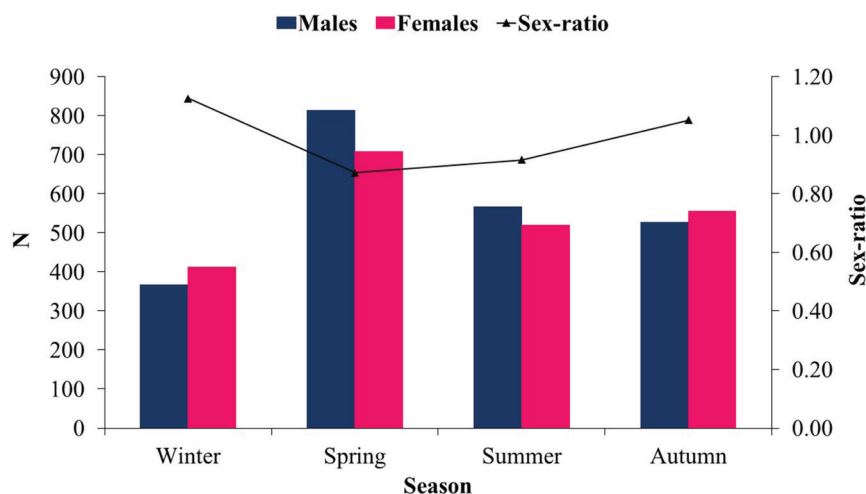
Where; a: condition factor, and b: allometric coefficient which represents the slope in the linear form of the equation.

The relationship between the number of oocytes and oocyte weight (OW) was estimated using a linear regression of the form  $N_o = a OW + b$ . A Student test (T- test) was applied to assess fecundity relationships.

### Results

#### Sex ratio

In the total population (4469 crabs), 2195 females and 2274 males were counted, which corresponded to 50.88% males (CI = 0.015) and 49.12% females (CI = 0.015). The mean carapace width values were 50.34 mm ( $\pm$ SD, 9.18) and 52.74 mm ( $\pm$ SD, 6.77) in males and females, respectively. The overall sex ratio was slightly male-biased (1: 0.97), variable across seasons (Fig. 2), and significantly different from the equal ratio ( $\chi^2_{cal} = 11.23 > \chi^2_{th} = 3.84$ ;  $df = 1$ ,  $p < 0.01$ ). During the hot season (spring and summer), the number of males was slightly higher than



**Fig. 2:** Seasonal sex-ratio variations in *L. dubia* from the Gulf of Gabès. Sex-ratio below 1: more males than females and vice versa.

that of females, while we recorded a significant female bias during the cold season (winter and autumn) ( $\chi^2_{\text{cal}} > \chi^2_{\text{th}} = 3.84; p < 0.01$ ).

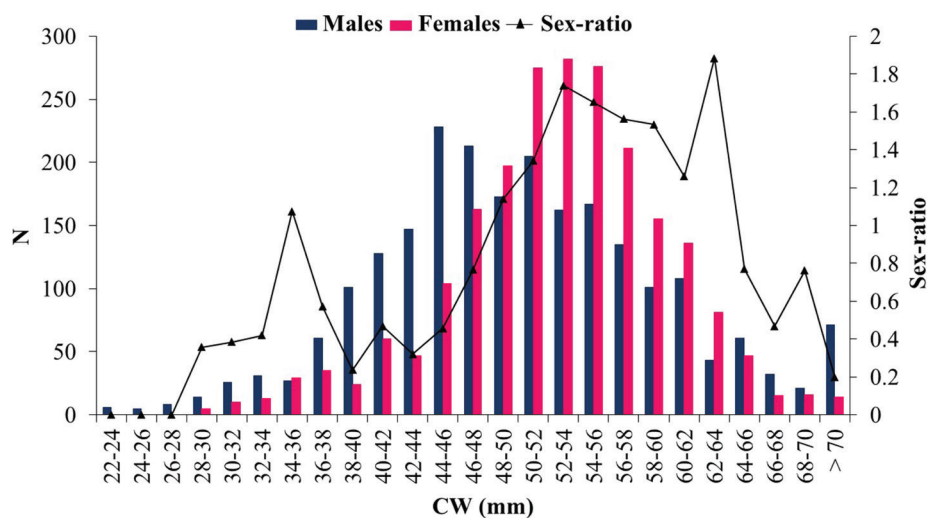
Sex ratio fluctuations related to size classes showed a significant male bias for small size classes (from 22 to 48 mm) as well as for large size classes (from 64 to more than 70 mm) ( $\chi^2_{\text{cal}} > \chi^2_{\text{th}} = 3.84; p < 0.01$ ), while females were significantly more numerous among medium size classes (48 to 64 mm) ( $\chi^2_{\text{cal}} > \chi^2_{\text{th}} = 3.84; p < 0.01$ ). Sexes were distributed almost equally for the size class of 34 to 36 mm (Fig. 3). The maximal total sex ratio (1.88) was recorded for the 62 to 64 mm size class and was zero for the first three size classes. Overall, the ratio varied significantly from 1:1 according to size class ( $\chi^2_{\text{cal}} = 28.15 > \chi^2_{\text{th}} = 3.84; p < 0.01$ ).

Most of the sampled population was composed of mature individuals, mainly concentrated during the cold season (September to February). Immature crabs were more numerous than mature ones during the hot period (March to August). The highest number of immature individuals was caught in the mid-breeding season (May), whereas the number of mature individuals was the highest in the

mid-cold season (November) (Fig. 4).

#### Monthly variations in GSI, HSI, K, and $K_{ch}$

Different stages of *L. dubia* reproductive cycle were described according to the GSI analysis. In females, the pre-maturation period occurred from December to mid-February, followed by a short period of early maturation until the end of February. The GSI values gradually increased from 2.06 to 2.92. The maturation phase was marked by a rapid increase in GSI during March, when it reached a peak of 6.48, followed by a spawning phase characterized by a fall in the GSI and announcing the beginning of the breeding season (March to the end of July). The post-laying phase occurred in August and September. During October and November, the ovaries entered the sexual rest phase. In males, the pre-maturation phase started in November and extended to the beginning of December. Maturation began in January and continued in February when the GSI moved from 1.26 to 1.79. Beyond these months, the crabs mated until the end



**Fig. 3:** Sex-ratio variations according to size classes in *L. dubia* from the Gulf of Gabès. Sex-ratio below 1: more males than females and vice versa.

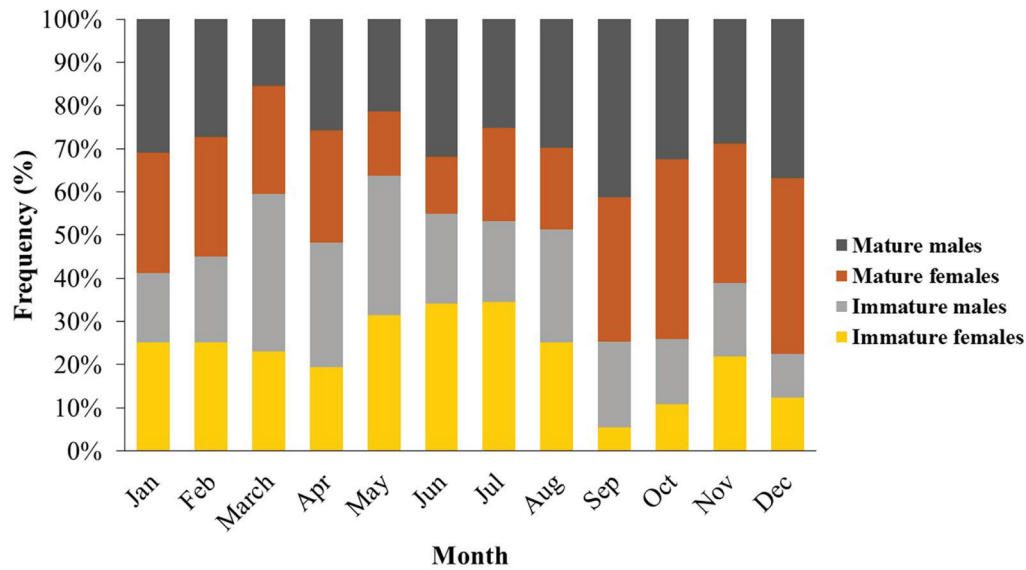


Fig. 4: Monthly frequencies of mature and immature *L. dubia* specimens from the Gulf of Gabès.

of August, after which the gonads rested from September to mid-November (Fig. 5A).

The mean monthly rates of hepatosomatic index (HSI) are shown in Figure 5B. In females, this rate reached a minimum during the post-laying period, from August onwards (2.79), in contrast to males, where a maximum of 7.39 was recorded in August. The condition coefficient, K, was not highly variable in females as it was in males. However, we recorded more variations for males in the  $K_{ch}$ , which showed that male chelipeds were much more developed than those of females (Fig. 5C, D).

#### Monthly variations in gonad maturity stages

Monthly variations in the gonad maturity stages are presented in Figure 6. For both sexes, the highest number of sexually mature crabs was observed in February, indicating that the breeding season was nearing. From March, the percentage of mature crabs started decreasing until the end of June for females and May for males. After July, this number gradually increased. Sexually immature crabs were the most common from April to July for females and from April to September for males. These results, in addition to the GSI analysis, showed that

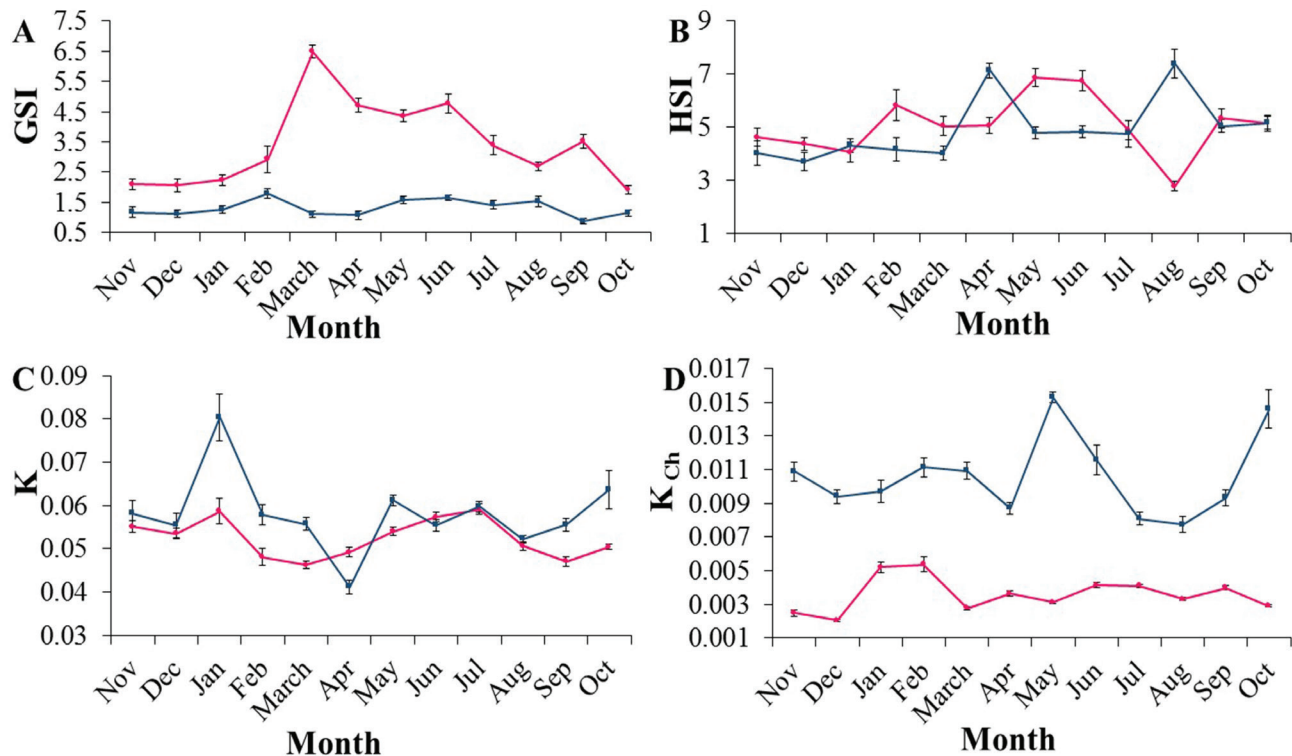
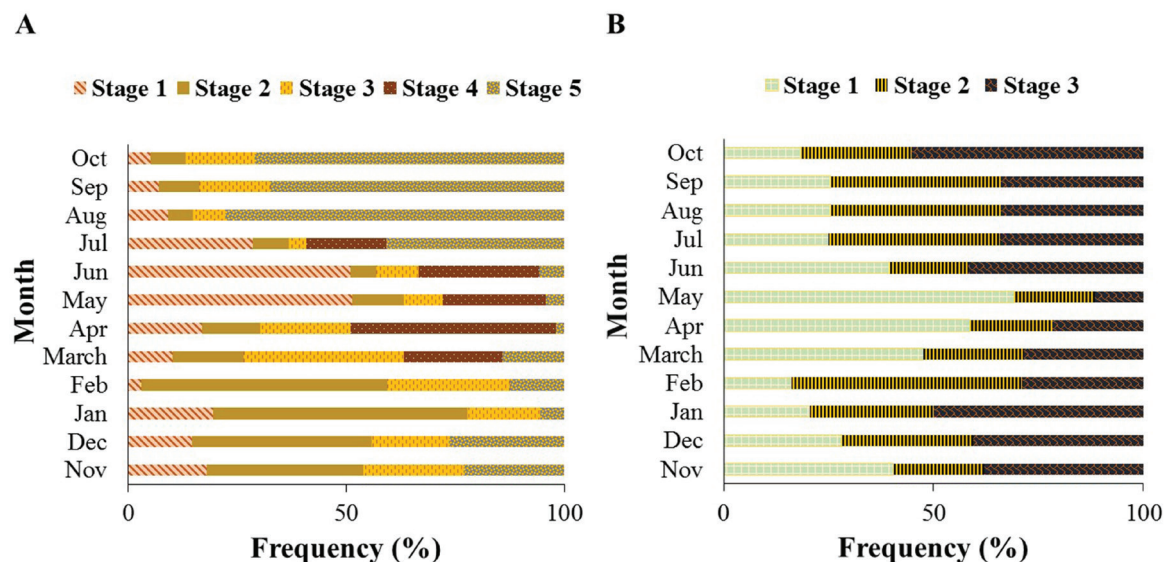


Fig. 5: Monthly evolution of the GSI (A), HSI (B), K (C) and  $K_{ch}$  (D) in *L. dubia* from the Gulf of Gabès. Pink: females, Blue: males. Error bars indicate two standard errors showing 95% confidence interval.





**Fig. 6:** Monthly evolution of the frequency of gonad macroscopic stages in *L. dubia* from the Gulf of Gabès. (A) Females, (B) Males.

the laying period of *L. dubia* lasted from March to July. The post-laying phase occurred in August and September. Beyond September, the females entered the sexual rest phase, and their gonads showed no activity. In males, mating began in March and continued until the end of August, after which the gonads entered the sexual rest phase for the next three months.

#### Relationship between GSI and gonad maturity stages

GSI was low at early stages of gonad maturation

(Stages 1 and 2) with white translucent ovaries and began to increase when yolk accumulation gradually shifted to stage 3 with light orange gonads. The highest mean GSI was observed in fully mature crabs with dark-orange ovaries (stage 4). Likewise, GSI was low in the early stages of gonad maturation (Stage 1) in males and began to decrease gradually to stages 2 and 3 with white differentiated testes. However, mean GSI values were less variable for males than for females. The Kruskal-Wallis test indicated that GSI values varied significantly in both sexes and for each stage ( $p < 0.001$ ), except for GSI values between 1 and 5 in females (Table 3).

**Table 3.** Relationship between GSI and gonad maturity stages in *L. dubia* from the Gulf of Gabès, a: calculated test statistic (H statistic).

GSI %	Females					Kruskal-Wallis test (H test), $df = 4$		
	Frequencies (%)					Rank sum <sup>a</sup>	Average rank sum	$p$
	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5			
< 1	17.65	17.76	1.12	0.0	2.56	120.66	63.14	< 0.001
1 to 5	68.9	70.09	57.31	32.0	83.97	6.75	3.15	0.14
> 5	13.45	12.15	41.57	68.0	13.47	41.88	21.17	< 0.001
No. of sample	119	107	89	75	156			
Mean GSI±SE (%)	2.84 ± 0.16	2.67 ± 0.19	4.39 ± 0.22	5.72 ± 0.21	3.36 ± 0.13			
GSI %	Males			Kruskal-Wallis test (H test), $df = 2$				
	Frequencies (%)			Rank sum <sup>a</sup>	Average rank sum	$p$		
	Stage 1	Stage 2	Stage 3					
< 1	64.74	37.58	20.24	60.29	31.46	< 0.001		
1 to 1.5	14.45	20.81	25.0	35.76	17.11	< 0.001		
> 1.5	20.81	41.61	54.76	18.41	8.75	< 0.001		
No. of sample	173	149	168					
Mean GSI±SE (%)	0.86 ± 0.044	1.37 ± 0.07	1.75 ± 0.072					

### Size at first sexual maturity

Size at onset gonadal sexual maturity in *L. dubia* from the Gulf of Gabès was  $38.86 \pm 0.07$  mm in females and  $40.73 \pm 0.03$  mm in males. The results of the  $\chi^2$  test showed that the function was well fitted with data from our study on gonadal sexual maturity. Furthermore, significant differences were recorded between the observed and estimated values based on logistic parameters of sexual maturity ( $\chi^2_{\text{cal}} > \chi^2_{\text{th}}$ ). The  $CW_{50}$  is plotted for females and males in Figure 7.

### Fecundity

The smallest ovigerous female exhibited a total weight of 33.41 g, 36.71 mm carapace width and was capable of producing about 1603 oocytes, whereas we recorded 23025 oocytes in the largest ovigerous female, with a size of 66.03 mm and a total weight of 149.31 g. The mean value of oocytes counted was  $10344.16 \pm 1418$  (Table 4). The number of oocytes ( $N_o$ ) versus size (CW), total weight (TW), and egg weight (OW) were highly correlated (Fig. 8).  $R_2$  values were close to 1, and Student's test revealed a highly significant correlation ( $t_{\text{cal}} > t_{\text{th}} = 1.96$ ,  $p < 0.001$ ) for the relationship of oocyte number with total weight ( $N_o = f(\text{TW})$ ).

### Discussion

Our study revealed variation in the mean carapace width recorded for females compared to males, which is

consistent with the results obtained for the spider crab *Mithrax tortugae* Rathbun, 1920 from the Brazilian coast (Cobo & Alves, 2009). This pattern diverges from common findings for brachyurans and may be explained by the absence of female samples belonging to significantly smaller size classes, which is likely due to fishing gear selectivity (Fischer & Wolff, 2006).

The sex-ratio in *L. dubia* from the Gulf of Gabès is close to 1, as is commonly observed in brachyurans (Hartnoll, 1978; Lawal-Are, 2010). This ratio was indicative of a slight male bias. A similar pattern was recorded for the Atlantic population of the species (O'Brien *et al.*, 1999), and the preliminary results obtained for the invasive population by Rjiba-Bahri *et al.* (2019). Generally, marine crustaceans have a skewed sex ratio, due to sex-specific habit segregation and single-sex migration (Wenner, 1972; Lin *et al.*, 2021). Biotic and abiotic changes can influence sex ratio; during the hot season (spring and summer), we found a clear dominance of males in the fishing area. This period coincided with the female brood season. Ovigerous females bury themselves and do not leave the bottom to protect the eggs. This behavior was previously confirmed by studying the species' trophic habits, where *L. dubia* ovigerous females presented empty stomachs and showed no feeding activity compared to non-ovigerous females and males (Chaffai *et al.*, 2020). It is important to mention that the samples used in this study came from coastal fishing activities; therefore, the low abundance (or even absence) of females during the hot period could also be explained by the clear preference of females for deep, high-salinity waters (Archambault *et al.*, 1990). In fact, they migrate to higher salinities to spawn (More, 1969; Perry, 1975). In our study, the sex

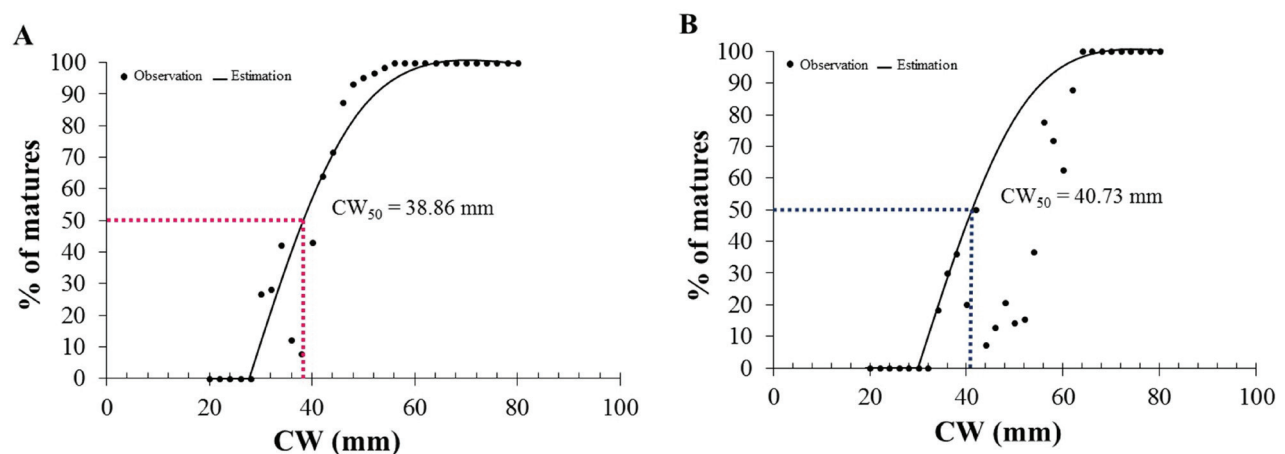
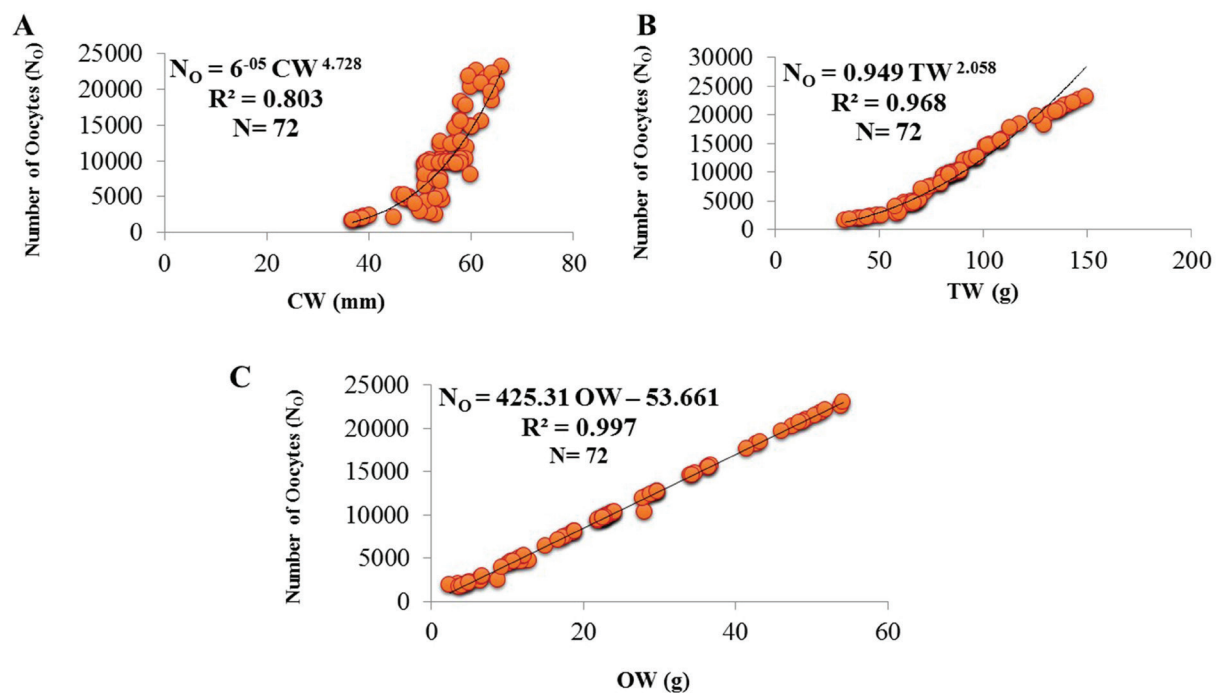


Fig. 7: Graphic representation of sexual maturity in *L. dubia* from the Gulf of Gabès. (A) Females, (B) Males.

Table 4. Absolute fecundity in *L. dubia* females from the Gulf of Gabès.

N = 72	CW (mm)	TW (g)	OW (g)	$N_o$
Min	36.71	33.41	3.76	1603
Max	66.03	149.31	54.11	23025
Mean $\pm$ CI	$53.89 \pm 1.61$	$87.02 \pm 6.64$	$24.32 \pm 3.33$	$10344.16 \pm 1418$



**Fig. 8:** Relationships between Number of oocytes ( $N_o$ ) versus Carapace Width (CW) (A), Total Weight (TW) (B) and Oocyte Weight (OW) (C).

ratio related to size classes revealed the absence of juvenile females belonging to the smallest size distribution in the fishing area. It was only possible to catch *L. dubia* female samples over 28 mm in size. This finding could be explained by the symbiotic relationships, especially with jellyfish. Juvenile crabs belonging to the genus *Libinia* Leach, 1815, are commonly involved in symbiotic relationships with several jellyfish species namely *Stomolophus meleagris*, *Chiropsalmus quadrumanus*, *Aurelia aurita*, and *Gonionemus* sp. (Jachowski, 1963; Phillips *et al.*, 1969; Tunberg & Reed, 2004; Sal Moyano *et al.*, 2012; Carman *et al.*, 2017; Gonçalves *et al.*, 2020). Spider crab juveniles can also be associated with the loggerhead sea turtle *C. caretta* (Frick *et al.*, 2004).

Spatial distribution patterns can influence species abundance in a given area (Gonçalves *et al.*, 2017; Gonçalves *et al.*, 2020). In our study, the total number of *L. dubia* caught decreased during the cold period, as recorded for the native population, for the congener *Libinia emarginata* Leach, 1815, and for the sea spider *Maja squinado* (Herbst, 1788) (O'Brien *et al.*, 1995; González-Gurriarán *et al.*, 2002). O'Brien *et al.* (1999) also reported a low abundance, even the absence of *L. dubia* and *L. emarginata*, in some months of the cold season, suggesting that crabs can enter a state of dormancy or migrate to other areas. O'Brien *et al.* (1995) explained this phenomenon, particularly in spider crabs, by migratory movements. Indeed, in *M. squinado*, newly molted individuals leave coastal areas and migrate to deep waters (> 50 m) during the cold season (González-Gurriarán *et al.*, 2002). In the present study, the sex-ratio was significant among sexes, seasons and size classes. However, the sample was not truly random and this sex ratio could have reflected a behavioral difference between the sexes related to the activity.

The maturity of any species depends on GSI varia-

tions. In our study, GSI was low in the early stages of maturation with white translucent gonads, in contrast to that observed in the advanced maturity stages. The GSI values were not inversely proportional to the HSI values. However, other studies reported a coincidence between minimum values of HSI and maximum values of GSI, and vice versa, suggesting metabolic reserve mobilization (López Greco & Rodríguez, 1999; Baklouti *et al.*, 2015). In *L. dubia* females, the hepatosomatic index reached a minimum during the post-laying period, in contrast to that in males. This could be explained by the depletion of stored lipid reserves in the hepatopancreas during the oocyte maturation and reproductive processes. In the present study, the condition coefficients  $K$  and  $K_{ch}$  showed no significant fluctuations when discussing reserve mobilization during the reproductive process, as observed in the spider crab *M. squinado* from the Gulf of Gabès (Baklouti *et al.*, 2015).

Knowledge of the gonad maturity stages is required for many purposes including the determination of mature individuals, size at first maturity, and reproductive potential of populations. Gonad color change during vitellogenesis allows for the rapid evaluation of the reproductive stage in time and space as well as the study of the reproductive cycle without performing microscopic analyses (Arculeo *et al.*, 1995). During the cold period, *L. dubia* presented mainly translucent uncolored gonads, which developed and gradually varied in color to mature during the hot season, due to the breeding period. The gonad maturity stages in *L. dubia* were five and three in females and males, respectively, as described in *Portunus segnis* (Forskål, 1775) from the Persian Gulf (Safaie *et al.*, 2013). In *L. ferreirae* from south-eastern Brazilian coast, Gonçalves *et al.* (2016) reported four gonad maturity stages in females with synchronicity for morphometric and gonadal maturity, and three in males with early

gonadal maturity compared with morphometric maturity. However, in the congener species *L. spinosa*, Sal Moyano *et al.* (2011) identified four stages of gonadal development in both males and females. Nonetheless, the number of gonadal development stages can be elevated to six in females and four in males in some crab species (Da Silva Castiglioni & Negreiros-Fransozo, 2006).

*Libinia dubia* sampled from the Gulf of Gabès showed a single spawning period (March to July), with the peak of the period occurring in May. Juveniles were numerous during the two months after the peak in ovigerous females. They grow in autumn and mature during winter. The recruitment peak was observed in the mid-hot season, when higher temperature levels were recorded, as observed for *L. ferreirae* (Gonçalves *et al.*, 2017). In our study, the sexual rest occurred in autumn, whereas in *L. emarginata*, the breeding period implied that sexual rest occurred mainly in winter (Hinsch, 1968) (Table 5). Indeed, a female spider crab does not require periodic copulae and can spawn for a long period of the year with only one spermatid mass. Therefore, sperm stored in the female spermatheca is used for the fertilization of eggs immediately prior to spawning (González-Gurriarán *et al.*, 1998).

In the current study, *L. dubia* females were precocious compared to males and reached gonad sexual maturity at a smaller size. The smallest ovigerous female size in our study was lower than that in CW<sub>50</sub>. In *L. ferreirae* from south-eastern Brazilian coast, gonadal sexual maturity was reached at carapace widths of 38.77 and 34.86 mm in females and males, respectively (Gonçalves *et al.*, 2016). In *L. spinosa*, the size at which 50% of females and males reached gonad maturity was 40.33 mm and 33.6 mm, respectively. Gonad maturity in males belonging to the genus *Libinia* is acquired prior to morphometric maturity (Sal Moyano *et al.*, 2011; Gonçalves *et al.*, 2016). According to Sampedro *et al.* (1999), the transition from the juvenile to the adult phase in males occurs over a wide range of CW, which may vary among cohorts, thus changing the size at maturity. Furthermore, spatial and

bathymetric segregation during migration can affect size at first sexual maturity (Baklouti *et al.*, 2015). This size can also be influenced by environmental factors such as temperature, salinity, or resource limitations (Fisher, 1999; Haig *et al.*, 2016).

The mean number of oocytes counted in our study was 10344.16± 1418, which is less than the mean fecundity estimated in the congener *L. spinosa* and in the spider crab *Leurocyclus tuberculatus*. For medium-sized *L. spinosa* female (≈50 mm), 30000 oocytes were recorded (González-Pisani & Greco, 2014). In the sea spider *M. squinado* from the Gulf of Gabès, Baklouti *et al.* (2015) counted 6430 eggs in a female with a CW size of approximately 43.65 mm, and 12800 eggs in a female of about 73.1 mm carapace width. Fecundity measures from the current study were well integrated into the fecundity interval previously established by Carmona-Osalde & Rodríguez-Serna (2012) for *L. dubia* ovigerous females under laboratory conditions. Overall, spider crabs have low fecundity compared to other brachyurans, particularly, crabs belonging to the portunid family (Safaie *et al.*, 2013; Yusli Wardiatno *et al.*, 2015). However, this can be offset by the large size of the eggs (Hines, 1982). In *L. dubia* from the Gulf of Gabès, fecundity was strongly correlated with body size and weight. Such correlations are common in brachyurans (Hines, 1982; Hartnoll, 1985; Cobo & Okamori 2008; Safaie *et al.*, 2013; Baklouti *et al.*, 2015).

Traits of invasive crustaceans include high growth rates, high fecundity, early sexual maturity, and small size at maturity (Best, 2015). These strategies help increase the population's reproductive output potential, which can accelerate the establishment of permanent populations (Wassick *et al.*, 2017). *Libinia dubia* seems to possess established traits common to invasive crustaceans. For example, it reached maturity at a smaller size than that recorded in the indigenous spider crab *M. squinado* (Baklouti *et al.*, 2015). The conditions of reproduction and growth (i.e., food availability) are favorable in the Gulf of Gabès (Chaffai *et al.*, 2020), which probably ex-

**Table 5.** Comparison of the reproductive cycle in some spider crab species.

Source	Area	Species	Spawning period	Peak breeding	Sexual rest
Current study	Gulf of Gabès (Tunisia)	<i>L. dubia</i>	Single, March to July	May	September to November
Sousa <i>et al.</i> (2020)	Southeastern Brazil	<i>L. spinosa</i>	Continuous	--	--
Baklouti <i>et al.</i> (2015)	Gulf of Gabès (Tunisia)	<i>M. squinado</i>	Single, September to February	November	April to September
El-Serehy <i>et al.</i> (2015)	Suez Canal, Egypt	<i>Schizophrys aspera</i> (H. Milne Edwards, 1834)	Continuous	May, December	--
Varisco & Vinuesa (2011)	Bustamante Bay, Argentina	<i>Leucippa pentagona</i> H. Milne Edwards, 1834	Single, June to February	--	March to May
Hinsch (1968)	Massachusetts, USA	<i>L. emarginata</i>	Single, March to September	--	December



plains the high dispersal ability and successful establishment of the species in the newly invaded environment for more than two decades. Size at onset sexual maturity data can subsequently support adaptation of management arrangements. In fact, *L. dubia* under-size individuals caught could limit them from reproducing and/or renewing the stock, which may help minimize the threats caused by the species.

## Conclusion

Developing a robust understanding of the reproductive biology of NIS is essential to refine management strategies. The current study provides crucial information on the peak breeding season and size at the onset sexual maturity of the invasive spider crab *L. dubia*, which are crucial for the accurate implementation of management policies. The reproductive performance of *L. dubia* might have assisted in the successful invasion of this species. Further research on the spatial distribution patterns in females of the invasive population is recommended. Moreover, testing habitat segregation during ontogenetic shifts is essential to describe species ecology and to improve our understanding of ovigerous female behavior. Such investigations will also support management practices to ensure that damage caused by the species is mitigated to the greatest possible extent. In addition, because of the low abundance of smaller juveniles recorded in the current study, further studies are needed to verify whether juveniles of the invasive population exhibit symbiotic life stages with locally available species as observed in the native range or in the congeneric species *L. ferreirae* and *L. spinosa*.

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