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Additonal and unusual records of bleu crabs *Portunus segnis* and *Callinectes sapidus* from the northeastern Tunisian waters (Central mediterranean sea)

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Abstract - The present paper deals with recent records of the blue crabs *Portunus segnis* (Forskål, 1775) and *Callinectes sapidus* (Rathbun, 1896) from the northeastern coast of Tunisia during September 2019. A total of 10 brachyura individuals including 4 blue swimming crabs and 6 Atlantic blue crabs were collected in shallow waters on sandy and muddy bottom by fishermen using gillnets and trammel nets from Bizerte and Ghar El Melh Lagoons. All morphometric measurements of these two species were studied. The difference between morphometric characters for *P. segnis* and *C. sapidus* were also examined. The concomitant occurrence of these two crab species will have probably unfavourable consequences for these restricted areas.

Key words: Biodiversity, Invasion, Brachyura, Tunisian Lagoons, Climate change, Socio-economic impacts.

1. Introduction

The alien decapods found in the Mediterranean Sea have mainly a lessepsian origin (77%) and *Portunidae* represent the highest number of Red Sea/ Indo-Pacific decapod aliens in the Mediterranean Sea (Klaoudatos and Kapris 2014). Among the invasive Portunidae, *Portunus segnis* and *Callinectes sapidus* were recorded from the southern Tunisia coast (Ben Souissi et al. 2017; Rabaoui 2015; Rifi et al. 2014) and exhibited a rapid geographic migration towards the northern areas.
The blue swimming crab *Portunus segnis* originated from Indo-west Pacific Ocean is one of the first Lessepsian species recorded and established in the Eastern Mediterranean (Zenetos et al. 2010). It was present in the Suez Canal since 1889 and reached Port Said (Egypt) in 1898 (Fox 1924). This species was until recently confused with its congener *Portunus pelagicus* (Linnaeus, 1758) and long considered "species nomenclature without ambiguity". This misidentification was revised by Lai et al. (2010) based on morphological and genetic studies.
Actually, *P. segnis* was reported in many Mediterranean countries passing from the Levantine basin to the east Aegean, eastern Sicily and then in northern Tyrrhenian (Galil and Zenetos 2002; Falsone et al. 2020). In Tunisia, the *P. segnis* first occurrence was in the Gulf of Gabes (southern Tunisian coasts) in 2014 (Rifi et al. 2014). Some months after this observation, it 'bloomed' in the southern of Tunisia (Crocetta et al. 2015) and expanded its geographical distribution to the northern coasts namely the Gulf of Hammamet in 2016 (Bdioui 2016) and in the Gulf of Tunis (Ben Abdallah-Ben Hadj Hamida 2019a).
Moreover, the Atlantic blue crab *Callinectes sapidus* (Rathbun, 1896) (Crustacea: Decapoda: Portunidae) is a shelf-estuarine important species originally distributed in the Eastern coasts of North America, widely distributed from southern Nova Scotia, Canada, to Argentina (Nehring 2011). This

crustacean is a euryhaline species commonly found on muddy and sandy bottoms, generally at depths < 35 m (Hill et al. 1989). This species was introduced in Europe through ballast waters (Mancinelli et al. 2017), where it progressively expanded from the Atlantic area, to the Mediterranean and the Black Seas (Suaria et al. 2017).The record of *Callinectes sapidus* was confirmed in the Mediterranean from 1948 with two specimens in the Northern Adriatic (Suaria et al. 2017). Since then, *C. sapidus* has been widely recorded in Mediterranean coastal waters (Daban et al. 2016; Galil et al. 2008; Galil 2011). According to Suaria et al. (2017), a population of blue crab is established now in the Adriatic Sea (Cilenti et al. 2015; Manfrin et al. 2016). In Tunisia, *Callinectes sapidus* has been recorded around Kerkennah Island (Southern Tunisia) by Ben Souissi et al. (2017). Two years after, this species continues to proliferate very rapidly in the north and was observed and collected in many localities (Ben Abdallah-Ben Hadj Hamida et al. 2019b).This work constitutes a biogeographical update of *Portunus segnis* and *Callinectes sapidus* distribution in the northern Tunisian waters mainly in Bizerte and Ghar El Melh lagoons. We reported also some observations on their biology and ecology in these areas.

2. Materials and methods

2.1. Study area and specimens sampling

Almost concomitantly, in September 2019, specimens of *P. segnis* and *C. sapidus* were accidentally collected in trammel nets at two Tunisian northern lagoons; Bizerte lagoon and Ghar El Melh lagoon as shown in the figure 1. At Bizerte lagoon, a total of four specimens of *Portunus segnis* and two ones of *Callinectes sapidus* were collected in 14th September by local fishermen using gillnets (Mesh size 22mm) on sandy-muddy bottom at depths ranging between 6 and 7 m. Personal communication with local fishermen confirmed confusion between the two blue crab species.
In addition, four individuals of *Callinectes sapidus* were caught in 20th September as a bycatch of trammel nets operated by small-scale fishing boats in Ghar El Melh lagoon in shallow waters between 4 and 6 m depth on muddy and sandy bottoms (Fig. 1). The fishermen immediately recognized the blue crab as an unusual capture of a conspicuous species.

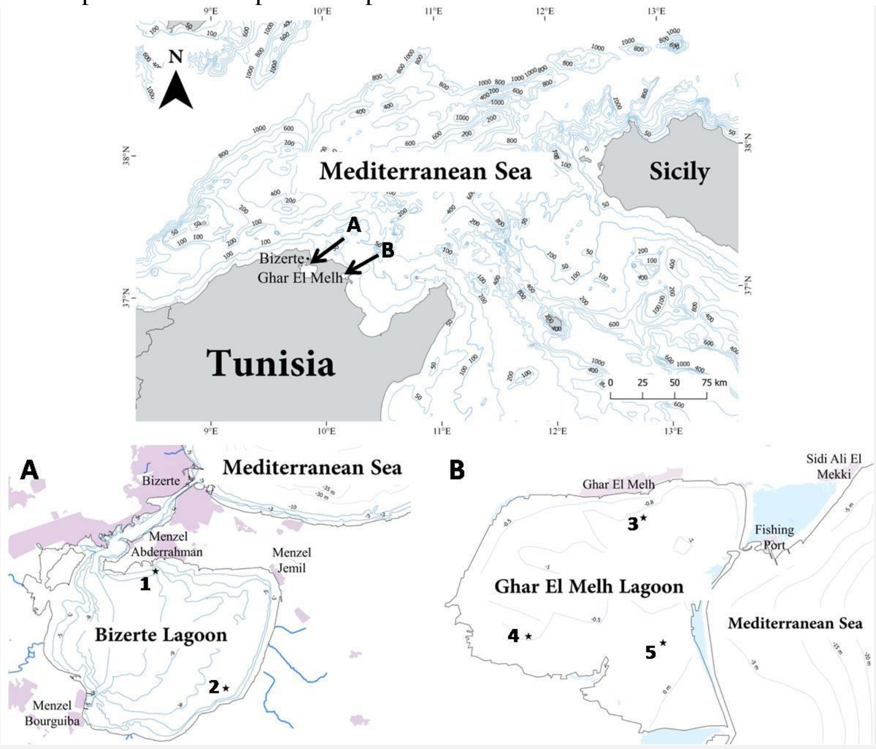

Figure 1. Locations of *Portunus segnis* (1 & 2) and *Callinectes sapidus* (1, 3, 4 & 5) records in Bizerte Lagoon (A) and Ghar El Melh Lagoon (B).

2.2. Morphological measurement procedures

In the laboratory, species were identified according to the description of Williams (1974) and Lai et al. (2010). All individuals collected were sexed and weighted (W) to the nearest 0.01 g using a precision balance. The morphological characteristics for each crab were measured to the nearest 0.1 mm using electronic callipers. Each specimen was photographed and subsequently preserved in 10% buffered formalin included in a mixed solution of ethanol and glycerol to avoid carapace hardening, and deposited in the faunal collection of the "Institut Supérieur de Pêche et d'Aquaculture de Bizerte".

Considering the limited number of specimens, males and females for each species were pooled together to determine the morphometric characters. A total of 18 different morphometric characters were measured following Giraldes et al. (2016), Carapace Length (CL), Width (excluding 9th anterior-lateral tooth-CW1/and including 9th anterior-lateral tooth-CW2), Posterior Margin (PM), Antero-Lateral Border (ALB) and Frontal Margin (FM). For right and left cheliped were obtained the Merus Length (MEL), Merus Width (MEW), Manus Length (MAL), Manus Width (MAW), Dactylus Length (DAL). In the 4th pereiopod merus were obtained the Length (4PL) and the Width (4PW). As well as in the dactylus at natatory leg were obtained the Length (NDL) and the Width (NDW). In the abdomen were the Penultimate segment Length (PL) and width (PW), and Telson Length (TL) and Width (TW) (Fig.2).

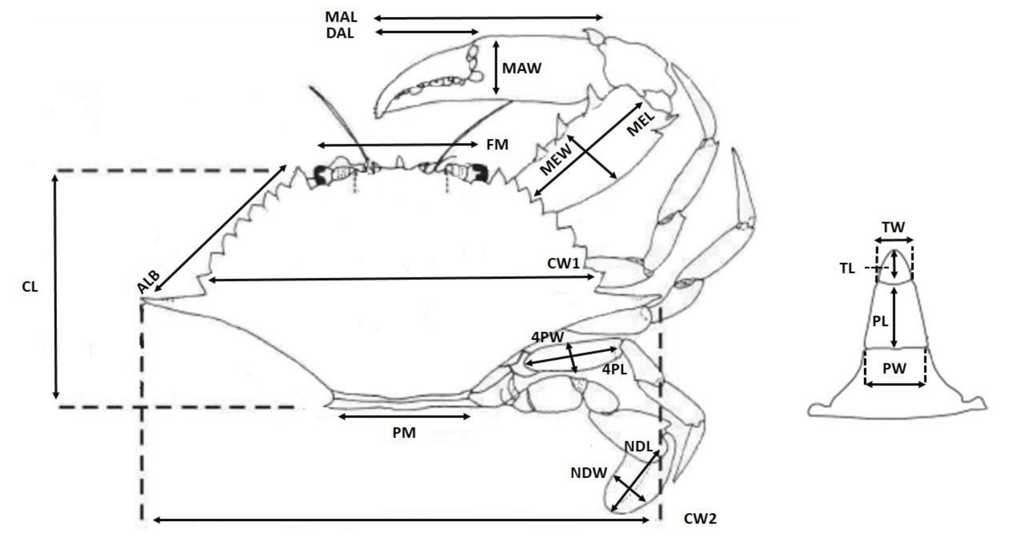

Figure 2.Morphological measurements taken for *Portunus segnis* and *Callinectes sapidus* caught in Tunisian waters

Analyses of character measured (W, FM, CL, PM, CW_1,CW_2, ALB, MAL, MAW, DAL, MEL, MEW, NDL, NDW, 4PL, 4PW, TL, TW, PL, PW) were performed for *Portunus segnis* and *Callinectes sapidus*. Student's t-test was utilized to assess deviations between the mean of each character measured, with significance level $\alpha = 0.05$ (Froese 2006).

3. Results and discussion

In this study, a total of 6 crabs of *C. sapidus* (3 females and 3 males) and 4 *P. segnis* (4 females including 3 breeding females) were analyzed (Fig. 3). According to (Safaie *et al.*, 2013), with reference to the coloring, arrangement and size of the ovary, the grained females are in stage 4. Indeed, the gonad covers most of the hepatic region with a dark brown color.

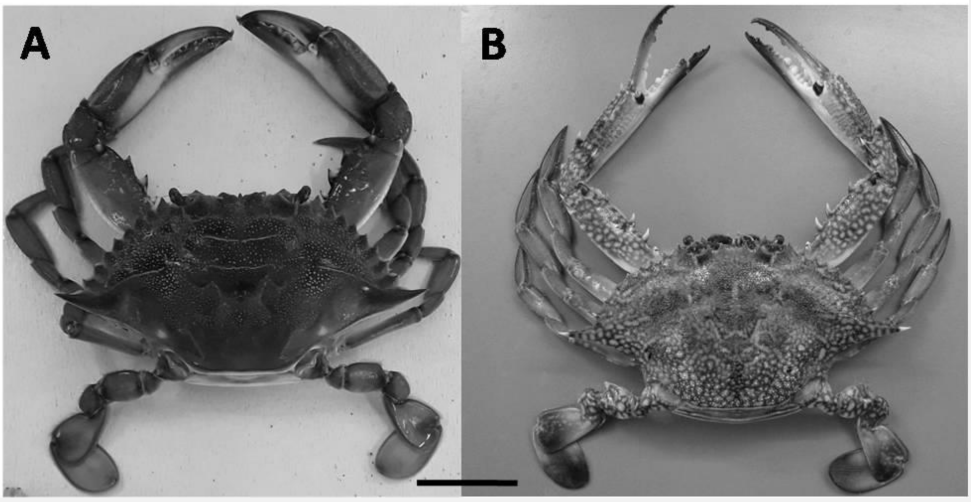

Figure 3. Specimen of *Callinectes sapidus* (A) and *Portunus segnis* (B) from Bizerte lagoon and Ghar El Melh lagoon, North Tunisian waters (September 2019). Scale bar: 25 mm

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Carapace length was ranging from 50.03 to 73.52 mm (mean 62.48 ± 7.69 mm) for *C. sapidus*, and from 57.06 to 75.45 mm (mean 66.60 ± 7.53 mm) in *P. segnis*. The mean total weight was 185.13 ± 82.82 g and 222.75 ± 68.86 g for *C. sapidus*, and *P. segnis* respectively. The average size of *P. segnis* was statistically similar to *C. sapidus* (Student's t-test = 0.78, p < 0.05) (Table I).
Morphometric measurements obtained for *C. sapidus* and *P. segnis* are indicated in table I. The morphometric measurements of all characters studied suggested that both crab species are similar for most characters measured (W, CL, PM, Cw1, CW2, ALB, DAL, NDW, 4PL, MAL, MAW, PW, MEL, MEW, TL and PL). In the case of TW and 4PW, a distinction between the species with a positive pattern for *P. segnis* (P >0.05) was identified. FM character shows a significant superiority in *C. sapidus* compared to *P. segnis* (P >0.05). For both species, and with regard to variance analysis, no significant difference was noted between 17 morphometric characters. However, 3 characters present a significant difference between *C. sapidus* and *P. segnis.*

Table I : Morphometric measurements for *P. segnis* and *C. sapidus* specimen caught in northern Tunisian lagoons (Bizerte and Ghar El Melh). n: number of specimens

Morphometric parameter	Species	n	Range	Mean± SD	*t-test*	*P-value*	Significance
W	*P. segnis*	4	160.00-317.82	222.75±68.86	0.70	0.50	-
	C. sapidus	6	91.10-322.20	185.13±82.82			
FM	*P. segnis*	4	22.75-28.75	25.42±2.52	-2.66	0.02	+
	C. sapidus	6	24.76-34.06	30.86±3.18			
CL	*P. segnis*	4	57.06-75.45	66.60±7.53	0.78	0.45	-
	C. sapidus	6	50.03-73.52	62.48±7.69			
PM	*P. segnis*	4	36.40-49.87	44.26±5.66	1.99	0.08	-
	C. sapidus	6	30.60-43.01	37.57±4.45			
CW_1	*P. segnis*	4	101.68-132.97	119.08±13.07	0.57	0.58	-
	C. sapidus	6	87.91-132.54	113.38±15.29			
CW_2	*P. segnis*	4	128.01-170.00	148.09±17.54	0.73	0.48	-
	C. sapidus	6	98.72-170.16	137.09±23.64			
ALB	*P. segnis*	4	51.77-67.38	60.66±6.58	1.12	0.29	-
	C. sapidus	5	36.81-67.01	53.19±10.52			
MAL	*P. segnis*	4	63.51-86.04	74.57±9.52	1.02	0.34	-
	C. sapidus	5	45.38-85.70	65.85±13.12			
MAW	*P. segnis*	4	17.76-25.65	21.49±3.24	-0.57	0.58	-
	C. sapidus	5	16.26-28.25	22.99±3.92			
DAL	*P. segnis*	4	33.98-46.69	41.59±5.50	1.45	0.18	-
	C. sapidus	5	26.10-45.13	35.47±6.09			
MEL	*P. segnis*	4	36.68-47.87	43.44±4.83	-0.10	0.91	-
	C. sapidus	5	37.60-50.31	43.78±4.16			
MEW	*P. segnis*	4	14.36-17.99	16.69±1.60	-0.29	0.77	-
	C. sapidus	5	13.93-18.54	17.22±2.91			
NDL	*P. segnis*	4	7.72-9.86	8.56±0.93	-1.21	0.25	-
	C. sapidus	6	6.78-31.27	15.39±9.99			
NDW	*P. segnis*	4	24.03-29.17	27.11±2.20	1.62	0.14	-
	C. sapidus	6	17.92-31.27	22.32±5.05			
4PL	*P. segnis*	4	25.88-35.76	32.61±4.58	1.68	0.13	-
	C. sapidus	6	20.65-36.70	26.90±5.13			
4PW	*P. segnis*	4	14.24-20.75	18.16±2.77	3.41	0.00	+
	C. sapidus	6	8.25-15.69	12.23±2.41			
TL	*P. segnis*	4	8.43-8.46	8.60±0.42	-0.70	0.50	-
	C. sapidus	6	5.42-12.70	9.46±2.17			
TW	*P. segnis*	4	8.53-11.41	10.38±1.27	3.26	0.01	+
	C. sapidus	6	4.46-9.63	6.85±1.71			
PL	*P. segnis*	4	14.03-17.1	15.82±1.28	-1.38	0.20	-
	C. sapidus	6	14.56-33.15	21.08±6.70			
PW	*P. segnis*	4	28.85-45.80	38.93±7.17	1.53	0.16	-
	C. sapidus	6	16.55-46.62	29.04±9.86			

Accidental and intentional introductions of carcinological alien fauna in Mediterranean Sea are the origin of profound disturbances (Zaouali et al. 2007). In Tunisia, non-native crabs represent 70.58% of total alien decapoda (Ounifi Ben Amor et al. 2016). *P. segnis* is one of the first NIS ranked with *Percnon gibbesi* as the "most successful invaders in the Mediterranean Sea" by Klaoudatos and Kapiris (2014) and listed, as *C. sapidus,* in the '100 worst invasive species' (Sterftaris and Zenetos 2006).
The Lessepsian *P. segnis* is an active predator and carnivorous species (Katsanevakis et al. 2014), with large populations recorded in the gulf of Gabes (Southern Tunisia) (Ben Abdallah-Ben Hadj Hamida et

al. 2019a). Few months after its first occurrence, the blue swimming crab bloomed and became a threat to artisanal fishermen with serious socio-economic impacts. Thus, Tunisian Ministry of Agriculture, Water Resources and Fisheries implemented a national plan for the exploitation and evaluation of the blue crab through grants for research, distribution of fishing gear to artisanal fishermen and tasting campaigns. This species is edible worldwide (Carpenter et al. 1997), and since its record in Tunisia, it has become frequent in all fish markets and in high demand from consumers. The expansion of the swimming blue crab into southern Tunisian waters (Gulf of Gabes) (Hajjej et al. 2016) has led to the development of fisheries addressed to catch this species. In this context, some new traps are experimented by the National Institute of Marine Sciences and Technologies for the capture of *P. segnis* (Bdioui and Missaoui, 2017). With regard to *C. sapidus*, although its range expansion in the Mediterranean Sea is very fast (Box et al. 2020) and its first occurrence in Tunisia dates from 2017 (Ben Souissi et al. 2017), the proliferation of this Atlantic crab has not been noted as for *P. segnis* which could be attributed to a confusion between the two species by Tunisian fishermen. Additionally Shaiek and Ben Haj (2019), suggest the presence of *P. segnis* in Bizerte lagoon since August-September 2018. Blue crabs were captured as a by-catch of trammel nets and invisible nets, targeting commercial shrimp species (*Penaeus keraturus* Forskål, 1775) and cuttlefish (*Sepia officinalis* Linnaeus, 1758), in northern Tunisian lagoons.

Records of these crab species in the vicinity of Bizerte port, suggests that the most probable introduction pathway is maritime traffic. This hypothesis has already been advanced by Crocetta (2006). In fact, and as explained by Klaudatos and Kapiris (2014), the maritime traffic is the most important pathway of introduction of alien decapods species as larvae can survive long time in ballast water. In addition, adult crabs could be found in sediments of ballast tanks and once introduced; these animals expand their geographic range through the planktonic larval stages (Klaudatos and Kapiris 2014).

Therefore, environmental conditions, larvae migration, recruitment pattern and/or anthropogenic factors such as shipping activities, could be behind the spread of this bio-invasion in the North of Tunisia. The strong potential of expansion of *P. Segnis* to higher latitudes could be attributed in part to the water temperature rise in the Mediterranean Sea. Indeed, several studies suggested that global warming speed up the increase of Lessepsian species in the Mediterranean Sea (Bianchi, 1997; Francour et al. 1994; Romano 2003, 2007). The blue crab recognised as strong swimmers showed a rapid proliferation and expansion of their biogeography unlike other species such as *Libinia dubia* H. Milne Edwards, 1834, reported in Tunisian waters since 1997.

The maximum CL for Blue crabs varies substantially among different populations (Giraldes et al. 2016). There is strong evidence that abiotic variables related to the local climate have the strongest influence on this parameter, as previously proven in the literature (Araújo et al. 2012; Ben Abdallah-Ben Hadj Hamida et al. 2019a; Hajjej et al. 2016,). Moreover, the occurrence of an important number of females as well as the high percentage of ovigerous ones leads to deduce that the blue crabs are well established in Bizerte and Ghar El Melh lagoons and forms a well-structured population. The sexual dimorphism in weight (male heavier than female) that was recorded in many studies is a typical pattern for many brachyuran crabs (Araújo and Lira 2012; Hosseini et al. 2014; Pinheiro and Fiscarelli 2009, Tadi et al. 2012, Noori et al. 2015).

Comparisons in morphometric ratios highlight some important differences between *C. sapidus* and *P. segnis* studied. The morphometric results presented in this work have not been previously undertaken in Tunisian waters, in particular the differences between the two species *C. sapidus* and *P. segnis*. The ratios presented here will be of use in future studies when comparing the sizes and ratios of these species; as well as comparisons for the same species in different regions and life stages. However, the number of specimens sampled for each species and in each lagoon should be enlarged in order to compare growth and reproductive parameters between species and lagoons.

When compared sizes of specimens collected in this study, *C. sapidus* is similar to *P. segnis* specimens ($P>0.05$).The comparison of *P. segnis* size in different localities in Tunisia shows that specimens from Northern Tunisian lagoon (Mean CL=66.60mm) were larger than those from the Gulf of Gabes located in the South of the country (Mean CL=64.1mm (Rabaoui et al. 2015); Mean CL=50.57mm (Hajjej et al. 2016); Mean CL=53.45mm (Ben Abdallah-Ben Hadj Hamida et al. 2019a). One important strategic adaptation of these species is the reproductive migration to deeper waters which is performed by females of crab species such as *P. segnis* and *C. sapidus* to ensure a higher rate of successful spawning. More favorable temperatures for the spawn are achieved in deeper waters where temperature remains at an almost constant (22-28 C) (Giraldes et al. 2016). This migration is performed as a strategy to ensure safe spawning without competition from aggressive males (Andrade et al. 2014; Le Vay and Falamarzi

2009). This behavior is not necessary for the expansion of the biomass of these crabs in Bizerte and Ghar El Melh lagoon where in these shallow coastal areas, temperatures fluctuate regularly and could not reach 32°C (Ramdani et al. 2009; Ziadi et al. 2015).

The lagoons of Bizerte and Ghar El Melh have been recognized as a sea in ecological decline with several contributing factors such as anthropogenic impacts, harsh environmental conditions, and overfishing, this is in combination with an important decline on fishery resources since the late 1980s (Ben Garali et al. 2009 ; Ramdani et al. 2009; Romdhane et al. 2019). References and personal communication with local fishermen revealed that a considerable number of fish species are disappearing from these lagoons. The largest fishery biomass removal has been through the implementation of a combination of fishing and aquaculture activities, these include gillnets and aquaculture structure placed along coastal areas. Fishing practice in these areas is particularly nonselective and is contributing to a significant drop of benthic resources. Observation on these activities leading to several tons of landings will increases in the future with the introduction of the blue crab species in these semi-closed areas. Additionally, despite evidences highlighting competitive interactions with native species (Gennaio et al. 2006; Mancinelli et al. 2013) and impacts on small-scale fisheries (Fuentes et al. 2019; Kampouris et al. 2019; Nehring 2011; Perdikaris et al. 2016; Ventura et al. 2018), the ecological and economic consequences of this invasion is poorly assessed and our understanding of the species distribution is limited.

Both, *P. segnis* and *C. sapidus* are considered among the “worst” Invasive Alien Species (IAS) in the Mediterranean Sea (Streftaris and Zenetos 2006), though appropriate monitoring protocols do not exist for these species neither other marine invaders. Nevertheless, increasing efforts are being made to engage local communities in the process of detecting and reporting newcomers, representing examples of usefulness of both citizen science and local ecological knowledge (Azzurro et al. 2019). These participatory approaches provide new and complementary options to detect and investigate biological introductions while promoting awareness among local communities.

P. segnis and *C. sapidus* are recognized as invasive new comers in the north of Tunisia (Bizerte Lagoon, Ghar El Melh Lagoon), the baseline information presented here may guide the implementation of management strategies and monitoring programs. These could be used as tools for the better understanding of the current standing stock of these species and their ecological relation with other species.

4. Conclusion

The Non-Indigenous *C. sapidus* and *P. segnis*, among the worst invasive species, impact negatively fishing activities and coastal ecosystems. The rapid expansion of these blue crabs in the North of Tunisia is due to an accelerated bio-invasion rate. Regular monitoring is crucial to identify the persistence pattern of these species and their impact in Bizerte and Ghar El Melh lagoons. These species are important in fishery around the world and their consumption is recommended for human diet due to their health-promoting characteristics. Accordingly, these threats should be transformed into benefits and an effective strategy should be developed for prevention against next introductions. Further researches on IAS biology and ecology and their impact on local fauna and ecosystems are of particular interest.

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