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Baseline

Baseline assessment of heavy metals content and trophic position of the invasive blue swimming crab *Portunus segnis* (Forskål, 1775) in the Gulf of Gabès (Tunisia)

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ABSTRACT

This study provides a baseline assessment of cadmium, copper, iron, manganese, lead, and zinc concentrations in muscles, gills, and exoskeleton of the Lessepsian blue swimming crab *Portunus segnis* captured in the Gulf of Gabès (Southern Mediterranean Sea, Tunisia) in November 2015. Furthermore, the species' trophic position is estimated using CN stable isotope analysis. The exoskeleton showed the lowest metal contents; in soft tissues, the essential Cu, Fe, and Zn and the non-essential Cd, Mn, and Pb showed the highest and lowest concentrations, respectively. The crab was characterized by a trophic position of 3.32, confirming its carnivorous trophic habits. Compared with literature data on invertebrate and fish species from the same area, Cd and Pb resulted remarkably low in the crab's soft tissues, while a food web-scale dilution effect was indicated. The need of a comprehensive assessment of the ecological impact of *Portunus segnis* in invaded Mediterranean waters is discussed.

The portunid blue swimming crab *Portunus segnis* (Forskål, 1775) is one of the earliest Lessepsian invaders (probably the first crustacean, Galil, 2011) recorded in Egyptian waters 30 years after the completion of the Suez Canal in 1868 (Fox, 1924). Subsequently, it has successfully established in coastal habitats of the Levantine basin, from Turkey to Egypt (Tureli and Yesilyurt, 2017 and literature cited). The species has been repeatedly recorded in other Mediterranean sectors, including the Aegean Sea, eastern Sicily and northern Tyrrhenian Sea (Rabaoui et al., 2015), and in recent years, in Tunisian coastal waters and neighbouring areas (e.g., Malta: Deidun and Sciberras, 2016). Specifically, *P. segnis* has been first recorded in Tunisia in 2014 in the Gulf of Gabès (Rifi et al., 2014); subsequently it expanded rapidly throughout the gulf, including Djerba Island and the Gulf of Hammamet; in addition, it has been episodically recorded in Libya [Bdioui, 2016; Ounifi Ben Amor et al., 2016; see Fig. 1A and Table A (Supplementary online material) for an updated review of the species occurrence in Mediterranean waters].

In the Persian Gulf and in other native habitats, *P. segnis* is of high economic importance (Hosseini et al., 2014); in the Mediterranean Sea, it is locally marketed in Turkey (Tureli and Yesilyurt, 2017), while in Tunisia the species is beginning to be considered a valuable shellfish product to be exported to EU countries. Consequently, a growing number of studies have focused on its occurrence, morphometrics, population structure (Rabaoui et al., 2015; Hajjej et al., 2016) as well as on its proximate composition and biochemistry, for alimentary and non alimentary purposes (Bejaoui et al., 2017; Hamdi et al., 2017). Noticeably, to date no assessment of heavy metal contamination has been performed for the species, notwithstanding the considerable pollution level characterizing some Tunisian coastal areas (e.g. the Gulf of Gabès: Rabaoui et al., 2014; El Zrelli et al., 2015). In addition, the crab is conventionally considered a predator with the potential of impacting local fisheries (Ounifi Ben Amor et al., 2016); yet, the trophic habits and functional role of the species within invaded benthic communities is currently unexplored.

The present study aims at providing a baseline assessment of heavy metal contamination and trophic position of *Portunus segnis* captured in the Gulf of Gabès (Tunisia) in winter 2015. To this end, cadmium, copper, iron, manganese, lead, and zinc concentrations were measured in muscle, gill, and exoskeleton tissues of adult crab specimens. The results are integrated with literature data on contamination levels in

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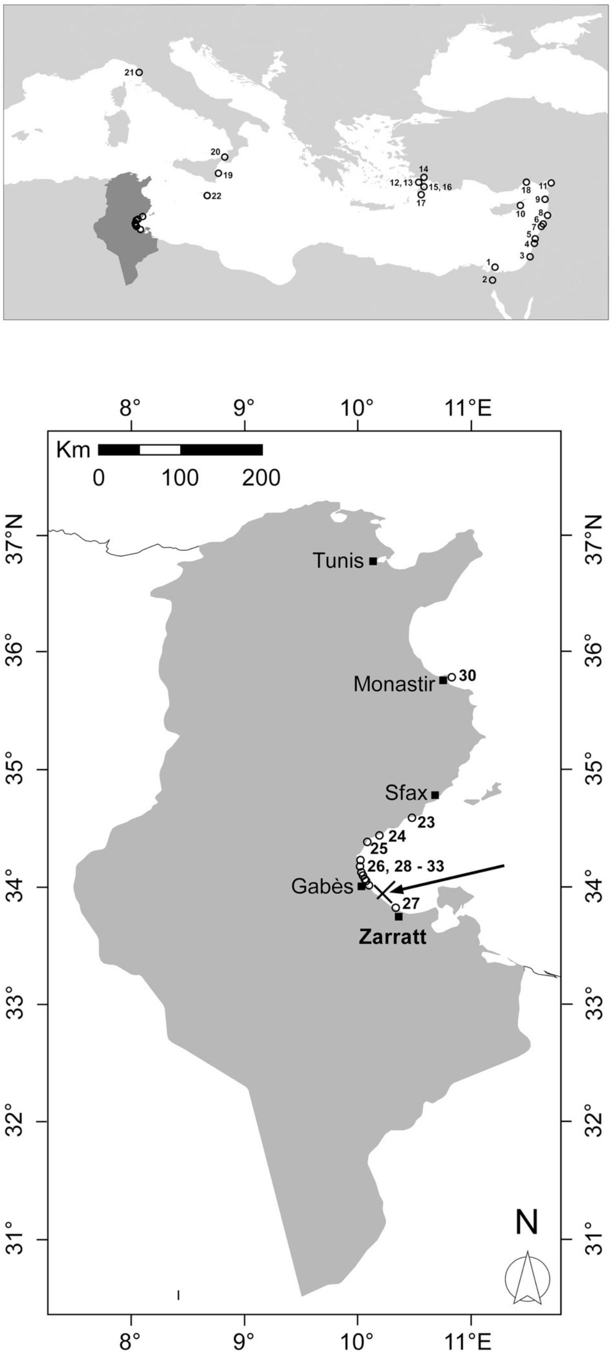

Fig. 1. (A) Records of *Portunus segnis* in the Mediterranean Sea (○). Data were collected between March and November 2017 by a literature survey performed in the online databases ISI Web of Science, Scopus, BioAbstracts, PubMed, and JSTOR adopting a multiple search criterion. The keywords "*Portunus segnis*" or "swimming blue crab" were used in combination with "Mediterranean Sea" or "Black Sea". The search produced a total of 23 references; 16 studies providing the coordinates of the record location or, alternatively, an univocal indication in a map (in this case, maps were digitized, georeferenced, and the location coordinates recorded) were selected. Table A in the supplementary online material reports details on locations.
(B) Location of the sampling site in the Gulf of Gabès, Tunisia (arrow), and occurrence of *P. segnis* in Tunisia (○). Table A in the supplementary online material reports details on locations.

other vertebrate and invertebrate species from the same area. In addition, carbon and nitrogen stable isotopes were measured in claw muscles, and used to calculate the trophic position of the crab. Estimations are compared with published information on dietary habits of the species in native habitats performed by conventional gut content analysis.

The Gulf of Gabès is located along the south-eastern coasts of Tunisia (Fig. 1B), extending for approximately 200 Km from the city of Sfax in the North to the Djerba Island in the South. The northern sector of the gulf is characterized by a high urbanization and a number of industrial activities ultimately causing a remarkable contamination of coastal biota by heavy metals and other pollutants (Barhoumi et al., 2009 and literature cited; Annabi et al., 2013). The southern coasts of the gulf are generally less impacted; the occurrence of chemical industries located close to Gabès city, however, coupled with the peculiar morphology and oceanography of the central area of the gulf (i.e., low sandy shores, shallow depths, strong tidal and wind-induced currents) determine local heavy metals pollution of water, sediments and biota in coastal and neighbouring freshwater ecosystems (Annabi et al., 2009, 2018; Rabaoui et al., 2014; Ayadi et al., 2015; El Zrelli et al., 2015).

In November 2015, adult *Portunus segnis* specimens (n = 9) were captured with the support of local fishermen approximately ten kilometers south of the city of Gabès (33°40′12″N; 10°21′0″E, Fig. 1). After collection, crabs were transferred alive in refrigerated containers to the laboratory, where they were sexed after examination of the shape of the abdomen apron. Consequently, each specimen had the carapax width (CW hereafter) measured with a vernier calliper as the distance (in mm) between the two outermost lateral spines, while their individual wet weight (WW) was determined with an electronic balance to the nearest 0.1 g.

Crabs were dissected with a ceramic scalpel and had portions of muscle, gill, and exoskeleton tissues removed. Muscle tissues were collected from the right claws. Subsequently, samples were dried to constant weight at 55 °C for 120 min, mineralized with 3 ml of nitric acid (1 M), and agitated for 48 h. The sample volume was eventually adjusted to 30 ml with deionized water. All samples were analyzed to determine cadmium, copper, lead, iron, manganese, and zinc concentrations using an Atomic Absorption Spectrophotometer (AAS, Avanta GBC spectrophotometer) equipped with Zeeman background correction. Metal concentrations were reported as $\mu g\,g^{-1}$ tissue dry weight.

Aliquots of the crab muscle were removed from the left claw, stored in falcon tubes, freeze-dried, and subsequently ground to a fine powder using a mortar and a pestle. Subsamples (0.81 ± 0.02 mg, mean ± SD) were pressed into Ultra-Pure tin capsules (Costech Analytical Technologies) and analyzed using an Isotope Ratio Mass Spectrometer (Thermo Scientific Delta Plus XP) connected to an Elemental Analyser (Thermo Scientific Flash EA 1112). Isotopic signatures were expressed in conventional δ notation (as parts per mil) in relation to international standards (PeeDee Belemnite for carbon and atmospheric N_2 for nitrogen). Analytical precision based on the standard deviation of replicates of internal standards (International Atomic Energy Agency IAEA-NO-3 for $\delta^{15}N$ and IAEA-CH-6 for $\delta^{13}C$) was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

The trophic position of *P. segnis* was estimated implementing a Bayesian approach within the R statistical environment (R Development Core Team, 2018) using the *tRophicPosition* package (version 0.7.5; Quezada-Romegialli et al., 2018a, 2018b). Analyses were performed using the $\delta^{13}C$ and $\delta^{15}N$ values of the crab, while the signatures of herbivorous isopods (undet.) and of the detritivorous Mediterranean rock shrimp *Sicyonia carinata* Brünnich, 1768 sampled in the Gulf of Gabès by Abdennadher et al. (2010) were adopted as isotopic baselines. To this end, original data were back-estimated using the means, standard deviations, and sample numerosity values reported for the two taxa (i.e., isopods: $\delta^{13}C = -11.9 \pm 0.8$, $\delta^{15}N = 4.6 \pm 0.5$, n = 3; *S. carinata*: $\delta^{13}C = -13.5 \pm 0.3$, $\delta^{15}N = 4.3 \pm 0.6$, n = 4) assuming a normal distribution. A full two-baseline, two-trophic discrimination factor Bayesian model was run with 2 parallel chains and 20,000 adaptive iterations assuming a trophic position λ of the two