







**Figure 1.** Location of the Ria Formosa lagoon (B) and of the Guadiana estuary (C) within the Iberian Peninsula (SW-Europe) (A). The sites where Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 specimens were collected are marked with white dots. In the Guadiana estuary, the black stars represent the sampling stations (S) where temperature and salinity data was measured during neap and spring tides. Images retrieved from Google Earth.

this study aims to describe the first report of the Atlantic blue crab in these ecosystems and to present hypotheses about its introduction. Finally, the hypotheses laid in the discussion of this paper may serve as a research framework delving on the invasion ecology of this new non-indigenous species in the SW-Iberian Peninsula.

## Materials and methods

### *Study areas*

The Ria Formosa is a mesotidal coastal lagoon separated from the Atlantic Ocean by a system of sand barrier islands and inlets (Figure 1B). The lagoon extends for 55 km along the southern Portuguese coast and has a maximum width of 6 km. The average depth is 3.0 m, the tidal amplitude varies from 1.3 m to 3.5 m, and the lagoon occupies an area of 84 km<sup>2</sup> at the high spring tide (Andrade 1990).

The Guadiana River estuary is a mesotidal estuary with an average depth of 6.5 m, occupying an area of 22 km<sup>2</sup>, and the tidal amplitudes range from 1.3 to 3.5 m. The estuary drains into the Gulf of Cadiz (Atlantic Ocean), extends inland for 70 km, and the last 50 km serve as the southern border of Portugal and Spain (Iberian Peninsula, Europe; Figure 1C). The Guadiana River flow varies substantially within and among years due to variation in rainfall. However, after the completion of the Alqueva dam in February 2002, the river discharge is usually lower than 20 m<sup>3</sup> s<sup>-1</sup> (Garel and Ferreira 2011), despite episodic flooding events (e.g., March–April 2013).



**Figure 2.** Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 specimen collected in the Guadiana estuary (SW-Iberian Peninsula, Europe) in July 2017. Photograph by Vânia Baptista.

#### *Capture and identification of Atlantic blue crab*

The Atlantic blue crab specimens collected in the Ria Formosa lagoon and in the Guadiana estuary were identified according to Williams (1984) (Figure 2). The total carapace width ( $\pm 0.1$  cm), fresh weight ( $\pm 1$  g), and sex were determined for some of the individuals captured by local fishermen since they kept most specimens for them. The Atlantic blue crab was a bycatch in both ecosystems and collected with trammel nets and traps (Supplementary material Table S1).

#### *Abiotic characterization of the sampling sites*

The water temperature and salinity were measured in the Guadiana estuary in the area colonized by the Atlantic blue crab, using a CTD (Valeport mini-CTD) recording at 8 Hz and logging data every 0.25 m along the water column. Two surveys were conducted, one at neap tide (August 31, 2017, tidal range 1.1 m) and one at spring tide (September 8, 2017, tidal range 3.0 m). Data were collected at high water slack (i.e., about one hour after high water level) at four stations (Figure 1C). There is no such data available for the Ria Formosa lagoon.

## **Results**

The first known accounts of the Atlantic blue crab in the Ria Formosa date back to 2016 and the first georeferenced capture site is located at 4 km from the coast (Table S1; Figure 1B). In the Guadiana estuary the first specimen was collected on July 1<sup>st</sup>, 2017, at Laranjeiras (Figure 1C; Table S1). As far as we are aware, the Atlantic blue crab occupies at least a 25-km stretch along the Guadiana estuary, from the low estuary at Vila Real de Santo

António and up to the lower upper estuary at Laranjeiras, located at 3 and 28 km away from the coast, respectively (Figure 1C). At least 11 Atlantic blue crab specimens were collected in the Ria Formosa lagoon, but we only have detailed information on one specimen (Table S1). However, in the Guadiana estuary the number of specimens reported to us is bigger and it ranged from 50 to 60 individuals (Table S1). In the Guadiana estuary, local fishermen collected 21 males and 5 females (the remaining were not sexed), the carapace width size ranged from 9.5 cm to 43.4 cm, and the total fresh weight varied between 65 g and 745 g (Table S1).

In the Guadiana, water stratification was weak during late summer, being slightly stronger at neap than at spring tides. Water temperature varied between 24.9 °C and 26.1 °C, and salinity varied between 14.4 and 27.6 at neap tide, and between 18.3 and 32.4 at spring tide (Table S2).

## Discussion

### *The Atlantic blue crab in the Ria Formosa lagoon and in the Guadiana estuary*

The Atlantic blue crab is one of two non-indigenous *Callinectes* species present in Europe (Mancinelli et al. 2017a), the other is rugose swimming crab *Callinectes exasperatus* (Gerstaecker, 1856) (Decapoda, Brachyura) – a single specimen was collected in the Bay of Cadiz (Cuesta et al. 2015).

The Atlantic blue crab has been reported from six sites/regions of the Iberian Peninsula. The Guadalquivir estuary is the closest site to the Guadiana estuary and to the Ria Formosa lagoon, which is located 120 km and 133 km away, respectively. So, four hypotheses may explain the presence of this species in these ecosystems. First, it might result from the dispersion of larvae from adjacent colonized ecosystems, as the Guadalquivir estuary. This hypothesis is supported by the occurrence of a westwards regional coastal current during ~ 40% of the time around the year (Garel et al. 2016). Second, adult individuals could have migrated westwards from the Guadalquivir estuary (Castejón and Guerao 2013), since this species can perform long-distance migrations (Hill et al. 1989). Third, the introduction could be due to the transport of live specimens in the fishing nets of boats coming from colonized ecosystems (e.g., Guadalquivir and/or Sado estuaries). Fourth, the colonization of the Ria Formosa lagoon and of the Guadiana estuary could represent another independent introduction of this species into European coastal waters via ballast water, however there is no transoceanic shipping in these sites. Therefore, the origin of these blue crab populations is uncertain and only a comprehensive genetic study can shed light on this matter. This genetic approach is essential for a future research framework on the Atlantic blue crab in these two ecosystems.

### *Changes in river flow and establishment of non-indigenous species in estuaries*

The disruption of natural river flow regimes affects biological communities along hundreds of kilometers across a spectrum of habitats, both aquatic and terrestrial, and even including adjacent coastal areas. Dams, water diversion, water extraction, and climate change are the factors most known for altering natural river flow regimes. The impacts caused by these factors on established ecological dynamics may create the opportunities for non-indigenous species to establish or to become invasive (McAllister et al. 2001).

Indeed, the Guadiana estuary underwent profound changes in all aspects of its geological, physical, chemical, and biological dynamics since the completion of the Alqueva dam in 2002 (Morais 2007, 2008; Morais et al. 2009; Domingues et al. 2011, 2014; Garel and D'Alimonte 2017). For example, the reduction of river flow increased the salinity along the estuary and the number of marine species present. Previously, the middle estuary was characterized by a reduced number of species mainly due to salinity constraints owing to marked salinity variations along the year at the estuarine turbidity maximum (Chícharo et al. 2001; Morais et al. 2009; Garel and Ferreira 2011). Nowadays, the estuarine turbidity maximum shifted more than 10 km upstream in comparison to pre-dam conditions and salinity conditions became more stable throughout the year (Morais 2007; Garel and D'Alimonte 2017). The new abiotic conditions resulted in empty ecological niches which coincided with an increase in the number of non-indigenous species identified over recent years in this part of the river: e.g., *Cordylophora caspia* (Pallas, 1771) (Cnidaria, Hydrozoa) (Seyer et al. 2017), *Synidotea laticauda* (Crustacea, Isopoda) (Mellado-Díaz et al. 2018), oriental shrimp *Palaemon macrodactylus* Rathbun, 1902 (Crustacea, Caridea) (Chícharo et al. 2009), weakfish *Cynoscion regalis* (Bloch and Schneider, 1801) (Pisces, Sciaenidae) (Morais and Teodósio 2016), and now the Atlantic blue crab (this study).

We hypothesize that the increased salinity observed in the middle and the lower reaches of the upper Guadiana estuary made these areas available for the colonization of native species, but it also presented an opportunity for non-indigenous species to establish viable populations (Seyer et al. 2017). The most likely mechanisms associated with the introduction of the Atlantic blue crab into the Guadiana estuary are probably due to the reduced abiotic and/or biotic resistance in these newly available niches (Procheş et al. 2008; Taylor and Duggan 2011; Papacostas et al. 2017), as we hypothesize that propagule pressure is not the cause for the introduction and putative establishment of the Atlantic blue crab in the Guadiana estuary. A reversed scenario was observed for the invasive porcelain crab *Petrolisthes armatus* (Gibbers, 1850) (Decapoda, Anomura)

in Georgia (USA) where the propagule pressure overwhelmed the native biotic resistance (Hollebone and Hay 2007).

Therefore, and as part of a future research framework, this hypothesis must be tested and the case of the porcelain crab used as an example of the opposite scenario.

#### *Overlap with native Brachyura*

The Ria Formosa lagoon is a mosaic of different benthic habitats, mainly of muddy and sandy bottoms and of submerged aquatic vegetation patches. Salinity changes across its extension are minimal, except during episodic rain events. Here, the most abundant Brachyurans are the West African fiddler crab *Uca tangeri* (Eydoux, 1835) (Wolfrath 1992), the marbled rock crab *Pachygrapsus marmoratus* (Fabricius, 1787), and the European green crab *Carcinus maenas* (Linnaeus, 1758) (Sprung 2001). Hypothetically, these species could hinder the invasion of the Atlantic blue crab if their niches overlap. However, the Atlantic blue crab has plenty of habitats available along the Ria Formosa lagoon which increases their chances of establishment, as it seems to be the case. Nonetheless, only a future systematic assessment across the entire lagoon can confirm or discredit this hypothesis.

In estuarine ecosystems like the Guadiana estuary, the diversity of species tends to decrease upstream towards oligohaline areas, and then to increase towards freshwater areas – Remane diagram *sensu* Whitfield et al. (2012). In this estuary, the Atlantic blue crab was collected mainly in the middle estuary (between 12 and 20 km from the river mouth) and in the lower section of the upper estuary (from 20 km upstream the river mouth). At least other three Brachyura species are present in the Guadiana estuary. The flying crab *Liocarcinus holsatus* (Fabricius, 1787) (Encarnaç o et al. 2013) and marbled rock crab *Pachygrapsus marmoratus* (Fabricius, 1787) are present exclusively in the lower estuary (up to 12 km upstream the river mouth), while the European green crab *Carcinus maenas* (Linnaeus, 1758) is present both in the lower and middle estuary (Encarnaç o et al. 2013). Occasionally, species of the genus *Liocarcinus* might be present near the river mouth. Thus, the Atlantic blue crab has a broader upstream distribution in this estuary than any other Brachyura. However, most of Atlantic blue crab currently known distribution range overlaps with the upper distribution range of the European green crab. Therefore, competition and functional redundancy could be high between the native European green crab and the Atlantic blue crab if this non-indigenous species will ever reach an invasive status. Nonetheless, the upper estuarine region is, without doubt, the area where the Atlantic blue crab will face less competition with native Brachyurans.

The co-existence of the Atlantic blue crab and European green crab in the Ria Formosa lagoon and in the Guadiana estuary, as well as in the

northwestern Atlantic is an open avenue for experimental studies on fundamental invasion ecology since both species are native and invasive in opposite regions of the North Atlantic. It is worth mentioning that the intraguild competition between native blue crab and non-indigenous green crab is modulated by temperature-dependent interactions as observed in a mesocosms experiment (Rogers et al. 2018). At low temperatures, green crabs preyed upon blue crabs, while at higher temperatures similar size blue and green crabs had similar competition capabilities but bigger blue crabs preyed upon green crabs (Rogers et al. 2018). A field study in the NW-Atlantic showed that the native blue crab controlled the green crab invasion, since the abundance of green crabs was controlled by the abundance of blue crabs (deRivera et al. 2005). Therefore, any extrapolations from these observations to the Ria Formosa lagoon and the Guadiana estuary—and to any other location where the Atlantic blue crab is non-indigenous—is highly speculative at least for three main reasons: 1) each site has its intrinsic abiotic and biotic specificities; 2) different lineages may be involved in the invasion so the outcome of species interactions vary (see Morais and Reichard (2018) for a review on cryptic invasions); 3) the time elapsed since the introduction of the NIS influences the naivety of native species towards the NIS behavior and therefore the outcomes of species interaction (Reichard et al. 2015). Thus, research on the fundamental invasion ecology of the Atlantic blue crab constitutes a third topic of our proposed research framework.

#### *The Atlantic blue crab as a fishing resource*

The introduction of the Atlantic blue crab increases the number of edible non-indigenous species present in the area, and particularly in the Guadiana estuary. Probably, the most notorious edible non-indigenous species present in this estuary is weakfish (Morais and Teodósio 2016; Morais et al. 2017). The use of the Atlantic blue crab as a new fishing resource in its non-indigenous range was already proposed and described extensively (Mancinelli et al. 2017b), which might become a reality if the species would ever reach an invasive status in the Ria Formosa lagoon or in the Guadiana estuary. However, there are some caveats in using non-indigenous species, and particularly invasive species, as a food resource, and how this use may mitigate their putative negative effects upon ecosystems (Nuñez et al. 2012). Thus, there are three main aspects to consider, as already pointed out for weakfish (Morais et al. 2017). First, fishing pressure must efficiently reduce the size and growth of the non-indigenous species population without affecting other native species (Nuñez et al. 2012). Second, the public must be made aware of the putative negative impacts of non-indigenous species and that introductions into non-invaded areas are not permitted (Nuñez et al. 2012). Third, the fishery



of an invasive species can never be managed to make it sustainable (Nuñez et al. 2012), which disables local communities from obtaining a long-term financial revenue. However, we advocate that the Atlantic blue crab fishery must be regulated in a near future if there is an opportunity to become sustainable, but only if it does not jeopardize existing fisheries and the ecosystem. Local communities should be the sole beneficiaries of this complementary revenue, which would avoid increased fishing pressure and other impacts induced by fishers coming from other regions. Any legal aspects regarding the use of non-indigenous species, and of invasive species in particular, should deserve an objective but thorough case-by-case assessment.

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### Supplementary material

The following supplementary material is available for this article:

**Table S1.** Records of Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 collected in the Ria Formosa lagoon and Guadiana estuary (SW Iberian Peninsula) by local fishermen in 2017 and up to June 2018. The number of specimens collected (N), carapace width (cm), total fresh weight (g), and sex (M – male, F – female, or undetermined) are described.

**Table S2.** Temperature and salinity measured in the Guadiana estuary at four sampling stations and three different depths during neap (August 31, 2017) and spring tides (September 8, 2017).

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