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The presence of *Mya arenaria* in the Ria de Aveiro is the third confirmed record of this invasive clam on the Portuguese coast

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The occurrence of the invasive clam Mya arenaria *is confirmed in the Ria de Aveiro (northern Portugal). Some live specimens were collected from the mid-upper intertidal zone. The population density was low (about 0.15 ind* m^{-2}*) and the individuals were large, of mean shell length 82.22* $\pm$ *14.85 mm. Large empty shells of* M. arenaria*, of mean shell length 85.32* $\pm$ *11.31 mm, were found in a life-like position, which indicates an episode of mass mortality in the sampling area and suggests that invasion by the clam in the Ria de Aveiro is not a recent event. Differences in the size-classes of empty shells of the invasive clam indicate that the clam is able to reproduce in this habitat. Occurrence of established populations of* M. arenaria *at different locations on the Portuguese coast, such as the Tagus estuary, the Lima estuary and the Ria de Aveiro, may be due to intentional introduction of* M. arenaria *in at least some cases.*

Keywords: *Mya arenaria*, established population, empty shells, Ria de Aveiro, Portugal

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INTRODUCTION

The clam *Mya arenaria* (Linnaeus 1758) is one of the oldest marine invaders of European coasts (Reise *et al.*, 1999). This invasive clam species is widespread throughout European seas (Strasser, 1999; Gollasch, 2006). Thus, *M. arenaria* is well established in the White Sea (Maximovich & Guerassimova, 2003), Norway (Winther & Gray, 1985), the Swedish coast (Möller & Rosenberg, 1983; Flach, 2003), the Baltic (Boström *et al.*, 2006; Zettler *et al.*, 2007), the German coast (Strasser *et al.*, 1999), in estuaries of The Netherlands (Ysebaert *et al.*, 1998), Belgium (Kerckhof *et al.*, 2007), the British isles (Seaward, 1990; Hughes *et al.*, 2000), Ireland (Seaward, 1990; Healy, 1997) and the Atlantic coast of France (Nicolas *et al.*, 2007). The clam has been described in the Basque Country, on the Spanish coast of the Bay of Biscay (Borja, 1988; Borja & Muxika, 2001). It has also been recorded south of this area in the Tagus estuary and in the Lima estuary (Conde *et al.*, 2010, 2011a), both of which are in Portugal. There is also some evidence of the occurrence of established populations of *M. arenaria* in other north-western areas of the Iberian Peninsula, such as the Douro estuary in Portugal (Oliveira, 2007) and Rianxo, in the Ria de Arousa, Spain (Rolán & Acuña, 2008). *Mya arenaria* does not outcompete the native fauna on the European coast where it coexists with endemic bivalve species (Bocher *et al.*, 2007; Conde *et al.*, 2011a).

The invasive clam is also established in the Mediterranean Sea, with records in France (Gulf of Lyon: see Stora *et al.*, 1995; Zenetos *et al.*, 2004), Italy (Sacca di Goro Lagoon: see Crocetta, 2011, 2012; Crocetta & Turolla, 2011) and the eastern Mediterranean Sea (Greece and Turkey: Zenetos *et al.*, 2004; Çinar *et al.*, 2011; Crocetta & Turolla, 2011), as well as in the Black Sea (Gomoiu & Petran, 1973), where it had been suggested to be able to outcompete the native bivalve *Lentidium mediterraneum* (O.G. Costa, 1829) along the Romanian shores (Gomoiu, 1981).

The occurrence of *M. arenaria* in the Ria de Aveiro has never been formally recognized. It has only been cited in the abstract of a note devoted to the macrofauna inhabiting local salt ponds (Vieira & Amat, 1997) and listed within the fauna sampled during the 2006 summer season (Nunes *et al.*, 2008). However, in the salt ponds, the salinity reaches as high as 54.4–220 g l^{-1} (Vieira & Amat, 1997), and is thus much higher than the range of tolerance of 0–30.5 g l^{-1} reported for the species by Matthiessen (1960), while the record of Nunes *et al.* (2008) was not confirmed in later extensive studies carried out in the area (Quintino *et al.*, 2011; Rodrigues *et al.*, 2011).

The aim of the present study was to find evidence of the current occurrence of *M. arenaria* in the Ria de Aveiro by surveying an area close to the sampling site described by Nunes *et al.* (2008).

MATERIALS AND METHODS

Study site

The Ria de Aveiro (northern Portugal) is a marine lagoon, which is linked to the Atlantic Ocean through an artificial

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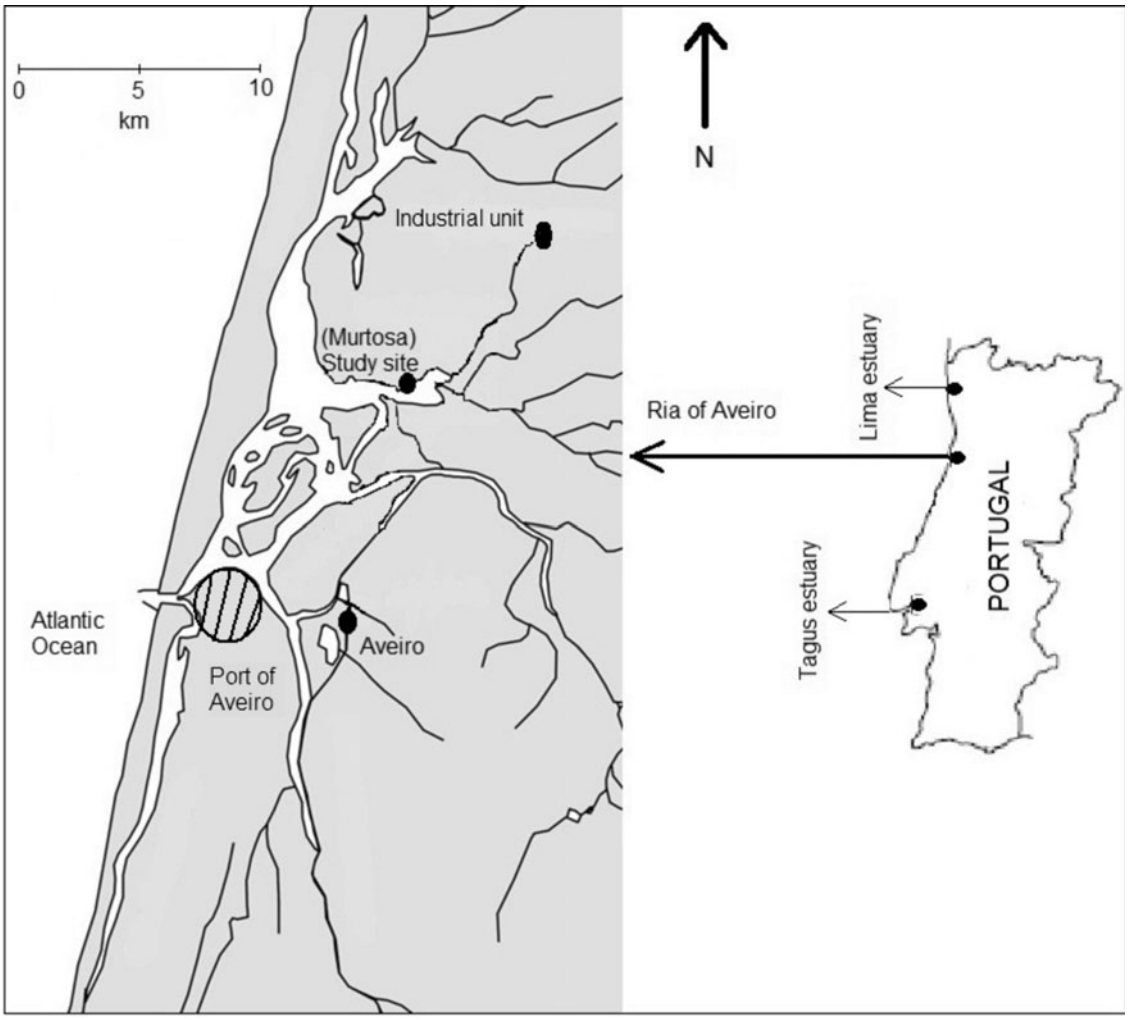

Fig. 1. Map of the Ria de Aveiro showing the location of the study site. The Tagus and the Lima estuaries are also shown on the right hand side of the figure.

inlet and is characterized by several narrow channels due to the existence of small river basins that drain into the ria. There is a commercial port close to the lagoon inlet. The large ria encompasses different habitats, such as intertidal sand flats, mud flats and salt marshes.

The study site is close to that described by Nunes *et al.* (2008) and is located in the north-western part of the lagoon (Figure 1), close to the village of Murtosa. The salinity fluctuates seasonally in this part of the lagoon, with conditions ranging from mesohaline to euhaline (Nunes *et al.*, 2008). The site is subject to anthropogenic pressure in the form of heavy metal pollution derived from an electrochemical plant located at the top of the Estarreja Channel (Pereira *et al.*, 1998) (Figure 1).

Field, laboratory procedures and data analysis

Sampling was carried out, during ebb tide, at the end of April 2012. A search for empty shells was conducted on an area of 2000 m^{-2} during 60 minutes, as described by Conde *et al.* (2011b). Interpretation of the historical evolution of populations of *M. arenaria* using empty shells followed the methods of Strasser *et al.* (1999) and Palacios *et al.* (2000). Collection of live individuals of *M. arenaria* was carried out when key-like holes were observed on the sediment surface. Random searches were conducted to find juvenile specimens of *M. arenaria*, by digging the sediment to a depth of 8 cm. The clams were transported in a cooled container and stored at 4°C until measurements were made later in the laboratory. Finally, macrobenthic species that were easily recognized by visual inspection within the search area were registered.

Shell length was measured in the left valve, with a Vernier caliper, to the nearest 1 mm. The fresh weight of live individuals was determined after blotting the clams on absorbent paper one day after collection. The *M. arenaria* specimens were grouped into size-classes with intervals of 10 mm. Empty shells of *M. arenaria* from the Ria de Aveiro were compared with those collected in the Tagus and Lima estuaries by Conde *et al.* (2011b). As the data were not normally distributed, a Wilcoxon's signed-rank test (W) was used for the comparisons. Empty shells from the Tagus and the Lima estuaries were collected in May 2010. It was assumed that empty shells from the Ria de Aveiro belong to dead animals at that time. The relationship between shell length and fresh weight was established by linear regression. Shell length and clam weight are expressed as mean $\pm$ standard deviation.

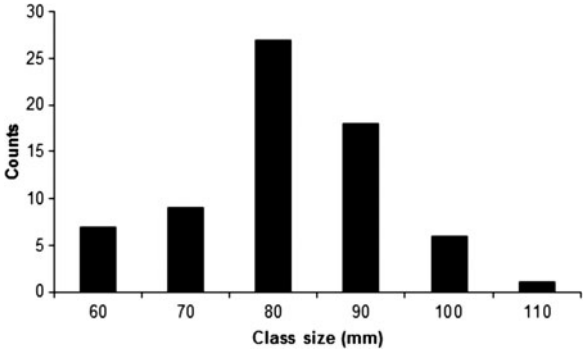

Fig. 2. Size-class distribution of empty shells of *Mya arenaria*.

RESULTS

A total of 68 empty *M. arenaria* shells were collected from the mid-upper part of the intertidal region. Most of the shells collected belonged to dead clams stuck in the sediment in a life-like position. The density of the dead population, calculated over three random plots of 1 m^2, was at least 2.33 $\pm$ 0.47 ind m^{-2}. The mean shell length of empty clams was 85.32 $\pm$ 11.31 mm. Maximum and minimum lengths of the empty shells were 119.3 mm and 60.6 mm respectively (Figure 2). The mean length of empty shells from the Ria de Aveiro is larger than the mean length of shells from the Tagus estuary (31.23 $\pm$ 10.81 mm; $W = 2992$, $P < 0.001$) and smaller than those from the Lima estuary (91.59 $\pm$ 12.45 mm; $W = 353$, $P = 0.0299$) (Conde *et al.*, 2011a; Figure 3). A similar conclusion was reached after carrying out a two-sample Kolmogorov–Smirnov test.

Signs of live individuals of *M. arenaria* on the sediment were scarce. In total, nine individuals were collected, and six of these were caught in an area of approximately 40 m^2, indicating a low density of 0.15 ind m^{-2}. All live individuals of *M. arenaria* were collected at approximately 70 cm from the mean high tide water mark. This intertidal location was slightly higher than the position occupied by dead assemblages of clams. Signs of the invasive clam were also observed at this intertidal position within seagrass (*Zoostera* sp.) meadows, but no attempts were made to collect specimens and the habitat was not disturbed. The mean shell length of *M. arenaria* was 82.22 $\pm$ 14.85 mm. Maximum and minimum shell lengths were 109.2 mm and 57.6 mm respectively (Figure 4A). The mean fresh weight of the clams was 63.03 $\pm$ 29.49 g. The length–weight relationship was linear and fitted well to the linear predictor ($R^2 = 0.92$; $F_{1,7} = 88.38$, $P < 0.001$), except in the case of one specimen (Figure 4B).

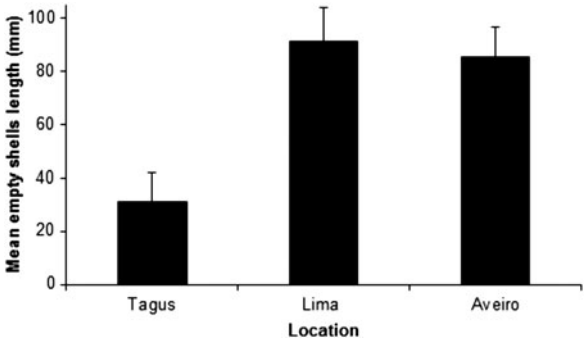

Fig. 3. Mean ($\pm$standard deviation) lengths of empty shells of *Mya arenaria* from three different locations in Portugal.

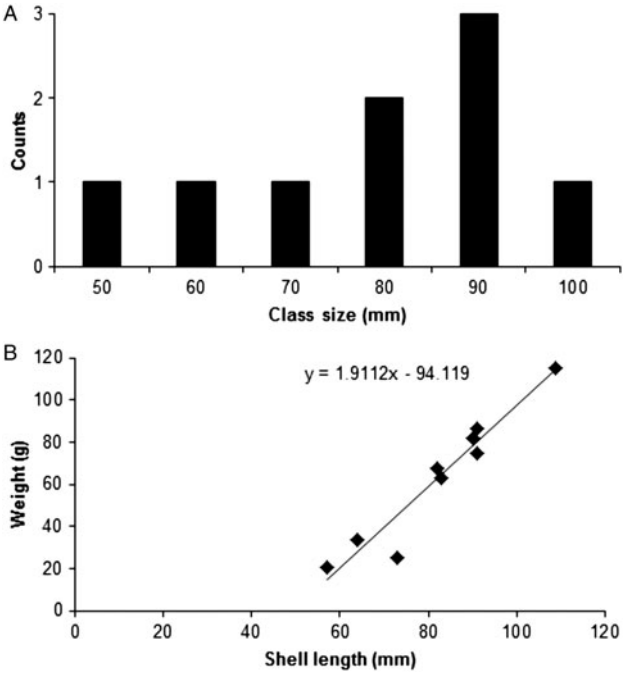

Fig. 4. (A) Size-class distribution of *Mya arenaria* and (B) length–weight relationship for the clams.

No juvenile specimens of *M. arenaria* were observed in the intertidal zone. The accompanying fauna observed by visual inspection at the survey site comprised the polychaetes *Diopatra neapolitana* Delle Chiaje, 1841 and *Hediste diversicolor* (O.F. Müller, 1776), the bivalves *Cerastoderma edule* (Linnaeus, 1758) and *Scrobicularia plana* (da Costa, 1778), the gastropod *Hydrobia ulvae* (Pennant, 1777) and the decapod *Carcinus maenas* (Linnaeus, 1758).

DISCUSSION

The occurrence of *M. arenaria* in the Ria de Aveiro increases records of this invasive clam to three confirmed locations on the Portuguese coast, apart from the invasions reported in the Tagus estuary (Conde *et al.*, 2010) and in the Lima estuary (Guimarães & Galhano, 1988; Conde *et al.*, 2011b).

The mean size of the empty shells suggests that the invasion occurred earlier in the Ria de Aveiro than in the Tagus estuary (Conde *et al.*, 2010). Therefore, the invasion on the Portuguese coast may have begun in the north of the country because the populations from the Lima estuary are also older than those from the Tagus estuary (Conde *et al.*, 2011b; Figure 3). Vieira & Amat (1997) and Nunes *et al.* (2008) described *M. arenaria* from among the fauna observed in their studies carried out in the Ria de Aveiro. However, a low density and a restricted distribution of the invasive clam may explain the absence of references to *M. arenaria* in other studies within the Ria de Aveiro (Quintino *et al.*, 2011; Rodrigues *et al.*, 2011). The invasive clam is found among estuarine macrobenthic assemblages on the

Portuguese coast (Nunes *et al.*, 2008; Conde *et al.*, 2010, 2011b) and on the northern coast of Europe (Ysebaert *et al.*, 1998; Hughes *et al.*, 2000).

The difference between the sizes of the empty *M. arenaria* shells suggests different years of recruitment. Nonetheless, it may be argued that these shells belong to individuals of the same cohort that died at different ages. However, a difference of 51.6 mm was also found between the maximum and the minimum shell length of the *M. arenaria* individuals collected from the study site. The growth rate of *M. arenaria* (Brousseau, 1979) indicates that the latter difference may only be explained by different recruitment times. Therefore, reproductive populations of the invasive clam appear to be established in the Ria de Aveiro.

The existence of dead assemblages of *M. arenaria* in the same position as live specimens also suggests that the invasion is not a recent event. Factors other than predation and wash out (Strasser *et al.*, 1999) may have caused mass mortality of the population of *M. arenaria* in the Ria de Aveiro. Water quality and the thermal tolerance of the clam (Strasser, 1999) may account for mass mortalities of southern populations.

One of the most common causes of marine invasions (Carlton, 1985) is transportation via ballast water. However, the occurrence of *M. arenaria* in three different locations of the Portuguese coast appears to be the result of intentional introduction, at least in some of these cases. This was suspected in Italian waters (Crocetta, 2012). The use of invasive species as bait and their use for aquaculture, ornamental and even scientific purposes have been suggested as means of introduction of such species in marine waters (Gollasch, 2006).

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