A field experiment on the reproductive success of the invasive clam *Mya arenaria* (Bivalvia) in the Tagus estuary: coexistence with the native clam *Scrobicularia plana*

ANXO CONDE [1,2], JÚLIO M. NOVAIS [1] and JORGE DOMÍNGUEZ [2]

[1] IBB-Institute for Biotechnology and Bioengineering, Center for Biological and Chemical Engineering, Instituto Superior Técnico (IST),1049-001, Lisbon, Portugal. E-mail: aconde@ist.utl.pt
[2] Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo, Vigo E-36310, España, Spain.

SUMMARY: A three month field experiment with tidal level (upper, middle, lower) and treatment (excavated and not excavated plots) as categorical experimental factors showed that the invasive clam *Mya arenaria* has reached a more advanced stage in the invasion process in the Tagus estuary. As we observed the smallest recruited juveniles of *Mya arenaria* (2 mm) throughout the study period, we concluded that the clam is capable of reproducing in the new habitat. Juveniles of both *Mya arenaria* and the bivalve *Scrobicularia plana* were found to avoid excavated experimental plots, showing a significantly higher abundance in the control plots. These data, strongly suggest that the recruited bivalves actively avoid unsuitable substrata. Juvenile specimens of *Mya arenaria* were more abundant in the mid-intertidal zone. However, juvenile specimens of *Scrobicularia plana* were mainly distributed in the upper intertidal level, which suggests that they have a different settlement behaviour from that observed for the juveniles of the invasive clam. Despite the divergent distribution between the juveniles of the two species in the study site, the possible interaction between these two species is considered and discussed.

Keywords: *Mya arenaria*, reproductive success, intertidal distribution, *Scrobicularia plana*, Tagus estuary.

RESUMEN: EXPERIMENTO DE CAMPO SOBRE LA CAPACIDAD REPRODUCTIVA DE LA ALMEJA INVASIVA *MYA ARENARIA* EN EL ESTUARIO DEL RIO TAJO: COEXISTENCIA CON LA ALMEJA NATIVA *SCROBICULARIA PLANA*. – La almeja invasiva *Mya arenaria* ha alcanzado un nuevo estadio invasivo en el estuario del Río Tajo, de acuerdo con los resultados de un experimento de campo que transcurrió durante 3 meses y en el que fueron considerados los factores categóricos elevación intermareal (superior, intermedia e inferior) y tratamiento (unidades experimentales excavadas y no excavadas). La presencia continua durante el tiempo de estudio de juveniles del menor tamaño observado (2 mm) nos permitió deducir que la almeja invasiva es capaz de reproducirse en el nuevo hábitat. Individuos juveniles de *Mya arenaria* y *Scrobicularia plana* evitaron las unidades experimentales excavadas, siendo más abundantes en las unidades de control. Esta observación sugiere que los juveniles de ambas especies evitan activamente sustratos poco adecuados. Los juveniles de *Mya arenaria* se distribuyeron principalmente en la zona intermareal media. Sin embargo, los juveniles de *Scrobicularia plana* se concentraron principalmente en la región intermareal superior, lo que sugiere un comportamiento de fijación distinto al observado en la almeja invasiva. A pesar de la distribución divergente entre los juveniles, la probable interacción entre las dos especies es considerada y discutida.

Palabras clave: *Mya arenaria*, éxito reproductivo, distribución intermareal, *Scrobicularia plana*, estuario del Tajo.

INTRODUCTION

The invasive clam *Mya arenaria* (Linnaeus, 1758) is thought to have been introduced by the Vikings into Europe from the east coast of North America, some

seven centuries ago (Petersen *et al*., 1992). It is known as one of the first macrobenthic invaders on the European coast (Reise *et al*., 1999). The distribution of *Mya arenaria* has widened on the European coast since its introduction. It has reached the White Sea at northern

latitudes (Maximovich and Guerassimova 2003), and the Tagus estuary in southern regions (Conde *et al.*, 2010).

The initial establishment of an invasive marine species is preceded by the invasive species being taken from the donor region, then transported and finally released into the recipient environment (Colautti and MacIsaac, 2004). After surviving these stages, the next step is the establishment of a self-reproducing population in the new habitat (Colautti and MacIsaac, 2004). Invasion processes can usually be considered as non-random events because the donor regions, transport vectors, non-random uptake and survival of the invasive species are particular features of a population. Propagule bias, i.e. a particular set of characteristics of invasive individuals in relation to the total variability of the species in the donor region, may therefore confound invasion patterns (e.g. Colautti *et al.*, 2006) as a result of inadequate assessment of invisibility, concerning the recipient habitat, or invasiveness, regarding the characteristics of the invasive species, among other misinterpreted patterns. Propagule pressure, defined as the inoculum size and the frequency of invasion attempts, is a key factor in explaining invasive processes (Lockwood *et al.*, 2005; Von Holle and Simberloff, 2005).

Recruitment of juvenile specimens of *Mya arenaria* involves events during the larval and post-larval stages. For example Strasser and Gunter (2001) suggested that reduced epibenthic predation on bivalve spat due to temporal mismatch between predator and prey may be a more important factor than larval supply in explaining high bivalve recruitment after severe winters, a frequently reported phenomenon on northern European coasts. Local hydrodynamism and bedload transport are also frequently cited as important factors in explaining post-larval distribution and recruitment of bivalves (Emerson and Grant, 1991; Armonies, 1996; Huxham and Richards, 2003), which are also determined by predation (Hunt and Mullineaux, 2002). Intra and interspecific competition have also been investigated in relation to variations in abundance and biomass of *Mya arenaria* populations (Möller, 1986).

The spawning period for *Mya arenaria* differs according to location. Some authors have reported a peak during the spring (van der Veer *et al.*, 1998; Strasser and Gunter, 2001) or summer in the northernmost regions (Maximovich and Guerassimova, 2003). Other authors have reported a second peak (Pfitzenmeyer, 1962; Roseberry *et al.*, 1991), and related the spawning periods to changes in the water temperature between seasons. The clam mainly inhabits intertidal locations although it is able to live in subtidal habitats (Glockzin and Zettler, 2008; Gomoiu and Petran, 1973). The invasive clam *Mya arenaria* may fully tolerate saline marine habitats; however, the clam has mainly been described as an important component of brackish communities on the European coast, contributing considerably to the biomass, in some cases along with *Scro-*

bicularia plana (da Costa, 1778) (Zwarts and Wanik, 1989; Ysebaert *et al.*, 1998; Bocher *et al.*, 2007; Conde *et al.*, 2010).

The main aim of the present study was to demonstrate that the established populations of *Mya arenaria* in the Tagus estuary are able to reproduce in the new habitat. Given the previous condition (the existence of a reproducing population), the intertidal distribution of juvenile specimens of *Mya arenaria* was studied and compared against the intertidal distribution of *Scrobicularia plana*. A field experiment was conducted to address these questions and also to assess the effect of disturbed excavated sediments on the abundance of juveniles of both bivalve species.

MATERIALS AND METHODS

The Tagus estuary (Fig. 1) is one of the largest European estuarine systems and covers an area of approximately 325 km^2. The Tagus is a mesotidal estuary with a tidal range of between 1 m at neap tides and 4 m at spring tides. Most of the southern bank is dominated by intertidal mudflats and salt marshes.

The study site was located on a small spit of land on the east bank of the Coina salt marsh (Fig. 1), approximately 1600 m in a straight line from the site where *Mya arenaria* was first recorded in the Tagus estuary (Conde *et al.*, 2010). This site was chosen because it was located in approximately the middle of the Coina salt marsh and was not comprised of deposited algal mats. The site is also easily accessed through a private property and is not frequently visited by locals. The topography of the study area was characterized by a more pronounced slope than those observed at surrounding sites, composed of an intertidal region extending over 130 metres between the limits of the high and low spring tides.
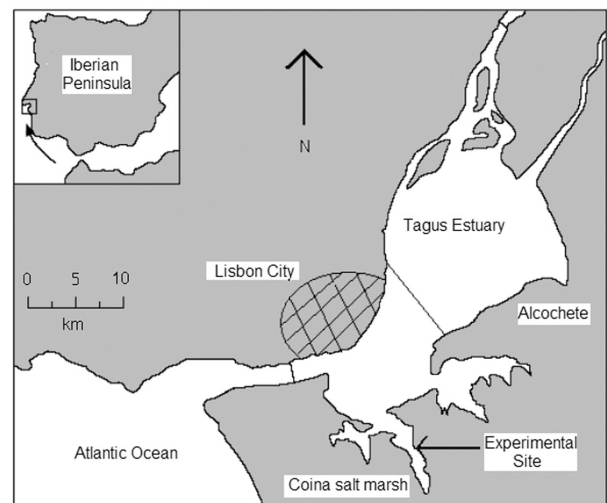

Fig. 1. – Map of the Tagus estuary. An arrow indicates the location where the intertidal experiment took place in the Coina salt marsh. The three transversal lines to the estuary axis are bridges.
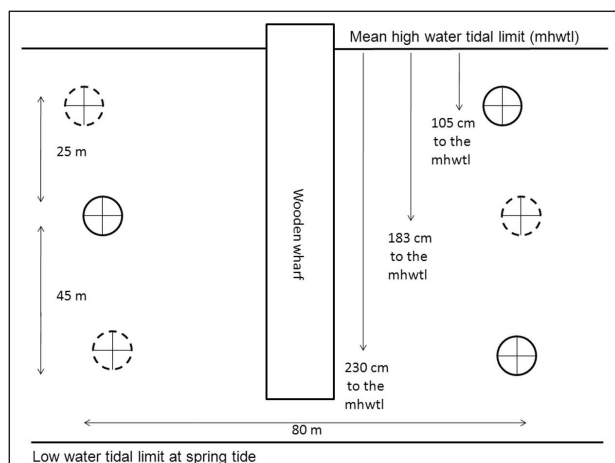
FIG. 2. – Schematic representation of the experimental design at the study site, Coina salt marsh. Excavated plots (open circles) and controls (solid-lined circles) are separated by a distance indicated by two-pointed arrows. The depth to the mean high water tidal level (mhwtd) of each experimental unit is indicated by a one-pointed arrow.

A field experiment was conducted at the site, in a total of six plots, evenly distributed at three intertidal levels (high, mid and low), situated at depths of 105 cm, 183 cm and 230 cm respectively under the mean high water tidal limit (mhwtl). The high and mid levels were separated by 25 m and the mid and low levels by 45 m (Fig. 2). Each plot was delimited by a circle with a diameter of 6 m. Each two plots at the same intertidal level were separated by approximately 80 m in two arrays of imperfectly aligned plots along the upper and lower limits of the intertidal zone. The two arrays of plots were separated by an abandoned wooden wharf, 110 m long and 3 m wide.

Treatment factor (excavation, non-excavation) was applied at random to the plots at each intertidal level. Both factors (intertidal level and treatment) were considered as fixed factors. The excavated plots were expected to enhance the deposition of bivalve spat if the settlement of bivalves was the result of a passive process. The unexcavated plots were therefore considered as controls. Time was not considered as a factor because the experiment took place during the autumn when *Mya arenaria* spat is known to settle in some southern locations (Pfitzenmeyer, 1962; Roseberry *et al.*, 1991). The treatment factor was left to act for one month. The excavated plots were dug out with a shovel to a depth of about 3 cm and the excavated substratum was discarded as far as possible from the plot in all directions to prevent the plot refilling.

Plots were sampled on three occasions, first on 17/10/2008 (on the same day that the treatments were applied), then on 14/11/2008 and finally on 12/12/2008. Each plot was divided into 4 quadrants, 3 of which were a priori randomly assigned for sampling fauna on each sampling occasion. The remaining quadrant was repeatedly used for field measurements of the physicochemical parameters and for obtaining sediment samples for analysis.

Temperature, salinity, oxygen, redox potential and pH were measured with a standard field probe in the interstitial water. Some values are missing as a result of equipment malfunction (oxygen concentration in the upper excavated plots in October; salinity in all plots in November; pH values in the upper unexcavated plots in December). The top 3 cm of the sediment were used for sediment analysis with the sieving method. The third quartile was used to characterize the grain size distribution rather than the median grain size because of the high percentage of coarse sand and pebbles in the low intertidal excavated plot. Total organic carbon (TOC) and total nitrogen (TN) were measured in a dried sample with an elemental analyzer (LECO CN2000) after the inorganic carbon was removed by acidification.

Sampling for infaunal organisms was carried out with a corer with an inner diameter of 9.5 cm (5 replicates=0.035 m^2) inserted to a depth of 25 cm. The hole left by the corer was inspected at a deeper level to search for any adult specimens of *Mya arenaria* that were buried more deeply. All samples were sieved through a 1 mm mesh (Sola, 1997). The retained material was preserved in 70% ethanol. Benthic animals were sorted under a dissecting microscope. The shell length (SL) of juvenile specimens of *Mya arenaria* was measured to the nearest 0.01 mm with Motic Image Plus 2.0 software, after taking photographs of the clams with a digital camera attached to a stereoscopic microscope. The individuals of *Scrobicularia plana* were considered juveniles up to a SL of 10 mm in accordance with Sola (1997). Carnivorous species that might be potential predators of juveniles of *Mya arenaria* (hereafter referred to as "predators") were identified to the lowest taxonomic level and counted.

Statistical analysis

The effects of the categorical factors tidal level (upper, middle, lower), treatment (excavation, non-excavation) and predators as covariates on the response variables juvenile specimens of *Mya arenaria* and *Scrobicularia plana* were analysed by fitting Generalized Linear Models (GLM) to the data with a log link function and a Poisson distribution for the error term (McCullagh and Nelder, 1989). The model accounted for the effect of the factors and the covariate, as well as for both the interaction between the categorical factors and the interaction between the covariate and the treatment factor, in accordance with the following linear model:

$$y_{ijk}=\mu+\alpha_i+\beta_j+X+(\alpha\beta)_{ij}+\beta_jX+e_{ijk}$$

where y_{ijk} is the response variable, μ is the overall population mean, α_i is the effect of ith level of factor "tidal level", β_j is the effect of jth level of factor "treatment", X is the covariate "predators", $(\alpha\beta)_{ij}$ is the interaction between the considered factors, β_jX is the interaction between the factor "treatment" and the covariate "predators" and e_{ijk} is the error term.

TABLE 1. – Physicochemical characteristics of the experimental plots pooled by months (and standard deviation) for each intertidal level (Up, upper; Mid, middle; Low, lower) and treatment (Excavated and Control).

Experimental unit	pH	Salinity (psu)	Temperature (ºC)	[O2] (mg/l)	Redox (mV)	Grain size (mm)	Fine fraction (%)	TOC (%)	TON (%)
Up Excavated	7.55 ±0.07	30.95 ±0.22	17.47 ±1.85	2.57 ±0.21	14.67 ±44.80	0.33 ±0.08	3.06 ±0.80	0.21 ±0.07	0.06 ±0.01
Up Control	7.67 ±0.12	30.91 ±0.23	17.98 ±2.24	1.60 ±0.09	-144.50±29.89	0.83 ±0.09	3.15 ±0.22	0.13 ±0.08	0.05 ±0.01
Mid Excavated	7.36 ±0.07	29.75 ±0.41	17.43 ±2.16	10.12±4.93	-12.02 ±50.98	0.78 ±0.04	14.65±4.41	1.40 ±0.42	0.21 ±0.06
Mid Control	7.52 ±0.12	30.10 ±0.25	18.47 ±1,11	7.66 ±1.38	-20.33 ±18.73	0.51 ±0.18	9.73 ±1.76	1.27 ±0.25	0.14 ±0.02
Low Excavated	7.32 ±0.09	30.65 ±0.22	16.20 ±1.55	6.99 ±3.48	-7.50 ±44.80	3.07 ±0.71	3.54 ±0.39	2.40 ±0.52	0.15 ±0.02
Low Control	7.11 ±0.09	29.40 ±0.55	15.47 ±1.79	4.98 ±2.84	-30.17 ±42.85	1.07 ±0.25	19.86±3.23	2.19 ±0.05	0.28 ±0.01

The discrepancy between the GLM model and the data is called "deviance". Deviance was used to test the goodness of fit of the model to the data. Samples were pooled over November and December prior to the GLM fitting.

Multiple comparisons were made and the p-values (P) adjusted by the Holm procedure whenever the null hypothesis for factor level was rejected. The relation between the juvenile specimens of *Scrobicularia plana* and the environmental parameters measured in the control plots was assessed by Spearman rank correlation.

All statistics were considered significant at $p<0.05$ and were calculated using the R package (R Development Core Team, 2007).

RESULTS

The experimental site was physicochemically characterized by having more homogeneous values of some environmental parameters, such as pH or salinity, while other parameters, such as redox potential and oxygen concentration, varied widely (Table 1). The intertidal zone underwent temperature changes due to heating from the sun between tides; therefore, the temperature varied considerably among plots on each sampling occasion. Temperature decreased over time, with a mean value of 22.1°C in October, 16.5°C in November and 11.9°C in December. Grain size was fairly uniform in the mid and upper regions of the intertidal zone, with coarser sediments in the lower level, especially in the excavated plot (Table 1; Fig. 3). The fine fraction of the sediment was less important in the upper layer of the intertidal zone, and organic matter, expressed as the percentage of TN or TOC, was distributed along the intertidal height gradient, with higher values at the lower level.

The number of juvenile bivalves and predators observed in each plot during the sampling period is shown in Table 2. Juvenile specimens of *Scrobicularia plana* were one order of magnitude more frequent than the *Mya arenaria* juveniles. Interestingly, juvenile specimens of the invasive clam were the second most frequently observed juvenile bivalve, and were more than twice as abundant as spat of *Cerastoderma edule* (Linnaeus, 1758). The presence of *Ruditapes decussatus* (Linnaeus, 1758) was almost negligible. A total of 46 predator individuals belonging to 6 different species were recorded, corresponding to an overall ratio
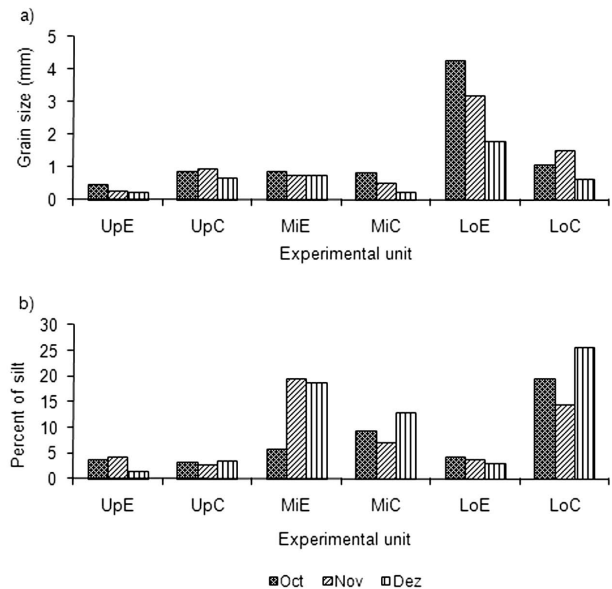

FIG. 3. – Evolution over time of changes in the third quartile of sediment grain size (a) and percentage of silt from each experimental unit (b). E, Excavated; C, Control. Intertidal levels: Up, Upper; Mi, Middle; Lo, Lower.

of almost 6 juvenile bivalves to 1 predator. The most numerous carnivorous species, with 26 individuals, was the polychaete *Nephtys hombergii* Savigny, 1818, mainly observed in the lower control plot. In addition, 8 unidentified nemerteans, 7 individuals of the polychaete *Mysta picta* (Quatrefagues, 1865), 3 specimens of the polychaete *Glycera tridactyla* (Schmarda, 1861) and 1 specimen each of the decapods *Crangon crangon* (Linnaeus, 1758) and *Carcinus maenas* (Linnaeus, 1758), also contributed to the pool of carnivorous species observed at the study site.
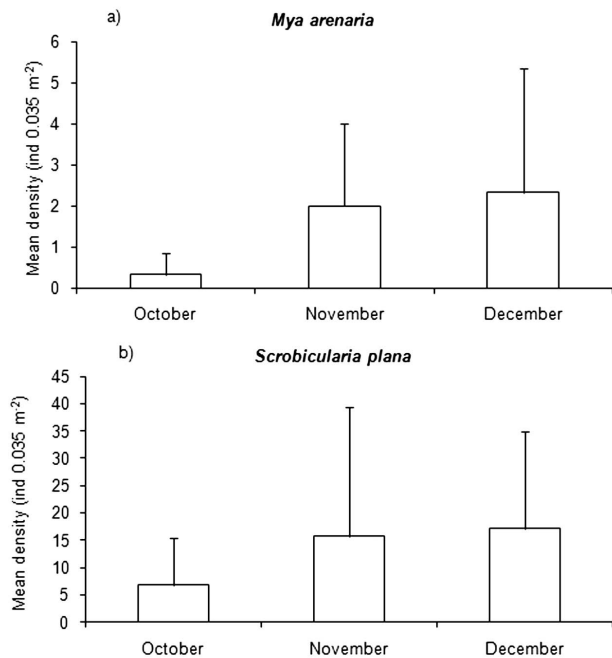
The benthic community was generally very similar to that described by Conde *et al.*, (2010), in which the gastropod *Hydrobia ulvae* (Pennant, 1777) was the most abundant species followed by the bivalve *Scrobicularia plana*, and the polychaetes *Capitella capitata* (Fabricius, 1780) and *Hediste diversicolor* (O.F. Müller, 1776).

The abundance of recruited *Mya arenaria* individuals increased during the study period (Fig. 4a), suggesting that peak recruitment may take place during late autumn or even early winter. Accordingly, the maximum number of *Mya arenaria* juveniles recorded

TABLE 2. – Total and relative number of juvenile bivalves and total number of predators observed in the experimental plots over time.

| Species | Month | Intertidal Level and Treatment | | | | | | Totals | Percent (%) |
| | | Up | | Mid | | Low | | | |
		Excavated	Control	Excavated	Control	Excavated	Control		
Scrobicularia plana	Oct	2	22	4	12	1			
	Nov	13	63	4	11	2	1	238	87.18
	Dec	21	48	7	23	2	2		
Mya arenaria	Oct	1				1			
	Nov	1	1		5		1	24	8.79
	Dec	3	1	2	8				
Cerastoderma edule	Oct				1	1			
	Nov		1		1	1		10	3.66
	Dec			2	3				
Tapes decussatus	Oct								
	Nov							1	0.37
	Dec				1				
Predators	Oct		3	1			4		
	Nov	1	1	1		3	12	46	
	Dec	2	3	3	5	1	6		

FIG. 4. – Mean density (and standard deviation) per plot (ind/0.035 m²) of *Mya arenaria* juveniles (a) and *Scrobicularia plana* juveniles (b), over time.

corresponded to 8 individuals observed in the mid control plot in December. The mean density value for this month was 2.3 ± 3.0 ind 0.035 m^{-2}, followed by a density of 2.0 ± 2.0 ind 0.035 m^{-2} observed in November. Only two juvenile specimens were observed in October, both in excavated plots (Table 2). The maximum observed density of *Scrobicularia plana* juveniles was 17.2 ± 17.7 ind 0.035 m^{-2} in December (Fig. 4b).

The size-frequency distribution indicates that the peak recruitment of *Mya arenaria* did not correspond to the first sampling occasion (October) because only clams belonging to the smaller size class (2 mm) were collected. Specimens in the 2 mm size class were observed on all three sampling occasions, again suggesting that the recruitment period probably extends beyond December. The 3 mm size class was the most abundant and possibly comprised older versions of the previously observed smaller classes as well as some immigrant individuals. A decrease in abundance between the 3 mm size class and the larger size classes was observed. The 2-3 mm size classes comprised 83.3% of the total observed juveniles of *Mya arenaria*. Size classes larger than 3 mm were more frequently observed in the mid-intertidal control plot.

The analysis of deviance (Table 3) revealed significant differences among tidal levels (deviance=15.076, df=2, P<0.001), treatments (deviance=4.717, df=1, P=0.030) and also a significant effect of the predator covariate (deviance=5.771, df=1, P=0.016) on the abundance of *Mya arenaria* juveniles. The number of predators and *Mya arenaria* abundance were inversely related. In the case of *Scrobicularia plana*, the analysis of deviance only revealed significant differences among tidal levels (deviance=140.93, df=2, P<0.001) and treatments (deviance=44.66, df=1, P<0.001). In the latter case, the error term was adjusted to a quasi-Poisson model (Crawley, 2007) because some overdispersion was observed (residual deviance/degrees of freedom= 1.25).

The mean number of *Mya arenaria* juveniles per sample increased from the lower to the upper level of the intertidal zone in the excavated plots (Fig. 5a). However, the same trend was not observed in the control plots, with a maximum mean number per sample in the mid-intertidal zone. The mean number of specimens was lower in the upper control plots than in the upper excavated plots, in agreement with the significant statistical interaction (deviance=6.004, df=2, P=0.049; Table 3, Fig. 5a). There was no signifi-

TABLE 3. – Analysis of deviance testing for the effect of intertidal level, treatment and predators as a covariate on the abundances of *Mya arenaria* and *Scrobicularia plana* juveniles as response variables. Significant *p*-values (P) are in bold.

Response variable	Source	df	Deviance	Residual df	Residual deviance	P (*Chi*)
Mya arenaria	Null			59	58.008	
	Level	2	15.076	57	42.933	**<0.001**
	Treatment	1	4.717	56	38.216	**0.030**
	Predator	1	5.771	55	32.445	**0.016**
	Level:Treatment	2	6.004	53	26.440	**0.049**
	Treatment:Predator	1	0.002	52	26.439	0.965
Scrobicularia plana	Null			59	285.371	
	Level	2	164.366	57	121.004	**<0.001**
	Treatment	1	52.091	56	68.913	**<0.001**
	Predator	1	0.182	55	68.731	0.695
	Level:Treatment	2	3.836	53	64.895	0.200
	Treatment:Predator	1	0.007	52	64.888	0.938
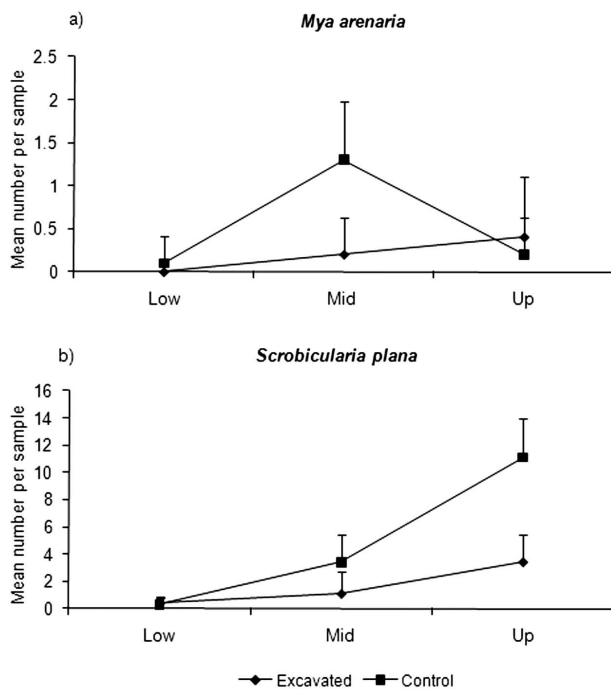
FIG. 5. – Interaction plot for the categorical factors expressed as the mean density per sample (and standard deviation) of the juveniles of *Mya arenaria* (a) and *Scrobicularia plana* (b).

cant interaction between the treatment effect and the predator covariate on the abundance of *Mya arenaria* (deviance=0.002, df=1, P=0.965), which suggests that predators were not selectively attracted by a specific treatment (excavated or control). Pairwise comparisons revealed significant differences in the abundance of *Mya arenaria* between the mid and both the lower and upper intertidal (P<0.001 and P=0.033 respectively), but no difference between the upper and lower intertidal (P=0.175). Juvenile specimens of *Scrobicularia plana* were significantly more abundant at the upper intertidal level than at the other two levels (P<0.001 in both cases, Fig. 5b). A significant difference in abundance of juvenile specimens of *Scrobicularia plana* was also found between the mid and lower intertidal levels (mid>lower level, P=0.047).

A total number of 46 adult specimens of *Scrobicularia plana* were collected during the study. The maximum mean density of *Scrobicularia plana* adults was observed in the mid-intertidal excavated plot, with a mean value of 5.7±1.5 ind 0.035 m^{-2}, followed by a mean density of 3.0±1.7 ind 0.035 m^{-2} in the lower excavated plot. No individuals were observed in the upper excavated plot. There were no records of adult individuals of other bivalve species, with the exception of one specimen of *Mya arenaria* (49 mm SL) recorded in the mid-control plot in December.

The environmental parameters did not show any statistically significant correlations in relation to the *Scrobicularia* plana juveniles, except in one case. The only significant negative correlation was between the abundance of *Scrobicularia plana* juveniles and the organic matter content of the sediment, considered as TOC (*rho*=-0.85, P=0.006) or TN (*rho*=-0.90, P=0.002). The correlations between *Mya arenaria* and the environmental parameters are not presented because zero value entries were frequent in the response variable. As a reference value, the best rho score was a negative correlation between grain size and the abundance of *Mya arenaria* juveniles (*rho*=-0.567, P=0.111).

DISCUSSION

The invasive process of *Mya arenaria* in the Tagus estuary has reached a further stage (following Colautti *et al.*, 2006) due to the reproductive success of the species in the new habitat. The reproduction of the clam was inferred from the continuous recruitment of the smaller size class (2 mm) and through an increasing pool of *Mya arenaria* juveniles throughout the study period. Propagule pressure has been described as a determining factor in explaining the successful establishment of invasive species in a recipient ecosystem (Lockwood *et al.*, 2005; Von Holle and Simberloff, 2005). *Mya arenaria* was the second most abundant bivalve species in the study site (Table 2), which suggests that propagule pressure favours the spread of this species within the estuary. In the present study, propagule pressure is not only considered at the initial stage of the invasion but also as a constant feature that promotes the invasion

process until its final stage (Lockwood *et al*., 2005). The maximum density of juveniles recorded in the study site is within the range reported by other authors in northern European populations (van der Veer *et al*., 1998; Strasser *et al*., 1999; Strasser and Gunter, 2001). In addition, the Tagus estuary ecosystem is similar to those in which *Mya arenaria* is common (Ysebaert *et al*., 1998; Bocher *et al*., 2007); therefore, the natural susceptibility of the recipient habitat to invasion may easily widen the distribution of the invader.

The intertidal distribution of *Mya arenaria* juveniles was observed to be concentrated in the mid-region of the intertidal zone. However, Conde *et al*. (2010) previously described a population of *Mya arenaria* in the upper intertidal level of the Coina salt marsh. A plausible explanation is that settlement depends on local hydrodynamics, as pointed out by other authors (Armonies, 1996; Hunt and Mullineaux, 2002). Therefore, the aggregation of juveniles may be observed in different levels of the intertidal region (Emerson and Grant, 1991; Strasser *et al*., 1999) depending on the dominant current regime. However, the distribution process of *Mya arenaria* juveniles may also depend to some extent on the active role of the species, which is suggested by the juveniles of the invasive clam avoiding the excavated plots. In this sense, in the case of *Mya arenaria,* we agree with the arguments of Huxham and Richards (2003) who proposed that if active behaviour occurs it is probably due to active rejection of the former substratum rather than an active choice of a particular substratum. It must be stressed that the initial hypothesis of the study, i.e. that the excavated plots would enhance the concentration of juvenile bivalves if passive processes were the main mechanism governing settlement, is not consistent with the finding that the juvenile bivalves were less abundant in the excavated plots. However, the number of *Mya arenaria* juveniles trapped in the excavated plots increased with the tidal height (Fig. 5a), which relates the emersion time directly to the possibility of the juveniles being relocated by currents. This, along with the significant statistical interaction between the upper tidal level and the excavated plot (Table 3), strongly suggests that *Mya arenaria* juveniles actively attempted to leave the disturbed plots while they were submersed.

Mya arenaria juveniles in the 2-3 mm size class have been reported to reach a critical period in survival (Gunther, 1992; Strasser *et al*., 1999; Hunt and Mullineaux, 2002; Jening and Hunt, 2009). The 3 mm size class was more abundant than the 2 mm size class in this study, which suggests that recruitment tends to take place at a length of 3 mm. Larger size classes were less abundant and there was no continuity of 3 mm clams in the size classes at successive sampling times. With due caution, the meaning of these results might be similar to those of Hunt and Mullineaux (2002), who hypothesized that clams under the critical size of 2 mm are mainly controlled by hydrodynamic forces and above the critical size there are more losses of *Mya arenaria* due to predation.

The preference of the native clam *Scrobicularia plana* for the upper level of the intertidal region suggests that a different mechanism explains its distribution from that previously discussed for *Mya arenaria*. Under the same hydrological conditions, the dispersal rates of different species may vary (Jennings and Hunt, 2009), but a common pattern would be recognized if passive forces drove dispersion in both species. The *Scrobicularia plana* juveniles mainly settled in the upper intertidal. Their abundance decreased with lower tidal heights, a pattern permanently observed during the study period (Table 2; Fig. 5b). Thus, the intertidal distribution of the juveniles of *Scrobicularia plana* appears to be related to active behaviour oriented to selecting a particular intertidal level, as described for instance for the tellinid bivalve *Macoma balthica* (Linnaeus, 1758) (Hiddink, 2003). The last author showed that *Macoma balthica* avoids shrimp predation by migrating to the high intertidal after early settlement.

França *et al*. (2009) reported higher densities of *Scrobicularia plana* juveniles in the upper intertidal part of a mudflat of the Tagus estuary. In accordance with the present study, França *et al*. (2009) reported a peak in recruitment from October to December, which resembles the recruitment pattern previously described by Sola (1997) in the Bidasoa estuary. Adult specimens of *Scrobicularia plana* are widely distributed within the estuary in all intertidal areas and the species is a key component of the community within the estuary (Calvário, 2001; Silva *et al*., 2006; Rodrigues *et al*., 2006; Dias *et al*., 2008; França *et al*., 2009; Conde *et al*., 2010). Although *Scrobicularia plana* is a deposit-feeder and *Mya arenaria* a filter-feeder (Ysebaert *et al*., 1998), interspecific competition between these two species appears to be inevitable in the Tagus estuary. Competition for space may be especially acute between the two species as they are buried at a common shallower depth in the sediment when they are younger, while adults occupy a different position in sediment depth (Zwarts and Wanik, 1989). Therefore, the occurrence of *Mya arenaria* in the estuary may exert further stress on the population growth of *Scrobicularia plana*. Similarly, on the Swedish west coast, Möller (1986) partly attributed a higher rate of mortality of *Cardium* (=*Cerastoderma*) *edule* to the interspecific competition of this species with *Mya arenaria*. Analogously, Gomoiu and Petran (1973) described the introduction and accelerated spread of *Mya arenaria* on the Romanian coast, Black Sea, Black Sea, and obtained high values in abundance and biomass. This apparently had no consequences for the native fauna, although *Mya arenaria* outcompeted most of the native bivalves some years later, mainly *Corbula mediterranea* Da Costa, due to competitive exclusion (Gomoiu, 1981).

The avoidance of disturbed sediment is a feature to be considered in the Tagus estuary where there are shellfish catchments and fishermen dig for bait (Dias *et al*., 2008). The activities of anglers and shellfish fishermen may increase the probability of a similar relocation of the two species away from the disturbed areas.

The results of the present study provide information on the initial invasion stage of the clam *Mya arenaria* and also on the biology of the species concerning recruitment periods, spat distribution and potential survival strategies. The number of individuals involved in relation to the total bivalve species observed indicates how important an invasive process may be in a recipient habitat, as well as the interactions between species. In addition, the data provide strong evidence of active behaviour of juvenile bivalves in relation to the substrate they inhabit, although the degree of importance of this behaviour is probably different between species. Further studies are necessary in order to investigate recruitment mechanisms, post-settlement migration and population dynamics in more depth, as well as ascertain the wider ecological implications of the presence of *Mya arenaria* in the Tagus estuary. Such studies would provide useful information for the proper management of the invasion.

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