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Longitudinal distribution and lateral pattern of megalopal settlement and juvenile recruitment of *Carcinus maenas* (L.) (Brachyura, Portunidae) in the Mira River Estuary, Portugal

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Abstract

Settlement is a critical process in the life history of crabs, and thus affecting the abundance, distribution and structure of estuarine communities. The spatial pattern of settlement of megalopae of the shore crab *Carcinus maenas* along a longitudinal estuarine gradient (Mira River Estuary, Portugal) was examined, as well as its effects on the juvenile population. To measure megalopal settlement, four replicate collectors were deployed in six equally spaced stations along the estuarine axis. Juveniles were collected on the same locations with a quadrat randomly deployed on the substrate. To assess fine-scale megalopal settlement within a curved region of the estuary, replicate collectors were deployed on both margins along Moinho da Asneira curve. Megalopae settled differently along the six longitudinal points, with a tendency to attenuate their settlement upstream. Within the curved region, megalopae preferentially settled on the left margin collectors, probably due to the weaker velocity speeds felt on this margin. Concerning the overall juvenile density, there were significant differences among the stations distributed along the estuary, but they did no reflect a longitudinal dispersion attenuation pattern. Size-frequency distribution of the juvenile population showed that the average size is higher on the left margin. Recruits (carapace length between 1.0 mm and 3.4 mm) were more abundant on the upstream stations. Density of early juveniles (3.4 mm–6.5 mm) and juveniles (6.5 mm–10 mm) was more stable throughout the estuary axis than that of recruits. This distribution pattern may result from tidal excursion processes or mechanisms to avoid biotic interactions, such as predation and competition.

Keywords: settlement; megalopae; juveniles; *Carcinus maenas*; Mira Estuary; longitudinal dispersion

1. Introduction

Recruitment is an important process in regulating marine benthic communities with complex life cycles and is determined by physical and biological processes that occur during the pelagic larval phase, settlement and juvenile growing phases (Keough and Downes, 1982; Eggleston and Armstrong, 1995). At benthic population densities below the carrying capacity of nursery areas, the supply of new settlers may determine the abundance of adult crab populations (Roughgarden et al., 1988; Loher and Armstrong, 2000).

Estuarine-dependent species exhibit two patterns of planktonic development in coastal waters: larval retention within the estuary (Paula, 1998; Bilton et al., 2002) or transport of newly-hatched stages from the estuary to oceanic waters where early development occurs (Goodrich et al., 1989; Tankersley and Forward, 1994; van Montfrans et al., 1995; Hasek and Rabalais, 2001; Bilton et al., 2002). Several mechanisms have been proposed for the onshore transport of larvae from the shelf towards the coast (Shanks, 1995; Almeida and Queiroga, 2003; and see Queiroga and Blanton, 2004), and for estuarine crabs it is the last larval stage, the megalopa, that re-invades

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the estuary and migrates upstream to the adult habitat (Olmi et al., 1990; O'Connor, 1993; DeVries et al., 1994), usually intertidal or shallow subtidal environments.

The transition from a planktonic to a benthic existence is a critical step in the life cycle of most benthic invertebrates that have a planktonic larval phase, including crabs (Welch et al., 1997). Settlement occurs when the postlarvae or the juveniles have reached a suitable habitat. This period in the life cycle of many benthic organisms can significantly influence the population abundance, mainly due to the high predation and cannibalistic pressure (e.g. Morgan et al., 1996; Moksnes et al., 1997; Adams et al., 2004).

Once in shallow habitats, settlement of the megalopae depends on the recognition of environmental cues at the appropriate areas that trigger metamorphosis (Rodríguez et al., 1993; Eggleston and Armstrong, 1995; Welch et al., 1997). Competent megalopae, which are those that are capable of exercising the habitat selection at the time of settlement (Jensen, 1991), show plasticity in the duration of this stage in response to environmental stimuli, such as sediment type and presence of adults of the same or other species (Paula et al., 2003). This suggests that crab megalopae can shorten or extend settlement and metamorphosis until appropriate environmental conditions are encountered (Jensen, 1991; Rodríguez et al., 1993; Wolcott and DeVries, 1994; Paula et al., 2001).

Several studies have shown that megalopae actively select their settlement grounds. *Callinectes sapidus* megalopae can distinguish among settlement sites using chemical cues, and their discrimination ability increases when reaching the premolt stage (Welch et al., 1997). These megalopae are attracted to odours from seagrass beds and repelled by those associated with potential predators (Welch et al., 1997). Other studies demonstrated that megalopae preferred structurally complex habitats, with interstitial spaces that provide refuge (O'Connor, 1993; Eggleston and Armstrong, 1995; Hedvall et al., 1998; Moksnes et al., 1998; Stevens and Kittaka, 1998). O'Connor (1993) found a correlation between *Uca* spp. settlement site and habitats occupied by conspecific adults. In other species, megalopae settle away from adults, in order to protect themselves from predation, cannibalism and competition. However, this strategy can also be disadvantageous because it may increase mortality and require extra energetic costs during the movement between habitats (O'Connor, 1993).

Settlement and the initial juvenile phase are considered critical periods because of the high mortality due to predation (Hedvall et al., 1998), causing a demographic bottleneck effect (Eggleston and Armstrong, 1995; Moksnes et al., 1998). High densities of juvenile stages of epibenthic mobile organisms are mainly encountered in structurally complex microhabitats that constitute refuge against predation (e.g. Dungeness crab *Cancer magister* in shell habitats: Fernandez et al., 1993; fiddler crabs *Uca pugnax* and *Uca pugilator* in marsh areas: O'Connor, 1993; blue crab *Callinectes sapidus* in seagrass beds: Moksnes et al., 1997; shore crab *Carcinus maenas* in mussel beds, shell debris, filamentous macroalgae and eelgrass habitats: Moksnes et al., 1998; Paula et al., 2006; red king crab *Paralithodes camtschaticus* in rocky cobble and shell-hash sites: Loher and Armstrong, 2000). Thus, complex habitat availability and its active selection by early stages seem to be decisive factors in settlement and post-settlement survival in these populations and also the major processes affecting the initial non-random distribution of postlarvae and young juvenile crabs (Eggleston and Armstrong, 1995; Loher and Armstrong, 2000; Moksnes, 2002). Furthermore, the results obtained by Hedvall et al. (1998) suggest that the initial distribution of megalopae of *C. maenas* in nursery habitats is rapidly modified by habitat specific predation rates, size-specific movements and habitat choice by juveniles.

The distribution of postlarvae and young juvenile crabs can be non-uniform along an available habitat gradient, concentrating in certain areas, as it was seen by O'Connor (1993) and Brodie et al. (2005). According to Olmi et al. (1990), such uneven distribution may result from: (1) differential settlement of postlarvae because of habitat selection or hydrography; (2) post-settlement dispersal of settling postlarvae and juvenile stages; and (3) differential mortality due to density-dependent factors (e.g. cannibalism). In fact, in the absence of suitable habitats, juvenile crabs can migrate upstream, to low salinity unstructured areas (Mense and Wenner, 1989), where predator diversity and abundance is lower (Posey et al., 2005).

Very few papers have addressed longitudinal patterns of settlement of crabs (e.g., Lipcius et al., 2005; Posey et al., 2005), although restricted to early juvenile phases, and none has addressed lateral patterns of settlement of megalopae. These studies concerning patterns of settlement and secondary dispersal of early juveniles are of major interest, since these spatial patterns of settlement and dispersal may explain distribution of the adults' population in the estuary.

The shore crab *Carcinus maenas* (L.) is one of the most common crabs that inhabits European estuaries and rocky shores. It has a complex life cycle that comprises an exported planktonic larval phase, which is rapidly flushed out to shelf waters by ebbing currents after release (Queiroga et al., 1994; Queiroga, 1996). Larvae take four to six weeks, depending on water temperature, to reach the megalopa stage in shelf waters (Dawirs, 1985). Megalopae migrate back to the estuary during spring tides, and transport is accomplished by selective tidal stream transport (Berril, 1982; Hedvall et al., 1998; Moksnes et al., 1998; Queiroga, 1998). Once they reach the estuarine environment, the megalopae of *C. maenas* settle in a variety of intertidal and subtidal habitats, showing, however, a preference for those that are structurally complex (Paula et al., 2006).

The Mira Estuary, located in the southwestern Portuguese coast, presents an ideal setup for studying longitudinal and lateral distribution of settlement of megalopae. It is a single-channelled, medium-sized estuary where the extension of the tidal excursion can be easily sampled, and unidirectional gradients can be followed. The Mira Estuary has also been used for studying numerous aspects of *Carcinus maenas* recruitment (Paula et al., 2006; Queiroga et al., 2006).

In the present study it was investigated the spatial pattern of settlement of megalopae of *Carcinus maenas* along the longitudinal axis of the Mira Estuary and its effects on the juvenile population density and structure. In addition, lateral differences in settlement along a curved region of the estuary were investigated on a finer spatial scale. The aim of this second study was to conduct field experiments to assess if settlement of megalopae was influenced by specific hydrological mechanisms occurring within a curve.

2. Materials and methods

2.1. Study site

The Mira Estuary is located in the southwestern Portuguese mainland coast (approximately 37°40′N and 8°45′W), extending for a length of about 40 km inland in a single channel (see Fig. 1). Tides are semi-diurnal, with an amplitude of around 1 m during neap tides and 3 m during spring tides. Tidal penetration varies between 7.5 km and 2.5 km during respectively average spring and neap tides (Paula, 1998). There is a relatively strong stratification of the water column during the neap tide period, whilst estuary waters are mixed during spring tides, with a vertical homogenisation of the water column due to turbulence (Paula, 1998; Blanton and Andrade, 2001).

The estuary presents a maximum of 400 m width and maximum depth is around 20 m at the Vila Nova de Milfontes bridge (approximately 2 km from the mouth). Due to low riverine input, the lower section of the estuary has a dominant marine character. Upstream, the single channel is about 8 to 10 m deep, becoming shallower inland (Queiroga et al., 2006). Saltmarshes occur as far as 20 km upstream. In the lower 8 km the intertidal zone is dominated by areas with varying density cover of the seagrass *Zostera noltii*, and bare sandy and muddy substrates. Near to *Z. noltii* habitats, the shallow subtidal zone is dominated by dense cover of the seagrass *Zostera marina*.

2.2. Field methods

2.2.1. Longitudinal pattern of megalopal settlement

Settlement of megalopae was studied by deploying 4 replicate benthic collectors on both margins at 6 sites regularly spaced along the estuarine length, up to 10 km from the mouth (see Fig. 1). Sites were chosen as to constitute similar areas with no vegetation, either marsh or seagrass, to allow appropriate comparisons (see Paula et al., 2006 for interactions between artificial collectors and surrounding substrate). The experiment was repeated in two different starting dates (May 4–10, 2002 and July 1–7, 2002), both during neap-tide periods, as this were the observed periods of maximum recruitment events (Queiroga et al., 2006). Collectors were changed each day, during the diurnal low tide, thus sampling consisted of two complete tidal cycles. The benthic collectors were made of ‘hog’s hair’ filter, with a dimension of 50 × 40 cm and 2.5 cm thick, attached to a metal frame and deployed flat on the substrate (see Paula et al., 2006 for diagrammatic top view of collector). Preliminary observations have shown that the weight of the metal frame was enough to prevent movement of the collectors with the currents. Collectors were placed in plastic bags and transported rapidly to the laboratory for immediate processing.

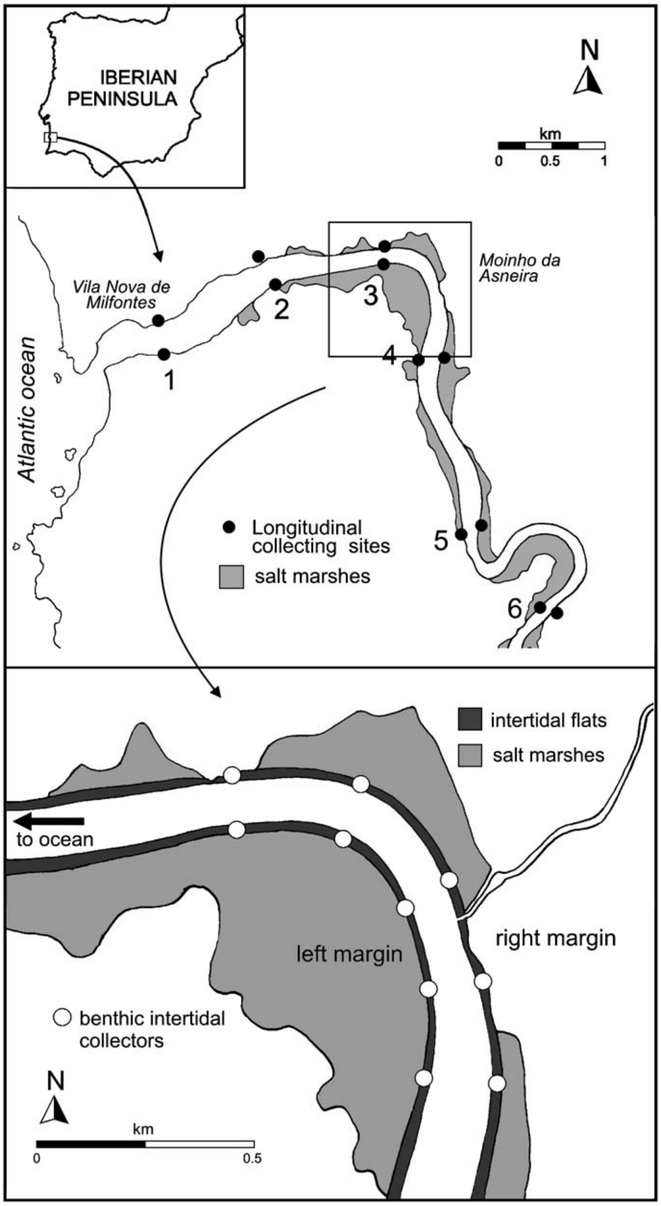

Fig. 1. Map of Mira Estuary showing: (a) position of sampling sites for longitudinal settlement patterns along a 10 km gradient; and (b) position of sampling sites for study of lateral pattern of settlement along Moinho da Asneira curve.

2.2.2. Lateral pattern of megalopal settlement

To assess detailed differential lateral settlement rates of megalopae on meanders of the estuary, an area with approximately 1 km in length (Moinho da Asneira curve) was chosen as a model area (see Fig. 1). As in the previous experiment, settlement was measured with the use of artificial collectors made with ‘hog’s hair’ filter material.

Sampling was conducted in two peak periods of megalopal settlement during neap tides, from June 19–23, 2002 and from July 3–6, 2002 (see Queiroga et al., 2006 for peak periods data of megalopal settlement). During the first experiment date, twenty collectors with dimensions 0.50 × 0.40 m and 2.5 cm thick were deployed along the Moinho da Asneira curve, lying at equidistant points from each other, during the diurnal low tide. In the second sampling date, for logistic reasons, only 18 replicate benthic collectors were deployed. Nevertheless, the same field methodology was undertaken. In both cases, collectors were deployed on similar intertidal areas on both margins (unvegetated muddy substrate). General transport procedures were equal to those described in the previous experiment.

2.2.3. Longitudinal juvenile population structure

At the same collecting sites defined for longitudinal settlement, the juvenile population of *Carcinus maenas* was sampled. Thus, six stations were chosen along the estuarine axis (see Fig. 1) and in each station were defined two parallel locations on both margins. Sampling was conducted on unvegetated muddy substrate. Due to the effort involved in obtaining a sufficient number of organisms to conduct size frequency analysis, the sampling was performed between May 31 and June 13 2002.

The sampling unit was a 0.5 × 0.5 m quadrat, deployed randomly on the substrate, where juveniles were collected by hand or with forceps. In each quatrat it was made an intensive search for juveniles and, at each site, sampling was considered terminated when reaching a total of at least 200 collected specimens in the overall replicate quadrats. All specimens from each station were preserved in the same vial in buffered 4% formalin and latter transferred to 70% alcohol.

2.3. Laboratory procedure

Megalopae of *Carcinus maenas* were recovered from the collectors' surface by rinsing in abundant freshwater and sorted out from the debris and algae with a binocular microscope. Megalopae were preserved in buffered 4% formalin, and identified using the descriptions by Rice and Ingle (1975) and Ingle (1992).

Juvenile carapace width (distance between tips of 5th pair of lateral spines) was measured under a binocular microscope with micrometric eye piece, and when necessary a vernier calliper was used to the nearest 0.1 mm. A total of 2600 specimens were collected, and of these, a random sub-sample of 110 was used for determination of stage and sex. Within this sub-sample carapace width range between 1.2 and 10.0 mm. Stage and sex were determined by detaching the abdomen and observing it under a compound microscope, using a key adapted from Shen (1935). Seven juvenile stages were identified among the sampled specimens. According to the identification key used, juvenile stages and sex were defined by different development of the pereopods (variations in size and shape of endopodites and exopodites). Thus, the first stage included juveniles with a maximum carapace width of 1.4 mm, the second stage juveniles with carapace width between 1.5 mm and 2.5 mm, the third stage juveniles between 2.6 mm and 4.5 mm, the fourth stage juveniles between 3.6 mm and 6.5 mm, the fifth stage juveniles between 5.6 mm and 8.0 mm, the sixth stage juveniles between 7.1 mm and 10.0 mm, and finally, the seventh stage, juvenile females with carapace width between 8.1 mm and 10.0 mm.

According to this classification, and for posterior analyses of different abundance patterns, these seven juvenile stages were grouped in three distinct classes: recruits (maximum carapace length between 1.0 mm and 3.4 mm), early juveniles (between 3.4 mm and 6.5 mm) and juveniles (carapace length bigger than 6.5 mm).

2.4. Data analysis

Due to strong heteroscedasticity of data, Kruskal–Wallis procedures were used to test for differences in postlarval abundance as a function of longitudinal collecting stations, margin and sampling period. For analysing the lateral differences in postlarval abundance along the Moinho da Asneira curve, the same statistical procedure was conducted. Dunn tests were used to compare the differences detected by Kruskal–Wallis test (Sokal and Rohlf, 1995).

Juvenile size-frequency distributions were decomposed in normal components with methods by Bhattacharya (1967), using the software FISAT (FAO-ICLARM stock Assessment Tools). The modal classes obtained were used to separate the population in classes of recruits (1.0–3.4 mm), early juveniles (3.4–6.5 mm) and juveniles (over 6.5 mm), as it was describe previously. Based on the morphological scale to differentiate among juvenile stages (Shen, 1935), each was assigned to a size-range up to the 7th stage (maximum length 10.0 mm).

For analysing the difference in density of total collected juveniles along the estuary, a 2-way ANOVA (margin and collecting station as fixed factors) was performed. Post-hoc Unequal N HSD test was used to determine under what conditions the factors in study differ. Mann–Whitney U-tests and t-tests were used for between-margin and among-stations comparisons of recruits, early juveniles and juveniles, since ANOVA's assumptions were not met for these comparisons.

3. Results

3.1. Longitudinal pattern of megalopal settlement

Fig. 2 presents the results of megalopal settlement along the collecting stations defined in the longitudinal axis of the estuary. Settlement of megalopae of *Carcinus maenas* shown significant differences among stations ($H = 38.518$; $P < 0.001$). According to the Dunn test performed, station 1 presented significantly higher settlement values than stations 3, 5 and 6, and station 2 differed significantly from station 6. In average, in station 1 settled 13.15 megalopae, while in stations 3, 5 and 6 settled 3.35, 4.61 and 3.95 megalopae, respectively. In stations 2 and 4 the mean number of settling megalopae was

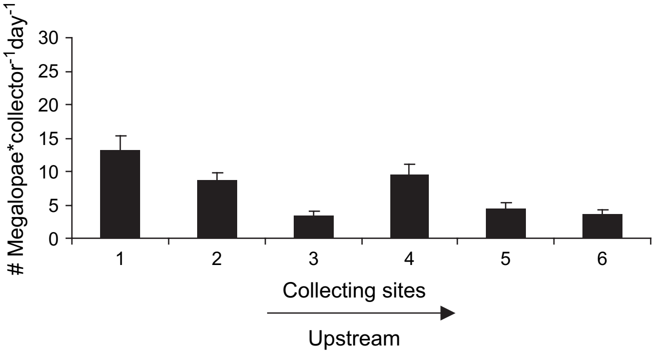

Fig. 2. Mean settlement rates of megalopae of *Carcinus maenas* along the longitudinal axis of the Mira estuary defined by the 6 collecting sites, on both margins. Vertical bars + SE.

8.7 and 9.19.The first start date was found to present significantly higher settlement rate than the second start date ($H = 85.196$; $P < 0.001$). Although relatively intense settlement was observed along the sampled area, there is a trend for a progressive attenuation of settlement along the estuary, and particularly if we consider independently the sub-areas stations 1–3 and 4–6.

3.2. Lateral pattern of megalopal settlement

Megalopal settlement was consistently higher on the left margin than on the right margin (Fig. 3). Left margin average settlement was 8.24 megalopae, whilst 5.22 megalopae settled, in average, on the right margin. Non-parametric results show that margin factor had significant effects on settlement ($H = 72.047$; $P < 0.001$). This statistical test also demonstrated that settlement was significantly higher in the first sampling period ($H = 68.786$; $P < 0.001$).

3.3. Longitudinal juvenile population structure

Fig. 4 presents the results of size-frequency distribution for the overall collected juvenile population, the corresponding modal curves and size range of the discrete juvenile moults. Within this sampling, the first seven juvenile stages were collected. In general, the average juvenile carapace width is higher on the left margin, with the exception of station 6 (Fig. 5 and Table 1). On the 2 most upstream stations, juveniles tended to be smaller, whilst at the remaining stations juveniles covered a wider range of carapace sizes (Fig. 5). This may indicate a probable attenuation of settlement rates along the estuary with dissipation of flood tidal excursion or higher post-settlement mortality.

Density of juvenile crabs was significantly different between both estuarine margins and also along the estuarine longitudinal axis (Table 2). Highest densities were observed at stations 1 and 2 at both margins, and the right margin presented consistently higher density (Fig. 6). ANOVA results indicate a significant interaction between margin and collecting station (Table 2), thus precluding analysis for individual factors and showing strong spatial heterogeneity along the estuary. According to the post-hoc test performed, density of juveniles in station 1 on the right margin was significantly higher than the density in any of the other collecting sites (Table 3). Station 1 on the left margin and station 2 on the

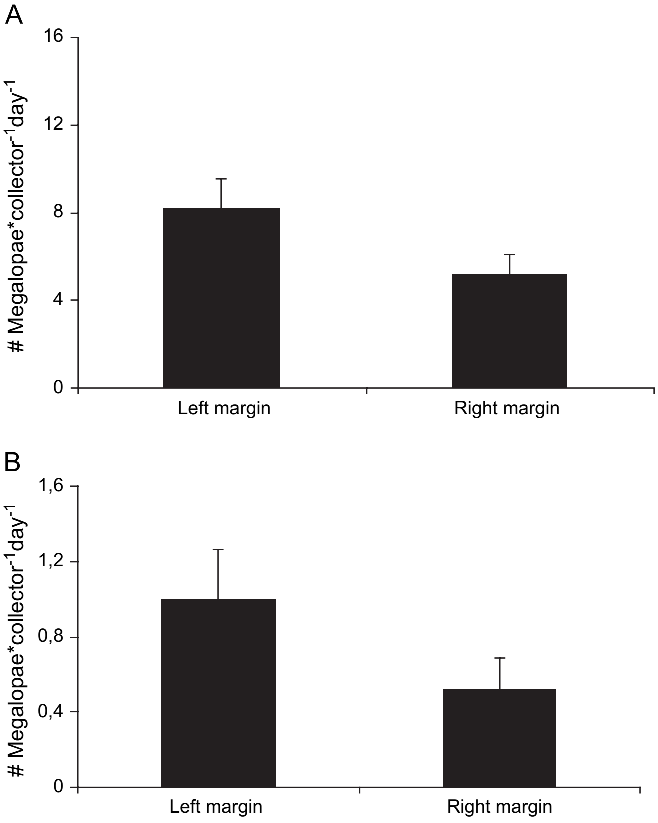

Fig. 3. Mean settlement rates of megalopae of *Carcinus maenas* along the Moinho da Asneira curve, on both margins, in the Mira Estuary. A – First sampling event; B – Second sampling event. Vertical bars + SE.

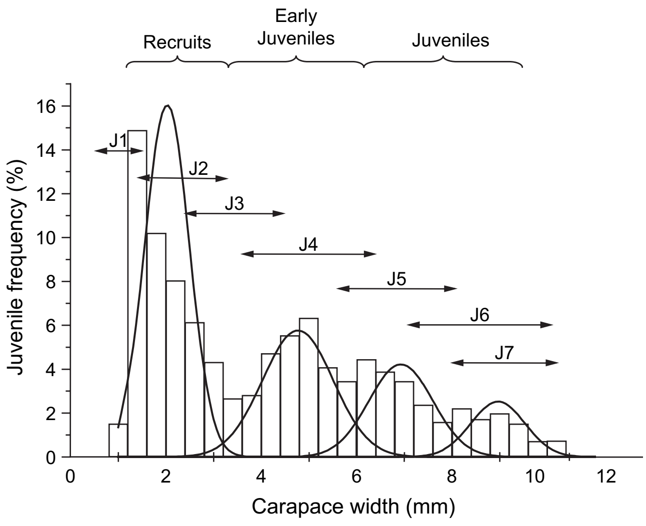

Fig. 4. Relative frequency distribution according to size classes for the overall juvenile population of *Carcinus maenas* of the Mira Estuary, and the different modal classes of this population along the estuary. Arrows indicate the size range for each juvenile stage.

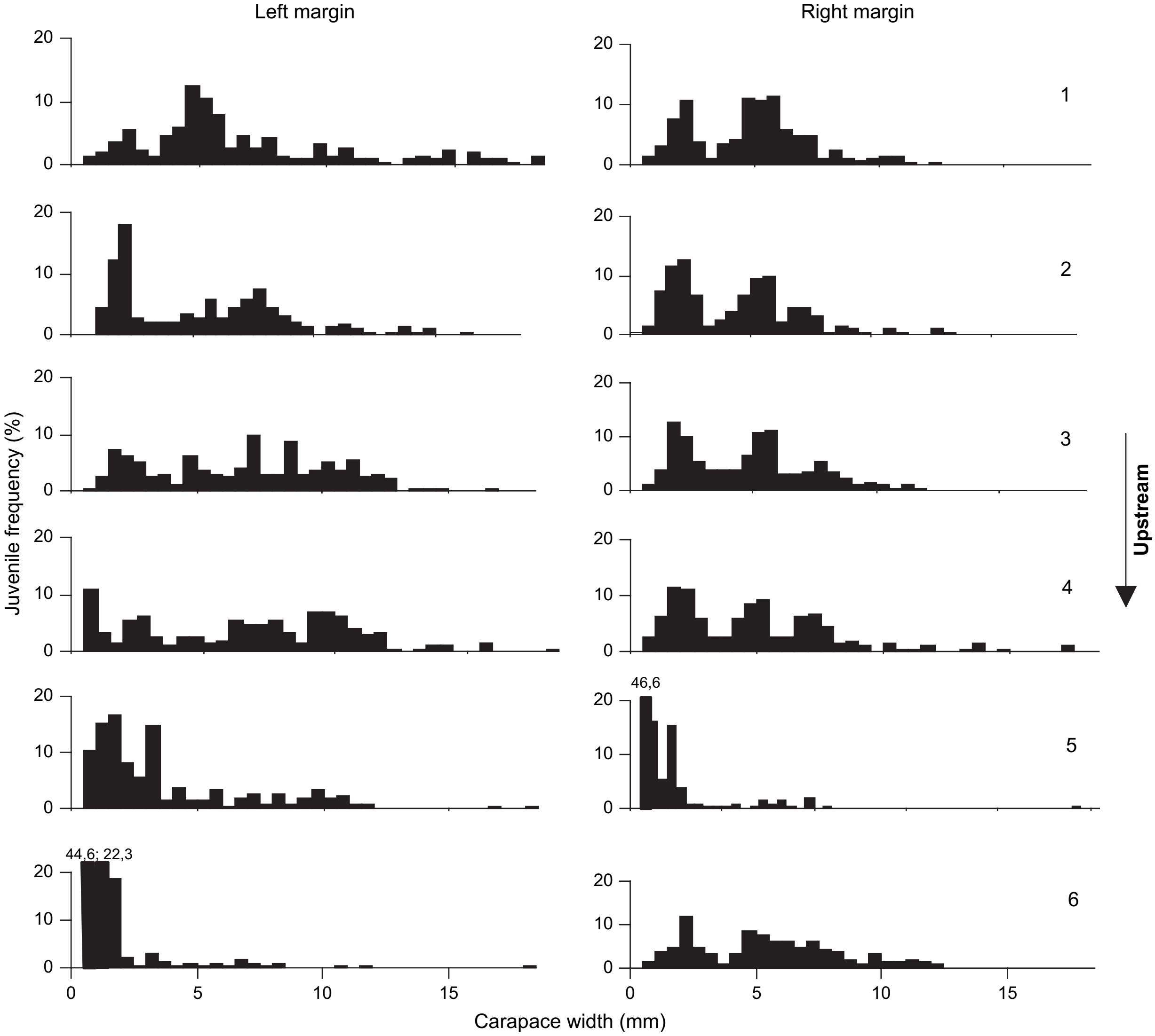

Fig. 5. Juvenile population structure of *Carcinus maenas* at longitudinal collecting sites, on both margins of the Mira Estuary.

right margin shown the same pattern of significant variability (Table 3). Although there were greater densities of juvenile crabs in these two stations, they did not differ from each other and from stations 2 and 5 on left and right margins, respectively. However, their results were significantly different from all other stations in study.

Patterns of frequency of different size groups are presented in Fig. 7, for both margins, along the estuarine longitudinal axis. These different size groups were made according to carapace length and with the modal classes as defined previously. Recruit major spatial peaks were located at the most upstream stations. All comparisons among size classes did not indicate significant differences. In any case, frequencies of early juveniles and juveniles was more homogeneous throughout the estuary than that of recruits. Moreover, the frequency of juveniles was lower on the right margin in the majority of the sampling sites (Fig. 7) and differed significantly from the density on left margin ($t = -2.379$, $P = 0.039$).

Table 1
Average juvenile carapace width (mm) along the longitudinal collecting stations, on both margins

	Collecting stations					
	1	2	3	4	5	6
Left margin	6.04	4.98	6.10	6.06	5.07	2.63
Right margin	4.88	4.40	4.60	4.69	2.82	3.80

Table 2
Results of 2-way ANOVA for total juvenile density along the estuarine longitudinal stations on both margins in the Mira Estuary

Effect	df	ms	*F*	*P*-level
Margin (M)	1	5.894	12.203	0.0006
Station (S)	5	2.381	4.931	0.0002
M × S	5	7.132	14.765	<0.0001

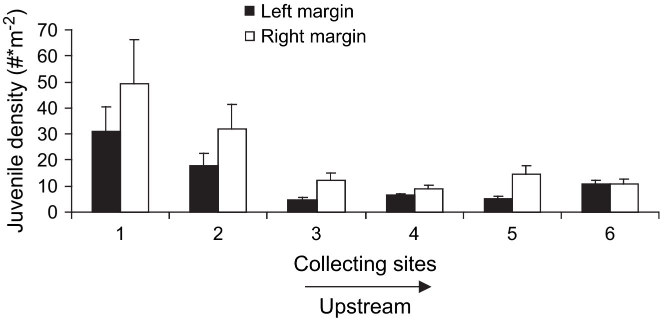

Fig. 6. Density of juveniles of *Carcinus maenas* along the longitudinal axis of the Mira Estuary defined by the 6 collecting sites, on both margins. Vertical bars + SE.

4. Discussion

According to Eggleston and Armstrong (1995) and Feldman et al. (1997) settlement is the process that mainly influences the distribution of decapods. However, the post-settlement processes have an important role in regulating populations and in the spatial organization of benthic communities (Etherington and Eggleston, 2000; Blackmon and Eggleston, 2001).

The settlement rates of *Carcinus maenas* megalopae did not show a continuous pattern of attenuation along the estuarine longitudinal axis, but there was a pattern of higher megalopa settlement at the lower part of the estuary. Although megalopae may migrate upstream in search of adequate settling habitats, studies on the blue crab *Callinectes sapidus* have shown that most do settle in the lower estuary (Boylan and Wenner, 1993; Wrona et al., 1995; Morgan et al., 1996). Dimension of the estuary and effective tidal excursion undoubtedly will affect transport magnitude, and in large systems (such as The Croatan-Albemarle-Pamlico Estuarine System in North Carolina, USA: Etherington and Eggleston, 2000) upstream transport of *C. sapidus* may be accomplished by secondary dispersal juveniles. The flood tide excursion during neap tides at the Mira Estuary does not reach beyond collecting station 4 (Paula, 1998), thus megalopae collected upstream of that area might be from previous tidal immigration from the shelf waters. This distribution past the area of tidal influence could also be due to wind driven transport, since postlarvae undergo active vertical movements during the tidal cycle in the water column, allowing transport by wind events when remaining in the surface waters (Queiroga, 1998). Abundance of megalopae prior to settlement, either in the plankton or on a re-suspension dynamics, can explain such upstream settlement, and in fact there are indications of a delay between peaks of immigration and of effective settlement intensity at the Mira estuary (Queiroga et al., 2006). Density-dependent secondary dispersal could be another factor influencing the longitudinal distribution along the estuarine axis in study. Megalopal moulting competency may be involved in this delay (Paula et al., 2003) but also varying settlement rates with current speed along the semi-lunar period.

Spatial heterogeneity of settlement is high, and results from the curve of Moinho da Asneira may show that meanders of the estuary, with associated heterogeneity in current patterns and passive sinking rates, induce lateral asymmetry in settlement intensity. The interaction of the estuarine morphology with the availability of postlarvae may have produced the observed variability of settlement intensity along the estuary. Also, patches of megalopae that entered the estuary in previous tidal cycles may mask the effects of the megalopal transport in a tidal immigration event. Nevertheless, the highest settlement was observed in straight and widest areas of the estuary (stations 1, 2 and 4) with presumably more dissipation of currents, and lower in the remaining stations that are closer to curves with higher current speed by the converging water mass. At Moinho da Asneira curve the lateral asymmetry of settlement was consistent, with high settlement rates on the left convex margin, with higher rate of passive sinking, which is evident in the larger development of salt marsh.

Considering the results of the juvenile population structure, it can be seen that there are some differences between the density of crabs on both margins. In spite of the results concerning recruits and early juveniles appear to indicate no settlement asymmetry, juveniles show a higher density on the left margin. This margin seems to have better refuge and nourishing

Table 3
Post-hoc Unequal N HSD test describing the differences among stations, on right (R) and left (L) margins, for the density of the overall collected juveniles

		Station 1		Station 2		Station 3		Station 4		Station 5		Station 6	
		R	L	R	L	R	L	R	L	R	L	R	L
1	R		0.00385	0.00899	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
1	L	0.00385		1.00000	0.53520	0.00964	0.00002	0.00004	0.00002	0.12988	0.00002	0.00116	0.00098
2	R	0.00899	1.00000		0.41460	0.00452	0.00002	0.00002	0.00002	0.07959	0.00002	0.00045	0.00038
2	L	0.00002	0.53520	0.41460		0.99831	0.55569	0.93938	0.76857	1.00000	0.62702	0.99269	0.99080
3	R	0.00002	0.00964	0.00452	0.99831		0.95565	0.99997	0.99552	1.00000	0.97600	1.00000	1.00000
3	L	0.00002	0.00002	0.00002	0.55569	0.95565		0.99881	1.00000	0.81450	1.00000	0.97506	0.98036
4	R	0.00002	0.00004	0.00002	0.93938	0.99997	0.99881		1.00000	0.99585	0.99972	1.00000	1.00000
4	L	0.00002	0.00002	0.00002	0.76857	0.99552	1.00000	1.00000		0.94666	1.00000	0.99867	0.99912
5	R	0.00002	0.12988	0.07959	1.00000	1.00000	0.81450	0.99585	0.94666		0.86760	0.99994	0.99991
5	L	0.00002	0.00002	0.00002	0.62702	0.97600	1.00000	0.99972	1.00000	0.86760		0.98846	0.99130
6	R	0.00002	0.00116	0.00045	0.99269	1.00000	0.97506	1.00000	0.99867	0.99994	0.98846		1.00000
6	L	0.00002	0.00098	0.00038	0.99080	1.00000	0.98036	1.00000	0.99912	0.99991	0.99130	1.00000	

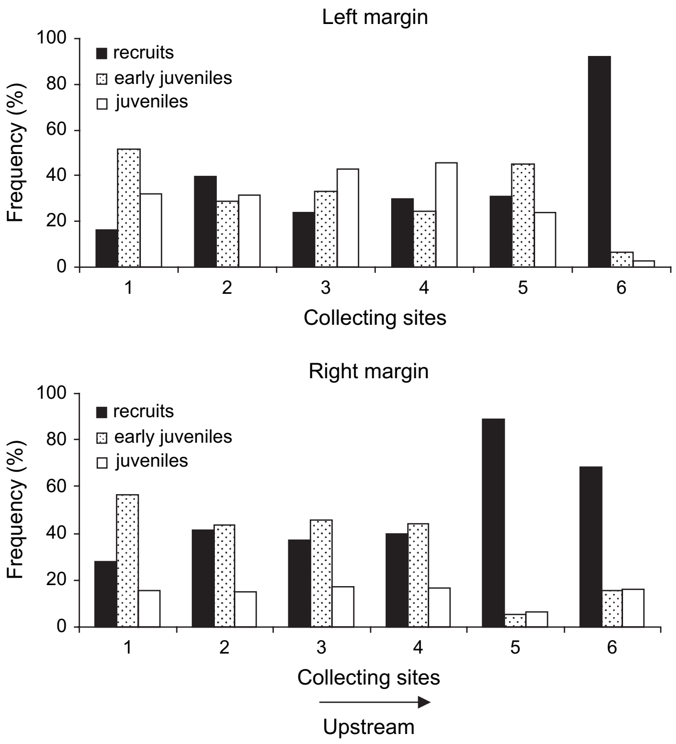

Fig. 7. Frequency (%) of recruits, early juveniles and juveniles of *Carcinus maenas* for both right and left margins along the estuarine longitudinal axis of the Mira Estuary.

conditions for this size class. Possibly, the hydrology of the estuary presents differences that promote an asymmetric sediment deposition on the margins, favouring the permanence of sub-adults on the left margin.

Through out the longitudinal gradient the population density differences were clear. In all sampled stations, the density of *Carcinus maenas* was always higher on the right margin and attenuating to upstream. This decrease of density along the longitudinal gradient is due to the numbers of early juveniles and juveniles obtained, and does not seem to be related with the density of recruits. Etherington and Eggleston (2000) demonstrate that the recruitment in *Callinectes sapidus* is, in part, dependent of the distance to the postlarval oceanic source. In this study, the spatial gradient does not seem to indicate a settlement attenuation. The highest densities of recruits observed in the upstream stations may be the result of consecutive tidal cycles or a mechanism for the recruits to avoid competition, predation and cannibalism with larger conspecifics. In fact, juveniles with cannibalistic behaviour are very efficient predators of small conspecifics, whereas the adult crabs have difficulties manipulating small prey (Moksnes et al., 1998); however, cannibalism is, in general, more pronounced between cohorts then within cohorts (Moksnes et al., 1998). Brodie et al. (2005) discovered a different distribution pattern within juvenile and adult populations of *Uca pugilator* and higher relative abundances of smaller juveniles in lower salinity areas were also observed by Posey et al. (2005). In this case, the lower salinity areas represented a distinct habitat for smaller juveniles, whilst larger juveniles and sub-adults did not show differences among salinity levels. This same abundance pattern was observed for the recruits, early juveniles and juveniles of *C. maenas* in the present study, with recruits being more abundant in the more upstream stations where the tidal influence is lower (Cartaxana, 1994; Paula, 1998). This distribution pattern may reflect a potential reduction of the predation pressure, as well as a more diverse and abundant prey populations' availability in mesohaline to oligohaline areas (Posey et al., 2005).

Once postlarvae move into estuaries using flood tide transport (Forward and Tankersley, 2001; Queiroga, 2003), they settle and metamorphose in nursery areas of aquatic vegetation and mussel beds (Moksnes et al., 1998). The inexistence of a clear attenuation pattern along the studied longitudinal gradient of recruits, early juveniles and juveniles may be due to post-settlement movements. This secondary dispersal of individuals may enhance connectivity between spatially separated habitats, as suggested by Etherington and Eggleston (2003), and mask possible settlement patterns. Recent studies show that early-staged blue crab juveniles may move into the water column and undergo horizontal transport (Blackmon and Eggleston, 2001; Forward et al., 2004). The reason why this transport occurs remains unknown, but can be associated with tidal or wind-generated currents (Forward et al., 2004). However, Reyns and Eggleston (2004) suggest that this pelagic movement may occur when conspecific densities increase in initial settlement habitats.

According to Forward et al. (2005), the early juvenile stages of *Callinectes sapidus* do not swim continuously during vertical migration, but instead undergo a series of hops in the water column. This discontinuous movement, probably caused by a circadian rhythm, may also contribute to the lack of post-settlement attenuation along the longitudinal gradient studied.

Megalopae show a high plasticity in selecting habitat and in obtaining refuge so they can explore different types of natural substrate (Hedvall et al., 1998). Nevertheless, the spatial variation in the habitat distribution may have a strong role in the intertidal crabs' recruitment and in the population dynamics (Moksnes, 2002). Is still unknown in which way the active selection of habitat by megalopae and juveniles of *Carcinus maenas* may gather the individuals and if the recruitment is regulated by the larval availability or by density-dependent post-settlement processes.

5. Conclusions

Estuaries play a fundamental role in the life history of many species as nursery habitats. The megalopae of *Carcinus maenas* reinvade these ecosystems to undergo their development until the adult stage. Significant differences in postlarval settlement were found among the six stations sampled along the longitudinal estuarine gradient. These differences reflected a trend of megalopae to settle in the lower part of the estuary. This distribution may also show the importance of the tidal gradient in the settlement processes of postlarvae. Considering lateral pattern of megalopal settlement, densities were higher on the left margin of the Moinho da Asneira curve. These results suggest that hydrological and hydrodynamic factors occurring in curves and meanders of the estuary influence the settlement rates of the megalopae of *C. maenas*.

This study also provided information on juvenile population structure of *Carcinus maenas*. Juvenile distribution along the longitudinal estuarine axis was consistent with those obtained for megalopae, as there was no clear density attenuation pattern. In fact, as it was also demonstrated by Etherington and Eggleston (2003), juvenile crab distribution and abundance patterns reflect the interaction between primary dispersal of postlarvae and secondary dispersal of early benthic juveniles. On the other hand, considering recruits, early juveniles and juveniles as components of the overall juvenile population survey, recruits showed a higher density on the more upstream stations. This may indicate that younger juveniles may seek refuge in low salinity areas. According to Posey et al. (2005), these areas are associated with greater survivorship, more rapid moulting, and higher crab dry weight compared to higher salinity areas, where larger and more competitive crabs are more common.

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