Carcinus maenas (Crustacea: Brachyura): Influence of artificial substrate type and patchiness on estimation of megalopae settlement

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Abstract

Settlement patterns of decapod crustaceans are influenced by size, shape and nature of substrates, as well as by species-specific behaviour patterns. Hog’s hair filter material is one of the most commonly used artificial substrates in the study of settlement rates. However, the use of hog’s hair collectors in settlement studies poses several problems: movement restriction of settled animals, relatively long and complicated laboratory processing time, and cost and decreasing availability. Despite widely used, no consistent investigation has focused on the influence of size and shape of hog’s hair collectors on rates of benthic settlement. A first experiment was set to investigate the effects of collector patchiness on settlement abundances of Carcinus maenas megalopae. Benthic hog’s hair collectors of different sizes were deployed intertidally in the lower Mira Estuary. Settlement was addressed as ind collector$^{-1}$ and ind m$^{-2}$ in relation to collector’ surface area and perimeter:area ratio. Results showed that collector patchiness significantly influenced settlement response of C. maenas, which differed according to settlement intensity and measure. Megalopae settlement responded to lower scales of habitat patchiness at high than at low intensity. Settlement as ind collector$^{-1}$ generally increased, while as ind m$^{-2}$ generally decreased, with increasing collector’ surface area (decreasing perimeter: area ratio). A second experiment investigated the efficiency of plastic grass, as a new type of artificial surface, in estimating settlement patterns of C. maenas megalopae, and compared it with that of hog’s hair. Collectors of both types were deployed daily and intertidally in the lower Mira Estuary. Settlement showed the same pattern and intensity on both collector types. Plastic grass collectors constitute a good alternative to those of hog’s hair, possessing several advantages over them.

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1. Introduction

The life cycle of many coastal and estuarine decapod crustaceans includes the transition of a planktonic larval phase to a benthic juvenile existence. This transition frequently produces bottleneck effects on the number of individuals, affecting the dynamics of juvenile and adult populations, with special relevance for fisheries and aquaculture management (e.g. Rabalais et al., 1995; Moksnes, 2004). The cryptic nature of megalopa and juvenile stages usually makes the study of respective recruitment and settlement patterns difficult. To overcome this constrain, several different types of artificial collectors have been developed in the recent decades specifically for certain organisms, and then adapted for several others. See Phillips and Booth (1994) and Phillips et al. (2001) for reviews of collectors’ designs. The function of all collector types is to enhance the thigmotactic response of megalopa and juvenile stages
by simulating their required settlement needs, allowing the assessment of settlement patterns and providing animals for aquaculture purposes.

Hog’s hair filter material has been the most broadly used settlement surface in different collector designs, especially for spiny lobsters and brachyuran crabs: Hunt’s collectors, a current version of Witham collectors, have been used for spiny lobsters Panulirus sp. (Phillips et al., 2005); cylindrical floating collectors have been used for the blue crab Callinectes sapidus (e.g. Olmi et al., 1990; Rabalais et al., 1995), Carcinus maenas (e.g. Moksnes and Wennhage, 2001; Moksnes et al., 2003) and other brachyuran species (Paula et al., 2001, 2003); flat collectors have been deployed in the water column (e.g. van Montfrans et al., 1990; Boylan and Wenner, 1993) or in the benthic zone for several brachyuran species (e.g. Paula et al., 2003, 2006).

Several studies have emphasized the importance of using artificial substrates to indicate the correlation between recruitment and settlement patterns of decapod crustaceans, when compared with those that are natural (e.g. Phillips and Booth, 1994; Metcalf et al., 1995; Eggleston et al., 1998). However, the physical and chemical nature of artificial substrates, as well as its interactions with the surrounding areas of deployment and with local biota, may in fact enhance or decrease propensity to settle (Goodrich et al., 1989; Moksnes and Wennhage, 2001; Paula et al., 2006). Furthermore, size, shape and complexity of settlement grounds may also have significant effects on recruitment intensity of crustacean decapods as well as other marine organisms. Studies in seagrass and other structured marine substrates have revealed that several individual small substrate patches may increase overall colonization rates when compared to larger patches with smaller perimeter: area ratios (e.g. Keough, 1984; McNNeill and Fairweather, 1993; Pineda and Caswell, 1997; Eggleston et al., 1998; Bologna and Heck, 2002; Hovel and Lipcius, 2002). However, for other marine organisms, recruitment and settlement rates vary positively with surface area (Attrill et al., 2000, 2005; Lee et al., 2001; Jenkins et al., 2002). Nevertheless, and despite the generalized use of hog’s hair collectors to assess settlement and recruitment patterns of brachyuran crabs, no specific study has addressed the influence of collector size and shape on such patterns.

The use of hog’s hair collectors presents several problems. The development of most brachyuran crabs includes a settling megalopa stage with strong abdominal swimming pleopods, and the structural design of hog’s hair fibers may restrict swimming movements of pleopods and trap spinulate pereiopods. This can mask real settlement patterns of species in which megalopae settlement may not be final. C. maenas and C. sapidus megalopae settle only temporarily if substrates are not suitable and metamorphose is not imminent (Zeng and Naylor, 1996; Welch et al., 1999; Tankersley et al., 2002; Moksnes et al., 2003). The necessary time taken to retrieve megalopae from hog’s hair fibers may also become a handicap in high sampling intensity experiments. Furthermore, hog’s hair material is becoming expensive and difficult to assess, as also noted by Phillips et al. (2005). Consequently, there is a demand for an alternative type of collector that allows free movement of settled megalopae, with easier and faster laboratory processing and economical viability.

This study was therefore designed with two objectives. The first was to evaluate the influence of hog’s hair collector size on settlement rates of C. maenas megalopae. The second objective was to test the efficiency of a new type of collector, plastic grass surfaces, in estimating daily settlement rates of C. maenas megalopae, when compared to that on hog’s hair collectors. In order to accomplish the first and second objective, hog’s hair collectors of different sizes in surface area and perimeter:area ratio were deployed intertidally during different settlement intensity periods, and plastic grass and hog’s hair collectors were deployed daily also in the intertidal zone of the lower Mira Estuary, respectively.

2. Materials and methods

2.1. Larval biology of C. maenas

C. maenas inhabits both hard and soft coastal and estuarine shallow habitats. After release, the planktonic zoeal phase is exported to coastal waters (Queiroga et al., 1994; Queiroga, 1996), where eventually the fourth zoeal stage undergoes metamorphosis to the megalopae phase. It is the megalopae that then reinvade estuarine waters by selective tidal stream transport and actively select their settlement grounds (Zeng and Naylor, 1996; Moksnes et al., 1998; Queiroga, 1998). Megalopae prefer relatively structured habitats, where they begin a benthic existence with metamorphosis to first crab stage (Hedvall et al., 1998; Moksnes et al., 2003). During spring and early summer in the Mira Estuary, settlement of C. maenas megalopae occurs with a semilunar periodicity during neap tides (around quarter moons), and variability in the intensity of settlement events also characterizes this settlement pattern (Paula et al., 2006; Queiroga et al., 2006).

2.2. Area description

All experiments were conducted on a sandy beach, located ~1 km inside the Mira Estuary. The Mira...
Estuary is a small mesotidal system located on the southwestern Portuguese mainland coast (37°40′ N, 8°40′ W). It presents a single river channel, with 400 m maximum width and average depth of 5 to 10 m. The tidal regime is typically semidiurnal, with tidal amplitude ranging between 1 and 3 m during neap and spring tides, respectively, and tidal influence extending to ~40 km inland (Paula et al., 2006). During neap tides, tidal penetration reaches 2.5 km and water column stratification occurs. During spring tides, tidal penetration reaches 7.5 km and homogenization of the water column occurs due to water turbulence (Paula, 1998; Blanton and Andrade, 2001). The estuary has a low, seasonal and limited freshwater input (due to Santa Clara dam located 60 km upstream), with the lower section presenting a marine dominance, characterized intertidally by extensive meadows of Zostera noltii, bare sandy areas and muddy substrates with boulders and pebbles. Bordering salt-marches occur as far as 20 km upstream.

2.3. Methods

2.3.1. Experiment 1

To assess the influence of collector size on the settlement rates of C. maenas megalopae, benthic hog’s hair artificial substrates varying in surface area and perimeter:area ratio were used. Collectors (n=4) of four different sizes were deployed on two days on each of two neap tide periods during the recruitment season (8, 10 and 23, 25 May 2003). All collectors were 2.5 cm thick, and were attached to the substrate with J-shaped metal stakes. The choice of collector sizes was based on a range of dimensions feasible for routine operation and these varied between half and double the size of the recommended standard surface area of 0.2 m² for benthic hog’s hair artificial substrates (Paula et al., 2003, 2006). Dimensions, surface area and perimeter:area ratio of the four collector sizes were respectively: size I — 0.25×0.40 m, 0.1 m², 13.0; size II — 0.50×0.40 m, 0.2 m², 9.0; size III — 0.75×0.40 m, 0.3 m², 7.7; size IV — 0.5×0.80 m, 0.4 m², 6.5 (an increase of 0.1 m²).

Significantly lower settlement intensity was recorded during the first than in the second neap tide period, with means of 11.4 and 83.3 ind m⁻², respectively (t=−12.36, p<0.001). Accordingly, the first period was considered of low settlement intensity and the second period of high settlement intensity, and days inside each period were pooled together by collector size, in order to assess possible influences of settlement intensity. The effects of collector size on settlement rates during each period were evaluated by one-way analysis of variances (ANOVA), considering two settlement measures: ind collector⁻¹ and ind m⁻². In all analyses, Cochran’s tests revealed homoscedasticity of variances, and Shapiro–Wilk’s tests revealed homogeneity of residuals. A posteriori comparisons between different collector sizes were performed by tests of Tukey’s honest-significant-differences (HSD). To estimate the collector size that would have maximized settlement during each intensity period a polynomial function was fitted to the numbers of ind collector⁻¹, using Least Mean Square comparisons.

2.3.2. Experiment 2

The efficiency of a new type of artificial surface, plastic grass, was tested and compared with hog’s hair collectors, in estimating daily benthic settlement of C. maenas megalopae. Collectors of both types (n=4) were deployed attached to similar metal frames to prevent movement caused by water currents (as in Paula et al., 2006), on 20 consecutive days during the recruitment season. The new collectors were 0.40×0.32 m in surface area and made from polyethylene green grass mats, composed of vertical 1×2×25 mm blades (6.8 blades cm⁻²) (Velcoc™, ref. 8000771610455). The vertical design of the blades allows free movement of megalopae, while conferring high complexity to the structure of the collector’ surface area. Hog’s hair collectors were 0.50×0.40 m in surface area and as described previously. Megalopae settlement was measured as ind m⁻² and comparisons of daily and overall settlement on both collector types were performed by Student’s t-tests.

All collectors where conditioned prior to use by immersion in seawater for one week. Collectors were deployed randomly on the intertidal zone, lying 2 to 4 m apart from each other, at the time of the diurnal low tide and recovered after 2 tidal cycles (25 h). After recovery, collectors were soaked in freshwater for 5 min, rinsed with freshwater jets through a 500 μm sieve, and crab megalopae and juveniles were retrieved.

3. Results

3.1. Experiment 1

Settlement of C. maenas megalopae showed significant effects of collector size during both low and high intensity periods, irrespective of settlement measure (ind collector⁻¹ or ind m⁻²), being more significant during the high intensity period (Table 1). These effects were somehow different according with settlement intensity. During the high intensity period, settlement as ind collector⁻¹ increased with increasing collector’ surface
area and decreasing perimeter:area ratio (Fig. 1A). Collector sizes III and IV presented the same and significantly higher values than those of sizes I and II ($p<0.001$ and $p<0.05$, respectively). The best polynomial fit to these data was a linear function that revealed that settlement maximization was achieved with the size IV collectors (Fig. 2A, $F=28.5, p<0.001$). Conversely, settlement as ind m$^{-2}$ showed a general decrease with increasing collector surface area and decreasing perimeter:area ratio (Fig. 1B). However, and despite size I collectors had caught significantly more megalopae than those of other sizes ($p<0.05$, $p<0.001$ and $p<0.001$ for collector size II, III and IV respectively), none of these showed significant differences between each other.

In the low intensity period, settlement as ind collector$^{-1}$ was significantly lower on size I than on size II and III collectors ($p<0.01$ and $p<0.05$, respectively), not being different between any other collector sizes (Fig. 1A). The best polynomial fit to these data was a quadratic function revealing a collector size between sizes II and III (0.27 m$^2$ in surface area) as the one that would have maximized settlement (Fig. 2B, $F=18.1, p<0.001$). Settlement as ind m$^{-2}$ decreased significantly with increasing surface area (decreasing perimeter:area ratio) between size II to size IV collectors ($p<0.05$), not showing significant differences between any other collector sizes (Fig. 1B).

### 3.2. Experiment 2

Settlement of *C. maenas* megalopae on plastic grass collectors showed the same pattern that on those of hog’s hair throughout all sampling: high settlement

<table>
<thead>
<tr>
<th>Settlement intensity</th>
<th>Measure</th>
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<th>MS</th>
<th>$F$</th>
<th>$p$</th>
</tr>
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<tbody>
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<td>Low</td>
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<td>1.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>ind collector$^{-1}$</td>
<td>Size</td>
<td>3</td>
<td>180.06</td>
<td>6</td>
<td>&lt;0.05</td>
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<td></td>
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<td>Error</td>
<td>28</td>
<td>31.38</td>
<td></td>
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<tr>
<td></td>
<td>ind m$^{-2}$</td>
<td>Size</td>
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<td></td>
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<td>Error</td>
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</table>

Fig. 1. *Carcinus maenas*. Average settlement of megalopae on the different size’ collectors at low and high settlement intensity periods (A) as ind collector$^{-1}$ and (B) as ind m$^{-2}$. Arrows represent increasing collector’ surface area and decreasing perimeter:area ratio with increasing collector size. Error bars: ±SE.

Fig. 2. *Carcinus maenas*. Fitness of a polynomial model to megalopae settlement, as ind collector$^{-1}$, on the different size’ collectors at (A) high and (B) low settlement intensity.
intensity from the 3rd to the 6th and from the 13th to the 18th days, and low values outside these periods (Fig. 3). Maximum settlement occurred in the same days for both collector types, being higher on those of hog’s hair on the 1st period, and on those of plastic grass on the 2nd period (Fig. 3). Overall mean daily settlement on plastic grass collectors was not significantly different from that on those of hog’s hair (9.3 ±5.3 and 11.0± 4.6 ind m\(^{-2}\), respectively; \(t = -0.888, p=0.376\)). Student’s \(t\) tests did not reveal any significant difference on settlement intensity between both collector types for any of the sampled days.

Laboratory processing of plastic grass collectors was considerably easier and less time consuming than that of hog’s hair collectors. Less soaking time and use of freshwater jets rinsing were necessary to remove megalopae. Average processing time of a plastic grass collector was half of that of a hog’s hair one, i.e. 6–7 min. Second and third rinsing did not retrieve more megalopae from plastic grass collectors, while it sometimes did (1–3 megalopae) from hog’s hair ones. Furthermore, immediate observation of retrieved megalopae, revealed that less damaged and higher rates of survived animals were obtained from plastic grass than from hog’ hair collectors.

4. Discussion

4.1. Influence of collector patchiness

According with Phillips et al. (2001) collector testing is only practical at times of high settlement intensity, since at other times results may be unreliable due to high variability in catch numbers. In this study, however, variability in settlement numbers was similar at high and low intensities, being also similar to those of other studies conducted recently in the same area (Paula et al., 2006; Queiroga et al., 2006), supporting the meaningfulness of results. However, caution must be taken when analyzing the present results, in the sense that it was not possible to dissociate the effect of collector’ surface area from collector’ perimeter:area ratio. In order to dissociate between these two effects, collectors varying in surface area but preserving perimeter:area ratio, and vice versa, should be investigated.

Abundances of \(C. maenas\) megalopae on hog’s hair collectors were significantly influenced by collector patchiness at scales between 0.1 and 0.4 m\(^2\) in surface area, irrespective of settlement intensity and settlement measure (ind collector\(^{-1}\), ind m\(^{-2}\)). However, responses were different with different settlement intensities and measures. At both high and low intensity settlement as ind collector\(^{-1}\) generally increased with increasing surface area (decreasing perimeter:area ratio) between size I and III collectors, stabilizing and decreasing on those of size IV at high and low intensity, respectively. These results seem to indicate that a collector size proximate to that of size III (0.3 m\(^2\) in surface area) would have maximized settlement numbers irrespective of settlement intensity. This is reinforced by fitness of polynomial functions to these data that revealed collector sizes of 0.4 and 0.27 m\(^2\) in surface area as those maximizing settlement at high and low settlement intensity, respectively.

Considering settlement as ind m\(^{-2}\), settlement generally tended to decrease with increasing collector’ surface area (decreasing perimeter:area ratio), with exception to size I collectors at low settlement intensity. However, this tendency differed with settlement intensity: settlement responded to lower scales of habitat patchiness at high than at low intensity, when only the highest and lowest values were significantly different. Different responses to habitat patchiness according to organism density, and also body size, have been previously reported in recruitment of grass shrimps \(Palaeomonetes\) spp. and blue crab \(C. sapidus\) (Eggleston et al., 1998). Nevertheless, the results generally agree with those of previous studies on several marine organisms that found higher settlement and recruitment densities on smaller natural patches when compared to larger patches, with higher and lower perimeter:area ratios, respectively (e.g. Keough, 1984; McNeill and Fairweather, 1993; Eggleston et al., 1998; Bologna and Heck, 2002; Hovel and Lipcius, 2002). Higher perimeter:area ratios usually enhance the probability of encounter rates by organisms, since more patch edge is exposed. This gains special relevance for organisms that possess a swimming capacity, as it is the case of \(C. maenas\) megalopae that actively search for suitable settlement substrates, usually
with a certain degree of complexity (Hedvall et al., 1998; Moksnes, 2002). In addition to what has hitherto been discussed, it is generally accepted that a more or less strong indissociable biological component is also involved in recruitment and settlement responses of marine organisms to habitat patchiness, further suggesting specificity in those responses (Eggleston et al., 1998; Jenkins et al., 2002; e.g. Atilla et al., 2005).

4.2. Influence of collector type

Plastic grass surfaces efficiently estimated benthic settlement of *C. maenas* megalopae when compared to that on hog’s hair collectors, presenting the same pattern, similar settlement densities and variability. As pointed out by Phillips et al. (2005), hog’s hair filter material is becoming difficult to access, and an appropriate substitute should allow comparisons with results from previous studies. The good homology between daily catches on the two types of collectors suggests that a conversion factor could be achieved with a proper sampling design. Besides a good settlement estimation, laboratory processing including the condition of retrieved animals, handling procedures and logistic requirements, overall weight and size, strength and durability, need for replacement, cost and effort of construction/acquisition are also important characteristics that may invalidate the use of a certain collector type. Laboratory processing of plastic grass collectors revealed to be easier and less time consuming (∼half the time) than that of hog’s hair ones. Megalopae retrieval was considerably more rapid and effective, resulting in less mechanical stress suffered by megalopae and higher percentage of live specimens being retrieved. Plastic grass collectors are entirely made of light flexible polyethylene, resulting in easy to handle and transportable structures resistant to wave action and sea exposure. This provides them with extreme durability and stability of surface area complexity when compared to those of hog’s hair, diminishing considerably the necessity for replacement. In fact, there is a continuous erosion of surface area complexity in hog’s hair collectors, since fibers are easily lost with wave action. Polyethylene green grass mats are widely available, being easier and cheaper to obtain than hog’s hair filters. We thus consider that plastic grass collectors may constitute a good alternative to those of hog’s hair.

In summary, this research indicates that settlement of *C. maenas* megalopae responded to patchiness of benthic hog’s hair collectors (0.1 to 0.4 m² in surface area), as a function of either the collectors’ surface area or perimeter:area ratio, irrespective of settlement intensity. However this response was contrasting with distinct settlement measures. Megalopae numbers would have been maximized with collector’ surface areas around 0.3 and 0.4 m² at low and high settlement intensities, respectively. The choice of hog’s hair collector’ size should thus be cautious and made according with the purpose of the study and the expected settlement intensity. Plastic grass collectors may constitute a good alternative to those of hog’s hair.

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