

# Settlement and post-larvae behaviour of *Mytilus galloprovincialis*: field and laboratory experiments

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**ABSTRACT:** Field sampling carried out in the Ría de Vigo (NW Spain) from 1991 to 1993 showed that *Mytilus galloprovincialis* settle directly from the plankton onto substrates exposed to various environmental conditions: exposed rocky shore; protected rocky shore; exposed, raft mussel-culture area; and protected, raft mussel-culture area. For direct settlement, competent larvae may use a wide variety of substrates: filamentous nylon ropes; the byssus and intricately arranged material in the bottom of mussel beds; filamentous, thallus and membranous algae; and rugosities on adult mussel shells. The peak of settlement occurred from spring to early autumn and differences in the settlement abundance among localities were influenced by currents. After this peak, the settlement of larger post-larvae continued, associated with the increase in storms during autumn that detach them from their original substrates. This dispersion phase allows for the possibility of colonising, or recruiting on other areas, even during the post-spawning season when the presence of small post-larvae is at its minimum. Laboratory experiments carried out with post-larval stages from 0.250 to 2.000 mm showed that under static water conditions they crawl and form clumps, but do not search for a specific substrate. If they are not disturbed, they may remain in their original place of settlement. Conversely, under moving water conditions they attach to a wide variety of substrates, particularly to byssal filaments and thalli of red algae *Ceramium rubrum*. The contact and attachment to substrates is carried out with a long mucous thread that also aids in forming clumps. The use of this mucus to settle results in a 'preference' for natural filamentous substrates but also in settlement on rugous hard surfaces. An alternative hypothesis to the primary and secondary settlement pattern previously described in the literature for *Mytilus edulis* is suggested.

**KEY WORDS:** Mussels · *Mytilus galloprovincialis* · Post-larvae · Behaviour · Settlement · Dispersion · Mucous threads

## INTRODUCTION

Bayne (1964) postulated that the competent larvae of *Mytilus edulis* smaller than 375 µm settle on filamentous substrates away from adult mussel beds. Following this primary settlement the post-larvae undergo a migratory phase, during which they are transported by water currents. During this latter phase they may more than once attach and detach themselves. On reaching a size between 900 and 1500 µm the post-larvae settle on adult mussel beds. Bayne named this settlement pattern 'pri-

mary and secondary settlement', suggesting that in *M. edulis* this is a mechanism that may reduce competition between post-larvae and adult mussels. Seed (1969) and Dare (1976) supported this hypothesis. The smallest post-larvae that they recorded among the adult mussel beds were 1000 to 2000 µm and 550 to 700 µm respectively. Dare (1976) and Dare et al. (1983) characterised mussels from 230 to 400 µm as primary settlers and those > 500 µm as secondary settlers.

Subsequent field studies have caused researchers to question this settlement pattern for *Mytilus edulis* in other regions, or in different mussel species, and have found that there is a wide variety of substrates upon which *Mytilus* species can settle. Suchanek (1978)

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described the settlement of *M. edulis* as occurring either sparsely on filamentous substrates or other intricately arranged material to acquire protection from predation, or massively on newly available rocky substrates. Petersen (1984) found that *M. edulis* settle directly on conspecific adults, several species of algae and occasionally in low densities on bare rock with scattered barnacles. MacGrath et al. (1988) and King et al. (1990) described how larvae of *M. edulis* settled directly on adult mussel beds without an initial primary settlement phase on filamentous algae. Andreu (1976) pointed out that for *M. galloprovincialis* the settlement pattern could be simpler than that postulated by Bayne. Cáceres-Martínez et al. (1993) found that *M. galloprovincialis* settled directly on adult mussel beds but also described an immigration of mussels that had previously settled on other substrates.

Laboratory studies have shown that *Mytilus edulis* can settle directly on conspecific adults (Petersen 1984). Fell & Balsamo (1985) recorded direct settlement of *M. edulis* on *Mercenaria mercenaria* clutches. Eyster & Pechenik (1987) found that *M. edulis* settled directly on adult mussel byssal threads.

The existence of different settlement modes in the genus *Mytilus* has been associated with genotypic differences between mussel populations and species, and also with environmental conditions (MacGrath et al. 1988, Cáceres-Martínez et al. 1993). However direct settlement has not been studied per se to contrast it

with the primary and secondary settlement pattern postulated by Bayne (1964). Until now there have been no intraspecific studies that compare the settlement pattern under different environmental conditions nor studies on the migration capacity of post-larvae in the laboratory.

The aim of the present work was to determine settlement and post-settlement behaviour of *Mytilus galloprovincialis* under different environmental conditions. We also wanted to determine the ability of post-larvae from 0.250 to 2.000 mm in length to select filamentous substrates for their settlement, and to see if post-larvae from 0.250 to 2.000 mm avoid or search adult conspecifics to settle.

## MATERIALS AND METHODS

**Field study.** Sampling was carried out in the Ría de Vigo (NW Spain) from March 1991 to April 1993. Four localities were chosen: Cabo Home, an exposed rocky shore; San Adrián, a protected shore; Liméns, an exposed raft mussel-culture area; and San Adrián, a protected raft mussel-culture area (Fig. 1). Filamentous nylon ropes of 35 cm length and 2 cm diameter (225 cm<sup>2</sup>) were used as spat collectors. A series of 12 collectors was attached to a stainless steel bar and hung at 1 m depth from each of the 2 rafts, along the side facing away from the shore. Each month, 1 collector was removed in order to produce a cumulative settlement record. Another collector was placed in the same structure and it was replaced each month as a monthly settlement record.

On the exposed rocky shore, 1 collector was placed on a stainless steel structure and cemented on the lower shore among the mussel bed, just above Low Water Spring Tide. On the unexposed shore, the structure with the collector was attached on the wall of a concrete pier, at the same level as those on the exposed rocky shore. These collectors were replaced monthly to obtain a settlement record. Simultaneously, standard areas of 225 cm<sup>2</sup>, equivalent to the rope collector surface area, were scraped directly from adult beds in both localities. Several species of algae were collected during the summer to determine the presence or absence of mussel post-larvae.

**Sample handling.** Collectors and samples obtained from the scraping of rocky areas were preserved in a solution of 70% ethanol. Subsequently the samples were

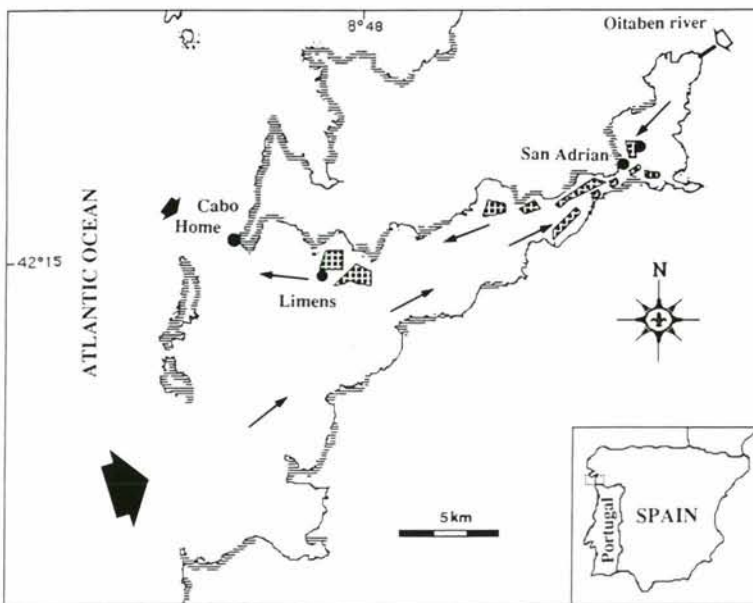


Fig. 1. Map showing the study area, indicating the locations of mussel raft-culture areas (▣) and natural mussel beds (■). Thin arrows indicate the flow inside the Ría, and wide arrows show the arrival of oceanic water (Prego et al. 1990, Prego & Fraga 1992)

immersed in a 10% solution of sodium hypochlorite (NaClO). Post-larvae were separated using a series of sieves (from 0.9 to 4.0 mm), dried, measured and counted (Cáceres-Martínez et al. 1993).

Algae were identified and the number of mussels per algae was recorded as no.  $g^{-1}$  of dried thalli (constant dry weight at 70°C for 24 h). Mussel post-larvae identification was done following the descriptions of Le Pennec (1978). Post-larvae with shell lengths from 0.250 to 0.470 mm were considered as direct settlers in accordance with the minimum and maximum values recorded for competent pediveliger larvae of *Mytilus edulis* (Rees 1954, Bayne 1964, Widdows 1991).

**Laboratory experiments. Specimens and experimental conditions:** Adults and post-larvae of *Mytilus galloprovincialis* were collected during summer 1993 in Cabo Home by scrapings from the adult mussel beds. After separation in a series of sieves using running sea water, post-larvae were grouped under a stereoscopic microscope (Nikon, SMZ-10) into 3 shell length groups (Table 1). Each group was maintained separately in aerated 2 l glass jars in a controlled temperature room ( $16 \pm 1^\circ C$ ). A 12 h light : 12 h dark cycle was maintained. The water was changed every 2 d using filtered sea water (3  $\mu m$  and 1  $\mu m$  cartridge filters). Salinity was maintained at 35 ppt. Post-larvae stocks were fed every 48 h with 50 ml of *Isochrysis galbana* ( $6 \times 10^5$  cells  $ml^{-1}$ ). The adults were held in 60 l aquarium with running sea water and fed following the same regime.

**Mucous threads:** Preliminary observations showed that *Mytilus galloprovincialis* may produce a long mucous thread which can be used to attach to substrates. To detect these hyaline mucous threads, activated charcoal powder was placed in Petri dishes with post-larvae of the different size-groups. The charcoal adhered to the mucus, making it visible, and the threads were photographed under a stereoscopic microscope (Nikon, SMZ-10, equipped with a Nikon FX-35DX camera).

**Substrates:** Cleaned thalli of *Ceramium rubrum*, byssal filaments, nylon net, and synthetic fibrous material (Scotchbrite™) were used as substrate. At the end of the experiments, all substrates were dried at 70°C for 24 h. Their surfaces were calculated by subsampling different sizes from each substrate, and by using semi-automatic image analysis (software 'VIDS V' for IBM PS/2). Afterwards, the subsamples were dried (70°C for 24 h) and weighed. A least squares regression equation between total area and dry weight of each kind of substrate was calculated. The following equations were determined: (area in  $mm^2$ , wt in mg) byssal filaments area =  $-0.865 + 0.208(wt)$ ,  $r = 0.90$ ,  $n = 18$ ; *Ceramium rubrum* area =  $-0.727 + 0.201(wt)$ ,  $r = 0.96$ ,  $n = 29$ ; fibre area =  $0.443 + 0.227(wt)$ ,  $r = 0.94$ ,

Table 1. *Mytilus galloprovincialis*. Shell length groups used for laboratory experiments showing the minimum and maximum values, mean and standard deviation

	Shell length (mm) groups		
	Group 1	Group 2	Group 3
Minimum	0.250	0.550	0.900
Maximum	0.375	0.975	1.975
Mean	0.333	0.805	1.348
SD	0.022	0.092	0.217
n	356	356	359

$n = 15$ ; nylon net area =  $-1.667 + 0.208(wt)$ ,  $r = 93$ ,  $n = 17$ . The total surface was determined for each substrate tested by using their dry weight and the equations described above. Results are expressed as percentage of mussel settled on each substrate.

**Substrate attraction — independent substrate tests:** To test substrate attraction under static and moving water conditions, 25 post-larvae from each length group were placed in separate plastic Petri dishes (Bibby Steriling, Ltd) of 87 mm diameter, filled with 55 ml of filtered sea water containing one of each substrate only. Three replicates were assayed for each experimental condition. To produce movement, Petri dishes were put on a Junior orbit shaker at 50 rpm. After 24 h, the number of post-larvae attached to the substrate was counted.

**Substrate preferences:** To test if post-larvae of different shell length groups may select a specific substrate and change from one substrate to another for better placement, 25 post-larvae from each shell length group were used as described above. In this experiment the 4 substrates were placed together. Under static water conditions the substrates were placed equidistant from each other and from the mussel post-larvae (in the centre of the Petri dish), and under moving water conditions substrates moved freely. The number of post-larvae attached on the substrates were counted at 12, 18 and 24 h. Three replicates were assayed for each experimental condition.

**Post-larvae settlement on living adult clumps:** To test if post-larvae of different shell length groups prefer to settle near or away from adult conspecifics, 8 adult mussels were washed in running sea water and carefully examined for the presence of post-larvae, then a clump of 4 adult mussels was allowed to attach to one of the sides of the aquarium ( $15 \times 30 \times 10$  cm). Once the clumps were formed, 125 post-larvae of each length group were placed on the opposite side. The experiments were carried out under moving water and static water conditions. For moving conditions, one aquarium was put on an orbital incubator (25 cycles  $min^{-1}$ ). Mussels and aquarium were examined 24 h

later for post-larvae and the place where they were found (byssal filaments, adult shell, barnacles on the shell, aquarium bottom) was recorded.

Aluminium foil was made to conform to the shape of mussel shells and their attached barnacles. The foil was carefully cut, dried (70 °C for 24 h) and weighed. Pieces of aluminium foil from 1 to 10 cm<sup>2</sup> were weighed, obtaining a least squares regression equation [aluminium area (cm<sup>2</sup>) = -52.248 + 29.742(wt, mg),  $r = 0.97$ ,  $n = 5$ ]. Then, the surface of mussel shells and barnacles was calculated by using the dry weight of the aluminium molds obtained.

**Statistical analysis.** A Kruskal-Wallis test was used to compare the percentages of post-larvae <0.470 mm from the total number of mussels with respect to the localities. This analysis was also used to compare the attachment of post-larvae groups on different substrates; differences were compared by nonparametric Tukey-type multiple comparisons test (Zar 1984). Wilcoxon's signed-ranks test was used to determine differences between moving water and static water conditions (Eyster & Pechenik 1987).

## RESULTS

### Field study

**Monthly sampling.** The monthly record indicated that settlement occurred throughout the study period (Fig. 2) and peaked between spring and early autumn. Settlement was more abundant in 1991 than 1992. The presence of post-larvae <0.470 mm indicated a direct settlement on collectors in all localities. Their number was larger from spring to mid-summer than during early autumn. The settlement was more abundant in the external than in the inner localities (Fig. 2) and more abundant in rocky shore areas than on rafts (Fig. 2). The Kruskal-Wallis test showed that there were no significant differences in the percentage of post-larvae <0.470 mm among localities ( $H = 57.079$ ,  $p > 0.05$ ). The largest mussels recorded were under 5.0 mm total shell length.

**Cumulative settlement.** Analysis of the scraping samples from the rocky areas showed that post-larvae <0.470 mm were found mainly in the bottom of the

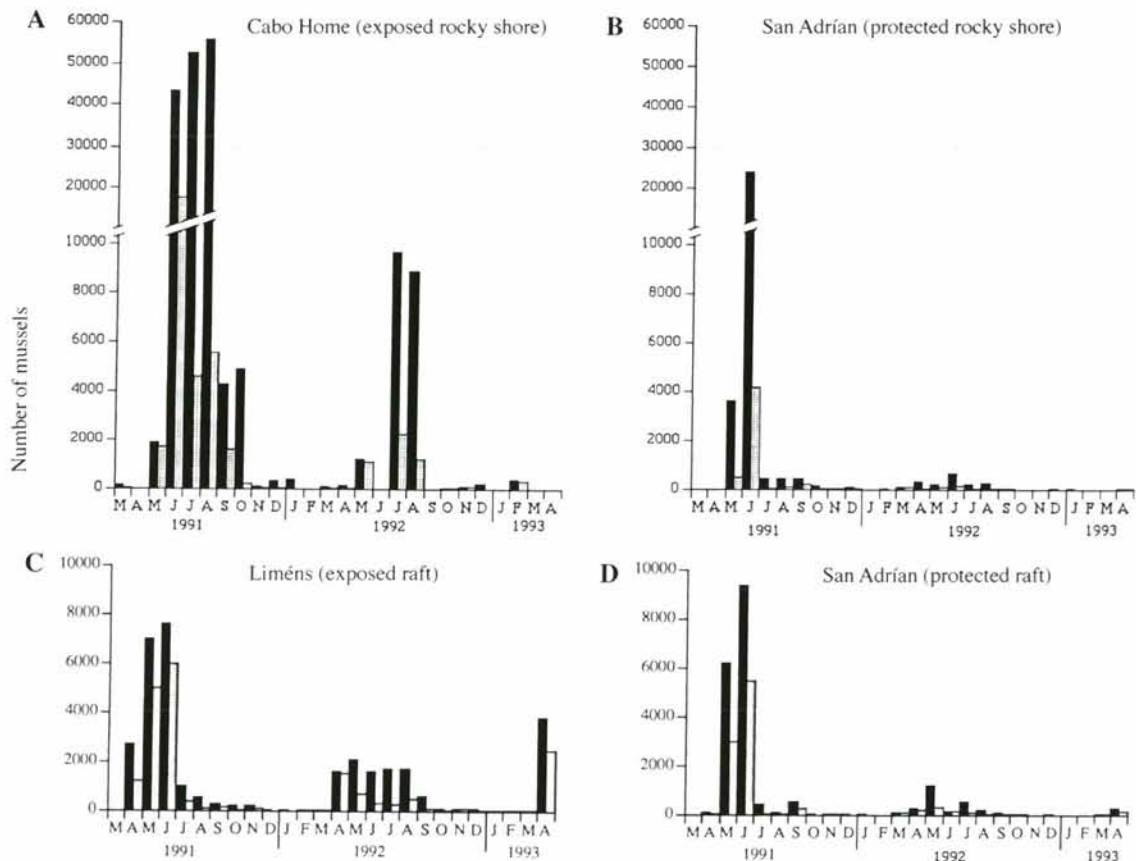


Fig. 2. *Mytilus galloprovincialis*. Total number of mussels (■) and total number of post-larvae <0.470 mm (▨) attached on inter-sampling rope collectors placed on (A) the exposed rocky shore of Cabo Home, (B) protected shore of San Adrián, (C) exposed raft of Liméns and (D) protected raft of San Adrián, Ría de Vigo, Spain, during 1991 to 1993

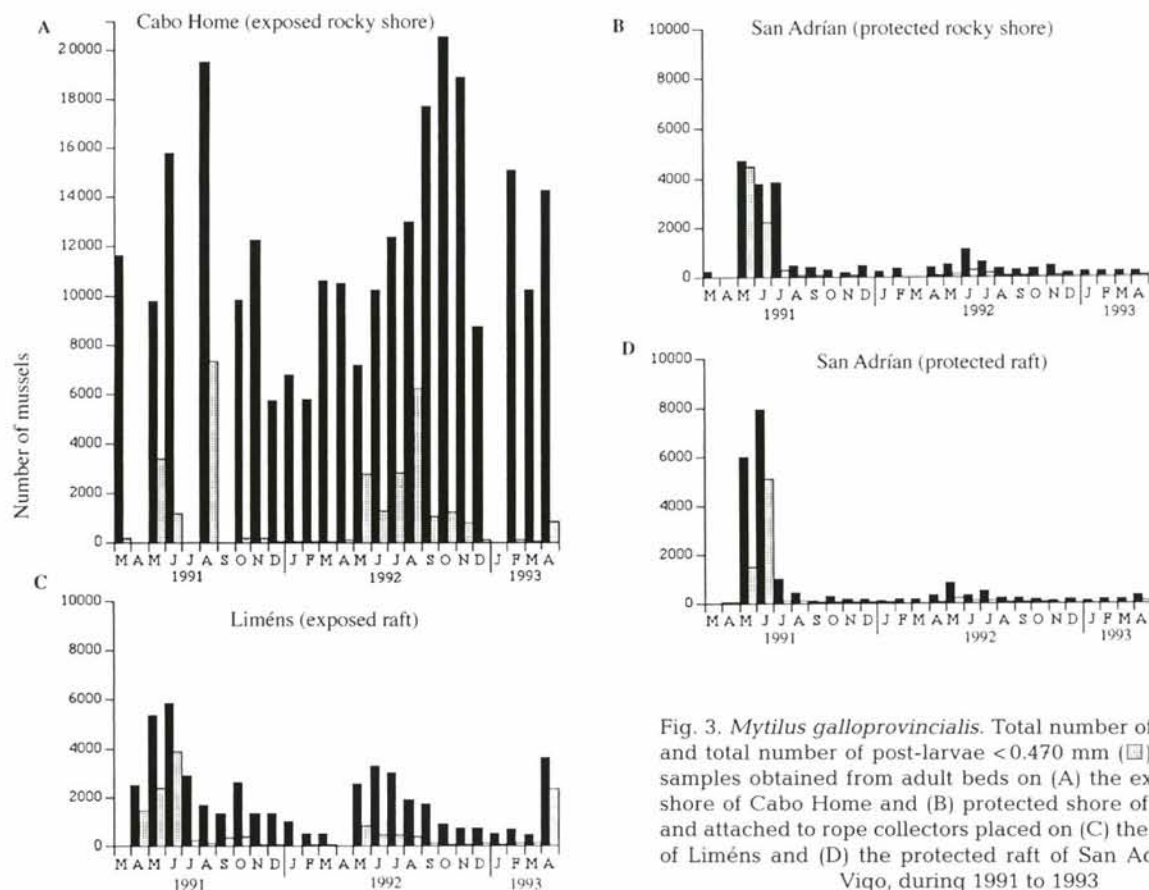


Fig. 3. *Mytilus galloprovincialis*. Total number of mussels (■) and total number of post-larvae <0.470 mm (□) recorded in samples obtained from adult beds on (A) the exposed rocky shore of Cabo Home and (B) protected shore of San Adrián, and attached to rope collectors placed on (C) the exposed raft of Liméns and (D) the protected raft of San Adrián, Ria de Vigo, during 1991 to 1993

sample among the byssus and intricately arranged material, and also on the rugosities and encrustations of the adult shells. As was the case in the monthly settlement record, the number of mussels was greater in the external than in the inner localities (Fig. 3), and in rocky shore areas than on rafts (Fig. 3). Presence of post-larvae <0.470 mm was detected in all localities, indicating direct settlement on adult conspecifics. In all study areas, with the exception of Cabo Home, post-larvae were more abundant in spring and mid-summer than in early autumn, showing a similar pattern as that seen in the monthly settlement record. The settlement peaked between spring and early autumn. In Cabo Home post-larvae <0.470 mm were abundant during spring and summer but also in late autumn, and the total number of mussels recorded showed a periodic pattern throughout the study period. However, taking as reference the number of post-larvae <0.470 mm, the settlement peak detected was similar to those obtained in the other localities (Fig. 3A). The Kruskal-Wallis test showed that there were no significant differences in the percentage of post-larvae <0.470 mm among localities ( $H = 50.181$ ,  $p > 0.05$ ).

The percentage of post-larvae <0.470 mm in the monthly settlement record was higher than in the

cumulative settlement record and this difference was significant (Kruskal-Wallis test,  $H = 118.8$ ,  $p < 0.01$ ).

**Algae.** Post-larvae <0.470 mm were distributed in all algae structures studied without any special trend. However the larger mussels (>0.470 mm) formed clumps on the rhizome or basal structures of algae.

In Table 2 the total number of mussels and percentage contribution of small post-larvae (<0.470 mm) on different algae species is shown. Mussel post-larvae were found on filamentous algae (*Ceramium rubrum* and *C. ciliatum*), thallus algae (*Chondrus crispus* and *Laurencia pinnatifida*), and membranous algae (*Loxmentaria articulata* and *Plocamium cartilagineum*).

### Laboratory experiments

**Substrate attraction.** In Fig. 4, the percentage of post-larvae attached to different substrates is shown. The moving water conditions greatly favoured the attachment of all post-larvae groups on different substrates ( $p < 0.01$ , Wilcoxon's signed ranks test, 4 paired trials, total of 600 post-larvae used). The statistical Kruskal-Wallis test did not show significant differences

Table 2. *Mytilus galloprovincialis*. Total number of mussels and percentage contribution of post-larvae <0.470 mm found attached per g of dried thalli of different algae species from the 4 localities studied. Values are the mean obtained for each species during summer 1992. n: no. of algae examined

Substratum	n	Total no. of mussels	<0.470 mm (%)
<b>Algae</b>			
<i>Laurencia pinnatifida</i>	8	215	30.5
<i>Chondrus crispus</i>	5	106	45.0
<i>Plocamium coccineum</i>	4	765	12.9
<i>Ceramium rubrum</i>	8	198	38.3
<i>Ceramium ciliatum</i>	5	45	10.0
<i>Lomentaria articulata</i>	5	1911	11.6
<i>Enteromorpha intestinalis</i>	4	34	43.7
<i>Caulacanthus ustulatus</i>	3	411	4.9
<i>Sargassum muticum</i>	4	4	20.4
<b>Hydroids</b>			
<i>Plumaria setacea</i>	3	207	42.1

( $p > 0.05$ ) among the attraction of different substrates: Group 1,  $H = 12.99$ ; Group 2,  $H = 8.87$ ; Group 3,  $H = 12.25$ . Nor were there significant differences among the 3 groups for the same substrates: byssal filaments,  $H = 2.00$ ; *Ceramium rubrum*,  $H = 1.78$ ; fibre,  $H = 6.67$ ; nylon net,  $H = 5.33$ . However Fig. 4 suggests some attraction of post-larvae groups to byssal filaments and *C. rubrum*.

**Substrate preferences.** The results obtained for the substrate of preference for settlement under static and moving water conditions are shown in Fig. 5. Settlement on the different substrates was higher under

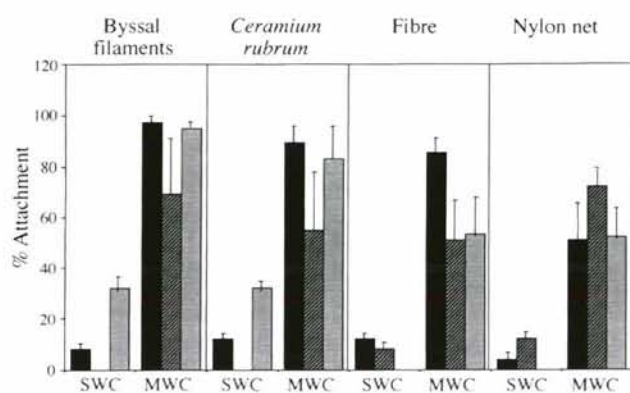


Fig. 4. *Mytilus galloprovincialis*. Percent ( $\pm$  SD) of post-larvae from Groups 1 (■), 2 (▨) and 3 (▩), attached per 1 cm<sup>2</sup> of different substrates, tested separately, in experiments carried out under static water conditions (SWC) and moving water conditions (MWC). In all trials the MWC favoured the attachment of all post-larvae groups ( $p < 0.01$ , Wilcoxon signed ranks test). Post-larvae did not show preference for any of the substrates tested in MWC and SWC ( $p > 0.05$ , Kruskal-Wallis test)

moving water conditions than under static water conditions ( $p < 0.001$ , Wilcoxon's signed ranks test, 5 paired trials, total of 750 post-larvae used).

Under moving water conditions, the most favourable substrates for the attachment of post-larvae from the 3 groups were mussel byssal filaments and *Ceramium rubrum* ( $H = 43.3$ ,  $p < 0.001$ , Kruskal-Wallis test followed by Tukey-type multiple comparisons). Some individuals changed from one substrate to another over time without any special trend (Fig. 5). The 3 groups showed similar percentages of attachment for

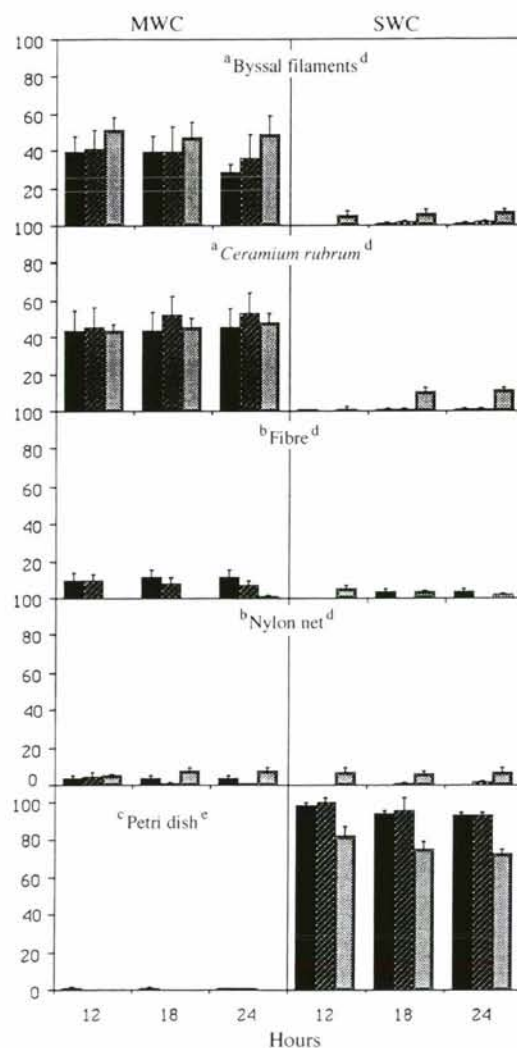


Fig. 5. *Mytilus galloprovincialis*. Percent ( $\pm$  SD) of post-larvae from Groups 1 (■), 2 (▨) and 3 (▩), attached per 1 cm<sup>2</sup> of different substrates in experiments carried out under static water conditions (SWC) and moving water conditions (MWC) recording each 6 h. In all trials the MWC favoured the attachment of all post-larvae groups ( $p < 0.01$ , Wilcoxon's signed ranks test). Dissimilar superscript letters to the left (MWC) and to the right (SWC) of the substrate type indicate a significant difference from each other ( $p < 0.05$ , Kruskal-Wallis test, followed by Tukey-type procedure)

the different substrates tested ( $H = 22.30$ ,  $p > 0.05$ , Kruskal-Wallis test).

Under static water conditions post-larvae preferred to spread by crawling on the bottom of the Petri dish and they did not show any preference for any of the substrates tested ( $H = 67.2$ ,  $p < 0.001$ , Kruskal-Wallis test followed by Tukey-type multiple comparisons). As under moving water conditions, the 3 groups showed similar percentages of attachment for the different substrates tested ( $H = 27.25$ ,  $p > 0.05$ , Kruskal-Wallis test). However, Group 3 appeared the most active (Fig. 5, SWC). Some individuals climbed the Petri dish walls and others formed clumps. There were changes from one substrate to another without any special trend. The majority of post-larvae remained in their original position throughout the experiment if they were not disturbed. When post-larvae were disturbed with a needle for counting, they crawled away.

**Mucous threads.** Secretion of long mucous threads was detected in the 3 groups. On many occasions these mucous threads attached to the needle during the manipulation of post-larvae, making their separation difficult. On some occasions the mucous threads were attached to the bottom of the Petri dish, like the mucus of a snail, as post-larvae crawled away (Fig. 6A). If, during crawling, post-larvae touched each other, they sometimes remained together, forming clumps from 2 to 23 mussels with the mucus surrounding them (Fig. 6B).

Under moving water conditions these mucous threads were used to make contact and attach to the

substrate (Fig. 6C, D). Some post-larvae were found hanging from the substrate with the mucous threads, individually or as clumps with up to 20 mussels.

**Post-larvae settlement on live adult clumps.** The number of post-larvae of each group attached to live adult mussel clumps under moving and static water conditions is shown in Table 3. The number of attached post-larvae on different substrates was significantly greater under moving than under static water conditions ( $p < 0.001$ , Wilcoxon's signed ranks test, 2 paired trials, total of 750 post-larvae used). Under moving water conditions, the 3 groups showed similar percentages of attachment to different substrates ( $H = 2.0$ ,  $p > 0.05$ , Kruskal-Wallis test). However, all post-larvae groups showed preference for the byssal filaments ( $H = 9.7$ ,  $p < 0.001$ , Kruskal-Wallis test followed by Tukey-type multiple comparisons). Under static water conditions, the 3 groups were attached to different substrates at similar percentages ( $H = 2.4$ ,  $p > 0.05$ , Kruskal-Wallis test), and all post-larvae showed a preference for remaining on the bottom of the aquarium ( $H = 8.9$ ,  $p < 0.001$ , Kruskal-Wallis test and Tukey-type multiple comparisons).

## DISCUSSION

The occurrence of settlement throughout the year, with an extended peak from spring to early autumn, was consistent with the presence of spawning mussels in the area throughout the year, with a peak from spring to summer (unpubl. data). It is also in accordance with the estimated duration of the planktonic life of *Mytilus edulis* (Widdows 1991).

The presence of post-larvae  $< 0.470$  mm on filamentous collectors, among adult conspecifics and on different species of algae, independent of the environmental conditions, indicated a direct settlement on this variety of substrates. On the other hand, the presence of post-larvae  $> 0.470$  mm suggest the arrival of mussels previously settled on other substrates or the growth of these direct settlers on collectors and substrates sampled during the study (King et al. 1990, Cáceres-Martínez et al. 1993).

The low number of post-larvae  $< 0.470$  mm from late summer to early autumn suggest a reduction of competent post-larvae in the plankton. However since the arrival of post-larvae  $> 0.470$  mm continued, this indicated that the dispersion of post-larvae continued, possibly favoured by the greater number of storms during autumn. At this time many algae and other sessile organisms are detached from the bottom and thrown on to littoral areas.

The importance of the currents and waves for dispersion of larvae and post-larvae, widely supported by

Table 3. *Mytilus galloprovincialis*. Number of post-larvae from different groups found attached to different substrates (per 10 cm<sup>2</sup>) during the experiment of post-larvae settlement on adult clumps under moving water conditions (MWC) and static water conditions (SWC). No difference in preferences were found between post-larvae groups for substrates under both MWC and SWC ( $p > 0.05$ , Kruskal-Wallis test). However, in MWC, all groups preferred to attach to byssal filaments and in SWC they preferred to remain on the bottom of the aquarium ( $p < 0.001$ , Kruskal-Wallis test followed by Tukey-type procedure). \*\*\* $p < 0.001$ .

Substrate	Group 1	Group 2	Group 3	Total
MWC				
Byssal filaments	12.25	10.75	12.00	35.00***
Mussel shell	1.64	1.50	2.40	5.54
Balanids	1.20	1.20	1.99	4.39
Aquarium	0.80	0.90	0.66	2.36
SWC				
Byssal filaments	0.25	0.38	0.20	0.83
Mussel shell	0.14	0.00	0.00	0.14
Balanids	0.00	0.26	0.26	0.52
Aquarium	2.64	2.67	2.64	7.9***

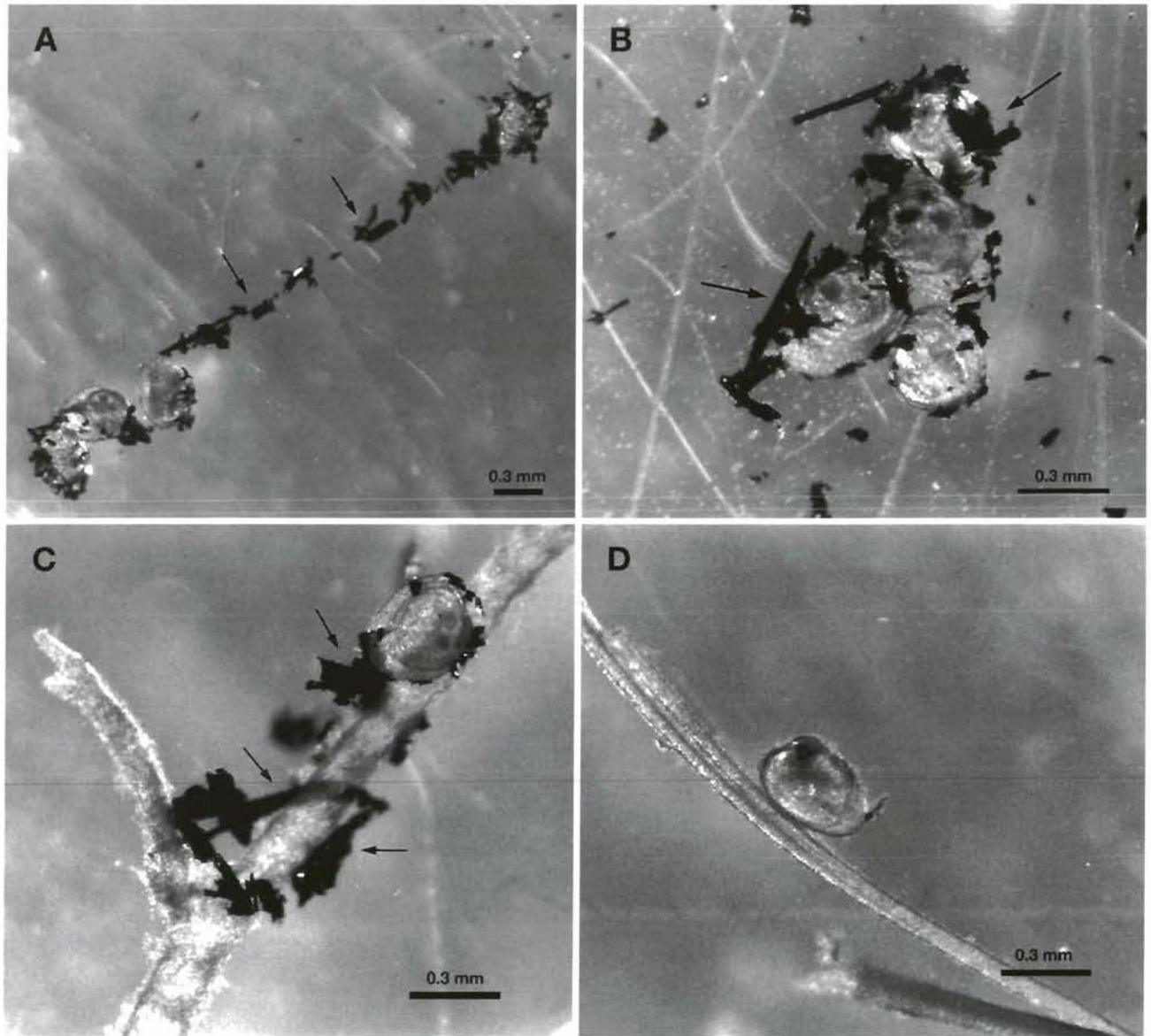


Fig. 6. *Mytilus galloprovincialis*. Examples of the use of mucous threads. Activated charcoal powder was added to the water in Petri dishes to allow mucous threads to be seen. Activated charcoal adhering to the mucus (indicated by arrows) of (A) post-larvae with their mucous thread attached to the bottom of the Petri dish, (B) a clump of post-larvae, with the mucus around them, in the bottom of the Petri dish, (C) post-larvae attached to *Ceramium rubrum* with their mucus surrounding the thalli. (D) Post-larvae attached on byssal filament. (In the absence of activated charcoal powder it is not possible to detect mucous thread)

several studies (Verwey 1952, Baggerman 1953, Wood & Hargis 1971, Wolf 1973, Seed & Suchanek 1992), could also explain the gradient in the total number of mussels detected, from the outer to the inner area and from rocky shores to floating rafts. In Ría de Vigo there is a natural outflow along the north shore due to the water discharge of Oitaben River ( $14 \text{ m}^3 \text{ s}^{-1}$ ) and by the major inflow of oceanic water along the south shore. Water flows throughout the Ría and returns to the Atlantic Ocean along the north coast (Prego et al. 1990,

Prego & Fraga 1992) (see Fig. 1). This flow can carry the larvae and post-larvae produced inside of the Ría to its external areas (Fig. 1) and the waves and tides could favour the arrival of larvae and post-larvae to the shore.

The previous existence of mussels in the samples scraped from natural beds of the exposed rocky shore made the detection of settlement peaks difficult and suggests that they may occur several times during the year. However, taking into account the number of post-



larvae <0.470 mm, the settlement peaks detected were relatively consistent with those which were seen for the other localities. Peaks in the total population reflect a very changeable structure, possibly favoured by the strong wave exposition.

The higher percentage of small post-larvae <0.470 mm seen in the monthly settlement record as compared to the cumulative settlement record and samples from adult beds suggests that (1) the substrate available was previously occupied, reducing the surface for attachment, and (2) competition between juveniles and adults could favour a major mortality of post-larvae <0.470 mm in these localities.

The post-larvae <0.470 mm detected on rugosities of adult shells and among balanids covering mussel shells show that at least some of the mussel larvae settled directly on hard substrates (Maas Geesteranus 1942, Suchanek 1978, Petersen 1984). Others settled directly on thallus, membranous and filamentous algae. In the samples from adult mussel beds, the major number of post-larvae <0.470 mm were found among byssal filaments of mussels, showing a 'preferential' settlement of mussel larvae on filamentous substrate, as has been stated by Blok & Geleen (1958).

The following preferred substrates existed in the adult mussel beds and permanent submerged rope collectors per se: byssus threads, hydroids, bryozoans, filamentous red algae and other kinds of algae, forming a characteristic community (see Lewis 1964, Newell 1970, Roman & Pérez 1979, Pérez-Cirera & Pacheco 1985, Seed & Suchanek 1992). In the postulate of primary and secondary settlement in *Mytilus edulis*, Bayne (1964) also pointed out that settlement occurs on filamentous substrates away from the adult mussel beds in order to avoid a negative competition. This statement suggested not only the recognition of filamentous substrates, but also the ability to perceive whether they are near or far away from mussel beds, implicating a great and powerful capacity of larvae to discriminate among the appropriate filamentous substrate. In the light of the present results, this seems limited.

Contrary to Bayne's (1964) statements, a wide range of sedentary marine species settle preferentially near adults of their own species: bivalve molluscs *Ostrea edulis*; gastropod molluscs *Rissoa splendida* and *Bitium reticulatum*; barnacles *Balanus amphirite*, *B. balanoides*, *B. crenatus* and *Eliminius modestus* (Meadows & Campbell 1972, Scheltema 1974, Crisp 1976). Gotelli (1990) summarised a number of benefits that accrue from the settlement in the proximity of conspecifics. Bayne (1964) has pointed out that competent mussel larvae could be filtered out by adults of their own species. If so, our field results suggested that the mortality of <0.470 mm post-larvae could be high for

cumulative rope collectors and from samples obtained from the scraping of rocky areas owing to the negative interaction between post-larvae and adults.

However, this negative interaction does not determine the occurrence of direct settlement on adult mussel beds, but can only determine post-larvae distribution patterns. In this sense, a particular record of small post-larvae in a specific locality seems to be related more with the existence and distribution of potential settlement surfaces and differential mortality than with different and powerful selective behaviour.

The studies of Maas Geesteranus (1942), Verwey (1952), Blok & Geleen (1958) and Bayne (1964) were in agreement in describing that mussel post-larvae could not remain on the substrate which they first contacted. This has been supported by studies on drifting using the long byssus threads, produced by *Mytilus edulis* from the late pediveliger stage up to at least 2.5 mm of length (Sigurdsson et al. 1976, Lane et al. 1985) and by other bivalves and gastropods (Beukema & de Vlas 1989, Martel & Chia 1991a, b). Verwey (1966) pointed out that mussel post-larvae spin long threads, which may facilitate their upward transport by turbulence and apparently help them in finding a new attachment. Blok & Tan Maas (1977) considered that the mucous threads of *Mytilus edulis* are involved chiefly in settlement by becoming ensnared on the substrate. Martel & Chia (1991a) proposed the same function in the mucus produced by the gastropod *Lacuna* spp. Our results showed that the mucous thread is used to make contact and attach to the substrate. Sigurdsson et al. (1976) suggested a possible acid mucopolysaccharide composition for the mucous threads. The mucus consistency explains the adherence of post-larvae to a dissection needle, the plastic Petri dishes and the different substrates tested. In this sense, its filamentous structure and consistency is easily caught by thin and ramified natural substrates: byssal filaments, filamentous algae, hydroids, artificial filamentous substrates, etc. The mucous thread can also permit the attachment to rough or discontinuous surfaces.

This settlement mechanism clearly explains the apparently contradictory results obtained in several studies on mussel settlement, because it does not depend on a chemical attraction or on a sophisticated searching and powerful selection behaviour in environments where the action of waves is strong and continuous. However this mechanism results in the distribution of post-larvae preferentially on filamentous substrates, including those provided by adult mussel beds.

Our results show that under static water conditions post-larvae from 0.250 to 2.000 mm crawl and spread on the Petri dishes and aquarium, but do not display a specific tendency to avoid or establish preferentially

on adult conspecifics, as we would expect from a primary and secondary settlement pattern (Bayne 1964). It was observed that if post-larvae were not disturbed, they could remain on their original point of attachment. Maas Geesteranus (1942) showed that young mussels under standing water conditions found a place for attachment after only 2 h and did not subsequently change their place of attachment. Senawong (1970) found that post-larvae of the littoral mussel *Hormomya mutabilis*, once firmly attached to the substrate, will never move unless an abrupt change in circumstances occurs. Tan (1975), studying the crawling behaviour in the green mussel *Mytilus viridis*, found that those mussels settling on the top of a rope stayed there even during an experimental tidal exposure time. The mussel under such circumstances does not make an effort to move downwards.

Under moving water conditions, the presence of post-larvae on all substrates studied and especially on those of natural filamentous structure suggests that the water current passively carried the post-larvae and that they randomly attached to an adequate substrate as a consequence of its structure. Eyster & Pechenik (1987) found that the attachment of *Mytilus edulis* larvae on algae and byssal filaments is dramatically enhanced by water agitation and suggested that water movement might increase contact frequency between larvae and various filaments provided, either by stimulating larval swimming or by increasing the frequency of random larval-filament collisions.

The occurrence of fewer post-larvae >0.470 mm on exposed structures of filamentous algae in the field could be due in such instances to their major length-weight ratio when that exceeds the supporting capacity of their byssus or of the substrate favouring their detachment by currents. This 'dispersion post-larvae phase' seems to be random, but results in the possibility to colonize other areas or recruit on established mussel beds even during the non-spawning season when the presence of competent larvae is minimal.

Under static water conditions, the tendency of post-larvae to crawl and form clumps when they are disturbed indicates that this could establish a favourable microdistribution on a substrate. The occurrence of some gregarious behaviour in post-larvae was also found by Maas Geesteranus (1942) in *Mytilus edulis*, Senawong (1970) in *Hormomya mutabilis* and Tan (1975) in *M. viridis*. Seed & Suchanek (1992) pointed out that the mussel beds tend to increase in size due to gregarious settlement and growth of individual mussels. Such behaviour could explain the presence of post-larvae mussel clumps on the basal structure of algae and among adult byssal filaments.

In the light of the results obtained, we can infer the sequence of steps required for the 'settlement and dis-

persion of mussel post-larvae' that can be considered as an alternative to the primary and secondary settlement described for *Mytilus edulis*:

(1) In Ría de Vigo, NW Spain, competent larvae of *Mytilus galloprovincialis* settle on all kinds of available substrates including those provided by adult mussel beds. The major occurrence of small post-larvae on filamentous substrates results from settlement through the 'contact mucous threads' secreted from the late pediveliger and post-larvae stages onto filamentous surfaces. It is not due to a selective behaviour to avoid the adult mussel beds.

(2) If a substrate affords the necessary protection against the currents, wave action, competition and predation, mussel post-larvae can become established, thereby colonizing new areas or recruiting on the existing mussel beds.

(3) If the substrate is undesirable, the mussel post-larvae are eliminated or dispersed by currents and waves, entering into a reoccurring settlement phase until they die or an optimal substrate is eventually reached. The increase in currents and waves during the non-spawning season, when the presence of post-larvae <0.470 mm is minimal, favours the dispersion and colonization by larger post-larvae into areas that may not be optimal for very small post-larvae.

(4) On the substrate, mussel post-larvae can crawl, reaching a better position and forming clumps by means of a gregarious behaviour.

The results and sequence referred to here could explain the apparently contradictory results among different settlement studies of primary and secondary settlement pattern described by Bayne (1964). The application of these sequence of events in the settlement to a general behaviour in the genus *Mytilus* must be confirmed in further studies.

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