The importance of larval supply, larval habitat selection and post-settlement mortality in determining intertidal adult abundance of the invasive gastropod *Crepidula fornicata*

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**A B S T R A C T**

Understanding the processes that drive the recruitment of invasive non-native species is of critical importance in evaluating their potential to colonise previously unoccupied habitats. The slipper limpet *Crepidula fornicata* has spread rapidly into most European waters since its first introduction from the North West Atlantic in the late 19th century. Its invasion success is thought to have been aided by its long larval phase and its tolerance towards a wide range of environmental conditions. The Milford Haven Waterway in Wales, U.K. supports a population with highly variable densities in the intertidal as well as the subtidal zone. In the present study, we tested a series of existing models to investigate the roles of larval supply, larval habitat selection and post-settlement mortality in determining the final distribution of *C. fornicata* in the intertidal zone of the Milford Haven Waterway. During the main reproductive season of 2011, data on total settlement rates and recruitment were collected by deploying slate panels for varying durations in the low intertidal zone, and data on larval abundances were obtained by taking frequent plankton samples at two sites with contrasting adult densities: Beggars Reach (~15±13 ind m$^{-2}$) and Pennar (~343±360 ind m$^{-2}$). Total larval densities were much higher at Pennar, but densities of late-stage larvae (i.e. larval supply) were similar at both sites, indicating that local hydrodynamics may have resulted in the spatial homogenisation of supply of late-stage, metamorphically competent larvae, despite the higher larval production at the high adult abundance site. Settlement rates also did not differ between sites. Seasonal recruitment was overall low, indicating that post-settlement mortality, likely as a consequence of exposure to intertidal conditions, is very high. The lack of a relationship between adult abundance and settlement rates indicates that the final distribution of *C. fornicata* in the intertidal may be a result of differential post-settlement mortality. Understanding recruitment patterns in non-native species is essential for developing management strategies for potentially harmful invaders such as *C. fornicata*.

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1. Introduction

The recruitment success of invertebrates with complex life cycles may be determined by a variety of processes affecting the different life cycle stages from the pelagic larval stage, through settlement and ultimately recruitment into the benthic population. The transition from the larval to the benthic stage during settlement is a critical period during which the larva must perform a number of complex behaviours, physiological adjustments and changes in morphology through metamorphosis (Jenkins et al., 2009). It has long been recognised that the supply of larvae is crucial to enable settlement at a site, and that levels of larval supply may be strongly correlated with settlement rates and in some cases the distribution of adults (Carlton, 2002; Gaines et al., 1985; Grosberg, 1982; Hurlbut, 1992; Jeffery and Underwood, 2000; Minchinton and Scheibling, 1991). However, numerous studies also found that adult distribution may be determined through processes acting during and after settlement (Delany et al., 2003; Gosselin and Qian, 1996; Jenkins, 2005; Jenkins and Hawkins, 2003; Jenkins et al., 2008; Kent et al., 2003; McGee and Targett, 1989; Power et al., 2006). Understanding how these processes interact in determining a species’ final distribution can be highly challenging, but is nevertheless important in understanding its population dynamics.

The spread of non-native species (NNS) through natural or human mediated vectors has received much attention in recent decades due to their potentially severe ecological and economic impacts in the receiving environments. Whilst much is known about the processes and factors that enable successful transport, release, establishment and initial spread of NNS in their non-native range, much less is known about how the species may interact with its environment once it has become established. Studies on the processes affecting the secondary spread of NNS have been under represented in research so far. It is possible that once established, the recruitment success of non native species may be determined by ecological processes in a similar manner to those operating on native species (Colautti and
MacIsaac, 2004; Davis et al., 2001). However, the patterns and processes that underlie the distribution of NNS need to be fully understood in order to predict their potential spread and impact.

The marine gastropod *Crepidula fornicata* (Linnaeus, 1758) is a NNS with well established impacts on native biota, including commercially important shellfish species (Thieltges, 2005; Thouzeau et al., 2000). It is native to the Atlantic coast of North America and has become a common component of the coastal fauna along most European shores shortly after its first introduction in the late 19th century. *C. fornicata*'s invasion success was greatly aided by its ability to colonise a wide range of hard substrata, including shellfish species such as *Crassostrea virginica*, *Crassostrea gigas* and *Mytilus edulis* (Blanchard, 1997; Korringa, 1942; McMillan, 1938) and ship hulls (Cole, 1952; Cole and Baird, 1953). Furthermore, its relatively long larval phase of ~2–4 weeks (Pechenik, 1980, 1984) suggests that larval transport is likely to be an important means of dispersal in its non-native range (Adam and Leloup, 1934; Korringa, 1942; Orton, 1915).

The success of *C. fornicata* as an invasive species has also been in part due to its high tolerance towards a variety of environmental conditions and its ability to thrive in a range of habitat types (Blanchard, 1997; Loomis and VanNieuwenhuyze, 1985). Nevertheless, *C. fornicata* is known to be habitat specific to some extent and although its initial establishment and spread in most European coastal waters happened within only ~70 years (Blanchard, 1997), its distribution and abundance seems to be strongly controlled in some areas. It thrives best in the shallow subtidal and low intertidal of sheltered bays, inlets or estuaries with a predominance of muddy–gravely substrates, and it appears to be rare in the open sea (Blanchard, 1997; Loomis and VanNieuwenhuyze, 1985), although Hinz et al. (2011) recorded high numbers in deep tidally scoured areas of the English Channel. Although it may colonise nearly any hard surfaces, *C. fornicata* is highly gregarious, and settlement has been found to correlate with adult abundance (McGee and Targett, 1989; Walne, 1956). Most likely this is in response to the presence of waterborne cues released by the adults that typically result in the formation of permanent associations, commonly referred to as chains or stacks. Stack formation, and hence the ability of the larvae to detect the presence of adults, is essential for the establishment of self-sustaining populations as adults reproduce through internal fertilisation. Recent studies have shown that larval supply may have a strong influence on the regional distribution of *C. fornicata* through advection of weakly swimming larvae away from the adult beds (Dupont et al., 2007; Rigel et al., 2010). It is likely that this may result in the transport of the larvae to areas with low adult abundance, hence minimising the chances of the larvae encountering already existing stacks for reproduction and lowering the chances of the species to establish self-sustaining populations beyond its original location of introduction.

Although *C. fornicata*’s relatively long pelagic larval phase together with its gregarious behaviour have long been recognised as important characteristics for controlling final adult distributions, the combined importance of these processes has not yet been studied. Field observations on post-settlement processes are almost completely lacking. The focus of the present study is to investigate which processes determine the final distributional patterns of the slipper limpet *C. fornicata* in the intertidal zone. In the first part of the study, we tested a series of three alternative, yet not necessarily mutually exclusive models developed by Jenkins (2005) that aim at explaining if intertidal adult abundance of benthic invertebrates is controlled through processes acting prior to (larval supply), during (larval habitat selection) or after settlement (post-settlement mortality). Firstly, the distribution of adults may be controlled at settlement as a result of differential larval supply. For example, local and regional hydrographic regimes may cause the differential supply of propagules to settlement sites (Gaines et al., 1983; Grosberg, 1982; Jeffery and Underwood, 2000; Minchinton and Scheibling, 1991). The first model hence predicts that the magnitude of larval supply will be reflected in the resulting settlement densities, and be correlated with final adult densities observed at the sites. Species may develop behaviour to counteract such passive larval transport. For example, the larvae of many species are able to actively choose a site for settlement (Jenkins, 2005; Kent et al., 2003; McGee and Targett, 1989), thus avoiding habitats that may be unsuitable for later growth and survival. A second model therefore predicts that the distribution of adults is determined at settlement, but as a result of larval choice instead of larval supply. In the case of gregarious species, one would expect to find higher settlement rates at sites with higher adult densities. Thirdly, adult distributional patterns may be determined after settlement has taken place, due to a variety of biological or physical factors causing differential post-settlement mortality (Delany et al., 2003; Gosselin and Qian, 1996; Power et al., 2006). A third model therefore predicts that settlement rates and adult abundance are not correlated, due to differing levels of post-settlement mortality between sites (Jenkins, 2005).

To test these models, we estimated the supply of late-stage larvae as well as rates of settlement during the main larval and settlement season (June/July–September, Bohn et al., 2012) at two study sites with contrasting densities of adult *C. fornicata*. Previous work suggests that the supply of late-stage larvae and subsequent settlement rates do not differ between these two study sites, despite a difference in adult densities (Bohn et al., 2012). However, those data were collected at monthly intervals and did not allow for a distinction between processes acting at various temporal scales. We therefore instigated a sampling programme during the main reproductive season of *C. fornicata* during 2011 that involved frequent collection of data on larval supply, settlement rates and levels of successful recruitment within one season, to estimate the importance of these processes in determining adult abundance more accurately. In a second part of the study, we quantified post-settlement mortality during the summer months, a process that might be highly important in controlling adult *C. fornicata* abundance in intertidal conditions.

2. Methods

2.1. Study sites

Field experiments and collection of *C. fornicata* broodstock for juvenile rearing were undertaken in the Milford Haven Waterway (MHW) in South West Wales, UK (Fig. 1). This natural ria holds the northernmost self-sustaining established population of *C. fornicata* since the first appearance of the species in Welsh coastal waters in the 1950s (Cole and Baird, 1953; Crothers, 1966) (Bohn unpubl.). The MHW is characterised by a rocky shore environment interspersed with muddy and gravelly banks (Nelson-Smith, 1965). Tides are semi-diurnal with a maximum range of nearly 8 m during spring tides. Low water during spring tides occurs around midday and midnight, resulting in the exposure of the lower-shore flora and fauna to contrasting air temperature extremes. All work was carried out along ~50 m transects running parallel to the shore line at a tidal height of ~1.2 m or ~1.8 m above Chart Datum at two sites within the MHW, Pennar (PE) and Beggars Reach (BR) (Fig. 1). Both sites are situated in the area of the MHW where *C. fornicata* is well established and were chosen to cover the extremes of *C. fornicata* density at that tidal height in the MHW (mean ± SD at BR: ~15 ± 13 ind m⁻², at PE: ~343 ± 360 ind m⁻², Bohn unpubl.). Freshwater input in the MHW is low (Nelson-Smith, 1965). At PE, salinities rarely fall below 30 psu and vertical mixing of salt and fresh water masses is nearly complete. At BR, located in the upper stretches of the ria, stratification of water masses may result in a maximum salinity difference of 8 between surface and bottom waters. Surface salinity here may be more variable than at PE, however marine conditions prevail until the upper stretches of the MHW, resulting in a diverse assemblage of marine species in the intertidal zone (Nelson-Smith, 1965, 1967). The low intertidal zone of both study sites is characterised by a mix of soft-sediments and gravel. Hard substrata (i.e. stones with grain size
of ~16–256 mm and shells) are more common at PE where ~45% of the intertidal seabed is covered by this substrata class, whilst only ~27% of the surface is covered by hard substrata at BR (Bohn unpubl.).

2.2. Field sampling to determine the processes controlling adult distribution

2.2.1. Larval supply

Frequent plankton sampling was undertaken between June and September 2011 to estimate the densities of late-stage, metamorphically competent larvae supplied to the intertidal zone at both study sites. This aimed at evaluating the role of larval supply in determining settlement rates and subsequent adult distributional patterns. Thirteen plankton samples were taken throughout the peak reproductive period at ~weekly intervals from each shore by wading through chest-deep water and towing a 250 μm plankton hand-net (diameter 35 cm) for ~2.5 min. A standard mechanical flowmeter (model 2030, General Oceanics Inc.) attached to the aperture of the net recorded that 1.3–5.0 m³ of seawater were filtered during each tow. All samples were preserved in 70% ethanol within 2 h of collection. They were analysed for total densities of C. fornicata larvae (i.e. counting all larvae across the whole size range), as well as for densities of only the late-stage, metamorphically competent larvae, using a dissecting microscope with an ocular micrometre (×63). Competent larvae were defined as those >650 μm with morphological characteristics that indicate metamorphic competency (flattened shell geometry and presence of a “brimmed shell”, see Pechenik, 1980; Pechenik and Lima, 1984). Densities were calculated as numbers of larvae m⁻³ using estimates of the number of larvae and the flowmeter readings.

2.2.2. Settlement rates and seasonal recruitment

Spatfall densities accumulating over varying time periods (2 weeks, 1 month, 3 months) were monitored at 1.2 m above C.D. at both study sites between June and September 2011. This aimed at estimating the magnitude of total settlement rates and subsequent levels of successful recruitment over the reproductive season at sites with contrasting settlement substrata. Artificial settlement substrata made out of ordinary gravel substra were exposed to air at the beginning of a spring tide week. This minimised the aerial exposure of new settlers to a maximum of ~16 mm with morphological characteristics of those >650 μm, see Pechenik, 1980). Previous work has demonstrated that spat densities on slate panels equal those on natural substrata under intertidal conditions (Bohn et al., 2012). Panels were first deployed in the middle of June 2011, and changed at three different intervals until mid September 2011. One set of twelve panels was deployed for two weeks at a time (“biweekly panels”), after which they were collected and replaced with clean slate panels. This was repeated at biweekly intervals until the panels were finally removed in mid September. Another set of twelve panels was also deployed in mid June, and replaced with clean slate panels each month until the end of the experiment (“monthly panels”).

Fig. 1. Map of the study sites Beggars Reach (BR) and Pennar (PE) in the Milford Haven Waterway (MHW) in the south west of the United Kingdom (top left).

A third set of 12 panels was deployed for three months, i.e. the whole duration of the experiment from mid June to mid September 2011 (“seasonal panels”). Slate panels were cut to a size of 11 cm × 11 cm and securely fastened to the intertidal zone by attaching them to metal frames (50 cm × 50 cm). Each frame had four panels attached using a single screw and wing nut per panel, and was anchored into the soft sediments with four 30 cm rods. Each frame held at least one panel of each of the three deployment durations. At the end of the deployment period, panels were transported to the laboratory by suspending them horizontally on metal stakes that rested in cooling boxes. Panels were separated with PVC rings (diameter 2 cm, height 1.5 cm) to avoid damage to the settled spat on either side of the panel during transport. All C. fornicata present on both sides of each panel were counted and measured under a dissecting microscope with an ocular micrometre (×63) in the laboratory.

The collection of monthly settlement rates may result in an underestimation of total settlement rates over the deployment periods (Bohn et al., 2012). This is likely due to the high mortality of the newly settled spat during exposure to air during spring low tide emersion or due to movement of the juveniles off the panels onto the surrounding substrata some point after settlement. In the present study, biweekly settlement rates over the settlement season were used as the best estimation of total settlement. Sampling was undertaken on the last days of neap tides, i.e. before the settlement substrata were exposed to air at the beginning of a spring tide week. This minimised the aerial exposure of new settlers to a maximum of three days during spring low tide conditions immediately after deployment. More frequent sampling of settlement panels was not possible due to the limited accessibility of the low shore transect during neap tides. The collection of settlement densities on substrata that had been deployed for longer time periods then allowed the comparison of total settlement rates (“biweekly panels”) with the magnitude of benthic recruitment occurring within one month or the whole season.

2.3. Field monitoring of post-settlement mortality

To quantify the mortality of juvenile C. fornicata that occurred following initial settlement, a known number of young juveniles were transplanted into the low intertidal zone (~1.8 m above C.D.) at BR in July 2011. A tidal height of 1.8 m was chosen instead of 1.2 m to maximise accessibility for frequent monitoring of juvenile survivorship. To overcome the difficulty of finding and maintaining a sufficient number of newly settled, and therefore very small, C. fornicata in the field, juveniles were reared in the laboratory. Adult C. fornicata broodstock were collected in March 2011 from the low intertidal zone in the MHW.
Adult stacks (~50 stacks per 25 L aquarium) were kept in constantly flowing seawater in aerated, 120 μm filtered seawater at ~15–16 °C and fed a diet of *Isochrysis galbana* (clone T-ISO). Larvae were collected from the adult tanks within 12 h following release and transferred to 1 μm-UV-filtered seawater at ~19–21 °C. Larvae were reared at densities of ~0.25–0.5 larvae mL$^{-1}$, and fed a diet of *I. galbana* (clone T-ISO, cell density ~1.8 x 10$^5$ cells mL$^{-1}$). Seawater was changed and at the same time food added every other day. All larvae from a single batch were released on the same day, but not necessarily from the same female. Larval growth was monitored to estimate larval development of each batch (data not shown). Once the larvae reached an average size of 650 μm, they were either exposed to an artificial inducer (20 mM K+ enriched filtered sea water, see Pechenik and Heyman, 1987) or natural substrata (biofilm metal shells or stones) for 6 h or 24 h to induce metamorphosis. The newly metamorphosed juveniles were kept under the same conditions as the larvae, but in ~150 mL containers at densities of ~0.5 juvenile mL$^{-1}$. Once juveniles attained a size > 3 mm, they were transplanted onto slate panels identical to those used to monitor settlement in the field. Using a glass pipette, seven juveniles from different batches were lifted onto each panel where they were left to re-position and re-attach. Prior to transplantation into the field, the colonised panels were suspended horizontally in 25 L tanks to minimise movement of the juveniles off the panels, and were kept under the same laboratory conditions as the adults. Juveniles were checked several times before transplantation into the field, and any dead, damaged or missing juveniles were replaced immediately. When the panels were deployed in the field, all the juveniles were ~7-10 wk old, and 4.5–8 mm in size.

In July 2011, 15 panels each with 7 juveniles attached were transported to the MHW in racks that were suspended in cooling boxes filled with aerated 1 μm- and UV-filtered seawater. Panels were installed in the field on five metal bars (three panels per bar) that were anchored in the soft sediment ~2 m apart from each other. To minimise the possibility of movement of the juveniles off the panel onto the surrounding substrata, each panel was suspended on the bars by resting it on a PVC ring (diameter 2 cm, height 2.5 cm). Of the 15 panels nine were caged to exclude potential predators of juveniles. Cages (14 cm x 14 cm x 10 cm in size; width x length x height) were constructed of plastic mesh (5 mm x 5 mm mesh size). The experiment was deployed during low water on 7th July 2011, the last day when the 1.8 m tidal height was accessible during the spring tide that week. Panels were first sampled 5 days later on 12th July, when they were uncovered for the first time during the following spring tide. Thereafter, panels were visited at least once at the beginning and once towards the end of each spring tide period for ten weeks until the experiment ended in mid September. During each visit the cages were opened carefully and the number of live juveniles attached to either side of the panels was noted on all study sites. For this, the two-factorial Scheirer–Ray–Hare test was used to compare differences in survivorship between caged and not caged panel treatments in the field study on EPSM monitoring.

3. Results

3.1. The processes controlling adult distribution

3.1.1. Larval supply and rates of settlement

Whilst total larval densities were higher at the high adult abundance site PE compared to the low adult abundance shore BR (One-way ANOVA on log10-transformed data, F$_1$ = 24.57, p < 0.001, Fig. 2a), densities of late-stage larvae (i.e. larval supply) did not differ between the low and high adult abundance sites (One-way ANOVA, F$_1$ = 0.02, p = 0.88, Fig. 2b). Late-stage, metamorphically competent larvae were rare throughout the study and densities never exceeded 0.8 ± 1.1 ind m$^{-3}$ (mean ± SD) (Fig. 2b). Two peaks in total larval density were observed, one at the beginning of July at PE and a second peak at both PE and BR approximately four weeks later towards the end of July (Fig. 2a). Both peaks in larvae were followed by peaks in settlement with a lag phase of approximately four weeks (Fig. 2c). Biweekly settlement rates varied greatly throughout the season at both locations. Differences between the two study sites were not consistent throughout the season (interaction site x sampling event: p < 0.001, Table 1).

3.1.2. Benthic recruitment

Spat densities recorded at the low adult abundance site (BR) and the high adult abundance site (PE) differed significantly on each sampling occasion (factor site: p < 0.05 for each sampling event, Table 2), irrespective of the duration of panel deployment (interaction site x duration: p > 0.05 for each sampling event, Table 2). However differences were not consistent throughout the season and spat densities were highest at BR in mid July and mid September, and at PE in mid August (Table 2, Figs. 3a, b and c). Levels of recruitment were not significantly different amongst the different deployment durations (Table 2, Figs. 3a, b and c), whether comparisons were made between two-week and one-month deployments in mid July and mid August, or between two-week, one-month and three-month deployments in mid September. This indicates that spat had not accumulated during panel deployment and that successful seasonal recruitment into the adult populations was negligible on both shores. This is supported by the fact that the majority of the juveniles were small (<1650 μm, Fig. 4a and b), suggesting that they were young and had settled recently. Juveniles on panels deployed for various durations were of similar sizes, suggesting that they were approximately the same age.

3.2. Post-settlement mortality during summer

Mortality of young *C. fornicata* spat during the summer of 2011 was high and differed significantly between the caged and un-caged treatments (log-rank test, χ$^2$ = 40.48, p < 0.001). Almost no mortality occurred in both treatments during the first five days following panel deployment (Fig. 5). This time corresponds with periods of neap tide conditions when the panels were continuously immersed. The majority of spat mortality occurred in the following three days, when the panels first became emersed during low water of spring tides. By
were estimated from the deployment of 12 slate settlement panels. Total larval densities as a measure of larval production were estimated from duplicate plankton samples, (b) Densities of late-stage, metamorphically competent larvae were usually observed during spring tide emersion. Biweekly settlement rates were estimated from the deployment of 12 slate settlement panels.

Fig. 2. Crepidula fornicata larval production (a), larval supply (b), and rates of settlement (c) in the intertidal zone (~1.2 m above C.D.) at Beggars Reach, a site with low abundance of adult C. fornicata, and Pennar, a site with high adult abundance, in the Milford Haven Waterway between mid June and mid September 2011. Mean±SD. (a) Total larval densities as a measure of larval production were estimated from duplicate plankton samples, (b) Densities of late-stage, metamorphically competent larvae were estimated from duplicate plankton samples, and (c) Biweekly settlement rates were estimated from the deployment of 12 slate settlement panels.

day 8, mortality was 100% on all uncaged panels, but only 41 ± 28% on the caged panels (mean ± SD). Mortality of the caged juveniles increased steadily until it reached 78 ± 18% at the end of the experiment. The largest increases in the percentages of dead juveniles were usually observed during spring tide emersion.

Table 1

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found, which is contrary to what might have been expected for a gregarious species such as *C. fornicata*: it is possible that active larval habitat selection prior to settlement results in the aggregation of large, late-stage larvae above adult beds. Similarly, no consistent differences in settlement rates were observed between sites where adult abundances differed. The lack of a relationship between settlement rates and adult abundance is especially surprising as gregarious settlement of *C. fornicata* has previously been observed in the field (McGee and Targett, 1989; Walne, 1956). Also, waterborne cues released by conspecifics were found to successfully induce metamorphosis of competent *C. fornicata* larvae (McGee and Targett, 1989; Pechenik, 1980; Pechenik and Heyman, 1987), which inevitably initiates settlement due to the associated loss of the velum, the swimming organ (Werner, 1955). We had postulated that the high adult densities at PE should have more readily resulted in the induction of metamorphosis and hence settlement of *C. fornicata* larvae compared to

### Table 2

Results of non-parametric 2-factorial Scheirer–Ray–Hare test on spat densities observed in mid July and mid September, and a parametric 2-way crossed ANOVA on spat densities recorded in mid August. Densities were recorded on panels deployed for two weeks or one month (mid July and mid August) and two weeks, one month or three months (mid September) at Pennar and Beggars Reach. SS: Sum of squares, MS: Mean squares, df: degrees of freedom. Significant differences are shown in bold (*p* < 0.05).

<table>
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<tr>
<td>Residual</td>
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![Fig. 3. Total settlement, monthly and seasonal recruitment of *Crepidula fornicata* in the intertidal of Beggars Reach and Pennar in the Milford Haven Waterway between June and September 2011. Densities of juveniles <5250 μm found on artificial settlement substrata are shown for (a) the first month (mid June–mid July), (b) the second month (mid July–mid August) and (c) the third month (mid August–mid September) of the experiment. Settlement substrata were first deployed in the middle of June and then either changed at biweekly intervals (black bars, sampled a total of 6 times during the experiment), changed at monthly intervals (light grey bars, sampled mid July, mid August, and mid September), or only sampled once after three months at the end of the experiment (i.e. seasonally, dark grey bars, sampled mid September). 5250 μm was used as a size limit as this corresponds to the maximum size of juvenile slipper limpets found on any of the panels. N.B.: No settlers were observed at Pennar at the end of June. Mean±SD, n=12.](image-url)
the location at BR where adult numbers are low and settlement cues are likely to be scarce.

It is likely that differences in settlement rates between sites had been nullified by factors causing early post-settlement mortality (EPSM). EPSM is known to be high for most benthic invertebrates and may greatly limit the distribution and abundance of species, especially in the intertidal zone where environmental conditions fluctuate extensively over very short time periods (Gosselin and Qian, 1997; Hunt and Scheibling, 1997). Settlement densities recorded in the present study suggest that this may be the case for *C. fornicata* in the intertidal zone of the MHW. No differences were found between densities and size frequencies of spat on panels that were deployed for varying lengths of time at either of the study sites. Even at the end of the experiment biweekly and seasonal spat densities did not differ, indicating that spat had not accumulated on panels over the three months of the experimental study. Large juveniles were rare, even on the seasonal panels. It is therefore more likely that spat had settled less than two weeks prior to sampling, independent of panel deployment duration. Further evidence for high EPSM acting on *C. fornicata* under intertidal conditions comes from observations from the transplantation experiment where

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**Fig. 4.** Seasonal variation in size frequencies (%) of *Crepidula fornicata* juveniles < 5250 μm on artificial settlement substrata at (a) Beggars Reach and (b) Pennar in the Milford Haven Waterway during summer 2011. Substrata were first deployed in mid June, and either sampled every two weeks (black bars), once a month (light grey bars) or after three months (dark grey bars) on the stated dates. Size frequencies from first collection (end of June) are not shown due to the very low numbers of juveniles present on the panels (Beggars Reach n = 2, < 1200 μm; Pennar absent).
mortality of the juveniles was as high as 100% after emersion at low water for only three days. Almost no mortality occurred during neap tides, for example between days 1 and 5 of the experiment when the colonised panels remained continuously immersed (Fig. 5). This also suggests that the settlement densities recorded from biweekly monitoring of the panels are a good estimator of total settlement rates occurring at the site, as sampling was always undertaken before exposure to intertidal conditions during the following spring tide, and therefore before exposure to potentially high levels of EPSM. Panels that were deployed for one or even three months however were exposed to intertidal conditions repeatedly for several days during spring tides. It is hence not surprising that spat did not accumulate on the monthly or seasonal panels. It is possible that patterns of juvenile distribution that were established at settlement have been affected by factors causing EPSM. Movement of the juveniles off the panels onto the surrounding substrates could also explain why we did not record more, and larger, juveniles on panels that were deployed for longer periods of time. However, successful juvenile movement is likely to depend on the availability of hard substrata, which are rare at the predominantly soft-sedimentary study sites. It is thus unlikely that post-settlement movement caused the low juvenile densities observed at both sites. Also, previous work undertaken at the same sites found no indication of an accumulation of juveniles on natural substrata compared to slate panels over the duration of a month (Bohn et al., 2012).

Common causes of EPSM include biological factors such as predation, competition for space and food, diseases and parasites, as well as abiotic factors such as desiccation and strongly fluctuating temperature or salinity regimes (see Gosselin and Qian, 1997; Hunt and Scheibling, 1997 for review). It is unclear the extent to which biotic factors are important in limiting the recruitment success of C. fornicata in the intertidal but it may be low. Field evidence for predation on juveniles is scarce, at least over its native range (Pechenik et al., 2010). Density dependent factors are also unlikely limiting. The attachment to congers during stack formation minimises intraspecific competition for space and its generalist feeding behaviour with a dual feeding mode during the juvenile stage enables feeding on a variety of food sources (Beninger et al., 2007; Decottignies et al., 2007; Pechenik et al., 1996). It is more likely that abiotic factors caused the high EPSM and resulted in the low recruitment observed in the intertidal of the MHW. The estuarine conditions in the MHW may present a highly stressful habitat due to variable salinity regimes as a result of tidal and weather conditions (Nelson-Smith, 1965) which, especially when combined with temperature stress may be a considerable threat to newly settled juveniles (Gosselin and Qian, 1997). Air temperatures may fluctuate by >22 °C within less than 24 h in the low intertidal zone of the MHW during spring tides (Bohn et al., 2012) as low water of spring tides in the MHW occurs during midday to early afternoon and midnight to early morning. This may potentially make the MHW a “hot spot”, i.e. an area where the body temperatures of intertidal organisms may be unexpectedly high for their latitudinal distribution, hence increasing the risk for increased physiological stress and juvenile mortality (Helmuth et al., 2006; Orton, 1915).

In considering the role of biotic or abiotic factors in determining the final distribution of C. fornicata, the results of the caging experiment are informative. Our findings suggest that predation can be excluded as the main cause for juvenile mortality. Although this experiment was lacking the necessary procedural control treatments to unambiguously investigate the effects of predation on juvenile distribution, we found that almost no mortality occurred between days 1 and 5, i.e. during the first neap tide. This was the case even on the un-caged panels. If predation however was the main cause for EPSM, this should have resulted in juvenile mortality in this treatment from the start of the experiment. Differences in survivorship between caged and un-caged treatments only became apparent after day 5 when survival was considerably higher in the caged panels. This is likely due to a microhabitat-effect of the cages, which may attenuate the effects of abiotic stressors, especially by providing shading or protection from other physical disturbances (Gosselin and Chia, 1995). This was not available on the bare, horizontal slate panels. Nevertheless, EPSM was generally high, also in the caged treatments, suggesting that despite the provision of a microhabitat, recruitment into the adult population remains low in the intertidal. Monthly settlement estimates recorded in 2010 at the same sites indicate that spat densities on slate panels equal those on natural substrata (Bohn et al., 2012) offering a variety of microhabitat structures, indicating that EPSM may result in low recruitment to the intertidal irrespective of substratum type.

We expected to find that the differing distribution of adults at the two sites in the MHW had resulted from varying levels of larval supply, the avoidance of larvae of certain habitat sites, or a combination of both. Results of this study clearly show that C. fornicata is highly susceptible to intertidal conditions during the juvenile phase and that post-settlement mortality may be the prime limiting factor for the recruitment success of C. fornicata in the intertidal zone. To unambiguously prove that EPSM caused the observed differential adult pattern in the intertidal zone of the MHW, a difference in levels of post-settlement mortality between both study sites needed to be evident. This was not the case, instead settlement rates were identical at both sites, and seasonal recruitment was in fact negligible. The high EPSM and very low seasonal recruitment likely masked the varying levels of mortality factors responsible for the final distribution of C. fornicata in the intertidal zone. It is likely that higher percentage surface cover of hard substrata at PE (45% gravel and shell) compared to BR (27%) result in higher levels of benthic recruitment due to the provision of more microhabitat structures in the otherwise soft-sedimentary habitats. A previous study from the same sites showed that the season during which settlement, and hence benthic recruitment takes place may be shorter by several weeks than the period during which larvae are present in the water, potentially limiting its spread in the area (Bohn et al., 2012). This shows the importance of including settlement and post-settlement processes into studies that are aimed at understanding the distribution and potential spread of C. fornicata. Also, the environmental tolerances of C. fornicata have not been sufficiently studied, especially with regards to the juvenile stage. Future research should be directed at investigating the processes affecting the various life cycle stages of C. fornicata in both the intertidal as well as the subtidal zone to allow accurate predictions about its potential spread and impacts in its non-native range.

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