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# The distribution and potential northwards spread of the non-native gastropod Crepidula fornicata in Welsh coastal waters

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# THE DISTRIBUTION AND POTENTIAL NORTHWARDS SPREAD OF THE NON-NATIVE GASTROPOD *CREPIDULA FORNICATA* IN WELSH COASTAL WATERS

**A THESIS PRESENTED TO BANGOR UNIVERSITY FOR**

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**BY**

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# THESIS SUMMARY

Understanding the processes that determine the spread of non-native species (NNS) is critical if their impacts on native species biodiversity and ecosystem processes are to be mitigated. The secondary spread of NNS may be controlled by a variety of factors including their dispersal potential, the availability of suitable habitats, and their ability to cope with biotic and abiotic conditions in the novel environment. This thesis investigates patterns of distribution and abundance of the non-native gastropod *Crepidula fornicata* in Welsh coastal waters. In a combination of field and laboratory observations of larval supply, larval settlement and post-settlement processes, combined with work on limiting factors such as low temperature, I investigated factors controlling its current adult distribution and potential for further northward colonisation from its current northernmost Welsh population, the Milford Haven Waterway (MHW). Results of this research project showed that *C. fornicata* is well established in the MHW, with locally superabundant aggregations and no indication for reduced reproductive success. It occurs across a variety of habitat types and the availability of certain hard substrata was found to most likely even facilitate population establishment. This indicates that limited habitat availability and decreased reproductive potential due to the exposure to sub-optimal seawater temperatures unlikely explain its absence from the coastal waters of Mid and North Wales. Benthic recruitment in the MHW, on the other hand, was generally low and occurred during a much shorter time period compared to the long larval season, indicating that settlement and post-settlement processes may be highly important in controlling adult distributional patterns. Early post-settlement mortality (EPSM) is likely important in determining patterns of adult distribution, whilst larval supply and associative larval settlement seem to be of minor importance. However, my results apply only to the distribution of adults in the intertidal, where exposure to harsher environmental conditions probably results in higher EPSM. Lastly, I found that the availability of certain microhabitats might attenuate the high levels of EPSM in the intertidal, thus having considerable impacts on fine-scale adult distributional patterns. The supply of late-stage larvae, in combination with hydrodynamic conditions and larval settlement behaviour, however, seems to be most important in limiting population spread at a regional scale, due to the likely presence of subtidal populations. This shows the importance of incorporating settlement and post-settlement processes into studies on recruitment success when aiming to predict the potential spread of a potentially harmful invader.

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# GENERAL INTRODUCTION

# 1.1 BACKGROUND

*Crepidula fornicata* (Linnaeus 1758) is an invasive non-native marine gastropod of the family Calyptraeidae that was first introduced into the coastal water of Europe in the late 19th Century. It received much attention and is widely studied because of its major impacts on the native fauna through modifications of soft and mixed sediment habitats, the resulting changes to the ecological balance of benthic communities, and its impacts on several commercial shellfish species by trophic competition (e.g. de Montaudouin and Sauriau, 1999; Grall and Hall-Spencer, 2003; Le Pape *et al.*, 2004; Thieltges, 2005b; Decottignies *et al.*, 2007a; Decottignies *et al.*, 2007b). Several life history traits have been shown to have greatly contributed to its successful introduction and rapid spread along European coasts, including:

- The high potential for natural dispersal during a pelagic larval life lasting several weeks, and its ability to prolong pelagic life even further when environmental conditions are unfavourable (Pechenik, 1980, 1984; Viard *et al.*, 2006),
- The high potential for dispersal through human-related activities, including discharge of ballast water (larvae), ship hull fouling (juvenile and adult form), and movements of shellfish for aquaculture purposes (juvenile and adult form) (Crouch, 1893; McMillan, 1938; Thieltges, 2005b; Mineur *et al.*, 2012),
- Its gregarious behaviour, which ensures that settlement occurs in appropriate environmental conditions and that there is ready access to mates (Dupont *et al.*, 2006),
- A relatively long reproductive season, enabling multiple spawnings per female in one year with more than 12,000 eggs released per spawning event (Richard *et al.*, 2006),
- The protection of the offspring during brooding by the female until the fully developed free-swimming veliger larvae hatch (Orton, 1912; Werner, 1955),
- Its high tolerance towards environmental conditions, especially to low and high temperature (Rigal, 2009; Diederich *et al.*, 2011; Schubert, 2011), and
- The epibiotism of *C. fornicata*, i.e. its ability to colonise nearly any hard surface, including conspecifics and other organisms, and the resulting advantage of being a strong competitor for space and food (Thieltges, 2005b; Mineur *et al.*, 2012).

In 2006, *C. fornicata* was accidentally introduced into the Menai Strait and Conwy Bay Special Area of Conservation (SAC) in North Wales, UK with a consignment of mussel spat (Morgan, 2007; Hewitt, 2008). This raised immediate concern amongst the Countryside Council for Wales (CCW) and the local aquaculture industry due to *C. fornicata*'s known potential for harmful impacts on native biota, including the cultured blue mussel *Mytilus edulis*. The successful mechanical removal of *C. fornicata* through the clearance of all infected mussel beds in 2007 and the manual removal of the few remaining *C. fornicata* specimens by 2008 prevented the introduction of the species to North Wales at that time. The northernmost self-sustaining population seems to remain within the Milford Haven Waterway (MHW) in South West Wales, a natural ria with established populations of *C. fornicata* since its first occurrence in Welsh waters in the 1950s (NBN Gateway at http://data.nbn.org.uk). Work in other parts of Europe suggests that its absence from Mid and North Wales may be due to the exposure to low, sub-optimal seawater or air temperatures at its northern range limit, causing high adult mortality during the winter months or hampering their reproductive success (Thieltges *et al*., 2004; Richard *et al.*, 2006). However, the persistence of some individuals for ~2 years after their introduction into North Wales in 2006 suggests that seawater temperatures are not the sole cause of its absence from Mid and North Wales, raising concern that *C. fornicata* may be able to establish self-sustaining populations to the north of its current distribution through repeated human-mediated introductions or natural larval dispersal in the near future.

In 2008 this Ph.D. research project, funded by CCW and the Bangor Mussel Producers Ltd, was started to investigate the potential of this species to expand its range to Mid and North Wales through natural dispersal mechanisms. In a series of field and laboratory studies, this study aimed at understanding the current adult distribution of *C. fornicata* at its northernmost self-sustaining population in Wales, the potential effects of low air or seawater temperatures on reproduction and recruitment success, and the processes that drive observed patterns of adult distribution. This introductory chapter summarises the theoretical background in invasion ecology especially with regards to established drivers of the invasion process. Also, the distribution and biology of *C. fornicata* are described to highlight potential factors and processes that may affect its invasive success.

## 1.2 THE ECOLOGY OF NON-NATIVE SPECIES: TERMINOLOGY, CONCEPTS AND PROCESSES

The introduction of non-native species (NNS) is ranked amongst one of greatest threats to biodiversity worldwide (IUCN, 2010). The prevention of the introduction of NNS and their management following successful introduction require detailed knowledge about patterns and processes that determine their invasion success. This further entails the necessity for the use of an unambiguous terminology and the clear distinction between the various steps of the invasion process, which has been subject to controversial debates in the last decades. Charles S. Elton, in his book *The Ecology of Invasions by Animals and Plants* (1958), refers to ecological invasions as "outbreaks of populations" of foreign species after invading another country. As such he considers cases of introduced species with harmful effects on other species or the human population, and have become "out of control" to some extent. Whilst Elton's views clearly were novel at the time, it is today well understood that terms such as *pest*, *weed*, *alien* or *invader*, often used ambiguously to refer to NNS of some sort, should instead be used with caution (Davis and Thompson, 2000; Colautti and MacIsaac, 2004). For example, Colautti and MacIsaac (2004) suggest that the term *invasive* should be used for NNS that successfully passed the main phases of the invasion process and became widespread in the non-native range (stages IVa and V, Fig. 1.1); this can be independent of the abundances reached in various locations. Other authors propose that the term *invasive* should be used for NNS that are known to adversely impact native biota or the human population in the introduced range (Davis and Thompson, 2000).

Following the definitions by Colautti and MacIsaac (2004), in this thesis I apply the term *non-native species* (NNS) to any species that was deliberately or accidentally transported to a particular location outside its native range through human-mediated processes, e.g. ballast water movement, ship hull fouling or movement of commercial species for aquaculture imports. There must be some indication for the establishment of the species at this particular location outside its native range. I use the term *invasive non-native species* (invasive NNS) for all NNS that, after successful establishment at a particular location in the non-native range, have spread beyond the location of first

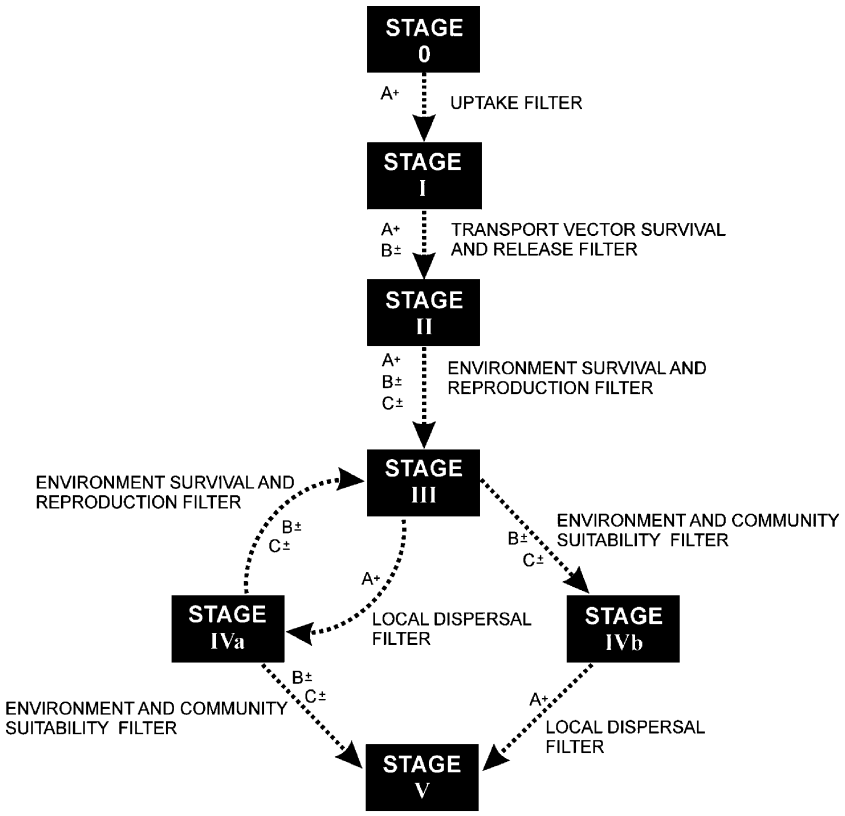



**Fig. 1.1:** The invasion model proposed by Colautti and MacIsaac (2004). In the donor region (stage 0), the propagules of potential invasive non-native species (NNS) will need to be taken up by a transport vector to pass into stage I. Transport vector survival (stage II) and survival + successful reproduction in the novel environment will lead to establishment of the NNS in a new region. The NNS may remain localized and rare (stage III), become widespread but remain rare at all locations (stage IVa), or become dominant but only locally established (stage IVb). Stage V is reached if both, regional spread and dominance, are achieved. Colautti and MacIsaac (2004) consider those NNS as invasive that are widespread (IVa and V), independently of their local abundances, whilst non-invasive NNS are those that remain localized. Propagule pressure (A), physico-chemical tolerances of the NNS with regards to the environment (B), and community interactions (C) may positively (+) or negatively (-)impact the likelihood that the propagule passes into the next stage.

introduction, have become a dominant component of the biota anywhere in this newly occupied region and have any detectable positive or negative impacts on the native biota or the human population. Most of the research presented in this thesis will deal with the potential recent or ongoing spread of *C. fornicata* after its release and rapid

establishment in Welsh coastal waters in the 1950s. I refer to this potential expansion of the geographical range of the species after establishment of local populations as *secondary spread* in subsequent chapters.

Colautti and MacIsaac's invasion model (2004) also describes how three major types of determinants affect the likelihood with which propagules may pass into the next stage of the invasion process, and thus potentially become invasive (Fig. 1.1). Propagule pressure refers to the number of non-native individuals that are released into the new environment (Carlton, 1996; Lockwood *et al.*, 2005). Especially the early invasion stages until initial establishment and subsequent local dispersal is positively affected by high propagule pressure (Colautti and MacIsaac, 2004; Colautti *et al.*, 2006). Secondly, the tolerances of the potential invasive NNS towards environmental conditions (referred to as physico-chemical tolerances of the NNS in Fig. 1.1) may affect most stages of the invasion process. For example, conditions during transport and in the novel environment may differ greatly to those in the native range. High tolerances towards a wide range of physical, chemical and biological conditions will thus increase the potential for the NNS to survive these early invasion stages. Thirdly, biological resistance of the receiving community, also referred to as community invasibility, will determine the likelihood with which later invasion stages are reached (Levine and D'Antonio, 1999; Alpert *et al.*, 2000; Colautti and MacIsaac, 2004).

Biotic and abiotic environmental conditions in the receiving environment, in combination with species traits, are thus particularly important in determining whether a species will become invasive at a location (i.e. become widespread, dominant and have some impact in the non-native range). Spread as well as impact of a particular NNS is expected to differ between areas in the introduced range due to varying environmental conditions, and a NNS may thus successfully establish at a particular location but not another, and be invasive elsewhere. It is thus important to refer to invasive NNS for particular stages of the invasion process, instead of classifying a species as invasive *per se* (Colautti and MacIsaac, 2004). The next section will introduce the American slipper impet *C. fornicata* and describe those environmental conditions and species traits of the various life cycle stages which have affected its spread in its European non-native range.

## 1.3 THE AMERICAN SLIPPER LIMPET *CREPIDULA FORNICATA*

### 1.3.1 DISTRIBUTION

Most species of the genus *Crepidula* have their native distribution along the coasts of America (Blanchard, 1997). *Crepidula fornicata* is native to the Atlantic coast of North America (Fig. 1.2), where it is widely distributed between the Gulf of St. Lawrence in Canada to the Gulf of Mexico (Walne, 1956; Fretter and Graham, 1981; Blanchard, 1997). Native populations are also reported from the Caribbean Islands of Puerto Rico, Cuba, Curacao and St. Thomas (Walne, 1956). It was first introduced into Europe ~135 years ago via movements of shellfish to Great Britain, and is now common along most European shores (Fig.1.2) (Blanchard, 1997).


**Fig. 1.2:** World-wide distribution of the *Crepidula fornicata* and steps of spread: **(1)** Native Range from Canadian border to Gulf of Mexico. **(2)** 1880-1910's - East coast of England. **(3)** 1910-1920's - Belgium and the Netherlands. **(4)** 1930's - Northwestern USA. **(5)** 1930-1940's - Denmark, Germany, South England and France. **(6)** 1950-1960's - Sweden and Norway. **(8)** 1970's - Spain and Mediterranean Sea. **(9)** 2000's - Northern Ireland (McNeill *et al.*, 2010). Its widely cited presence in Japan since the 1970s **(7)** has been a misidentification, making the Northwestern USA the only location with non-native populations outside Europe. Adapted from (Blanchard, 1997)

#### 1.3.1.1 INTRODUCTION TO EUROPE VIA ENGLAND AND ITS EARLY SPREAD

The first record of *C. fornicata* in its non-native range is from 1872 in Liverpool Bay, England (McMillan, 1938). It was most likely introduced attached to the American clam *Venus (= Mercenaria) mercenaria* or the American oyster *Crassostrea virginica* that were imported to this area at that time. *Crepidula fornicata* was also found in Beaumaris in North Wales around ~1886, associated with *C. virginica*. Interestingly, although these are the first reports of the occurrence of *C. fornicata* in British waters, no further records of its presence in these locations exist until today (besides those reporting the accidental introduction of *C. fornicata* onto commercial mussel beds in the Menai Strait in North Wales in 2006 that will be discussed later). It seems that these populations did not persist (Barnes *et al*., 1973), and *C. fornicata* was not recorded from the British west coast until the 1950s (Robson, 1929; Cole and Baird, 1953).

Recurrent movements of shellfish such as *C. virginica* were undertaken between the 1870s and 1920s to promote the British oyster trade after the collapse of stocks of the native oyster *Ostrea edulis* (Korringa, 1942; Blanchard, 1997). This resulted in a series of introductions of adult specimens of *C. fornicata* attached to the imported oysters into British waters, in particular to the east and south coasts of England. Between 1887-1893, several live and dead shells were found in the rivers Colne, Crouch and Roach in Lincolnshire and Essex (Crouch, 1893; Adam and Leloup, 1934). Within a few years, *C. fornicata* became locally abundant. Cole (1952) later concludes that the source populations must have been in Essex where oysters were re-laid. He also suggests that *C. fornicata*'s range expanded north and south from there.

*Crepidula fornicata* became a component of the fauna of the English south coast soon after and gradually extended its range westwards through the English Channel (Orton, 1915). Between 1908/1909 and 1915, it had spread between East Sussex and the Isle of Wight (Robson, 1929) and by 1946, it was already recorded from Cornwall (Cole, 1952). Orton (1915) writes that there is no indication that any adult *C. fornicata* were moved to these locations. He therefore states that it "furnishes an excellent example of the efficacy of a free-swimming larva in extending the domain of a sea-dwelling animal". Cole (1952) disagreed and argued that hull fouling of merchant or war ships that had

remained in infested areas on the English east coast and were moved to the south coast for repairs was the most likely vector of introductions to the English south coast. At about the same time, *C. fornicata* spread further north from the Essex populations. In 1946, several individuals were found attached to a German ship that was broken up in Northumberland and soon after, *C. fornicata* became established there (Cole, 1952). By the early 1950s, *C. fornicata's* distribution in England was thus ranging from Northumberland to the south coast of Cornwall (Orton, 1950; Cole, 1952).

#### 1.3.1.2 CURRENT DISTRIBUTION AND POPULATION STATUS IN ENGLAND

Today, *C. fornicata* is a common component of the fauna of the east, south and south west coasts of Great Britain (Utting and Spencer, 1992; Eno *et al.*, 1997). Database searches imply that slipper limpets can be found as far north as Yorkshire on the east coast of England (NBN Gateway at http://data.nbn.org.uk, Fig. 1.3). Highest densities can be found in the Essex estuaries with more than 2000 individuals $m^{-2}$ in some areas (FitzGerald, 2007). *Crepidula fornicata* is also present along the full extent of the English Channel (Hinz *et al.*, 2011). High abundances are also reported from the Solent, with densities up to ~200-400 individuals $m^{-2}$ (FitzGerald, 2007). Other areas with established populations include Poole Harbour, Portland Harbour, Weymouth, Lyme Bay, Plymouth Sound and adjacent estuaries (FitzGerald, 2007). Little is known about the presence and population status of *C. fornicata* on the English west coast, but a single record exists from the north coast of Devon (see Fig. 1.3). *Crepidula fornicata* seems absent from North West England. Records can also be found from Scotland (NBN Gateway at http://data.nbn.org.uk, Fig. 1.3). Informal enquiries made in 2009 about the source of these records could not verify these alleged occurrences (Bohn unpubl.).

#### 1.3.1.3 SPREAD AND POPULATION STATUS IN IRELAND, MAINLAND EUROPE AND THE PACIFIC

*IRELAND* - The first finding of *C. fornicata* in its non-native range outside British waters is from Galway, Republic of Ireland from 1905, associated with the American oyster *C. virginica* imported from Essex (Minchin *et al.*, 1995). Repeated introductions to Irish

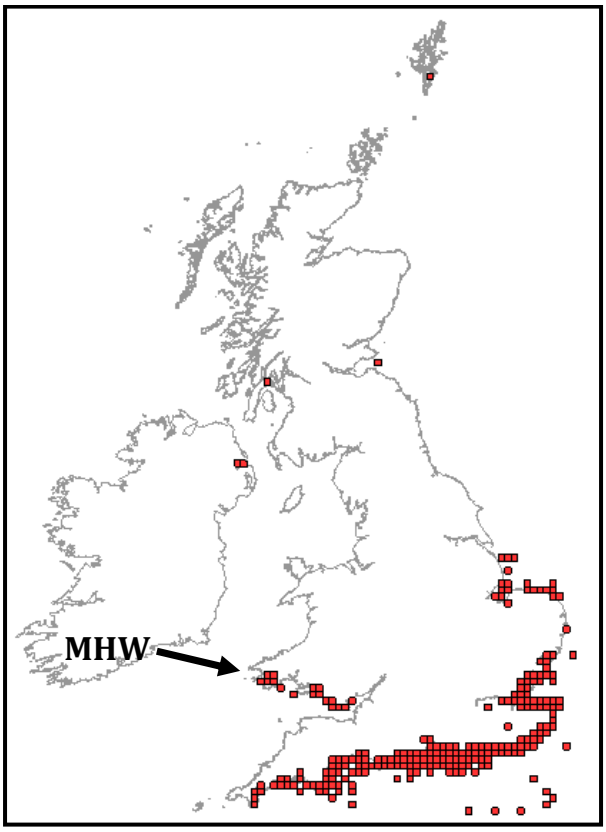



**Fig. 1.3:** Map of the UK and the Republic of Ireland showing the current distribution of *Crepidula fornicata* as available from records from the NBN-Gateway (available at http://data.nbn.org.uk, last accessed 15th October 2012). *MHW* – Milford Haven Waterway in South West Wales, UK.

waters are documented (Minchin *et al.*, 1995). It may have persisted for some time in two locations, Kilmakilloge Harbour and Clew Bay, but the very cold winter in 1962/ 1963 may have caused high mortality in these populations, possibly explaining why *C. fornicata* did not become established in Ireland until very recently. Most likely, all of these introductions were due to imports of oysters from Great Britain or mainland Europe, or the movement of ships from British harbours that had slipper limpets attached to their hulls (Arnold, 1960; Minchin *et al.*, 1995). In 2009, the presence of *C. fornicata* was confirmed from Belfast Lough, Northern Ireland where 20+ specimens were found intertidally. It is not fully understood how and when *C. fornicata* was introduced here, but likely this had happened prior to 2004 (McNeill *et al.*, 2010). *Crepidula fornicata* is now well established at this location.

*BELGIUM*- The first record from the coasts of mainland Europe is from 1911, when a live specimen was found on an oyster bank in Ostende in Belgium, followed by a second specimen that was found attached to *Buccinum undatum* in 1923 (Adam and Leloup, 1934; Blanchard, 1997). It is not known how *C. fornicata* was introduced, but it was likely due to a human-mediated introduction. By 1934, slipper limpets were found frequently amongst oysters and mussels in Ostende and Blankenberghe, imported from the Netherlands (Adam and Leloup, 1934). Most of the Belgian coast does not provide suitable habitats for *C. fornicata* due to the prevalence of sandy shores. Considerable numbers however can still be found at Ostende Harbour and where oyster farming is carried out (Blanchard, 1997).

*THE NETHERLANDS* - In 1926, live slipper limpets were found in Zandvoort, after dead shells had already been found in Bergen aan Zee in 1922 (Korringa, 1942; Blanchard, 1997). *Crepidula fornicata*'s range expansion happened quickly and seems to be still ongoing on oyster and mussel beds in Oosterschelde and Lake Grevelingen, where they constituted approximately half of the biomass of the macrozoobenthos in 1988 (Blanchard, 1995). Similarly to the debate on how *C. fornicata* had spread in British waters, there is some controversy over how *C. fornicata* was introduced to the Netherlands. Whereas some authors think that *C. fornicata* had spread from the the British populations to the Dutch coast by larval dispersal through the English Channel (Orton, 1915; Adam and Leloup, 1934), others consider that the transport of adults attached to shellfish, ships or wreckage is more likely (Korringa, 1942, 1951; Cole, 1952). Korringa (1942; 1951) bases the later assumption on the fact that stack formation is required for reproduction through internal fertilisation and hence a successful establishment of populations. She states that "a few widely scattered larvae cannot effectively extend the distribution of the species".

*DENMARK* - *Crepidula fornicata* was first recorded from Danish waters in 1932 in the Limfjord where it rapidly increased in numbers (Blanchard, 1997). It was associated with oyster seed imported from the Netherlands, and spread to the north-western Kattegat by 1949. Today, *C. fornicata* can still be found on farmed oysters in the Limfjord and the west coast of Jutland. It is assumed that in the 1950s, *C. fornicata* spread to Scandinavia from the Danish populations.

*GERMANY* - *Crepidula fornicata* was first found in German waters in 1934 amongst oyster beds in the Sylt-Romo basin (Werner, 1948). Oysters had been imported from the Netherlands for cultivation in the 1930s and 1940s, most likely with attached *C. fornicata*. By 1944, *C. fornicata* already occurred on five oyster beds (Werner, 1948). A recent study on its population status in the German Wadden Sea found that *C. fornicata* is most abundant on mussel beds in the low intertidal and shallow subtidal with an average abundance of 141 individuals $m^{-2}$ (Thieltges *et al.*, 2003). Although densities have increased substantially since the first introduction, they remain relatively low compared to British or French populations. Low winter temperatures may control the expansion of *C. fornicata* in the northern Wadden Sea (Thieltges *et al.*, 2004).

*FRANCE* - In 1949, *C. fornicata* was found in the province Calvados in Normandy and in the harbour of Brest in Brittany, attached to mussels and *Pecten maximus*, respectively (Cole, 1952). The introduction and the very fast spread of *C. fornicata* along the French coast are most likely due to multiple processes, including the accidental introduction attached to Allied ships at the end of the World War II (Blanchard, 1995, 1997). Also, the import of *Crassostrea gigas* during the 1970s was followed by the occurence of large numbers of *C. fornicata* at all oyster cultivation sites. By 1978, *C. fornicata* completely covered the seabed in some areas of the Bay of Brest. In the the Bay of Marennes-Oleron it reached ~700 t in 1982, only 13 years after its first occurrence. The French populations seem to contain the highest densities of *C. fornicata* in Europe. In 2004, the biomass of *C. fornicata* in the Bay of Mount Saint-Michel was ~150,000 t (Blanchard *et al.*, 2006), compared to 8,000 t of *C. gigas*, 3,000 t of *O. edulis* and 10,000 t of *M. edulis* (Blanchard *et al.*, 2008). It was soon recognised as a serious competitor of the native oyster (Minchin *et al.*, 1995).

*SCANDINAVIA (SWEDEN AND NORWAY)* - Live specimens were found in 1950 in the coastal waters of the province Bohuslän in Sweden, attached to *B. undatum* or *Cyprina* sp. (Blanchard, 1997). These individuals may have arrived as larvae transported with currents from Denmark. In 1963, *C. fornicata* was recorded from the coast in Skagerrak in Norway. Larval dispersal or hull fouling were the most likely vector here, as there was no oyster farming activity. The Oslo Fjord seems to be the northern limit of *C. fornicata's* distribution (see Fig. 1.2 and 1.4).

*MEDITERRANEAN SEA (ITALY AND MALTA)* - *Crepidula fornicata* was recorded from the Mediterranean Sea in 1966 for the first time, when 3 young individuals were found on the north coast of Malta (Blanchard, 1997). It is assumed that the introduction was due to hull fouling. In Italy, dead and live shells were first found in 1973-1974 from the east coast of Sicily, attached to *M. edulis* that were introduced from Portugal or the Netherlands. Marsaxlokk Bay in Malta, where slipper limpets were first found in 1973, is still the southern limit of *C. fornicata*'s range in Europe.

*SPAIN AND PORTUGAL* - The first Spanish record of *C. fornicata* is from the late 1970s in the Ria of Aldan in Galicia (Blanchard, 1997). It was introduced with the pacific oyster *C. gigas* from Ireland and France. Today, *C. fornicata* is commonly found in Galician estuaries. Its range seems to be restricted to these estuaries, probably due to the lack of sheltered habitats on the open Atlantic coast. This may also explain why *C. fornicata* is still absent from the Portuguese coasts.

*PACIFIC* - In the 1930s, *C. fornicata* was introduced to the Pacific coast of North America through movements of oysters with associated slipper limpets, whereupon it became a common component of the fauna along the Washington State coastline (Hoagland, 1974; Blanchard, 1997). This seems to remain the only confirmed non-native population of *C. fornicata* outside Europe. Its documented presence in the bays of Tokyo and Sagami in Japan since 1968 (Minchin *et al.*, 1995) seems to be a result of a misidentification (F. Viard pers. comm.).

### 1.3.2 THE CASE STUDY OF *CREPIDULA FORNICATA* IN WALES

Besides the above mentioned records from Liverpool Bay and the Menai Strait from the 1880s, *C. fornicata* had not been reported from the west coast of Great Britain until 1953 when six individuals were found in Pennar Gut in the MHW, Wales (Fig 1.3) (Cole and Baird, 1953). Stacks and egg-brooding females were found, which implies that, although few in numbers, these were already capable of reproducing. Most likely, these specimens had also been attached to the bottom of naval and merchant's ships that were brought to the MHW for repairs and breaking up after remaining in *Crepidula*-

infested areas on the east and south coast of England for many years (Cole, 1952; Cole and Baird, 1953). Numbers of both intertidal and subtidal populations increased quickly thereafter and by 1960-1961, up to 150 stacks were brought up in an average dredge haul (Crothers, 1966). By October 1962, slipper limpets were present almost anywhere between Hazelbeach and Landshipping Quay. The first live specimen on Dale Beach, located at the mouth of the estuary, was found in April 1964.

*Crepidula fornicata* is now well established in South and South West Wales. It has locally reached very high abundances in the MHW, its original location of introduction into Wales. It is also common in Swansea Bay (pers. obs., Fig. 1.3). However little seems to be known about the densities it may achieve and whether its introduction to the south is due to range expansion from the Milford Haven populations by larval dispersal, or through human-mediated introductions. There is no conclusive evidence that *C. fornicata* has established self-sustaining populations anywhere to the north of the MHW (Fig. 1.3; L. Allen, A. Bunker, B. Sampson and others pers. comm.). It is frequently

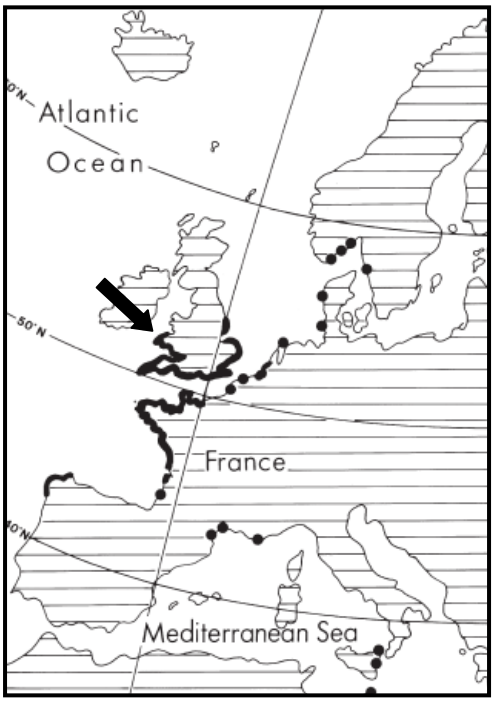



**Fig. 1.4:** Map showing the range of *Crepidula fornicata* in European waters (black outlines). Although *C. fornicata* is shown to be present Cardigan Bay (arrow) for many years, this may not be true (M. Blanchard pers. comm.). Modified from Blanchard (1997).

stated that *C. fornicata* is present in South Cardigan Bay in South West Wales (Blanchard, 1997; Rayment, 2008), but this seems to be incorrect due to a misinterpretation of Fig. 1.4 (M. Blanchard pers. comm.). Enquiries to local fishermen and fisheries officers were made in 2009 to investigate the current range of *C. fornicata* along the Welsh coast line. It was reported that *C. fornicata* was occasionally found attached to scallops that are dredged in South Cardigan Bay, but in very low numbers. These records could not be verified. Also, some individuals of *C. fornicata* have been found within the Skomer Marine Nature Reserve (SMNR) in South West Wales since 2008, all of those attached to scallops (Newman *et al.*, 2009; M. Burton pers. comm.).

In 2006, *C. fornicata* was accidentally introduced into the Menai Strait and Conwy Bay Special Area of Conservation (SAC) in North Wales, most likely with a consignment of mussel spat imported from the English Channel to commercial mussel beds in the north east of Bangor Pier (Morgan, 2007; Hewitt, 2008). The presence of *C. fornicata* was confirmed in February 2007. In March 2007 the affected area was dredged to remove all mussels with associated slipper limpets. A few live *C. fornicata* were found in the affected area during surveys that were carried out the same month to investigate the success of the removal procedure. It was thus decided to relay clean mussels onto the affected areas to smother any remaining slipper limpets. This procedure seems to have been effective and no live or dead specimens of *C. fornicata* were found during intertidal surveys carried out in 2008 (Hewitt, 2008; K. Smith pers. comm.). Some of the females that were collected during the 2007 surveys were bearing eggs, indicating that between the introduction event in 2006 and their removal in 2007, the slipper limpets were reproducing and possibly also releasing larvae (Morgan, 2007). Until the completion of this thesis, the most northern established self-sustaining populations of *C. fornicata* along the British coastline however seems to remain within the MHW (but see Chapter 2 for more detail on its current Welsh distribution).

### 1.3.3 THE BIOLOGY OF *CREPIDULA FORNICATA*

This section summarises the life cycle of *C. fornicata* (Fig. 1.5) and discusses the biology and environmental tolerances of the different life stages. *Crepidula fornicata* begins its

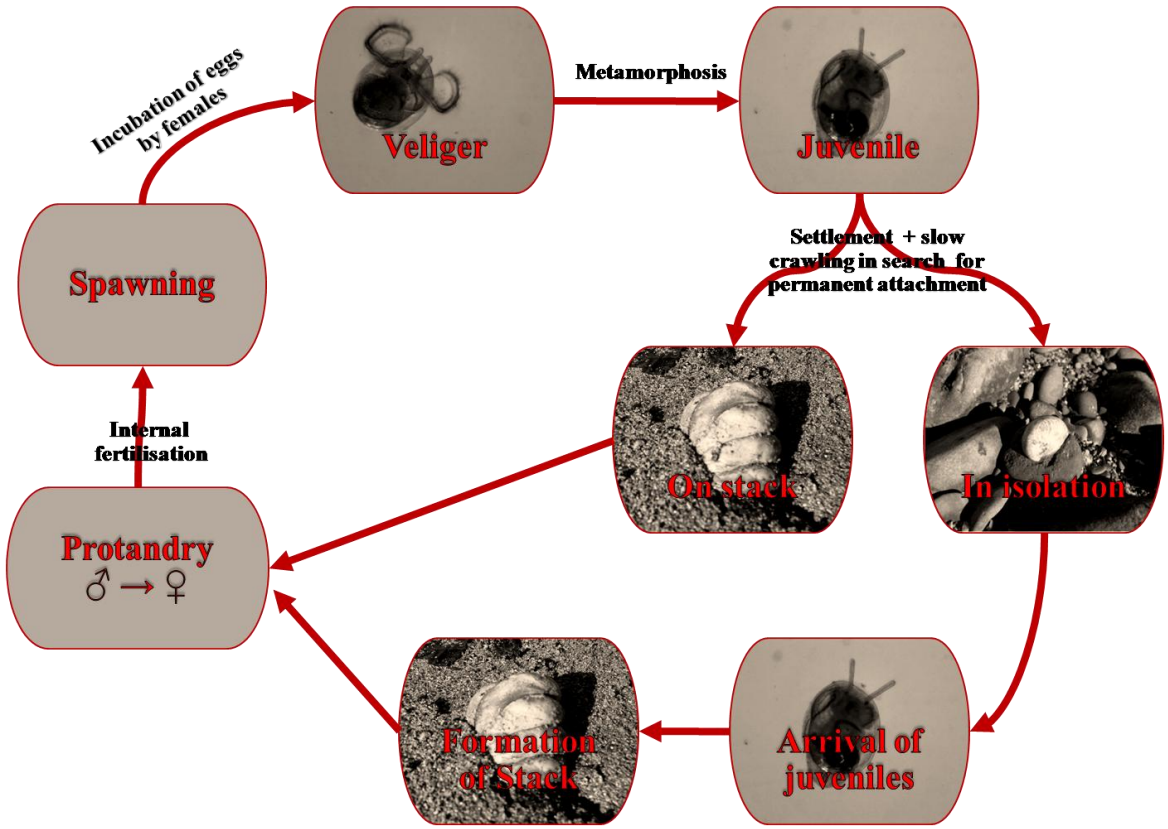



**Fig. 1.5:** Summary of the life cycle of Crepidula *fornicata*.

life as free swimming veliger larva directly after hatching. After spending ~2 to 4 weeks in the plankton, the larvae undergo metamorphosis which is associated with the loss of the swimming organ, the velum. The newly metamorphosed juveniles hence leave the pelagic realm and start their benthic life. Whilst being capable of slow crawling for some time following metamorphosis, the juveniles will soon find a permanent substratum for attachment. This is ideally an already existing stack consisting of several adult *C. fornicata*. Settlement in isolation is possible and often initiates the formation of a new stack. Juveniles usually reach male maturity fairly early in their lives. The arrival of new males in the stack and the resulting change of the sex ratio in the stack allow the bottom most male to gradually develop into a female. Internal fertilisation may occur as soon as both male and female animals are present in the same stack. If fertilisation was successful, the females will brood the eggs for several weeks, until the veliger larvae hatch.

#### 1.3.3.1 THE ADULT STAGE - STACK FORMATION AND PROTANDRIC HERMAPHRODITISM

*Crepidula fornicata* is highly gregarious and typically occurs in stacks. Stacks are formed by settlement of the larvae on top of conspecifics following metamorphosis into the juvenile form. Following settlement, young members of the chain may still change their position or move between stacks or other substrates, whereas the adult members of the stack do not actively separate (Conklin, 1897; Orton, 1909). Up to 29 individuals may form a stack if all juveniles and 'side chains' are included (pers. obs.). The bottom-most individual fixes the stack to a hard substrate such as stones or the shells of live or dead animals (Orton, 1909; Fretter and Graham, 1981).

*Crepidula fornicata* is a protandrous hermaphrodite, i.e. will first pass the male phase before changing sex into the female form. The youngest members of the stacks, i.e. the latest settlers that are found on the top of the chains, are thus usually juveniles or males, whereas the females are found at the bottom of the stacks, are the older members of the stack and usually also larger in size (Fig. 1.6). Individuals in the middle of the stacks often are in the process of sex change (Conklin, 1897; Orton, 1909; Collin, 1995). Male maturity is very often reached in less than one year (Werner, 1955). The subsequent sexual differentiation into the female phase is strongly related to the association of the

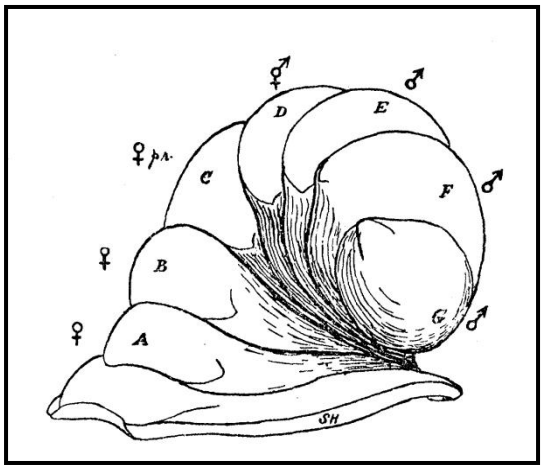



**Fig. 1.6:** Stack of seven individuals of *C. fornicata* on an oyster shell (SH). Female animals (A, B and C) are marked with the symbol ♀, males (E, F and G) with ♂ and the animal in the process of sex change with ⚥ (D). From Orton 1909.

animal with the congeners in the same stack, its 'social environment' (Coe, 1938). When settlement occurs on an already existing stack, the male phase may be prolonged and last for up to 6 years, probably due to pheromones secreted by the female members in the stack (Fretter and Graham, 1981). However, if settlement takes place in isolation and thus in the absence of females, the animal may only be slightly older than one year and as small as 15-20mm when reaching the female phase (Werner, 1955). Such a rapid development to the female stage ensures that the next juvenile, the second member of the chain, may develop quickly into a male and remain as such due to the presence of the female in the newly formed stack. Reproduction between the two members of the stack is now possible. Stacks can therefore be viewed as independent mating groups due to the presence of both sexes (Collin, 1995). Richard *et al.* (2006) consider a single stack a self-sufficient reproductive unit and state that a single stack may be sufficient to start the invasion process if it is introduced to a previously uninvaded location.

#### 1.3.3.2 REPRODUCTION - INTERNAL FERTILISATION, SPAWNING AND EGG BROODING

Reproduction of *C. fornicata* happens through internal fertilisation. Copulation occurs mainly between members of the same stack (Dupont *et al.*, 2006; Le Cam *et al.*, 2009). Males can fertilise females several animals below them by inserting their penis into the pallial oviduct of the female. Between 50 and 80 fertilised egg capsules may be laid by one female at a single spawning event and are brooded under the foot of the female (Orton, 1912; Werner, 1955; Deslous-Paoli and Heral, 1986). Each capsule contains 200-400 eggs (Orton, 1912; Chipperfield, 1951; Werner, 1955) and fecundity is thus relatively high (Table 1.1) (Conklin, 1897; Deslous-Paoli and Heral, 1986; Richard *et al.*, 2006). Eggs are laid between spring and early autumn (Table 1.1). The timing of the reproductive period seems to be triggered by seawater temperature as spawning first occurs at ~6-10°C (Richard *et al.*, 2006). Females were found to first spawn in their third year (Deslous-Paoli and Heral, 1986). They may spawn up to four times per year in the Bay of Brest, but only twice in England, probably because of a shorter reproductive season due to lower seawater temperatures (Chipperfield, 1951; Richard *et al.*, 2006). A prolonged spawning season as a result of climate-change induced increases in seawater temperature was recorded from the French coast (Valdizan *et al.*, 2011).

**Table 1.1:** Summary on the reproductive periods of *Crepidula fornicata* in several parts of the world. Adapted from Richard *et al.* (2006)

| Site | Spawning Period | Duration of Egg Brooding by Female | Time of Larvae in the Water Column | Duration of Larval Life | Water T at First Larval Appearance | Period of Spatfall | No. of Eggs per Female | No. of Spawnings per Female per Year | Authors |
|---|---|---|---|---|---|---|---|---|---|
| **USA –** | Early summer – Mid August [1] | | | ~2-3 wks [1] | | | 13,200 [1] | | Conklin 1897 [1] |
| **New England[1,2]** | April/ May - ? [2] | ~ 4 weeks [2] | | ~2 wks [2] | | | | | Orton 1912a [2] |
| **UK –** | Mid April – Sept [3] (Peak: May – early July) | 21-28 days [3] | 21-28d after spawning - ? [3] | 35 days [3] | 10°C [3] | June to Sept [3] | | 2 (irregular, concerted) [3] | Chipperfield 1951 [3] |
| **River Blackwater[3] Lyme Bay[4] River Crouch[5]** | Mid April – Sept[4] | | April – Oct [4] | | | | | | Orton 1950 [4] |
| | March – October [5] | | | | | June – August (Peak July) [5] | | | Walne 1956 [5] |
| **Germany –** | April – Sept [6] | | May – October [6] | | 6-7 °C [6] | | | | Thieltges *et al.* 2004 [6] |
| **Sylt Island[6,7]** | April – October [7] | | | | 6-7 °C [7] | | | | Werner 1948 [7] |
| **France –** | March – Oct[8] | | | | | | | | Lubet and Le Gall 1972 [8] (in Richard *et al.* 2006) |
| **Banc do Quihot[8] Bay of Brest[9,10] Bay of Marennes-Oleron [11]** | March – August [9] | | | | | Peak: July [9] | 4600-12000 [9] | | Coum 1979 (in Richard *et al.* 2006) [9] |
| | Mid Feb – Sept [10] | | | | | | 10102-14695 (annual mean Feb-Sept) [10] | 3 – 4 [10] | Richard *et al.* 2006 [10] |
| | End Feb – Oct[11] | | | | | Peak: June [11] | 11247 [11] | | Dealous-Paoli and Heral 1986 [11] |

#### 1.3.3.3 THE PELAGIC LARVAL PHASE

After four weeks of brooding, the free swimming veliger larvae hatch at a size of ~350-400 µm (Werner, 1955; Pechenik, 1980, 1984; Pechenik *et al.*, 2002). *Crepidula fornicata* larvae grow relatively fast, and daily growth rates vary between 15-100 µm $day^{-1}$ under laboratory conditions (Pechenik *et al.*, 1996c; Hilbish *et al.*, 1999; Pechenik and Levine, 2007). Temperature affects larval development and survival. Larval growth rates increase at higher temperatures (Lucas and Costlow, 1979; Pechenik, 1984; Pechenik and Lima, 1984), and 15°C and 29-35°C have been shown to be close to the lower and upper temperature tolerance limits of the larvae, respectively, with significantly higher mortality of the larvae or the post-metamorphic individuals (Lucas and Costlow, 1979; Pechenik and Lima, 1984). It has thus been assumed that successful recruitment occurs above 15°C in the field. However, recent laboratory studies conducted in France report survival of larvae reared at 9°C, although mortality was high (~90%, Rigal, 2009). Also, in the German Wadden Sea, in UK waters and in the Bay of Morlaix in France larvae are present at temperatures below 15°C (Table 1.1) (Werner, 1948; Chipperfield, 1951; Thieltges *et al.*, 2004; Rigal, 2009). Larval densities during the peak of the reproductive season in different locations have been reported to vary from 23-8000 individuals $m^{-3}$ (Thieltges *et al.*, 2004; Rigal, 2009).

*Crepidula fornicata* larvae are weak swimmers and passive larval transport with prevailing currents is thus more likely the main mechanism for its natural dispersal. The larvae may travel several kilometres a day and up to 150km in total during the larval period (Blanchard, 1997). The resultant high dispersal of *C. fornicata* larvae causes that regional spread and population connectivity may be high, but are strongly dependent on the hydrodynamic conditions (Dupont *et al.*, 2003; Viard *et al.*, 2006; Dupont *et al.*, 2007b). For example, benthic recruitment may be low as a result of high dispersal in the open ocean, where larvae may be exported away from the adult beds with the prevailing hydrodynamic conditions (Rigal *et al.*, 2010). However, at least at a regional scale, the long larval phase explains *C. fornicata*'s fast and successful spread in many areas (Viard *et al.*, 2006). This also supports the assumption that *C. fornicata* reached the coasts of Scandinavia from the Danish populations and the Dutch coasts from England as larvae (Blanchard, 1997). It is not known if the larvae are capable of active vertical migration

through the water column and if this could affect larval dispersal, which was shown to be the case for many other invertebrates with a pelagic phase (Cronin and Forward, 1986; Shanks, 1986; DiBacco *et al.*, 2001; Marta-Almeida *et al.*, 2006).

#### 1.3.3.4 THE TRANSITION TO THE JUVENILE BENTHIC STAGE - METAMORPHOSIS AND SETTLEMENT

The pelagic life most commonly ends after 2-4 weeks when the larvae undergo metamorphosis during which they lose the velum, the swimming organ (Conklin, 1897; Werner, 1955; Pechenik, 1980, 1984). Several requirements need to be fulfilled so that metamorphosis can occur. This includes optimal environmental conditions, the presence of a metamorphosis-inducing cue and the differentiation of the larvae to the fully developed stage (i.e. attainment of metamorphic competence by the larvae). The change of the spiralled to the flattened shell geometry and the presence of a shelf at the rear of the shell (commonly referred to as 'brim') are distinctive morphological features of the latter (Pechenik and Lima, 1984). Larvae rarely metamorphose before reaching a size of 700 µm. The formation of the brim structure can first be observed at about the same size, making it a good indicator for metamorphic competence of the larva (Pechenik, 1980).

Size at metamorphosis however can be very variable, due to for example the ability of the larvae to delay metamorphosis for more than 30 days in the absence of metamorphosis-inducing cues or under unfavourable environmental conditions (Pechenik, 1980; Pechenik and Lima, 1984; Rigal, 2009). After a delay period of more than 30 days, the larva usually dies or metamorphoses spontaneously, that is it will metamorphose although metamorphosis-inducing cues are absent or conditions unfavourable (Pechenik, 1984). Temperature has been shown to impact the relationship between shell length and age at metamorphosis: larvae grow faster under higher temperatures and full morphological differentiation, metamorphic competence and spontaneous metamorphoses occur earlier (Pechenik, 1984). It can thus be assumed that the duration of the pelagic larval phase in the field depends strongly on the seawater temperature the larvae are exposed to, which in turn will affect the dispersal potential of *C. fornicata* (Rigal, 2009).

Several artificial and natural cues have been found to induce metamorphosis, including the presence of shells of conspecifics and other shellfish species, adult-conditioned seawater and elevated KCl concentrations (Pechenik, 1980; Pechenik and Heyman, 1987; McGee and Targett, 1989; Pechenik and Gee, 1993). The latter was found to be the most successful inducer and is thus regarded as a good test to establish the attainment of metamorphic competence, to determine size or age at competence of *C. fornicata* or to obtain juveniles for subsequent experiments (Pechenik and Heyman, 1987; Pechenik *et al.*, 1996b; Rigal, 2009).

Little is known on ecological processes that affect juvenile survival and subsequent benthic recruitment and field observational data on settlement and post-settlement patterns are largely lacking. Data on the timing and duration of the settlement season only exist from the East coast of the UK. Here, the main settlement season extends from the end of June to the end of August at relatively high (~20°C) seawater temperatures, compared to temperatures required for spawning (Chipperfield 1951, Walne 1956). Studies in the native range documented that post-colonisation processes may be highly important in shaping adult distribution of *C. fornicata*, thus altering the distribution of the early settlers that is established during settlement (Shenk and Karlson, 1986; McGee and Targett, 1989).

## 1.4 STUDY AIMS AND THESIS OUTLINE

The previous section summarised biological traits of all life cycle stages that may be important in facilitating the successful invasion of *C. fornicata* in its non-native range. Vector uptake and transport (stage I in the invasion model developed by Colautti and MacIsaac, 2004) are largely facilitated by the fact that *C. fornicata* is an epibiont to several commercially important shellfish species, including the American oyster *C. virginica*, the Pacific oyster *C. gigas*, the European oyster *O. edulis*, the blue mussel *M. edulis*, the king scallop *P. maximus* and the common whelk *B. undatum*. Transport of the larvae with ship ballast water most likely also aided its transoceanic movement. Transport survival (stage II) and establishment (stage III) in its non-native range in

Europe require high environmental tolerances of all life cycle stages; studies are here partly lacking, especially on stress tolerances of the juveniles stage. Several studies have shown that *C. fornicata*'s further spread within its European range (stage IVa) occurred through repeated introductions of adults on ship hulls and with consignments of aquaculture species. This was also the case in all three documented introduction events to Welsh waters: with aquaculture imports to Beaumaris in 1886 and the Menai Strait in 2006, and to the MHW attached to ships prior to 1953. Natural larval dispersal may have contributed to its spread in its non-native range, although this has been controversially discussed. Establishment and population increase (stages IVb and V) are clearly also facilitated by the relatively high fecundity, resulting in high propagule pressure and good potential for high recruitment. Stack formation may also benefit population establishment, as it maximises reproductive success and ensures larvae settle in suitable conditions for survival.

Although clearly a very successful coloniser of new environments, *C. fornicata* does not always proliferate after introduction (i.e. remains at low population densities) – populations may not surpass stages IVa or IVb. High winter mortality of intertidal adult beds (Thieltges *et al.*, 2003; Thieltges *et al.*, 2004), limited habitat availability (de Montaudouin *et al.*, 2001), restricted reproductive success due to low summer seawater temperatures (Richard *et al.*, 2006) and low larval supply as a result of high larval export away from potential mates in adult beds (Rigal *et al.*, 2010) have all been identified as potential limiting causes. None of these studies, however, incorporated settlement and post-settlement processes into their investigations, despite these processes being known to strongly affect adult distributional patterns of other marine invertebrates (Pawlik, 1992; Gosselin and Qian, 1997; Hunt and Scheibling, 1997; Jenkins, 2005). The failure of *C. fornicata* to expand northwards from the MHW, its original location of introduction to Wales, may be a consequence of any one of the above mentioned impediments on the larval or adult stage, or so far unknown effects on benthic recruitment via the transition to the juvenile stage and subsequent survival. This thesis will deal with the potential secondary spread of the American slipper limpet *C. fornicata* in Welsh coastal waters and investigate potential limiting environmental conditions (seawater temperature, habitat composition and availability) and biological processes (larval supply, larval habitat selection, and post-settlement processes).

Chapter 2 aims to confirm if the MHW still holds the northernmost population of *C. fornicata* in Wales. Its local distribution along the vertical shore gradient and between habitat types at this established most northern population are described.

Chapter 3 investigates if restricted reproductive or recruitment success as a result of exposure to unsuitable air or seawater temperatures may explain the failing northwards spread of *C. fornicata* from the MHW, through monthly monitoring of the larval, juvenile and adult stages. This chapter has been published in Marine Biology:

- Bohn K, Richardson CA, Jenkins SR (2012) The invasive gastropod *Crepidula fornicata*: reproduction and recruitment in the intertidal at its northernmost range in Wales, U.K. and implications for its secondary spread. Mar Biol 159: 2091-2103

Chapter 4 addresses the importance of larval supply, larval habitat selection and post-settlement processes in determining intertidal adult abundances of *C. fornicata* in the MHW, to highlight the most important drivers of its distribution in an established population. This paper has been published in the Journal of Experimental Marine Biology and Ecology:

- Bohn K, Richardson CA, Jenkins SR (2013) The importance of larval supply, larval habitat selection and post-settlement mortality in determining intertidal adult abundance of the invasive gastropod *Crepidula fornicata.* J Exp Mar Biol Ecol 440: 132-140

Chapter 5 includes a series of experimental laboratory and field settlement studies that aim at answering if adult distributional patterns are established as a result of larval microhabitat associations. The importance of microhabitat structure in improving juvenile survivability and thus resulting in differential adult distribution are investigated. This chapter is in preparation for submission.

- Bohn K, Richardson CA, Jenkins SR (in prep) Larval microhabitat association of the non-native gastropod *Crepidula fornicata* and effects on recruitment success in the intertidal zone.

Chapter 6 summarises the main findings of this Ph.D. research project. I aim to determine if introductions to North Wales are likely to re-occur, especially through natural larval dispersal, and if establishment and spread could be successful in the Menai Strait and Conwy Bay SAC. Conclusive remarks include advice for future research work and for good practices of management and preventive measures.

# CHAPTER 2 -

## The northernmost population of the non-native gastropod *Crepidula fornicata* in Welsh coastal waters: distribution and habitat associations in the Milford Haven Waterway

## 2.1 ABSTRACT

The American slipper limpet *Crepidula fornicata* was first recorded from Welsh coastal waters in 1952 when single individuals were found in the low intertidal in the Milford Haven Waterway (MHW), South West Wales. Its establishment and local spread happened rapidly. However, there was no indication of a northwards range extension until 2008 when two individuals were found in the Skomer Marine Nature Reserve (SMNR) just north of the MHW. This study aimed at confirming if the MHW still contains the northernmost population of *C. fornicata* in Wales in a series of intertidal and subtidal surveys. Population densities and habitat associations were studied in areas with established populations. We did not find any live *C. fornicata* outside its known northern range limit in Wales (the MHW). However, *C. fornicata* was found to be well established in most of the MHW, occurring in local very abundant aggregations, intertidally and subtidally, with maximum densities of 2750 and 1150 individuals $m^{-2}$, respectively. No individuals were found at the entrance of the MHW and habitat data suggest that this may be due to the absence of suitable hard substrata for attachment. Subtidally, highest densities were attained in areas with high content of gravel (grain sizes ~16-256 mm), suggesting that the availability of hard substrata encourages its establishment at a site. In the intertidal, there was no such relationship. In fact high gravel content was indicative of low *C. fornicata* abundance, possibly because gravelly shores intertidally are an indicator of more exposed conditions. Sheltered conditions are likely to be more important for *C. fornicata* establishment in the intertidal than the subtidal, due to generally harsher environmental condition in the intertidal that cause higher levels of early post-settlement mortality (EPSM) during intertidal exposure. The presence of substantial subtidal populations suggest that availability of certain hard substrata may facilitate *C. fornicata*'s population growth and potential expansion in Welsh coastal waters, but that other processes, especially EPSM, may affect the distribution of the species in the intertidal.

Keywords: distribution, northern range limit, Milford Haven Waterway, Wales, habitat association, vertical zonation

## 2.2 INTRODUCTION

The introduction of invasive non-native species (NNS) is ranked amongst one of the greatest threats to global biodiversity worldwide due to their severe ecological and economic impacts in the recipient environment (Grosholz, 2002). Several attempts have been made at developing a common terminology in invasion ecology, often by suggesting classifications of the various stages of the invasion process (Richardson *et al.*, 2000; Kolar and Lodge, 2001; Sakai *et al.*, 2001; Colautti and MacIsaac, 2004). Most commonly, a NNS is considered as invasive only once it has passed all stages of the invasion process. These include the uptake of the species in its native habitat by a transport vector, the release of the NNS into the recipient habitat after survival of the transport, and subsequent establishment and spread in the new environment (Richardson *et al.*, 2000; Kolar and Lodge, 2001; Sakai *et al.*, 2001; Colautti and MacIsaac, 2004). The invasion success of NNS therefore depends on a variety of biotic and abiotic factors that may affect any one of these stages.

Much research has been undertaken on processes and factors that facilitate the early invasion stages. For example, it is well understood that low biotic resistance of the receiving community, high environmental tolerance of the NNS and high propagule pressure are advantageous to support survival, reproduction and thus establishment of a population after initial arrival in the new environment (Levine and D'Antonio, 1999; Colautti and MacIsaac, 2004). In comparison, little is known about how NNS spread beyond the first location of introduction. It seems evident however that the availability of specific habitats in adjacent areas and a high dispersal potential of the NNS are particularly important in determining the success rate at which species spread once initially established. The secondary spread of NNS through natural dispersal mechanisms is therefore likely subjected to similar ecological principles that determine the distributions of species in their native range (Davis *et al.*, 2001).

The invasive gastropod *Crepidula fornicata*, native to the coastal waters of the West Atlantic, was first introduced to European coastal waters in the 1880s/ 1890s, attached to the American oyster *Crassostrea virginica* that was imported into the UK for

aquaculture at that time (Crouch, 1893; McMillan, 1938; Korringa, 1942; Blanchard, 1997). Establishment happened rapidly and by the early 1950s, *C. fornicata's* distribution in the UK was already ranging from Northumberland in the North East to the south coast of Cornwall (Orton, 1950; Cole, 1952). Populations in Belgium, the Netherlands, Denmark, Germany and France became established during the same time, followed by later records from more southern and more northern locations (Scandinavia, Spain and the Mediterranean Sea) (Blanchard, 1997).

A variety of biological traits may explain this rapid establishment across European shores. *Crepidula fornicata* colonises most natural and man-made hard substrata, including stones, shellfish and artificial structures such as glass, ship hulls and marina pontoons (Loomis and VanNieuwenhuyze, 1985; McGee and Targett, 1989; Mineur *et al.*, 2012). Its introduction is thus assumed to be at least partly due to the repeated accidental introductions of adult *C. fornicata* attached to ships, wreckage or transported shellfish species such as *Crassostrea gigas, C. virginica* and *Mytilus edulis* (Korringa, 1942, 1951; Cole and Baird, 1953). Transport of the free-swimming larvae with ballast water may have also occurred due to the relatively long pelagic larval phase that lasts ~2-4 weeks (Pechenik, 1980, 1984). Furthermore, a variety of studies have demonstrated that the larvae, juveniles as well as the adults are relatively euryhaline and eurythermal (Pechenik and Lima, 1984; Pechenik and Eyster, 1989; Rigal, 2009; Diederich *et al.*, 2011; Schubert, 2011), hence increasing the chances for survival once exposed to the changeable environmental conditions between the donor region, the transport vector and release into the new environment. *Crepidula fornicata* therefore may thrive in a variety of environmental conditions and habitat types (Loomis and VanNieuwenhuyze, 1985; Blanchard, 1997). This includes estuaries and bays that are known to be amongst the most heavily invaded coastal ecosystems due to, for example, the repeated transfers of organisms for aquaculture and high intensity of shipping (Cohen and Carlton, 1998; Ruiz *et al.*, 2000).

Although *C. fornicata* is now well established in Europe, its local and regional spread varied a lot between locations. It spread quickly through the full east-west extent of the English channel within ~40 years (Orton, 1915; Cole, 1952). Hinz *et al.* (2011) re-visited a number of sites in the English channel for which data on the benthic communities

were available from the late 1950s. The authors found that both geographical spread and local densities of *C. fornicata* increased noticeably (Holme, 1961; Hinz *et al.*, 2011). Today, densities of >1000 individuals $m^{-2}$ are often attained in the coastal waters of the UK and France (FitzGerald, 2007), with maximum densities of >4700 individuals $m^{-2}$ reported from the Bay of Marennes-Oléron, France (de Montaudouin and Sauriau, 1999).

At other locations, however, geographical spread and population densities have been more restricted. In the German Wadden Sea, *C. fornicata* is clearly well established but abundances remain relatively low at ~141 individuals $m^{-2}$ ~70 years after its first introduction into the area (Thieltges *et al.*, 2003). Its low proliferation may be due to a combination of climate-induced mortality events and the limited availability of suitable habitats. *Crepidula fornicata* is mainly confined to the intertidal-subtidal transition zone in this area, most likely as *M. edulis* and *C. gigas* beds co-occur at this tidal height and provide the most suitable attachment substrata in the otherwise sandy-muddy habitats. Colonisation of the intertidal zone however will result in the frequent exposure to freezing air temperatures during spring tide emersion in winter. Resultant high adult mortality events thus may be responsible for the limited increase of the population since establishment (Thieltges *et al.*, 2004). Similarly, limited habitat availability is thought to be one of the main causes for *C. fornicata*'s modest densities in Arcachon Bay, France (de Montaudouin *et al.*, 2001). The low biomass and geographical spread ~30 years after the first introduction of *C. fornicata* in the bay is attributed to the extensive coverage of the bay by *Zostera marina* beds. Several other environmental parameters may impact slipper limpet expansion in certain areas. Seawater temperature for example affects the length of the reproductive season (Valdizan *et al.*, 2011) and the growth and survival of the larvae (Rigal, 2009), especially at the low (<12°C) temperature ranges. However biotic interactions such as predation or competition for food are unlikely causes for its limited population increase (Thieltges *et al.*, 2004; Thieltges, 2005a; Beninger *et al.*, 2007; Decottignies *et al.*, 2007a).

The role of natural larval dispersal in determining the secondary spread of *C. fornicata* has also been widely debated (Orton, 1915; Adam and Leloup, 1934; Korringa, 1942, 1951). Its relatively long larval phase of 2-4 weeks (Pechenik, 1980, 1984) allows for

potentially very high long distance dispersal. It has even been suggested that *C. fornicata*'s spread from the UK to the Netherlands in the 1920s was a result of larval dispersal (Orton, 1915; Adam and Leloup, 1934). However, the specific association of *C. fornicata* with hard substrata and, if to successfully reproduce, conspecifics means that the expansion of reproducing, self-sustaining *C. fornicata* populations may be limited, as the transport of larvae with the prevailing hydrodynamic conditions might decrease the likelihood of successful attachment on conspecifics. The necessity of gregarious settlement after a long pelagic phase is thus more likely to limit its regional spread, even if gregarious settlement is advantageous in other respects, for example as it ensures reproductive success and suitable environmental conditions. The availability of certain hard substrata (especially conspecifics), on the other hand, is likely essential for successful settlement, stack formation and hence reproduction and establishment, likely making habitat composition one of the most important factors affecting the distribution of self-sustaining adult populations of *C. fornicata*.

The first confirmed record of *C. fornicata* from the west coast of the UK is from 1953, when individuals were first found in the Milford Haven Waterway (MHW, Fig. 2.1 and 2.2), a natural ria in South West Wales (Cole and Baird, 1953). Establishment and spread along most of the ria happened within ~10 years after the species was first recorded (Crothers, 1966). Whilst colonisation to the south has been successful (Mettam, 1979, see NBN Gateway at http://www.nbn.org.uk), there is only little indication of a northwards range extension (but see summary of rare findings in study area description). The reasons for its failing northwards spread despite its relatively long presence in the MHW are unknown. This study therefore aimed at answering the following objectives:

i) To determine the northernmost distributional limit of *C. fornicata* in Wales, UK in a series of intertidal and subtidal surveys.
ii) To understand *C. fornicata*'s fine-scale distribution between habitat types and along the vertical shore gradient in areas where it is successfully established.
iii) To suggest potential causative factors and processes for the observed distributional patterns between habitat types and along the vertical shore gradient that will be foci of studies summarised in Chapters 3-5.

# 2.3 METHODS

## 2.3.1 STUDY AREAS

### 2.3.1.1 THE MILFORD HAVEN WATERWAY (MHW)

Sampling effort of all intertidal and subtidal surveys of the present study concentrated in particular on the MHW in South West Wales, UK (Fig. 2.1 and 2.2) as this ria is known to contain well established populations of *C. fornicata* (see NBN Gateway at http://www.nbn.org.uk). The MHW is a natural ria that was formed post-glacially after flooding of eroded river beds (Nelson-Smith, 1965; CCW, 2009). It is the largest ria-estuary complex in the UK, covering an area of ~55 km², 30% of which is intertidal (CCW, 2009). The MHW is characterised by very wave-sheltered conditions and a strong tidal regime. The maximum tidal range during spring tides may be nearly 8 m, resulting in extensive movements of water masses. The volume of the MHW is thus highly variable, ranging between ~345,000 M L during extreme high water spring tides (full volume) and only 34,000 M L at extreme low water spring tide (empty volume) (Nelson-Smith, 1965). The Eastern and the Western Cleddau, the Pembroke River (Fig. 2.2) and a series of smaller streams drain into the ria. The main source for freshwater discharge into the MHW are the Cleddau rivers with an annual estimated freshwater discharge of >450,000 M L (Nelson-Smith, 1965). Freshwater influx is thus relatively low, resulting in fully marine conditions (salinity >30) until the middle reaches of the MHW and almost complete vertical mixing. Estuarine conditions are confined to the east and the upper reaches of the ria with a more variable salinity regime. For example, surface salinity at Beggars Reach may vary between 18-34, depending on weather and tidal conditions. Vertical stratification of water masses here results in salinity differences of up to 8 between surface and bottom waters (Nelson-Smith, 1965, Bohn pers. obs.).

The relatively shallow waters (depth <20 m) of the MHW are surrounded by a shoreline of ~170 km. Towards the mouth of the ria, eroded rocky reefs are common in the slightly more exposed locations whilst sandy beaches can be found in sheltered bays

(Nelson-Smith, 1965). Muddy-gravelly banks and mud flats interspersed with rocky shores are typical features towards the east and the upper reaches of the MHW where conditions are fully sheltered. Due to the low influx of freshwater, a diverse assemblage of marine habitats prevail intertidally as well as subtidally, including sheltered and moderately exposed rocky shores dominated by macroalgal communities and filter feeders, mud and sand flats, beds of *M. edulis* and saltmarshes (Nelson-Smith, 1967). Many of these features are of conservation importance and most of the MHW is thus part of the Pembrokeshire Marine Special Area of Conservation (SAC) (Relevant Authorities Group, 2008; CCW, 2009).

The MHW is also the biggest harbour in Wales and one of the five biggest ports of the UK. This leads to a series of chronic anthropogenic pressures, especially capital and maintenance dredging, shipping movements, ballast water discharge and port waste management, as well as acute disturbance events such as the Sea Empress oil spill in 1996 (Relevant Authorities Group, 2008). Shipping related activities in particular may have resulted in the high number of NNS that can be found in the MHW. For example, merchant and naval ships were often brought to Pennar in the MHW (Fig. 2.2) in the years following WWII for repairs and breaking up. It is likely that *C. fornicata* was introduced to the MHW attached to the hulls of these vessels, as first records of *C. fornicata* in the MHW are from Pennar in 1953 (Cole and Baird, 1953). *Crepidula fornicata* has spread rapidly throughout the ria thereafter. By 1954, solitary individuals and "small" stacks were already found in the low intertidal of Hazelbeach and Pwllcrochan (Fig. 2.2) (Crothers, 1966). At Lawrenny, a shore close to Jenkins Point, *C. fornicata* was first recorded in 1959. Crothers (1966) states that by 1962, populations at Lawrenny had increased to >200 individuals $m^{-2}$ and were "very abundant" at Beggars Reach. Slipper limpets were present almost everywhere between Hazelbeach and Landshipping Quay, a shore located to the north of Black Tar Point (Fig. 2.2). The first live specimen on Dale Beach, located at the mouth of the estuary, was found in April 1964 (Crothers, 1966). Today, *C. fornicata* is well established within the MHW (see NBN Gateway at http://www.nbn.org.uk). It is considered a nuisance to commercial shellfish species, especially the native oyster *Ostrea edulis* that used to form natural beds in the MHW (Woolmer *et al.*, 2011). However, no detailed description of *C. fornicata*'s current population status in the MHW exists to date.

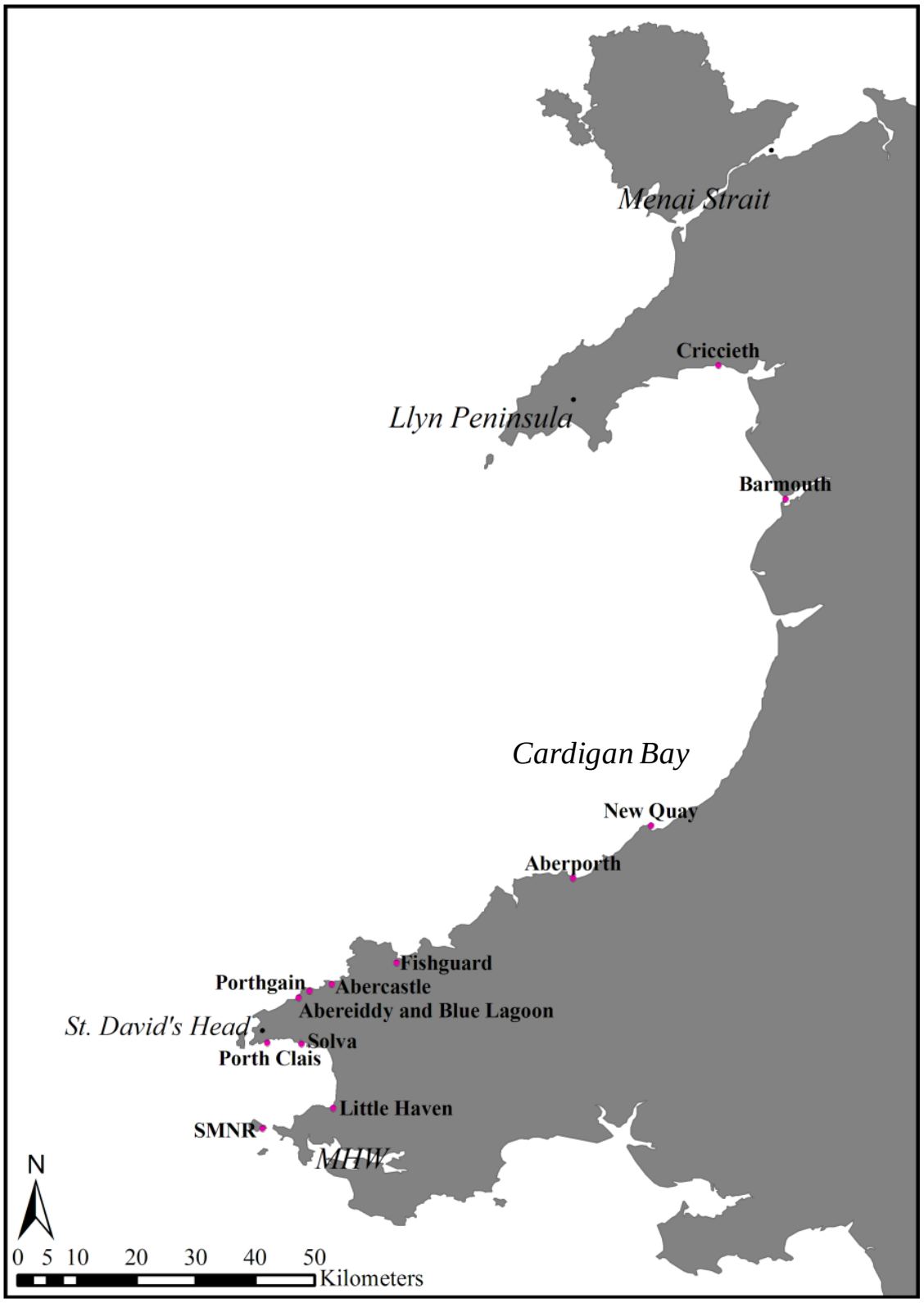



**Fig. 2.1:** Map of Wales, UK with all intertidal sites in Cardigan Bay (bold) and further geographic features of interest that were mentioned in the text (italics). SMNR – Skomer Marine Nature Reserve, where two intertidal sites were surveyed (Skomer North Haven and Skomer South Haven). MHW – Milford Haven Waterway, where 10 intertidal sites were surveyed (see Fig. 2.2 for detailed map).

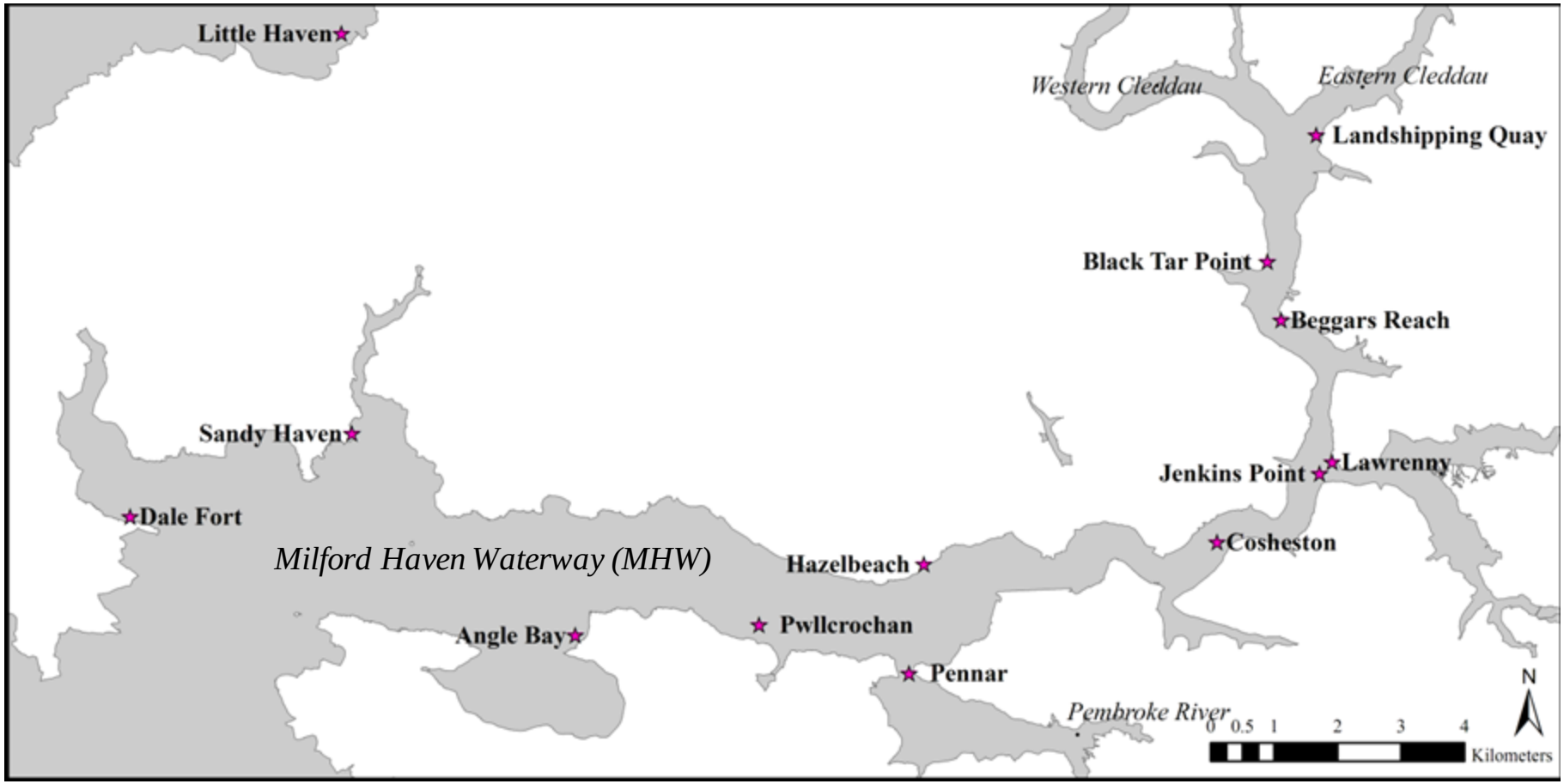



**Fig. 2.2:** Map of the Milford Haven Waterway (MHW) with all ten intertidal sites (Dale Fort, Sandy Haven, Angle Bay, Pwllcrochan, Hazelbeach, Pennar, Cosheston, Jenkins Point, Beggars Reach, Black Tar Point) and further sites of interests mentioned in the text (Lawrenny and Landshipping Quay). The Western Cleddau, the Eastern Cleddau and the Pembroke River drain into the ria and are the main sources of freshwater of the MHW.

#### 2.3.1.2 THE SKOMER MARINE NATURE RESERVE (SMNR)

Surveys for this present chapter were also undertaken in specific target areas in Welsh coastal waters outside and towards the north of the MHW where there had been some evidence for the presence of *C. fornicata*. Target areas include the Skomer Marine Nature Reserve (SMNR, Fig. 2.1, 2.3a), a statutory reserve that was designated as such in 1991 under the UK Wildlife and Countryside Act (1981) (Relevant Authorities Group, 2008). The SMNR also lies within the boundaries of the Pembrokeshire Marine SAC. The reserve is ~13.24 km$^2$ in size and located ~10 km to the north-west of the MHW (CCW, 2012). The boundaries of the SMNR include the island Skomer, ~3 km$^2$ in size. Conservation features include sublittoral reefs, sea caves and breeding populations of grey seals (Relevant Authorities Group, 2008). Little published data exist on its physical environment. It is a fully marine and wave-exposed environment due to its location in the open ocean. Prevailing habitat types to the north of the island Skomer are mixed sediments whilst rocky substrata dominate in the south (M. Sciberras, M. Burton pers. comm.).

*Crepidula fornicata* remained absent from areas within or adjacent to the boundaries of the SMNR until very recently when, in 2008, two individuals were found attached to live king scallops *Pecten maximus* during the four-yearly scallop surveys undertaken by the Countryside Council for Wales (CCW) (Newman *et al.*, 2009, M. Burton pers. comm.). More recent findings emerged after the completion of the survey work that will be presented in this chapter. A single individual (maximum shell length 2.5 cm) was found in 2011 attached to *P. maximus* during routine survey work (Newman *et al.*, 2012, M. Burton pers. comm.). In July 2012, the next scallop survey was undertaken and a total of ten stacks were found, all attached to *P. maximus* or *Aequipecten opercularis*. Individuals were solitary or occurred in stacks of two, and at least three of the bottom-most individuals were carrying eggs, i.e. had reached sexual maturity and reproduced successfully. Total sampling effort of this survey was high with 1074 scallops studied (M. Burton pers. comm.) and densities of *C. fornicata* were thus most likely very low.

#### 2.3.1.3 CARDIGAN BAY SAC

Cardigan Bay in Mid Wales (Fig. 2.1, 2.3a) is the largest bay in the UK, measuring >100 km from its northernmost extent at the Llŷn Peninsula to St. David's Head in the south (CCW, 2005). Its semi-enclosed coastline results in moderate exposure to wave action. Cardigan Bay, as well as adjacent areas of the Irish Sea, is exposed to prevailing south-westerly winds and flow of oceanic water from the Atlantic (Evans, 1995). Strong tidal streams across most of the Irish Sea cause that most of the water column in Cardigan Bay is well mixed, except during calm weather in summer. Water flow is northward in average over a year, but weak (Evans, 1995). An area of 960 km$^2$ in Cardigan Bay forms a designated SAC. Conservation features include the presence of rocky and biogenic reefs, sea-caves and sand banks as well as its populations of bottlenose dolphins and grey seals. Detailed published data on prevalent habitat types are lacking, but it is known that the SAC is characterised by heterogeneous habitats that are mainly sedimentary with mosaics of mud, sand, gravel and pebble patches (CCW, 2005).

Informal enquiries were undertaken in 2008 during which local Welsh fishermen reported repeated catches of *P. maximus* with attached *C. fornicata* from the area in and around the SAC (anonymous source). None of these findings could be confirmed, but the presence of large quantities of empty *C. fornicata* shells at a beach in New Quay, Mid Wales suggest that live specimens could be present in areas adjacent to New Quay (pers. obs., B. Sampson pers. comm., Fig. 2.1 and 2.3a).

### 2.3.2 SUBTIDAL SURVEYS

#### 2.3.2.1 CAMERA SURVEYS IN THE MHW AND THE SMNR

*SURVEY METHODOLOGY*

The subtidal distribution of *C. fornicata* was monitored during two research cruises in areas in and adjacent to the MHW and the SMNR (Fig. 2.3 a) using an underwater still images camera (model Canon EOS 400D Digital SLR, 10 megapixels) in waterproof housing. The camera was mounted on a sled at a fixed height of 54 cm pointing

downwards and was towed behind a survey vessel moving at low speed (0.5-1.5 kn). The area of the seabed that was photographed on each picture thus remained consistent in size and was estimated by attaching a measuring tape between the skies of the sled during one tow. Images of the seabed (0.44 m x 0.30 m) were recorded every 10 s. This way, when towed at an average speed of ~1 kn, an area of ~0.13 $m^2$ of the seabed was photographed ~every 5 m. The camera was deployed for ~10 min at each station, resulting in ~60 images that in total cover ~7.8 $m^2$ of the seabed at each deployment.

Between August 2nd and 6th 2010 the MHW and areas just outside the mouth of the ria were surveyed from the survey vessel Pedryn (CCW). 42 camera tows were undertaken in a grid formation ~1 km apart between the mouth and the upper reaches of the MHW. An additional 26 tows were placed in the shallow subtidal parallel to ten intertidal sites (see below) at varying distances from the shore, with two or three tows running parallel to each intertidal survey site (see Appendix 2.1 for details). This aimed at studying the vertical distribution of *C. fornicata* from the low intertidal to the middle of the channel in the subtidal zone. All of these parallel subtidal transects were relatively shallow (1-15 m below C.D.). Differences between the two or three parallel tows at each station varied (e.g. depth remained similar between the three parallel transects at Angle Bay at ~1 m below C.D., but differed between 1-15 m at Pwllcrochan, Appendix 2.1). Additional nine tows were placed along a 1-2 km grid extending from the entrance to just outside the MHW. All tows were 150-210 m in length.

Between May 5th and 12th 2010 a total of 25 tows were opportunistically surveyed in and adjacent to the SMNR during a research cruise onboard the survey vessel Skalmey (CCW). Ten tows were undertaken within the boundaries of the SMNR and another 15 tows in close proximity to the SMNR. Tows were 250 m in length. More details on survey design and methodology can be found in Sciberras (2012).

### *STILL IMAGES ANALYSIS*

All images recorded during both surveys were thoroughly checked for the presence of *C. fornicata*. For all tows where *C. fornicata* was found to be present on any of the pictures, 30 pictures were randomly chosen for quantitative analysis, excluding all

images with poor visibility and where surface canopy macroalgae (especially *Laminaria* spp., *Ulva* spp.) covered more than ~33% of the image area. All *C. fornicata* in the selected images were counted.

The habitat type was described for each tow inside the MHW by analysing 20 of the previously selected pictures. A grid with 8 x 6 equidistant points was placed on the image using the software Adobe Photoshop v7 and the substratum type found underneath each point was recorded. Grain sizes were determined using the length of the grids (~50 mm) as a reference scale. The fine grain sizes (mud, sand, fine and medium gravel) had to be pooled due to the difficulty of determining grain sizes accurately from the digital images, resulting in 6 different substrata types:

- *Sediment* includes mud, sand, fine and medium gravel; grain size up to ~16 mm
- *Gravel* includes coarse gravel and cobble; grain size between ~16-256 mm
- *Boulder* grain size >256 mm
- *Shells* all empty shells
- *Live habitat-forming species* live *Mytilus edulis*, *Ostrea edulis* and scallops
- *Crepidula fornicata* *live Crepidula fornicata*

In case the field of view under the intercept point was obstructed by macroalgae, these points were excluded from all analyses and the percentage for each remaining grid point was adjusted. Also, when studying the relationship between substrata composition and *C. fornicata* abundance, the substrata class 'live *Crepidula fornicata*' was excluded in the same way, to avoid autocorrelation between *Crepidula*-abundance and habitat composition. The substrata composition of each of the surveyed sites was classified into one of the following habitat types depending on the percentage cover of each of the substrata classes, using the calculated averages from all 20 pictures per tow:

- *Sediment* >80% sediment
- *Sediment with gravel* >70% sediment and 10-30% gravel
- *Sediment with shell* >70% sediment and 10-30% shell
- *Gravel* >80% gravel
- *Gravel with boulder* >60% gravel and 10-30% boulder

- *Boulder* >80% boulder
- *Mix of sediment and gravel* 30-70% sediment and 30-70% gravel
- *Mix of sediment and shell* 30-70% sediment and 30-70% shell
- *Mix of sediment, gravel and shell* >10% each
- *Mix of sediment, gravel and boulder* >10% each
- *Mussel bed mixed with sediment, gravel and shell* >10% each

#### 2.3.2.2 DESTRUCTIVE SURVEYS IN CARDIGAN BAY

38 destructive samples were opportunistically taken during two research cruises in Cardigan Bay onboard the RVs Mya and Prince Madog (School of Ocean Sciences, Bangor University) between 15th and 26th August 2009 close to New Quay in Cardigan Bay. Samples were either 0.5-5 min hauls using a mussel dredge (mesh size 2.5 cm, frame width 0.9 m) or 10 min hauls using a beam trawl (mesh size 4 cm, frame width 2 m). All samples were thoroughly checked for the presence of live or dead *C. fornicata*.

### 2.3.3 INTERTIDAL SURVEYS

Between February 2009 and October 2010, the low intertidal of 24 sites along the Welsh coast line were (Fig. 2.1 and 2.2) quantitatively surveyed for the presence/ absence of *C. fornicata*, and when present, to describe its population characteristics (density, stack sizes, habitat utilised). Ten of the 24 surveyed sites were located within the MHW (Fig. 2.2). Here, sites were randomly chosen across a variety of habitat types and the full extent of the ria from the mouth to the upper reaches to understand its local distribution within the ria. Only sites in the upper stretches of the Haven could not be surveyed as accessibility was restricted. To ascertain the absence of *C. fornicata* outside and to the north of the MHW, 14 of the 24 survey sites were located within the SMNR and in Cardigan Bay (Fig. 2.1), where there had been only anecdotal evidence of the rare occurrence of *C. fornicata*. Here, we targeted specific sites to maximise the chances of finding *C. fornicata* beyond its known northern limit in the MHW. All sites were

therefore either in very close proximity to the locations with rare previous findings of *C. fornicata* (e.g. North Haven and South Haven in the SMNR) or sheltered bays, inlets or small estuaries which are habitat types known to be suitable to support high densities of adult *C. fornicata* (Loomis and VanNieuwenhuyze, 1985; Blanchard, 1997).

Three horizontal transects were sampled at each of the 24 sites except at Little Haven, Porth Clais, Porthgain, Abereiddy, the Blue Lagoon, Skomer North Haven and Skomer South Haven where the extent of the shore only allowed placement of 1 or 2 transects (see Appendix 2.1 for details on survey locations). Transects were ~100 m in length and ran parallel to the water line at a tidal height of 1.0-1.3 m above Chart Datum, established by marking the water line when the tidal prediction programme Tide Plotter vs 5.5 (Belfield Software Ltd 1997-2008) predicted the 1.2 m tidal height for the nearest port. Also, the vertical distribution of *C. fornicata* was surveyed at one transect of four study sites within the MHW (Pennar, Hazelbeach, Cosheston, Beggars Reach, Fig 2.2, Appendix 2.1). For this, densities were estimated at the tidal heights of 0.5-0.7 m and 1.5-1.8 m a. C.D. that were established using the same approach as for the 1.0-1.3 m tidal height. Densities were estimated by searching ten randomly placed 1 $m^2$ quadrats per transect for live and dead *C. fornicata*. In areas with very high densities of *C. fornicata*, random subsamples of the standard 1 $m^2$ quadrats were taken using a 0.25 $m^2$, 0.1 $m^2$ or 0.05 $m^2$ quadrat. When no or very few slipper limpets were found, 30 min timed searches beyond the vertical and horizontal extent of the transects were added to confirm the absence/rarity of *C. fornicata*. All individuals found inside the quadrats or during the timed search were counted and the number of individuals per stack and the primary substratum used for attachment (i.e. used by the bottom-most individual) were noted. Due to the difficulty of spotting small individuals in the field, all *C. fornicata* <5 mm were excluded.

The substrate composition of the intertidal sites was determined from five digital images taken of 0.25 $m^2$ quadrats that were randomly placed along each transect. The area was cleared of macroalgae prior to taking the image to enable identification of the substrata underneath. Pictures were analysed in the same way as for the subtidal sites, but using a grid with 7 x 7 equidistant points.

### 2.3.4 STATISTICAL ANALYSIS

Data were analysed to study the effects of habitat composition on the density and distribution of adult *C. fornicata*. Statistical analyses were undertaken on the relationships between the percentage cover values for various substrata classes and adult densities, stack sizes or types of substrata used for primary attachment, using average values from each intertidal or subtidal transect where *C. fornicata* was present. An accurate estimation of densities was only possible during quadrat counts in intertidal surveys. Subtidal still image analyses however only allowed the reliable estimation of surface-dwelling organisms, resulting in an underestimation of subtidal *C. fornicata* densities due to the three-dimensional structure of the stacks. Analyses were therefore run separately for the intertidal (1.0-1.3 m a. C.D. transects) and subtidal stations. Regression analyses were run between percentage cover of gravel and recorded densities for both the intertidal and subtidal stations. For the intertidal stations, regressions were also undertaken between percentage cover of gravel and stack sizes, as well as between percentage cover of gravel and the frequency with which gravel was recorded as the primary attachment substratum. No such analyses were possible for the subtidal stations as the non-destructive survey technique did not allow collection of data on stack sizes and primary attachment substrata. For the intertidal, analyses were also run between percentage cover of live *Mytilus edulis* and the percentage of primary attachment substratum that were live *M. edulis*. All data were checked for normality, homogeneity of variances and linearity before linear regressions were carried out. Percentage cover data were always arcsine square-root transformed and density data log10 transformed to fulfil assumptions of linear regressions.

The original survey design was intended to investigate the vertical distribution of *C. fornicata* from the low intertidal (1.0-1.3 m a. C.D.) to the shallow subtidal and the middle of the channel at ten sites, by combining data collected during intertidal and subtidal surveys. Due to the above mentioned differences in levels of accuracy between density estimation methods for the intertidal and subtidal stations, it was decided not to combine data for statistical analyses. Results for all ten study sites are summarised in Appendix 2.1. However, at the four sites (Pennar, Hazelbeach, Cosheston, Beggars Reach) where three intertidal heights and a minimum of two subtidal transects were

surveyed, statistical comparisons were undertaken separately for the intertidal and subtidal transects in a two-factorial design (intertidal: site x tidal height, subtidal: site x distance to shore). The number of transects that could be placed in the subtidal varied between the four sites, so that subtidal densities were analysed only between two transects (subtidal I and II). The non-parametric, two-factorial Scheirer-Ray-Hare test was chosen for this due to heterogeneity of variances that could not be removed with data transformations. Mann-Whitney pairwise comparisons were used when significant main effects were detected.

## 2.4 RESULTS

### 2.4.1 CONFIRMATION OF THE NORTHERN RANGE LIMIT IN WALES

Intertidal and subtidal surveys undertaken between February 2009 and October 2010 found no indication of a northwards spread of *C. fornicata* beyond its known northernmost distribution in the MHW in South West Wales, UK. No live individuals were found whilst surveying the coastal waters of the SMNR, Cardigan Bay and areas just adjacent to the MHW (Fig. 2.3b). This is despite the fact that some of these areas were targeted areas due to previous, rare findings of live or dead *C. fornicata* shells (Fig. 2.3a). However, dead shells were found during intertidal surveys at New Quay beach in Cardigan Bay and on a single image recorded during the subtidal survey of the SMNR (Fig. 2.3b).

### 2.4.2 POPULATION STATUS IN THE MILFORD HAVEN WATERWAY

Intertidal and subtidal surveys of the MHW found that *C. fornicata* is well established along most of the extent of the MHW, occurring in the low intertidal and shallow subtidal between Dale Fort close to the mouth and the upper reaches at Black Tar Point (Fig. 2.4). *Crepidula fornicata* was not recorded at the entrance and the upper-most reaches of the ria just south to where the Eastern and Western Cleddau merge to form

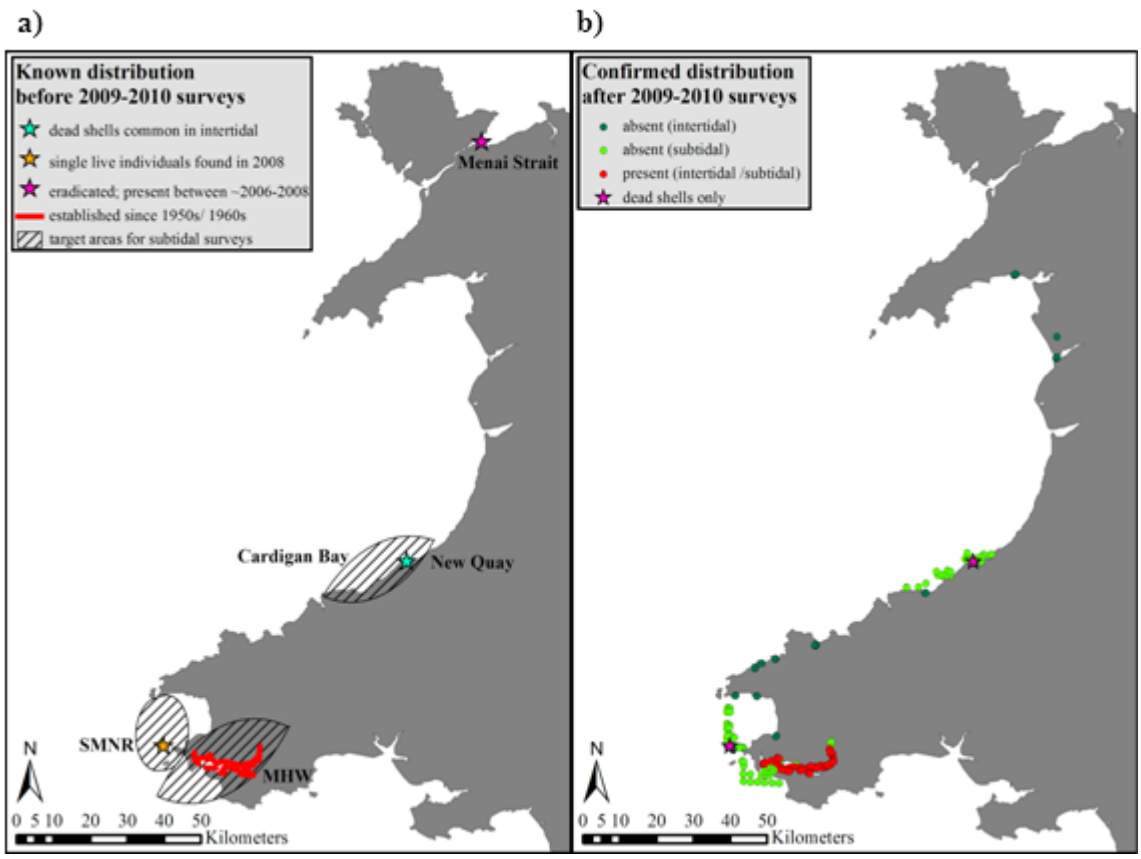



**Fig. 2.3:** Map of Wales, UK with a) the known distribution of *Crepidula fornicata* before surveys of the present study were undertaken in 2009 and 2010 in specific target areas (hatched areas), and b) the distribution of *C. fornicata* as confirmed during the intertidal and subtidal surveys from 2009 and 2010. Each marker represents one sampling site. *SMNR* – Skomer Marine Nature Reserve, *MHW* – Milford Haven Waterway.

the main channel. Highest intertidal and subtidal densities are reached in the middle stretches where *C. fornicata* occurs across a variety of habitat types (Fig. 2.4 and 2.5). Subtidally, *C. fornicata* was most abundant in the shallow waters at Pennar with 1152±881 individuals $m^{-2}$ (mean±SD, Fig. 2.4). Extremely high intertidal densities were recorded at Pwllcrochan (1.0-1.3 m tidal height) with mean densities of 2748±3859 individuals $m^{-2}$ at transect 3 (Fig. 2.6). Especially in the intertidal, a remarkable decline in densities from the middle stretches of the ria towards the mouth and towards the upper reaches is apparent: whilst medium to high densities were still found at Pennar and Hazelbeach, densities at the intertidal sites of Cosheston, Jenkins Point, Beggars Reach and Black Tar Point in the upper reaches were relatively low (Fig. 2.4 and 2.6, Appendix 2.1). At the mouth of the ria, lowest intertidal densities were recorded at

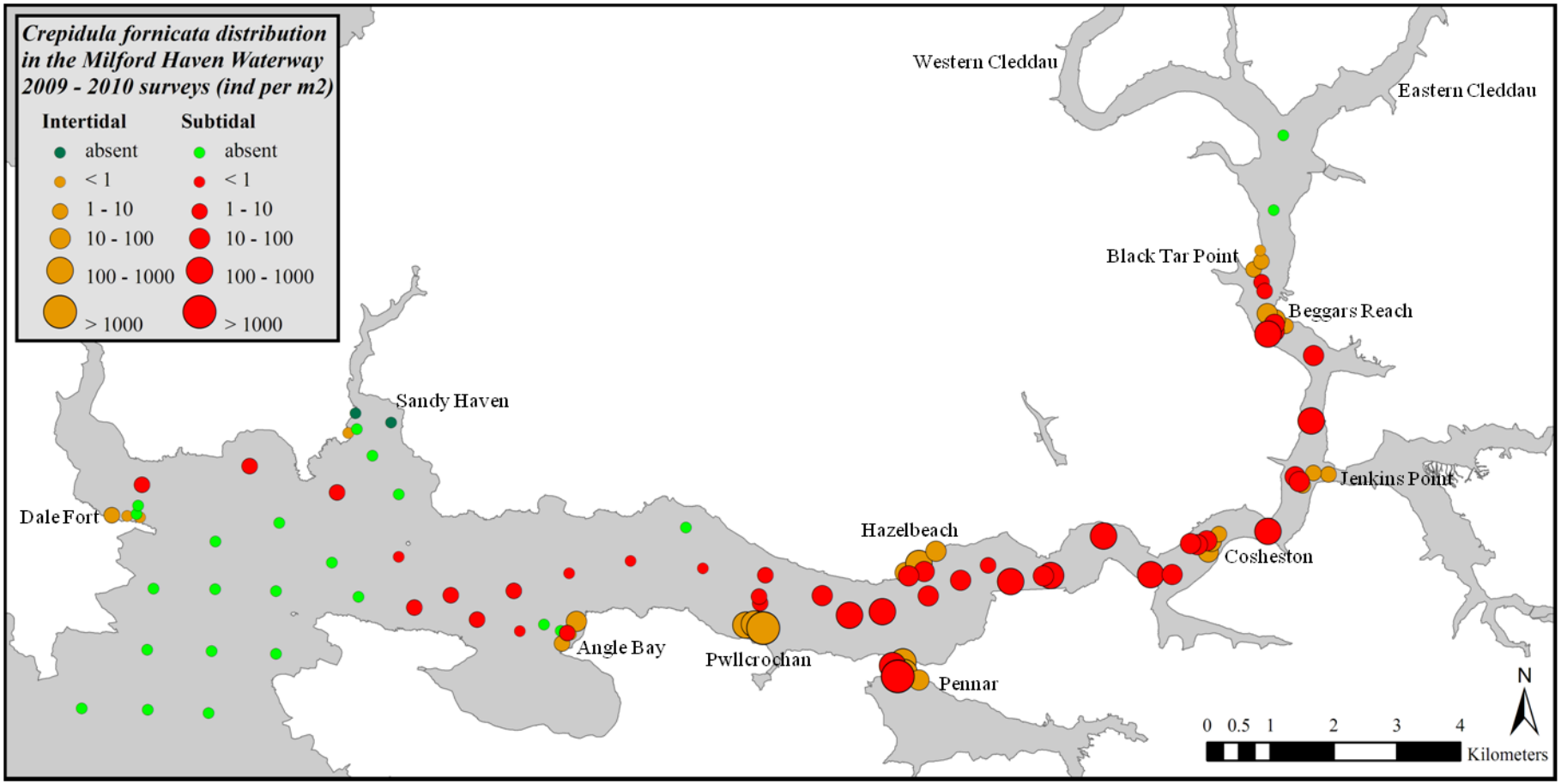



**Fig. 2.4:** Distribution of *Crepidula fornicata* in the Milford Haven Waterway, its confirmed northern-most geographical location in Wales, UK. Each marker represents the start coordinates of a transect that was surveyed during the surveys of 2009 and 2010. Intertidal densities (orange markers) are calculated averages of *C. fornicata* counts in ten 1 $m^2$ quadrats per surveyed ~100 m transect (1.0-1.3 m above C.D.). Subtidal densities (red markers) are averages of counts from 30 still images, each covering ~0.13 $m^2$ of the seabed, taken along ~150-210 m transects using an underwater camera mounted on a vessel-towed sled. Bright and dark green markers are stations were no *C. fornicata* were found subtidally or intertidally, respectively.

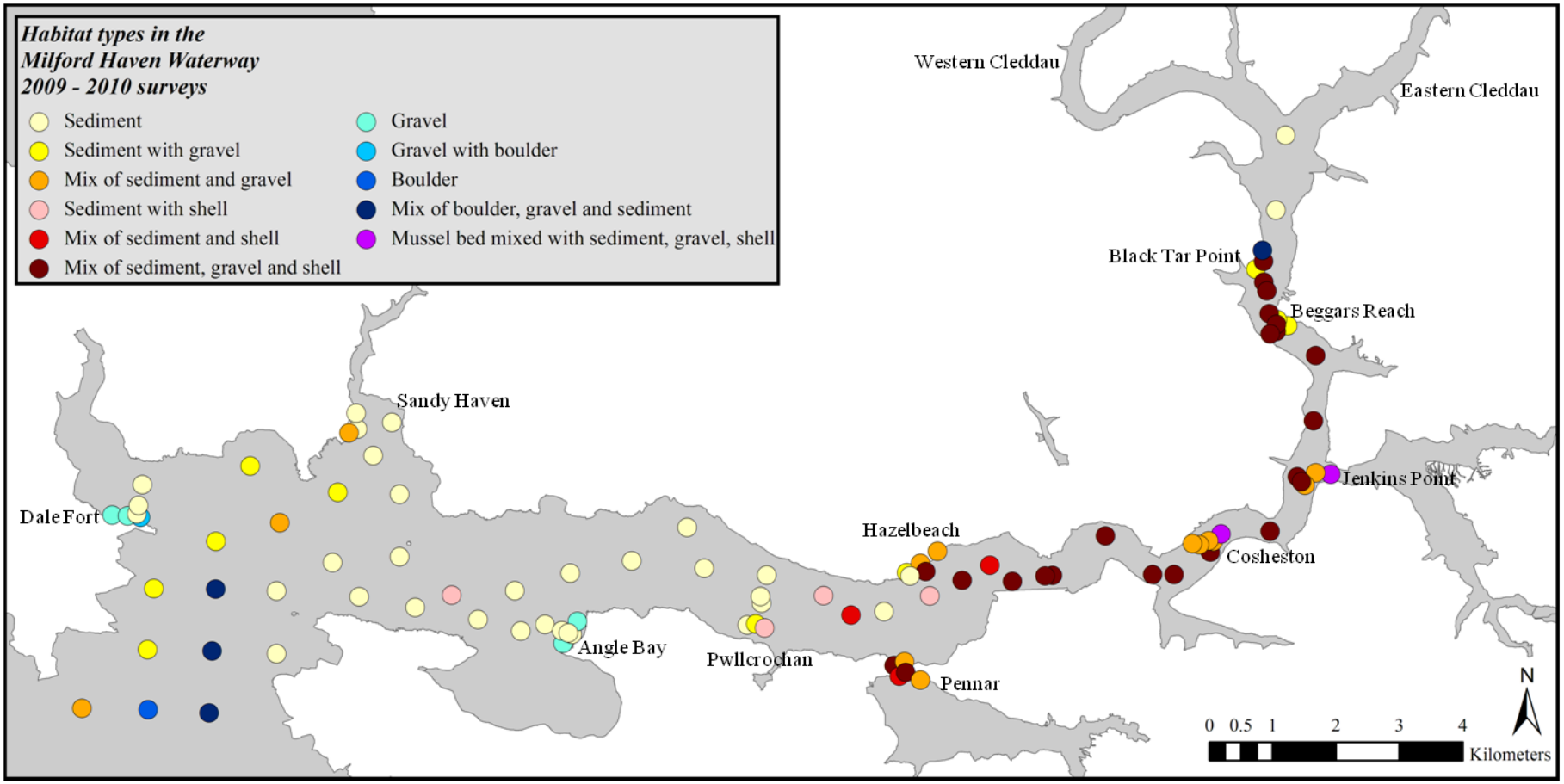



**Fig. 2.5:** Habitat distribution in the Milford Haven Waterway. Habitat types were classified by grouping average percentage surface cover of 6 different substrata classes (*Sediment*, *Gravel*, *Boulder*, *Shell*, *Live habitat-forming species*, *Crepidula fornicata*) that were either determined from 20 randomly selected still images of the seabed taken during the subtidal survey in August 2010 using a sled-mounted underwater still images camera, or from 5 digital images taken during intertidal surveys in 2009-2010. See methods for details on habitat classes.

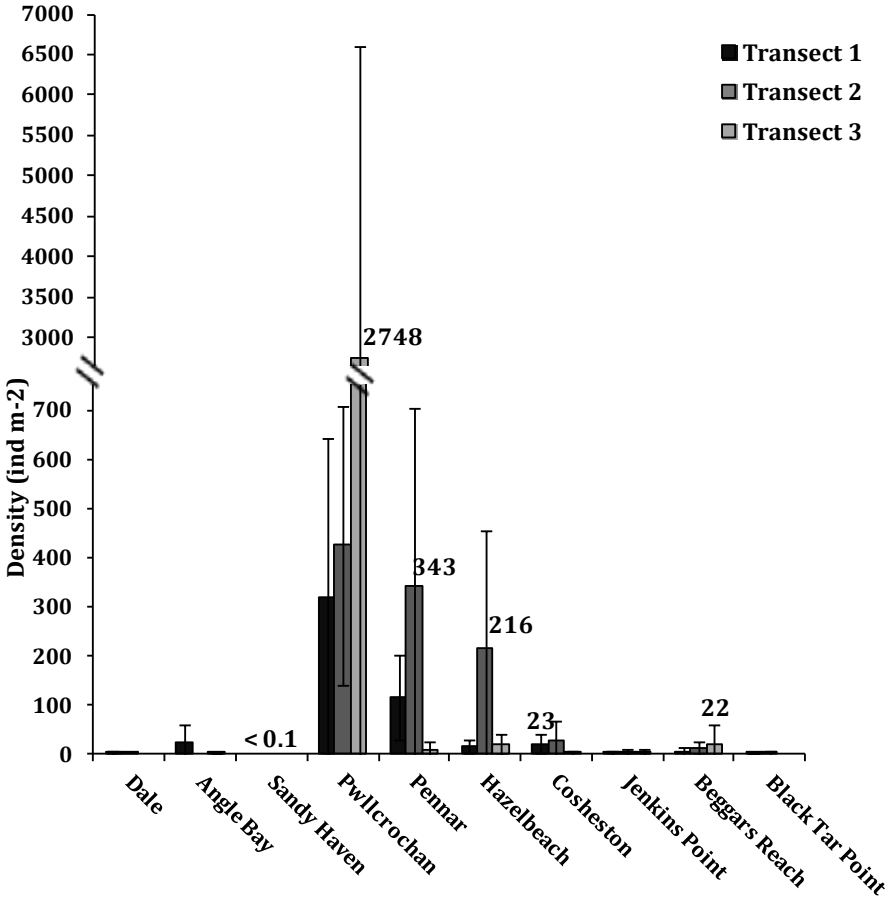



**Fig. 2.6:** Densities of *Crepidula fornicata* at three transects at each of ten intertidal sites in the Milford Haven Waterway (1.0-1.3 m above C.D.). Data labels are mean densities of transects with highest (Pwllcrochan) and lowest densities (Sandy Haven) as well as the study sites for later experimental work (see chapters 3-5). Note break and change in scale in y-axis.

Sandy Haven, where individuals were only found during the timed search but not in thequantitative survey, indicating that average densities are <0.1 individuals $m^{-2}$ (Fig. 2.6). This decline in densities from the middle to the mouth and the upper reaches is not quite as pronounced in the subtidal, where densities in the area between Pennar and Beggars Reach are less variable compared to the intertidal (Fig. 2.4).

### 2.4.3 DISTRIBUTION ACROSS HABITAT TYPES

Still image analyses showed that, subtidally, *C. fornicata* occurs in most habitat types, but is absent in areas with a high content of boulders (Fig. 2.4 and 2.5). Densities remain low in homogenous habitats dominated by sediment (<16 mm). Highest densities were

found in areas where sediment had a high content of hard substrata (i.e. mix of sediment and shell, mix of sediment and gravel, or mix of sediment, gravel and shell, Fig. 2.4 and 2.5).

Percentage cover of sediment and gravel were highly positively correlated (Pearson product-moment correlation, $r_{intertidal}$=-0.878, $r_{subtidal}$=-0.858, p<0.001, Fig. 2.7a). All analyses were therefore undertaken only on one substrata class (*Gravel*). Gravel percentage cover was significantly related to both intertidal as well as subtidal densities of *C. fornicata*, but this relationship was negative in the intertidal and positive in the subtidal zone (Fig. 2.7b and 2.7c). Furthermore, gravel surface cover was negatively related to the average number of individuals found in the stacks in the intertidal (Fig. 2.7d) and positively related to the frequency of primary attachment substrata that were gravel (Fig 2.7e).

Nine intertidal sites were found to support live *Mytilus edulis*, with 0.7-23% of the total surface of the site (1.0-1.3 m above C.D.) covered in this substratum type. In contrast to what was observed for the substratum class gravel, the availability of live mussels at a site did not result in the utilisation of live mussels as primary attachment substratum for *C. fornicata* stacks (Fig. 2.7f). For example, 23% of the surface of Cosheston transect 3 consisted of live *M. edulis*, but no *C. fornicata* was found attached to mussels.

### 2.4.4 DISTRIBUTION ACROSS THE VERTICAL SHORE GRADIENT

Intertidal densities differed significantly between the three intertidal heights and the four sites that were surveyed for the vertical distribution of *C. fornicata* (both main effects: p<0.001, Table 2.1). Differences between the tidal heights were consistent between sites (interaction site x tidal height: p>0.05, Table 2.1). Densities were highest at Pennar and lowest at Beggars Reach (Table 2.1, Fig. 2.8). The lowest intertidal height always contained highest densities, with maximum densities of 1031±943 individuals $m^{-2}$ (mean±SD) reported at ~0.5-0.7 m above C.D. at Pennar (Table 2.1, Fig. 2.8).

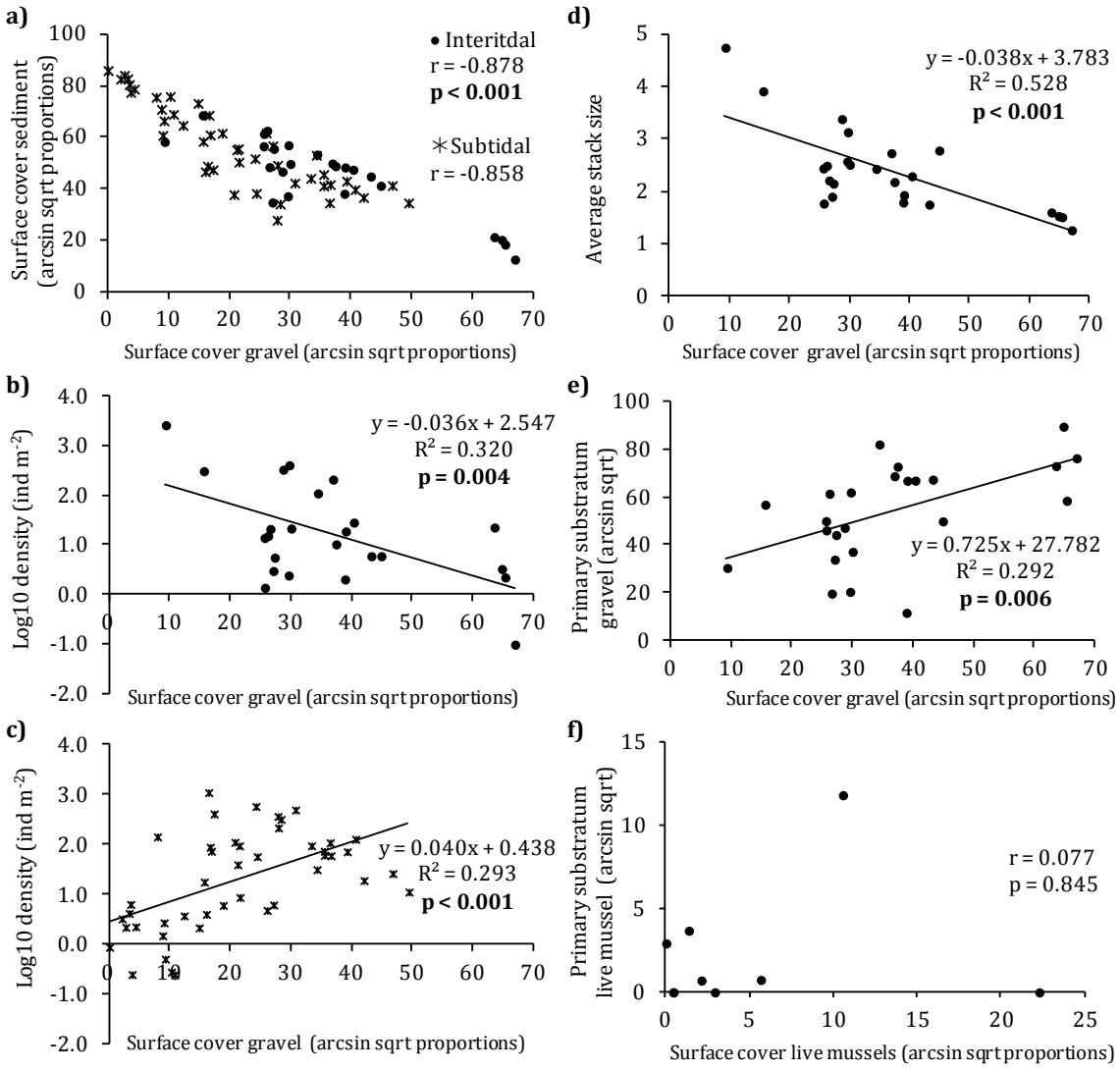



**Fig. 2.7:** Effect of habitat composition on adult *Crepidula fornicata* densities and dispersal in the Milford Haven Waterway. **a)** Correlation between two dominant substrata classes (sediment, grain size <16 mm, and gravel, grain size 16-256 mm) in the intertidal and subtidal transects. **b)** Intertidal percentage cover of gravel vs. average *C. fornicata* density of each intertidal transect. **c)** Subtidal percentage cover of gravel vs. average density of each subtidal transect. **d)** Intertidal percentage cover of gravel vs. average stack sizes. **e)** Intertidal percentage cover of gravel vs. the percentage of primary substrata that were gravel. **f)** Intertidal percentage cover of live mussels *Mytilus edulis* vs. the percentage of primary attachment substrata that were live mussels. Markers are means of all transect with densities >0.1 individuals $m^{-2}$. All percentage data was arc sine square root transformed and densities log10 transformed to fulfill assumptions of homoscedasticity and normality.

*Crepidula fornicata* densities also differed between the four study sites in the subtidal (factor site: $p<0.001$, Table 2.2). Densities at Pennar were highest, whilst there were generally no differences between the other three sites (Table 2.2). No differences were observed between the parallel transects surveyed with varying distance from 0 m above C.D. (factor distance from shore: $p>0.05$, Table 2.2). This was consistent between study sites (interaction site x distance from shore: $p>0.05$, Table 2.2). No clear relationship between intertidal and subtidal densities was apparent, as revealed by visual inspection of the data (Fig. 2.8). For example, whilst densities are high at Pennar in the intertidal as well as the subtidal, this is not the case for Hazelbeach or Cosheston, where densities were highest in the lowest intertidal height, but relatively low in the subtidal transects.

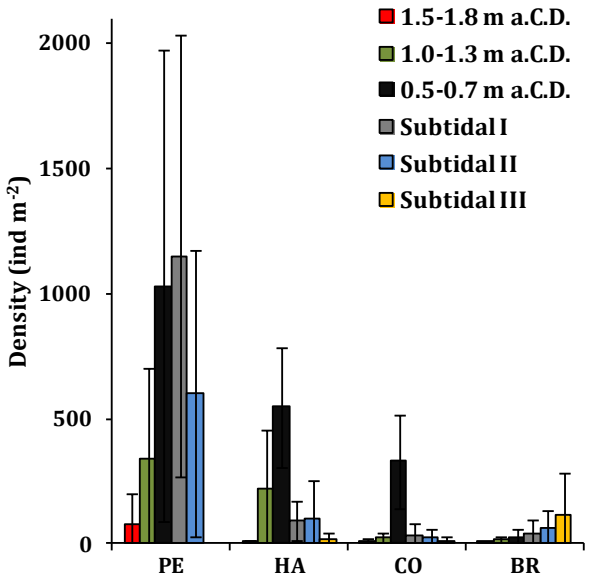



**Fig. 2.8:** Vertical distribution of *Crepidula fornicata* at Pennar (PE), Hazelbeach (HA), Cosheston (CO) and Beggars Reach (BR) in the Milford Haven Waterway. Bars are means (±SD) from ten 1 $m^2$ quadrats or 30 still images taken using a sled-mounted underwater still camera. Subtidal I: 50 m distance from ~0 m above C.D. Subtidal II: 150 m distance, Subtidal III: middle of the channel and ~250-500 m distance (n.a. for PE due to narrow width of channel at this site). Field work for subsequent data chapters was undertaken in the intertidal of a minimum of one of these four sites (see chapters 3-5).

**Table 2.1:** Vertical distribution of *Crepidula fornicata* in the intertidal zone of the Milford Haven Waterway in South Wales, UK. Results of non-parametric two-way crossed Scheirer-Ray-Hare test and Mann-Whitney pairwise comparisons to test for differences in *C. fornicata* densities between three tidal heights (*0.6 m* – 0.5-0.7 m above Chart Datum; *1.2 m* – 1.0-1.3 m a. C.D. and *1.8 m* –1.5-1.8 m a. C.D.) at four study sites (*BR* – Beggars Reach, *CO* – Cosheston, *HA* – Hazelbeach and *PE* – Pennar). Non-parametric tests were chosen due to heterogeneity in variances that could not be removed with data transformations. Significant differences are shown in bold.

| | | SS | $SS/MS_{total}$ | df | p |
|---|---|---|---|---|---|
| *site* | | 17540.0 | 33.8 | 3 | <**0.001** |
| *tidal height* | | 24980.6 | 48.2 | 2 | <**0.001** |
| *site x tidal height* | | 5525.9 | 10.7 | 6 | 0.100 |
| | | | *BR* | *CO* | *HA* |
| Mann-Whitney factor *site* | | *PE* | <**0.001** | **0.002** | 0.162 |
| | | *HA* | <**0.001** | 0.110 | |
| | | *CO* | **0.005** | | |
| | | | *1.8 m* | *1.2 m* | |
| Mann-Whitney factor *tidal height* | | *0.6 m* | <**0.001** | **0.001** | |
| | | *1.2 m* | <**0.001** | | |

**Table 2.2:** Distribution of *Crepidula fornicata* in the subtidal zone of the Milford Haven Waterway in South Wales, UK. Results of non-parametric two-way crossed Scheirer-Ray-Hare test and Mann-Whitney pairwise comparisons to test for differences in *Crepidula fornicata* densities between two parallel transects with varying distances to 0 m above C.D. at four study sites (*BR* – Beggars Reach, *CO* – Cosheston, *HA* – Hazelbeach and *PE* – Pennar). Non-parametric tests were chosen due to heterogeneity in variances that could not be removed with data transformations. Significant differences are shown in bold.

| | | SS | $SS/MS_{total}$ | df | p |
|---|---|---|---|---|---|
| *site* | | 99977.3 | 95.4 | 3 | <**0.001** |
| *distance to 0m a. C.D.* | | 476.0 | 0.5 | 1 | 0.500 |
| *site x distance* | | 2048.0 | 2.0 | 3 | 0.582 |
| | | | *BR* | *CO* | *HA* |
| Mann-Whitney factor *site* | | *PE* | <**0.001** | <**0.001** | <**0.001** |
| | | *HA* | 0.060 | <**0.001** | |
| | | *CO* | 0.133 | | |

## 2.5 DISCUSSION

Results of our intertidal and subtidal surveys suggest that the northernmost Welsh self-sustaining population of the American slipper limpet *C. fornicata* remains within the boundaries of the MHW, the location of its first introduction to Welsh coastal waters in the 1950s (Cole and Baird, 1953). We only found little evidence for a northwards range expansion: during our surveys, no live individuals were found outside the MHW. However, *C. fornicata* has been recorded from the SMNR, where several individuals were found in 2008 and then again in 2011 and July 2012 after the completion of the survey work that is presented in this chapter. Most likely, *C. fornicata* is very rare in the SMNR and the sampling effort employed in our study could not detect its presence at such low densities. Also, *C. fornicata* may only have started expanding its range in recent years, which would explain the increased frequency of its occurrence in the SMNR between 2008-2012 and its absence in the samples taken during our surveys in 2009.

We also found that, although densities inside the MHW are highly variable, local super-abundances are reached in the middle stretches of the ria, indicating that the prevailing regional environmental conditions are unlikely limiting its abundance at its northern range. The secondary spread of NNS following initial introduction and establishment in a region may be limited by the availability of suitable habitats (Colautti and MacIsaac, 2004). Inside the MHW, *C. fornicata* occurs across a variety of habitats. This observation makes it unlikely that *C. fornicata*'s absence to the north of the MHW is solely due to the absence of suitable habitat types. However, we found that *C. fornicata* only occurs in very low densities or is fully absent in certain homogeneous habitats such as boulder and sediment dominated areas. These were primarily located at the entrance of the MHW, possibly forming a dispersal barrier and hampering a rapid expansion of the species along the Welsh coast. *Crepidula fornicata*'s larval phase lasts ~2-4 weeks (Pechenik, 1980, 1984) which certainly aids its long distance dispersal from a source population to surrounding areas under prevailing local hydrodynamic conditions. Larval transport from the MHW to Mid Wales is likely, due to the prevailing northward direction of the water masses across the Irish Sea (Evans, 1995). A lack of optimal settlement substrata could however restrict settlement, potentially resulting in low

adult densities in an area and in a highly dispersed distribution of adults in the open coast. It is likely that this would minimise the chances for gregarious attachment and thus the establishment of a self-sustaining, breeding population beyond its original location of introduction, which may at least in part explain the absence of detectable numbers of *C. fornicata* in Mid and North Wales.

In its native range, *C. fornicata* is known to reach highest densities in shell-rich areas (Driscoll, 1967) and areas with high surface cover of hard substrata (Loomis and VanNieuwenhuyze, 1985). This seems self-evident, considering that *C. fornicata* needs hard substrata for settlement. Results of our study partly support this. In the subtidal survey stations, highest abundances were observed in mixed habitat types with high content of gravel and shell. We found a significant positive relationship between the percentage cover of gravel (grain size ~16-256 mm) and *C. fornicata* densities in the subtidal, indicating that this substrata type may provide suitable conditions for settlement and subsequent juvenile and adult survival.

Surprisingly, however, percentage cover of gravel and *C. fornicata* densities were negatively related in the intertidal stations. We can only speculate what causes this opposing pattern between intertidal and subtidal sampling stations. Besides habitats that are rich in hard substrata, high *C. fornicata* densities are often associated with high content of silt and clay (Driscoll, 1967; Barnes *et al.*, 1973), probably because high content of fine sediments is an indication of particularly sheltered conditions that are very suitable habitats for *C. fornicata*. The methodology for habitat classification we employed in our study resulted in a highly negative correlation between the percentage surface cover of the two most dominant substrata classes (sediment and gravel), as both had been recorded from the same images. The occurrence of high numbers of *C. fornicata* in areas with low content of gravel may thus in fact be an indication of a positive correlation with content of fine sediment. In the intertidal zone, where environmental conditions are generally more stressful compared to in the subtidal, sheltered conditions may be more important to support survival and establishment of *C. fornicata*. Thus, it is possible that the establishment of large numbers of *C. fornicata* in high energy environments (and therefore less sedimentary but more gravelly habitats)

is possible in the subtidal but not the intertidal, which would explain the opposing patterns observed between both zones.

In the intertidal, we found a highly positive relationship between the percentage cover of gravel and the utilisation of gravel as the attachment substratum of the bottom-most individual (as opposed to other substrata that were found to be present, such as live and dead shells). This may be interpreted as a trend that the availability of gravel results in dispersed (i.e. less clustered) distribution of adults, resulting in the more frequent creation of 'new' stacks when such settlement space is freely available. In contrast, in areas dominated by fine sediments, attachment might be "forced" to take place on top of conspecifics, as other substrata are scarce. This is in accordance with our observation that average stack size decreases with higher surface cover of gravel. It is possible that the availability of this substratum type does not only affect the local, clumped distribution of *C. fornicata*, but also the success with which the species disperses into previously un-colonised areas through the formation of 'pioneer stacks' by settling in isolation. In contrast to what has been observed for the substrata class gravel, the availability of live *M. edulis* did not cause an increase in the frequency with which live *M. edulis* were utilised as primary attachment substratum. This is in contrast to what has been found in the German Wadden Sea where *C. fornicata* is most commonly associated with mussel beds (Thieltges *et al.*, 2003). It is possible though that this is a reflection of the limited availability of other suitable hard attachment substrata in that study area. In contrast, our results imply that the availability of gravelly substrata, but not necessarily other hard substrata such as shellfish species, are an important factor in determining the distribution and potentially even facilitating the spread of *C. fornicata*, at least under intertidal conditions.

Post-colonisation processes such as juvenile movement and mortality have previously been found to be highly important in re-structuring the distribution of *C. fornicata* after settlement and therefore in determining adult distributional patterns (Shenk and Karlson, 1986; McGee and Targett, 1989). Early post-settlement mortality (EPSM) in particular may be crucial in determining adult distributional patterns, particularly in the intertidal zone where environmental conditions tend to be more stressful compared to the subtidal (Gosselin and Qian, 1997; Hunt and Scheibling, 1997). Support that EPSM

is highly important in determining the distribution of *C. fornicata* in the intertidal zone stems from comparisons of densities along the vertical shore gradient. Highest densities were always attained at the very low intertidal below ~0.7 m above C.D. and the species was almost absent above ~1.8 m above C.D., irrespective of overall nearby abundance. Whether the strong vertical zonation is due to the differential supply of larvae to these intertidal heights, selective settlement of the larvae, re-location of the juveniles after settlement, or differential mortality acting on the juvenile or the adult phase is unknown. However, densities in the subtidal zone never varied as strongly as densities between the different intertidal heights, which suggests that EPSM as a result of tidal elevation is high in the intertidal, likely causing the observed differential adult distributional patterns. Other factors are more unlikely: juvenile movement between discrete hard substrata is unlikely in sediment-rich shores. Also, the long larval phase decreases the likelihood of differential supply at a scale of meters, especially when weak-swimming larvae are faced with a strong tidal regime as in the MHW (Nelson-Smith, 1965). Selective settlement behaviour of *C. fornicata* larvae may affect adult distributional patterns. Previous work has shown that highest numbers of juveniles are commonly found in areas with high adult abundance, thus also likely determining adult distributional patterns (Walne, 1956; Hoagland, 1978; McGee and Targett, 1989). Data from our study, however, show that even if gregarious settlement takes place, this is not reflected in the intertidal distribution of adults. Stack sizes are relatively low, especially in gravel-rich areas, and gravel availability seems to determine adult distribution between substrata types, suggesting that gregarious settlement choice is of minor importance in determining the distribution of adults, compared to other factors or processes such as gravel availability and EPSM. The presence of dense *C. fornicata* beds in the subtidal, where EPSM is less strong, however make it unlikely that these processes explain *C. fornicata*'s failing northwards expansion. In fact, our data even suggest that the availability of gravel may increase dispersal potential and colonisation of *C. fornicata* to areas that were previously un-occupied.

In our study, we could not confirm the presence of *C. fornicata* in the SMNR or Cardigan Bay, despite the fact that live and dead shells have been found in previous years and very recently after the surveys for this chapter had been finished. This could be due to limited sampling effort employed in our surveys and the resultant failure to detect

*C. fornicata* if it occurred at extremely low densities. However, live individuals have only been found on three occasions in the SMNR, despite the very frequent monitoring work that is carried out by CCW, making it unlikely that a dense population is already fully established. It is more likely that the expansion of the species into areas north of the MHW is at its start now. This would be surprisingly late as its initial introduction occurred prior to 1953 (Cole and Baird, 1953). In comparison, *C. fornicata* spread within only 40 years along the full extent of the UK coast of the English channel, where it was first observed in East Sussex in 1908/ 1909 (Orton, 1915) and by 1946 in Cornwall (Cole, 1952). Although human mediated vectors such as fouled ships' hulls and transport of larvae in ballast water may have facilitated its spread, it is assumed that a main reason for this fast range expansion was natural larval dispersal (Orton, 1915). Similarly, larval dispersal is also likely to occur from the MHW to the north with the prevailing oceanographic conditions in the Irish Sea. However, directly limiting factors such as unsuitable prevailing seawater temperatures (Thieltges *et al.*, 2004; Richard *et al.*, 2006; Valdizan *et al.*, 2011) could explain the failing northwards spread of *C. fornicata* from the MHW, which will be focus of Chapter 3. The results from this present chapter suggest that habitat availability, larval habitat choice and EPSM may be crucial processes in explaining the dispersal potential and secondary spread success of this potentially harmful NNS. Later chapters will focus more closely on some these processes, especially the relative importance of larval supply, larval habitat selection and EPSM in shaping the distribution of adults in the intertidal zone (see Chapters 4 and 5).

## APPENDIX 2.1: SUMMARY OF SURVEY STATIONS

All stations in the Milford Haven Waterway in South West Wales, UK, in which a minimum of three intertidal and two subtidal transects were surveyed for the presence and abundance of *Crepidula fornicata*. Start and end coordinates are in decimal degrees. Study sites for Chapter 3-5 are highlighted in grey.

| Site | Intertidal Height/ Distance from Shore+Depth | *Crepidula fornicata* density (mean±SD) | Habitat type | Start Coordinate | | End Coordinate | | Survey Method + Date |
|---|---|---|---|---|---|---|---|---|
| | | | | Long | Lat | Long | Lat | |
| Dale Intertidal 1 | 1.0–1.3 m a. C.D. | 3.4±3.6 | Gravel | 51.70430 | -5.15901 | 51.70448 | -5.15642 | Intertidal, quadrat, 26/02/2009 |
| Dale Intertidal 2 | 1.0–1.3 m a. C.D. | 0.1±0.3 | Gravel | 51.70426 | -5.15550 | 51.70411 | -5.15288 | Intertidal, quadrat, 26/02/2009 |
| Dale Intertidal 3 | 1.0–1.3 m a. C.D. | 0 | Gravel with boulder | 51.70350 | -5.15107 | 51.70414 | -5.15256 | Intertidal, quadrat, 26/02/2009 |
| Dale Subtidal I | 50 m distance, 2 m below C.D. | 0 | Sediment | 51.70462 | -5.15337 | 51.7042 | -5.15132 | Subtidal, underwater stills camera |
| Dale Subtidal II | 150 m distance, 3 m below C.D | 0 | Sediment | 51.70575 | -5.15308 | 51.70515 | -5.15108 | Subtidal, underwater stills camera |
| Dale Subtidal III | 500 m distance, 4 m below C.D. | 2.2±6.0 | Sediment | 51.70882 | -5.15235 | 51.70758 | -5.15052 | Subtidal, underwater stills camera |
| Sandy Haven Intertidal 1 | 1.0–1.3 m a. C.D. | 0 | Sediment | 51.72412 | -5.10683 | 51.72023 | -5.10436 | Intertidal, quadrat, 09/03/2009 |
| Sandy Haven Intertidal 2 | 1.0–1.3 m a. C.D. | present | Mix of sediment and gravel | 51.71737 | -5.10583 | 51.71787 | -5.10645 | Intertidal, quadrat, 09/03/2009 |
| Sandy Haven Intertidal 3 | 1.0–1.3 m a. C.D. | 0 | Sediment | 51.71787 | -5.10645 | 51.71911 | -5.09612 | Intertidal, quadrat, 09/03/2009 |
| Sandy Haven Subtidal I | 50 m distance, 2 m below C.D. | 0 | Sediment | 51.71798 | -5.10393 | 51.71653 | -5.10433 | Subtidal, underwater stills camera |
| Sandy Haven Subtidal II | n.a. (obstructed by rocks) | --- | --- | --- | --- | --- | --- | --- |
| Sandy Haven Subtidal III | 500 m distance, 5 m below C.D. | 0 | Sediment | 51.71433 | -5.10012 | 51.71302 | -5.10105 | Subtidal, underwater stills camera |

| Site | Intertidal Height/ Distance from Shore+Depth | *Crepidula fornicata* density (mean±SD) | Habitat type | Start Coordinate | | End Coordinate | | Survey Method + Date |
|---|---|---|---|---|---|---|---|---|
| | | | | Long | Lat | Long | Lat | |
| Angle Bay Intertidal 1 | 1.0–1.3 m a. C.D. | 23.6±38.1 | Gravel | 51.69339 | -5.05150 | 51.69202 | -5.05190 | Intertidal, quadrat, 25/02/2009 |
| Angle Bay Intertidal 2 | 1.0–1.3 m a. C.D. | 0 | Sediment | 51.69168 | -5.05203 | 51.69014 | -5.05297 | Intertidal, quadrat, 25/02/2009 |
| Angle Bay Intertidal 3 | 1.0–1.3 m a. C.D. | 2.3±2.6 | Gravel | 51.68985 | -5.05329 | 51.68882 | -5.05494 | Intertidal, quadrat, 25/02/2009 |
| Angle Bay Subtidal I | 50 m distance, 1 m below C.D. | 2.2±7.0 | Sediment | 51.69033 | -5.05393 | 51.69323 | -5.05327 | Subtidal, underwater stills camera |
| Angle Bay Subtidal II | 150 m distance, 1 m below C.D. | 0 | Sediment | 51.69062 | -5.05535 | 51.69188 | -5.05445 | Subtidal, underwater stills camera |
| Angle Bay Subtidal III | 500 m distance, 1 m below C.D. | 0 | Sediment | 51.69137 | -5.05932 | 51.68998 | -5.06108 | Subtidal, underwater stills camera |
| Pwllcrochan Intertidal I | 1.0–1.3 m a. C.D. | 321.0±320.7 | Sediment | 51.41549 | -5.00791 | 51.41563 | -5.00713 | Intertidal, quadrat, 11/04/2009 |
| Pwllcrochan Intertidal II | 1.0–1.3 m a. C.D. | 424.8±281.9 | Sediment with gravel | 51.41562 | -5.00674 | 51.41553 | -5.00613 | Intertidal, quadrat, 11/04/2009 |
| Pwllcrochan Intertidal III | 1.0–1.3 m a. C.D. | 2747.8±3859.3 | Sediment with shell | 51.41538 | -5.00391 | 51.41518 | -5.00550 | Intertidal, quadrat, 11/04/2009 |
| Pwllcrochan Subtidal I | 50 m distance, 1 m below C.D. | 3.3±12.4 | Sediment | 51.69573 | -5.01007 | 51.6958 | -5.01255 | Subtidal, underwater stills camera |
| Pwllcrochan Subtidal II | 150 m distance, 1 m below C.D. | 4.3±15.6 | Sediment | 51.69662 | -5.01043 | 51.69692 | -5.01270 | Subtidal, underwater stills camera |
| Pwllcrochan Subtidal III | 500 m distance, 15 m below C.D. | 3.8±10.3 | Sediment | 51.69965 | -5.00918 | 51.70005 | -5.01168 | Subtidal, underwater stills camera |

| Site | Intertidal Height/ Distance from Shore+Depth | *Crepidula fornicata* density (mean±SD) | Habitat type | Start Coordinate | | End Coordinate | | Survey Method + Date |
|---|---|---|---|---|---|---|---|---|
| | | | | Long | Lat | Long | Lat | |
| Pennar Intertidal 1 | 1.0–1.3. m a. C.D. | 115.6±85.8 | Mix of sediment and gravel | 51.68890 | -4.97481 | 51.68819 | -4.97695 | Intertidal, quadrat, 10/03/2009 |
| Pennar Intertidal 3 | 1.0–1.3 m a. C.D. | 10.8±16.3 | Mix of sediment and gravel | 51.68601 | -4.97467 | 51.68567 | -4.97316 | Intertidal, quadrat, 10/03/2009 |
| **Pennar Intertidal 2 (high)** | **1.5-1.8 m a. C.D.** | **76±124.7** | **Mix of sediment, gravel and shell** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **Intertidal, quadrat, 19/09/2009** |
| **Pennar Intertidal 2 (mid)** | **1.0–1.3 m a. C.D.** | **343.0±359.7** | **Mix of sediment, gravel and shell** | **51.68795** | **-4.97707** | **51.68671** | **-4.97661** | **Intertidal, quadrat, 10/03/2009** |
| **Pennar Intertidal 2 (low)** | **0.5-0.7 m a. C.D.** | **1031.4±943.4** | **Mix of sediment, gravel and shell** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **Intertidal, quadrat, 19/09/2009** |
| Pennar Subtidal I | 50 m distance, 5 m below C.D. | 1151.8±881.1 | Mix of sediment and shell | 51.68612 | -4.97803 | 51.68743 | -4.97832 | Subtidal, underwater stills camera |
| Pennar Subtidal II | 150 m distance, 5 m below C.D. | 601.2±576.3 | Mix of sediment, gravel and shell | 51.68757 | -4.9793 | 51.68943 | -4.97983 | Subtidal, underwater stills camera |
| Pennar Subtidal III | n.a. channel too narrow | --- | --- | --- | --- | --- | --- | --- |
| Hazelbeach Intertidal 1 | 1.0–1.3 m a. C.D. | 15.8±13.8 | Sediment with gravel | 51.70025 | -4.97946 | 51.70087 | -4.97731 | Intertidal, quadrat, 11/03/2009 |
| Hazelbeach Intertidal 3 | 1.0–1.3 m a. C.D. | 19.7±20.7 | Mix of sediment and gravel | 51.70336 | -4.97144 | 51.70405 | -4.97045 | Intertidal, quadrat, 11/03/2009 |
| **Hazelbeach Intertidal 2 (high)** | **1.5-1.8 m a. C.D.** | **4.4±6.5** | **Mix of sediment and gravel** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **Intertidal, quadrat, 10/10/2010** |
| **Hazelbeach Intertidal 2 (mid)** | **1.0–1.3 m a. C.D.** | **216.3±239.7** | **Mix of sediment and gravel** | **51.70124** | **-4.97610** | **51.70224** | **-4.97422** | **Intertidal, quadrat, 11/03/2009** |
| **Hazelbeach Intertidal 2 (low)** | **0.5-0.7 m a. C.D.** | **546.8±238.4** | **Mix of sediment, gravel and shell** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **Intertidal, quadrat, 10/10/2010** |
| Hazelbeach Subtidal I | 50 m distance, 2 m below C.D. | 91.0±80.0 | Sediment | 51.70043 | -4.97645 | 51.70183 | -4.9741 | Subtidal, underwater stills camera |
| Hazelbeach Subtidal II | 150 m distance, 5 m below C.D. | 97.5±158.6 | Mix of sediment, gravel and shell | 51.70107 | -4.97303 | 51.70002 | -4.97463 | Subtidal, underwater stills camera |
| Hazelbeach Subtidal III | 500 m distance, 10 m below C.D. | 18.3±24.4 | Sediment with shell | 51.69773 | -4.97178 | 51.69697 | -4.97388 | Subtidal, underwater stills camera |

| Site | Intertidal Height/ Distance from Shore+Depth | *Crepidula fornicata* density (mean±SD) | Habitat type | Start Coordinate | | End Coordinate | | Survey Method + Date |
|---|---|---|---|---|---|---|---|---|
| | | | | Long | Lat | Long | Lat | |
| Cosheston Intertidal 2 | 1.0–1.3 m a. C.D. | 29.4±39.3 | Mix of sediment and gravel | 51.70622 | -4.90802 | 51.70703 | -4.90763 | Intertidal, quadrat, 09/04/2009 |
| Cosheston Intertidal 3 | 1.0–1.3 m a. C.D. | 2.5±5.4 | Mussel bed mixed with sediment, gravel, shell | 51.70775 | -4.90702 | 51.70812 | -4.90597 | Intertidal, quadrat, 09/04/2009 |
| **Cosheston Intertidal 1 (high)** | **1.5-1.8 m a. C.D.** | **8.8±14.8** | **Mix of sediment, gravel and shell** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **Intertidal, quadrat, 20/09/2009** |
| **Cosheston Intertidal 1 (mid)** | **1.0–1.3 m a. C.D.** | **22.5±17.5** | **Mix of sediment, gravel and shell** | **51.70465** | **-4.90882** | **51.70550** | **-4.90825** | **Intertidal, quadrat, 09/04/2009** |
| **Cosheston Intertidal 1 (low)** | **0.5-0.7 m a. C.D.** | **328.8±188.0** | **Mix of sediment, gravel and shell** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **Intertidal, quadrat, 20/09/2009** |
| Cosheston Subtidal I | 50 m distance, 1 m below C.D. | 32.2±48.4 | Mix of sediment and gravel | 51.7071 | -4.90873 | 51.7059 | -4.91007 | Subtidal, underwater stills camera |
| Cosheston Subtidal II | 150 m distance, 2 m below C.D. | 26.9±30.6 | Mix of sediment and gravel | 51.70645 | -4.91082 | 51.70772 | -4.90867 | Subtidal, underwater stills camera |
| Cosheston Subtidal III | 250 m distance, 6 m below C.D. | 11.4±15.4 | Mix of sediment and gravel | 51.70658 | -4.91237 | 51.70798 | -4.91043 | Subtidal, underwater stills camera |
| Jenkins Point Intertidal 1 | 1.0–1.3 m a. C.D. | 3.10±4.79 | Mussel bed mixed with sediment, gravel, shell | 51.71698 | -4.87970 | 51.71722 | -4.88150 | Intertidal, quadrat, 10/04/2009 |
| Jenkins Point Intertidal 2 | 1.0–1.3 m a. C.D. | 6.2±6.3 | Mix of sediment and gravel | 51.71740 | -4.88310 | 51.71735 | -4.88493 | Intertidal, quadrat, 10/04/2009 |
| Jenkins Point Intertidal 3 | 1.0–1.3 m a. C.D. | 6.1±5.1 | Mix of sediment and gravel | 51.71653 | -4.88678 | 51.71552 | -4.88727 | Intertidal, quadrat, 10/04/2009 |
| Jenkins Point Subtidal I | 50 m distance, 5 m below C.D. | 63.3±40.3 | Mix of sediment, gravel and shell | 51.71595 | -4.8881 | 51.71777 | -4.88708 | Subtidal, underwater stills camera |
| Jenkins Point Subtidal II | 150 m distance, 10 m below C.D. | 61.1±70.9 | Mix of sediment, gravel and shell | 51.71665 | -4.88912 | 51.71833 | -4.88793 | Subtidal, underwater stills camera |
| Jenkins Point Subtidal III | n.a. channel too narrow | --- | --- | --- | --- | --- | --- | --- |

| Site | Intertidal Height/ Distance from Shore+Depth | *Crepidula fornicata* density (mean±SD) | Habitat type | Start Coordinate | | End Coordinate | | Survey Method + Date |
|---|---|---|---|---|---|---|---|---|
| | | | | Long | Lat | Long | Lat | |
| Beggars Reach Intertidal 1 | 1.0–1.3 m a. C.D. | 5.7±9.1 | Sediment with gravel | 51.73807 | -4.89267 | 51.73840 | -4.89412 | Intertidal, quadrat, 12/04/2009 |
| Beggars Reach Intertidal 3 | 1.0–1.3 m a. C.D. | 21.8±38.9 | Mix of sediment, gravel and shell | 51.73967 | -4.89695 | 51.74013 | -4.89753 | Intertidal, quadrat, 12/04/2009 |
| **Beggars Reach Intertidal 2 (high)** | **1.5-1.8 m a. C.D.** | **0.2±0.6** | **Sediment with gravel** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **Intertidal, quadrat, 07/10/2010** |
| **Beggars Reach Intertidal 2 (mid)** | **1.0–1.3 m a. C.D.** | **14.6±13.3** | **Sediment with gravel** | **51.73885** | **-4.89510** | **51.73938** | **-4.89650** | **Intertidal, quadrat, 12/04/2009** |
| **Beggars Reach Intertidal 2 (low)** | **0.5-0.7 m a. C.D.** | **23.8±34.3** | **Mix of sediment, gravel and shell** | **n.a.** | **n.a.** | **n.a.** | **n.a.** | **Intertidal, quadrat, 07/10/2010** |
| Beggars Reach Subtidal I | 50 m distance, 2 m below C.D. | 40.9±57.1 | Mix of sediment, gravel and shell | 51.73817 | -4.89518 | 51.73913 | -4.89765 | Subtidal, underwater stills camera |
| Beggars Reach Subtidal II | 150 m distance, 6 m below C.D. | 59.6±74.2 | Mix of sediment, gravel and shell | 51.73718 | -4.89518 | 51.73822 | -4.89775 | Subtidal, underwater stills camera |
| Beggars Reach Subtidal III | 230 m distance, 5 m below C.D. | 114.7±169.4 | Mix of sediment, gravel and shell | 51.73683 | -4.89655 | 51.73783 | -4.89903 | Subtidal, underwater stills camera |
| Black Tar Intertidal 1 | 1.0–1.3 m a. C.D. | 1.4±2.1 | Sediment with gravel | 51.74588 | -4.90045 | 51.74660 | -4.89932 | Intertidal, quadrat, 07/04/2009 |
| Black Tar Intertidal 2 | 1.0–1.3 m a. C.D. | 2.1±4.6 | Mix of sediment, gravel and shell | 51.74708 | -4.89872 | 51.74792 | -4.89870 | Intertidal, quadrat, 07/04/2009 |
| Black Tar Intertidal 3 | 1.0–1.3 m a. C.D. | 0 | Mix of boulder, gravel and sediment | 51.74863 | -4.89912 | 51.74952 | -4.89957 | Intertidal, quadrat, 07/04/2009 |
| Black Tar Subtidal I | n.a. obstructed by moorings | --- | --- | --- | --- | --- | --- | --- |
| Black Tar Subtidal II | 150 m distance, 1 m below C.D. | 9.0±15.9 | Mix of sediment, gravel and shell | 51.74413 | -4.89845 | 51.7458 | -4.89710 | Subtidal, underwater stills camera |
| Black Tar Subtidal III | 350 m distance, 4 m below C.D. | 6.1±12.3 | Mix of sediment, gravel and shell | 51.74287 | -4.89768 | 51.7445 | -4.89615 | Subtidal, underwater stills camera |

# CHAPTER 3 -

## The invasive gastropod *Crepidula fornicata*: Reproduction and recruitment in the intertidal at its northernmost range in Wales, UK and implications for its secondary spread

## 3.1 Abstract

The establishment and spread of a non-native species in an introduced range depends to a large extent on the performance of the species under the prevailing environmental conditions. The spawning, larval, and spatfall periods of the invasive gastropod *Crepidula fornicata* were monitored in the intertidal zone at its northernmost range in Wales, UK between February 2010 and January 2011. The duration of the reproductive season was similar to that recorded from more southerly European populations. Spawning and larval release occurred throughout most of the year even at low seawater temperatures of <7°C, but benthic recruitment was observed over a much shorter period at seawater temperatures >16°C. Recruitment was low and likely controlled by post-settlement mortality. These observations suggest that *C. fornicata*'s northwards spread in Welsh waters will not be limited by seawater temperature negatively affecting reproduction, but by processes acting after larval release. These data show the importance of incorporating settlement and post-settlement processes into studies on recruitment success when aiming to predict the potential spread of a potentially harmful invader such as *C. fornicata*.

Keywords: reproduction, recruitment, temperature, secondary spread, northwards range extension

## 3.2 Introduction

The spread of non-native species (NNS) poses a serious environmental threat worldwide due to their negative impacts on native species biodiversity and ecosystem processes in the recipient habitats (Grosholz, 2002; Stachowicz *et al.*, 2002). Understanding the processes that determine the success of NNS is critical if their ecological and economic impacts are to be mitigated. After initial introduction and successful establishment, the secondary spread of NNS may be controlled by a variety of factors including their dispersal potential, the availability of suitable habitats, and their ability to cope with biotic and abiotic conditions in the novel environment (Colautti and MacIsaac, 2004). Amongst the latter, prevailing seawater temperatures may be one of the most important factors in determining the range a marine NNS may occupy in the novel area. Exposure to temperatures close to the thermal tolerance limits of the species may restrict its local abundance and geographical spread in the new environment (Chapman, 2000; Colautti and MacIsaac, 2004).

Once established, the expansion of NNS may be limited by similar processes to those acting on native species. Warming seawater temperatures in North West Europe are thought to have resulted in increases in abundance and northward expansion of a number of southern Lusitanean species (Hiscock *et al.*, 2004; Mieszkowska *et al.*, 2005; Mieszkowska *et al.*, 2006; Hawkins *et al.*, 2008; Masuda, 2008). Temperature-induced phenological changes such as an extended reproductive season (Moore *et al.*, 2011; Valdizan *et al.*, 2011) may be one important mechanism by which breeding populations can establish beyond their normal northern range limit (Hawkins *et al.*, 2008). The expected rising seawater temperatures as a consequence of global climate change are therefore expected to facilitate the spread of NNS, by providing more suitable conditions during all stages of the invasion process, including their secondary spread (Stachowicz *et al.*, 2002; Walther *et al.*, 2002; Rahel and Olden, 2008; Walther *et al.*, 2009). However the effects of temperature often act through extreme events, such as extremely cold winters that may cause high mortality especially of intertidal species and may also restrict their distributions and abundances. For example, the exceptionally cold winter of 1962-1963 led to the high mortality and sudden decline in the abundance

of several southern intertidal species along the British Isles, especially along their northern range limit (Crisp, 1964). Such mass mortality events were also found to greatly affect the abundance of several NNS, especially at their northern distributional limit (Thieltges *et al.*, 2004; Buettger *et al.*, 2011; Canning-Clode *et al.*, 2011; Firth *et al.*, 2011).

Temperature effects on a NNS at its range limit may act on several if not all stages of its life cycle. Mass mortality events have been documented in adults, for example of the Asian green mussel *Perna viridis* (Firth *et al.*, 2011)and the Pacific oyster *Crassostrea gigas* (Buettger *et al.*, 2011), as a result of extreme low air temperatures during winter. However early life stages are generally more vulnerable (deRivera *et al.*, 2007). For example sub-optimal temperatures were found to negatively affect spawning, larval development, spatfall and subsequent juvenile survival in *C. gigas* and this has led to recruitment failure in some regions (Spencer *et al.*, 1994; Child and Laing, 1998; Diederich *et al.*, 2005; Dutertre *et al.*, 2010). Conditions however need to be suitable for all life cycle stages so that recruitment, and hence spread of the species into a new environment, can be successful.

The non-native American slipper limpet *Crepidula fornicata* spread rapidly throughout most European waters following its first introduction from the North American Atlantic coast in the late 19$^{th}$ century. Its non-native range now spans from the Mediterranean Sea in the south to the Irish Sea and Norwegian coastal waters in the north (Blanchard, 1997; McNeill *et al.*, 2010). Whilst abundances at its northern and southern geographical range limit are relatively low, very dense populations can be found in France and the UK (Barnes *et al.*, 1973; Blanchard, 1997, 2009; Hinz *et al.*, 2011). Several recent studies have shown that the low proliferation of *C. fornicata* in some areas and its rapid population increase at other locations may be strongly linked to the prevailing climatic conditions affecting the adult as well as the larval life cycle stage (Thieltges *et al.*, 2004; Richard *et al.*, 2006; Rigal, 2009; Valdizan *et al.*, 2011). For example, the reproduction of adult *C. fornicata* is strongly regulated by seawater temperature with spawning occurring above 6-7°C or 10°C, depending on location (Werner, 1948; Chipperfield, 1951; Hoagland, 1979; Thieltges *et al.*, 2004; Richard *et al.*, 2006; Valdizan *et al.*, 2011). During the last decade, warmer seawater temperatures

along the French coast have resulted in the earlier appearance of egg-brooding females, resulting in an extended brooding period (Valdizan *et al.*, 2011). The authors suggest that this may also prolong the time period in which benthic recruitment is possible and may have caused the recent increases in adult abundances observed in the area (Richard *et al.*, 2006; Valdizan *et al.*, 2011). Besides these limiting effects of spring and summer seawater temperature on reproductive output, there is also evidence that low winter air temperatures may limit *C. fornicata* populations directly through enhanced mortality of the adults. This was observed in the German Wadden Sea, where populations are mainly restricted to the low intertidal and shallow subtidal zone, making them highly susceptible to direct or indirect effects of freezing air temperatures (Thieltges *et al.*, 2004). Reproduction however was unaffected in this area - summer and spring seawater temperatures are well above those required to induce spawning (Thieltges *et al.*, 2004). Milder winters in recent years are thought to have been the main reason for the observed increase in abundance in the area (Nehls *et al.*, 2006). Lastly, low seawater temperatures have also been shown to negatively impact the larval stage, through negative effects on larval development and mortality reaching almost 100% at seawater temperatures <12°C (Rigal, 2009).

The Milford Haven Waterway (MHW) in South West Wales, UK, is a natural ria with populations of *C. fornicata* firmly established since the species first occurred in Welsh waters in the 1950s (Cole and Baird, 1953). *Crepidula fornicata* spread rapidly within the ria (Crothers, 1966), and now reaches local abundances of >1000 individuals $m^{-2}$ in both the intertidal and subtidal zone (Chapter 2). 60 years after *C. fornicata*'s initial colonisation of the MHW there has been no observed northward spread until today, and the MHW still seems to contain the northernmost self-sustaining population of the species along the Welsh coastline. Colonisation to the south of the MHW however has been successful (Mettam, 1979, NBN Gateway at http://www.nbn.org.uk). In order to understand the limiting factors preventing the northward spread of *C. fornicata* from the MHW a series of investigations were undertaken to determine patterns of reproduction and recruitment at this northern limit in relation to ambient air and sea temperatures over a one year period. Whilst most current evaluations of *C. fornicata*'s invasion success have been based upon studies on the length of the larval and spawning periods, and the effects of low air or seawater temperatures on adult or larval

survivability, studies have rarely investigated all life cycle stages simultaneously. Settlement and post-settlement processes have received little attention so far, despite the fact that they are known to be highly important in determining adult abundances and distributions of other benthic invertebrates (see Hunt and Scheibling, 1997 for review). This study is the first to quantitatively monitor the length of the reproductive and recruitment season of *C. fornicata* by simultaneously studying three main processes of the early life cycle stages. Firstly, the spawning period was determined by monitoring adults for the presence of females with laid egg capsules. Previous studies have shown that this is a good predictor of the length of the reproductive season, as periods of egg-brooding correspond well with periods of gametogenic activity, followed by ovoposition (Beninger *et al.*, 2010a, 2010b). The same samples were used to determine the adult sex ratios at these locations as an estimate of recruitment dynamics of the populations. Secondly, the larval period was determined through monthly plankton sampling. Thirdly, the period during which benthic recruitment occurred was monitored by installing artificial slate settlement substrata. Their reliability in estimating spat densities was assured by comparisons with spat densities collected on natural substrata. These data on reproductive output and recruitment were matched with temperature data available from two sites inside the MHW where *C. fornicata* is present, and two sites in South West and North Wales where *C. fornicata* is absent (the Skomer Marine Nature Reserve (SMNR) and the Menai Strait, respectively, Fig. 3.1).

## 3.3 MATERIALS AND METHODS

### 3.3.1 STUDY SITE

Biological data were collected from the low intertidal zone (~1.0-1.3 m above C.D.) of four sites in the MHW in South Wales, UK: Beggars Reach (BR, 51°44'22 N, 4°53'46 W), Cosheston (CO, 51°42'17 N, 4°54'30 W), Hazelbeach (HA, 51°42'04 N, 4°58'31 W), and Pennar (PE, 51°41'13 N, 4°58'37 W) (Fig. 3.1). The sites are located in the middle stretches of the ria where *C. fornicata* is well established and were chosen to cover the high variation of abundances of *C. fornicata* representative for that tidal height in the

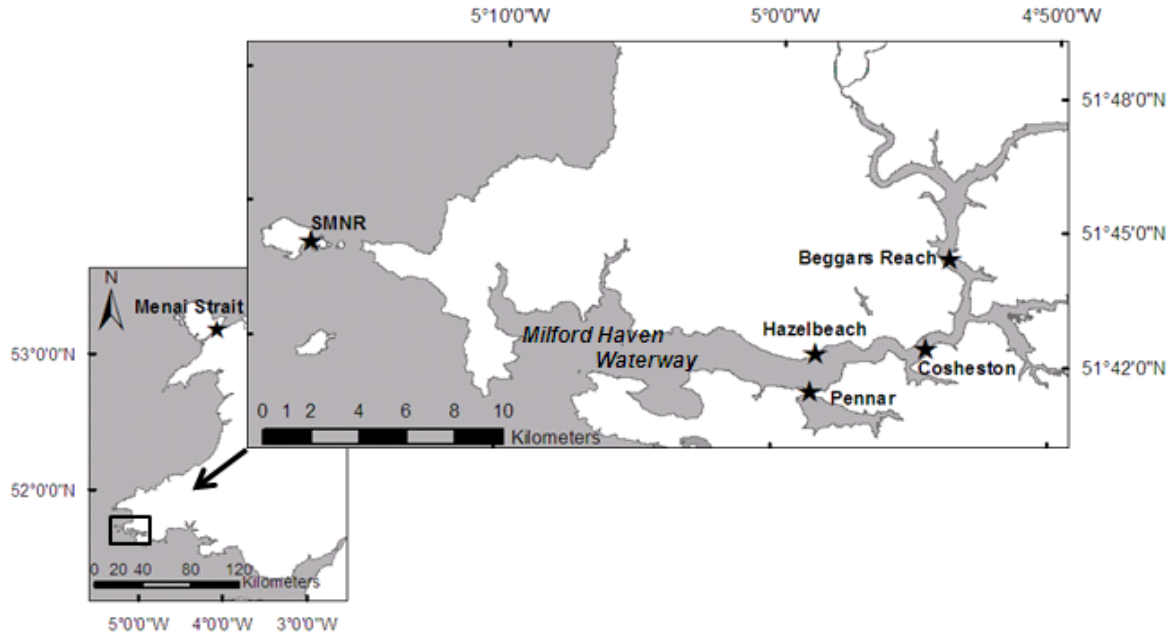



**Fig. 3.1:** Map of Wales, UK (left) and the Milford Haven Waterway (MHW) in South West Wales (right) with the four study sites (Pennar - PE, Hazelbeach - HA, Cosheston - CO and Beggars Reach - BR). Temperature loggers were installed in the low intertidal at Hazelbeach and Beggars Reach in the Milford Haven Waterway, as well as the Skomer Marine Nature Reserve (SMNR, South West Wales) and the Menai Strait (North Wales)

MHW (mean±SD individuals $m^{-2}$ at BR: 15±13, CO: 23±18, HA: 216±240, PE: 343±360, Chapter 2). The low intertidal of all four study sites consists of muddy-gravelly substrates. Due to the relatively low input of freshwater into the MHW, marine conditions prevail in the ria and support a diverse assemblage of marine flora and fauna (for details on the biological environment, see Nelson-Smith, 1967). Salinity in the study area ranges from fully saline conditions of ~34-35 psu at PE and HA closer to the mouth of the ria to a more variable salinity regime in the upper reaches of the MHW (Nelson-Smith, 1965; Bohn pers. obs.). For example, at BR, located furthest up the ria, surface salinity varies between ~18-34 psu, depending on tidal and weather conditions. Where appropriate, comparisons were made among locations to assess the generality of response over these spatial scales, rather than to test any specific hypothesis about the particular locations used.

### 3.3.2 TEMPERATURE MONITORING

Temperature loggers (DS1921Z-F5 Thermochrom, iButton®) were installed to record every 30-60 min at BR and HA in the MHW at the same intertidal height where the monitoring work was undertaken. The static loggers recorded the seawater temperature when submersed as well as the air temperature during emersion at low water. Daily average seawater temperatures were calculated for each location by removing data recorded during emersion at low tide. Minimum and maximum temperatures were extracted per 24 h using all data (i.e. including recordings during emersion). For comparison, seawater temperature data were obtained from two locations outside the MHW: the SMNR and the Menai Strait (Fig. 1). In the SMNR, seawater temperature was measured in the same way as in the MHW (i.e. through installation of static temperature loggers in the low intertidal zone and by removing data recorded during emersion at low water). Weekly recordings of sea surface temperature were available for the Menai Strait in North Wales.

### 3.3.3 SEXUAL STAGES, SPAWNING PERIOD AND EMBRYONIC DEVELOPMENT

A minimum of 100 *C. fornicata* were collected at CO and HA by carefully searching four to ten randomly placed 0.25 $m^2$ quadrats for any live *C. fornicata*. Individuals <5mm were excluded from later analyses due to the difficulty of spotting small slipper limpets in the field. Samples from both sites were first taken at the beginning of February 2010 and monthly thereafter until sampling was terminated after egg-brooding females were no longer found in October 2010 (see results). Following collection, all individuals were carefully removed from their attachment substrata to determine the sexual stages of each individual and to check for the presence of laid egg capsules. Sexual stages of all collected specimens were determined under a dissecting microscope using the following categories (adapted from Orton, 1909; Richard *et al.*, 2006):

1) Juvenile – absence of a well-developed penis, uterus and genital papillae
2) Male – presence of a well-developed penis
3) Intermediate stage – presence of a penis as well as an uterus and/or genital papillae
4) Female – presence of a well-developed uterus and genital papillae

Using the numbers of males and females identified in each sample, the sex-ratio was calculated as the proportion of males of the total number of males and females (e.g. Collin, 2006), for each site and sampling event. Juvenile proportions were calculated as the number of juveniles of all sampled individuals (i.e. the total number of juveniles, males, intermediates and females) for each study site and sampling event. The sex ratios, as well as the juvenile proportions, were compared between both study sites with independent t-tests using the calculated sex ratios or juvenile proportions of the different sampling events as replicates. Normality and homogeneity of variances were established using the Kolmogorov-Smirnov- and Levene's test, respectively. Arcsine square root transformation was applied to the juvenile proportions to achieve homogeneous variances.

The duration of the active reproductive period was estimated from the presence of laid egg masses under the females. The embryonic development of all broods was determined to estimate the onset of spawning more precisely than would be possible by four-weekly sampling for the presence of laid egg masses alone. For this, egg capsules from each female were collected and individually preserved in 4% buffered formalin. Five egg capsules from each brood were later opened under a dissecting microscope and one of three developmental stages was assigned to the brood from each female, adapted from Chipperfield (1951) and Richard *et al.* (2006):

1) Morula
2) Trochophore
3) Veliger

To estimate the approximate beginning and end of the spawning period, we assumed that encapsulation lasts ~20-30 days in total (Brante *et al.*, 2009), with the morula and trochophore stages being completed during the first half of that time period (Maeda-Martinez, 2008). As noted in Richard *et al*. (2006), we also found that assigning the same category to all egg capsules from a female was appropriate as the development of all eggs was similar.

### 3.3.4 LARVAL PERIOD

Plankton samples were taken 12 times between February 2010 and January 2011 (i.e. at ~monthly intervals) parallel to and <100 m away from the intertidal sites at BR, CO, HA and PE (depth~5-10 m). A single five minute surface tow was taken at each site by deploying an un-weighted 200 µm plankton net from a vessel moving at ~1.0-1.5 kn. A standard mechanical flowmeter (model 2030, General Oceanics Inc.) was attached to the aperture of the net; this showed that between 24 $m^3$ and 68 $m^3$ were filtered during each tow. On each occasion, all four stations were sampled within a 3 h period around high water. Samples were preserved in 4% buffered formalin within 4 h after sample collection.

Densities of *C. fornicata* larvae across the full size range (hereafter "total larval density") were determined for each sample by counting all *C. fornicata* larvae in the whole sample or subsamples thereof (between ~3.13% and 100% of the sample). Samples were split into subsamples using a Folsom plankton splitter. Counts were converted into numbers of larvae $m^{-3}$ using the flowmeter readings of volume sampled. Maximum shell lengths of up to 100 larvae per sample were measured with an ocular micrometer (x 40 or 63). In addition to total larval density, densities of metamorphically competent *C. fornicata* larvae were determined for each sample in order to estimate the supply of late-stage, "ready-to-settle" larvae to the intertidal sites. Also here, subsamples between 3.13% and 100% were analysed, but only larvae were counted with morphological characteristics that indicate metamorphic competency (maximum shell length >650 nm, flattened shell geometry and presence of a "brimmed shell", see Pechenik, 1980; Pechenik and Lima, 1984). Mean densities of metamorphically competent larvae from the four study sites were compared statistically using competent larval densities from all sampling events where metamorphically competent larvae were present in any of the four samples (May-October). The non-parametric Kruskal-Wallis test was chosen due to the presence of many zero values resulting in heterogeneous variances that could not be removed by transforming the data.

## 3.3.5 RECRUITMENT SEASON

### 3.3.5.1 RECRUITMENT ON ARTIFICIAL SUBSTRATA

Recruitment panels were installed at the same tidal height where adult monitoring was undertaken at each of the four sites in the MHW. Panels were changed once a month to measure monthly recruitment of *C. fornicata*. Panels were made of ordinary roofing slate cut to a size of 11 cm x 11 cm. They were kept in position on the shore by screwing them to anchored metal frames (50 cm x 50 cm) with a single screw and wing nut per panel. Four panels were attached to each frame, and five frames were installed at each site (i.e. 20 panels per site). The first set of panels was deployed at the beginning of February 2010. Panels were changed every four weeks thereafter for clean slate panels. The last set was collected at the beginning of November 2010. Each month the four sites were visited at low water of spring tides in the same order on four subsequent days (day 1: BR, day 2: PE, day 3: CO, day 4: HA). Panels were transported to the laboratory in racks stored in cooling boxes and were analysed in the laboratory within 5 h of collection. All *C. fornicata* present on both sides of each panel were counted under a dissecting microscope (x 40 or 63), and from August onwards size measurements were also taken with an ocular micrometer. Due to the presence of many zero values and resulting heterogeneous variances, the non-parametric, 2-way Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Dytham, 2011) was chosen to compare spat densities between sites throughout the season, using only data from sampling events when spat were found at any of the study sites (July-November).

Juvenile and young male slipper limpets are still mobile, so they may be able to colonise or leave the panels after settlement by moving off their initial settlement substrata. To minimise the possibility of post-metamorphic movement onto or off the panels, the area surrounding each frame was cleaned of any slipper limpet stacks and hard substrata at each visit. However, even adults were occasionally found attached to the panels during our study. For example, we once found a fully developed, but relatively small female (27 mm), and two stacks of two males (26 mm + 19 mm; 23 mm + 15 mm). This clearly shows that some movement of *C. fornicata* older than four weeks took place onto the panels. Metamorphosis and settlement occur at a size of ~1 mm (e.g. Pechenik, 1980),

and post-metamorphic growth rates are at least 0.1 mm $day^{-1}$ under laboratory conditions (Eyster and Pechenik, 1988; Pechenik and Eyster, 1989). In the field, growth rates of ~2 mm $month^{-1}$ have been reported (Walne, 1956). To ensure monthly recruitment rates were not biased by the possible movement of post-metamorphic slipper limpets onto the panels during the four week period of panel deployment only individuals with a maximum shell length of <4 mm were counted to reduce the risk that older settled *C. fornicata* might be erroneously included.

#### 3.3.5.2 RECRUITMENT ON NATURAL SUBSTRATA

To determine if the numbers of spat found on the panels reliably reflected monthly recruitment occurring at the sites, natural hard substrata were quantitatively sampled for densities of juvenile slipper limpets (<4 mm) once a month between June and September 2010 at BR and PE in close proximity to the recruitment panels. All hard substrata >0.5 cm2 (including live and dead *C. fornicata* shells) were collected in five randomly placed 0.1 m2 quadrats. Samples were returned to the laboratory in cooling boxes and processed within 16 h of collection. All slipper limpets were measured using a microscope (x 40 or 63) with an ocular micrometer and those <4 mm were counted. Due to the different sampling techniques applied when monitoring recruitment on natural and artificial substrata, graphical comparisons were made between the two substrates rather than applying any statistical tests.

## 3.4 RESULTS

### 3.4.1 TEMPERATURE

In the MHW, the lowest temperature was recorded on 25th of December 2010, a particularly cold day with freezing temperatures of <-5°C during emersion at low tide, and average seawater temperatures of 3.4°C at BR and 5.2°C at HA (Fig. 3.2a and 3.2b). Highest daily average seawater temperatures were ~18.1°C at BR (26th June 2010) and

17.1°C at HA (7th August 2010). Temperature was generally higher at BR than at HA between the end of March and the end of September 2010, but this trend was reversed during the winter. Similarly, seawater temperatures during the summer months were slightly warmer inside MHW compared to the sites outside the MHW (Fig. 3.3).

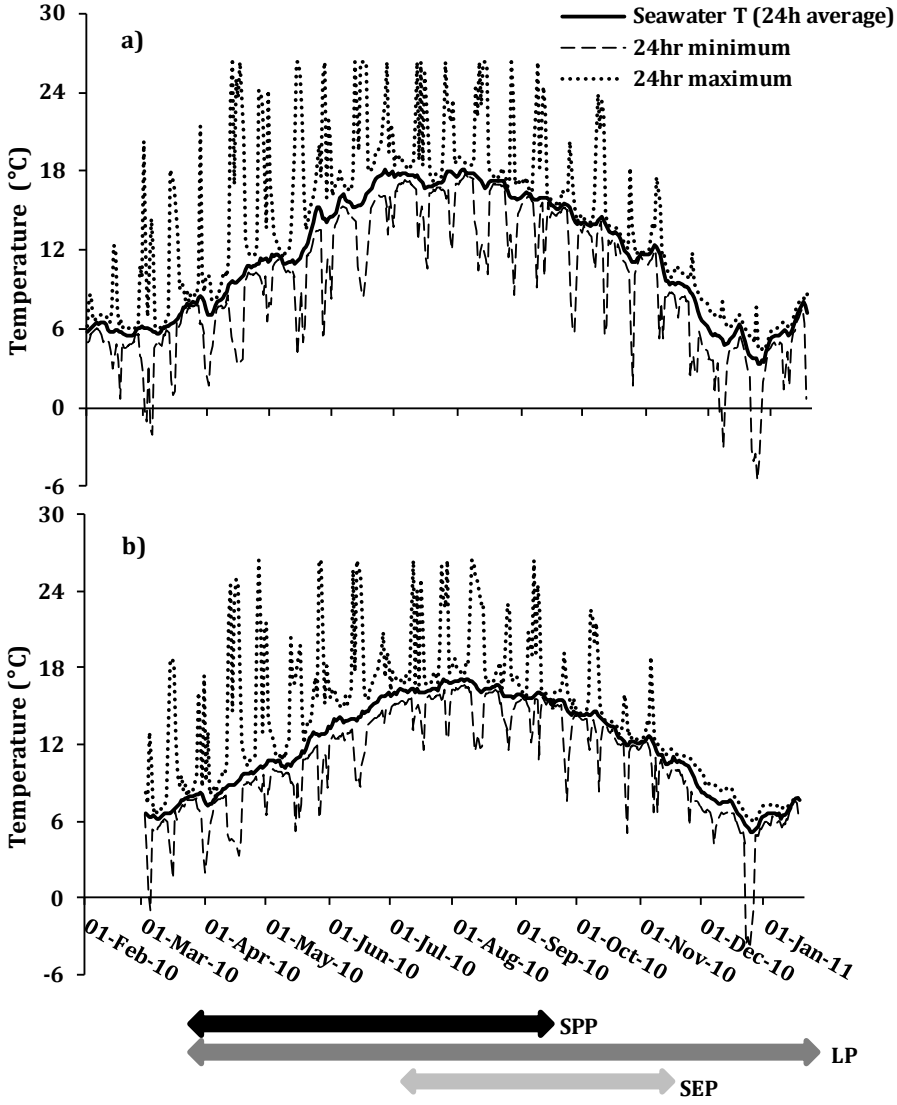



**Fig. 3.2:** Daily average seawater temperatures (i.e. average from all recordings excluding data recorded whilst data loggers were emersed during low water at spring tides) and daily minimum and maximum temperatures (including all data) for Beggars Reach (a) and Hazelbeach (b) in the Milford Haven Waterway, South West Wales. Data loggers were installed ~1.0-1.3 m above C.D. The data loggers record a temperature range between a minimum temperature of -5.5°C and a maximum temperature of 26.4°C. No data available for February 2010 at Hazelbeach as data loggers were lost. Arrows indicate the approximate length of spawning (SPP), larval (LP) and settlement periods (SEP) (see results)

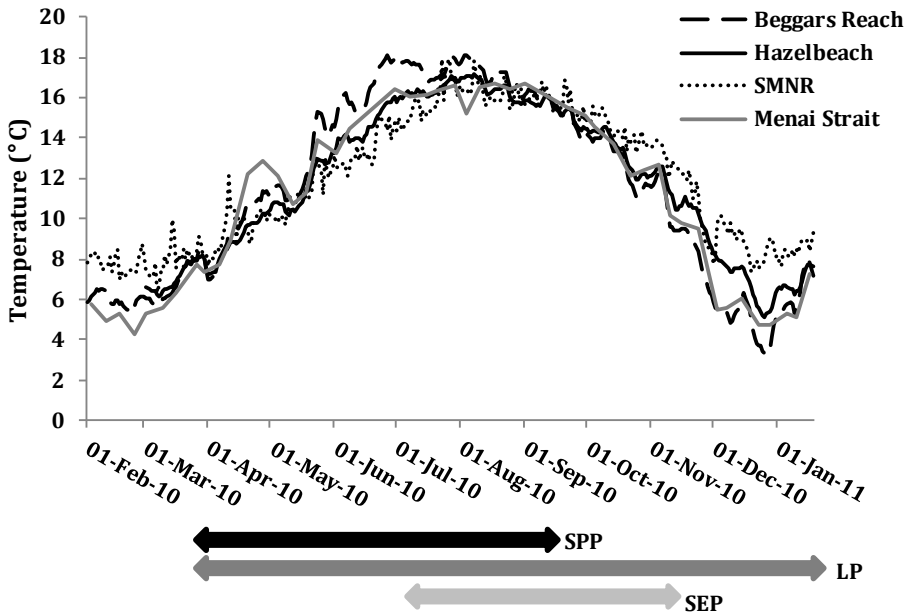



**Fig. 3.3:** Average daily seawater temperatures at Beggars Reach and Hazelbeach in the Milford Haven Waterway, South West Wales. For comparison, temperature data from the Skomer Marine Nature Reserve (SMNR, South West Wales) and the Menai Strait (North Wales) outside the Milford Haven Waterway are shown. Data loggers were installed ~1.0-1.3 m above C.D. No data available for February 2010 at Hazelbeach as loggers were lost. Arrows indicate approximate length of spawning (SPP), larval (LP) and settlement period (SEP) (see results)

### 3.4.2 SEXUAL STAGES, SPAWNING PERIOD AND EMBRYONIC DEVELOPMENT

There were no apparent seasonal trends in the occurrence of female and male frequencies at HA and CO throughout the study (Fig. 3.4). Furthermore, the proportions of males and females in the populations at each study site were balanced, resulting in an average sex ratio (calculated as the proportion of males of the total number of males and females) of 0.51±0.02 and 0.45±0.04 at HA and CO, respectively (mean±SD across all sampling events). The mean sex ratio was significantly higher at HA than at CO (t-test, $t_{1,16}$=3.84, p=0.001). Juveniles were rare throughout the study, especially at CO where the juvenile frequency was consistently lower compared to HA (Fig. 3.4, t-test, $t_{1,16}$=3.77, p=0.002). Few individuals were in the process of sex change (0% to 6%), and they were found throughout the whole year (data not shown).

The inspection of females for the presence of laid egg capsules indicated that the spawning period in 2010 lasted from ~mid March to September. Egg-brooding females were first found at the end of March when all eggs were in early embryonic development, i.e. the morula stage (Fig. 3.5a and 3.5b). Spawning had therefore most likely commenced <2 wks before sampling had been undertaken, i.e. after mid March (Maeda-Martinez, 2008; Brante *et al.*, 2009). Average daily seawater temperatures varied between ~ 6.5-8.0°C during that time (Fig. 3.2a and 3.2b). The percentage of egg-brooding females steadily increased until the middle of June, when 78% of the females were bearing eggs at CO and 92% at HA. Egg capsules were last found at the beginning of September, with only 6% (CO) and 4% (HA) of the eggs being in the morula stage. No egg-brooding females were found four weeks later during October. Spawning and egg-brooding had therefore stopped in the majority of females between the beginning of September and the beginning of October at both sites, when daily average seawater temperatures had declined from 16.0°C to 14.5°C (Fig. 3.2a and 3.2b). The interval of >4 wks between sampling events was more than the time required for the completion of intracapsular development of a single brood. It can therefore be inferred that females in the MHW were capable of multiple spawning in 2010 and had spawned at least 2.2 times at CO and 2.9 times at HA (sum of all egg-brooding female frequencies at CO = 224%, HA = 289%).

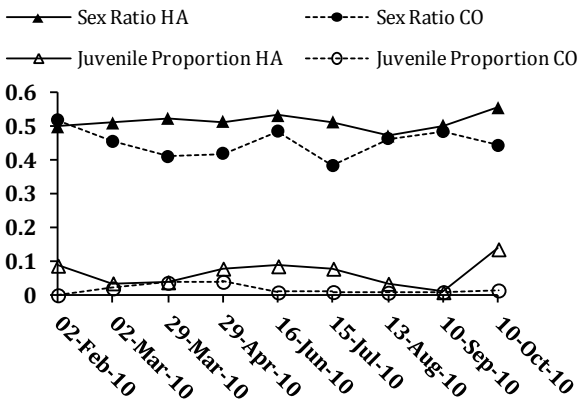



**Fig. 3.4:** The sex ratio (number of males / total number males+females) and the proportion of juveniles of the whole population (number juveniles / total number of males+females+ intermediates+juveniles) at Cosheston (CO) and Hazelbeach (HA) in the Milford Haven Waterway for each sampling event between February and October 2010

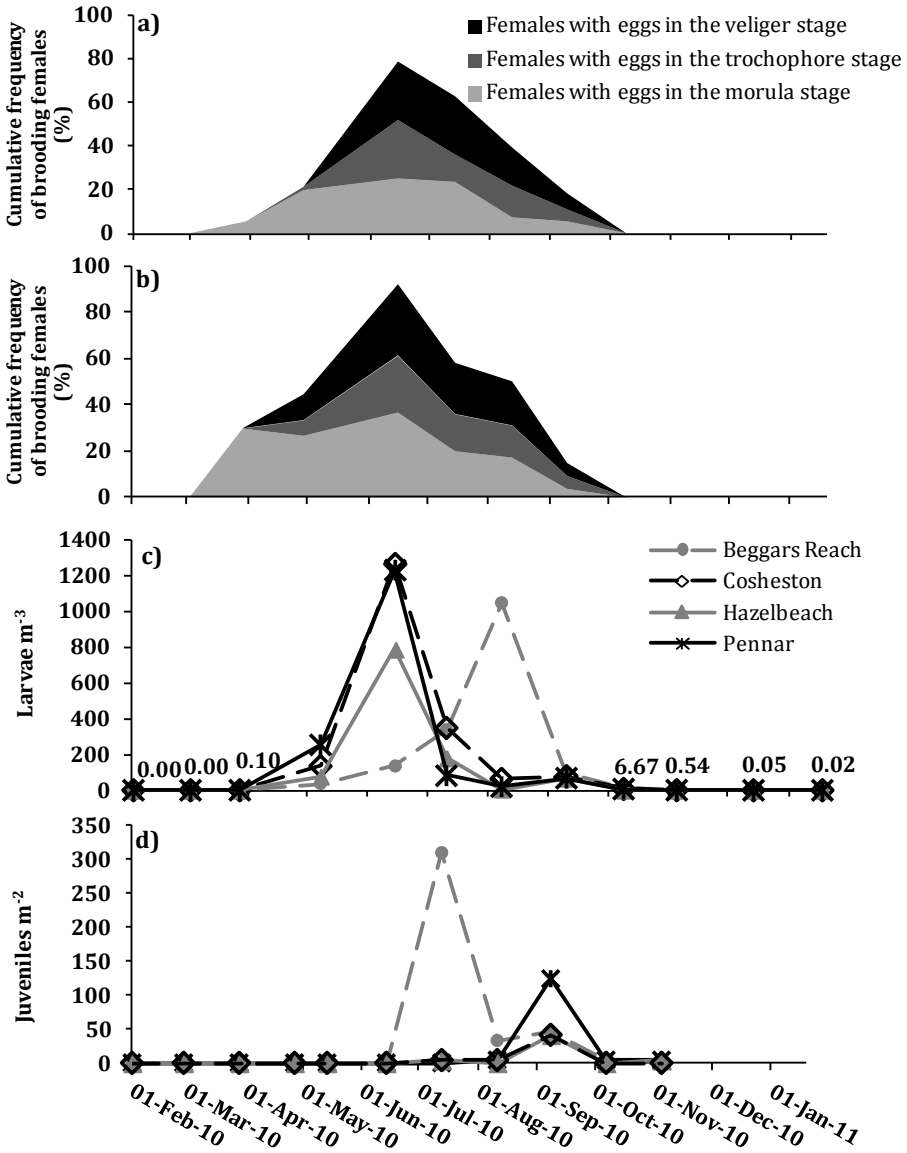



**Fig. 3.5:** Seasonal reproduction of *Crepidula fornicata* in the Milford Haven Waterway between February 2010 and January 2011. **a)** and **b)** Cumulative frequency of females found with broods of eggs in the different categories of embryonic development at Cosheston (a) and Hazelbeach (b), sampled between February and October 2010. **c)** Densities of *Crepidula fornicata* larvae found in monthly plankton samples at all four study sites, sampled between February 2010 and January 2011. Numbers above the Pennar data series are data labels to visualise very low densities. **d)** Mean densities of juvenile *Crepidula fornicata* <4 mm on artificial settlement substrata (slate panels) at all four study sites, sampled between February and November 2010. Error bars omitted for clarity

### 3.4.3 LARVAL PERIOD

Densities of *C. fornicata* larvae varied considerably between sampling events and locations in 2010 (Fig. 3.5c). Larvae were first found in low concentrations on 28th March 2010 (<0.1 individuals $m^{-3}$ at PE, CO and HA, but absent at BR). Larvae were still present in very low numbers in samples collected on 14th December 2010 and 18th January 2011 (December 2010: 0.02-0.09 individuals $m^{-3}$ at all four sites; January 2011: 0.02 individuals $m^{-3}$ CO and PE, absent at HA and BR), despite the fact that daily average seawater temperatures were only 7.5°C at HA and 5.7°C at BR on the days the samples were taken (Fig. 3.2a and 3.2b). Larval abundances were highest during the summer months, with maximum densities of >1200 individuals $m^{-3}$ at CO and PE in June, and two months later at BR with similar densities. The majority of the larvae measured were small in length: ~95% of the sampled larvae were <480 µm (see supplemental figure in Appendix 3.1 for larval size distributions), suggesting that the larvae had only hatched a few days before sampling had taken place. Only very few metamorphically competent larvae were observed in any of the plankton samples, and densities of late stage larvae did not differ between study sites (Fig. 3.6, Kruskal-Wallis test, $H_3$=3.78, p=0.30).

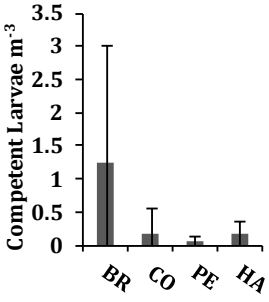



**Fig. 3.6:** Densities of metamorphically competent *Crepidula fornicata* larvae in plankton samples from all four study sites (BR – Beggars Reach, CO – Cosheston, PE – Pennar, HA – Hazelbeach) in the Milford Haven Waterway in 2010. Bars are mean (±SD) densities from all sampling events where metamorphically competent larvae were present in any of the four samples (May to October)

### 3.4.4 RECRUITMENT SEASON

Regular monitoring of artificial substrata (slate panels) indicated that the recruitment season of 2010 began between mid June and mid July (Fig. 3.5d). Confirmation of the lack of recruitment prior to this time was made by observing no recruits on natural substrata at BR and PE in June. Afterwards, spat were found on the panels at each monthly visit until they were removed in November 2010, and on the natural substrata that were collected in July, August and September at BR and PE (Fig. 3.7). Differences in monthly recruitment among sites depended on the time of sampling events (interaction site x sampling event Scheirer-Ray-Hare 2-way ANOVA, $H_{12,380}=138.0$, $p<0.001$). Highest densities were found at BR in July with 310±242 individuals $m^{-2}$ and 142±15 individuals $m^{-2}$ on artificial and natural substrata, respectively (mean±SD). Observed recruitment at the other three sites were very low during that month. A peak in recruitment was also recorded two months later at PE (mean±SD artificial substrata: 124±91 individuals $m^{-2}$, natural substrata: 164±35 individuals $m^{-2}$). Recruitment at HA and CO remained low throughout the whole spatfall season in 2010.

Seawater temperatures exceeded 16°C when spat were first found at the beginning of the season and almost no spat were found when seawater temperatures consistently fell below ~15°C in September (Fig. 3.2a and 3.2b). Spat densities on artificial and natural substrata collected at PE and BR were consistently similar (Fig. 3.7), confirming that the

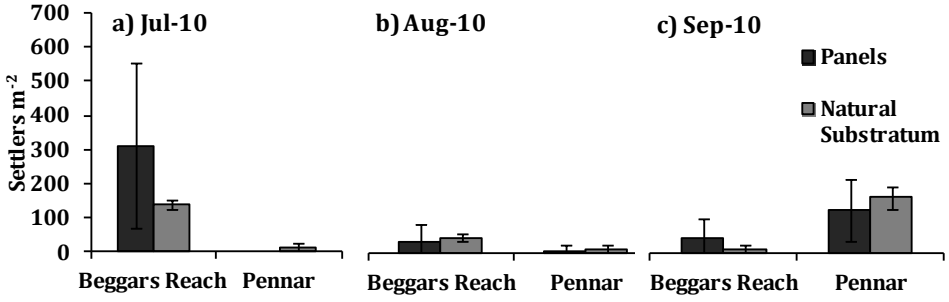



**Fig. 3.7:** Densities of juvenile *Crepidula fornicata* (<4 mm) on natural substrata and artificial substrata (panels) at Beggars Reach and Pennar in July **(a)**, August **(b)** and September 2010 **(c)**. Mean±SD

**Table 3.1:** Average sizes of *Crepidula fornicata* spat <4 mm found on the different substrata types (artificial or natural) in the Milford Haven Waterway for each sampling event during the settlement season of 2010

| | August | | September | | October | November |
|---|---|---|---|---|---|---|
| Site | Artificial | Natural | Artificial | Natural | Artificial | Artificial |
| Beggars Reach | 1.2±0.5 (n=8) | 2.1±0.8 (n=38) | 1.2±0.6 (n=11) | 2.8±1.1 (n=16) | 1.3 (n=1) | --- |
| Pennar | 1.1 (n=1) | 2.8±0.9 (n=11) | 1.0±0.1 (n=30) | 1.2±0.3 (n=82) | 1.2±0.1 (n=2) | 1.56 (n=1) |
| Cosheston | 1 (n=1) | n.a. | 0.9±0.1 (n=10) | n.a. | --- | --- |
| Hazelbeach | --- | n.a. | 1±0.1 (n=10) | n.a. | --- | 1.25 (n=1) |

Data are means (mm) ±SD; Settlement on natural substrata was never monitored at Cosheston and Hazelbeach (n.a.); No data available for July; --- no spat found on substrata during that sampling event

spat densities on the chosen artificial substrata reliably reflected settlement occurring on the shore. The size of the majority of the spat found on either substratum type was well below the 4 mm threshold set to distinguish spat that had recruited within the four week panel deployment period from older recruits that may have migrated onto the panels (Table 3.1).

## 3.5 DISCUSSION

Although *C. fornicata* has spread rapidly within the MHW since its first recorded occurrence in this ria in 1953 (Cole and Baird, 1953; Crothers, 1966) and can be found in superabundant aggregations in some areas today, our observations suggest it has not expanded its distribution to Mid and North Wales (Chapter 2). Data from the present study show that the lack of northward expansion is unlikely to be due to sub-optimal prevailing spring and summer seawater temperatures hampering reproduction of the species in Welsh waters. The population in the MHW is characterised by a long reproductive season that lasts at least from ~mid March to September, which corresponds well with lengths previously reported from other European study sites (Richard *et al.*, 2006). Also, seawater temperatures in the MHW at the beginning of the

spawning season in 2010 were 2-3°C lower than the reported 10°C-threshold necessary to trigger spawning elsewhere (UK East coast: Chipperfield, 1951; US: Hoagland, 1979; France: Richard *et al.*, 2006; Valdizan *et al.*, 2011). Spawning at such low temperatures has only been observed at one other study location (German Wadden Sea: Werner, 1948; Thieltges *et al.*, 2004). This, together with the occurrence of multiple spawning events (cumulative egg-brooding female frequency >220% at both sites) and the high larval densities (maximum densities >1200 larvae $m^{-3}$) during the peak reproductive period indicates that reproductive activity of *C. fornicata* is not compromised in the MHW, its northern range limit in Wales. Furthermore, spring and summer seawater temperatures in the SMNR and the Menai Strait, where currently there are no populations of *C. fornicata*, are above those required to elicit spawning and larval release (Fig. 3.3). This suggests that seawater temperatures to the north of the MHW are not acting as a strong limiting factor for the northwards spread of *C. fornicata* in Wales through negative effects on its reproductive output. Benthic recruitment in the MHW however was restricted to a much shorter time window than the long larval period. Spatfall was first observed at seawater temperatures of ~16°C, differing by ~10°C to the minimum temperature at which larvae were present in the water, and the peak recruitment season occurred between mid July and mid September. Field observations on settlement and recruitment at other study sites are scarce, but the settlement season on the east coast of the UK was found to be similar (end of June to end of August at seawater temperatures of ~20 C, Chipperfield, 1951; Walne, 1956). Seawater temperatures during the peak recruitment season in the SMNR and the Menai Strait were slightly lower compared to inside the MHW (Fig 3.3). It is therefore possible that benthic recruitment of *C. fornicata* would be restricted to a much shorter time period if larvae were transported outside of the MHW, possibly restricting its abundance and local spread in Welsh coastal waters, even if conditions are generally suitable for reproduction.

Although no egg-brooding females were found during the final sampling event in October, larvae were still present at low densities (<0.1 individuals $m^{-3}$) in plankton samples that were collected in December 2010 and January 2011, indicating that larval release had not ceased completely despite the very low seawater temperatures. There are several possible explanations for this observed mismatch between the duration of

the spawning period and the larval period, none of which are mutually exclusive: 1) very few females may continue spawning in the winter months at any one location in the MHW, which would not be detectable with sample sizes and frequencies employed in studies such as ours, 2) egg-brooding, not necessarily egg-laying, of very few females may be extended at low seawater temperatures, leading to a delayed release of larvae, or 3) larvae released into the water column towards the end of the recorded reproductive season undergo very slow development after release, leading to a prolonged planktonic phase. We think the latter is most unlikely. Larval mortality reaches almost 100% at temperatures <12°C and metamorphic competency is hardly ever attained (Rigal, 2009). Daily average seawater temperatures during the plankton sampling events in winter 2010-2011 were well below temperatures suitable for larval development of *C. fornicata*, and all larvae were <435µm which is similar to the size at hatching (Pechenik, 1980). It is therefore more likely that those larvae were released only shortly before sampling had taken place, either as a result of rare and "late" spawning and/ or prolonged egg-brooding occurring in autumn and winter. No other studies have found evidence of egg-laying during that time (Thieltges *et al.*, 2004; Richard *et al.*, 2006; Valdizan *et al.*, 2011) nor the presence of planktonic larvae (although typically plankton samples collected in other studies have been much lower in volume than those we took, e.g. only 0.01 $m^3$ in Thieltges *et al.* (2004), compared to 50 $m^3$ in the present study).

Late-stage larvae were rare throughout the study. *Crepidula fornicata* has a pelagic phase of ~2-4 wk (Pechenik, 1980, 1984) and during that time, larvae are likely to be exported away from their parental adult beds (Rigal *et al.*, 2010). Work by Dupont *et al.* (2007b) and Rigal *et al.* (2010) show that the "fate" of the larvae after release is greatly influenced by local hydrodynamic conditions that may result in a dispersal of ~100 km. The majority of the larvae we sampled throughout the year were small and hence likely young and collected whilst still being in close proximity to the parental stacks. Where the larvae are transported to after release in the MHW cannot be estimated without fine scale hydrodynamic modelling, but it is possible that larval export to the outside of the MHW is high. Of course, it is possible that we missed a large proportion of the late-stage larvae in our study due to the employment of horizontal, surface plankton tows. These would not account for vertical differences in larval abundance of different age classes

that are common in many marine benthic species with a pelagic larval phase (Cronin and Forward, 1986; Shanks, 1986; Dobretsov and Miron, 2001) Work that will be presented in Chapter 4 however showed that late-stage larvae of *C. fornicata* are also rare in samples taken in close proximity to the sea bed. As already mentioned, larval mortality is likely high, especially when larval release occurs at the beginning and end of the spawning season when seawater temperatures are below the ~12°C required for larval development and survival (Rigal, 2009). If the prevalent temperatures in the MHW favour spawning and larval release, but not necessarily larval development, this would further explain the absence of large numbers of late-stage larvae, despite the high total larval densities we observed during the peak reproductive season.

Monthly recruitment was generally low and moderate recruitment was only observed at two sites (BR and PE), both having opposing adult densities. *Crepidula fornicata* is known to display strong gregarious behaviour (Hoagland, 1978; McGee and Targett, 1989), and selective initial settlement and/ or post-settlement movement of a gregarious species should result in higher densities of juveniles in areas with high adult densities (Jenkins, 2005). Our data shows that this is not necessarily the case in the intertidal populations of *C. fornicata* in the MHW. The observed spatfall densities on both artificial and natural substrata were unrelated to the densities of the adults. We think it is likely that the selective larval settlement behaviour or post-settlement movement of juvenile *C. fornicata* may be concealed by high early post-settlement mortality acting on the newly metamorphosed spat. The collection of monthly data on recruitment does not allow us to distinguish between initial settlement rates and subsequent survival rates of the juveniles (Minchinton and Scheibling, 1993), but the small sizes of the juveniles we found on the settlement substrata, irrespective of substrata type, suggest that the majority of the spat may have settled only several days before sampling took place. Total settlement rates over the four week periods the panels were deployed may have been much higher. Particularly in the intertidal zone, early post-settlement mortality may be high due to the occurrence of discrete physical disturbance events such as short periods of exposure to extreme temperatures during emersion at low water. If this is the case, recruitment at the four different sites should be compared with caution. At the sites that were visited last each month (CO and HA) spat were exposed to intertidal conditions twice a day for 2-3 days in the week sampling

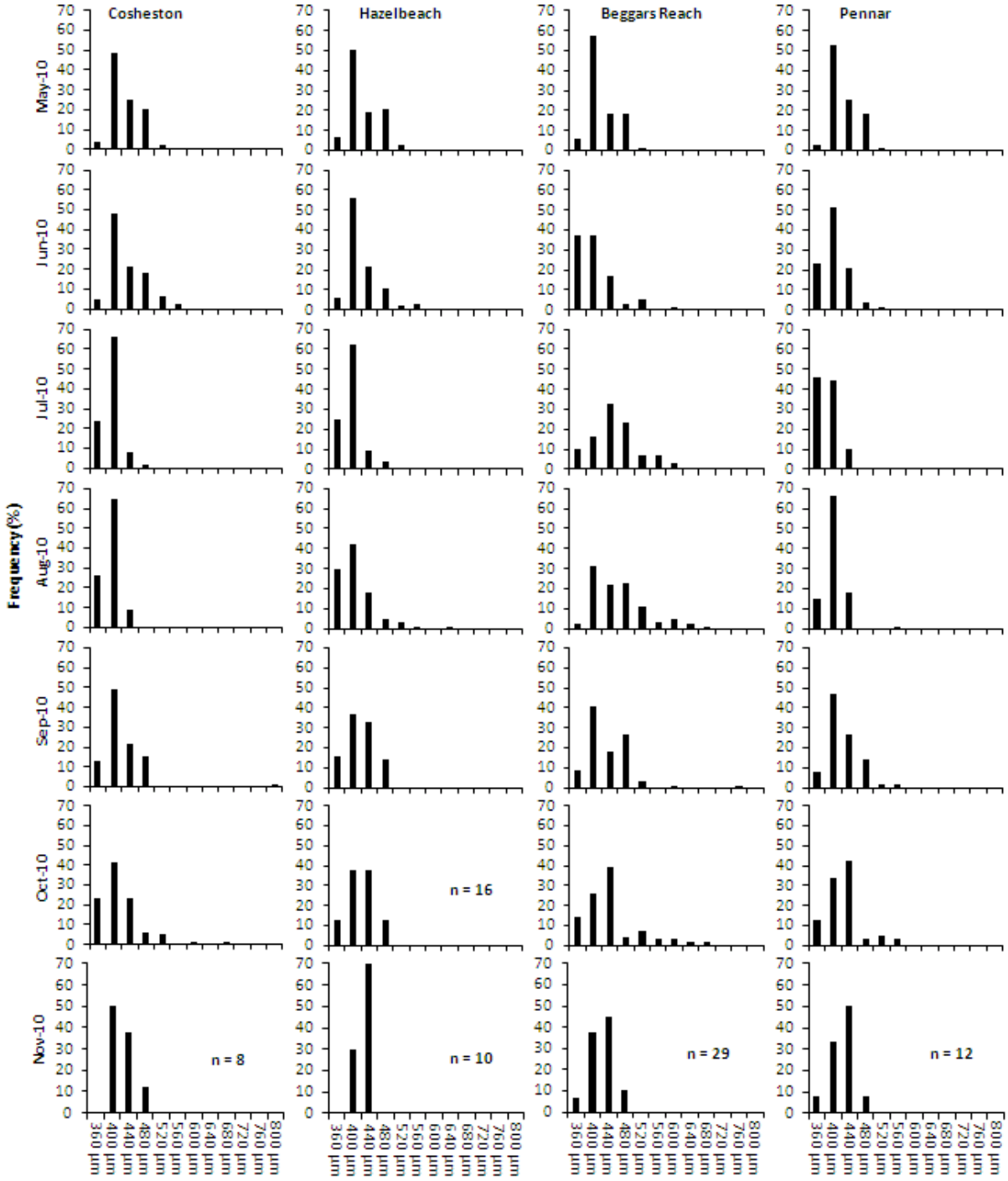
was undertaken, possibly resulting in high levels of mortality and explaining the very low colonisation of *C. fornicata* on the panels at these two sites. Chapter 4 will present work that aims at quantifying levels of early post-settlement mortality to differentiate between levels of total settlement and recruitment into the adult population within one season, by comparing juvenile densities on panels that were installed for varying durations.

The relatively low monthly recruitment most likely resulted in low overall recruitment of juveniles into the intertidal adult populations of the MHW in 2010. The small percentage of juveniles and the relatively low sex ratio in the MHW compared to most other native and non-native populations (Wilczynski, 1955; Hoagland, 1978; Collin, 1995, 2006; Dupont *et al.*, 2006; Dupont *et al.*, 2007a; but see also Hoch and Cahill, 2012) provide evidence that recruitment into the adult populations has been low in the previous year too, indicating that early post-settlement mortality in the intertidal zone of the MHW may generally be high. This pattern was even more pronounced at one of the locations investigated (CO) where both male and juvenile frequencies were particularly low, suggesting that the magnitude of early post-settlement mortality varies over spatial scales of kilometres. Little is known about which environmental factors may affect juvenile survival and recruitment into the adult population of *C. fornicata*. It is likely however that such factors differ spatially (between sites or tidal height) and/ or temporally (i.e. depending on whether settlement occurs close to neap or spring tide conditions), which would explain the differential distribution of settlers, and consequently adults, that were observed in the MHW.

The absence of detectable numbers of adult *C. fornicata* outside the MHW indicates that recruitment from the source population (the MHW) into the open coast is negligible or absent. It has been suggested that *C. fornicata*'s secondary spread at its northern limit at other European study sites may be restricted by high adult winter mortality in primarily intertidal conditions where animals are exposed to low air temperatures (Thieltges *et al.*, 2003; Thieltges *et al.*, 2004). The presence of substantial subtidal populations in the MHW means this process is highly unlikely to be important in limiting *C. fornicata* at its northern range limit in Wales. It is worth noting however that adult mortality events in the intertidal were never observed during our study in the MHW, not even after freezing

air temperatures occurred for several days in December 2010. Also, Crisp (1964) and co-workers observed only ~25% mortality in *C. fornicata* populations along the UK south coast during the much harsher winter of 1962-1963, one of the coldest winters on record last century. This implies that adults are generally tolerant towards exposure to the minimum air temperatures experienced at their northern range limit in Wales. Here, we reason that instead, settlement and post-settlement processes may be highly important in controlling adult abundances and possibly the secondary spread of the species, rather than unsuitable conditions for adult reproduction or high winter mortality. Although our data were collected during one year only and hence do not allow us to exclude the possibility of inter-annual variability in the timing and length of the reproductive season as has been shown elsewhere (Valdizan *et al*., 2011), we show that there is a large difference in the seawater temperatures at which larvae and spat were first observed in the year the study was undertaken. Consequently, the recruitment season in the MHW may have been restricted to a much shorter time period compared to the brooding period. This implies that benthic recruitment might be the process most susceptible to changes in prevailing seawater temperatures the species would be exposed to in case of a beginning northwards range expansion. Conversely, it can be assumed that the expected rise in seawater temperature as a result of global warming may facilitate *C. fornicata*'s spread along the Welsh coast, by providing better conditions for successful benthic recruitment. Furthermore, our data suggest that early post-settlement mortality may be a strong limiting process for recruitment into the adult population, at least under intertidal conditions. This demonstrates the significance of incorporating settlement and post-settlement processes into predictions of *C. fornicata* recruitment success in its invasive range. This has previously been done by mainly studying the length of the spawning period, female fecundity, larval development and adult winter mortality only (e.g. Thieltges *et al*., 2004; Richard *et al*., 2006; e.g. Valdizan *et al*., 2011). Further work is required that examines the conditions necessary for successful settlement of *C. fornicata*, and which factors may restrict recruitment due to early post-settlement mortality, in both intertidal and subtidal populations. The importance of post-settlement mortality will be focus of the next chapter that presents results of an intensive sampling programme that aimed at studying how processes acting before, during or after settlement are affecting distributional patterns of *C. fornicata* under intertidal conditions.

## APPENDIX 3.1: LARVAL SIZE FREQUENCIES




**Supplementary Figure:** Size frequencies of larvae collected at all four study sites in the Milford Haven Waterway between May and November 2010. A subsample of 100 larvae was measured in each sample, unless otherwise stated for low density-samples in which case all larvae were measured (n). Size frequencies for samples from 28th March 2010, 14th December 2010 and 18th January 2011 are not shown due to the low numbers of larvae (14th March 2010: <475 µm, n=10 at all four sites; 14th December 2010: <435 µm, n=10; 18th January 2011: <375 µm, n=1)

# CHAPTER 4 -

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

## 4.1 ABSTRACT

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 individuals $m^{-2}$) and Pennar (~ 343±360 individuals $m^{-2}$). 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*.

Keywords: *Crepidula fornicata*, recruitment, larval supply, settlement, post-settlement mortality

## 4.2 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 (Grosberg, 1982; Gaines *et al.*, 1985; Minchinton and Scheibling, 1991; Hurlbut, 1992; Jeffery and Underwood, 2000; Carlon, 2002). However, numerous studies also found that adult distribution may be determined through processes acting during and after settlement (McGee and Targett, 1989; Gosselin and Qian, 1996; Delany *et al.*, 2003; Jenkins and Hawkins, 2003; Kent *et al.*, 2003; Jenkins, 2005; Power *et al.*, 2006; Jenkins *et al.*, 2008). 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 likely 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 (Davis *et al.*, 2001; Colautti and MacIsaac, 2004). 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 (Thouzeau *et al.*, 2000; Thieltges, 2005b). 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. *Crepidula 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* (McMillan, 1938; Korringa, 1942; Blanchard, 1997) and ship hulls (Cole, 1952; Cole and Baird, 1953). Furthermore, its relatively long larval phase of ~2-4 weeks (Pechenik, 1980, 1984) suggests larval transport is likely to be an important means of dispersal in its non-native range (Orton, 1915; Adam and Leloup, 1934; Korringa, 1942).

The success of *C. fornicata* as an invasive species has also been in part due to its high tolerance of a variety of environmental conditions and its ability to thrive in a range of habitat types (Loomis and VanNieuwenhuyze, 1985; Blanchard, 1997). 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-gravelly substrates, and it appears to be rare in the open sea (Loomis and VanNieuwenhuyze, 1985; Blanchard, 1997), although Hinz *et al.* (2011) recorded high numbers in deep tide 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 (Walne, 1956; McGee and Targett, 1989). 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.*, 2007b; Rigal *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 to explain if intertidal adult abundance of benthic invertebrates is controlled through processes acting prior to settlement (larval supply), during settlement (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 (Grosberg, 1982; Gaines *et al.*, 1985; Minchinton and Scheibling, 1991; Jeffery and Underwood, 2000). 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 (McGee and Targett, 1989; Kent *et al.*, 2003; Jenkins, 2005), 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 (Gosselin and Qian, 1996; Delany *et al.*, 2003; 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 and recruitment during the main larval and settlement season (June/ July–September, Chapter 3) 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, (Chapter 3). 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. We refer to settlement as the point in time at which *C. fornicata* larvae leave the pelagic phase and adapt the benthic mode through loss of the swimming organ at metamorphosis and consequent attachment to a hard substratum (Pechenik, 1980; Keough and Downes, 1982). We use the term recruitment for the number of individuals that have survived for any length of time following settlement, thus being a result of both total levels of settlement and subsequent losses due to post-settlement mortality (Jenkins, 2005). 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.

## 4.3 METHODS

### 4.3.1 STUDY SITES

Field sampling and collection of *C. fornicata* broodstock for juvenile rearing were undertaken in the Milford Haven Waterway (MHW) in South West Wales, UK (Fig. 4.1). This natural ria holds the northernmost self-sustaining established population of

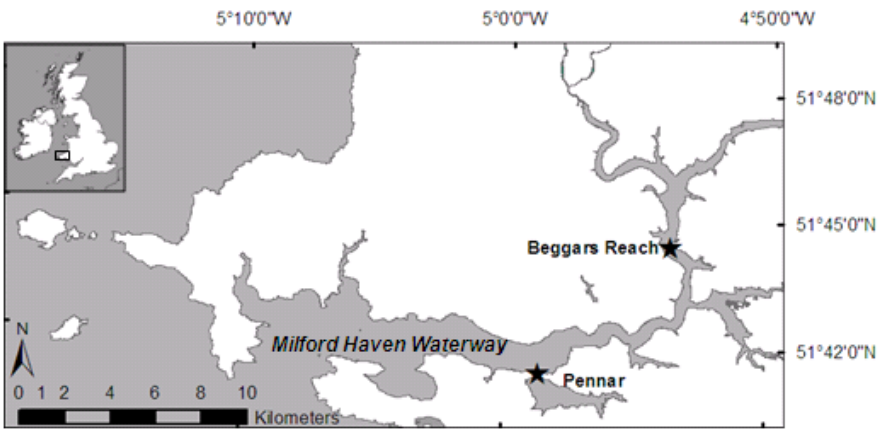



**Fig. 4.1:** Map of the study sites Beggars Reach (BR) and Pennar (PE) in the Milford Haven Waterway (MHW) in the south west of the United Kingdom (top left)

*C. fornicata* since the first appearance of the species in Welsh coastal waters in the 1950's (Cole and Baird, 1953; Crothers, 1966) (Chapter 2). 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 either ~1.2 m or ~1.8 m above Chart Datum at two sites within the MHW, Pennar (PE) and Beggars Reach (BR) (Fig. 4.1). 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 individuals $m^{-2}$; at PE: ~343±360 individuals $m^{-2}$, Chapter 2). 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 (data not shown).

### 4.3.2 FIELD SAMPLING TO DETERMINE THE PROCESSES CONTROLLING ADULT DISTRIBUTION

#### 4.3.2.1 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. Duplicate plankton samples were taken thirteen times 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 $m^3$ of seawater were filtered during each tow. All samples were taken up to 1.5 h after low water during the flood tide, from the body of water above the transects where settlement panels were deployed (see below). 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 micrometer (x 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.

#### 4.3.2.2 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 adult densities. Artificial settlement substrata made out of ordinary roofing slate were used to enable the fast collection and subsequent measurements of settlement rates in the laboratory. The reliable estimation of settlement densities on natural substrata was found to be difficult due to the prevalence of soft sediments amongst the mud-gravel substrata and the small minimum size of the early settlers (~ 0.7 mm, see Pechenik, 1980). Previous work has demonstrated that spat densities on slate panels equal those on natural substrata under intertidal conditions (Chapter 3). 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 sampling programme ("monthly panels"). A third set of 12 panels was deployed for three months, i.e. the whole duration of the sampling programme from mid June to mid September 2011 ("three-monthly panels"). Slate panels were cut to a size of 11 cm x 11 cm and securely fastened to the intertidal zone by attaching them to metal frames (50 cm x 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 micrometer (x 63) in the laboratory.

Previous work has shown that the collection of monthly settlement rates may result in an underestimation of total settlement rates over the deployment periods (Chapter 3). 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.

### 4.3.3 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 a. 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 aquaria) 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). Regular monitoring of the larval batches assured that 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 * $10^5$ 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 (biofilmed shell fragments 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 4-8 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 panels 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 7$^{th}$ 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 12$^{th}$ July, when they were uncovered for the first time again 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 in both treatments.

### 4.3.4 STATISTICAL ANALYSES

Most data were analysed using two-factorial ANOVA or the equivalent non-parametric Scheirer-Ray-Hare test if data transformations could not establish homogeneous variances or normality (Dytham, 2011). All data were tested for homogeneity of variances and normality using Levene's test and the Kolmogorov-Smirnov test, respectively. Total settlement rates, estimated by measuring biweekly settlement rates, were compared between study sites over the season using the two-factorial Scheirer-Ray-Hare test, using study site and sampling events as factors. Spat densities recorded on biweekly, monthly and three-monthly panels were compared to distinguish total settlement rates from levels of recruitment over varying time periods at both shores for each sampling event separately (factors panel deployment duration and site). For this, the two-factorial Scheirer-Ray-Hare test was used to compare biweekly and monthly settlement rates recorded during the mid-July sampling event, and a two-factorial ANOVA for data recorded during the mid-August sampling event. At the end of the sampling programme in mid September, when three-monthly panels were sampled in addition, the Scheirer-Ray-Hare test was used to compare data from all three deployment durations.

One-way ANOVA was used to compare total larval densities as well as densities of late-stage larvae between study sites. Calculated average densities obtained from duplicate samples for each sampling event were used as replicates; comparisons between sampling events at the two study sites were not made as samples of both study sites had to be taken on different days and sometimes at different intervals. Total larval densities were log10-transformed to achieve homogeneity in variances.

'Study site' and 'sampling events' were considered random factors for all tests to allow assessment of generality of results over these spatial and temporal scales, instead of testing hypotheses about the particular sites or sampling occasions. Panel deployment

duration was considered a fixed factor to allow comparisons about total settlement rates (biweekly panels), monthly recruitment (monthly panels) and recruitment occurring over the whole duration of the study (three-monthly panels). The log-rank test (Machin *et al.*, 2006) was used to compare differences in survivorship between caged and not caged panel treatments in the field study on post-settlement mortality monitoring.

# 4.4 RESULTS

## 4.4.1 THE PROCESSES CONTROLLING ADULT DISTRIBUTION

### 4.4.1.1 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, $F_{1, 24}$=24.57, p<0.001, Fig. 4.2a), densities of late-stage larvae (i.e. larval supply) did not differ between the low and high adult abundance sites (One-way ANOVA, $F_{1, 24}$=0.02, p=0.88, Fig. 4.2b). Late-stage, metamorphically competent larvae were rare throughout the study and densities never exceeded 0.8±1.1 ind m-3 (mean±SD) (Fig. 4.2b). 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. 4.2a). Both peaks in larvae were followed by peaks in settlement with a lag phase of approximately four weeks (Fig. 4.2c). Biweekly settlement rates varied greatly throughout the season at both locations. Differences between the two study sites were not consistent throughout the season (Two-way Scheirer-Ray-Hare test, interaction site x sampling event: p<0.001, Table 4.1).

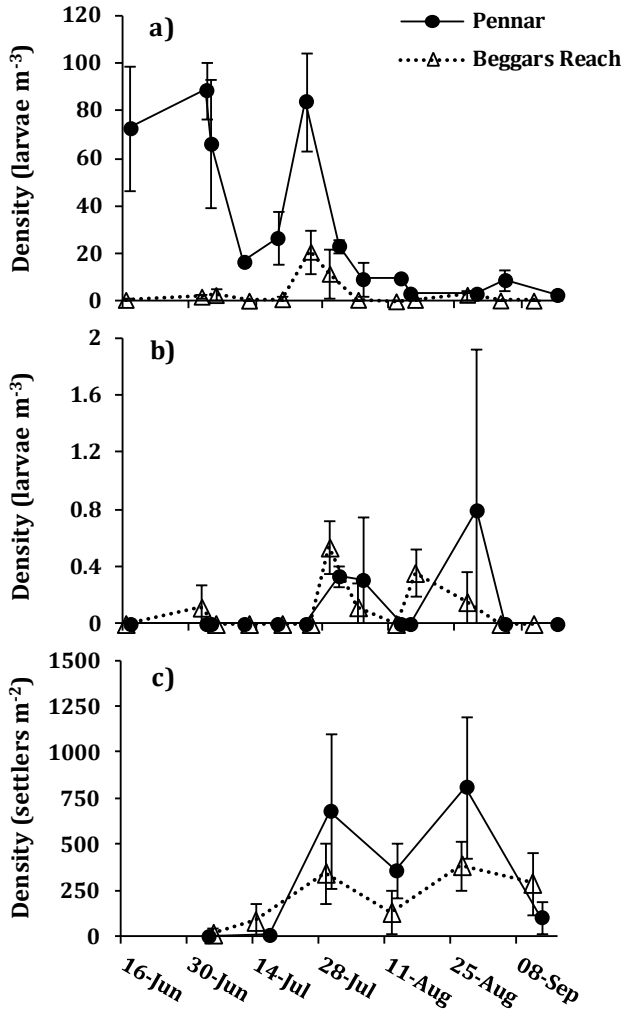



**Fig. 4.2:** *Crepidula fornicata* larval production **(a)**, larval supply **(b)**, and rates of settlement **(c)** in the intertidal zone (~1.2 m above C.D.) at Beggars Reach, a site with low abundance of adult *C. fornicata*, and Pennar, a site with high adult abundance, in the Milford Haven Waterway 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.

**Table 4.1**: Results of non-parametric 2-factorial Scheirer-Ray-Hare test on differences in biweekly settlement densities at Pennar and Beggars Reach in the Milford Haven Waterway at 6 sampling events during the settlement season of 2011. Densities were recorded on panels deployed for two weeks at Pennar and Beggars Reach. SS: Sum of squares, MS: Mean squares, df: degrees of freedom. Significant differences are shown in bold

| | SS | $SS/MS_{total}$ | df | p |
|---|---|---|---|---|
| site | 60.06 | 4.57 | 1 | **0.03** |
| sampling event | 954.95 | 72.66 | 5 | **<0.01** |
| site x sampling event | 232.23 | 17.67 | 5 | **<0.01** |
| Residual | - | - | | |

#### 4.4.1.2 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 4.2), irrespective of the duration of panel deployment (interaction site x duration: $p>0.05$ for each sampling event, Table 4.2). However, the direction of the differences between both sites varied between sampling events: monthly recruitment was highest at BR in mid July and mid September, and at PE in mid August (Table 4.2, Fig. 4.3a, b and c). Levels of recruitment were not significantly different amongst the different deployment durations (Table 4.2, Fig. 4.3a, 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. 4.4a and 4.4b), 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.

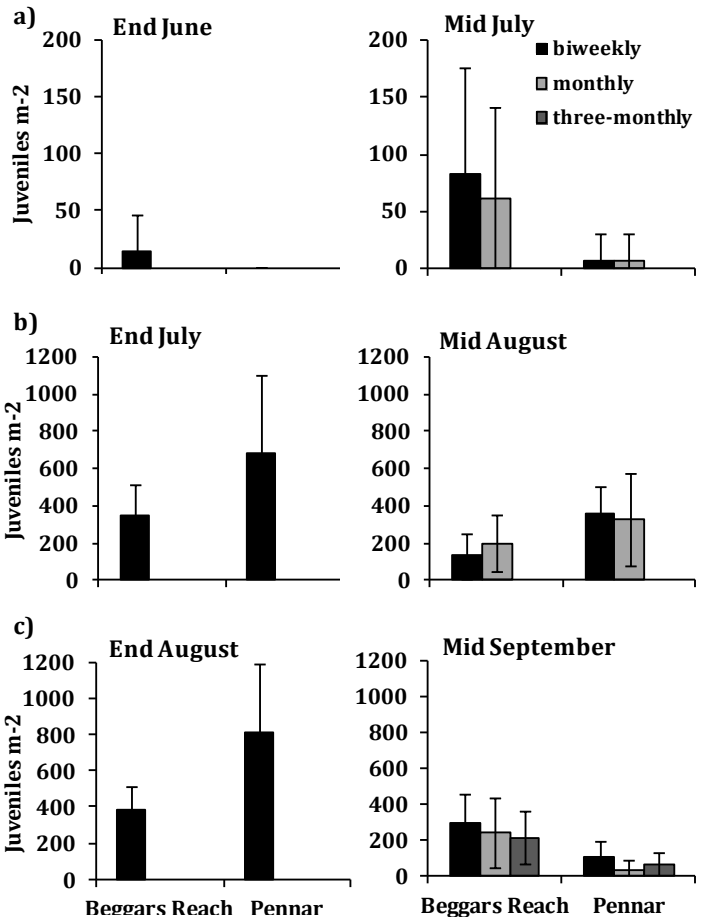



**Fig. 4.3:** Total settlement, monthly and seasonal recruitment of *Crepidula fornicata* in the intertidal of Beggars Reach and Pennar in the Milford Haven Waterway between June and September 2011. Densities of juveniles <5250 µm found on artificial settlement substrata (n=12) are shown for **(a)** Month 1, **(b)** Month 2 and **(c)** Month 3 of the sampling programme. 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 (dark grey bars, sampled mid September). Mean±SD.

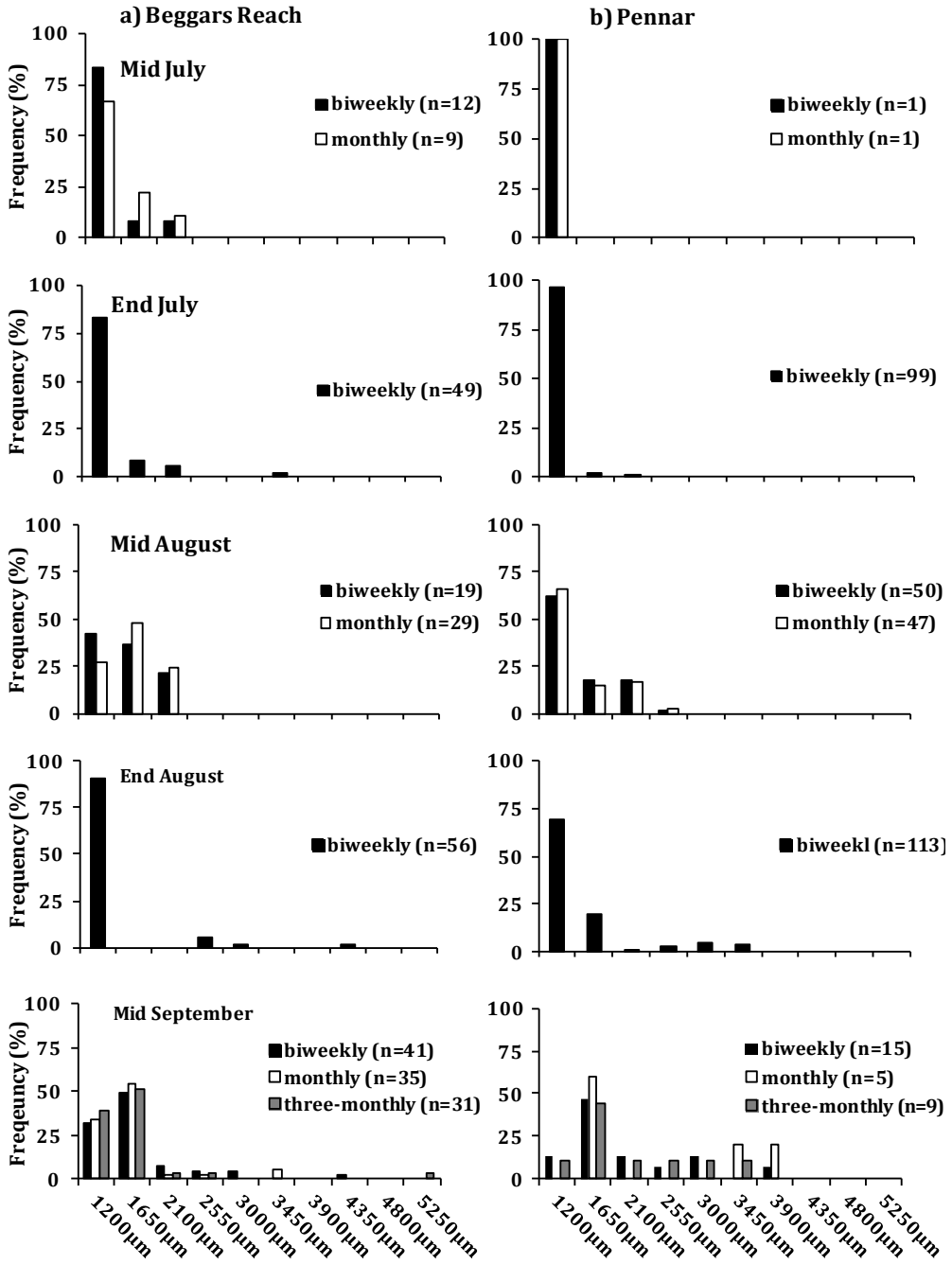



**Fig. 4.4:** Seasonal variation in size frequencies (%) of *Crepidula fornicata* juveniles <5250 µm on artificial settlement substrata at **(a)** Beggars Reach and **(b)** Pennar in the Milford Haven Waterway during summer 2011. Substrata were first deployed in mid June, and either sampled every two 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 (beginning of July) are not shown due to the very low numbers of juveniles present on the panels (Beggars Reach n=2, <1200 µm; Pennar absent)

**Table 4.2**: Results of non-parametric 2-way crossed 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. Significant differences are shown in bold

| | mid July | | | | mid August | | | | mid September | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SS | SS/ $MS_{total}$ | df | p | df | MS | F | p | SS | SS/ $MS_{total}$ | df | p |
| site | 7.52 | 10.40 | 1 | **<0.001** | 1 | 370107.92 | 12.33 | **0.001** | 82.35 | 24.38 | 1 | **<0.001** |
| duration | 0.19 | 0.26 | 1 | 0.610 | 1 | 3557.36 | 0.12 | 0.732 | 7.58 | 2.25 | 2 | 0.325 |
| site x duration | 0.19 | 0.26 | 1 | 0.610 | 1 | 32016.26 | 1.07 | 0.307 | 1.03 | 0.30 | 2 | 0.859 |
| Residual | - | - | | | 44 | 30024.13 | | | - | - | | |

### 4.4.2 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, $\chi^2$=40.48, p<0.001). Almost no mortality occurred in both treatments during the first five days following panel deployment (Fig. 4.5). 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 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.

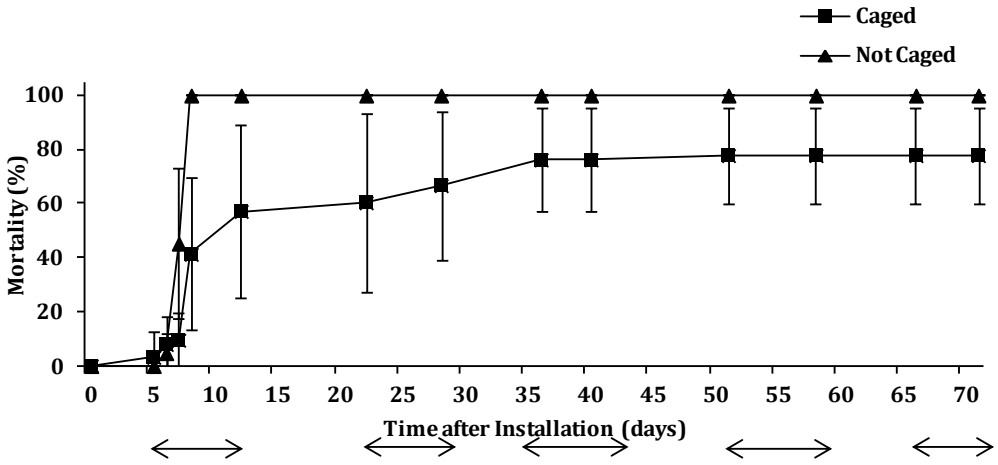



**Fig. 4.5:** Mortality (%) of juvenile *Crepidula fornicata* at Beggars Reach during summer 2011. Fifteen slate panels with 7 laboratory-reared juveniles attached were transplanted into the low intertidal (~1.8 m a. C.D.) in July 2011. Panels were either caged (n=9) or uncaged (n=6). The number of live juveniles found attached to each panel was recorded at least twice during each spring low tide until the end of the experiment on day 71. Data points are average mortality (%) calculated for each sampling event and treatment (mean±SD). Arrows show the periods of spring tides when juveniles were emersed twice a day.

## 4.5 DISCUSSION

In this study, we have simultaneously examined the importance of larval supply, larval settlement and post larval settlement processes in controlling successful recruitment of the American slipper limpet *C. fornicata* into the intertidal in the MHW. Results demonstrate that adult abundance and distribution may primarily be determined following settlement, rather than through larval supply or settlement behaviour. At two sites with highly contrasting adult abundances in the low intertidal zone no obvious differences in the levels of supply of late-stage larvae to the sites were found, and differences in estimates of total settlement rates between both sites were not consistent throughout the season. Post-settlement mortality was very high, probably resulting in negligible recruitment into the adult population within one season. However, we did not observe any consistent differences in mortality between both sites, which would have been necessary to support the model that differing levels of post-settlement mortality caused the observed differential patterns in adult abundance. Nevertheless, results from

the present study clearly show that this process can be critical in limiting recruitment success in the intertidal and is a likely cause for the observed differential adult distribution.

Pelagic larval dispersal is a common mechanism through which marine non-native species disperse. This was early recognised as an important vector for the spread of *C. fornicata* into European waters. For example, Orton (1915) mentioned that "*Crepidula* furnishes an excellent example of the efficacy of a free-swimming larva in extending the domain of a sea-dwelling animal". More recently however it has been shown that the importance of larval supply in determining the final distribution and spread of *C. fornicata* may greatly depend on local and regional hydrodynamics, geographical features of the area, and the location of adult spawning grounds (Viard *et al.*, 2006; Dupont *et al.*, 2007b; Rigal *et al.*, 2010). We found that at the two sites monitored larval supply was not important in determining the final abundance of *C. fornicata* in the intertidal zone. Although total larval densities were much higher at the high adult abundance shore (PE), densities of large, late-stage larvae (i.e. larval supply) did not differ between the high and low adult abundance sites. This suggests that larval production might be higher at the site with higher adult densities (PE), but that the distribution of late-stage larvae may be independent of the location of original larval release as a result of larval export away from the adult beds. The majority of the larvae collected were small and hence likely sampled shortly after release in close proximity to their parental stacks. This is in accordance with data recorded in 2010 from the same sites (Chapter 3). Rigal *et al.* (2010) also found that metamorphically competent larvae were rare, but when they were present they occurred at sites furthest away from the adult beds, most likely due to the export of larvae into the open ocean. Similarly, transport of larvae via the strong tidal and current regime in the MHW (Nelson-Smith, 1965) may have caused the spatial homogenisation of the pool of late-stage larvae in the present study, much as Jenkins (2005) observed in barnacle larvae over similar scales. However, it is important to note that densities of late-stage larvae were generally very low. Differential supply may have thus been difficult to detect.

Both peaks in total larval abundance (likely originating from peaks in larval production) were followed by peaks in settlement densities after approximately four weeks,

corresponding well with the expected duration of the larval phase from release to settlement (Conklin, 1897; Werner, 1955; Pechenik, 1980, 1984). This indicates that the timing of larval release may to some extent control settlement. Despite this potential relationship between the larval phase and settlement, data of the present study suggest that neither larval supply nor habitat selection explain the observed difference in adult abundance between sites, implying that the final distribution of *C. fornicata* is determined following settlement of the larvae. No relationship between late-stage larval densities and adult abundance was 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 (Walne, 1956; McGee and Targett, 1989). Also, waterborne cues released by conspecifics were found to successfully induce metamorphosis of competent *C. fornicata* larvae (Pechenik, 1980; Pechenik and Heyman, 1987; McGee and Targett, 1989), 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 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 sampling programme biweekly and three-monthly spat densities did not differ, indicating that spat had not

accumulated on panels over the three months of the study. Large juveniles were rare, even on the three-monthly 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 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. 4.6). 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 three-monthly 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, work that is presented in Chapter 3 and was 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.

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 congeners 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 (Pechenik *et al.*, 1996a; Beninger *et al.*, 2007; Decottignies *et al.*, 2007a). 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 (Chapter 3) 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).

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 is unlikely 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 recruitment estimates recorded in 2010 at the same sites indicate that spat densities on slate panels correspond to those

on natural substrata (Chapter 3) offering a variety of microhabitat structures, indicating that EPSM may result in low recruitment to the intertidal irrespective of substratum type. However, these data were collected at monthly intervals only and thus do not allow the distinction between recruitment dynamics over varying time scales. This will be addressed in the next chapter.

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 demonstrate 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 recruitment over the entire period of the study 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 (Chapter 3). 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*. This will focus of Chapter 5 that investigates the importance of larval and early juvenile microhabitat associations, and how such associations may affect the distribution of the later life stages. 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.

# CHAPTER 5 -

## Larval microhabitat associations of the non-native gastropod *Crepidula fornicata* and effects on recruitment success in the intertidal zone

## 5.1 ABSTRACT

Habitat-specific distributions of marine benthic invertebrates can be caused by several processes acting prior to, during or after settlement, including differential settlement and varying levels of mortality between habitat types following adaptation of the benthic mode. The non-native gastropod *Crepidula fornicata* is known for its gregarious settlement patterns, yet associations with other shellfish species are also common. In the present study, a series of no-choice and choice laboratory assays were undertaken in which larvae were offered different settlement substrata, separately and simultaneously, to investigate whether differential settlement of *C. fornicata* larvae occurs in favour of specific microhabitat types. A field experiment was also designed to test if recruitment success in the intertidal differed between microhabitat types, by comparing densities of young (<2 weeks) and older (<8 weeks) settlers. The laboratory studies indicated that settlement occurs in larger numbers in association with certain habitats, likely due to passive processes. However, settlement in association with specific microhabitat types was not observed in the intertidal. Instead, the distribution of *C. fornicata* recruits is established after settlement, as the distribution of older recruits, but not younger ones, differed between microhabitat types. Our findings show that the availability of certain complex structures in the intertidal zone is highly important in determining survival success of the species, due to varying levels of early post-settlement mortality (EPSM).

Keywords: *Crepidula fornicata*, larval settlement, habitat selection, microhabitat, post-settlement mortality, recruitment

## 5.2 INTRODUCTION

The associations of species with specific habitat types can be the consequence of a variety of settlement and post-settlement processes. For example, active selective settlement behaviour of larvae or passive processes such as varying levels of 'accessibility' of the settlement surface may occur in favour of certain habitat types (Olabarria *et al.*, 2002; Hale *et al.*, 2008; 2009; Tait and Hovel, 2012). Gregarious settlement in close proximity to conspecifics or associative settlement with other species, including microbial biofilms, may result in non-random distributions (McGee and Targett, 1989; Pawlik, 1992; Kent *et al.*, 2003; Jenkins, 2005). However, settlement may also occur randomly across various habitat types, and in this case, habitat-specific distributions may be established at a later stage due to differences in the habitats' suitability to support later growth and survival of the settled organism. For example, differences in foraging opportunities, food availability or protection from biological and physical stressors may cause highly variable levels of early post-settlement mortality (EPSM) (Gosselin and Chia, 1995a, 1995b; Moksnes *et al.*, 1998; Loher and Armstrong, 2000), which especially under intertidal conditions tends to be high due to the exposure to extreme environmental conditions (Gosselin and Qian, 1996, 1997; Hunt and Scheibling, 1997; Delany *et al.*, 2003; Power *et al.*, 2006). Associative settlement occurring with certain microhabitats, but not others, can thus drastically improve the survival of an individual. The effects of settlement and post-settlement processes on species distributions are therefore unlikely acting in isolation, making it highly challenging to distinguish between their relative importance when predicting recruitment dynamics of a species in certain habitat types.

The American slipper limpet *Crepidula fornicata*, a sessile gastropod native to the North West Atlantic coast, has now established non-native populations in most European waters (Blanchard, 1997). It is highly gregarious and juvenile abundance has been found to be positively correlated with adult abundance (Walne, 1956; Hoagland, 1978; McGee and Targett, 1989, but see Chapter 4). Settlement of planktonic larvae can be followed by relocation of crawling juveniles and males up to a few weeks old (Werner, 1955). Whether established by the larval or the juvenile stage, permanent settlement

often occurs on top of conspecifics, resulting in the formation of stacks that are a necessary prerequisite for internal fertilisation to occur. Stacks with >10 individuals are common (pers. obs.). However, *C. fornicata* is often found attached to other hard substrata (Mineur *et al*., 2012), including several commercially important shellfish species (McMillan, 1938; Korringa, 1942; Thieltges, 2005b). This has greatly aided its first introductions and subsequent secondary spread in its non-native range. The first documented specimens in European waters were found amongst the American oyster *Crassostrea virginica* (Crouch, 1893; McMillan, 1938), and the further transportation of *Mytilus edulis*, *Crassostrea gigas* and *C. virginica* with attendant epibont *C. fornicata* through aquaculture practices has facilitated its spread within its non-native range (Korringa, 1942; Blanchard, 1997; Mineur *et al*., 2012). Potential resulting direct and indirect negative effects on its basibionts are well documented (Thouzeau *et al.*, 2000; Riera *et al.*, 2002; Thieltges, 2005b; Decottignies *et al.*, 2007a; 2007b; Blanchard *et al.*, 2008).

Settlement of *C. fornicata* occurs as a direct consequence of metamorphosis following the loss of the swimming organ, the velum (Werner, 1955). Several chemical, physical and/ or biological cues may induce metamorphosis in well developed, late-stage competent *C. fornicata* larvae, including the presence of juvenile *C. fornicata* and fragments of *Mercenaria mercenaria* shells (Pechenik, 1980), adult-conditioned seawater (Pechenik and Heyman, 1987; McGee and Targett, 1989), the presence of the species *Crepidula plana* and the hermit crab *Pagurus pollicaris* occupying shells of *Busycon carica* (McGee and Targett, 1989), short exposure to heat shocks (Gaudette *et al.*, 2001), a naturally-produced halogenated compound (Taris *et al.*, 2010) and elevated KCl concentrations (Pechenik and Heyman, 1987; Pechenik and Gee, 1993). However, it remains unclear if the above mentioned aggregative distribution of juveniles and adults amongst conspecifics and other basibionts is a result of differential patterns established directly at settlement of the larvae due to physiological or behavioural responses to any one of these cues. Alternatively, the documented clumped distribution of juveniles may be a result of passive deposition of the larvae following metamorphosis, triggered after contact with a settlement inducing cue.

It is also possible that recruitment differs between certain microhabitat types due to post-metamorphic movement or mortality (Crowe and Underwood, 1998). Previous studies showed that the distribution of *C. fornicata* is modified some time after settlement due to such post-colonisation processes (Shenk and Karlson, 1986; McGee and Targett, 1989). Particularly in the intertidal, recruitment of *C. fornicata* is highly variable, possibly due to the exposure of the newly settled individuals to sub-optimal conditions causing high EPSM (Chapter 3). Previous work has also shown that this might be attenuated when microhabitats provide protection from such physical stressors (Chapter 4).

In the present study, we conducted a series of laboratory experiments to investigate if settlement of the larvae of *C. fornicata* differs between various microhabitats, i.e. shells of conspecifics and shells of a second mollusc species (*Mytlus edulis*). We also determined whether the early distribution of juvenile *C. fornicata* changes due to post-metamorphic movement. Lastly, a field experiment was designed to monitor settlement rates in association with certain microhabitat types, and if recruitment success differs between these.

## 5.3 METHODS

### 5.3.1 STUDY SITE

Field experiments and collection of adult *C. fornicata* broodstock for larval rearing were undertaken in the low intertidal zone (~1.0-1.3 m above Chart Datum) at Pennar in the Milford Haven Waterway (MHW) in South West Wales, UK (Fig. 5.1). The low intertidal of this natural ria is characterised by muddy-gravel banks that are interspersed with sandy beaches and rocky outcrops (Nelson-Smith, 1965). Marine conditions prevail in the ria due to the low input of fresh water, resulting in the occurrence of a diverse marine flora and fauna (Nelson-Smith, 1967). Tides are semi-diurnal and low water during spring tides occurs around mid-day and mid-night, causing the regular exposure of the lower shore biota to extreme environmental conditions during spring tide

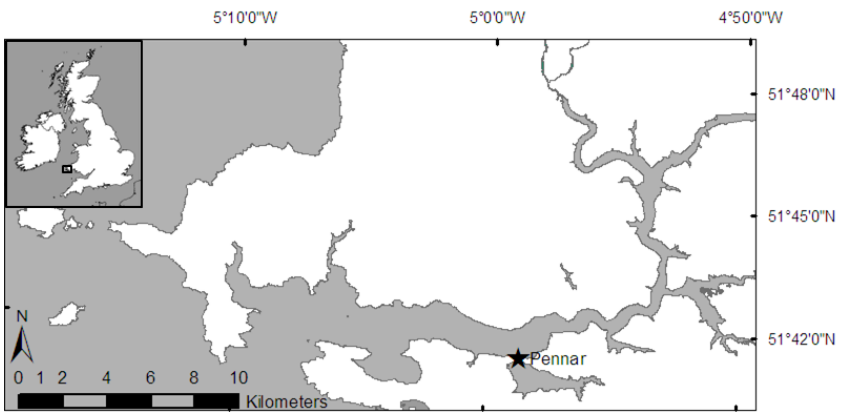



**Fig 5.1:** Map of the Milford Haven Waterway (MHW) in South West Wales, UK (top left inset). Field experiments and collection of adult broodstock were undertaken at ~1.0-1.3 m above C.D. at Pennar.

emersion. Further details on the physical and biological environment can be found elsewhere (Nelson-Smith, 1965, 1967).

*Crepidula fornicata* was first observed in the MHW in the early 1950s (Cole and Baird, 1953), has spread rapidly across most of the ria by the 1960s (Crothers, 1966) and now reaches localised densities of >1000 individuals $m^{-2}$ in the low intertidal and subtidal zone of the middle reaches of the ria (Chapter 2). Densities vary greatly at a scale of meters and between habitat types. At Pennar, a shore typical for the MHW due to the composition of the substrata of mud and gravel, a vertical gradient in adult densities of 1031±943, 343±360 and 76±125 (mean±SD) has been observed at tidal heights of ~0.6, 1.2 and 1.8 m above C.D., respectively (Chapter2). The reproductive season of *C. fornicata* in the MHW extends from early spring to late autumn with the main period of settlement occurring between the end of June/ July and September (Chapter 3).

### 5.3.2 LABORATORY SETTLEMENT ASSAYS

A series of no-choice and choice assays were undertaken in which larvae of *C. fornicata* were offered several substratum types separately or simultaneously to test whether larval settlement occurs in greater numbers on certain substratum types. No-choice assays were done to test the effectiveness of experimental treatments in inducing

metamorphosis (and thus settlement) and at assuring the absence of artefact effects of experimental conditions. For these, larvae were offered only one of each shell types (i.e. *M. edulis* or *C. fornicata* shell). Choice experiments were undertaken by exposing the larvae to both shell types simultaneously to assess whether differential settlement between various shell types may occur.

Olabarria *et al.* (2002) suggest that equally designed choice and no-choice assays should be undertaken simultaneously to unambiguously distinguish behavioural processes (i.e. larval preference of a specific settlement site) from passive processes due to differences in accessibility (e.g. the simplicity with which attachment occurs over a given time). If following this design, active selective settlement behaviour of the larvae can be established when the proportion of larvae observed on each substratum type differs when larvae are offered a choice compared to when larvae are offered no choice (Olabarria *et al.*, 2002). This was not possible within the scope of this study due to difficulties in rearing sufficient amounts of larvae for the simultaneous undertaking of choice and no-choice assays. These experiments are thus designed to identify patterns in settlement (and the potential for these early processes in determining the observed juvenile and adult distribution in the field), but not to identify causative mechanisms for the observed patterns (i.e. if due to passive or active larval settlement).

#### 5.3.2.1 LARVAL REARING

Larvae for all experiments were reared in the laboratory. An adult *C. fornicata* broodstock was collected at Pennar. Stacks (>6 adults each) were individually placed in cylindrical tubes (diameter ~12cm) with 250 µm-mesh attached to the bottom to ensure the retention of the released larvae and enable the identification of the parental stack of each larval brood. Each adult stack was supplied with constantly flowing, aerated and 120 µm-filtered seawater at ~15-16 °C and fed a diet of *Isochrysis galbana* (clone T-ISO). Larvae were collected within 12 h following release and immediately transferred to 1 µm- and UV- filtered seawater at ~19-21 °C. Larvae were reared in batch cultures at densities of ~0.25-0.5 larvae $mL^{-1}$, and fed a diet of *I. galbana* (clone T-ISO, cell density ~1.8 * $10^5$ cells $mL^{-1}$). Seawater and food were changed every other

day. Larvae from a single batch were released on the same day and were from the same stack, but not necessarily from the same female.

To estimate the development of each batch of larvae, growth rates and morphological development of 15 randomly chosen larvae were monitored every other day (data not shown). Once a batch reached an average size of >650 µm and showed first signs of morphological differentiation to the competent stage (flattened shell geometry and presence of a "brimmed shell", see Pechenik, 1980; Pechenik and Lima, 1984), subsamples of 40 larvae, divided into 4 replicates of 10 larvae, were frequently exposed to an artificial inducer (20 mM K+ enriched filtered sea water, see Pechenik and Heyman, 1987) for 4-6 h to establish the percentage of metamorphically competent larvae in the batch. When metamorphic competency of a batch was nearing 50%, a no-choice or choice settlement experiment was started within the next 48 h. All larvae were between 14-19 days old at the beginning of the experiments. Experiments were run under the same conditions as the larval rearing, but in the absence of food to avoid the presence of a potentially confounding cue. Each larva was only used once.

#### 5.3.2.2 NO-CHOICE ASSAYS

Metamorphically competent larvae were exposed either to an experimental treatment (i.e. various shell types) or a control treatment (i.e. no shell present) for 6 h to compare larval settlement behaviour in the absence of choice. We aimed at standardising and maximising the total number of larvae metamorphosing in the experimental treatments to improve comparability between groups and allow assessment of active larval choice. Experimental treatments were therefore run in seawater conditioned by adult *C. fornicata* stacks (adult conditioned seawater, hereafter ACSW) which is known to be effective in inducing metamorphosis (Pechenik and Heyman, 1987; McGee and Targett, 1989). Seawater was conditioned by putting three stacks (5-7 adults each) in 2 L of 1 µm- and UV- filtered seawater for 2.5-3 h. ACSW was passed through a 100 µm filter to remove any debris and faeces. Experimental treatments were undertaken in glass dishes (diameter ~7 cm, height ~3.5 cm) filled with 50-70 ml ACSW. Pilot trials showed that *C. fornicata* larvae may settle on the glass dish instead of the substrata. To

encourage settlement of the larvae on top of the substrata, instead of the surrounding glass the bottom of the dish was covered to a depth of ~1 cm of sand, which we considered an unsuitable settlement substratum for *C. fornicata*. The sand was collected from the low intertidal in the Menai Strait, North Wales, washed and sieved to 125-500 µm.

Empty *C. fornicata* shells (treatment I - *Crepidula*) or empty *M. edulis* shells (treatment II - Mussel) were used as substratum types in the experimental treatments, to offer settlement surfaces identical to those typically encountered by the larvae in the field (i.e. treatment I - conspecifics during stack formation, treatment II - a shellfish species that is abundant in the same habitat and is often colonised). An individual shell was placed in the middle of each dish and gently pushed into the sand until the shell margins were covered. Live *C. fornicata* or *M. edulis* were not used because suspension feeding adults are known to ingest *C. fornicata* larvae (Pechenik *et al*., 2004). Only "fresh" and clean shells of each species were used after removing the animal and all other epibiota from the shell. All shells were of similar size (~4.5-5.5 cm maximum length).

Several control treatments were run in parallel to establish metamorphic competency of the larvae at the time the experiment was undertaken and to control for potential effects of experimental conditions on metamorphosis and settlement behaviour of the larvae. Using the same glass dishes, the efficacy of ACSW in providing the cue that induces metamorphosis was investigated by exposing larvae to ACSW alone, not using substrata or sand (treatment III - ACSW). Also, a positive control and a negative control were established by exposing larvae to 20 mM excess KCl to establish percentage competency of the batch (treatment IV - K+), or filtered seawater only (treatment V - FSW), respectively. To ascertain that the sand did not contain cues that might induce metamorphosis and attract settlement of the larvae, we established another treatment in which the bottom of the glass dish was covered with sand in the same way as in the experimental treatments, but using FSW and offering no substrata (treatment VI - Sand).

Four replicates were used for each experimental or control treatment. Fifteen larvae were introduced to each replicate at the beginning of the experiment by pipetting them

evenly across the water surface. The dishes were left undisturbed for 6 h to allow sufficient time for metamorphosis (and hence settlement) to occur. After 6 h, the glass dishes were examined under a dissecting microscope (x 40 or 63). The number of settled individuals on each surface type (shell, glass or sand) and the number of free-swimming larvae were noted. This experiment was run four times.

Data were analysed using one-way ANOVA to test for differences among experimental and control treatments following Levene's test to establish homogeneity of variances. The proportions of larvae settled on top of the shells (instead of the glass dishes and the sand, hereafter referred to as 'other substrata') between treatments I and II were compared each time the experiment was run. One-way ANOVA was also used on the proportions of the total number of settled larvae, regardless of whether settlement had occurred on top of the shells or the 'other substrata'. This aimed at testing if cues in the experimental treatments (ACSW, shell types offered) were efficient in inducing metamorphosis and adoption of a benthic mode in competent larvae by comparing proportions with the overall level of metamorphic competency, which was estimated by the percentage of larvae metamorphosing in response to the K+-treatment. Also, this tested for potential artefact effects of experimental conditions (sand, glass dishes) on metamorphosis and settlement behaviour. Data transformations never achieved homogeneity of variances for the first and second run of the experiment due to the presence of only zero values in certain treatments (FSW-treatment in run 1, ACSW-treatment in run 2), so these treatments were removed for statistical analyses. Data from the third and fourth run were arcsine-square root transformed to achieve homogeneous variances. Tukey's post-hoc test established which treatments differed.

#### 5.3.2.3 CHOICE ASSAYS

Shells of *C. fornicata* and *M. edulis*, prepared in the same way as for the no-choice assays, were simultaneously offered to *C. fornicata* larvae in 8 experimental mesocosms (12.5 cm x 12.5 cm x 6 cm, length x width x height) that were filled with sand (depth ~1 cm) and ~350 ml ACSW. Sand and ASCW were prepared as described for the no-choice assays. Two shells from each species were added to each mesocosm and arranged in a

mosaic pattern with gaps >1 cm between shell margins. At the beginning of the experiment, 50 larvae were introduced by evenly distributing them across the whole surface of the mesocosm. Four mesocosms were sampled after 6 h and the remaining four mesocosms after 24 h in order to determine whether the distribution of early settlers differed to that of older juveniles, as a result of post-metamorphic movement. As in the no-choice assays, the number of individuals that had settled on each surface type (*C. fornicata* shells, *M. edulis* shells, the mesocosm wall, sand) were noted as well as the number of free-swimming larvae. Metamorphic competencies of the larval batches used in these experiments were monitored in parallel by exposing 15 larvae to excess KCl for 6 h or 24 h, proceeding in the same way as in the no-choice assays. This experiment was run twice.

Replicated *G*-tests of goodness-of-fit were undertaken to compare the magnitudes of settlement on both substratum types (Sokal and Rohlf, 1995; Lee *et al*., 2004). We tested the hypothesis that the distribution of settlers between *Crepidula* shells and mussel shells is uneven (i.e. deviates from an expected ratio of 1:1). For this, analyses were run separately for each trial (i.e. for 6 h and 24 h exposure for each of the two runs of the experiment). A second hypothesis was tested which predicts that the distribution of settlers may change with length of exposure to experimental conditions, due to post-metamorphic movement. Here, for each run separately, we tested the distribution of settlers after 24 h against the distribution after 6 h, instead of using an expected ratio of 1:1 (even distribution). All replicated *G*-tests of goodness-of-fit were run by undertaking *G*-tests on the replicates separately (Replicates 1-4), followed by a *G*-test on all the data from each replicated experiment together to test whether the expected ratio is fit by all data ($G_{total}$), on the pooled data set of each replicated experiment ($G_{pooled}$) and an heterogeneity test ($G_{hetero}$) (Sokal and Rohlf, 1995; Lee *et al*., 2004).

### 5.3.3 FIELD SETTLEMENT EXPERIMENT

A field experiment was undertaken at Pennar (~1.0-1.3 m above C.D.) to investigate whether rates of settlement and recruitment varied between different microhabitat types under intertidal conditions. For this, 2 sets of 16 slate plates (11 cm x 11 cm) were

deployed in mid July 2011 during spring low tides. The surfaces of the plates were manipulated to simulate various naturally occurring microhabitat types. Whilst 2 x 4 plates were left bare (referred to as treatment 'Panel' hereafter), the same amount of plates were covered in flat stones ('Stone'), empty *C. fornicata* shells ('*Crepidula*') or empty *M. edulis* shells ('Mussel'). Stones and shells (unbroken and not eroded) used for the experiments were collected at the same site and cleaned thoroughly of all epibionts the day before deployment. Wired mesh (mesh size 2 cm x 2 cm) was wrapped around the plates with the stones or shells aligned on top to keep the substrata in position. Plates were deployed on metal frames identical to those used in previous studies at the same site(Chapters 3 and 4), with one panel of each microhabitat type attached to each frame. One set of the plates was retrieved and replaced with new plates every two weeks to measure the densities of young (<2 weeks) *C. fornicata* settlers on the various microhabitat types (hereafter referred to as 'biweekly'). The other set was sampled only once at the end of the experiment in mid September to estimate the densities of older *C. fornicata* recruits (<8 weeks, 'two-monthly') (n=4 per substrata type and panel deployment duration). At the end of deployment in the field, plates were collected and transported to the laboratory by suspending them on horizontal metal stakes that rested in cooling boxes. All *C. fornicata* present on the panels as well as on the substrata were counted and measured under a dissecting microscope with an ocular micrometer (x 63).

One-way ANOVA was undertaken on the calculated densities for each sampling event and panel deployment duration separately to test for potential differences in settlement rates (biweekly plates) and recruitment success (seasonal plates) between the four different microhabitat types. Levene's test was used to check for homogeneity in variances and transformed where appropriate. Tukey post-hoc testing was performed where significant differences were detected.

# 5.4 RESULTS

## 5.4.1 LABORATORY SETTLEMENT ASSAYS

### 5.4.1.1 NO-CHOICE ASSAYS

Comparisons among treatments and controls for the general settlement response (i.e. the total number of larvae metamorphosing and settling, irrespective of settlement site) showed variable patterns over the four different runs of the no-choice experiment (Table 5.1, Fig. 5.2a). This occurred even though metamorphic competency of the larval batches was generally high (percentage metamorphosis K+-treatment >60% in all four runs, Fig. 5.2a), demonstrating that all larval batches used during the experiments were in a developmental stage suitable to observe settlement if metamorphosis-inducing cues were present.

Treatments I and II (*Crepidula* shell and mussel shell) were generally effective in inducing metamorphosis and settlement, although the percentage metamorphosis in these treatments was relatively low in run 1 and run 2. ACSW was only effective in

**Table 5.1:** No-choice assays. Results of one-way ANOVA to test for the effects of different treatments (*Crepidula* shell, mussel shell, ACSW, K+, FSW, sand) on the percentage of *Crepidula fornicata* larvae metamorphosing within 6 h. Treatments with no metamorphosed larvae were removed for statistical analyses as there were no variances to calculate (FSW in run 1 and ACSW in run 2). Data from Run 3 and Run 4 were arcsine-square root transformed to achieve homogeneous variances. Significant differences are shown in bold.

| | df | MS | F | p |
|---|---|---|---|---|
| *Run 1* | 4 | 0.216 | 27.514 | **<0.001** |
| Residual | 15 | 0.008 | | |
| *Run 2* | 4 | 0.293 | 72.439 | **<0.001** |
| Residual | 15 | 0.004 | | |
| *Run 3* | 5 | 2400.381 | 14.513 | **<0.001** |
| Residual | 18 | 165.401 | | |
| *Run 4* | 5 | 4263.235 | 29.651 | **<0.001** |
| Residual | 18 | 143.780 | | |

**Table 5.2:** No-choice assays. Results of one-way ANOVA to test for the attractiveness of *Crepidula* shells (treatment I) or mussel shells (treatment II) as settlement substrata for *Crepidula fornicata* larvae. Tests were done on the proportions of the newly metamorphosed juveniles that were observed on the shells instead of the surrounding substrata (glass or sand) after 6 h. Significant differences are shown in bold.

| | df | MS | F | p |
|---|---|---|---|---|
| *Run 1* | 1 | 0.146 | 0.538 | 0.496 |
| Residual | 5 | 0.271 | | |
| *Run 2* | 1 | 0.516 | 39.450 | **0.002** |
| Residual | 5 | 0.013 | | |
| *Run 3* | 1 | 0.424 | 10.415 | **0.018** |
| Residual | 6 | 0.041 | | |
| *Run 4* | 1 | 0.114 | 1.210 | 0.314 |
| Residual | 6 | 0.094 | | |

inducing metamorphosis in competent larvae in large numbers in run 3 when percentage metamorphosis was similar to the K+-treatment (Tukey $p>0.05$, Fig. 5.2a). Although the low response to ACSW indicated that experimental conditions were not always successful in maximising settlement of competent larvae, the number of larvae metamorphosing in response to treatments I and II did not differ (Tukey $p>0.05$ in all four runs, Fig. 5.2a). This showed that the cues present in both treatments were similarly effective in inducing metamorphosis, even if this was not due to the provision of ACSW. Only few larvae responded when exposed to FSW (treatment V) and sand (treatment VI), ensuring that the experimental conditions did not induce larval metamorphosis and settlement.

Although similar numbers of larvae metamorphosed when presented with either shell type, there was a trend that larger proportions of larvae in the *Crepidula* treatment settled on top of the shell instead of on the surrounding substrata compared to in the mussel shell treatment. This was significant in run 2 and 3 (Table 5.2, Fig 5.2b). In total, 76 larvae settled on *Crepidula* shells and 37 on mussel shells.

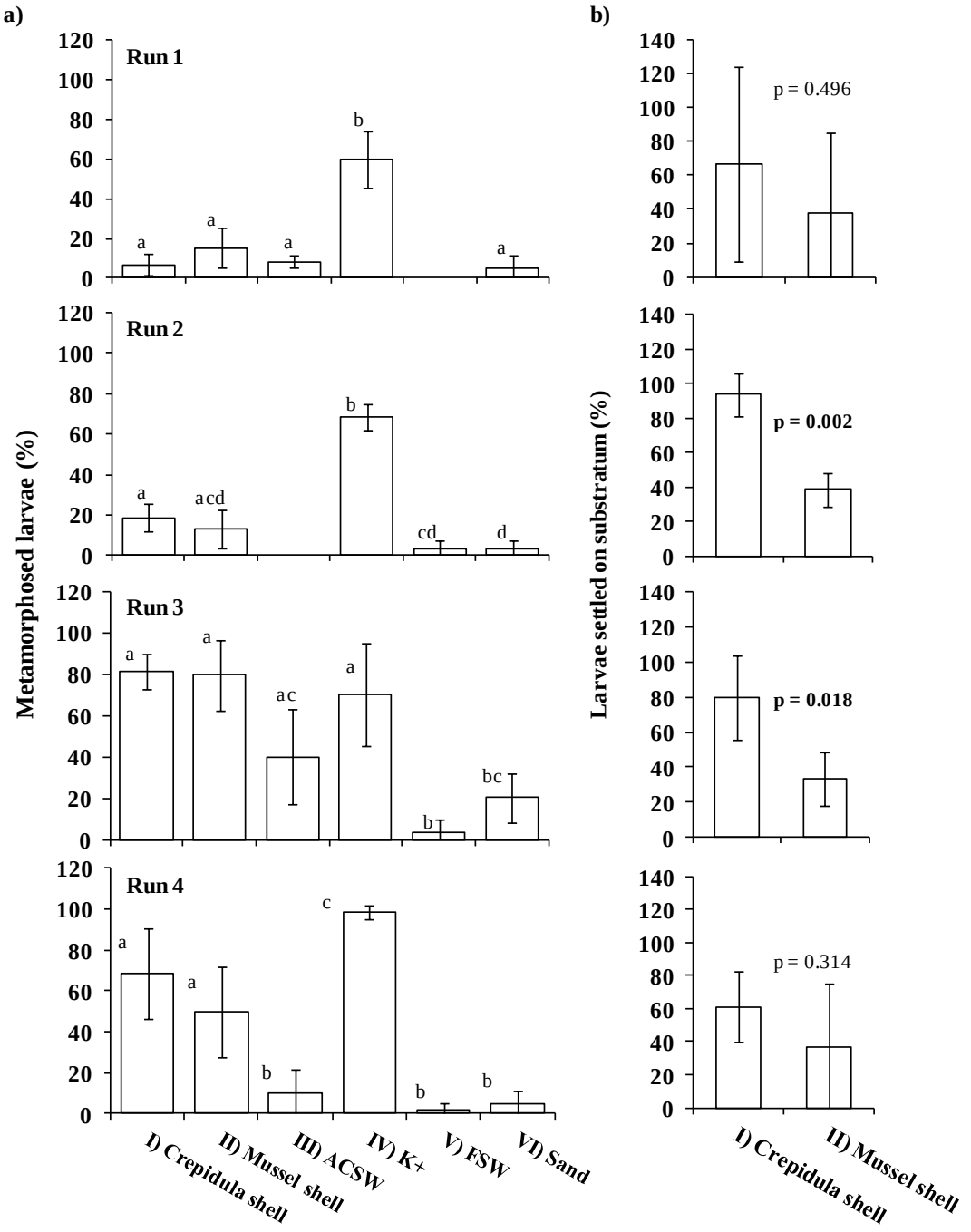



**Fig. 5.2:** Results from no-choice settlement assays where *Crepidula fornicata* larvae were exposed for 6 h to one of two experimental treatments: I) *Crepidula* shell, II) mussel shell, or one of four control treatments: III) adult conditioned seawater (ACSW), IV) 20 mM K+ enriched filtered sea water (K+), V) filtered seawater (FSW) or VI) sand. **a)** Percentage of all larvae that had metamorphosed, including those that had settled on the sand or glass dishes. Different letters above bars indicate significant differences between treatments. **b)** Percentage of newly metamorphosed juveniles that had settled on top of the empty *Crepidula* shell (treatment I) or the empty mussel shell (treatment II), instead of the sand or glass, of all metamorphosed larvae. Proportions were significantly different in run 2 and run 3. Mean±SD.

### 5.4.1.2 CHOICE SETTLEMENT ASSAYS

Replicates 1-4 from each trial always fitted the same ratios, resulting in non-significant heterogeneity tests ($G_{hetero}$, Table 5.3) and thus allowing the pooling of all the data of each experiment (Sokal and Rohlf, 1995). There was a general trend that more larvae had settled on the *Crepidula* shells, with approximately twice as many settled larvae observed on top of the *Crepidula* shells than on top of the mussel shells (Fig. 5.3a and 5.3b). *G*-tests on the pooled data ($G_{pooled}$) from each trial revealed that deviations from an expected even distribution were significant twice: in run 1 after 24 h and in run 2 after 6 h (Fig. 5.3 a and 5.3 b, Table 5.3).

In run 2 after 24 h, similar numbers had settled on the mussel shells (36±14% of all settled larvae, mean±SD) and the *Crepidula* shells (42±5%), but this had not been the case after 6 h when proportions differed between the substratum types (Fig. 5.3, Table 5.3). This indicates that here, the newly metamorphosed juveniles made an active choice towards the mussel shells between 6 h and 24 h. These temporal changes were marginally significant at p=0.05 (Table 5.4). No changes over time were observed during run 1.

**Table 5.3:** Choice assays. Replicated *G*-test of goodness-of-fit to test the hypothesis of differential settlement of *Crepidula fornicata* larvae on *Crepidula* shells and mussel shells after 6 h or 24 h in two separate runs of the experiment. Comparisons were done against the expected ratio of an even distribution of settlers between substratum types (1:1). See Sokal & Rohlf 1995 for details. Significant differences are shown in bold.

| *Experiment* | | Replicates | | | | $G_{total}$ | $G_{pooled}$ | $G_{hetero}$ |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | | |
| *Run 1* - | d.f. | 1 | 1 | 1 | 1 | 4 | 1 | 3 |
| 6 h vs even | *G* | 0.287 | 2.039 | 0.335 | 0.077 | 2.737 | 2.136 | 0.601 |
| distribution | p | 0.592 | 0.153 | 0.563 | 0.781 | 0.603 | 0.144 | 0.896 |
| *Run 1* - | d.f. | 1 | 1 | 1 | 1 | 4 | 1 | 3 |
| 24 h vs even | *G* | 0.699 | 1.828 | 4.717 | 5.017 | 12.260 | 11.181 | 1.079 |
| distribution | p | 0.403 | 0.176 | **0.030** | **0.025** | **0.016** | **<0.001** | 0.782 |
| *Run 2* - | d.f. | 1 | 1 | 1 | 1 | 4 | 1 | 3 |
| 6 h vs even | *G* | 5.062 | 2.039 | 0.335 | 0.077 | 7.513 | 4.478 | 3.035 |
| distribution | p | **0.024** | 0.153 | 0.563 | 0.781 | 0.111 | **0.034** | 0.386 |
| *Run 2*- | d.f. | 1 | 1 | 1 | 1 | 4 | 1 | 3 |
| 24 h vs even | *G* | 0.476 | 1.007 | 0.430 | 2.165 | 4.077 | 0.728 | 3.349 |
| distribution | p | 0.490 | 0.316 | 0.512 | 0.141 | 0.396 | 0.393 | 0.341 |

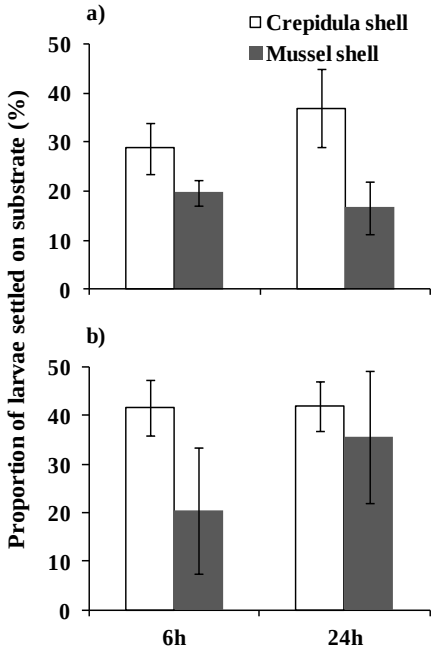



**Fig. 5.3:** Proportions of newly metamorphosed *Crepidula fornicata* juveniles settled on the empty *Crepidula* shells and the empty mussel shells of the total number of metamorphosed juveniles in the first run **(a)** and the second run **(b)** of the choice settlement assays. Larvae were simultaneously offered the choice between both substratum types for 6 h or 24 h. Metamorphic competency of the larval batches was always >90%. Mean±SD.

**Table 5.4:** Choice assays. Replicated *G*-test of goodness-of-fit to test the hypothesis of temporal changes in the distribution of newly metamorphosed *Crepidula fornicata* larvae between 6 h and 24 h of exposure to *Crepidula* shells and mussel shells in two separate runs of the experiment. The observed distribution of settlers between both substrata types after 24 h was tested against an expected ratio that was derived from the observed distribution after 6 h (*Crepidula*:mussel = 34:23 in Run 1, 33:18 in Run 2). See Sokal and Rohlf 1995 for details. Significant differences are shown in bold.

| *Experiment* | | Replicates | | | | $G_{total}$ | $G_{pooled}$ | $G_{hetero}$ |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | | | |
| *Run 1* - 6 h vs. 24 h | d.f. | 1 | 1 | 1 | 1 | 4 | 1 | 3 |
| | *G* | 0.019 | 0.242 | 1.643 | 2.144 | 4.048 | 2.970 | 1.078 |
| | p | 0.889 | 0.623 | 0.200 | 0.143 | 0.400 | 0.085 | 0.782 |
| *Run 2* - 6 h vs. 24 h | d.f. | 1 | 1 | 1 | 1 | 4 | 1 | 3 |
| | *G* | 0.376 | 6.299 | 0.512 | 0.003 | 7.190 | 3.841 | 3.349 |
| | p | 0.540 | **0.012** | 0.474 | 0.959 | 0.126 | **0.050** | 0.341 |

### 5.4.2 FIELD EXPERIMENTS

Biweekly settlement densities never differed between the four different substratum types in the field trials (Table 5.5, Fig. 5.4). At the end of the experiment, however, densities of juvenile slipper limpets differed between substratum types on the two-monthly panels (Table 5.5, Fig. 5.4). Post-hoc testing revealed that densities on the two-monthly bare slate panels were significantly lower compared to those on the two-monthly mussel shell panels (Tukey $p=0.05$).

## 5.5 DISCUSSION

The combined laboratory and field studies of the present study showed that although settlement of the American slipper limpet *C. fornicata* occurs in greater numbers in association with certain habitat types under laboratory conditions, the intertidal distribution of juvenile settlers is determined during processes acting after settlement. We found that settlement of *C. fornicata* larvae tended to be higher on *Crepidula* shells than on mussel shells in laboratory assays. Interestingly, however, such associations with specific microhabitat types are not obvious soon (<2 weeks) after settlement under intertidal conditions. Post-colonisation processes were found to re-structure juvenile distribution, as differences in juvenile densities between microhabitat types were observed for older (<8 weeks) but not younger recruits (<2 weeks). EPSM is therefore highly important in shaping the intertidal distribution of the species. Our results thus indicate that although associative settlement occurs, adult distributional patterns in the intertidal zone may be established post-settlement.

The non-random aggregation of newly metamorphosed *C. fornicata* juveniles that was observed during our laboratory assays may be caused by several processes. For example, associative settlement may have occurred due to the passive deposition of the metamorphosed individuals after contact with one of the waterborne cues that initiate metamorphosis. The results from our laboratory experiments indicate that this is unlikely. The proportions of metamorphosed individuals that had settled on the shells

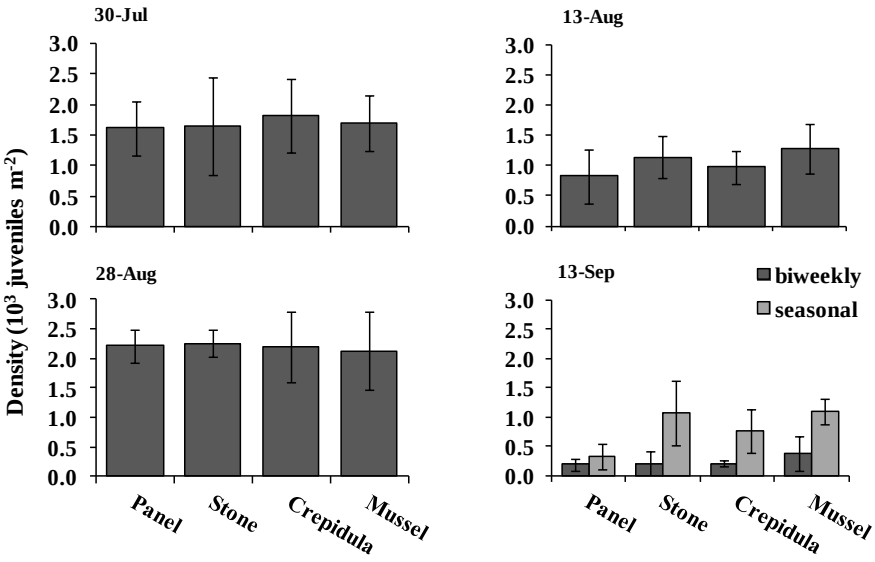



**Fig. 5.4:** Juvenile *Crepidula fornicata* densities on the different substrata types that were deployed for two weeks (biweekly) or eight weeks (seasonal) at Pennar in the Milford Haven Waterway (MHW) during the settlement season of 2011. Ordinary roofing slates were used as a base and either left bare ('Panel'), or were covered in flat stones ('Stone'), empty *Crepidula fornicata* shells ('Crepidula') or empty *Mytilus edulis* shells ('Mussel'). Mean±SD.

**Table 5.5:** Field experiment. One-way ANOVA to test for differences in juvenile *Crepidula fornicata* densities between microhabitat types after two weeks (biweekly) and eight weeks (seasonal). Slate plates were manipulated to simulate different microhabitats by attaching stones, *Crepidula* shells or mussel shells, or were left bare. Data from biweekly panels on 13th September were arcsine-square root transformed to achieve homogeneous variances. Significant differences are shown in bold.

| | df | MS | F | p |
|---|---|---|---|---|
| *Biweekly 1(30th July)* | 3 | 31872.595 | 0.091 | 0.963 |
| Residual | 12 | 349474.989 | | |
| *Biweekly 2 (13th August)* | 3 | 155954.076 | 1.094 | 0.389 |
| Residual | 12 | 142578.551 | | |
| *Biweekly 3 (28th August)* | 3 | 10672.267 | 0.470 | 0.986 |
| Residual | 12 | 224967.464 | | |
| *Biweekly 4 (13th September)* | 3 | 26.089 | 0.622 | 0.614 |
| Residual | 12 | 41.926 | | |
| *Seasonal (13th September)* | 3 | 510557.910 | 3.809 | **0.040*** |
| Residual | 12 | 134042.149 | | |
| *Tukey | panel < mussel | | | |

differed between *Crepidula* shells and mussel shells in the no-choice experiments, although the total numbers of larvae metamorphosing in either treatment were similar. Fully passive fall-out of the larvae should have resulted in a completely random distribution across all surface types in both treatments, including the sand, where however only <3.5% of the settled individuals were observed in the experimental treatments of each run. This indicates that the underlying process of metamorphosis and the availability or efficacy of the inducing cue did not determine the distribution patterns of settlers amongst these substrata types. Active searching behaviour of the larvae could also result in an aggregated distribution of settlers with specific substrata types. Our study was not designed to test for this using the approach suggested by Olabarria *et al.* (2002). The very similar results in settlement ratios on both substrata types between the choice and the no-choice assays however suggests that larvae showed no active preference *sensu* Olabarria *et al.* (2002).

Differences in settlement between both shell types were most likely due to differences in 'accessibility'. Olabarria *et al.* (2002) refers to this as the 'ease with which a microhabitat can be found or be occupied'. In our study, unsuitable surface complexity, for example, may have reduced the numbers of larvae that could successfully attach to the mussel shell during settlement, even if equally 'liked' compared to other potential surfaces and presenting similar metamorphosis-stimulating cues. Differences in the levels of settlement on certain surface structures over others have been widely reported from barnacles (Raimondi, 1988; Hills and Thomason, 1998; Berntsson *et al.*, 2000), even if the process (behavioural vs. passive) has not always been explicitly tested. The aim of the present study was not to identify cues that may facilitate metamorphosis and cause increased settlement on specific shell types. The experimental conditions were standardised between both treatments (i.e. the use of similarly sized and treated shells, sand, and ACSW), so any differences are most likely due to characteristics intrinsic to the shell types, such as specific biofilms or surface complexity.

We have however found some evidence that the distribution established at settlement changes during the first day following settlement. In run 2 of the choice experiment, changes in settlement patterns between 6 h and 24 h were apparent, indicating that post-metamorphic movement alters the dispersal of settlers within 24 h. Surprisingly,

the change here occurred in favour towards a 'more even' distribution. After 24 h, settlers were observed in equal proportions between both shell types, as indicated by non-significant deviations from an expected even (1:1) distribution. However, deviations were apparent from the expected distribution determined after 6 h, indicating that some time after metamorphosis a choice is made by the juveniles in favour of the mussel shells, levelling out any differences established during settlement due to better accessibility of the *Crepidula* shells. Differential mortality can be excluded as the post-colonisation process under the experimental conditions of our laboratory studies. Mortality was overall low and 93% of all introduced individuals were recovered alive at the end of this trial, either as settled or as free-swimming larvae.

Interestingly, any evidence for aggregative settlement from laboratory experiments was not reflected in our observations from the field, where biweekly settlement rates never differed between microhabitat types. A lack of discrimination of microhabitat in field trials in contrast to what is predicted from laboratory experiments has also been observed in barnacles (Thompson *et al.*, 1998). It is likely that local hydrodynamics inhibit settlement in association with certain microhabitat types under field conditions, even on the most accessible surfaces (i.e. the *Crepidula* shells, as laboratory assays suggest), resulting in a random deposition of the larvae. Juvenile movement between habitat types, although a potentially important process as revealed by our laboratory studies, is unlikely the main reason for this observed distribution, as the set-up used in our experiment would have required the juveniles to move from the panels onto the surrounding sediments, providing a large obstacle for juvenile movement. EPSM, although previously shown to be high at the same study site (Chapter 4), is also unlikely to have caused the distribution among microhabitat types on the biweekly panels. Whilst submersed, EPSM is low (Chapter 4), and sampling of the manipulated panels in the present study had always been undertaken on the first day of spring tides when the panels were accessible again, decreasing the potential loss of juveniles due to mortality in this treatment.

Work by Shenk & Karlson (1986) and McGee & Targett (1989) showed that the distribution of *C. fornicata* established during settlement is altered by post-settlement processes, resulting in a differential spread of early settlers to that of older juveniles

and adults. Findings from the present study confirm this. At the end of the field experiment, more larvae were observed on the mussel microhabitat panels compared to the bare panels, suggesting that the mussel shells offered a more suitable habitat for survival during the first days of intertidal exposure. Our combined results from laboratory and field studies thus show that although settlement occurs in association with specific habitat types (shells of conspecifics) under laboratory conditions, its fine-scale distribution in the intertidal is not determined by such processes, but by variable levels of EPSM between microhabitat types following an even dispersion of settling larvae across habitat types. Microhabitat choice by the early life cycle stages of a species may be essential in determining its recruitment success, especially if the early stages are highly susceptible to mortality (Gosselin and Chia, 1995a). If larvae lack the ability to distinguish between microhabitats encountered during the process of metamorphosis, this may have detrimental effects on later survival and population growth. Interestingly, *C. fornicata* attains highest densities in the subtidal and low intertidal (Blanchard, 1997, Chapter 2). It is likely that the species is not well adapted to intertidal conditions, and also has not evolved the behavioural response necessary to counteract such passive processes through controlled settlement in association with microhabitats that would support successful recruitment of the species. If this is the case, reproduction would also be impaired in the intertidal, due to difficulties of larvae or juveniles to locate pre-existing stacks or EPSM overruling any potential associations in favour of settlement on conspecifics. It is highly likely that processes that determine the fine-scale distribution of *C. fornicata* under subtidal conditions, where the species is exposed to less physical stressors, differ from those acting under intertidal conditions. Further work is needed to investigate whether the different processes acting under intertidal and subtidal conditions may also cause variations in *C. fornicata*'s fine-scale distribution.

# CHAPTER 6 -

# GENERAL DISCUSSION

# 6.1 THE DISTRIBUTION OF *CREPIDULA FORNICATA* AT DIFFERENT SPATIAL SCALES

The preceding four chapters presented a series of studies that investigated the distribution of the non-native gastropod *Crepidula fornicata* in Welsh coastal waters, and how its distribution is affected by processes operating at various spatial scales. In this final chapter, I will discuss the main factors that I found to be important in, firstly, limiting the regional distribution of *C. fornicata* in Welsh waters, with special emphasis on its lack of northwards spread in Wales, and secondly, determining its fine-scale distribution among microhabitat types and across the vertical shore gradient.

## 6.1.1 THE NORTHERNMOST WELSH DISTRIBUTION AND FACTORS LIMITING ITS NORTHWARDS SPREAD

Chapters 2 and 3 investigated the potential for a northwards range expansion of *C. fornicata* in Welsh coastal waters. I found little evidence of a northwards spread of *C. fornicata* from its first location of introduction in Welsh coastal waters, the Milford Haven Waterway (MHW) during the intertidal and subtidal surveys undertaken between 2009 and 2010. The northernmost, established self-sustaining Welsh population still seems to reside within the MHW, although occasional findings from outside the MHW emerged between 2008 and 2012 when a few individuals were found attached to great scallops *Pecten maximus* in the Skomer Marine Nature Reserve (SMNR). Mobility of *P. maximus* is highly restricted and its movement by humans is prohibited within the boundaries of the SMNR. This suggests that these individuals had settled as larvae after natural dispersal into the SMNR. Some of the stacks that were found during the monitoring work of the Countryside Council for Wales (CCW) in 2012 included egg-brooding females. Since females begin to lay eggs in their third year (Deslous-Paoli and Heral, 1986), first settlement in the SMNR must have already occurred prior to 2010. In Chapter 3, I showed that females in South West Wales spawn multiple times. It is thus possible that the females in the SMNR had already released larvae prior to their removal by CCW, increasing the likelihood that larvae have already dispersed even further.

However, total numbers of *C. fornicata* found outside the MHW remain extremely low, despite the high monitoring effort undertaken by CCW and during the survey work of this research project in the last four years. Although I aimed to maximise the chances of finding *C. fornicata* outside its confirmed range (the MHW) by targeting specific survey areas, it is likely that I did not cover its full potential non-native range in Mid and North Wales and that sampling effort was not large enough to detect a population at such low densities. Also, most records appeared after the survey work presented in Chapter 2 was undertaken, indicating that the northwards range extension beyond the MHW may only have occurred very recently, lowering the chances for its detection. Thus given that *C. fornicata* was recorded in this ria as early as 1953 (Cole and Baird, 1953), natural expansion from the area has been extremely slow and limited in extent, a surprising observation in the light of my observations of effective reproductive output in these populations. Limited and slow dispersal is not necessarily a feature of *C. fornicata* in other parts of its introduced range. For example natural dispersal along and possibly across the English Channel has occurred rapidly (Robson, 1929; Orton, 1950; Cole, 1952).

A combination of factors including prevailing environmental conditions (especially temperature, habitat availability and hydrodynamic conditions), the species' physiological tolerances and biotic interactions determines the geographic range of marine invertebrates. Northern range limits in particular are usually set by sub-optimal prevailing seawater temperatures and sometimes geographic dispersal barriers. Similar processes may restrict the secondary spread of non-native species (NNS) after successful introduction to a novel region (Davis *et al.*, 2001; Colautti and MacIsaac, 2004). Results presented in Chapters 2 and 3 show that restricted reproductive success as a result of low summer seawater temperatures or an insufficient availability of suitable habitat types are unlikely the main reasons for *C. fornicata*'s limited northwards spread. Instead, the species occurs across a variety of habitat types in the MHW and occurs in highest numbers in areas with high content of gravel, which is sufficiently available in the coastal waters of Wales (CCW, 2005, 2009). The relatively long spawning and larval season at a wide range of seawater temperatures, the occurrence of multiple spawning events per female and the high larval densities suggest that reproduction and the early life cycle stages are not limited in the MHW and in areas with similar seawater

temperatures (the SMNR and the Menai Strait). However, I found that spatfall is restricted to a shorter time period compared to the long larval period. This implies that benthic recruitment only occurs at warmer seawater temperatures which could limit its northwards spread if larvae were introduced to areas with cooler seawater temperatures.

### 6.1.2 FINE-SCALE DISTRIBUTION AND PROCESSES LIMITING INTERTIDAL RECRUITMENT

Factors other than environmental conditions or physiological tolerances of the species tend to affect species distributions at a much finer scale. For example, selective larval settlement behaviour, differential larval supply and differential post-settlement mortality or migration can determine the distribution of species among microhabitat types. Although these processes usually operate at a scale of meters, the rejection of certain substrata types by the larvae during settlement or the microhabitat's insufficiency to support juvenile survival may in some cases also explain the absence or limited proliferation of a species in a larger area (Strathmann *et al.*, 1981; Hunt and Scheibling, 1997; O'Riordan *et al.*, 2010). In case of *C. fornicata*, gregarious settlement behaviour is thought to result in the aggregated distribution of adults, enabling reproduction after stack formation. Understanding the importance of pre-settlement, settlement and post-settlement processes in determining the distribution of adults is important when aiming to understand if variable densities between locations are due to environmental conditions, behavioural preferences or a combination of both. Work presented in Chapters 4 and 5 therefore aimed to increase the knowledge about the roles of larval supply, larval settlement behaviour, microhabitat suitability and post-settlement processes in establishing the final distribution of *C. fornicata*. Surprisingly, I found no indication for active larval choice during settlement in the laboratory assays, although differential settlement occurred between substrata types (Chapter 5). Also, levels of seasonal recruitment as well as biweekly and monthly settlement densities (Chapters 3 and 4) were similar at several intertidal shores with varying adult abundances. This suggests that processes before and during settlement are of minor importance in determining the intertidal distribution of *C. fornicata*. Instead, its final

distribution is probably determined through processes occurring after settlement. I found that early post-settlement mortality (EPSM) is high due to repeated exposure of the newly settled individuals to intertidal conditions during spring tide emersion, which is shown by the high levels of juvenile mortality I recorded during the caging experiment in 2011 (Chapter 4). High EPSM may thus be the reason for the low intertidal recruitment observed in both settlement seasons of 2010 and 2011, and also explain the relatively low intertidal sex ratios and stack sizes. If gregarious settlement takes place intertidally, any aggregation of juveniles that may be established during settlement by the larvae is most likely overruled by EPSM.

### 6.1.3 INTERTIDAL VERSUS SUBTIDAL PROCESSES

The findings that were discussed in the preceding sections show that most processes presented in this thesis mainly apply to the distribution of *C. fornicata* in the intertidal zone, where EPSM is high and a strong vertical gradient in adult densities is apparent. Subtidally, adult patterns are likely determined through other processes, as EPSM is likely to be less intense. This is supported by the fact that adult densities between subtidal transects were found to be less variable compared to densities between the different intertidal heights, suggesting that subtidal recruitment is less variable. Work on settlement and post-settlement processes in the subtidal zone was not possible during this research project. Previous work however suggests that larval supply can be important in determining the spatial distribution and potential spread of *C. fornicata* in the subtidal. Rigal *et al.* (2010) found that larval supply, influenced by local hydrodynamic conditions and the location of adult spawning grounds, can strongly limit the proliferation of *C. fornicata* in the open coast, due to transport of the larvae away from conspecifics. It is unlikely that this applies to the distribution of *C. fornicata* in the MHW, intertidally as well as subtidally. I found that larval supply generally did not differ between the intertidal study sites, irrespective of total numbers of larvae released at the location. This indicates that strong mixing of the larval pool takes place after release, resulting in homogenous supply between locations, a result matching the observations of Jenkins (2005) on intertidal barnacles over similar spatial scales. Patterns of supply to the subtidal seabed outside the MHW are clearly an important component in

understanding the limited northward spread of *C. fornicata*. Strong tidal currents may result in high dispersal of the larvae and minimise the chances for gregarious attachment, thus slowing down the establishment of self-sustaining populations outside the MHW. The very recent recurrent findings of *C. fornicata* in the SMNR suggest that establishment outside the MHW only occurs now after a long lag-phase of ~50 years, possibly due to high larval dispersal that resulted in low supply of ready-to-settle larvae. The combination of supply of late-stage larvae, hydrodynamic conditions, larval settlement behaviour and the necessity for reproduction through internal fertilization are therefore likely affecting successful stack formation and population spread. EPSM on the other hand, whilst surely important in structuring the vertical distribution and its distribution between microhabitat types, probably is less important in restricting *C. fornicata*'s geographic spread in Wales, as subtidal populations are likely to form that are unaffected by high levels of EPSM.

The roles of habitat availability and composition are also likely to differ between intertidal and subtidal conditions. For example, subtidal densities were positively related to a higher content of the substrata class gravel, most likely as it provides a suitable surface for settlement and aids the creation of new stacks. In the intertidal, on the other hand, I found that high gravel content was indicative of low *C. fornicata* abundance. This may be because gravelly intertidal shores are an indicator of more exposed conditions which are less suitable environment for *C. fornicata* establishment. The negative effect of high energy environments on *C. fornicata* abundance is likely more pronounced in the intertidal, where environmental conditions are more stressful, due to high levels of EPSM. However, I found that intertidally, microhabitat structures can increase survivorship of the juveniles, as was shown by overall lower mortality on the caged panels compared to the uncaged panels in the monitoring experiment presented in Chapter 4. Furthermore, during the experiment in which settlement surfaces were manipulated to resemble natural settlement substrata (presented in Chapter 5), seasonal recruitment was higher on certain substrata types (that is, *Mytilus edulis* shells) compared to, for example, the artificial flat slate panels. This was despite the fact that, as previously mentioned, there was no indication for substrata choice made by the larva and differential settlement in favour for shells of conspecifics. Recruitment success of *C. fornicata* in the intertidal therefore increases on

specific microhabitat structures, probably as some types offer protection from desiccation and heat stress.

Lastly, it is important to point out that these findings on the role of microhabitat structures in the intertidal also show that the horizontal flat slate panels I employed for most of the settlement work may have underestimated *C. fornicata*'s recruitment success at the study sites. Other substrata types may support higher seasonal settlement densities. However, as discussed in Chapter 3, monthly settlement rates did not differ between fully natural (i.e. all substrata types occurring at a site) and artificial (i.e. the slate panels) substrata, and only one substrata type of the manipulated panels had significantly higher seasonal recruitment associated with it. The juvenile densities recorded on the slate panels may therefore still be a relatively good estimate for overall settlement and recruitment densities in the intertidal, where recruitment simply is extremely low.

## 6.2 ON ITS WAY NORTH? THE POTENTIAL NORTHWARDS SPREAD OF *CREPIDULA FORNICATA* AND ADVICE FOR FUTURE WORK

In the last sections I have summarised the main factors that my research found to be the most likely causes for the observed regional and fine-scale distribution of adult *C. fornicata.* Ultimately, this research project was designed to understand its absence from certain areas. This is particularly challenging as evidence on whether a NNS could potentially establish and spread outside its current range would ultimately require to base research in so far uninvaded areas. This, of course, is impossible due to obvious associated risks of an introduction of the NNS to that area. This only leaves the possibility of excluding potential causative factors through investigations undertaken at other study areas. In this research project, I provide evidence that the population inside the MHW is not negatively affected by two of the main processes that usually set the northern range of marine invertebrates: sub-optimal seawater temperatures and habitat availability. My findings further indicate that conditions (that is, summer seawater temperature and habitat availability/ composition) outside the MHW are

relatively similar and thus not the main reason for the absence of large numbers of *C. fornicata* in these areas. Other dispersal barriers may exist, for example hydrodynamic conditions that restrict the potential for stack formation through high larval dispersal. However, it is likely this will only delay its establishment to the north of the MHW, until propagule pressure is large enough after repeated inoculations with larvae so that successful gregarious settlement can occur. From the result of this PhD research I thus infer that population establishment to the north of the MHW is not unlikely, if transport of larvae or adults through repeated human-mediated introductions or natural larval dispersal occurred repeatedly.

If population establishment of *C. fornicata* in Mid and North Wales in fact is possible, it is crucial to use results from studies such as this one to derive advice for future monitoring practices and research work. Firstly, much of the monitoring and research that was undertaken in the past, including the studies presented in this thesis, have been undertaken in the intertidal. However, in this thesis I showed that the intertidal zone represents a particularly stressful environment for *C. fornicata* and may thus not be suitable to sustain high levels of recruitment. Future monitoring work should focus on subtidal areas in particular, as these are the most likely areas occupied by *C. fornicata*. Also, a large amount of research is still lacking on processes that are determining the distribution of *C. fornicata* subtidally, and whether patterns of recruitment and the resulting spatial distribution differ to those in the intertidal.

Secondly, the role of larval supply and how this is influenced by hydrodynamic conditions is not fully understood. This would require detailed knowledge on larval swimming behaviour in relation to prevailing hydrodynamic patterns. This information is currently lacking for the case study of *C. fornicata* in South West Wales, UK and research on this topic was beyond the scope of the present study. Future research however would benefit if this was incorporated into studies.

Thirdly, I presented the importance of microhabitat structures and availability of certain habitat types for *C. fornicata* establishment. Although generally ubiquitous in its distribution, it is likely to occur in higher densities in gravel-rich areas subtidally, but not intertidally. Also, I found that different microhabitat types may differ in their

suitability to support recruitment. These differences should be kept in mind when targeting specific sites for routine monitoring.

This thesis provided some first insights into environmental conditions, processes and species traits that could explain the limited spread of the potentially harmful non-native gastropod *C. fornicata* in Wales, UK. Work carried out in an area with well established populations (the MHW) showed little indication for environmental limitations of its spread. Future work should be directed at investigations into the differential recruitment patterns in intertidal and subtidal habitats, which stressors affect juvenile survival in the intertidal, and how larval transport and swimming and settlement behaviour determine settlement patterns.

## Other fields may need further work, too:

### *FRESH COOKING*

*Slipper limpets should not be prepared as bivalves and bear a closer relationship to other gastropod flesh (e.g. abalone) which must either be cooked quickly or over a long period at low heat.*

*RAPID COOK - Shucked slipper limpet flesh flash fried in garlic butter for 30 seconds yielded a clean and good tasting dish. This short cooking method could possibly be restricted to appropriately safe slipper limpet sources as discussed in Section E.3.1.4.*

*MEDIUM COOK - Slipper limpets were initially cooked in the shell in the same way as mussels in garlic butter. Final product was rubbery and a little contaminated by dirt on the shell which could not be adequately cleaned from the shells when fresh.*

*SLOW COOK – A sweet chilli chowder was prepared covered and cooked at gas mark 1 for 5 hours. Although the dish did not taste particularly good the texture of the flesh was very soft confirming that slow cooking does produce a favourable texture. Rick Steins is enthusiastic in his recent book about a featured ormer (abalone) recipe with cinnamon and shiitake mushrooms that requires a long low cook. It would be interesting to perform a blind taste test between ormers and slipper limpets!*

*Direct quote from FitzGerald, 2007*

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