A molecular approach to the study of a mussel hybrid zone on the west coast of Ireland

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I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of MSc is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abstract

The shores of the northwestern Atlantic have two indigenous mussel species, the Blue mussel, *Mytilus edulis* and the Mediterranean mussel, *Mytilus galloprovincialis*. These two species once isolated by the last ice age, are now occurring sympatrically along 1500 miles of the northwestern Atlantic coast. They regularly interbreed and hybridise, producing a patchwork of mixed pure species and hybrid populations, their distribution mainly defined by environmental factors.

In hybrid populations on exposed shores in Ireland mussels higher up the shore are more likely to be *M. galloprovincialis*. This study set out to test two hypotheses: is this pattern due to preferentially settlement of larvae at a given shore height, or is it due to blanket settlement of larvae followed by preferential secondary settlement or post-settlement selective mortality.

The *Me15/16* DNA marker was used to analyse the genetic composition of newly-settled spat onto artificial substrates, which were placed on the mid and low shore areas of two exposed shores in Galway Bay over the period May-October 2002. Samples of adults were collected at the same time, with additional samples collected from a nearby sheltered shore. This DNA marker can differentiate between *M. edulis*, *M. galloprovincialis* and hybrids.

There was no evidence for preferential settlement by any of the three genotypes on exposed shores. Neither was there evidence of post settlement mortality, as adults from exposed shores were genetically similar to settling spat. Contrary to the results from published studies, there was no significant difference in the genetic structure of adult mussel populations with tidal height. However, in adult exposed shores samples there was a significantly higher frequency of *M. galloprovincialis* among larger individuals in the population, possibly indicating a selective advantage for the genotype in this environment.

Adult mussels from a nearby sheltered site were found to be significantly different to exposed shore adults, due to higher frequencies of *M. edulis* and hybrid genotypes. There was no difference in the proportions of the three genotypes in small versus large mussels at this site. The difference between adults on exposed and sheltered shores may be due to the competitive advantage of *M. galloprovincialis* on exposed shore sites, e.g., resistance to wave action and faster growth rates.

Introduction

The genus *Mytilus* is of relatively recent origin, with the first fossils appearing in North Atlantic Pliocene strata about 5 million years ago (Vermeij 1992). The genus includes three mussels with distinct evolutionary lineages, *Mytilus edulis* Linnaeus (1758), *Mytilus galloprovincialis* Lamarck (1819) and *Mytilus trossulus* Gould (1850). It is generally acknowledged that *M. edulis* is the ancestral species from which *M. galloprovincialis* and *M. trossulus* have evolved (Seed 1992).

Speciation among these three closely related species was most likely allopatric. *M. trossulus* is more distantly related to the other two species and has probably been diverging for the longest time (Hilbish et al. 2000). This species is believed to have originated in the North Pacific, which was colonised by *Mytilus* after the opening of the Bering Strait about 3.5 mya. During the Pleistocene 1-2 mya, two invasions of the North Atlantic from the Pacific by *M. trossulus* are believed to have occurred: one in the western Atlantic (Canadian Maritimes), and the other in the eastern Atlantic (Scandanavia) (Riginos and Cunningham 2005). *M. galloprovincialis* is thought to have originated during one of Pleistocene ice ages, when the Mediterranean was cut off from the Atlantic (Barsotti and Meluzzi 1968). The warmer conditions in the Mediterranean Sea and reduced contact with the Atlantic, probably favoured the process of differentiation. Since the Pleistocene *M. galloprovincialis* has extended its range northwards onto the coasts of NW Europe. Southern hemisphere populations of *Mytilus* species probably derive from two migration events, one colonising through the Atlantic during the Pleistocene, and another more recent migration, also through the Atlantic (Hilbish et al. 2000).

Geographic Distribution

Until relatively recently, geographic distributions of *M. edulis* and *M. galloprovincialis* have been mapped solely on the basis of external shell characters. With the advent of molecular markers the distributions of both *M. edulis* and *M. galloprovincialis* have had to be revised, either by extending or reducing previously reported geographic ranges. In the case of *M. trossulus* in the north Atlantic, its distribution has been mapped solely using molecular markers. *M. edulis* is widely distributed throughout the temperate latitudes in the northern and southern

hemispheres. The species is distributed in European waters from Russia to the Atlantic coast of southern France, but is absent from the Baltic and the Mediterranean (Gosling 1992). *M. edulis* is reported around Iceland and on the east coast of America, where its range extends from Labrador to North Carolina (Varvio et al. 1988, McDonald et al. 1991). It is absent from the North Pacific but is present around the lower latitudes of South America (Pacific and Atlantic) and the Falkland Islands (McDonald et al. 1991).

M. galloprovincialis also occurs in temperate waters of both hemispheres but its range extends into much warmer latitudes than *M. edulis*. *M. galloprovincialis* is found in the Black Sea, on Mediterranean coasts and on the Atlantic coasts of Spain, Portugal, Morocco, Ireland and France. In Britain it is found in SW and NE England, the south coast of Wales, N. Scotland as far as the Shetland and Orkney islands (Gosling 1992). The species is also found in New Zealand, Tasmania and Australia and on the east China coast as far north as Korea. *M. galloprovincialis* is believed to have spread to the southern hemisphere thorough the Atlantic during the Pleistocene, and its presence in ancient middens and fossil records in New Zealand implies a long time occurrence in that area (Hilbish et al. 2000, Gardner 2004). *M. galloprovincialis* has probably been introduced accidentally into South Africa, Japan, California and Hong Kong (Gosling 1984, 1992, Seed 1992). In Japan its introduction has been accredited to the increase in trans oceanic traffic in the early twentieth century (Inoue et al. 1997).

M. trossulus is distributed in the colder waters of the northern hemisphere, along both sides of the Atlantic and Pacific Oceans. It is found on the Pacific coast of North America from Alaska to Central California, along the Pacific coast of Russia to the Kamchatka peninsula, and in the Canadian Maritimes and Baltic Sea (Gosling 1992) So far, *M. trossulus* has not been found in the Southern Hemisphere. Accurately defining the geographic distributions of the three taxa can be problematic. Although, large areas are indeed composed of mono-specific populations, wherever two taxa occur in sympatry, they interbreed readily.

In the following sections the focus will be, primarily, on *M. edulis* and *M. galloprovincialis* in NW Europe, although research findings on *M. trossulus* will be cited, where appropriate.

Taxonomy of Mytilus edulis and M. galloprovincialis

The systematic status of *M. galloprovincialis* the "Mediterranean" or "Padstow" mussel has been the subject of considerable discussion since the 1860s, with some regarding it as a distinct species (Seed 1978) and others considering it merely as a variety in the larger *M. edulis* species complex (Gosling 1984). This controversy stems from the fact that there is considerable overlap in shell characters, which are the main characters used in bivalve taxonomy.

There are several shell characters that have been used to distinguish between *M. edulis* and *M. galloprovincialis*. The anterior end of the shell of *M. galloprovincialis* is distinctly beaked or incurved, while that of *M. edulis* has a more snub-nosed appearance (Fig. 1). The shell of *M. galloprovincialis* tends to be higher and flatter than in *M. edulis*, giving different transverse profiles in the two forms. The anterior adductor muscle scar is small and circular in *M. galloprovincialis*, whereas in *M. edulis* it is narrow and elongated. The hinge plate in *M. edulis* is a gently curving structure while in *M. galloprovincialis* it forms a much tighter arc (Fig. 1).

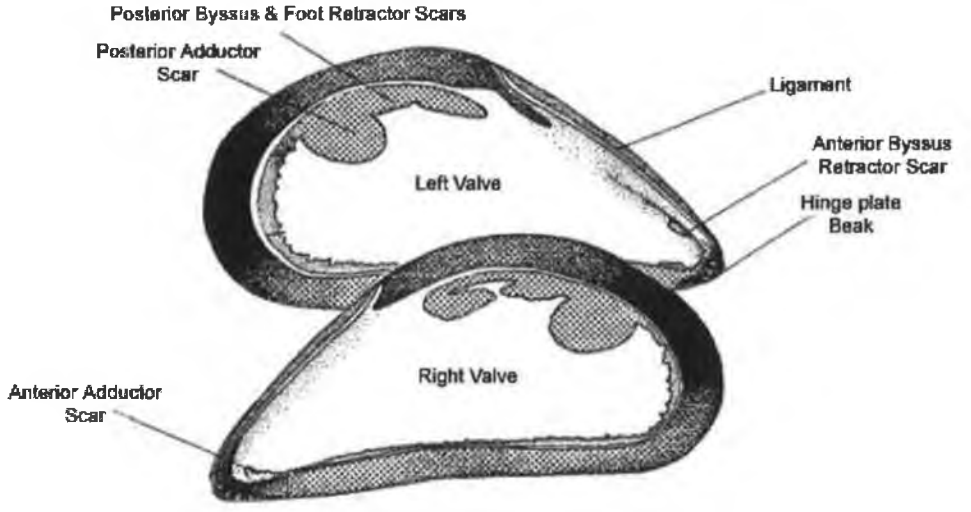

Fig 1. *M. edulis* showing shell characteristics used in identification of *M. edulis* and *M. galloprovincialis*.

The colour of the mantle edge is typically purple-violet in *M. galloprovincialis* and yellow-brown in *M. edulis* (Seed 1978, Gosling 1984, Seed and Suchanek 1992). Using these characters it is sometimes easy to separate the two taxa. However, in

regions where hybridisation and introgression (backcrossing of hybrids to parental species) is occurring, separation of the two is exceedingly difficult. Separation can also be confounded by ontogenetic changes in shell allometry, population density, and the effect of environmental factors, such as exposure to wave action.

Therefore, systematic information that is relatively free of environmentally-induced changes is highly desirable. The introduction of enzyme (allozyme) electrophoresis, coupled with traditional morphometrics, has assisted greatly in separating *Mytilus* taxa (Varvio et al. 1988, McDonald et al. 1991). There are 5-6 allozyme loci that, when used in combination, can differentiate between *M. edulis* and *M. galloprovincialis* (Gosling 1992). To date, there is no single locus that is truly diagnostic i.e. assignment of an individual to the correct species with a probability >0.99 (Avise 1974). The lack of diagnostic loci, the possible effects of selection on some loci, and the difficulty of unambiguously identifying hybrids and backcrossed individuals in hybrid zones, meant that the development of markers that were free of these shortcoming would be invaluable in advancing understanding of *Mytilus* systematics.

Several DNA markers have been developed that can, when used in combination, differentiate between *M. edulis* and *M.galloprovincialis*, and have contributed to our understanding of patterns of hybridisation and introgression in complex mosaic hybrid zones (Bierne et al. 2002a, Bierne et al. 2003a, Bierne et al. 2003b, Bierne et al. 2003c). Two of these markers *Glu-5'* and *Me15/16* are located within the nuclear gene encoding a polyphenolic adhesive protein, a key component in the attachment of mussels to the substrate (Inoue et al. 1995, Rawson et al. 1996). Only one of these markers, *Me15/16*, can unambiguously differentiate between *M. edulis*, *M. galloprovincialis* and *M. trossulus*. PCR amplification of *Me15/16* using the primer sequences *Me15* and *Me16* (Inoue et al. 1995) produces a species-specific band of about 180bp, 168bp and 126bp for *M. edulis*, *M trossulus* and *M. galloprovincialis*, respectively. Hybrid individuals have both parental bands. The Mendelian inheritance of the *Me15/16* marker has recently been confirmed in laboratory crosses of the two taxa (Wood et al. 2003).

Reproduction, Settlement and Growth

Most *Mytilus* populations exhibit a seasonal pattern of reproduction with a spring/summer and an autumn spawning (Seed 1969, King et al. 1989). There are, however, a number of variations on this pattern, some populations exhibit a single short, spawning period of a few weeks, while others have a protracted spawning period of as much as six months, and in cultured populations in Ireland winter spawning (January-February), followed by a late spring spawning is a common phenomenon (see Gosling 1981 for references). In Ireland, spring spawning coincides with rising water temperature while autumn spawning occurs when temperatures are falling (Rodhouse et al. 1984).

In SW England Seed (1971) found temporal differences in the spawning of sympatric populations of *M. edulis* and *M. galloprovincialis*. However, a further study in SW England (Gardner and Skibinski 1990) reported significant overlap in spawning time between *M. edulis* and *M. galloprovincialis*, which clearly would facilitate interbreeding.

There is no suggestion in the literature of gamete incompatibility between the two mussel taxa. Laboratory crosses of *M. edulis* and *M. galloprovincialis* produced viable hybrids, although hybrid larvae were slower-growing than either parent species (Beaumont et al. 2004). In an earlier study Beaumont et al. (1993), found that while hybrid larvae had initially higher mortality, this was offset by their faster growth rate compared to pure bred larvae. An even earlier study (Lubet et al. 1984), however, reported no reduction in viability, growth or mortality rates when hybrids were compared to parental species crosses; hybrids were found to be fertile. When gamete competition was allowed i.e., when there was a choice of conspecific and heterospecific gametes, Bierne et al. (2002) reported a reduction in the proportion of hybrids between *M. edulis* and *M. galloprovincialis*, which they attributed to assortative fertilisation. Overall, there is general agreement that there are no barriers to fertilisation between the two taxa, but to date there is no consensus on the superiority, or otherwise, of hybrid larvae compared to purebred reared under laboratory conditions.

Mussels have external fertilisation, and larvae remain in the water column for up to 4-8 weeks. In temperate waters, the larvae are generally abundant throughout the spring and summer months, although some studies (Seed 1969, Rodhouse et al. 1984) have recorded *M. edulis* larvae in the plankton throughout much of the year. The

larvae become competent to settle at ~250 µm shell length (SL). However, they are able to delay metamorphosis and may remain in the plankton until they reach 350-400 µm SL (Seed 1969, Sprung 1992). Therefore, newly settled larvae can vary in size between 230 µm and 400 µm SL (King et al. 1989, King et al. 1990). Larvae can therefore significantly extend their dispersal potential, and, by using surface currents, they have the potential to settle hundreds of kilometres away from their progenitors.

Bayne (1965) defines settlement as "... the descent of larvae from the plankton to the bottom substrate and the behaviour just preceding attachment". Settling spat either settle first on filamentous substrates such as hydroids or algae and move (via byssus drifting or crawling) to the adult beds (Bayne 1965), or settle gregariously onto the adult mussel beds fixing to the byssus threads of adults (McGrath et al. 1988). Numbers of *Mytilus* recruiting to the shore can vary considerably between geographically close locations (Lutz and Kennish 1992), due to factors such as mortality during the planktonic phase, availability of suitable substrates, hydrographic conditions and predation (Lutz and Kennish 1992, Dobretsov and Wahl 2001).

There are conflicting results on growth rates of *M. edulis* and *M. galloprovincialis*. Seed (1971) reported that under laboratory conditions *M. edulis* grew up to four times faster than *M. galloprovincialis*. However, other authors have reported no significant difference in growth rates between the two taxa (Skibinski and Beardmore 1979, Lubet et al. 1984, Rodhouse et al. 1984). When growth rates of hybrids and parent species from laboratory crosses were compared the results were conflicting, with evidence for heterosis (hybrid superiority) in some studies (Beaumont et al. 1993, Bierne et al. 2002) and not in others (Lubet et al. 1984, Beaumont et al. 2004). In hybrid populations in SW England, *M. galloprovincialis* had a faster growth rate than *M. edulis*, and hybrids had intermediate growth rates (Skibinski et al. 1983, Gardner et al. 1993, Wilhelm and Hilbish 1998). Several hypotheses have been advanced to explain the results including differential susceptibility to thermal stress (Hilbish et al. 1994) and wave shock; *M. galloprovincialis* genotypes have greater byssal attachment, and are thus less likely to be dislodged by wave action, than *M. edulis* (Gardner and Skibinski 1991, Willis and Skibinski 1992). Clearly, more studies are needed to see if this growth advantage in *M. galloprovincialis* is replicated at other sites.

Substantial differences in viability have been observed in populations of *Mytilus* in SW England. *M. galloprovincialis* had higher viability than *M. edulis*, and hybrids were intermediate to the two parental forms (Gardner et al. 1993, Wilhelm and Hilbish 1998). This may explain the higher frequency of *M. galloprovincialis* in larger, older mussels in hybrid populations (Skibinski and Roderick 1991). A later study linked these viability differences to differences in strength of attachment with *M. galloprovincialis* being more strongly adhered to the substrate than *M. edulis* or (Willis and Skibinski 1992).

Maintenance of the *Mytilus* Hybrid Zone

Hybrid zones are areas where two genetically distinct species overlap, mate, reproduce and produce viable progeny (Barton and Hewitt 1989). *M. edulis* and *M. galloprovincialis* occur widely over much of Northern Europe, and wherever the two occur in sympatry they hybridise (Gosling 1992, Gilg and Hilbish 2000, Hilbish et al. 2003). This hybrid zone stretches for about 1400 km of coastline (Orkney Islands to the Bay of Biscay) and has a mosaic structure, with some areas populated by mono-specific populations of *M. edulis*, and other areas by varying mixtures of *M. edulis*, *M. galloprovincialis* and hybrids (Skibinski et al. 1983). Laboratory experiments have shown there is no barrier to interbreeding between *M. edulis* and *M. galloprovincialis* (Beaumont et al. 1993, Beaumont et al. 2004) and fertile F_1 hybrids have been reported (Lubet et al. 1984). In addition, in some areas, e.g. the Atlantic coasts of Ireland, Scotland and France there is evidence (using allozyme markers) that introgression is substantial (Gosling and Wilkins 1981, Skibinski et al. 1983). This has recently been confirmed for French Atlantic coast sites, using DNA markers (Bierne et al. 2003a).

The processes that may be preventing *M. edulis* and *M. galloprovincialis* from forming a single hybrid swarm, or a genetically homogenous species are now being actively debated. Stable hybrid zones are maintained through a balance of dispersal and selection against hybrids. The competition between the magnitude of dispersal and the intensity of selection determines the width of the hybrid zone. In terrestrial species such as the fire bellied toads and crickets (Barton and Hewitt 1989) with limited dispersal potential, hybrid zones are narrow (10-15 km), compared to marine organisms with significant dispersal potential, such as *Mytilus*, where the hybrid zone in NW Europe is very broad (~1400 km).

Two types of selection may operate against hybrids. Endogenous selection, or tension zone models, hypothesize that selection against hybrids is intrinsic, due to the incompatibility of the two differentiated parental genomes (Barton and Bengtsson 1986, Barton and Hewitt 1989). Exogenous selection, or environmental gradient models, contend that selection acts either for or against hybrids, depending on the environment (Schilthuizen 2000a, b). Both endogenous and exogenous selection can operate in a hybrid zone, although Jiggins and Mallet (2000a, b) contend that the former may play a lesser role, implying that exogenous selection, combined with assortative mating, is the primary factor maintaining hybrid zone stability.

Deciding which of these models, tension zone or environmental gradient, best explain the maintenance of a particular hybrid zone involves detailed analysis of the dynamics of selection within the zone. Unfortunately, published data on fitness comparisons of *Mytilus* species and hybrids are sorely lacking (Wilhelm and Hilbish 1998).

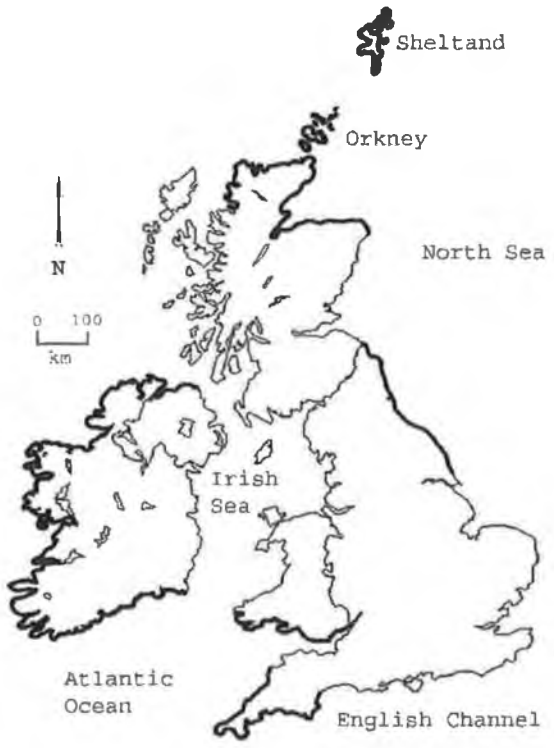

Fig 2. Map showing in heavy outline the geographic areas in the British Isles where *M. galloprovincialis* has been detected using morphological and electrophoretic markers (Gosling 1984).

Microgeographic Distribution of *Mytilus* and Hybridisation in the British Isles

The geographic distribution of *M. edulis* and *M. galloprovincialis* around the British Isles has been reported on the basis of shell morphometrics and allozyme markers (Gosling and Wilkins 1981). *M. edulis* is present on all coasts but *M. galloprovincialis* has a more restricted distribution (Fig.2).

The latter species has been reported on the South, West and North coasts of Ireland, the South coast of Wales, the South West Peninsula of England, the North East of England, the North East of Scotland and the Orkneys and Shetland Islands.

It is believed that the absence of *M. galloprovincialis* from the Irish Sea may be related to the hydrographic conditions at the entrance to the sea, preventing the influx of larvae into the area (Gosling and Wilkins 1981). Summer heating has been postulated as the causal factor for the reduced exchange of water between the Celtic and Irish Seas (Brown et al. 2003). Solar heating stratifies the water column and reduces water exchange between the stratified Celtic Sea and the mixed Irish Sea.

In areas where the two taxa are sympatric they hybridise. In some areas, e.g., the Atlantic coasts of Ireland, hybridisation and introgression between the taxa is much more extensive than in other areas, e.g., SW England (Skibinski et al. 1983).

The most studied mussel hybrid zone in Western Europe is in SW England (Skibinski et al. 1983, Wilhelm and Hilbish 1998, Gilg and Hilbish 2003a, b, Hilbish et al. 2003). The hybrid "patch" extends for 180 km between north Cornwall and south Devon, and separates two relatively pure populations of *M. edulis* and *M. galloprovincialis* (Hilbish et al. 2002). Oceanographic features maintain the hybrid zone by allowing spat from pure populations to enter, but prevent hybrid larvae from leaving the area (Gilg and Hilbish 2003a). Within the zone *M. galloprovincialis* alleles and genotypes are favoured and there is a decline in the frequency of *M. edulis* alleles with increase in size (Gilg and Hilbish 2003a). *M. galloprovincialis* individuals are more resistant to wave exposure and dislodgement than *M. edulis* (Willis and Skibinski 1992). Hilbish *et al.,* (2003) have shown that the general decline in *M. edulis* type alleles with increasing shell size does not occur at sheltered estuarine sites. They hypothesised that increased wave exposure, or a covariant, leads to the observed decrease in *M. edulis* type alleles with increase in size. Supporting an exogenous selection mechanism based on differential dislodgement or co-variant by wave action. In turn, selection pressure against *M. edulis* type alleles in the population is offset by influxes of larvae that contain a high frequency of *M. edulis* type alleles (Wilhelm and

Hilbish 1998, Gilg and Hilbish 2000). The integrity of the hybrid zone is maintained by a balance between directional selection against *M. edulis* type alleles and large influxes of *M. edulis* type alleles in incoming larvae. It would appear that this hybrid zone is long-standing with a stable age structure of genotype and allele frequencies, which has remained constant for at least several generations (Skibinski et al. 1983, Hilbish et al. 2002). The Atlantic coast of France has a similar hybrid zone stretching from Normandy to Biarritz with a noticeably discontinuous transition between *M. galloprovincialis* populations in the Iberian Peninsula and *M. edulis* populations in the North Sea (Bierne et al. 2003b).

In the British Isles, *M. galloprovincialis* appears to favour more exposed, full salinity conditions, while *M. edulis* is found in more sheltered estuarine or reduced salinity areas (Skibinski et al. 1983, Gosling and Wilkins 1985). However, wave exposure score alone cannot predict the occurrence of *M. edulis*, *M. galloprovincialis* or their hybrids (Gosling and Wilkins 1981).

Studies from SW England and the west coast of Ireland have detected significant differences based on allozymes within the adult mussel populations from exposed shores at different tidal heights (Gardner and Skibinski 1988, Gosling and McGrath 1990). Individuals from the upper shore were reportedly more *M. galloprovincialis*-like than those found lower on the shore. Selective pressures such as the ability of *M. galloprovincialis* to withstand emersion, their resistances to wave shock, and possible selective larval settlement have been proposed as reasons for this distribution (Gardner and Skibinski 1990, Gosling and McGrath 1990, Gilg and Hilbish 2003c).

Using the *Glu-5'* DNA marker, Gilg and Hilbish (2000) investigated settlement of primary and secondary settlers onto pads placed in Whitsands Bay, SW England. They found no evidence for preferential settlement with respect to genotype, and concluded that the genetic composition of adult mussel populations with respect to tidal height is probably due to differential selection intensity. It should be noted that the mussels analysed by Gosling and McGrath (1990) were from shores with high exposure, unlike the mussels investigated by Gilg and Hilbish (2000).

The aims of this study were to:

- Reinvestigate the population genetic structure of adult *Mytilus* at Ballynahown and Carraroe, the same exposed west of Ireland shores investigated by Gosling and McGrath (1990).
- To use the diagnostic *Me15/16* DNA marker on newly settled mussel spat to test two hypotheses that may account for the genetic differentiation with tidal height in hybrid mussel populations:

  - *Preferential primary settlement*: assumes that larval segregation occurs before or during settlement. Either larvae segregate in the plankton and this variation is reflected in settlement or larvae settle preferentially at a given shore height. Prediction is that larvae settling higher on the shore will have a higher frequency of the *M. galloprovincialis* allele.

  - *Preferential secondary settlement / post settlement mortality*: assumes that larvae settle homogenously. Initial settlement may be random with respect to tidal height but resettlement or mortality may be genotype dependent. Prediction is that genotype frequencies at time of primary settlement will be independent of tidal height but differences will emerge later on in adults as a result of selection.

- To compare the population structure of spat and adult mussels at Ballynahown and Carraroe exposed shores
- To compare the relationship between spat shell length at settlement and genotype.
- To compare the genetic structure of adults at Ballynahown and Carraroe with adults from a nearby sheltered shore.

Methods

Sampling Sites

The sampling sites Carraroe (53° 14.05′N, 9° 35.29′W) and Ballynahown (53° 13.35′N, 9° 31.63′W) are located on the northern shore of Galway Bay. The two sites are separated by Cashla Bay (Fig 3). Both are south facing with Ballynahown described as an exposed rocky shore with a exposure gradient of 2 on the Ballantine scale (King et al. 1989). The shore at Carraroe has an exposure rating of 2 on the Ballantine scale (personal communication, D.McGrath, 2004). A sheltered site, situated very close (900 m) to the Ballynahown exposed shore site (Fig 3), with an exposure rating of 5 on the Ballantine scale (personal communication, D. McGrath, 2004), was also sampled.

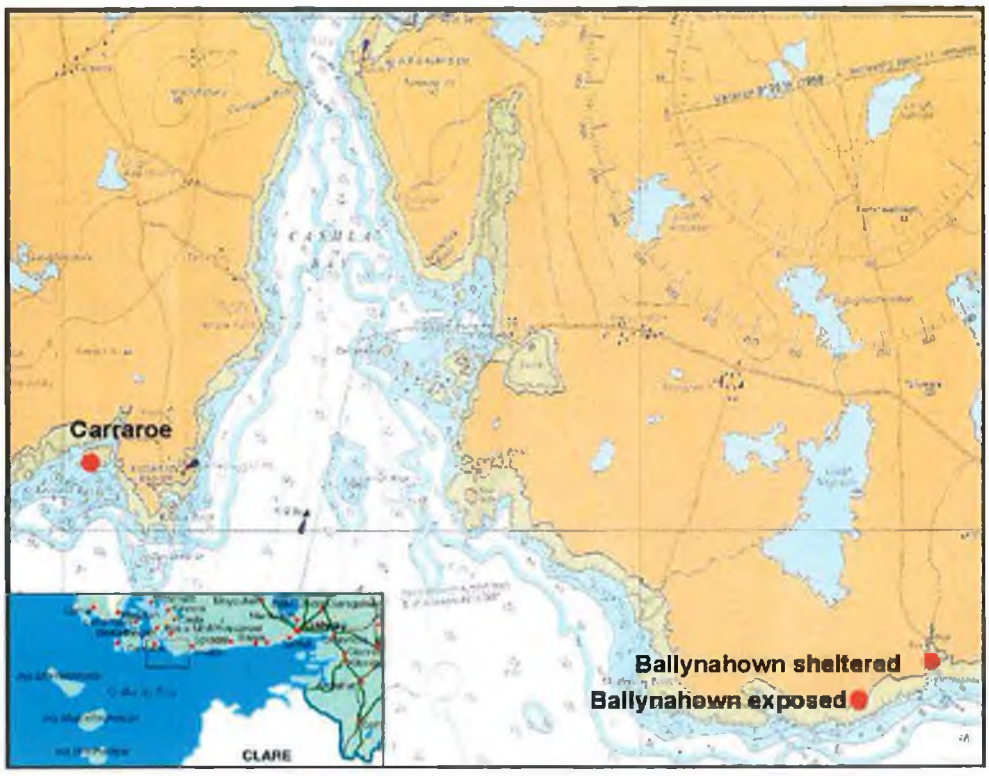

Fig 3. Showing the position of the two exposed Carraroe and Ballynahown sites, and the single sheltered site on the north shore of Galway Bay

The two exposed sites were selected because of previous allozyme studies (Gosling and McGrath 1990), their close proximity to one another (less than 5 km) and because the two shores are similar in terms of wave exposure and aspect to wave action. The sheltered Ballynahown site was selected in order to compare the genetic structure of sheltered shore mussels with nearby exposed shore mussels.

Sample Collection

On exposed shores deployment of collection pads for newly-settled mussels started on the 13 May 2003 and pads were collected and replaced every two weeks until the 24 October 2003, with a total of 22 visits made during the sampling period.

On both shores, pads were placed on the low and mid-shore (0.5 and 2 m above mean low water springs, respectively), where mussels are most abundant, with a vertical distance of 1.5 m between the two groups of pads. Continuous sloping rock ledges that were free of lose or movable rocks, were chosen for pad deployment. Six sampling pads and one redundant pad (a backup in case of pad loss) were placed along a 10 m transect of rock ledge at each tidal height using random number tables to position each pad (Fig. 4). Each pad was made from a 50 mm x 90 mm piece of a Killeen Easy Clean™ pot-scouring pad. The pads were fixed to perspex holders 90 mm long, 70 mm wide and 5 mm thick (Fig. 5). These holders had a central bolthole of 6 mm in diameter to allow attachment to the rock, and eight 4 mm diameter holes, which were used to fix the scouring pad to the Perspex holder using plastic cable ties. The holder, scouring pad and cable ties were assembled prior to deployment in the field. A De Walt™ portable drill was used to make a 6 mm diameter hole with a masonry drill bit, and the pad was then fixed to the rock with a medium weight Rawlok™ Brickwork bolt. Once the pad holder was bolted to the rock the cable ties were fastened, thus keeping the scouring pad securely in place. Collection of the settlement pads involved cutting the cable ties from each holder and placing the scouring pad into a pre-labelled 30 ml container. The pad holder was then removed and replaced with a new one complete with pad and cable ties.

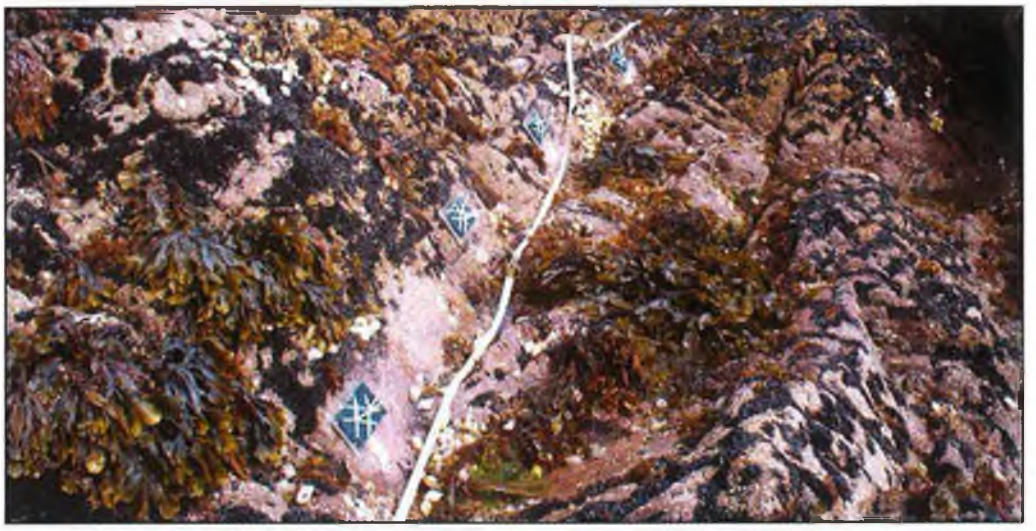

Fig 4. Collection pads deployed on the lower intertidal area of the exposed shore at Carraroe, Galway

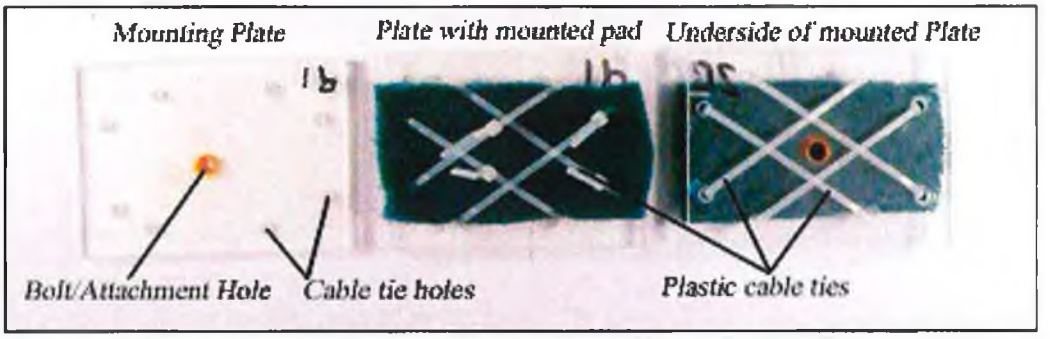

Fig 5. Perspex mounting plates with scouring pads attached by plastic cable ties

On return to the laboratory settlement pads were submerged in 80% alcohol, care being taken not to allow bubbles to remain at the bottom of the 30 ml universal containers, as this could result in some samples being improperly preserved.

Adult mussels were also collected at settlement pad deployment/collection. A 25cm^2 section of mussel mat (e.g. cluster of mussels as in Fig 4) was removed using a knife, from pre-determined, randomised positions along the settlement pad deployment transect. Six replicates were taken at each tidal height. Each sample was placed in a labelled 100 ml plastic beaker (Sarstedt) and later preserved in 100% ethanol. One hundred present ethanol was used, as water trapped in the mussel mantel cavity dilutes the preserving ethanol, reducing it preservative ability.

Ballynahown sheltered shore adult mussels were collected on the 27 September 2004. Mid and low shore mussels were sampled by taking three replicates at each tidal height, and maintaining a vertical distance of about 1.5 m between the two heights, similar to the sampling strategy adopted for the Ballynahown exposed site. About 40-60 individuals for each replicate were removed with a knife and were later preserved in 100% ethanol.

Shell measurement

Newly settled mussels were separated from the settlement pad by removing 9 cm^2 of the central section of each pad and shaking it in a beaker of distilled water for 30 s. The removed section was then torn in two (splitting horizontally) and each side of the split section was shaken for a further 60 s. The section was then examined to ensure complete removal of mussels. The resulting material at the bottom of the beaker was then filtered through a 200 µm sieve. This material was then examined under a dissecting microscope fitted with an ocular micrometer (X 35 magnification).

In this study settlers have been divided into three size groups; primary settlers (<550 µm shell length (SL)), secondary settlers (551 to 1500 µm SL) and youth of the year (>1.5mm SL) (Gilg and Hilbish 2000). All mussel spat (<1500 µm) were measured from the anterior hinge to the posterior edge of the shell under a dissecting microscope fitted with an ocular micrometer (X 35 magnification). Youth of the year that were < 2.6 mm SL were measured under the dissecting microscope, and larger individuals were measured from the anterior hinge to the posterior edge of the shell to the nearest 0.1 mm using a Vernier callipers (see Appendix). The measured mussels were then placed in 95% alcohol in a 7 ml plastic container (Sarstedt) to await genetic analysis.

DNA Extraction

Adults

DNA, from what are considered to be pure populations of *Mytilus edulis* Aarhus, Denmark (56°12'W 10° 20'N) and *Mytilus galloprovincialis* Rio Bensafrim, Lagos, Portugal (8°40`W 37° 6`N), was extracted from adult alcohol-preserved mussels (20-30 cm shell length) using a Chelex extraction method (Sambrook et al. 1989).

Approximately 2 mm^3 of tissue was removed from the posterior adductor muscle. The tissue was then washed and blotted in sterile water to remove the preserving alcohol, then squashed using the flat edge of a sterile scalpel. The resulting tissue flake was placed in a 1.5 ml tube with 500 µl of 10% Chelex (Sigma) solution (1 g Chelex to 10 ml sterile $_{dd}H_2O$) and 7 µl of proteinase K (20 µl/ml), vortexed for 20 s and then placed in a rotary shaker and incubated at 56°C for 2 h. Samples were then vortexed again, 20 µl of ribonuclease A (20 µl/ml) was added, and the samples were placed in a 36°C oven and shaken twice in one hour. Samples were then placed in a 100°C oven for 30 min and vortexed once after 15 min. The samples were then either used directly or stored at -20°C.

Prior to PCR processing the sample was spun at 10 000 rpm for 3 min to separate the mixture of tissue, supernatant and Chelex. Three µl of the supernatant was then added to the PCR mix. When adding this solution to the PCR mix care must be taken not to add any of the Chelex, as it inhibits the PCR process.

Spat

Genetic analysis of primary settlers was only carried out on pads collected on the 14 July and the 24 October. The 14 July was selected as it represents peak summer settlement, while the 24 October represents a peak in autumn settlement at Carraroe for the sampling period, but with reasonably high numbers still settling at Ballynahown. At both Carraroe and Ballynahown three replicates were sampled from the mid and low shore.

Random sampling of measured spat was achieved by spilling the contents of the 7 ml sample bottle into a petri dish, which had a grid drawn on the bottom lid. The grid consisted of 56 whole squares (5 x 5 mm^2). The grid was then sampled using random number tables until 36 individuals between 250-500 µm were collected. Each spat was measured and placed in an individual 1.5 ml Eppendorf micro-centrifuge tube (Eppendorf AG). Spat were measured for total length as before (Fig 6), and for first settlement ring. This first settlement ring coincides with the time of settlement in *M. edulis* and is described as the prodissoconch-dissoconch boundary, which marks the transition from a simple monolayer larval shell to a complex multi-layered post larval shell (Lutz and Kennish 1992).

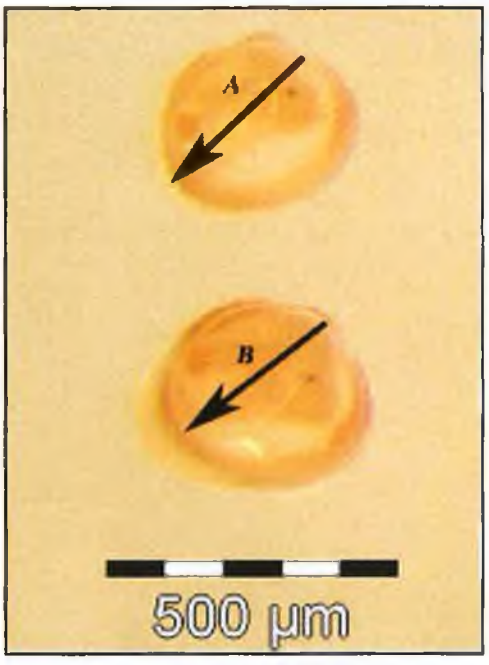

Fig. 6 *Mytilus* spat showing (A) post-settlement shell length and (B) pre-settlement shell length, with the prodissoconch-dissoconch boundary clearly evident.

Ten µl of lysis solution (7.5 mM Tris-HCl, pH 8.3; 3.75 mM NH_4Cl; 3.75 mM KCl; 1.5 mM $MgCl_2$; 2 µg proteinase K) (Sutherland et al. 1998) was added to each tube, which was then incubated at 37°C for 2 h. Samples were boiled for 10 min in a water bath (Blanco) to inactivate proteinase K and were then either processed through the Polymerase Chain Reaction (PCR) procedure or stored at -20°C overnight.

PCR amplification and electrophoresis

PCR reagents

The solutions for PCR were made up x 38 to reduce sampling error when measuring small volumes. Chemicals for PCR were added in the same order as in Table 1. The 10x Buffer plays an essential role in preventing chemical damage to the deoxynucleotide mix (dNTPs) and primers. When the REDTaq™ was added the solution was kept on ice to prevent activation of the enzyme. This x 38 master mix was then pipetted 10.2 µl at a time into 36 0.5 ml amplification strip tubes (Sarstedt). These strips were kept on ice while 3 µl of lysed mussel sample was added to each cup and mixed using the tip of the pipette.

Table 1. Concentrations for PCR Mix

Chemicals	x 1 (µl)	x 38 (µl)
Distilled, sterile water	3.1	117.8
10x Buffer*	1.0	38
$MgCl_2$* (2.5 mM)	1.5	57
dNTPs* (0.6 mM)	2.0	76
Forward Oligo Primer$ (6 mM)	1.0	38
Reverse Oligo Primer$ (6 mM)	1.0	38
RedTaq™Polymerase*(1ul = unit)	0.6	22.8
Total	10.2	387.6

*Chemical produced by Sigma

$ MWG Biotech AG

The primer sequences *Me15* and *Me16* (Inoue et al. 1995) (Table 2) have been developed for a genetic marker *Me15/16* which is located within the nuclear gene encoding a polyphenolic adhesive protein, a key component in the attachment of mussels to the substrate. PCR amplification of *Me15/16* produces a species-specific band of about 180 bp for *M. edulis* and a 126 bp band for *M. galloprovincialis*; hybrid individuals have both bands (Fig 7).

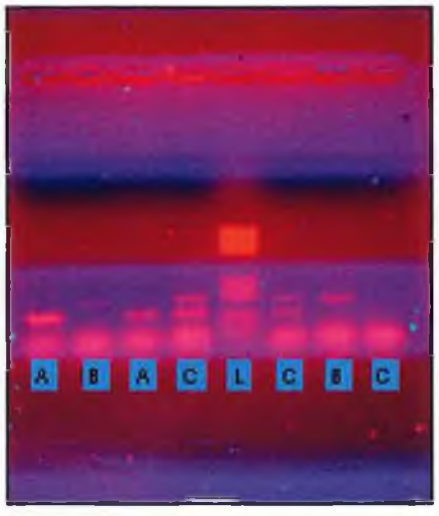

Fig 7. Agarose gel showing bands representing *M. galloprovincialis* (A), *M. edulis* (B), hybrid individuals (C) and Ladder (L) (Bands below * are unused primers).

Table 2. Primer sequences for *Me 15* and *Me 16*.

Me 15 CCA GTA TAC AAA CCT GTG AAG A (Forward)
Me 16 TGT TGT CTT AAT AGG TTT GTA AGA (Reverse)

PCR conditions

PCR was carried out using a Biometra™ T1 Thermal Cycler, using the cycling times in Table 3. This programming allows the files to run one after another so the machine can be left to cycle over night. The central step cycle file repeats 30 times creating multiple copies of the primer sequences, using all the available DNTPs. The soak file holds the samples at -1°C for an indefinite time so the machine can be left unattended after the cycles have been completed. After PCR the samples were run immediately on agarose gels or stored at -20°C.

The thermocycling protocol (Table 3) consisted of an initial denaturation step at 95°C for 4 min, followed by 30 "step cycle file" cycles, consisting of 94°C for 45 s, 56°C for 30 s and 70°C for 90 s, and a final extension of 6 min 40 s at 72°C. PCR products were separated on 2% agarose gels to which ethidium bromide (10 mg/ml) had been added. A DNA ladder (pBR322 Hae III DIGEST, Sigma) and one reference individual were run on each gel. The reference individual was either *Mytilus edulis* from Denmark or *Mytilus galloprovincialis* from Portugal. DNA fragments were visualised under UV light and photographed with a digital camera.

Table 3. PCR Protocol

Incubator files	Temperature (°C)	Time
Time delay file (Denaturing)	95	4 min
Step cycle file (Denaturing)	94	45 s
(Annealing)	56	30 s
(Primer extension)	70	90 s
Final extension time delay file	72	6 min 40s
Soak file	-1	~

Agarose gel electrophoresis

Agarose gels were run using a wide mini-sub cell GT electrophoresis cell (Bio-Rad). For each gel 92 ml of 1x TEB (Tris-Borate EDTA) Buffer (10x Tris-Borate EDTA, 108 g Trizma base, 55 g Boric acid, 9.5 g EDTA disodium salt, 750 ml distilled H_2O) and 1.84 g of Agarose wide range/routine 3:1 powder (Sigma) was added. This solution was placed on a hotplate until the agarose powder had melted and the solution was clear, at which point 5 µl of ethidium bromide (10 mg/ml) was added.

This solution was then poured into a level gel mould, with two 20-sample combs positioned (top and centre) after the gel had cooled slightly. The gel mould was then left to cool for 10 min before being placed at 4°C for 30 min.

The PCR samples were prepared for electrophoresis by adding 1 µl of 6x gel loading solution (Sigma) to each tube.

The agarose gel was submerged in 600 ml of TBE running buffer (600 ml 1 x TBE and 30 µl of ethidium bromide (10 mg/ml) and the wells were visualised by placing a background strip of black plastic under the tray. The gel was then loaded with 13.5 µl of sample per well. Along with the samples one ladder (pBR322 Hae III DIGEST, Sigma) and one reference individual was added to each lane.

Fifty volts was applied to the gel for ~2 h or until the loading solution had run ¾ of the way down the gel. The gel was then removed from the gel rig and visualised on an ultra violet (UV) light box (Hoefer, Mighty Bright) at high intensity (240 nm). The combination of ethidium bromide and nucleic acid glow under UV light and the resulting fluorescing banding was recorded using a digital camera (Kodak, Easy Share CX4300). The image was then downloaded onto a desktop computer and enhanced using Adobe Photoshop™ 7.0.

Data Analysis

Data were converted into computer format using Microsoft Excel™ (Microsoft Office 2000 Professional). For recruiting mussels, their numbers, sizes and size frequencies were analysed using Minitab 14.0. Minitab™ was used for Mann-Whitney tests, *t*-tests, Kruskal-Wallis tests, and for Anderson-Darling tests for normality.

Genetic data were analysed using Microsoft Excel™ and GENETIX 4.0.5.2 (Belkhir et al. 2003). *G*-tests were carried out using PopTools (Hood 2004), an add-in for Microsoft Excel™.

The G –test (likelihood ratio test) was used to compare allele and genotype frequency instead of the Genepop exact test (Chi square test). This was done as both are approximately the same, being based on log likelihood ratio tests, testing for differences in frequency distributions. However, the G-test has several theoretical advantages over the chi square test including, when the expected value is small and/or the sample has a small number of observations. In such cases it is found to be more appropriate to use the G-test (Sokal and Rohlf, 1994).

Results

Settlement

Primary spat settled continually at the two exposed sites throughout the sampling period May to October 2003 (Fig 8). The highest numbers were observed over the summer period (end June-end July) on both shores. Numbers of settlers remained low at Carraroe after July, but seemed to be increasing again towards the end of the sampling period in October. At Ballynahown numbers of spat increased in August, and again at the end of September-beginning of October. On both shores, numbers of primary settlers were generally higher on the low shore.

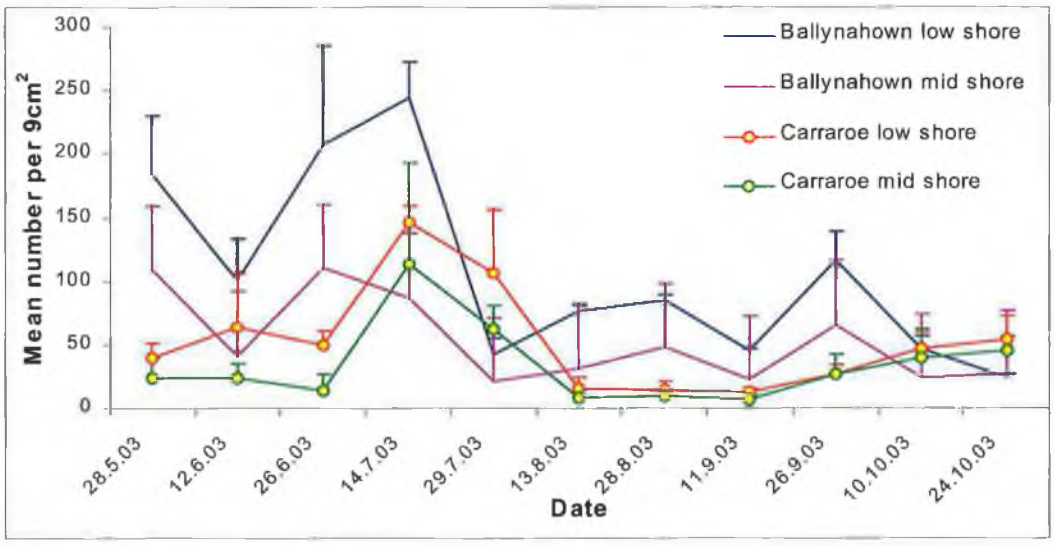

Fig 8. Mean numbers of *Mytilus* primary settlers and SD recruiting onto pads between May and October 2003 at the Ballynahown and Carraroe exposed shore sites.

Genetics of settling spat

Genetic analysis of primary spat was only carried out on pads collected on the 14 July and 24 October. The 14 July was selected as it represents peak summer settlement, while the 24 October represents the period with highest numbers of autumn settlers at Carraroe. Reasonably high numbers of spat were still settling at this time at Ballynahown. At both Carraroe and Ballynahown, three replicates selected at random were sampled from the mid and low shore. The summer and autumn periods were

chosen at both sites in order to test spatial and/or temporal variability in the genetic composition of recruits.

G-tests of independence were used to analyse genotype and allele frequencies. For tidal height replicates, when the expected numbers of genotypes was < 5 for an individual pad, generally the case for numbers of *M. edulis*, analysis was only carried out on *M. galloprovincialis* and hybrid genotype frequencies.

Ballynahown

There was no significant difference in allele (Table 5) or genotype frequencies between replicates at each tidal height (G-test $P > 0.05$), so data from the three replicates were pooled for each tidal height. Table 5 shows uniformity of allele frequency within replicates at each tidal height and date, indicating uniform settlement at each tidal height.

Table 5. Frequency of the *Me15/16 M. galloprovincialis* allele in primary settlers onto pads for July and October 2003 at Ballynahown. Replicates are represented by Roman numerals; sample size in brackets.

14 July	Mid shore	Low shore
I	0.66 (31)	0.53 (24)
II	0.55 (25)	0.60 (27)
III	0.59 (26)	0.58 (26)
Average	0.60 (82)	0.57 (77)

24 October	Mid shore	Low shore
I	0.61 (27)	0.64 (26)
II	0.55 (28)	0.70 (18)
III	0.51 (28)	0.61 (32)
Average	0.56 (83)	0.65 (76)

There was no significant difference (G-test $P > 0.05$) in allele or genotype frequencies in *M. edulis*, *M. galloprovincialis* and hybrid spat when the July mid and

low shore samples were compared (Fig 9). However, there was a significant difference in allele and genotype frequency (Genotypes G= 7.51 P = 0.02; Alleles G= 6.21 P = 0.015) between the mid and low shore October comparison (Fig 10), due to higher frequencies of *M. galloprovincialis* spat in the low shore samples. Frequencies of *M. edulis* were never more than 11% in any sample.

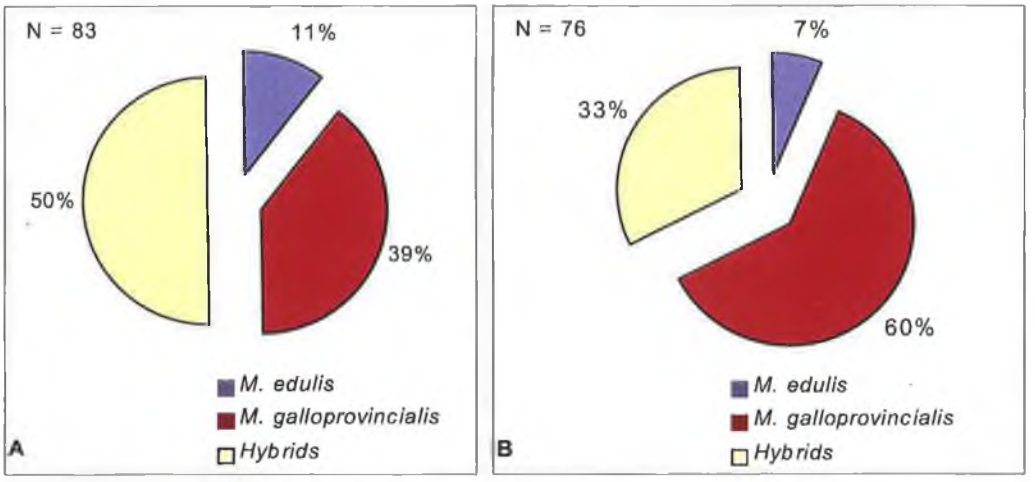

Fig 9. Percentage frequencies of *M. edulis, M. galloprovincialis* and hybrids settling onto pads in the mid (A) and low (B) shore at Ballynahown, 14 July 2003. N = sample size.

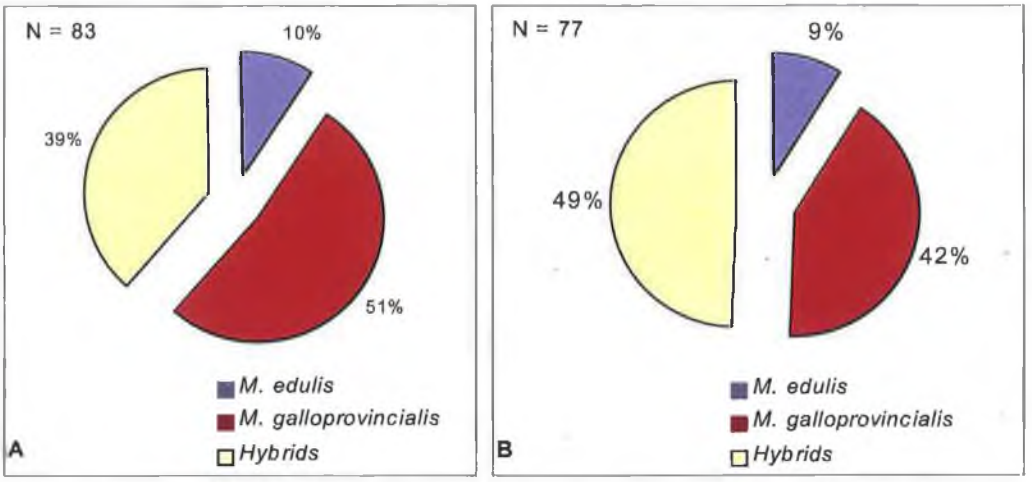

Fig 10. Percentage frequencies of *M. edulis, M. galloprovincialis* and hybrids settling onto pads in the mid (A) and low (B) shore at Ballynahown, October 2003. N = sample size.

When July and October low shore samples from Ballynahown were compared no significant differences in allele or genotype frequencies was detected. When July and October mid shore samples from Ballynahown were compared no significant differences in allele or genotype frequencies was detected.

Carraroe

There was no significant difference in allele or genotype frequencies (G-Test $P > 0.05$) between replicates at each tidal height, so data were pooled for tidal heights. Uniform allele frequencies at each shore height were observed between replicates indicating uniform spat settlement at each date and tidal height (Table 6)

Table 6. Frequency of the *Me15/16 M. galloprovincialis* allele in primary settlers onto pads for July and October 2003 at Carraroe. Replicates are represented by Roman numerals; sample size in brackets.

14 July	Mid shore	Low shore
I	0.77 (30)	0.69 (36)
II	0.71 (36)	0.78 (24)
III	0.75 (34)	0.70 (36)
Average	0.74 (100)	0.73 (96)

24 October	Mid shore	Low shore
I	0.80 (35)	0.63 (34)
II	0.63 (33)	0.63 (30)
III	0.68 (34)	0.73 (31)
Average	0.70 (102)	0.66 (95)

There was no significant difference in allele or genotype frequencies of *M. edulis*, *M. galloprovincialis* and hybrid spat between the pooled mid and pooled low shore in the July or October samples (Figs 11 and 12). Pooled July and pooled October samples were significantly different in allele and genotype frequencies (Genotypes G = 6.93 P = 0.03; Alleles G = 7.12 P = 0.015), due to the significantly higher frequency of *M. edulis* and hybrids in the low shore October sample (G = 6.54 P = 0.04).

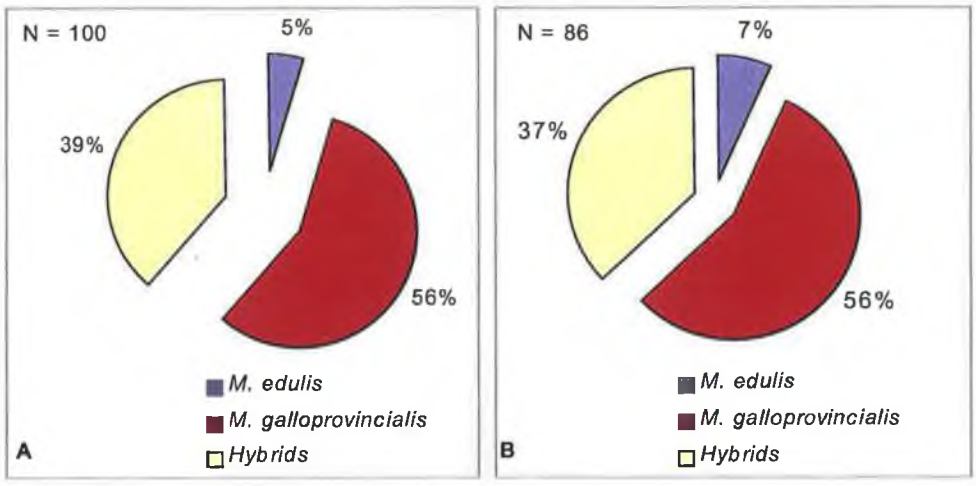

Fig 11. Percentage frequencies of *M. edulis, M. galloprovincialis* and hybrids settling onto pads in the mid (A) and low (B) shore at Carraroe, July 2003. N = sample size.

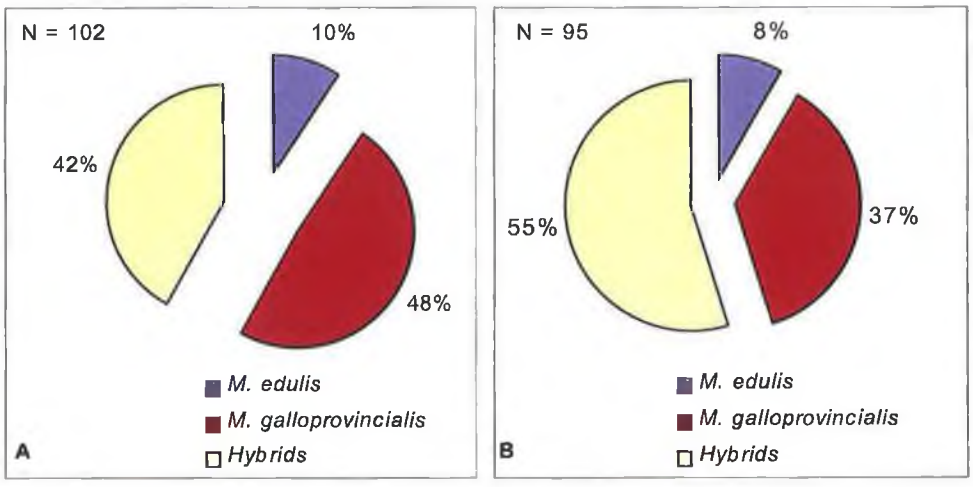

Fig 12. Percentage frequencies of *M. edulis, M. galloprovincialis* and hybrids settling onto pads in the mid (A) and low (B) shore at Carraroe, October 2003. N = sample size.

Ballynahown and Carraroe

Allele and genotype frequencies of primary settlers were compared between the two shores. No significant differences were detected for either mid shore or low shore July comparisons or mid shore October comparisons. However, low shore comparisons for October were significantly different (Genotype G = 9.52 P = 0.01, Allele G = 8.45 P = 0.014), due to the higher number of. *M. edulis* and hybrids present in the low shore Carraroe sample.

Relationship between spat shell length and genotype

Genetic analysis was carried out on spat varying from 230-525 µm total shell length In the October sampling, spat up to 525 µm were genetically analysed to increase sample numbers from settlement pads with low numbers of primary settlers; these individuals (> 500µm) made up no more than 10% of the samples. Size data were tested for normality and in all cases were found to have a non-normal distribution (Anderson–Darling normality test $P < 0.05$). Therefore, a non-parametric Mann-Whitney *U* test was used for analysis.

In the Ballynahown July sample, there were no significant differences in larval size between the six mid and low shore replicates, so replicate data from the two tidal heights were pooled. The October sample was also pooled with no significant difference in primary settlement sizes between the mid and lower shores. Therefore, all size data for July and October Ballynahown primary settlement were compared. The October sample had significantly ($P = 0.011$) larger larval median lengths (390 µm) than the July sample (360 µm).

At Carraroe, there were no significant differences in larval size between replicates for the mid or low shore pads, so replicate data were pooled. Although no significant differences were found between shore heights in the October samples, significant differences were observed in July ($P = 0.001$) between the pooled mid (340 µm) and pooled lower (390 µm) shore spat medians.

Spat on pads at Ballynahown (B) were significantly larger than at Carraroe (C) in July (C: median 340 µm; B: 380 µm; $P = 0.014$) and October (C: median 370 µm; B: 390 µm; $P = 0.003$).

There was no significant difference in the median shell length of *M. edulis*, *M. galloprovincialis* and hybrids in pooled replicates from mid and low shore samples for the Ballynahown or Carraroe July or October samples, indicating that spat size is independent of genotype.

Relationship between shell length at settlement and genotype

To determine if there was a difference in size at settlement between genotypes the distance from the hinge to the prodissoconch-dissoconch boundary was measured in all spat for genetic analysis.

Larvae began to settle at ~230 µm, with most settlement occurring at a size of 270-290 µm. All shell length data (N = 701) for primary settlers were divided into size classes and the percentage frequency of *M. edulis, M. galloprovincialis* and hybrid in the different size classes was determined (Fig. 13). *M. edulis* and *M. galloprovincialis* had a median hinge to prodissoconch-dissoconch boundary length of 300 µm, while hybrids had a lower, but not significantly different, median length of 280 µm. There was no evidence for a relationship between size at settlement and genotype.

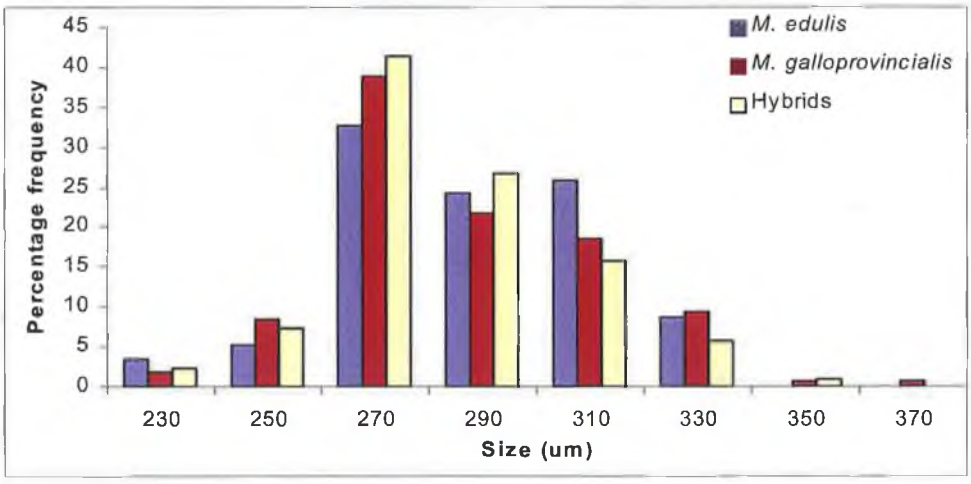

Fig 13. Percentage frequency of *M. edulis, M. galloprovincialis* and hybrid spat (N = 701) in different settlement size classes at Ballynahown and Carraroe, July and October 2003.

Using the same shell length data, it was possible to calculate the length of time that spat had been on the pads, by means of spat growth rate estimates of Bayne (1964), and Gilg and Hilbish (2000). These authors calculated a mean growth rate for *Mytilus* of 30 μm d^{-1} in temperate waters. Measuring the length of shell after the first settlement ring and dividing this by 30 μm gave an estimate of the number of days that spat had been on the pads. Although some spat were on the pads for as long as 10-11 days numbers were too low for statistical analysis. The analysis focussed instead on spat that had been on the pads between 6-7 days where numbers were higher. Fig. 15 shows the numbers of spat settling onto pads over the 14 days that pads were left on the shore at Ballynahown and Carraroe in July and October. The majority of spat found on the pads arrived in two pulses, 4 days, and 1-2 days before pad collection. Fig. 15 illustrates the percentage frequency of *M. edulis*, *M. galloprovincialis* and hybrids that have been on the pads between 0 (date of pad collection) and 6.0 days, the period when maximum numbers of spat settle onto pads.

The relative frequencies of *M. edulis*, *M. galloprovincialis* and hybrids are reasonably constant with no indication of pulses of settlement for any one genotype i.e. larvae settling on the pads, at least for the 6 days prior to collection, appear to be a homogenous mixture of all three genotypes. The proportions of which mirror very closely the proportions observed for all spat samples from the two exposed shores.

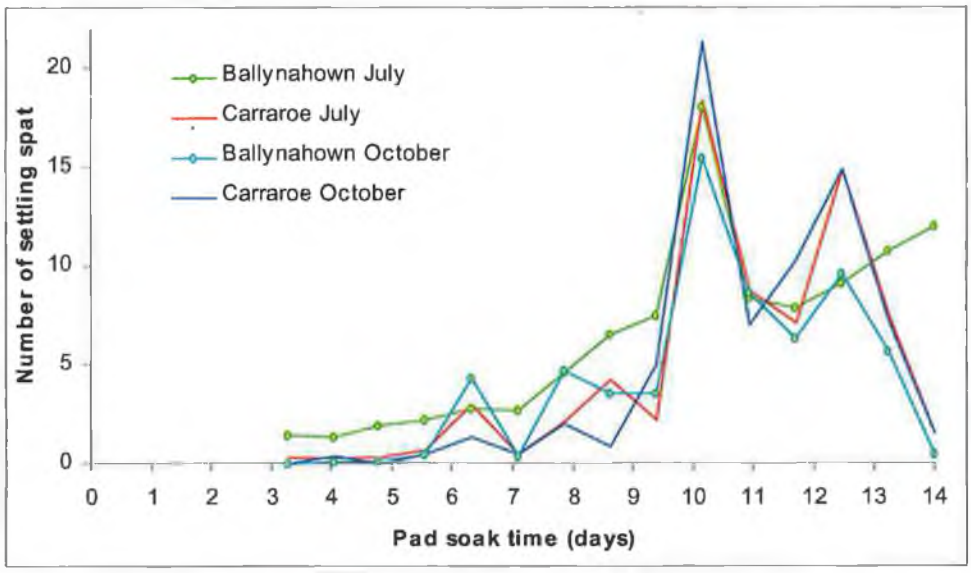

Fig 14. Numbers of *Mytilus* spat settling onto pads during a 14-day period in July and October 2003 at the exposed shore sites at Carraroe and Ballynahown.

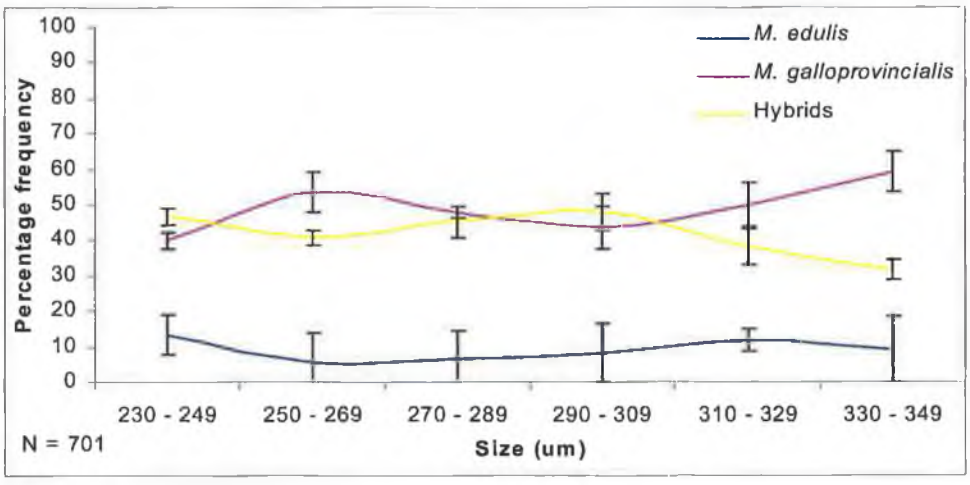

Fig 15. Relative frequency (%) and SD of *M. edulis*, *M. galloprovincialis* and hybrids from pooled spat data (N = 701) from pads placed on the exposed Ballynahown and Carraroe shores in July and October 2003.

Genetics of adult mussels

G-tests of independence were used to analyse allele and genotype frequencies in samples of adult *M. edulis*, *M. galloprovincialis* and hybrids. For tidal-height-date samples when the expected numbers of genotypes was < 5 for an individual pad - generally the case for numbers of *M. edulis* - analysis was only carried out on genotype frequencies in *M. galloprovincialis* and hybrids.

Ballynahown exposed shore adults

In conjunction with pad collection/deployment six replicate samples of adult mussels were also collected between May and October 2003 from the mid and low shore regions of the exposed shore at Ballynahown. One of the six replicates was selected at random for genetic analysis from samples collected on the 26 June, 13 August and 10 October 2003, dates which are evenly spaced over the sampling period May-October.

Table 7. Frequency of *Me15/16 M. galloprovincialis* allele in adult mussels from the exposed (ES) shore Ballynahown; sample size in brackets.

Site	Sampling Date	Mid Shore	Low Shore
ES	26.6.03	0.72 (32)	0.78 (36)
ES	13.8.03	0.67 (33)	0.76 (35)
ES	10.10.03	0.66 (35)	0.68 (36)
Average		0.68 (100)	0.74 (107)

Frequencies of the *M. galloprovincialis* allele of adults at the Ballynahown exposed shore are presented in Table 7. Genotype and allele frequencies in mid shore samples were not significantly different for the three sampling dates. This was the same situation for low shore samples. Therefore, all mid shore samples, and all low shore samples were pooled. When genotype and allele frequencies for pooled mid, and pooled low shore mussels were compared, there were no significant genetic differences between mussels at the two tidal levels (Fig 16 and Table 7).

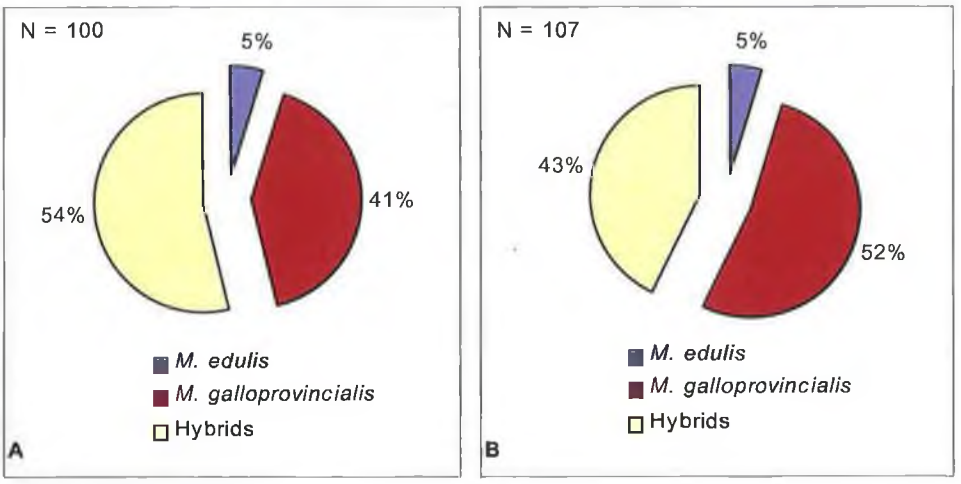

Fig 16. Percentage frequency of *M. edulis, M. galloprovincialis* and hybrid genotypes in samples of adults from the mid shore (A) and low shore (B) at the Ballynahown exposed shore site.

Ballynahown sheltered shore adults

Adult mussels were collected on the 27 September 2004 from the mid and low shore regions of a sheltered site situated very close (900 m) to the exposed shore site at Ballynahown. Two replicates out of three samples were genetically analysed at each tidal height; the total sample size was 141 individuals. There was no significant difference in allele or genotype frequencies between replicates for either the mid or low shore samples. Replicates were then pooled to compare genotype and allele frequencies between tidal heights and no significant differences were observed between them (Fig 17 and Table 8).

Table 8. Frequency of *Me15/16 M. galloprovincialis* allele in adult mussels from the Ballynahown sheltered shore (SS); sample size in brackets.

Site	Sampling Date	Mid Shore	Low Shore
SS	27.9.04	0.68 (33)	0.58 (36)
SS	27.9.04	0.51 (36)	0.53 (36)
Average		0.65 (69)	0.56 (72)

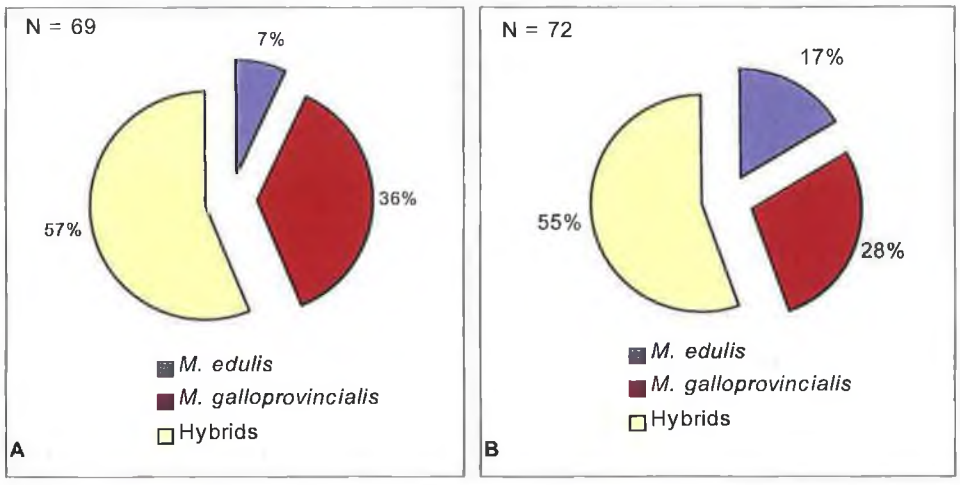

Fig 17. Percentage frequency of *M. edulis, M. galloprovincialis* and hybrid genotypes in samples of adults from the mid shore (A) and low shore (B) at a sheltered site close (900 m) to the Ballynahown exposed shore.

When the pooled data from the Ballynahown exposed site were compared with the pooled data from the nearby sheltered site there was a significant difference in genotype and allele frequencies between the two shores ($G = 6.21$ $P < 0.001$, $G = 8.51$ $P < 0.001$), due to the higher frequency (16%) of *M. edulis* and hybrid individuals on the sheltered shore.

Carraroe exposed shore adults

In conjunction with pad collection/deployment six replicate samples of adult mussels were also collected between May and October 2003 from the mid and low shore regions of the exposed shore at Carraroe. Similar to the exposed shore at Ballynahown, one out of six replicates was selected at random for genetic analysis from samples collected on the 26 June, 13 August and 10 October 2003.

While genotype and allele frequencies were similar for the June and August mid shore samples significant differences were observed between the October and June ($G = 8.12$ $P < 0.05$, $G = 5.10$ $P < 0.05$) and October and August ($G = 9.23$ $P < 0.05$, $G = 3.82$ $P < 0.05$) samples, due to the higher frequency of hybrid individuals in the October sample. The difference between this sample and the other two may be related to size: mean shell length was 11.1mm (SD ± 2.9 mm), with 62% under 10

mm, while the mean shell length was 15 mm (SD ± 2.3 mm) and 16.6 mm (SD ± 2.4 mm) for the June and August samples, respectively. Later in this section, a relationship between genotype frequency and shell length will be shown, with smaller individuals more likely to be hybrids. Although the October settlement sample was significantly different, when samples from the three dates (Table 9) were pooled no significant difference in allele or genotype frequencies were detected between the three low shore samples, or between the pooled mid and the pooled low shore samples (Table 9 and Fig 18).

Table 9. Frequency of *M. galloprovincialis* alleles in adult mussels from the Carraroe exposed sites (ES); sample size in brackets

Site	Date	Mid Shore	Low Shore
ES	26.6.03	0.86 (28)	0.69 (27)
ES	13.8.03	0.87 (30)	0.75 (22)
ES	10.10.03	0.68 (34)	0.71 (36)
Average		0.80 (92)	0.71 (85)

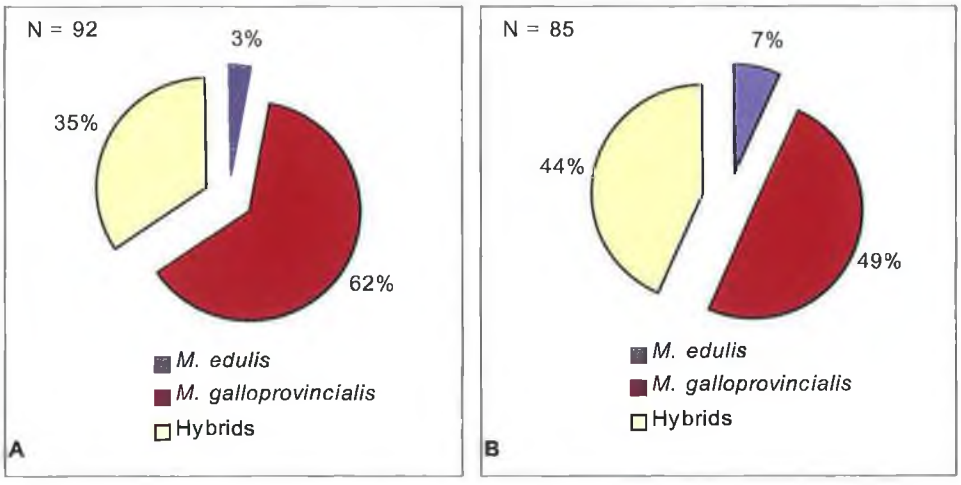

Fig 18. Percentage frequency of *M. edulis, M. galloprovincialis* and hybrid genotypes in samples of adults from the mid shore (A) and low shore (B) at the Carraroe exposed shore site.

There were no significant differences in allele or genotype proportions between samples of adults from Ballynahown and Carraroe exposed sites. However, similar to the results obtained for the Ballynahown exposed and sheltered shore comparison, significant differences in genotypes and allele frequencies ($P < 0.001$) were also observed between the Carraroe exposed and sheltered Ballynahown samples (Fig 19).

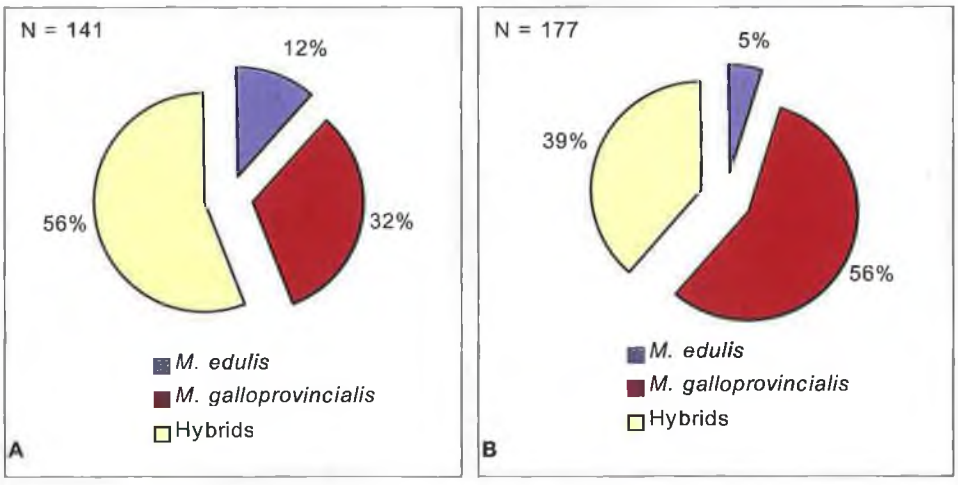

Fig 19. Percentage frequency of *M. edulis, M. galloprovincialis* and hybrid genotypes in samples of adults from the Ballynahown sheltered shore (A) and Carraroe exposed shore (B).

Comparison of the genetic structure of adults and spat from the Ballynahown and Carraroe exposed shore sites

Ballynahown

Genotype and allele proportions between pooled mid shore adults from the three sampling dates, June, August and October 2003 were compared with pooled mid shore 14 July spat and pooled mid shore 24 October spat (Fig 20). There were no significant differences in genotypic or allelic frequencies between adult or spat samples. Neither were there significant differences in genotypic or allelic frequencies between pooled adults, or pooled low shore July, or pooled low shore October spat (Fig 21). The genetic composition of the adult population at Ballynahown directly

reflects the genetic composition of primary settlers on this shore, at least for the sampling dates in the study.

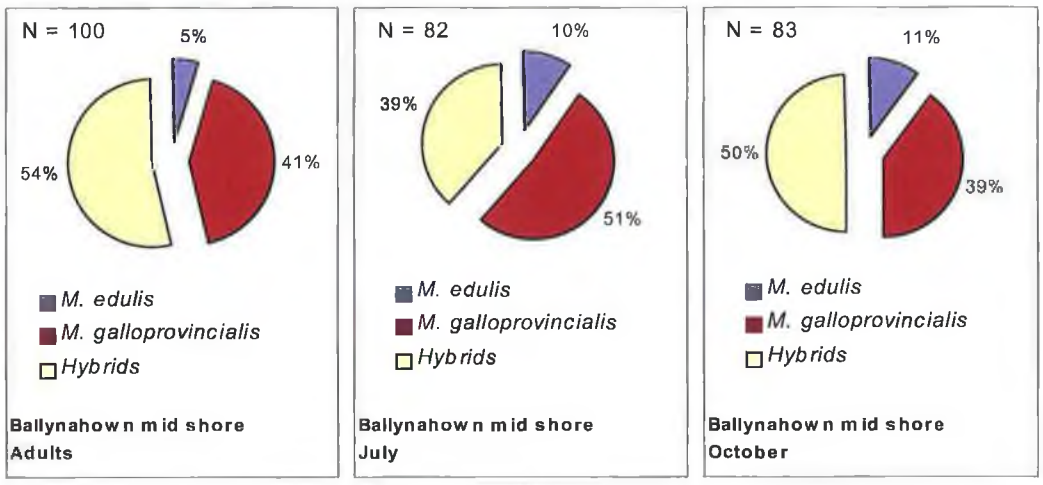

Fig 20. Percentage frequency of *M. edulis*, *M. galloprovincialis* and hybrids in pooled adult samples (June, August, October 2003) and pooled mid shore July, and pooled mid shore October 2003 samples of spat at the Ballynahown exposed shore site.

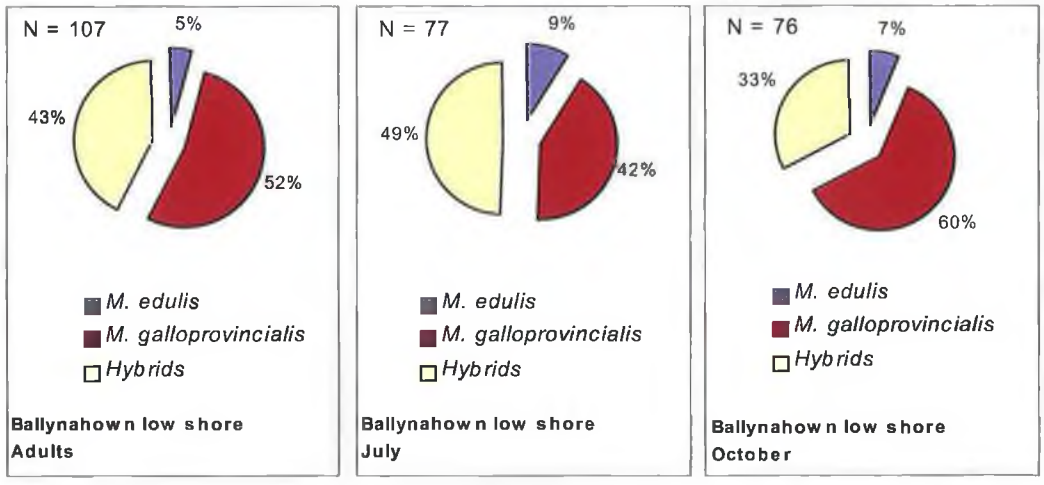

Fig 21. Percentage frequency of *M. edulis*, *M. galloprovincialis* and hybrids in pooled adult samples (June, August, October 2003) and pooled low shore July, and pooled low shore October 2003 samples of spat at the exposed shore, Ballynahown.

Carraroe

Similar results were obtained for comparisons between adult and spat samples at Carraroe (Figs 22 and 23), indicating that the adults at this site have a similar genetic composition to primary settlers.

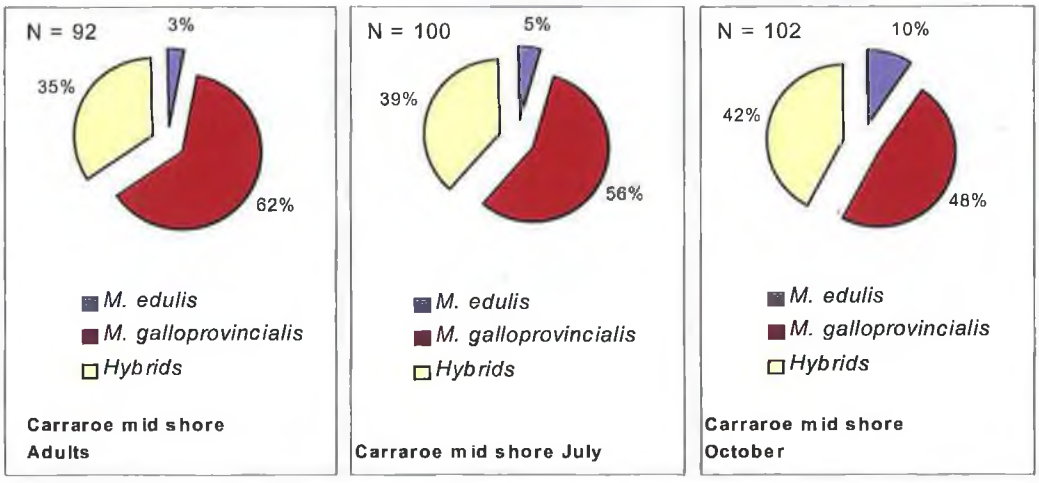

Fig 22. Percentage frequency of *M. edulis*, *M. galloprovincialis* and hybrids in pooled adult samples (June, August, October 2003) and pooled mid shore July, and pooled mid shore October 2003 samples of spat at the exposed shore, Carraroe.

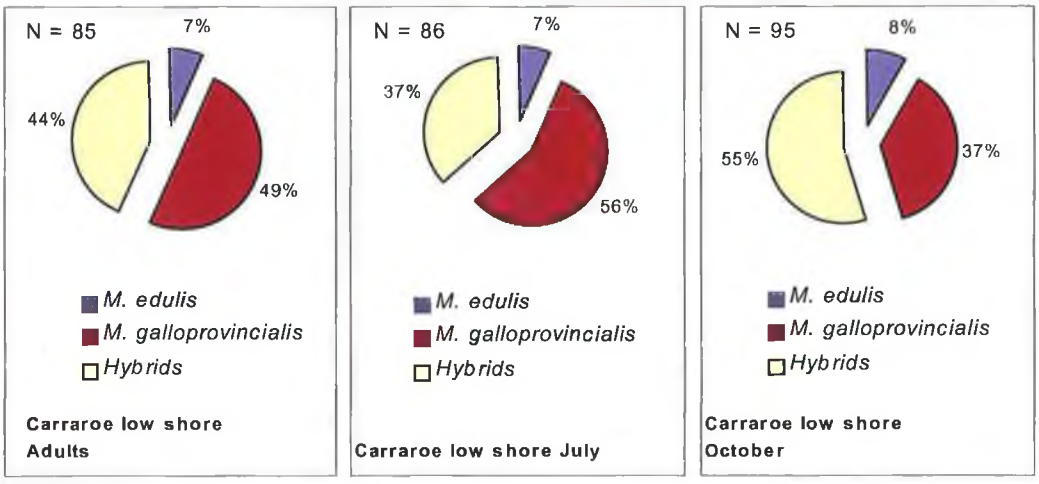

Fig 23. Percentage frequency of *M. edulis*, *M. galloprovincialis* and hybrids in pooled adults (June, August, October 2003) and pooled low shore July, and pooled low shore October 2003 samples of spat at the exposed shore, Carraroe.

Adult population structure

Ballynahown and Carraroe exposed shores

Genotype and allele frequencies of adult mussels from the mid and low shore at the Ballynahown and Carraroe exposed shores were not significantly different therefore, genotype and size data were pooled for the three sampling dates 26 June, 13 August and 10 October 2003. Data were sorted into size frequency classes to calculate relative frequency of *M. edulis*, *M. galloprovincialis* and hybrids in the different size classes (Fig. 24).

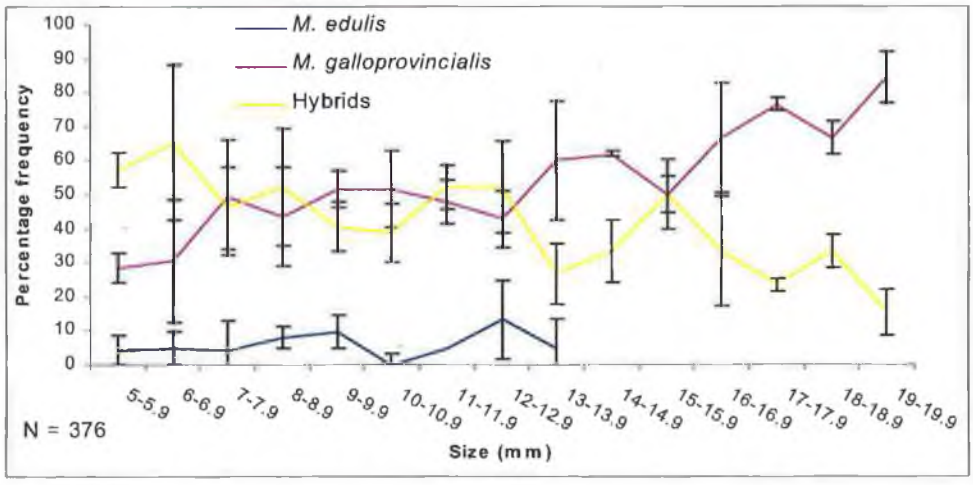

Fig 24. Relative frequency (%) ±SD of *M. edulis*, *M. galloprovincialis* and hybrid genotypes in different size classes of adults in the combined Ballynahown and Carraroe exposed shore samples for 26 June, 13 August and 10 October 2003. Size classes in the SL range 20-24.9 mm had < 10 individuals and were omitted.

The frequency of *M. galloprovincialis* increased from 30% in the smallest size classes to nearly 80% in the largest size class analysed. There was a corresponding decrease (from 55% to 20%) in the frequency of hybrids (Fig 24). The frequency of *M. edulis* was low (<10%) in all size a class in the SL range 5-13.9 mm and only one individual was recorded above 15 mm at 21.8 mm.

Five size classes were created to investigate the relationship between allele and genotype frequencies, and SL. Allele frequencies of the different size classes are given in Table 10. The size class 20-24.9 mm was omitted because of low numbers

(only 8 individuals). When one size class was compared to the next in sequence, there was no significant difference in allele or genotype proportions. However, when the smallest (5-7.9 mm) was compared to the largest (17-19.9 mm) the differences in allele (P = 0.045) and genotype (P = 0.001) proportions were significantly different. Table 10 shows the change in the frequency of the *M. galloprovincialis* allele with size; larger individuals had a higher frequency of the allele than smaller ones.

Table 10. Frequency of the *Me15/16 M. galloprovincialis* allele in five shell-length classes at Ballynahown and Carraroe exposed shores.

Size (mm)	G - Allele frequency	Sample size
5 - 7.9	0.69	93
8 - 10.9	0.71	139
11 - 13.9	0.72	59
14 - 16.9	0.79	46
17 - 19.9	0.86	40

Ballynahown sheltered shore

The percentage frequency of *M. edulis*, *M. galloprovincialis* and hybrid genotypes in different size classes are given in Fig 25. The size class 5- 9.9 mm (SL) had < 10 individuals and was therefore omitted from the analysis.

The frequency of *M. edulis*, *M. galloprovincialis* and hybrid genotypes remain reasonably constant over the sampled size range (Fig 25). Five size classes were created to assess the relationship between allele and genotype frequencies, and SL (Table 11). No significant differences in either allele or genotype proportions were observed between any of the five SL classes and when the smallest (10 – 19.9 mm) was compared to the largest (>50 mm) no differences in allele or genotype proportions were detected.

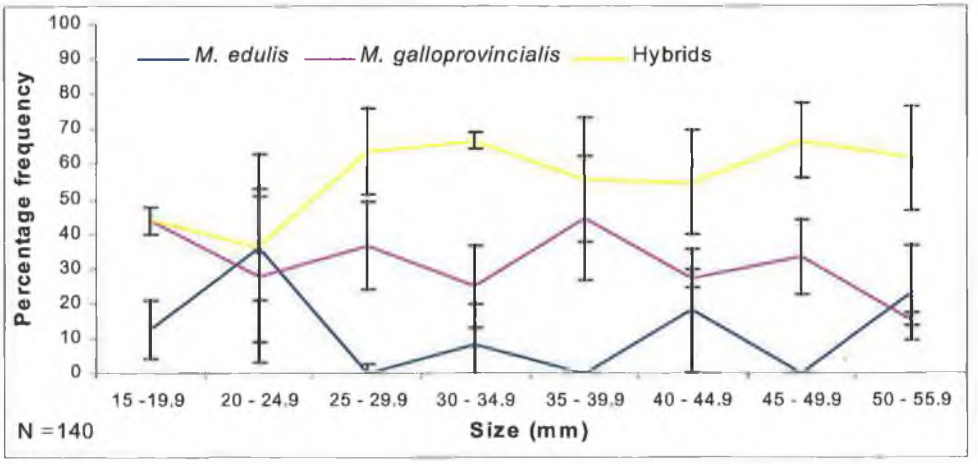

Fig 25. Relative frequency (%) ± SD of *M. edulis*, *M. galloprovincialis* and hybrid genotypes in different size classes of adults from the Ballynahown sheltered site, September 2004.

Table 11. Frequency of the *Me15/16 M. galloprovincialis* allele in five shell-length classes at the Ballynahown sheltered shore, September 2004.

Size (mm)	G - Allele frequency	Sample size
10 – 19.9	0.65	30
20 – 29.9	0.60	101
30 – 39.9	0.64	34
40 – 49.9	0.60	32
> 50	0.46	21

Comparison of the genetic structure of spat, and adults of different shell lengths from Ballynahown and Carraroe exposed shores

Genotype and allele data from spat settlement in July and October at the two exposed shores was pooled and compared with pooled data from adults collected on 26 June, 13 August and 10 October at the same sites. The relative frequency of spat of *M. edulis*, *M. galloprovincialis* and hybrids in spat and five size classes of adults are shown in Fig 26. There was no significant difference in genotype proportions between

settling spat and adult size classes between 5 and 16.9 mm. However, significant differences were detected between spat and adults over 17 mm (P = 0.025). No significant difference in allele frequencies was detected between spat and any adult size classes.

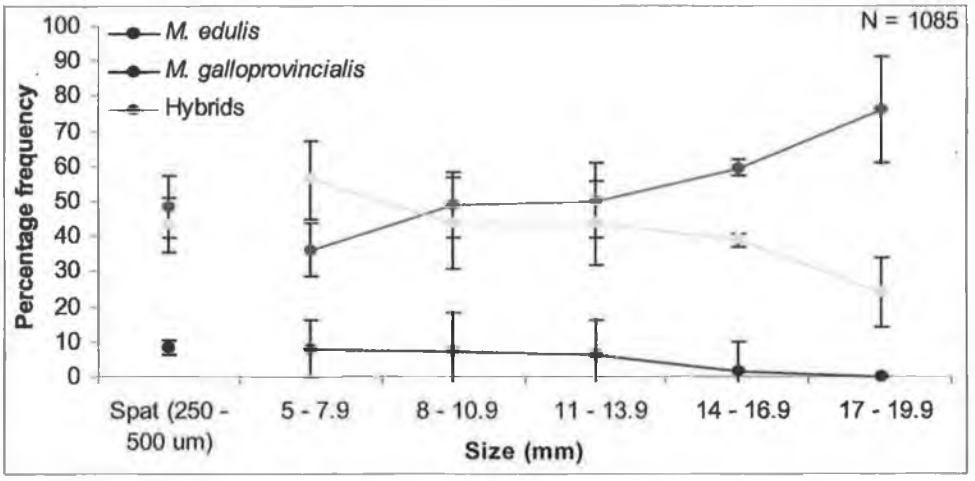

Fig 26. Relative frequency (%) ± SD of *M. edulis*, *M. galloprovincialis* and hybrid genotypes in settling spat and five different size classes of adults from Ballynahown and Carraroe exposed sites 2003. Size classes in the SL range 20 - 24.9 mm had < 10 individuals and were omitted.

Discussion

Settlement

Spat settlement was continuous throughout the summer and autumn at Carraroe and Ballynahown. Observed numbers were higher on both shores in the June-July period, with a smaller peak in October at Ballynahown, which was not observed at Carraroe. The pattern of settlement agrees with previously published results for Ballynahown (King et al. 1990), although these authors observed a much larger peak in October than in June-July. It is possible that the increasing numbers settling at the end of October at Carraroe might be signally the beginning of a large settlement event, perhaps larger than the summer peak. What is clear, from the results of this study and those of King et al. (1990), is that settlement patterns vary on a temporal scale both within and between years, and also on a spatial scale, between shores.

The presence of recruiting spat throughout the sampling period indicates continual spawning of the adult population in the Galway bay area. Extended spawning of mussel populations has also been reported for sites in the UK and France (Seed 1969, Gilg and Hilbish 2000, Bierne et al. 2003a). Larvae recruiting outside major spawning events are probably the result of adults spawning asynchronously within the population, or individual larvae that have remained planktonic for an extended period of time. Larvae can remain planktonic for up to 8 weeks (Bayne 1965) or even longer through byssus drifting (Lane et al. 1985) in unfavourable conditions, thus adding uncertainty to the spawned time and origin of settling larvae.

On both sites, settlement of spat on the lower shore appeared higher than on the mid shore. This difference is probably a combination of both behavioural and environmental factors. Larvae may actively choose to settle on the lower shore and it has been shown that *M. edulis* larvae settle selectively at a depth of 1.5 m in the White Sea (Dobretsov and Miron 2001). A more likely reason for greater numbers settling on the low shore is that the low shore is submerged and therefore exposed to settlers for greater periods.

The overall higher numbers of settling spat at Ballynahown suggests that it is a more suitable settlement site, or that it has greater exposure to settling spat. *Mytilus* larvae use macroscopic algae, adult mussel beds (McGrath et al. 1988) and almost any available hard substrate as primary settling strata. The upper and lower shores at

Ballynahown are relatively devoid of macroscopic algae and this may lead to increased levels of settlement onto artificial substrate such as settlement pads. The reverse is true for Carraroe where the settlement pads have to compete with macroscopic algae for settling larvae (Fig 4).

Spat genetics

Spat settling onto pads in July and October at Ballynahown and Carraroe comprised ~ 50% *M. galloprovincialis*, 40% hybrids and 10% *M. edulis*. These values contrast with those of Gosling and Wilkins (1981) who reported hybridisation estimates, based on allozyme markers, of 7-19% for nine exposed sites on Irish Atlantic coasts. The values are also higher than those reported (< 20%) by Gilg and Hilbish (2003) for sites in SW England. In several cases it has been difficult to accurately assess the number of settling spat with the hybrid genotype as authors, using the diagnostic nuclear markers *Glu*-5', reported allele but not genotype frequencies (Gilg and Hilbish 2000, Bierne et al. 2002a,b, Gilg and Hilbish 2003c, Hilbish et al. 2003). Incidentally, there are no published accounts, to date, of the use of the *Me15/16* marker in genetic analysis of hybrid zone mussels in western Europe.

The frequency of the *M. galloprovincialis* allele ranged between 0.53 - 0.80, depending on the sample. In SW England Gilg and Hilbish (2000b) analysed newly settled spat in a hybrid population and reported frequencies of *M. galloprovincialis Glu-5'* alleles of less than 0.12. However, on the west coast of France, Bierne et al. (2003) have reported frequencies of the compound *G* allele (averaged over three DNA markers, *Glu-5'*, *mac-1* and *Efbis*) very similar to the frequencies observed in the present study.

There are probably no barriers to gene flow between *M. edulis* and *M. galloprovincialis* so that mating is random among the three genotypes (Bierne et al. 2003a, Bierne et al. 2003b) It is reasonable to suggest that F_1 hybrids are breeding among themselves and backcrossing with *M. edulis* and *M. galloprovincialis*. If F_2 hybrids are also fertile then populations on exposed shores probably are highly introgressed. Using allozyme loci or a selection of nuclear DNA loci significant departures from HWE have been detected in populations from W. France and SW England (Skibinski et al. 1983, Coustau et al. 1991, Daguin et al. 2001, Bierne et al. 2003c, Gilg and Hilbish 2003c). Bierne et al. (2003b) suggest that, while hybridisation does occur on the west coast of France, isolation mechanisms (either

pre- or postzygotic) are strong and prevent complete mixing of the *M. edulis* and *M. galloprovincialis* gene pools. Gilg and Hilbish (2003) suggest that in SW England introgression is inhibited by a combination of local hydrography and directional selection (see later) What factor(s) may be responsible for the breakdown in reproductive isolation between the two taxa on Irish exposed shores is at present unclear. One reason might be that there are no pure populations of *M. galloprovincialis* in Ireland. At individual sites where *M. galloprovincialis* has been reported it is always intermixed with *M. edulis*, although the proportion of the latter is often small (this study, Gosling and Wilkins 1981, Doherty 2005).

The genetic composition of July primary settlers did not differ between tidal heights at either Ballynahown or Carraroe. Therefore, the results do not support the hypothesis of preferential primary settlement. It is likely in view of the proximity (~ 6 km) of the two shores, that the primary settlers originated from the same parental population, or that they comprised a mixture from several different locations that produced a similar genetic signature at each site. This settlement event may represent a synchronous spawning of a large proportion of mussels over the greater Galway Bay area (Fig 3 insert).

The results support those of Gilg and Hilbish (2000), who found no difference in *Glu-5'* allele frequencies in cohorts of newly settled mussels in SW England. But they are contradictory to those of Gosling and McGrath (1990) who found a significantly higher frequency of *M. galloprovincialis* alleles in midshore than in low shore spat. However, the experimental design of Gosling and McGrath (1990) did not allow them to distinguish between primary and secondary settlers or to evaluate the possibility that selection occurred in the five months that spat were grown on in the laboratory. In addition, they used allozyme loci *Odh* and *Est-D* that are only partially diagnostic for *M. galloprovincialis* and *M edulis* and do not allow the unambiguous identification of hybrids. However, there is also the possibility that the genetic composition of exposed shore mussels has changed in the intervening years. Supportive evidence comes from a reanalysis, using *Me15/16*, of sites surveyed by Gosling and Wilkins (1981), which showed that *M. edulis* has decreased in frequency, or was absent, on some sheltered and exposed Atlantic coast sites (Doherty 2005, Haniffy 2005).

In contrast to the results observed for the July settlement, significant differences were observed in the genetic composition of primary settlers in the October mid and low shore samples at both Ballynahown and Carraroe. These differences were due to

elevated frequencies of *M. galloprovincialis* on the low shore at Ballynahown, and to elevated frequencies of *M. edulis* and hybrid genotypes on the low shore at Carraroe; no differences were observed for mid shore comparisons between shores. This low shore variation is probably due to asynchronous spawning of adult mussels at this time of year and/or random variations in spat settlement.

When mid shore July and mid shore October samples were compared there was no evidence for temporal variability in the genetic composition of primary settlers at either site; only mid shore samples were compared in view of the anomalies observed in the October low shore samples. These results agree with those of Gilg and Hilbish (2000) who observed primary settling cohorts to be genetically homogeneous over time at three tidal heights at Whitsand Bay, SW England although their sampling was carried out only over the summer months.

Overall, there was little evidence from the genetic composition of primary settlers on pads to suggest that *M. edulis* and *M. galloprovincialis* adult populations spawn at different times in Galway Bay. Spawning asynchrony has been observed or inferred in hybrid populations (Gardner and Skibinski 1990, Secor et al. 2001, Bierne et al. 2003a) and is believed to be one of the factors that prevents complete introgression of *M. edulis* and *M. galloprovincialis* in SW England and NW France (Bierne et al. 2003b). It is possible that the genetic composition of spat on the settlement pads that were deployed, but not analysed, could be different to that observed for the 14 July and 24 October pads. To answer this it is important that analysis of all pads over the complete sampling period (May-October) be analysed. In addition, it would be worthwhile to investigate the reproductive cycle, and reproductive effort, of *M. edulis*, *M. galloprovincialis* and hybrids at Irish sites.

The lack of significant differences in median shell lengths of *M. edulis*, *M. galloprovincialis* and hybrids in pooled replicates from mid and low shore samples for the Ballynahown or Carraroe July or October samples, indicates that spat size is independent of genotype i.e., there is no evidence for differential growth among the three genotypes over the spat size range analysed. To date, there are no data on wild spat for comparison. However, if the results are compared with growth rates of hybrids and parent species from laboratory crosses the picture is confusing, with some studies (Beaumont et al. 1993) (Bierne et al. 2002b) indicating that hybrids have the faster growth and others finding no evidence for heterosis (Lubet et al. 1984,

Beaumont et al. 2004). Evidence for hybrid heterosis in the laboratory could be variable due to 'family' effects.

Exposed shore adults

There were no significant differences in the genetic structure of adult mussels from the mid and low shore areas of the two exposed shores. This differs from results published by other authors (Skibinski et al. 1983, Gardner and Skibinski 1988, Gosling and McGrath 1990) who reported greater frequencies of *M. galloprovincialis* alleles higher on the shore, due to the species being more resistant to wave exposure and dislodgement than *M. edulis* (Willis and Skibinski 1992). Adults on both exposed shores were genetically similar to each other and also to primary settlers at the *Me15/16* locus i.e., the composition of adults directly reflected that of newly settled spat. This does not support the prediction that allele frequencies at time of primary settlement are independent of tidal height, but that differences emerge later in adults. The difference in genotype and allele frequency between adults and primary settlers could be from selective mussel movement, mortality due to wave exposure, desiccation or predation at different tidal levels. These results agree with Gilg and Hilbish (2000), who found no difference in the genetic composition of primary settlers and juvenile mussels in SW England, and with those of Bierne et al. (2003a) for south Brittany, France, where larvae directly reflected the genetic structure of adult populations. In the present study no significant departures from HWE were observed in any of the adult samples, thus indicating that adults, like spat, are probably highly introgressed on these shores.

Although there were no significant genetic differences between primary settlers and adults at the *Me15/16* locus, the frequency of *M. galloprovincialis* increased from 30% in the smallest size classes to nearly 80% in the largest size class and there was a corresponding decrease (from 55% to 20%) in the frequency of hybrids, with no *M. edulis* observed in the larger size classes. This agrees with several studies in SW England (Gardner and Skibinski 1991, Willis and Skibinski 1992, Gardner et al. 1993) who showed that *M. galloprovincialis* had a faster growth rate than *M. edulis*, and hybrids had intermediate growth rates. The clear growth advantage of *M. galloprovincialis* is believed to be due to its greater ability to withstand dislodgement by wave action in exposed locations (Skibinski et al. 1983). This may explain the high percentage of *M. galloprovincialis* at exposed locations, and its high frequency among

older mussels at such sites. However, *M. galloprovincialis* is also present at high frequency on many sheltered shores, where strong wave action is not an important factor.

Sheltered shore adults

Allele and genotype frequencies in sheltered and exposed Ballynahown mussels were significantly different, due to the higher frequency of *M. edulis* and hybrid individuals on the sheltered shore. The higher incidence of *M. edulis* on sheltered shores has been well documented in the British Isles (Gosling and Wilkins 1981, Skibinski et al. 1983) and W. France (Bierne et al. 2003a). Hilbish *et al.* (2003) have suggested that mussels at sheltered sites are protected from the selective agent that produces strong associations between allele frequency and size in open-coast populations. However, the frequency of *M. edulis* on Irish sheltered shores varies considerably (0-100%), depending on location (Doherty 2005, Haniffy 2005). It seems likely, in view of the genetic similarity between Carraroe and Ballynahown exposed shore populations, and the relatively high frequency of *M. galloprovincialis* and hybrids on the Ballynahown sheltered shore, that these three shores which are situated within 6 km of each other, share a common larval source. It is not clear why there are higher frequencies of *M. edulis* on the sheltered shore unless *M. edulis* has some selective advantage in this environment. The presence of *M. edulis* in all size classes at this site, unlike the exposed site where *M. edulis* was only present in smaller size classes, supports this suggestion. Bierne et al. (2003a), however, found that in a hybrid zone in NW France, *M. galloprovincialis* and hybrids seemed unable to settle in a sheltered area, resulting in greater numbers of *M. edulis*, while on a nearby exposed shore (< 200 m) all three genotypes were present. This does not appear to be the case for Irish populations. There is no doubt that the genetic structure of hybrid populations is remarkably complex and that there is no consensus on population structure between one geographic region and the next within the NW Europe hybrid zone.

Adult/spat relationship

The level of hybridisation at Ballynahown and Carraroe indicates that there is no hydrographical or geographical barrier to gamete mixing between populations of *M. galloprovincialis* and *M. edulis*. A detailed study of the hydrodynamics affecting Galway Bay is needed to evaluate the origin of larvae settling at Ballynahown and

Carraroe. In the hybrid zone in SW England local hydrography prevents the entry of *M. edulis* and *M. galloprovincialis* larvae into the hybrid zone, although hybrid populations can export larvae into both parental populations; larvae settling within the hybrid zone most likely originate within the zone (Gilg and Hilbish 2003a). Within the zone, selection in favour *M. edulis* alleles at the larval stage is effectively balanced by directional selection in favour of *M. galloprovincialis* alleles in adults.

Maintenance of mussel hybrid zones

The relative roles of endogenous or exogenous selection in the maintenance of the mussel hybrid zone in NW Europe is a topic for active debate in recent years (Hilbish et al. 2002, Gilg and Hilbish 2003a, b). Widespread hybridisation, and introgression in some areas of the hybrid zone, together with little evidence of hybrid inferiority from laboratory crosses, suggests that endogenous selection is not a major factor in the maintenance of the hybrid zone (Gilg and Hilbish 2003a). Attention has therefore focussed more and more on the identification of environmental variables that might affect the relative fitness of hybrid and parental genotypes.

In SW England the hybrid zone is situated between pure *M. galloprovincialis* on open coast sites, and pure *M. edulis* populations in sheltered estuarine sites. Within the zone, selection in favour of *M. edulis* alleles at the larval stage is effectively balanced by directional selection in favour of *M. galloprovincialis* alleles in adults, due to differential dislodgement by waves. In NW France there is also partial ecological segregation between adults of *M. edulis* (occupying sheltered habitats with freshwater influence) and *M. galloprovincilais* (predominantly on oceanic exposed sites which is responsible for the small scale patchiness of this mosaic hybrid zone (Bierne et al. 2003a). These authors suggest that larvae actively select their habitat depending on their genotype; this has the advantage of sparing the cost of local adaptation. In addition, they have found that *M. edulis* settles earlier (March-April), while *M. galloprovincialis* and hybrids settle later (May-June). Hybrid larvae also settle at exposed locations leaving *M. edulis* alone in sheltered environments. Bierne et al. (2003a) contend, however, that other factors besides ecological segregation are limiting interbreeding.

There are features of Irish hybrid populations that differentiate them from the SW England and NW France situations: (1) no evidence of pre-settlement selection in larvae; (2) no evidence for asynchronous settlement; and (3) lack of distinct

partitioning of the taxa between exposed and sheltered locations. There are also some similarities: higher frequencies of the *M. galloprovincialis* allele at exposed locations, and evidence to suggest that *M. galloprovincialis* has a selective advantage in that environment.

An intriguing question is whether *M. edulis* and *M. galloprovincialis* are in the process of complete introgression, since there is little evidence for any reproductive barriers between them in the west of Ireland. Another question is whether the frequency of *M. galloprovincilais* in increasing, not just on Irish exposed sites, but more strikingly, on sheltered shores. A supposed increase might be explained by the increase in sea surface temperature (0.6°C) in the North Atlantic over the last century (Jones et al. 2001). In the NE Atlantic cold-water species of phytoplankton and zooplankton have retreated northwards and warm-water species have moved after them; the changes have been so dramatic in the last 20 years that marine scientists are referring to this as 'a regime shift' and one clearly forced by global warming (Michael Viney *Irish Times* May 21 2005). Other plausible explanations for an increase could be movement of mussel seed for aquaculture. Exposed shore mussels, which have a high frequency of *M. galloprovincialis* and hybrids, are the primary source for seeding ropes in sheltered sites on Atlantic coasts. To test if *M. galloprovincialis* has increased in frequency over the past 25-30 years would involve re-sampling a selection of the shores analysed by Gosling and Wilkins (1981), using the same partially diagnostic allozyme markers that they used in the study. Since tissue samples were not archived the re-analysis could not employ *Me15/16*. Despite the lower diagnostic power of the allozyme markers, it should still be possible to determine whether or not there has been a change in the frequency of *M. galloprovincialis* at these sites.

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