

PATTERNS OF LARVAL DISPERSAL AND THEIR EFFECT ON THE MAINTENANCE OF A BLUE MUSSEL HYBRID ZONE IN SOUTHWESTERN ENGLAND

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Abstract.—The blue mussels *Mytilus edulis* and *M. galloprovincialis* hybridize in southwestern England. Within this hybrid zone environmentally based directional selection favors individuals with alleles specific to *M. galloprovincialis*. What forces are countering this directional selection and allowing for the maintenance of a stable hybrid population are unknown. We used both the genetics of recently settled larvae and a fine-scale model of the physical oceanography of the region to determine the patterns of larval dispersal throughout the hybrid zone and the bordering parental populations. Evidence from both the model and the genetics suggests that the hybrid zone lies between two barriers to dispersal. Start Point separates the *M. edulis* population from the hybrid zone and allows minimal dispersal from the hybrid zone into the *M. edulis* population, but none in the other direction. Likewise, the *M. galloprovincialis* populations along the northern coast of Cornwall regularly receive immigrating larvae from the hybrid zone, but larvae from the *M. galloprovincialis* population do not enter the hybrid zone. However, larvae settling at hybrid zone sites have high frequencies of alleles specific to *M. edulis*, suggesting that reproductive barriers, selection in the larval stage, or gene flow from an undetermined source is effectively balancing the directional selection observed in the adults.

Key words.—Dispersal, hybridization, hybrid zone, larvae, *Mytilus*, selection.

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Hybrid zones are locations where two diagnostically distinct populations meet and interbreed producing offspring of mixed ancestry (Harrison 1993; Arnold 1997). Hybridization has several potential evolutionary outcomes, including: completion of reproductive isolation, merging of the two species into one, displacement of one parental species, or the formation of a third, evolutionarily distinct, “hybrid” lineage (Arnold 1997). The dynamic nature of hybrid zones makes them excellent locations for studying a multitude of patterns and processes involved in speciation. Although hybrid zones tend to be a conflux of a number of evolutionary processes, in many cases hybrid zones can remain locally stable over long periods of time on a human scale. Therefore, it is important to understand how such stability can be maintained when there are many potentially conflicting processes at work.

Most models of hybrid zones assume that selection against hybrids occurs within the zone and hybrid genotypes are ultimately replaced by dispersal from both parental populations into the zone of contact. Tension zone models assume that the selection against hybrids is intrinsic, due to the incompatibility of the two differentiated genomes (Barton and Hewitt 1985, 1989). On the other hand, mosaic hybrid zone models assume that hybrids have lower fitness in either parental habitat (Howard 1982, 1986; Harrison 1986, 1990). However, the patterns of selection in many hybrid zones do not follow the predictions of either of the above models (Arnold 1997). In some cases, hybrids are actually favored by natural selection in the zone of contact (e.g., hybrid superiority, Moore 1977), whereas in others the relative fitness of F_1 , F_2 , or backcross hybrid genotypes may be very different with some hybrid genotypes being as fit as parental genotypes (Arnold 1997). Regardless of the mechanism of formation, the stability of a hybrid zone is at least partially determined by the patterns of dispersal throughout the zone of contact and the type of selection occurring in the region.

In this study we focused on a hybrid zone between two species of marine mussels, *Mytilus edulis* and *M. galloprovincialis*. This hybrid zone is a mixture of patches containing pure parental populations of each species and areas where individuals of mixed ancestry are common. Hybrid populations are known to exist along the western coast of France (Coustau et al. 1991; Daguin et al. 2001), the west coast of Ireland (Gosling and McGrath 1990; Skibinski et al. 1983), and southwest England (Skibinski et al. 1983; Skibinski and Roderick 1991; Hilbish et al. 2002). Environmentally dependent selection has been documented in some of these hybrid populations and suggested in others (Gardner et al. 1993; Wilhelm and Hilbish 1998). The presence of selection, along with the unique opportunity hybrid populations afford to measure dispersal in marine systems (Gilg and Hilbish 2003a), make these hybrid populations intriguing areas to study the evolutionary dynamics involved in maintaining stability in marine hybrid zones.

The most extensively studied of these hybrid populations is located in southwest England along the coasts of Devon and Cornwall (Skibinski et al. 1983; Gardner and Skibinski 1988; Gardner et al. 1993; Hilbish et al. 1994; Wilhelm and Hilbish 1998; Hilbish et al. 2002). Part of this region is inhabited by pure populations of *M. edulis* (the southern coast of Devon east of Start Point) while another portion is inhabited by relatively pure populations of *M. galloprovincialis* that have frequencies of alleles specific to *M. edulis* of <0.1 (northern Cornwall, northwest of St. Ives) (Fig. 1). Between these regions lie populations of mussels of mixed ancestry that extend nearly 200 km (Fig. 1, Hilbish et al. 2002). Mussel populations within this region form a “hybrid swarm” containing intermediate frequencies of *M. edulis* and *M. galloprovincialis*-specific alleles (0.1–0.9) and an array of genotypes, including those resulting from advanced levels of introgression (Skibinski et al. 1983; Gardner 1994; Hilbish et

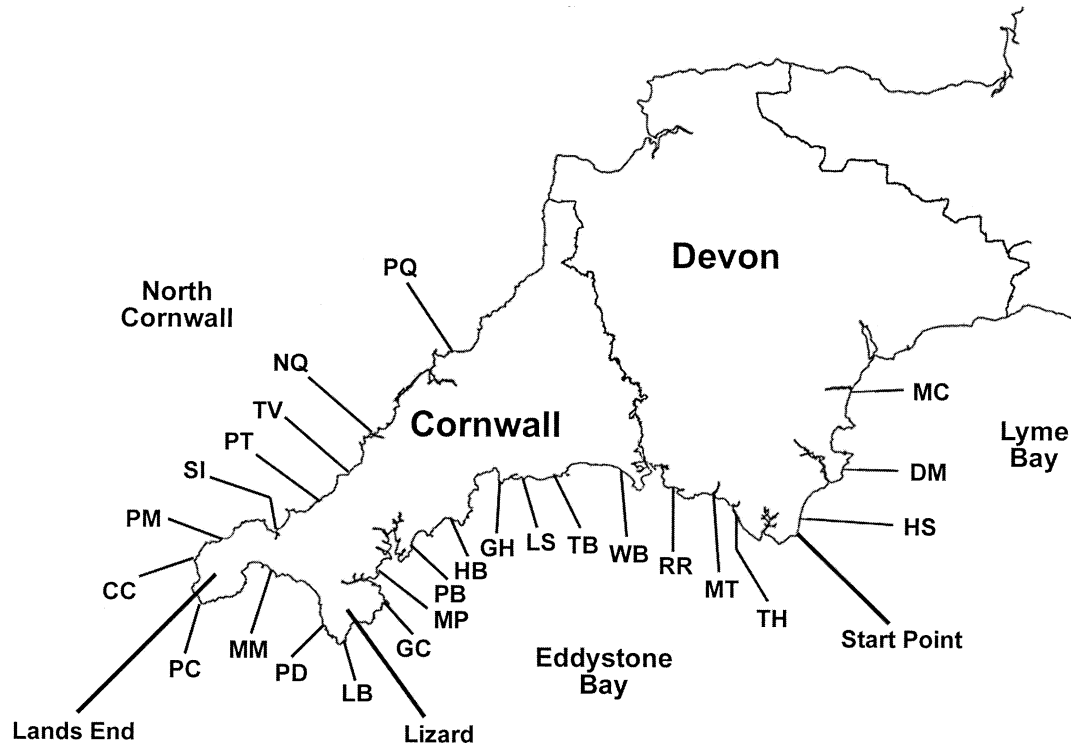


FIG. 1. A map of southwestern England showing sites where mussel spat were collected and other key locations. Populations of pure *Mytilus edulis* are found at locations east of Start Point. Relatively pure populations of *M. galloprovincialis* inhabit the northern coast of Cornwall northeast of St. Ives. Hybrid populations are found at all locations between Start Point and St. Ives.

al. 1994; Wilhelm and Hilbish 1998). Additionally, these hybrid populations typically show a pattern of directional selection in which mussels with *M. galloprovincialis*-like genotypes are favored over those with *M. edulis*-like genotypes and mussels with intermediate genotypes have intermediate fitness (Gardner et al. 1993; Wilhelm and Hilbish 1998). Adult mussels in the smallest size classes (youngest) have high frequencies (>0.8) of alleles specific to *M. edulis*. With increasing age and size the frequency of *M. edulis* alleles declines until the population becomes dominated by mussels with a high frequency of *M. galloprovincialis* alleles (Skibinski 1983; Gardner and Skibinski 1988; Gardner et al. 1993; Wilhelm and Hilbish 1998; Hilbish et al. 2002). Selection is environmentally dependent (Wilhelm and Hilbish 1998) and varies in strength among locations (Hilbish et al. 2002). The patterns of selection within this hybrid zone have been studied for the last 20 years and during that time the location of the hybrid zone and the size/allele frequency structure of the populations have remained remarkably stable (Skibinski et al. 1983; Gardner 1994; Hilbish et al. 1994; Hilbish et al. 2002). The stability of this system leads to the questions of how this hybrid zone is being maintained when selection should be leading to the displacement of *M. edulis* from the region?

Previous studies have suggested that selective elimination of *M. edulis* alleles from the hybrid zone may be balanced by preferential migration of *M. edulis* into the zone (Edwards and Skibinski 1987; Wilhelm and Hilbish 1998). Wilhelm and Hilbish (1998) suggested that over 80% of the larvae settling at Whitsand Bay, a location within the hybrid zone,

must have originated in a pure *M. edulis* population to produce the multilocus genotype frequencies observed in the adults at that site. Although Whitsand Bay is only 50 km from Start Point and the nearest pure *M. edulis* population, the rest of the hybrid zone populations show similar patterns, including sites over 150 km from Start Point (Hilbish et al. 2002). Therefore, similar proportions of larvae would have to immigrate from pure populations of *M. edulis* throughout the hybrid zone to maintain its stability, requiring the movement of massive numbers of larvae from east of Start Point into the hybrid zone.

Mussel larvae, like those of many marine invertebrates, develop in the plankton for several weeks to more than two months depending on the water temperature, salinity, and food availability (Bayne 1965). During their larval stage they are transported as passive particles with the ocean currents giving them the potential for dispersal over long distances. Direct measurement of dispersal, however, is difficult (Levin 1990; Nathan 2001) and it is relatively unknown how far mussel larvae tend to disperse (but see McQuaid and Phillips 2000; and Gilg and Hilbish 2003a). In this study we will address the hypothesis that asymmetrical larval dispersal is countering the selection favoring *M. galloprovincialis*-like genotypes in the mussel hybrid zone in southwestern England. The larval dispersal patterns were investigated from two perspectives. First, we assessed the population genetics of recently settled mussel larvae (spat) from several hybrid zone locations. By comparing the genetics of the spat to the expected allele frequencies calculated from the local adult populations, we can determine if the larvae recruit from local

populations or are immigrants from genetically distinct populations. Second, we used a model of coastal circulation to determine the patterns of water movement throughout the region. This will allow us to determine whether genetic patterns of newly recruited mussels correspond well to the local physical oceanography. These results allow us to determine the contribution of dispersal to the maintenance of this hybrid zone and each of the parental populations.

METHODS

Adult Population Structure

To determine whether the mussel larvae settling at a location are a product of the adults in the area, we first estimated the background allele frequency that each mussel population produces. The *M. edulis* population located east of Start Point is characterized as having 100% *M. edulis* specific alleles at the marker *Glu-5'* (Hilbish et al. 1994, 2002). The *M. galloprovincialis* populations in north Cornwall contain about 5% *M. edulis*-specific alleles (Hilbish et al. 1994, 2002). These frequencies are the same for all size classes giving a reliable standard by which to compare allele frequencies of settling larvae. In contrast, the hybrid zone is not so straightforward. The frequency of species-specific alleles is strongly age and size dependent in mussel populations within the hybrid zone. Likewise fecundity is strongly dependent upon size. Therefore, any estimation of a hybrid zone allele frequency "signature" must take the size structure, the size-specific allele frequency, and the relative fecundity of various size classes into account.

The size structure of the adult populations at several hybrid zone sites were investigated to estimate the population genetics of the larvae that would be produced by the hybrid zone mussel populations. This expected allele frequency was estimated by the following equation:

$$F_E = \sum (F_i R_i P_i),$$

where F_i is the frequency of a diagnostic allele in a given size class, R_i is the relative fecundity of that size class, and P_i is the proportion of the population that is in that size class. The equation is solved for both alleles and, therefore, calculates the relative proportions of both "*M. edulis*" and "*M. galloprovincialis*" gametes, giving an allele frequency estimate for the population. Because allele frequencies, fecundity, and relative numbers of individuals all differ among size classes, we obtained estimates of all three factors for each size class.

Adult mussels were collected from eight hybrid zone sites that have large adult mussel populations, including: Whitsand Bay (WB), Lansallos (LS), Hemmick Beach (HB), Pendower Beach (PB), Maenporth (MP), Poldu Cove (PD), St. Michael's Mount (MM), and Porthcurno (PC) in 1998 (Fig. 1). At each location, four 0.25 m² plots were scraped of all mussels (except at WB where eight plots were scraped). The mussels were placed in labeled Whirl-Paks (NASCO, Fort Atkinson, WI) for transport. Mussels from each of the plots were then sorted into 5-mm size classes based on shell length and the proportion of mussels in each size class was determined. Allele frequencies for the *Glu-5'* gene for each size class were taken from Hilbish et al. (2002). The fecundity

of mussels in each size class was estimated from fecundity curves in Gardner and Skibinski (1990) and assumed to be the same for all genotypes. Both the allele frequencies and the relative fecundity estimates are given in Appendix A.

Spat Collection Sites

Recently settled mussel larvae (spat) were collected from a total of 18 sites in 1996, and from 20 sites in both 1998 and 1999. Collections in all three years were made from three sites within the *M. edulis* parental populations east of Start Point, including: Maidencombe (MC), Dartmouth Castle (DM), and Hallsands (HS) (Fig. 1). Spat collections were made from four sites within the *M. galloprovincialis* populations along the northern coast of Cornwall in 1998 and 1999, including Portreath (PT), Trevaunance (TV), Newquay (NQ), and Port Quin (PQ). Collections were made from 15 hybrid zone sites in 1996 and from 13 hybrid zone sites in 1998 and 1999. Sites were approximately 15 km apart and were chosen based on the presence of adult mussels and accessibility. Collection sites in 1996 included (from east to west): Thurleston (TH), Renny Rock (RR), Whitsand Bay (WB), Talland Bay (TB), Gribbon Head (GH), Hemmick Beach (HB), Pendower Beach (PB), Gillen Cove (GC), Lizard Lifeboat Station (LB), Poldu Cove (PD), St. Michael's Mount (MM), Porthcurno (PC), Cape Cornwall (CC), Porthmeor (PM), and St. Ives (SI). In 1998 and 1999 several hybrid zone sites were changed, decreasing the overall number of hybrid zone sites in an effort to increase the efficiency of collections and account for the addition of sites in north Cornwall. TH and RR were replaced by Mothecombe (MT), TB and GH were replaced by Lansallos (LS), and GC was replaced by Maenporth (MP). Most of the collection sites are in small embayments along the open coast with very little freshwater influence. The sites are considered representative of the mosaic nature of the hybrid zone and include sites that have shown varying degrees of selection favoring *M. edulis* in the adult populations (Hilbish et al. 2002).

All collections were made at low tide during spring tides (at approximately two week intervals) from June through August in 1996, from mid-May through August with one collection in mid-October in 1998, and from mid-May through mid-September with one collection in October in 1999. Spat were collected using both artificial and natural substrates. ECKO brand scouring pads and pieces of burlap sack (approximately 20-cm square) that were folded in thirds in each direction were screwed into plastic inserts placed into holes drilled with a gasoline-powered roto-hammer in intertidal rocks. Whenever possible, artificial substrates were placed in the midst of adult mussel beds. Four to six artificial substrates were placed at each collection site. In 1996 ECKO brand scouring pads were used exclusively, whereas in 1998 and 1999 pads made from burlap were the predominant artificial substrate as they seemed to be better collectors of spat and were less noticeable to potential vandals. During each collection, artificial substrates were removed from the inserts and placed in Whirl-Paks. New artificial substrates were placed in the same locations. Although mussel larvae would settle on the artificial substrates, most of the spat collected came from algae within the mussel beds. *Ceramium rubrum*

was the primary alga collected, but other species were often included in the collections. Algae were collected from as many microsites as possible at each collection site and placed in a labeled Whirl-Pak. These microsites typically included both exposed and relatively protected areas. Upon returning to the laboratory, spat were physically removed from the substrates under a dissecting microscope and placed in 95% ethanol for genetic analysis.

Genetic Analysis

Each individual spat was measured for shell length under a dissecting microscope and then whole genomic DNA was extracted as described in Gilg and Hilbish (2000). Each spat was then genotyped by polymerase chain reaction (PCR) of the 5' end of the *Glu* gene as described by Rawson et al. (1996), except using primers Me-15 and Me-16 developed by Inoue et al. (1997). The *Glu-5'* gene diagnostically distinguishes alleles specific to *M. edulis* and *M. galloprovincialis* by a 55 bp insertion/deletion polymorphism. Therefore, the PCR products can be used directly to distinguish alleles from each species without the use of restriction enzymes or sequencing techniques. Single locus genotype and allele frequencies were then calculated for spat settling at each collection site and each collection date. The use of a single locus allowed analysis of large numbers of spat and because there is strong linkage disequilibria among genetic markers that distinguish *M. edulis* and *M. galloprovincialis* (Wilhelm and Hilbish 1998; Rawson et al. 1996) the *Glu-5'* marker provides a strong indication of the genetic origins of a cohort of spat.

Hydrographic Model

A 2D model of the north Atlantic was used to determine circulation patterns around southwestern England for the settlement periods contained in this study. This model, developed by Robin Pingree at the Marine Biological Association of the United Kingdom, Plymouth, England (Pingree and Griffiths 1980; Sinha and Pingree 1997), calculates tidal currents by solving the depth integrated hydrodynamic equations and then incorporates wind stresses as a surface boundary condition. Tidal currents and wind stresses from specific time periods when larvae were predicted to be in the plankton were used to calculate a vector of water movement within 5-km square plots of a numerical grid. The grid contains 50,000 such plots and encompasses most of the northern Atlantic. This vector of water movement can then be used to trace the dispersal of neutral particles over a given time period. The model was run for specific settlement periods observed from the spat collections.

Periods of larval settlement were determined by the size frequency structure of the collected spat. Mussel larvae tend to settle at 250- μ m shell length (Bayne 1964). Based on larval growth rates (Bayne 1965; Gilg and Hilbish 2000), spat that are less than 500- μ m shell length most certainly settled in the two weeks since the previous collection and can be considered new settlers, or primary spat. Therefore, collections that contained large numbers of spat between 250- μ m and 500- μ m shell length were considered "settlement dates." For each settlement date, three estimates of developmental time were calculated based on the water temperature the mussel

larvae encountered prior to settlement. Weekly night time mean sea surface temperatures were acquired from the National Environment Research Council, Dundee Satellite Receiving Station, Dundee, Scotland and processed by the Plymouth Marine Laboratory Remote Sensing Group, Plymouth, England. These temperatures were then used to calculate a minimum required developmental time based on larval growth rates determined by Bayne (1965). The minimum developmental time was considered our "short" estimate. Adding the maximum metamorphic delay (Bayne 1965) to the "short" estimate gave the "long" estimate, and the mean of the two estimates was considered the "mid" estimate. These estimates ranged from a maximum of 83 days early in the season to a minimum of 27 days by late summer.

Particles were released into the model on a specific birth date (determined by the developmental estimates above) and then followed until the settlement date. These particles were simultaneously released into the model from 20 locations throughout southwestern England. The 20 collection sites from 1998 and 1999 (13 within the hybrid zone, three *M. edulis* sites, and four *M. galloprovincialis* sites) were used as the release points for the model. Daily wind stresses for the model were obtained from the British Atmospheric Data Centre, Oxfordshire, England (European Centre for Medium-Range Weather Forecasts, Reading, England) for the three years of the study. A trace of the entire path of the particle was plotted onto a map and could then be used to obtain net dispersal distance, the direction of movement, and the population in which the particle potentially settled.

Statistical Tests

All comparisons of observed allele frequencies across time and space were conducted using an RxC G-Test of independence as described by Sokal and Rohlf (1981). To control for a bias of the test, in cases where samples only contained one of the two possible alleles, a value of one was inserted for the absent allele. Chi-square tests for goodness of fit were used to compare observed to expected allele frequencies while an exact test was used to test for deviations of observed genotype frequencies from Hardy-Weinberg expectations because the exact test is more reliable when expected numbers of some genotypes are <5 .

RESULTS

Adult Allele Frequency Estimates

Size structures of adult mussel populations at hybrid zone sites were highly variable (Fig. 2). At most sample sites, the smallest size classes were the most abundant and the maximum size was about 40 to 45 mm in shell length (SL). Two sites (SI and MP), however, had mussels that were >50 mm SL and three sites (MM, PD, and SI) did not show an abundance of animals in the smallest size classes. Relative fecundity estimates for each size class and allele frequencies for each size class at each site are given in Appendix A. The differences in size structure and the differences in allele frequency patterns of the adults at these sites resulted in different estimates of the "background" allele frequency of the hybrid zone (Table 1). Individual *M. edulis* specific allele frequency

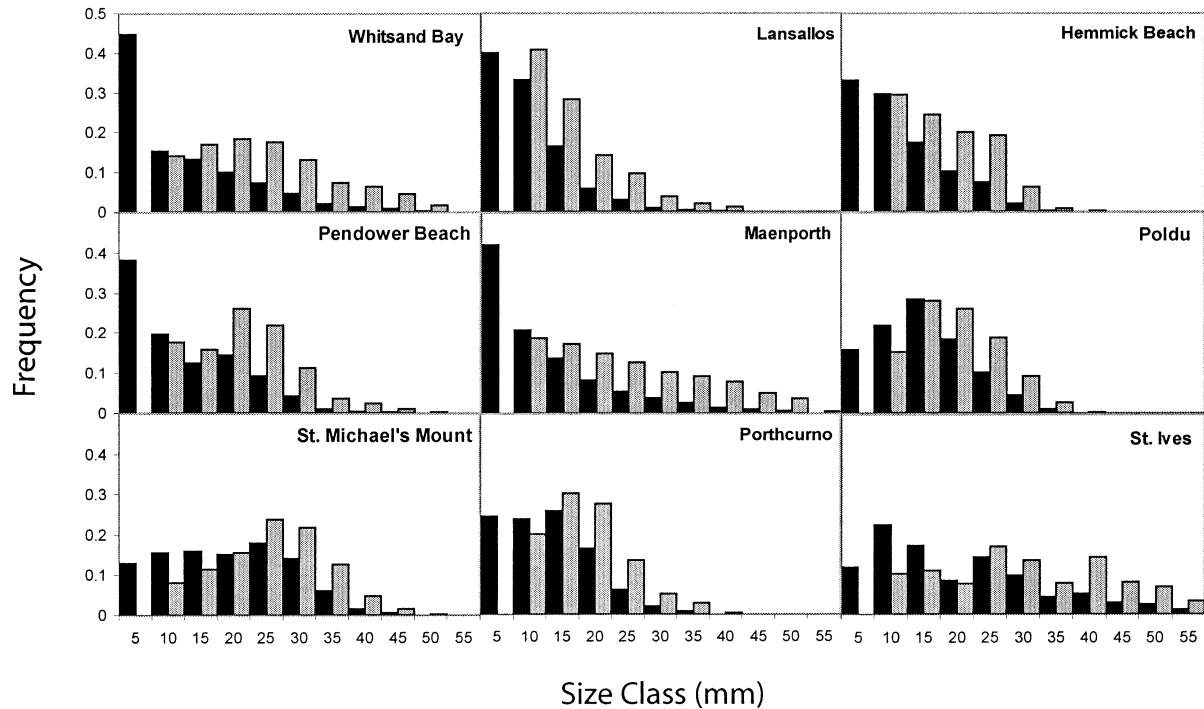


FIG. 2. Size-frequency histograms of adult hybrid zone populations (black bars) and relative contribution of each size class to the gene pool (gray bars).

estimates within a site ranged from a low of 0.42 at PB (plot 4) to a high of 0.88 at MP (plot 3). We then pooled the number of individuals in each size class across all plots into a single estimate for each site. The allele frequency estimates of these pooled values ranged from a low of 0.51 at PB to a high of 0.82 at MM. Because the potential of long range dispersal is high in mussels, spat settling at a site are not likely to be derived from the same site (Gilg and Hilbish 2003a). Instead, the mean value of the pooled totals from each site was used to determine an estimate for the larvae the entire adult population of the hybrid zone would produce. Using this calculation, the estimated frequency of *M. edulis* specific alleles in larvae produced by the hybrid zone is 0.71 with a standard deviation of ± 0.11 .

Larval Settlement

Some level of either primary or secondary settlement was observed during every collection period. We define primary spat as being $\leq 500\text{-}\mu\text{m}$ shell length since they most likely settled within the two weeks since the previous settlement (Bayne 1965; Gilg and Hilbish 2000). Secondary spat are those individuals that are $> 500\text{-}\mu\text{m}$ shell length. Not all sites, however, received spat. Out of 18 sites in 1996, both primary and secondary spat were collected at only 13. Of 20 possible sites, primary spat were collected at 13 sites in 1998 and 11 in 1999. Including collections with secondary spat brings the total to 16 sites for both 1998 and 1999. Typically, the same sites received settlement reliably in both years. Settlement was heaviest at sites in Lyme Bay (MC, DM, HS), Eddystone Bay (WB, LS, HB, PB, MP), and in north Cornwall (PT, TV, NQ, PQ). Sites around the Lizard and Lands End generally received few or no spat with a few exceptions (PD, MM,

PC). Settlement, however, did not occur throughout the range of collection sites at the same time (Table 2, Fig. 3). This is best illustrated in the collections from 1998 and 1999 since they contain samples from all three regions and for a longer span of time. In most cases, larval settlement was observed earliest at the sites located east of Start Point in the *M. edulis* population. These sites typically received larvae from May through July. Sites within the *M. galloprovincialis* population typically did not receive spat until August. The pattern of settlement within the hybrid zone tended to be intermediate to that of the parental populations; with settlement occurring from May through August (Table 2, Fig. 3).

Spat Genetics

Despite the differences in the timing of settlement among the populations, very little temporal variation in allele frequency was observed in any of the collection years. In 1996, significant temporal variation in allele frequency was observed only among the five cohorts of primary spat collected at WB ($G = 13.8$, $df = 4$, $P < 0.05$) (Table 2). The last cohort at WB that year had a significantly higher frequency of *M. edulis* specific alleles than all others (removal of final cohort from G -test results in: $G = 7.3$, $df = 4$, ns). In 1998, two hybrid zone sites (WB and MP) and one *M. galloprovincialis* site in North Cornwall (TV) showed significant temporal variation in the allele frequency of primary spat (WB: $G = 23.1$, $df = 5$; MP: $G = 20.3$, $df = 5$; TV: $G = 14.5$, $df = 2$; $P < 0.05$ in all cases). The first two settlements at WB had significantly lower *M. edulis* allele frequencies than the other four ($G = 1.6$, $df = 3$, ns after removal of first two cohorts), while the 23 July and 9 August settlements at MP had significantly lower *M. edulis* allele frequencies than the

TABLE 1. Estimates of *Mytilus edulis* specific allele frequencies from the adult populations of various hybrid zone sites. Estimates are made incorporating the proportion of individuals in each reproductive size class, the observed allele frequency of each size class at each site, and the relative fecundity of individuals in each size class.

Sample	WB1	WB2	LS	HB	PB	MP	PD	MM	PC
Plot 1 estimate	0.614	0.650	0.689	0.873	0.513	0.784	0.792	0.803	0.706
Plot 2 estimate	0.534	0.562	0.672	0.817	0.520	0.785	0.662	0.827	0.686
Plot 3 estimate	0.728	0.538	0.692	0.832	0.541	0.876	0.742	0.839	0.696
Plot 4 estimate	0.667	0.614	0.628	0.776	0.419	0.801	0.706	0.832	0.603
Pooled estimate	0.624	0.624	0.665	0.824	0.507	0.806	0.729	0.823	0.668
Mean/all sites	0.706 ± 0.112								

other four settlements ($G = 5.0$, $df = 3$, ns after removal of 23 July and 9 August). The final settlement at TV in 1998 had a significantly lower *M. edulis* allele frequency than the two previous settlements ($G = 1.2$, $df = 1$, ns after removal of final cohort). In 1999, only WB and MP showed evidence of significant temporal variation in the allele frequencies of primary spat (WB: $G = 38.2$, $df = 6$; MP: $G = 11.95$, $df = 5$; $P < 0.05$ in both cases). At WB, the first settlement again had the lowest *M. edulis* allele frequency ($G = 8.32$, $df = 5$, ns after first cohort removed), while at MP the third and sixth settlements had significantly higher *M. edulis* allele fre-

quencies than the other collections ($G = 1.8$, $df = 3$, ns if cohorts 3 and 6 removed).

Allele frequencies of primary and secondary spat typically did not differ and were consequently pooled for further analysis (Gilg and Hilbish 2003b). When primary and secondary spat from all collection dates are pooled several trends are apparent. Spat collected from sites east of Start Point generally have *M. edulis* allele frequencies >0.99 (Table 3, Fig. 4). The spat allele frequencies from the three *M. edulis* sites do not differ significantly from each other (1996: $G = 3.1$; 1998: $G = 0.6$; 1999: $G = 1.8$, $df = 2$ in all cases) and do not differ significantly from the expected *M. edulis* allele frequency of 1.00 (Table 3, Fig. 4). In most cases, however, alleles specific to *M. galloprovincialis* are found in some spat settling at the three sites east of Start Point, albeit in very low numbers.

Spat settling within the hybrid zone typically have *M. edulis* allele frequencies that are extremely elevated in comparison to the predicted allele frequency based on the adult populations (Table 3, Fig. 4). In 26 of 27 cases, the *M. edulis* allele frequency of the spat was significantly higher than the mean expected allele frequency. In fact, in 20 of 27 cases, the *M. edulis* allele frequency of the spat was significantly higher than one standard deviation above the expected mean (Table 3, Fig. 5). Sites within the hybrid zone also show some evidence of spatial heterogeneity in spat allele frequencies. In general, sites from Eddystone Bay (RR, WB, TB, LS, GH, HB, PB, GC, MP) tend to receive spat with relatively higher frequencies of *M. edulis* alleles than do sites around the Lizard and Lands End (PD, MM, PC). The only site that did not show a significantly higher *M. edulis* allele frequency than expected was PD in 1999, and six of the seven cases where the *M. edulis* allele frequency was not significantly higher than one standard deviation above the mean expected value were from sites around the Lizard and Lands End. In 1996 and 1999 this trend seems to be due to a gradual decrease in *M. edulis* allele frequency with distance from Start Point. In 1998, on the other hand, the change is rather abrupt, with more similarity in allele frequency among sites within Eddystone Bay and among sites around the Lizard and Lands End, but not between them. Although they tended to be similar, allele frequencies of spat collected at sites within Eddystone Bay differed significantly from one another (1996: $G = 24.7$, $df = 6$; 1998: $G = 37.3$, $df = 4$; 1999: $G = 189.1$, $df = 5$). Conversely, allele frequencies of spat collected from sites around the Lizard and Lands End did not differ significantly in either 1996 ($G = 1.3$, $df = 2$) or 1998 ($G = 1.3$,

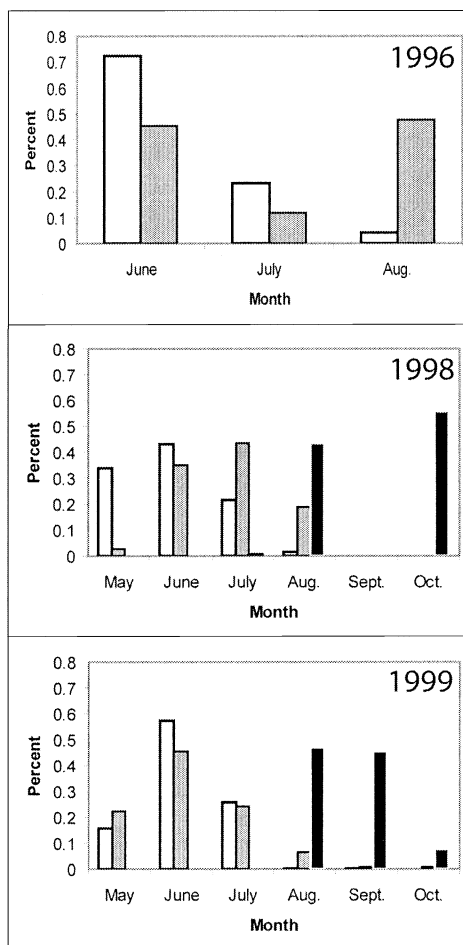


FIG. 3. The proportion of primary spat collected at sites with either *Mytilus edulis* (white bars), *M. galloprovincialis* (black bars), or hybrid (gray bars) adult populations during each month of collection.

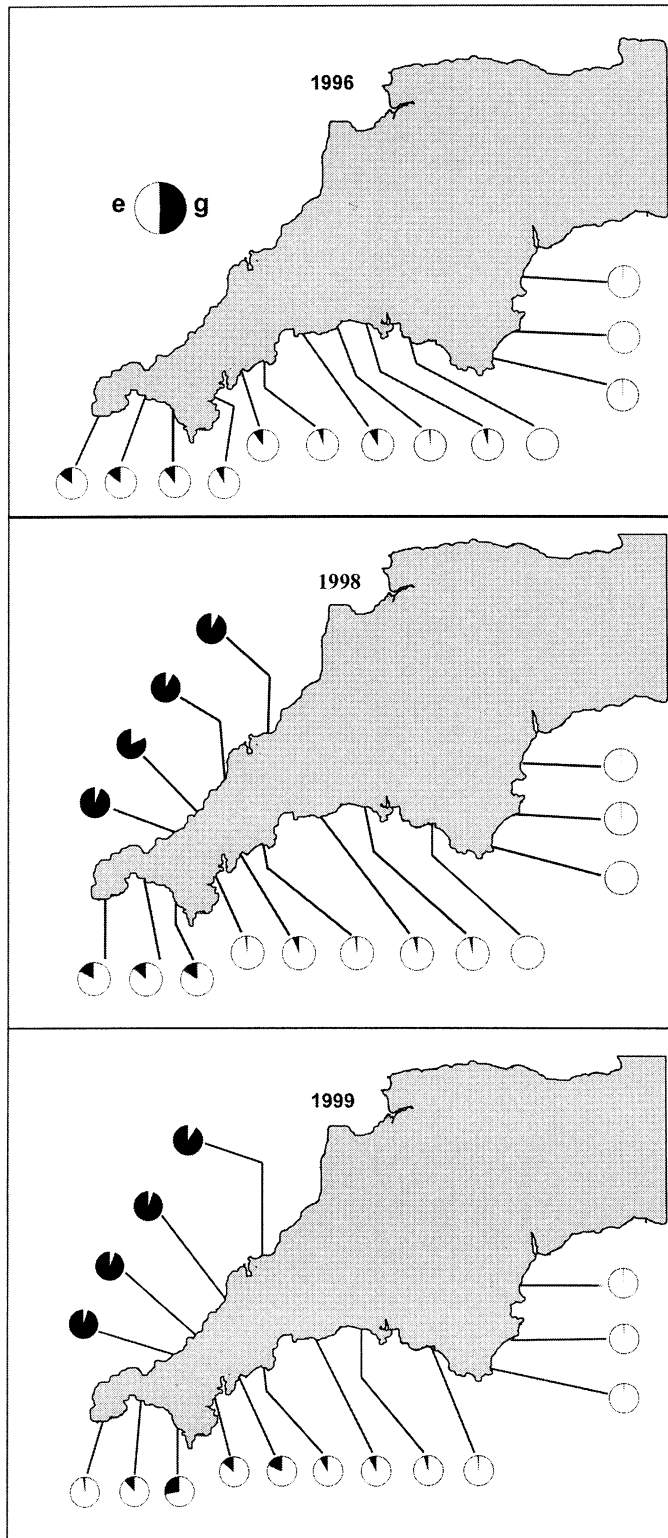


FIG. 4. *Glu-5'* allele frequencies of pooled primary and secondary spat collected throughout southwestern England. White and black colors represent *Mytilus edulis*-specific (e = white) and *M. galloprovincialis*-specific (g = black) alleles, respectively.

df = 2), but did differ significantly in 1999 ($G = 16.4$, df = 2).

Allele frequencies of spat collected at sites within the *M. galloprovincialis* populations along the northern coast of Cornwall frequently differed significantly from the expected *M. edulis* allele frequency of 0.05 (Table 3). Primary spat settling on 9 August 1998 had significantly higher *M. edulis* allele frequencies than expected at three of the four sites (PT: $\chi^2 = 5.3$ ($P < 0.05$); TV: $\chi^2 = 36.1$ ($P < 0.001$); NQ: $\chi^2 = 0.01$ ($P > 0.05$); PQ: $\chi^2 = 5.5$ ($P < 0.05$); df = 1 in all cases) (Table 2). The 25 August 1998 and the 29 August 1999 settlements at TV also had significantly higher *M. edulis* allele frequencies than expectations based on the adult populations (1998: $\chi^2 = 10.5$ ($P < 0.05$); 1999: $\chi^2 = 7.1$ ($P < 0.05$)) (Table 2). However, when primary and secondary spat from all collection dates are pooled, the allele frequencies do not typically differ from the expected value (Table 3, Fig. 4). The only exception was at TV in 1998, where the *M. edulis* allele frequency of pooled spat was significantly higher than the expected value.

Allele frequencies of spat also differed among years, especially within the hybrid zone (Table 3). Of eight hybrid zone sites that had a minimum of 10 spat collected from them in each of at least two years, seven showed significant variation in allele frequencies. In general, spat collected in 1998 tended to have higher frequencies of *M. edulis* specific alleles than those collected in either 1996 or 1999. The decrease in *M. edulis* allele frequency in the westernmost hybrid zone sites also differed among years. In 1996 and 1998, consistent *M. edulis* allele frequencies below 0.9 are observed only at sites west of MP. In 1999, on the other hand, the observed allele frequency drops below 0.9 at PB, more than 40 km further east.

Genotype frequencies of spat differed significantly from Hardy-Weinberg expectations at 27%, 78%, and 67% of the hybrid zone sites in 1996, 1998, and 1999, respectively (Table 4). These deviations were due primarily to a lack of heterozygotes and an excess of *M. galloprovincialis*/*M. galloprovincialis* homozygotes. Similarly, all collections from *M. galloprovincialis* sites in northern Cornwall differed significantly from Hardy-Weinberg expectations except at PQ in 1999 ($P = 0.069$). On the other hand, none of the spat collections from pure *M. edulis* sites differed from Hardy-Weinberg expectations.

Circulation Model

Neither developmental time nor year affected the general results of the circulation model; particles traveled in similar directions and showed similar dispersal patterns when released from the same point at different times. Therefore, we pooled termination points for all years and all developmental estimates for particles released from the same location (Fig. 6). Particles released from sites located east of Start Point tend to remain within that region; none of the particles ever crossed Start Point to where they could potentially settle within the hybrid zone. The fate of particles released within the hybrid zone depended on the release point. Particles released from sites within Eddystone Bay (MT, WB, LS, HB, PB) almost always traveled in an eastward direction. Al-

TABLE 2. Allele frequencies of *Mytilus edulis* specific alleles at the *Glu* gene of primary spat (250–500- μ m shell length) from each collection date and location. Sample sizes are shown underneath the allele frequency. Significant *G* values showing temporal variation among cohorts are labeled with an asterisk. Data where allele frequencies differ significantly from those of other collections at the same location are marked by two asterisks. Dashed lines represent collections that did not include any primary spat.

1996									
	June 5	June 16	July 5	July 18	Aug. 3	Aug. 30	<i>G</i>		
East of Start Point									
MC	0.993 75	0.989 46	1.00 15	1.00 17	—	—	1.74		
DM	—	—	—	—	1.00 8	—	NA		
HS	—	1.00 22	—	1.00 6	—	—	0.00		
Hybrid Zone									
RR	—	—	—	—	—	1.00 17	NA		
WB	0.889 140	0.898 88	—	0.923 65	0.954 97	1.00** 34	13.80*		
TB	—	—	—	—	0.994 77	1.00 1	NA		
GH	—	1.00 1	1.00 2	—	0.862 65	1.00 3	NA		
HB	—	—	1.00 2	1.00 2	1.00 3	0.938 16	NA		
PB	—	—	—	—	0.853 17	1.00 1	NA		
GC	—	—	—	—	1.00 1	—	NA		
PD	—	—	—	—	1.00 3	—	NA		
MM	—	—	—	—	1.00 10	0.900 15	0.44		
PC	—	—	—	—	1.00 2	0.875 4	NA		
1998									
	May 22	June 8	June 23	July 1	July 23	Aug. 9	Aug. 21	Oct. 6	<i>G</i>
East of Start Point									
MC	1.00 191	1.00 94	0.996 121	1.00 48	—	—	1.00 11	—	3.54
DM	0.981 27	1.00 32	1.00 54	—	—	—	—	—	0.38
HS	—	1.00 17	—	1.00 12	—	—	—	—	2.89
Hybrid Zone									
WB	0.926** 54	0.909** 93	0.981 130	1.00 42	—	1.00 63	0.968 31	—	23.10*
LS	—	—	—	—	1.00 17	0.981 53	0.972 18	—	0.16
HB	—	0.974 38	0.982 54	0.983 89	0.991 58	1.00 11	—	—	1.64
PB	—	—	0.955 22	0.970 67	0.970 84	0.917 12	—	—	1.59
MP	—	0.965 86	0.988 205	0.991 214	0.948** 77	0.921** 38	0.991 57	—	20.3*
MM	—	—	—	—	0.853 17	0.861 72	0.828 32	—	0.25
North Cornwall									
PT	—	—	—	—	—	0.167 9	—	0.048 63	2.91
TV	—	—	—	—	—	0.217 30	0.143 28	0.00** 31	14.50*
NQ	—	—	—	—	—	0.046 11	0.063 8	0.040 100	0.11
PQ	—	—	—	—	—	0.132 19	0.069 51	0.00 6	1.31

TABLE 2. Continued.

	1999											G
	May 19	May 28	June 13	June 29	July 11	July 29	Aug 10	Aug 29	Sept 10	Sept 25	Oct 21	
	East of Start Point											
MC	1.00 46	1.00 36	1.00 128	1.00 40	0.981 26	1.00 11	—	—	—	—	—	3.19
DM	—	—	0.986 35	1.00 11	1.00 39	1.00 15	—	—	—	—	—	0.96
HS	—	—	0.990 49	1.00 10	1.00 14	1.00 48	—	—	—	—	—	1.82
	Hybrid Zone											
WB	0.898** 187	0.981 162	0.969 161	0.951 132	1.00 14	0.990 52	1.00 51	—	—	—	—	38.20*
LS	—	—	—	0.925 106	—	0.978 23	0.962 13	—	—	—	—	2.42
HB	—	—	0.941 17	0.964 28	—	0.908 38	—	—	—	—	—	0.89
PB	—	—	—	0.850 10	0.750 12	0.907 43	—	—	—	—	—	3.76
MP	—	0.846 65	0.842 187	0.918** 141	0.864 66	0.878 123	0.931** 29	—	—	—	—	11.95*
MM	—	—	—	0.833 9	—	0.912 17	0.938 32	0.944 9	—	—	—	1.82
	North Cornwall											
TV	—	—	—	—	—	—	—	0.167 12	0.00 9	—	—	1.46
NQ	—	—	—	—	—	—	—	0.038 105	0.011 47	0.00 8	0.125 8	4.74

though most of these particles remained within Eddystone Bay, some particles terminated east of Start Point. However, most of these particles were advected offshore where the probability of successful settlement is very low. However, a small number of projected dispersal paths, terminated within 10 km of shore where the possibility of settlement is high. All of the particles from the hybrid zone that terminated near shore in Lyme Bay penetrated 35 km or less past Start Point.

Particles released from sites around the Lizard and Lands End (LB, PD, MM, PC, CC, PM, SI), on the other hand, generally traveled westward, moving around Lands End before dispersing in a northeasterly direction along the northern coast of Cornwall. These particles often ended either offshore or northeast of SI in the *M. galloprovincialis* parental population. Dispersal distance of particles from the hybrid zone that terminated in *M. galloprovincialis* populations were gen-

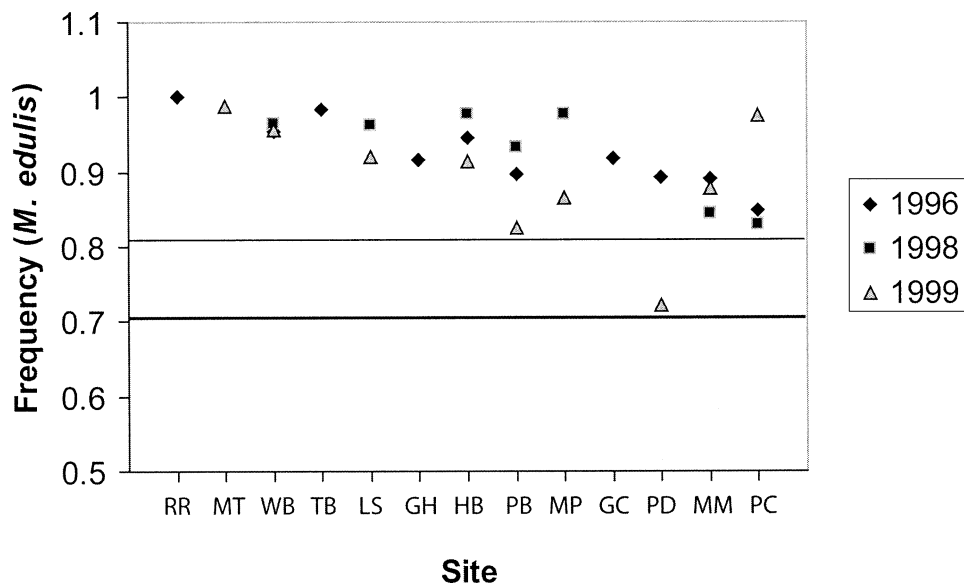


FIG. 5. Frequency of *Mytilus edulis* specific alleles at the *Glu-5'* marker of pooled primary and secondary spat collected from hybrid zone sites. Sites are listed in order from east to west. The bold black line represents the mean expected allele frequency of larvae produced by the hybrid zone. The smaller black line is one standard deviation above the mean.

TABLE 3. *Mytilus edulis* specific allele frequencies for pooled primary and secondary spat from each site and each collection year. Chi-squared values are from comparisons of observed allele frequencies to the expected frequencies of the adult population of the region in which the site is located. For hybrid zone sites two chi-squared values are given; the first is a comparison to the mean expected allele frequency while the second is a comparison to 1 SD above the mean expected allele frequency. Sample sizes are number of individuals. *G*-values are results of comparisons of allele frequencies within sites and among years. Asterisks denote significant variation in allele frequency at the 0.05 level.

Site	1996			1998			1999			<i>G</i>
	<i>f</i>	χ^2	<i>n</i>	<i>f</i>	χ^2	<i>n</i>	<i>f</i>	χ^2	<i>n</i>	
MC	0.997	0.6	523	0.999	0.6	1275	0.998	0.0	623	1.2
DM	1.000	0.0	30	0.998	0.0	259	0.995	0.6	314	2.1
HS	0.989	0.00	87	1.000	0.0	157	0.996	0.0	239	1.4
MT	—	—	—	—	—	—	0.989	34.8*** 17.7***	45	—
RR	1.000	14.2*** 7.6**	17	—	—	—	—	—	—	—
WB	0.952	660.9*** 273.7***	1130	0.966	577.4*** 258.3***	855	0.956	878.3*** 372.6***	1457	3.7
TB	0.985	121.2*** 60.4***	162	—	—	—	—	—	—	—
LS	—	—	—	0.962	176.9*** 76.9***	292	0.923	204.3*** 65.5***	460	10.4**
GH	0.910	48.9*** 13.9***	122	—	—	—	—	—	—	—
HB	0.944	69.0*** 27.1***	126	0.977	525.2*** 252.9***	655	0.914	91.3*** 22.3***	216	29.8***
PB	0.900	21.8*** 5.5*	60	0.932	180.5*** 64.4***	355	0.823	30.8*** 0.1	232	33.5***
MP	—	—	—	0.977	871.4*** 419.2***	1201	0.865	298.0*** 36.6***	1217	229.7***
GC	0.919	16.2*** 5.1*	37	—	—	—	—	—	—	—
PD	0.892	17.0*** 3.8	51	0.833	4.8* 0.1	30	0.721	0.1 4.3	34	8.1**
MM	0.860	13.0*** 1.4	57	0.844	69.8*** 7.8**	246	0.878	68.0*** 11.6***	238	7.9**
PC	0.851	35.8*** 2.6	175	0.826	9.6** 0.1	69	0.975	14.0*** 6.7**	20	12.4***
PT	—	—	—	0.062	1.1	127	0.032	0.1	126	3.0
TV	—	—	—	0.181	105.4***	155	0.029	0.3	171	44.4***
NQ	—	—	—	0.071	3.5	169	0.055	1.5	661	0.9
PQ	—	—	—	0.077	3.5	98	0.046	1.4	44	0.7

* $P = 0.05$, ** $P = 0.01$; *** $P = 0.001$.

erally greater than those observed around Start Point. Mean dispersal distance of those that were within 10 km of the shore was 53.9 km with some particles traveling more than 130 km. Finally, particles released from sites in north Cornwall typically traveled to the northeast along the coast, projecting away from the hybrid zone.

Exceptions to the dispersal trends were observed primarily at two sites. Particles released from MM never moved more than a few kilometers in any direction. On the other hand, the most dynamic hybrid zone site was probably MP, which seemed to show dispersal patterns similar to those observed at sites within Eddystone Bay and sites from the Lizard/Lands End areas. Particles released from MP went either east or west potentially settling in Eddystone Bay, the Lizard, Lands End, or north Cornwall. A couple of particles released from MP even spent some time along the coast of Lyme Bay.

These projections suggest four hydrographic regions around southwest England, each with distinct dispersal patterns (Fig. 6). Larvae that originate in the *M. edulis* populations in Lyme Bay (MC, DM, HS) will most likely settle within Lyme Bay. Larvae originating from hybrid zone sites east of MP in Eddystone Bay will travel eastward and most

likely will settle within Eddystone Bay or get advected offshore, especially around Start Point. Larvae arising from hybrid zone populations west of MP will usually travel westward and settle along the coasts of Lands End, the Lizard, or on the northern coast of Cornwall in the *M. galloprovincialis* parental populations. Finally, larvae produced in populations located on the north Cornish coast will be transported to the northeast and will remain within the *M. galloprovincialis* populations.

DISCUSSION

The structure of this hybrid zone appears to be unique when compared to previously described hybrid zones. Although most hybrid zones tend to be the product of dispersal from parental populations into the area of contact, this hybrid zone appears to lie in between two barriers to dispersal. Start Point and the northwestern shore of Lands End define the edges of this hybrid zone and tend to disrupt dispersal. However, this disruption is not complete and is asymmetric, favoring movement of larvae from the hybrid zone into the *M. galloprovincialis* populations in north Cornwall. In both cases, there

TABLE 4. Single locus genotype frequencies (*Glu-5'*), sample sizes, and χ^2 values from Hardy-Weinberg equilibrium comparisons of all size classes of spat collected. E and G represent the *Mytilus edulis* and *M. galloprovincialis* specific alleles at the *Glu* gene, respectively. An asterisk notes samples that differ significantly from Hardy-Weinberg expectations. Expected genotype frequencies calculated from the local adult populations are given for each region.

	E/E	E/G	G/G	n	χ^2
East of Start Point					
Expected	1.000	0.000	0.000		
1996					
MC	0.994	0.006	0.000	523	0.008
DM	1.000	0.000	0.000	30	0.000
HS	0.977	0.023	0.000	87	0.010
1998					
MC	0.998	0.002	0.000	1278	0.003
DM	0.996	0.004	0.000	262	0.003
HS	1.000	0.000	0.000	158	0.000
1999					
MC	0.997	0.003	0.000	624	0.102
DM	0.994	0.003	0.003	320	166.0*
HS	0.992	0.008	0.000	239	0.004
Hybrid Zone					
Expected	0.490	0.420	0.090		
1996					
RR	1.000	0.000	0.000	17	0.00
WB	0.924	0.057	0.020	1130	159.8*
TB	0.982	0.006	0.012	162	110.2*
GH	0.836	0.148	0.016	122	1.21
HB	0.929	0.032	0.040	126	59.5*
PB	0.833	0.133	0.033	60	4.03*
GC	0.865	0.108	0.027	37	2.55
PD	0.784	0.216	0.000	51	0.76
MM	0.772	0.175	0.053	57	4.41*
PC	0.743	0.217	0.040	175	3.44
1998					
MT	1.000	0.000	0.000	9	0.00
WB	0.933	0.062	0.005	897	8.20*
LS	0.926	0.067	0.007	285	4.94*
HB	0.961	0.034	0.005	738	34.10*
PB	0.890	0.085	0.025	364	39.10*
MP	0.959	0.037	0.004	1225	33.70*
PD	0.767	0.133	0.100	30	8.50*
MM	0.761	0.202	0.037	297	6.70*
PC	0.739	0.174	0.087	69	10.60*
1999					
MT	0.978	0.022	0.000	45	0.006
WB	0.922	0.068	0.010	1459	55.40*
LS	0.864	0.114	0.022	457	21.70*
HB	0.857	0.115	0.028	217	14.50*
PB	0.711	0.224	0.065	232	12.40*
MP	0.761	0.209	0.030	1217	13.40*
PD	0.529	0.382	0.088	34	0.074
MM	0.803	0.151	0.046	238	20.70*
PC	0.950	0.050	0.000	20	0.010
North Cornwall					
Expected	0.003	0.095	0.903		
1998					
PT	0.023	0.077	0.900	130	14.20*
TV	0.123	0.116	0.761	154	56.70*
NQ	0.046	0.069	0.886	175	50.00*
PQ	0.041	0.071	0.888	98	22.80*
1999					
PT	0.016	0.032	0.952	126	18.30*
TV	0.018	0.023	0.959	171	18.60*
NQ	0.030	0.048	0.921	661	172.1*
PQ	0.023	0.046	0.932	44	9.20*

appears to be little or no movement of larvae from the parental populations into the hybrid zone. Instead, hybrid populations are exporting larvae into both parental populations.

Spat collected from sites within the *M. edulis* parental population have allele frequencies similar to the adults in that region. While the allele frequencies of the spat were not significantly different from the expected frequency of 1.00, there were alleles specific to *M. galloprovincialis* found in spat at every site and in all years. Alleles specific to *M. galloprovincialis* have not been found in any of the adult mussel populations east of Start Point in any previous samples (Hilbish et al. 1994, 2002). Even though the number of hybrid spat found east of Start Point was extremely small, this finding suggests they are the result of migration from the hybrid zone. Hybrid larvae made up 0.4% of the total sample collected from *M. edulis* sites. Using the allele frequency estimate of the hybrid zone adult populations suggests that 1.3%, 0.4%, and 1.0% of the larvae settling east of Start Point originated from the hybrid zone in 1996, 1998, and 1999, respectively. Therefore, the *M. edulis* parental population seems to be receiving a small fraction of larvae from the hybrid zone on a consistent basis. It is unlikely that these hybrid spat originated from populations within Lyme Bay because we have genotyped more than 1000 adults from that region in previous years and have never found a single individual with a *M. galloprovincialis* specific allele.

The results of the model correspond very well with the genetic results. The hydrographic model suggests that most of the larvae settling in Lyme Bay originated in Lyme Bay. Projections from sites east of Start Point were either lost offshore or "settled" somewhere along the coast within Lyme Bay. A number of the projections originating at locations within the hybrid zone migrated east of Start Point. However, most of these were advected offshore at Start Point where they were most likely lost. Only a few projections from hybrid zone sites actually made it around Start Point and terminated close enough to shore where they had a possibility of successful settlement. This suggests that Start Point is a strong barrier to dispersal, especially from east to west. If conditions are right, some larvae from the hybrid zone may penetrate into populations east of Start Point, but the occurrence of such events seems to be rare.

Sites located along the northern coast of Cornwall in the *M. galloprovincialis* populations also show evidence of gene flow from the hybrid zone. Individual settlement events show convincing evidence of genetic intrusions during some time periods. One settlement each at PT and PQ and three at TV (two in 1998, one in 1999) had significantly higher *M. edulis* allele frequencies than expected. When pooled over the entire settlement season, however, the signature of hybrid immigration becomes weaker, as pooled spat at most sites tended to have an allele frequency signature similar to the adults in the region. An exception was TV in 1998, where the *M. edulis* specific allele frequency of all spat collected for the year was more than three times greater than that seen in the adults. This suggests that a large portion (20.1%) of the larvae that settled at TV in 1998 originated from the hybrid zone. These data suggest that these sites frequently receive larvae from the hybrid zone and, in some cases, the proportion immigrating from the hybrid zone can be relatively large. Unfor-

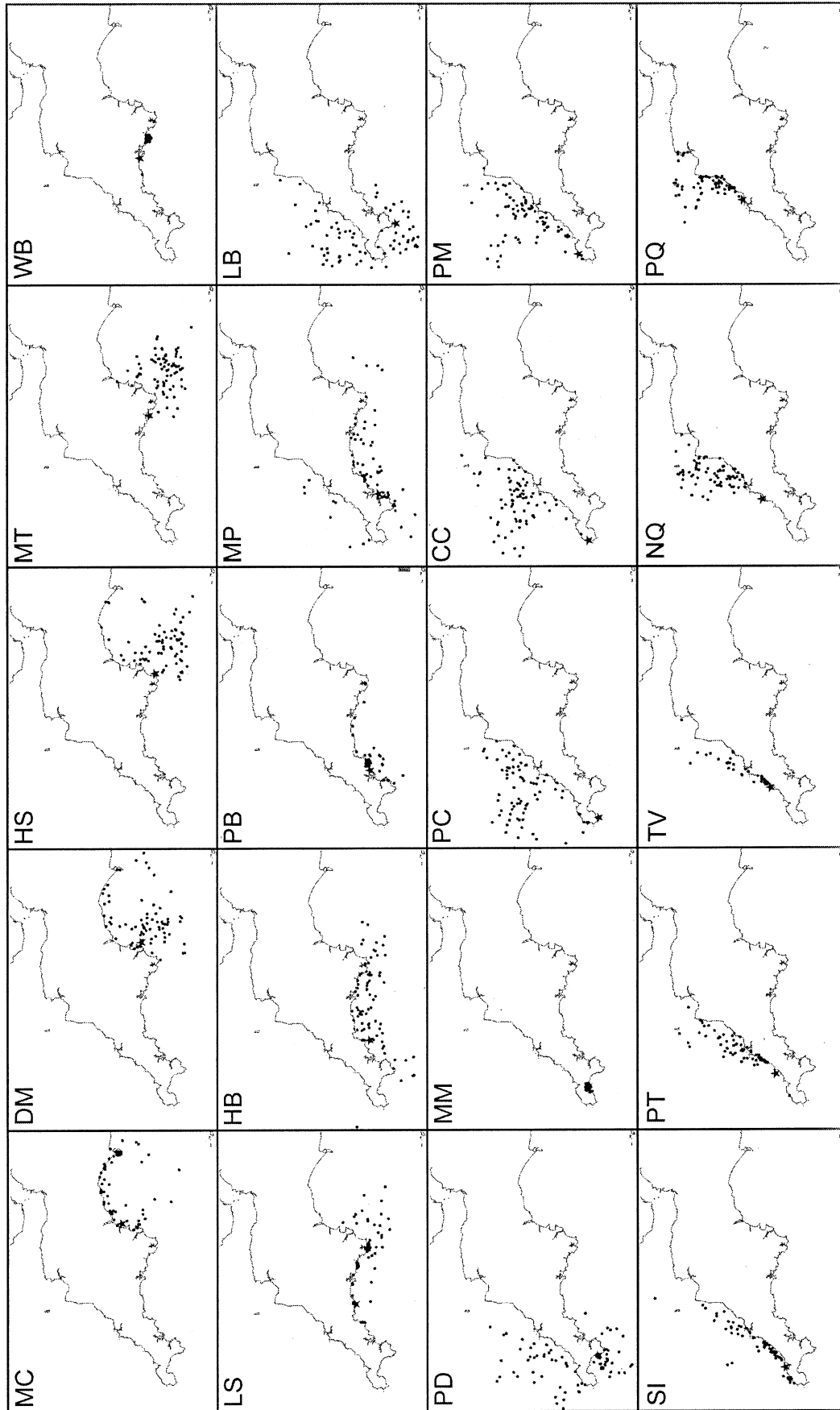


FIG. 6. Results of a transport model based on the physical oceanography of the region. Each panel shows all termination points of particles released from a given location; stars represent the release point.

unately, the genetic structure of the mussel populations in north Cornwall makes it difficult to observe the intrusion of foreign larvae. An immigrating hybrid is much easier to find amongst a fixed genetic background (like east of Start Point) than it is against the background of a consistently low, but nonzero allele frequency. Therefore, it is quite possible that the larval pool of many, if not all of these sites, consistently contained larvae from the hybrid zone but their presence could not be statistically detected.

The results of the model again correspond very well to the genetic results. Particles originating at sites within the *M. galloprovincialis* population along the northern coast of Cornwall typically project to the northeast along the coast, remaining within the *M. galloprovincialis* population. There was only one case of a particle from a site in north Cornwall projecting to the southwest and into the hybrid zone. On the other hand, a large proportion of the particles released from locations within the hybrid zone around the Lizard and Lands End crossed the population boundary at St. Ives and terminated in north Cornwall. This suggests that dispersal from the hybrid zone into the *M. galloprovincialis* population is potentially great, but that dispersal in the opposite direction happens rarely, if at all.

The different hydrographic patterns around Lands End and Start Point could very well explain the continued presence of *M. edulis* alleles at low frequencies in the mussel populations in north Cornwall, and the absence of alleles specific to *M. galloprovincialis* in Lyme Bay. Although some immigration from the hybrid zone is evident in both cases, it appears that Start Point is a much stronger barrier to dispersal than is Lands End. The observed patterns of dispersal also suggest the presence of selection favoring each of the parental types within their respective regions. The continuous flow, however small, of larvae from the hybrid zone would result in steadily increasing frequencies of *M. edulis* specific alleles in north Cornwall and of *M. galloprovincialis* specific alleles east of Start Point. The temporal stability of allele frequencies in each of these parental populations can only be explained by a selective force countering the gene flow.

Although evidence of dispersal from the hybrid zone into each of the parental populations is strong from both the genetic and the hydrographic data, the same cannot be said about evidence for dispersal from the parental populations into the hybrid zone. Larvae that settled within the hybrid zone generally had *M. edulis* allele frequencies that were much greater than the expectations based on the local adult populations. Not only were the *M. edulis* allele frequencies generally higher than the mean expected value, but most were significantly higher than one standard deviation above the mean. A potential problem, of course, is the unknown actual allele frequency of a larval cohort produced by adults within the hybrid zone. A reliable estimate is difficult due to variation in both fecundity and allele frequency with size, and differences in both size and genetic structure with location. Still, the allele frequencies observed in the spat seem inordinately high to have been produced by the populations within the hybrid zone. Even if the hybrid zone populations produce larvae with a *M. edulis* allele frequency signature of 0.87 (the highest allele frequency estimate of all adult pop-

ulations), the bulk of the spat samples from hybrid zone sites still have a significantly greater allele frequency.

This suggests that the hybrid zone is receiving a large proportion of its larvae from a pure, or relatively pure, *M. edulis* population. In fact, for the allele frequencies to be elevated so greatly above what the hybrid zone populations are capable of producing would require that upwards of 80% of the larvae settling at some of the hybrid zone sites would need to be from a pure *M. edulis* source population. This estimate is the same as that shown by Wilhelm and Hilbish (1998). Even at some of the sites that consistently received spat with slightly lower allele frequencies, the proportion from a *M. edulis* source would still need to be approximately 50%. Furthermore, the allele frequencies observed in the spat are very similar to those observed in the smallest size classes (<10 mm shell length) of adult mussels at hybrid zone sites (Hilbish et al 2002; Gilg and Hilbish 2003b; also see Appendix). Of course, one potential source of larvae with high frequencies of *M. edulis* alleles would be the population located east of Start Point.

The spatial variation in allele frequencies from east to west offers limited support for the immigration hypothesis. If much of the larval pool settling within the hybrid zone originated from east of Start Point, one would predict that the frequency of *M. edulis* alleles would decrease with distance from Start Point. This is typically observed, because spat collected from sites around the Lizard and Lands End typically have lower *M. edulis* allele frequencies than do sites within Eddystone Bay. Interestingly, the allele frequencies of spat in the hybrid zone never drop to a state where auto recruitment is the most probable answer. This means that even at PC, 180 km from Start Point, upwards of 50% of the settling larvae would have to originate from east of Start Point. This does not seem likely as previous estimates of mussel dispersal distances are much lower (McQuaid and Phillips 2000; Gilg and Hilbish 2003a).

Another confounding problem is the lack of significant temporal variation in allele frequencies at hybrid zone sites. If spat settling within the hybrid zone are the result of a mixing of larvae originating from east of Start Point and larvae produced within the hybrid zone, the mixing fractions should differ over time. The dynamic nature of winds and physical oceanographic patterns within years results in high variance in dispersal distances, which should cause temporal variation in the population mixing fractions. Spat collected at most hybrid zone sites have approximately the same allele frequency no matter when they settle. However, in the rare events that temporal variation is observed, it does not correspond to the patterns of settlement times among the populations. Spat tend to settle much earlier east of Start Point (May–July) than they do in north Cornwall (August–October), suggesting that the *M. edulis* populations are reproducing before the *M. galloprovincialis* populations. Therefore, we would, predict that spat settling within the hybrid zone should have higher frequencies of *M. edulis* alleles in May–July, and that once the mussel populations east of Start Point complete spawning in mid–late summer, we should observe a decrease in *M. edulis* allele frequency. Yet spat with highly elevated frequencies of *M. edulis* alleles are settling throughout the hybrid zone with no observable change over time.

This is true even of spat collected from hybrid zone sites when none are being found concurrently at sites east of Start Point. If anything, the consistency of the allele frequencies suggests that the larvae settling at sites within the hybrid zone are always being produced by the same genetic population.

The dispersal patterns shown in the model projections also reject the hypothesis that large numbers of larvae are migrating from east of Start Point into the hybrid zone. Projections starting from east of Start Point never crossed into the hybrid zone. Therefore, the circulation patterns of the region suggest that larvae settling within the hybrid zone most likely originated within the hybrid zone. In fact, the patterns of circulation suggest that the hybrid zone can be divided into two regions: Eddystone Bay and the Lizard/Lands End. Projections originating in Eddystone Bay typically travel to the east, while projections originating in the Lizard/Lands End area typically go west. The difference in circulation patterns may result in the observed differences in allele frequencies between spat settling in the two areas. Still, projections from a site intermediate to the two areas (MP) show a mixture of east bound and west bound projections, suggesting regular gene flow between the regions.

Similarly, there is little evidence for gene flow from north Cornwall into the hybrid zone. Again, settlement in the two regions occurs at different times. Most of the primary settlement within the hybrid zone is finished by the time the first settlements are observed at sites in north Cornwall. If *M. galloprovincialis* larvae are dispersing into the hybrid zone we would expect that the later settlements would have higher frequencies of *M. galloprovincialis* alleles than the earlier settlements. This is obviously not the case because none of the hybrid zone sites showed this trend. Also, none of the cohorts settling within the hybrid zone showed highly elevated frequencies of *M. galloprovincialis* alleles or *M. galloprovincialis*/*M. galloprovincialis* homozygotes. While it is a common trend for the spat settling at hybrid zone sites to have a slightly elevated frequency of *M. galloprovincialis*/*M. galloprovincialis* homozygotes for the observed allele frequency, it never exceeds 10%. This is not much higher than we would expect using the estimate of the mean hybrid zone adult population under Hardy-Weinberg equilibrium, suggesting that the *M. galloprovincialis*/*M. galloprovincialis* homozygotes are likely the result of hybrid matings. Finally, the model shows that particles originating in north Cornwall actually travel away from the hybrid zone. Only one projection from north Cornwall terminated in the hybrid zone, and it only penetrated a few kilometers. Therefore, dispersal of larvae from *M. galloprovincialis* populations in northern Cornwall into the hybrid zone appears highly unlikely.

If larvae settling within the hybrid zone are not coming from either east of Start Point or northern Cornwall, there must be alternative explanations for the elevation in the frequency of *M. edulis* specific alleles and the relative lack of heterozygotes. We have three hypotheses. The first is some form of gametic incompatibility or disruption of gamete recognition among some hybrid crosses. Differences in fertilization success rates or larval survivability of various hybrid crosses is a potential mechanism for explaining the lack of heterozygotes and differences in allele frequency. Although

evidence of strong assortative mating has been reported from crosses between *M. edulis* and *M. galloprovincialis* (Bierne et al. 2002), little is known about fertilization success rates among hybrid crosses. Reproductive incompatibilities between gametes could potentially produce a pattern of genotype frequencies similar to that observed in spat collected from the hybrid zone. In fact, using a model assuming that conspecific crosses have 100% success while all other hybrid crosses (F_1 , F_2 , backcrosses) have only 25% success results in frequencies of 0.759, 0.176, and 0.065 for *M. edulis*/*M. edulis* homozygotes, heterozygotes, and *M. galloprovincialis*/*M. galloprovincialis* homozygotes, respectively. This model yields a *M. edulis* allele frequency of 0.85. Unfortunately, such models of gametic incompatibilities cannot explain *M. edulis* allele frequencies above 0.90. Therefore, another mechanism would be required to fully explain the genetic data from spat settling within much of the hybrid zone, especially Eddystone Bay.

A second possibility is selection in the larval stage favoring individuals with *M. edulis* alleles. Bierne et al. (2002) showed some evidence for selection against F_1 hybrids in laboratory crosses, but the results were inconsistent. Selection against hybrids is quite common in hybrid zone systems (Howard 1993; Barton and Hewitt 1985) and can be both environmentally (exogenous) or nonenvironmentally based (endogenous). It is possible that hybrid mussels are under endogenous selection during the larval stage resulting in a radical change in both allele frequencies and genotype frequencies. Still, it would be difficult to explain the spatial variation in *M. edulis* allele frequencies by an environmentally independent selection model. Juvenile mussels from the westernmost hybrid zone sites consistently have lower frequencies of *M. edulis* specific alleles than those from the easternmost sites (Hilbish et al. 2002). Spat settling in the hybrid zone have allele frequencies similar to juveniles collected from the same sites and show the same pattern of lower *M. edulis* allele frequencies in the west in all three years of this study. This suggests that the pattern in the juveniles is not the product of postsettlement selection but instead reflects the genetics of the larvae at settlement (Gilg and Hilbish 2003b). On the other hand, the estimates of larval allele frequencies produced by the adult populations within the hybrid zone do not differ in a geographically dependent manner. Therefore, the spatial variation would have to be produced by selection prior to settlement that favors individuals with *M. edulis* alleles and that is stronger in the east than in the west. Consequently, the selection in the plankton would need to be environmentally dependent in order to produce the observed patterns.

It is unknown what may be selecting larvae with *M. edulis* alleles throughout the hybrid population. One clue may come from the differences in spawning times among the populations. It is possible that *M. galloprovincialis*-like larvae do not survive early in the season when mussels from the hybrid zone populations are reproducing. Because *M. galloprovincialis* populations in this region do not appear to spawn until mid or late summer, their larvae may have more successful development and settlement when the water temperatures are warmer. However, if that is the case, we would predict greater temporal variation in allele frequencies at hybrid zone sites with higher frequencies of *M. galloprovincialis* alleles in the

later samples, which is not observed. Another possibility is that there are genotype-dependent differences in size-specific fecundities. In our estimates of larval allele frequencies produced by the hybrid zone populations we assumed that size-specific fecundity was the same across all genotypes. If *M. galloprovincialis*-like mussels have much lower fecundity than *M. edulis*-like mussels our estimates would be biased toward lower frequencies of *M. edulis* alleles. This also seems unlikely because in order to produce the observed allele frequencies, *M. edulis*-like mussels would need to be three times as fecund as *M. galloprovincialis*-like mussels. Neither Gardner and Skibinski (1990) nor Secor (1995) showed genotype dependent differences in size-specific fecundity of this magnitude in southwestern England.

Finally, immigration from another predominantly *M. edulis* source population in the area is another possible mechanism by which *M. edulis* allele frequencies could be elevated. Potential source populations must have three characteristics. First, they must have frequencies of *M. edulis* alleles that are at least as great as the highest allele frequencies observed in spat settlements. Second, they must lie within a probable dispersal path based on the hydrodynamics of the region. Third, they must have prolonged reproductive periods that overlap with those of the hybrid zone populations. Therefore, the list of known potential sources is limited.

Two possible source populations are the Fal and Tamar estuaries. Both of these estuaries lie geographically within the hybrid zone and the mussel populations in both typically have high frequencies of *M. edulis* specific alleles (Hilbish et al. 2003). Unfortunately, the *M. edulis* allele frequencies of these populations are very similar to that observed in the spat at some sites (0.85–0.93), but do not exceed the highest frequencies observed in the spat. Also, both estuaries lie within Eddystone Bay making it unlikely that they are supplying larvae to populations around Lands End and the Lizard due to the west to east direction of water circulation in that area.

A second possible source population are subtidal mussel populations. Only one subtidal mussel population is known in the area, although we have not searched extensively. Mussels have been found attached to buoy chains off Plymouth Sound in the English Channel. These mussels have a *M. edulis* allele frequency of 0.98 (Hilbish et al. 2003). Therefore, this population fits the first criteria because allele frequencies of spat settling within the hybrid zone generally do not exceed 0.98. Because the model suggests movement from west to east in this region, a large subtidal population extending from Lands End to a location short of Start Point could potentially produce the spatial allele frequency patterns observed in the spat. The west to east current would provide more larvae to the easternmost hybrid zone sites than to those around Lands End and the Lizard. However, this model does not seem likely, for several reasons. First, it requires that nearly all of the recruitment in the intertidal populations originates from the subtidal populations, making the intertidal populations either complete sinks or numerically negligible compared to the subtidal populations. The intertidal populations in the hybrid zone are very extensive with population sizes in the tens of millions, if not more. The size and distribution of subtidal mussel populations in southwestern England are unknown but subtidal mussels typically suffer high rates of

predation and are typically rare (Seed and Suchanek 1992). Thus, it is unlikely that subtidal mussel populations in southwest England numerically dwarf the intertidal populations. Also, it is unknown how consistent high *M. edulis* allele frequencies are in subtidal populations in this region because, to our knowledge, only one has been sampled. In addition, we would again predict that mixing of larvae from subtidal and intertidal populations would result in much more temporal variation in allele frequencies than we observed.

It is unlikely that further investigation using a second or even third species-specific marker would help resolve how the frequency of *M. edulis* alleles is being increased relative to expectations. A strict mixing model based on immigration from a predominately *M. edulis* source population should produce similar allele frequencies at all loci. However, if selection is substantially stronger at some loci than others, the allele frequencies at each locus could be quite different. Unfortunately, selection could also produce very similar allele frequencies at all loci (as it does in the adults) and if the allele frequencies of the spat are the result of both selection and immigration, extra loci would not help clarify the results. To date each of the species-specific markers that have been investigated have shown similar patterns of allele frequency variation both among and within each of the populations (Rawson et al. 1996; Wilhelm and Hilbish 1998). Hybrid zone populations show high frequencies of *M. edulis*-specific alleles at all markers in small adult and juvenile mussels, which suggests that analysis of additional loci in the spat would give us the same answer at all available markers. Still, other markers are currently available and may be of some use in future analyses (Ohresser et al. 1997; Bierne et al. 2002).

The structure of the mussel hybrid zone in southwestern England is quite unique in several aspects. Hybrid-zone theory typically assumes dispersal from each of the bordering parental populations into the zone of contact. While asymmetrical patterns of supply from the parental populations were previously assumed for this system, it is quite apparent that dispersal from the parental populations into the hybrid zone is not the typical pattern of larval movement in this region. Although the hybrid zone in this study has strong barriers to dispersal from both parental populations, consistent, though light, dispersal from the hybrid zone into each parental population is observed. Therefore, the locational stability of the hybrid zone is due at least partially to the limited exchange of larvae among the populations. On the other hand, many of the results open avenues for new questions regarding the stability of this hybrid zone and the bordering parental populations. The relative success rates of hybrid crosses (F_1 , F_2 , and backcrosses), the possibility of selection in the larval stage, and the population genetic structure of potentially contributing populations all still need to be addressed in order to understand how *M. edulis* alleles are being maintained in the hybrid zone populations. Furthermore, fitness estimates of different genotypes in each region are also needed in order to determine why the hybrid zone is not expanding.

Yet another important question is how stable are the dispersal patterns over larger time scales? The dispersal model used in this study clearly shows consistent dispersal patterns throughout the region in all three years analyzed. Do these

dispersal patterns occasionally break down, and did such a break down in the circulation patterns result in the origin of this hybrid population? It appears as if the distribution of genetically different mussel populations occupying the coasts of western Europe are the result of the complex interplay of environmentally based selection in the adult stage, the local dispersal patterns, and apparently some kind of genetic bias during spawning or the larval stage. While the effects of selection in the adult stage have been documented in several cases and suspected in others in this region, the local dispersal patterns and the potential for differential mating success or selection in the larval stage have only recently been investigated. Future studies addressing these questions will likely lead to a more complete understanding of the continued presence of distinct hybrid and parental mussel populations throughout western Europe and provide insight into patterns of hybridization and speciation in marine systems.

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APPENDIX

Mytilus edulis-specific allele frequencies at *Glu-5'* reproduced from Hilbish et al. 2002. Relative fecundity estimated from fecundity curves by Gardner and Skibinski 1990.

Size class	Allele frequency by site								Relative fecundity
	WB	LS	HB	PB	MP	PD	MM	PC	
10–15 mm	0.85	0.62	0.92	0.70	0.94	1.00	0.93	0.77	0.5
15–20 mm	0.82	0.81	0.88	0.60	0.94	0.90	0.79	0.75	0.7
20–25 mm	0.70	0.75	0.96	0.50	0.76	0.70	0.83	0.71	1.0
25–30 mm	0.50	0.46	0.67	0.42	0.90	0.55	0.96	0.54	1.3
30–35 mm	0.38	0.50	0.28	0.44	0.56	0.40	0.73	0.18	1.5
35–40 mm	0.30	0.45	0.20	0.22	0.58	0.14	0.74	0.20	2.0
40–45 mm	0.25	0.45	0.22	0.22	0.76	0.14	0.73	0.20	3.0