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Dispersal vs. retention: correspondence of species-specific reproductive cycles and settlement periods in a blue mussel hybrid zone

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ABSTRACT: While long distance dispersal of planktotrophic larvae in marine organisms has traditionally been considered the norm, several recent studies have shown that local retention of larvae and hydrodynamic barriers to dispersal often exist. This study focuses on the question of whether genetically distinct populations within a blue mussel hybrid zone typically exchange larvae or if larvae are often retained within the population of origin. Larvae were tracked to their point of settlement using a genetic marker and analyzed for correlations with differential timing of reproduction and settlement among 3 genetically distinct populations (*Mytilus edulis*, *M. galloprovincialis* and hybrid). Correspondence of allele frequencies to local reproduction and settlement suggest that larvae settling within the *M. edulis* and the hybrid zone populations originated from those locations. On the other hand, larvae settling within the *M. galloprovincialis* populations often contain significant proportions of immigrants from the hybrid zone. The observed patterns are consistent with previous studies of the local hydrodynamics, suggesting that physical barriers to dispersal often exist and result in retention of larvae.

KEY WORDS: Larval dispersal · Retention · *Mytilus* · Hybrid zone

INTRODUCTION

Populations of marine organisms with planktotrophic larvae have traditionally been viewed as open systems with high connectivity (Thorson 1950, Strathmann 1985). It is generally assumed that larvae with the capacity for dispersal typically disperse away from their population of origin and settle some distance from their source. A number of recent studies have verified the movement and subsequent settlement of larvae over relatively long distances (up to 100 km) (Gilg & Hilbish 2003a,b, or see reviews by Palumbi 2001, 2003, Hellberg et al. 2002, Levin 2006). These studies have typically focused on intrusion of non-local larvae into a region containing a genetically different population. Several studies, however, have shown that the assumption of high connectivity is not always correct. Significant barriers to dispersal have been found in several study systems that can lead to asymmetric connectivity (Gilg & Hilbish 2003a,b, Veliz et al. 2006) or even local larval retention (reviewed in Warner & Cowen 2002, Levin 2006, and see Becker et al. 2007). The degree to which larvae with pelagic dispersal are retained within their population of origin, and the causes and consequences of dispersal and retention are still not well understood. Here we address the question of larval retention within a blue mussel hybrid zone located in southwestern England.

The blue mussels *Mytilus edulis* and *M. galloprovincialis* overlap in range and hybridize readily at several locations along the Atlantic coast of western Europe (Skibinski 1983, Daguin et al. 2001, Hilbish et al. 2002). In southwestern England a hybrid zone is located along the southern coasts of the counties of Devon and

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Cornwall between Start Point and the town of St. Ives (Fig. 1). The hybrid population is a true hybrid swarm with multi-locus genotypes representative of 'pure' individuals of both species as well as F1 and various multi-generational hybrids. Within the hybrid zone, frequencies of *M. edulis*-specific alleles at diagnostic loci differ among size classes; smaller mussels tend to have higher frequencies of *M. edulis*-specific alleles than do larger mussels at the same site due to selection favoring *M. galloprovincialis*-like genotypes. Estimates using adult demography suggest adult mussels within the hybrid zone should produce larvae with *M. edulis*-specific allele frequencies of approximately 0.71 ± 0.112 (Gilg & Hilbish 2003a). *Mytilus edulis* populations, where adult populations are fixed for *M. edulis*-specific alleles at diagnostic loci, occupy sites east of Start Point, whereas predominantly *M. galloprovincialis* populations, with frequencies of *M. galloprovincialis*-specific alleles >0.9 in adult populations, reside north of St. Ives (Hilbish et al. 2002). The distinctions between each population are quite abrupt (<5 km) which could be due to either disruption of dispersal leading to larval retention within each population, or to post-settlement selection, or both. Previous studies in this region have focused on measuring the movement of dispersing larvae using the genetic differences among the populations and a 2D model of the physical oceanography (Gilg & Hilbish 2003a,b). An observed difference in the timing of larval settlement within each of the populations now allows us to investigate whether local larvae are often retained in each of these populations, or whether the observed genetic structure may be due to differences in local selection pressures.

In the present study we test whether any of the 3 genetically distinct populations (*M. edulis*, *M. galloprovincialis* and hybrid) show signs of significant larval retention by comparing the reproductive cycles, the timing of larval settlement and the population genetics of the spat (recently settled larvae). Larval retention should have a distinct signature. First of all, variation among populations in the timing of larval settlement should correspond to differences in their reproductive peaks. Second, the population genetics of the spat should be similar to that of the local adult population and should not deviate from Hardy-Weinberg equilibrium. Third, since the larvae settling at a site come from a consistent source we should see little genetic variation over time. Larval import, on the other hand, will show a quite different signature with genetic variation between adults and spat, deviation from Hardy-Weinberg expectations, temporal genetic variation and potential differences between the timing of reproduction and the timing of settlement.

MATERIALS AND METHODS

Spat collection. Spat were collected from a total of 20 sites spanning the 3 genetically distinct populations throughout southwestern England in 1998, 1999 and 2000 (Fig. 1). Sites include Maidencombe (MC), Dartmouth (DM), Hallsands (HS), Mothecombe (MT), Whitsand Bay (WB), Lansallos (LS), Hemmick Beach (HB),

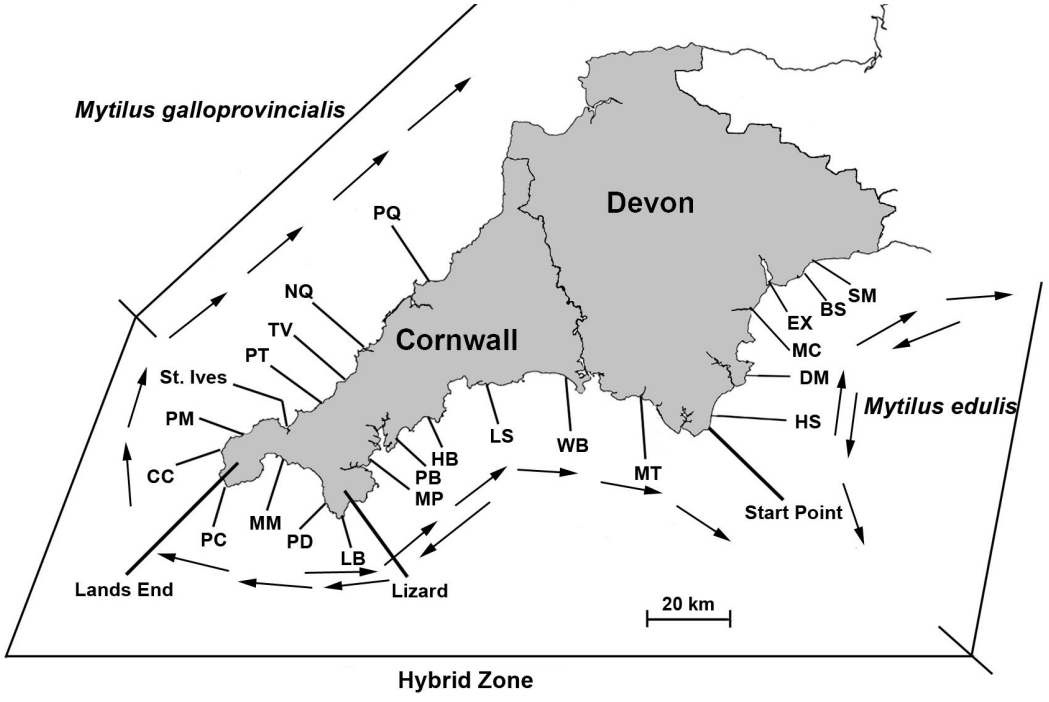

Fig. 1. Southwestern England showing collection sites and important geographical locations. Arrows denote typical directions of water movement as shown from a 2D hydrographic model described in Gilg & Hilbish (2003b)

Pendower Beach (PB), Maenporth (MP), Lizard Lifeboat Station (LB), Poldu (PD), St. Michael's Mount (MM), Porthcurno (PC), Cape Cornwall (CC), Porthmeor (PM), St. Ives (SI), Portreath (PT), Trevaunance (TV), Newquay (NQ) and Port Quin (PQ). The predominant method was to collect various species of red alga (e.g. *Ceramium ruburium*) which are a natural substrate for mussel larvae. Additionally, 4 to 6 artificial substrates (ECKO scouring pads and patches of burlap) were deployed at each site. Collections were made at low tide during each spring tide throughout the collection period. In 1998, spat were collected from mid-May through August with 1 additional collection in October. In 1999, spat were collected from mid-May through September with a final collection at the end of October. No dates between mid-May and mid-October were missed in the 2000 collections. All substrates were placed in Whirl-paks for storage until they could be processed. Upon returning to the laboratory, spat were physically removed from the substrates using jeweler's forceps under a dissecting microscope and placed in 95% ethanol for genetic analysis.

Collected spat were divided into 2 general age classes: primary and secondary. Primary spat were those individuals that were ≤500 µm shell length while secondary spat were >500 µm shell length. Mussel larvae settle when approximately 250 µm in shell length and can grow up to 30 µm per day following settlement (Bayne 1965, Gilg & Hilbish 2000). Therefore, spat that are ≤500 µm shell length most likely settled within the 2 wk period since the previous collection. By focusing this study on primary spat we can minimize the potential for the observed patterns to be affected by post-settlement processes.

Reproductive cycles. A total of 24 adult mussels between 30 and 40 mm shell length were collected monthly from each of 10 locations throughout southwestern England for assessment of their reproductive cycles. Mussels were collected from 4 sites within the *Mytilus edulis* population: Exmouth (EX), Budleigh Salterton (BS), Maidencombe (MC) and Sidmouth (SM) (Fig. 1). Samples were collected from MC only at the beginning of the study, but, due to a lack of adults in the proper size class, later collections came from BS. The hybrid zone was represented by collections from Whitsand Bay (WB), Maenporth (MP) and St. Michael's Mount (MM). Collections from the *M. galloprovincialis* population were made from Trevaunance (TV), Newquay (NQ), and Port Quin (PQ). Each site was sampled during the first spring tide cycle of each month from March through November of 2000. A piece of tissue ~0.25 cm^2 in size was extracted from the mantle of each individual and preserved in 10% Baker's formalin. Histological analysis was performed as in Secor et al. (2001) to discern the fractions of mature and immature sperm or eggs, adipogranular cells, vesicular connective tissue and intrafolliculuar space within the tissue.

Genetic analysis. Individual spat were measured for shell length under a dissecting microscope and then placed in a simple lysis buffer to extract whole genomic DNA as described in Gilg & Hilbish (2000). Each spat was genotyped by 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 *Mytilus edulis* and *M. galloprovincialis* by a 55 bp insertion/deletion polymorphism such that species-specific PCR products are easily resolved on an agarose gel. In both 1999 and 2000, several samples of spat had low PCR efficiency. In cases of troublesome PCR reactions, the addition of 0.065 µl of Bovine Serum Albumin to each 12 µl reaction mix often considerably increased the proportion of successfully amplified samples (see Palumbi 1996). Single locus genotype and allele frequencies were then calculated for spat settling at each collection site on each collection date. Proportions of collected spat originating from different populations were estimated using a simple 2-population admixture model as follows:

$$p_{spat} = X(p_A) + (1 - X)(p_B)$$

where X and (1 – X) represent the proportions of the settlers originating from the 2 contributing populations with allele frequencies p_A and p_B.

Statistical analysis. Comparisons of Mature Gamete Fractions, Immature Gamete Fractions, Glycogen Storage Cell Fractions, Protein Storage Cell Fractions and Gamete Volume Fraction (sum of mature and immature gamete fractions) among sites within region and among regions were done using a nested ANOVA with the *Mytilus edulis*, *M. galloprovincialis* and hybrid populations being considered different regions. Comparisons of allele frequencies among age classes and across collection dates were made using an R × C *G*-test of independence (Sokal & Rohlf 1981). To control for bias of the test, in cases where samples only contained one of the two possible alleles, a value of 1 was inserted for the absent allele. Goodness of fit to Hardy-Weinberg expectations was calculated using an exact test since many analyses included expected numbers of <5 for some genotypes.

RESULTS

Primary settlement was observed at some locations on every collection date in each year, but settlement patterns were quite disparate among the 3 populations (Fig. 2). Most of the spat settlement within the *Mytilus edulis* population occurred in May and June. Settle-

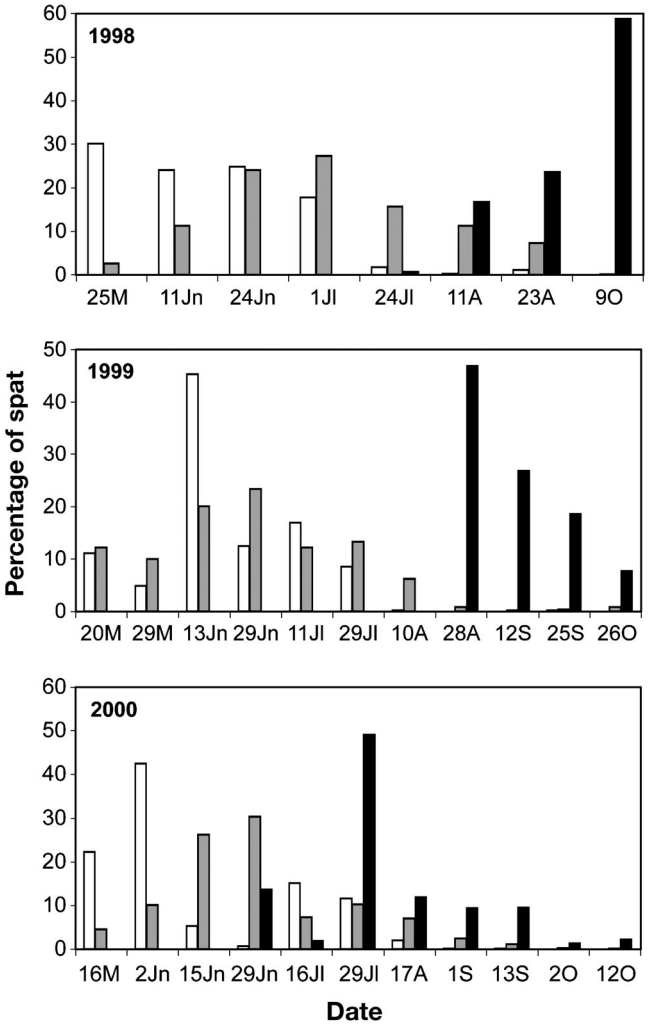

Fig. 2. *Mytilus* spp. Percentage of primary (≤500 µm) spat collected during each collection period from 20 sites within *M. edulis* (white bars), hybrid (grey bars) and *M. galloprovincialis* (black bars) populations. Values include spat that were not successfully genotyped

ment at hybrid zone sites tended to peak in June and July but also had the longest duration, overlapping with the settlement periods of both parental populations. Timing of larval settlement at both *M. edulis* and hybrid zone sites were consistent in all 3 years of the study. Settlement at *M. galloprovincialis* sites, on the other hand, showed substantial differences among years with settlement occurring approximately 1 mo earlier in 2000 than observed in either 1998 or 1999. Settlement occurred from August through October in both 1998 and 1999, but large settlement events were observed in late June in 2000 and continued through October.

Histological analysis tends showed strong regional differences in reproduction similar to the settlement patterns observed. Gamete Volume Fractions (GVF, total volume of both mature and immature gametes) tended to be higher in *Mytilus edulis* and hybrid populations in early spring and plummeted by mid-summer suggesting that reproduction typically ended prior to August in these populations (Fig. 3). In contrast, GVF in *M. galloprovincialis* populations tended to increase through the spring and early summer, reaching its peak in July and August followed by dramatic decreases to very low values by October. Results of a nested ANOVA to test for significant differences in GVF among regions and among sites within regions shows significant differences among sites within regions in every month except September. Therefore, we used the site within region mean square as the error term to make a more conservative test for differences among regions (Table 1). Significant regional differences were observed in early spring and late summer/early fall. GVF in *M. edulis* and hybrid zone populations were higher than those observed in *M. galloprovincialis* sites in March but significantly lower from July through October. Similar patterns were observed when analyses were conducted on specific tissue types, including mature gametes, immature

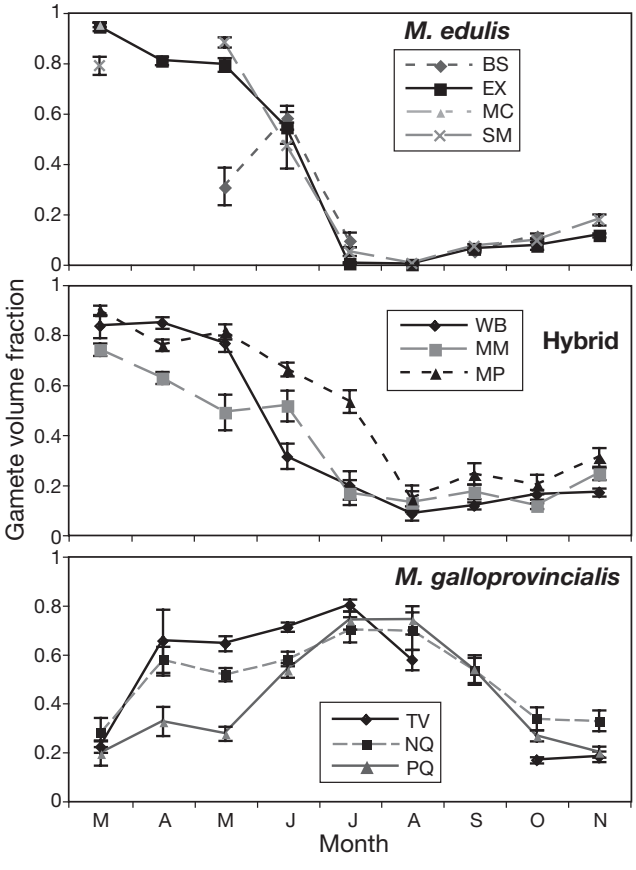

Fig. 3. *Mytilus* spp. Total gamete volume fractions from samples collected in 2000 from 3 different sites within each of the *M. edulis*, hybrid and *M. galloprovincialis* populations

Table 1. *Mytilus* spp. Monthly comparisons of gamete volume fraction (GVF) among regions. Site within region mean square error of a nested ANOVA was used to test for significant differences among regions

Month	DF	Type III SS	Mean square	*F*	p
March	2	25.585	12.792	32.40	0.0006
April	2	1.631	0.816	1.64	0.3018
May	2	3.714	1.857	1.11	0.3875
June	2	0.417	0.209	0.54	0.6093
July	2	21.729	10.864	31.64	0.0006
August	2	17.781	8.890	58.99	0.0003
September	2	8.270	4.135	71.18	0.0002
October	2	1.106	0.553	5.37	0.0461
November	2	0.272	0.136	1.02	0.4244

gametes, protein storage cells and glycogen storage cells (data not shown).

In the cases of both the *Mytilus edulis* and the hybrid zone population, GVF tended to be highest in the months immediately preceding the strongest settlement events (March, April and May) and a decrease in GVF tended to coincide with spat settlement. Populations at both *M. edulis* and hybrid zone sites were typically depleted (GVF < 0.2) by August and little settlement occurred in those regions after August. Given typical water temperatures from March through May (10 to 12°C) mussel larvae likely remain in the plankton for at least 6 wk and potentially up to nearly 12 wk (Bayne 1965). Therefore, reproductive events occurring in March and April will result in settlement primarily in May and June. Spat were always present at some sites in the *M. edulis* and hybrid populations by mid-May with stronger and more consistent settlements occurring in June. Larval development is much quicker in mid-summer when water temperatures are greater (up to 17°C), lasting between 4 and 6 wk. Since the last evidence of substantial mature gametes in *M. edulis* populations are from June samples, this would correspond to settlement ending in late July or early August. Hybrid zone populations tend to still have some mature gametes in samples from July suggesting that settlement may continue slightly longer than observed at *M. edulis* sites. These estimates correspond well to the observed settlement patterns in these regions (Fig. 2).

Populations within the *Mytilus galloprovincialis* region did not show such a clean correspondence between GVF and settlement. Fractions of mature gametes tended to increase through June and peaked in July and August. GVF did not start to decrease at any of the sites until August, and GVF values >0.2 were still apparent in some *M. galloprovincialis* populations in November. These data suggest that most reproduction in this region likely occurred from June through September, but primarily after July. Spawning in mid-June would result in settlement no earlier than mid- or late-July. This would suggest that most settlement within the *M. galloprovincialis* population should occur after July. In 1998 and 1999 spawning was not observed until August, but in 2000 a strong settlement event occurred in late June with a smaller but still substantial settlement in mid-July. In other words, spat settlement occurred more than 1 mo prior to the observed decrease in GVF at *M. galloprovincialis* sites in 2000. These data suggest that either substantial reproduction was occurring in the *M. galloprovincialis* population while the GVF was still increasing, or that the early settlers at those sites are from a different population.

Allele frequencies of primary spat pooled by region are provided in Table 2 with more complete data sets of all primary spat collections at each site and date in online supplementary material (Appendices 1, 2 & 3 available at: www.int.res/articles/suppl/m351p151_app.pdf). Patterns among regions were typically stable over years. Spat collected from sites within the *Mytilus edulis* population had frequencies of *M. edulis*-specific alleles of 1.00 in most situations. A small subset of collections in each year contained some individuals with *M. galloprovincialis* alleles, suggesting they were likely immigrants from the hybrid zone. Allele frequencies of spat from within the *M. edulis* population did not differ significantly across time within sites or among sites on the same date.

Spat collected from hybrid zone sites also had very high frequencies of *Mytilus edulis*-specific alleles, typically above 0.9 (Table 2). In 17 of 23 cases, the *M. edulis*-specific allele frequency of primary spat pooled across dates from the same site were significantly higher than the expected allele frequency of 0.71 ± 0.112 (Gilg & Hilbish 2003a, Table 3). In nearly half of these instances the observed allele frequencies were significantly higher than 1 standard deviation above the expected allele frequency. Collections of primary spat from some sites show significant temporal variation, but the variation does not follow a consistent pattern. Sometimes the lowest allele frequencies occur in early settlements, sometimes late and sometimes at intermediate dates. The pattern also differs among years at the sites. Spatial variation, on the other hand, tends to be a bit more consistent. There is not significant spatial variation in allele frequencies of spat collected at hybrid zone sites on all collection dates, but when it exists the trend is for a decrease in *M. edulis*-specific allele frequency from east to west (Table 3). Still, even at the sites closest to the *M. galloprovincialis* population where lower frequencies of *M. edulis*-specific alleles would be expected in models of larval export, *M. edulis*-specific allele frequencies are typically >0.85.

Table 2. *Mytilus* spp. Frequencies of *M. edulis*-specific alleles at the *Glu* gene of primary spat (250 to 500 µm shell length) from each collection date pooled over sites within regions (E: *M. edulis*; HZ: hybrid zone; G: *M. galloprovincialis*). (*): significant *G*-values showing temporal variation among cohorts; (–): collections that included <5 primary spat

1998	May 22	Jun 8	Jun 23	Jul 1	Jul 23	Aug 9	Aug 21	Oct 6				*G*
E-pooled	0.998	1.000	0.997	1.000	1.000	–	1.000	–				6.8
	218	143	175	67	10		11					
HZ-pooled	0.932	0.944	0.982	0.986	0.959	0.938	0.948	–				41.9*
	59	223	420	418	266	251	143					
G-pooled	–	–	–	–	–	0.159	0.061	0.041				14.9*
						69	82	88				
1999	May 19	May 28	Jun 13	Jun 29	Jul 11	Jul 29	Aug 10	Aug 29	Sep 10	Sep 25	Oct 21	
E-pooled	1.000	1.000	0.995	1.000	0.994	1.000	–	–	–	–	–	0.8
	50	40	212	61	79	74						
HZ-pooled	0.909	0.937	0.902	0.930	0.885	0.918	0.966	0.974	0.700	0.944	–	25.4
	165	237	382	427	104	304	131	19	5	9		
G-pooled	–	–	–	–	–	–	–	0.051	0.009	0.034	0.100	6.1
								118	56	44	10	
2000	May 16	Jun 1	Jun 15	Jun 29	Jul 16	Jul 27	Aug 17	Aug 30	Sep 12			
E-pooled	1.000	0.984	1.000	1.000	1.000	1.000	0.900	–	–			10.3
	95	153	19	5	80	82	10					
HZ-pooled	0.946	0.976	0.961	0.957	0.958	0.972	0.927	1.000	–			5.0
	56	85	90	94	60	89	41	5				
G-pooled	–	–	–	0.330	0.125	0.180	0.289	0.052	0.026			59.4*
				97	8	194	90	29	39			

Primary spat from *Mytilus galloprovincialis* sites perhaps show the most interesting patterns in allele frequency. Allele frequencies at *M. galloprovincialis* sites ranged from 0.00 to 0.60 (Appendices 1 to 3). When primary spat were pooled across dates at the same site, 5 of 10 samples had *M. edulis*-specific allele frequencies that were significantly higher than expected to be produced by the adult population at those sites (~0.05) (Table 2). Interestingly, 4 of the 5 significant samples were collected in 2000. These results suggest a relatively large contribution of spat from an outside source. While most of the allele frequencies of pooled primary and secondary spat samples in 1998 and 1999 do not show significant deviation from the expected allele frequencies (Table 3), individual settlement events do show evidence of significant larval contribution from the hybrid zone. The settlement event prior to 9 August 1998 had *M. edulis*-specific allele frequencies significantly >0.05 in 3 of 4 cases (PT: $\chi^2 = 5.3$; TV: $\chi^2 = 36.1$; NQ: $\chi^2 = 0.01$; PQ: $\chi^2 = 5.5$). Spat settling at TV on 21 August 1998 and 29 August 1999 also showed significant deviations from expected allele frequencies ($\chi^2_{1998} = 10.5$; $\chi^2_{1999} = 7.1$). In 2000, 6 of 11 samples with $n > 5$ individuals had significantly higher frequencies of *M. edulis*-specific alleles than expected. These data suggest that spat settling at *M. galloprovincialis* sites often contain migrants from a population with high frequencies of *M. edulis*-specific alleles.

Samples of primary spat from *Mytilus galloprovincialis* sites also show a consistent pattern of temporal variation. In both 1998 and 2000, the earliest samples have significantly greater frequencies of *M. edulis*-specific alleles than do samples collected later in the season. The pattern is not observed in 1999, but that year had the least evidence of migration of the 3 yr of the study. Spatial variation in allele frequencies exists on some sampling dates but does not display an apparent pattern with distance from the hybrid zone.

Genotype frequencies in primary spat do not deviate from Hardy-Weinberg proportions (HWP) at *Mytilus edulis* sites in any year (Table 4). Hybrid zone sites show significant deviations from HWP at 9 out of 20 site/date combinations and typically show a heterozygote deficiency although some samples have an overabundance of heterozygotes. Primary spat from the *M. galloprovincialis* sites deviate significantly from HWP in 9 of 11 cases and sites that deviated from HWP always have fewer heterozygotes than expected.

DISCUSSION

In the current study, we took evidence from multiple data sets in order to determine whether certain regions within a *Mytilus* spp. hybrid zone were characterized as areas of larval retention instead of import from other sources as typically expected of marine organisms with a pelagic larval stage. As stated in the introduction, areas typified by larval retention should show correspondence of settlement periods with reproductive

Table 3. *Mytilus* spp. *M. edulis* specific allele frequencies (f) 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 2 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. (n): no. of individuals; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. For site locations see Fig. 1

Site	1998			1999			2000		
	f	χ^2	n	f	χ^2	n	f	χ^2	n
MC	0.999	0.0	476	0.998	0.0	289	0.981	0.1	180
DM	0.996	0.0	121	0.995	0.0	109	1.000	0.0	118
HS	1.000	0.0	29	0.996	0.0	121	1.000	0.0	146
MT	–	–	–	1.000	2.6	11	1.000	7.1**	30
					0.9			2.4	
WB	0.962	75.6***	422	0.959	128.8***	739	0.987	23.9***	111
		20.8***			34.7***			7.5*	
LS	0.983	18.9***	90	0.943	24.2***	158	0.923	3.3	26
		5.9*			5.8*			0.7	
HB	0.984	54.3***	256	0.925	12.1***	93	0.958	10.2**	59
		16.9***			2.5			2.7	
PB	0.966	34.8***	189	0.871	5.1*	70	0.922	4.1*	32
		9.8**			0.5			0.8	
MP	0.978	137.0***	678	0.873	46.3***	616	0.964	44.9***	248
		41.2***			4.3*			12.5***	
PD	–	–	–	0.850	0.6	10	–	–	–
					0.0				
MM	0.858	7.99**	130	0.895	8.3**	86	0.788	0.2	14
		0.5			1.2			0.0	
PC	0.909	1.2	11	1.000	1.4	6	–	–	–
		0.2			0.5				
PT	0.063	0.45	72	0.039	0.2	38	0.082	3.9*	98
TV	0.142	17.8***	53	0.091	1.5	22	0.208	60.2***	60
NQ	0.033	0.5	45	0.033	2.0	167	0.219	299.2***	263
PQ	0.086	3.6	70	–	–	–	0.463	280.3***	41

cycles in that area and the genetics of the spat should be similar to that observed in the adults. In this light we would argue that the hybrid zone investigated in this study contains 2 regions of larval retention and 1 with consistent and sometimes heavy supply of larvae from other sources.

The *Mytilus edulis* population tends to retain its own larvae with little evidence for significant contributions from other populations. Settlement of spat corresponds well to the reproductive timing of that population with settlement occurring several weeks after reproduction appears to begin. It should be noted that spat were collected in all 3 yr of the study, but reproductive cycles were only measured in 2000. Still, the settlement data from 2000 matches the reproductive cycles very well. The larvae that settle at *M. edulis* sites consistently have very high frequencies of *M. edulis*-specific alleles although they are not always fixed. A small number of hybrid larvae are observed settling at these sites and some are observed each year. Allele frequencies pooled across sites and dates can be used to determine the proportion of spat settling at *M. edulis* sites that originated from the hybrid zone using the 2-population admixture model (see 'Materials and methods'). The estimates below assume that the frequency of *M. edulis*-specific alleles within the hybrid zone is 0.71 based on adult demography as described in Gilg & Hilbish (2003a). Using this calculation, estimated migration rates were extremely low over the course of the study with the proportion of migrants calculated at 0.005, 0.010 and 0.026 for 1998, 1999 and 2000, respectively.

At first appearance, the allele frequencies observed in the spat settling within the hybrid zone suggest a large influx of larvae from a pure *Mytilus edulis* source population. Spat collected at all hybrid zone sites have very high frequencies of *M. edulis*-specific alleles, often more than 1 standard deviation above the mean expected frequency estimated to be produced by the local adult populations. In fact, the allele frequencies at most sites require that >80% of the larvae settling originated from a pure *M. edulis* source population (also see Wilhelm & Hilbish 1998). Even the sites with the lowest frequencies of *M. edulis*-specific alleles require a 50% or greater contribution from a pure *M. edulis* source population. Therefore, the question becomes what is the source of these excess *M. edulis* alleles?

Table 4. *Mytilus* spp. Genotype frequencies, results of an exact test for Hardy-Weinberg equilibrium and Wright's *F* statistic of deviations from expected heterozygosity. Significant deviations from Hardy-Weinberg proportions are shown in bold face. E: *M. edulis*; G: *M. galloprovincialis.* For site locations see Fig. 1

Site	E/E	E/G	G/G	n	p	*F*
1998						
MC	0.998	0.002	0.000	476	1.000	0.000
DM	0.992	0.008	0.000	121	1.000	0.000
HS	1.000	0.000	0.000	29	1.000	NA
WB	0.929	0.066	0.005	422	0.096	0.091
LS	0.967	0.033	0.000	90	0.983	0.000
HB	0.973	0.023	0.004	256	0.053	0.274
PB	0.947	0.037	0.016	189	**<0.001**	0.439
MP	0.959	0.038	0.003	678	**0.035**	0.117
MM	0.769	0.177	0.054	130	**0.004**	0.275
PT	0.014	0.097	0.889	72	0.216	0.176
TV	0.057	0.170	0.774	53	**0.046**	0.304
NQ	0.000	0.067	0.933	45	0.966	−0.042
PQ	0.057	0.057	0.886	70	**<0.001**	0.638
1999						
MC	0.997	0.003	0.000	289	1.000	0.000
DM	0.991	0.009	0.000	109	1.000	0.000
HS	0.992	0.008	0.000	121	1.000	0.000
MT	1.000	0.000	0.000	11	1.000	NA
WB	0.924	0.069	0.007	739	**0.005**	0.126
LS	0.899	0.089	0.013	158	0.069	0.180
HB	0.882	0.086	0.032	93	**0.006**	0.381
PB	0.771	0.200	0.029	70	0.229	0.111
MP	0.774	0.198	0.028	616	**0.006**	0.108
MM	0.849	0.093	0.058	86	**<0.001**	0.505
PT	0.026	0.026	0.947	38	**0.040**	0.649
TV	0.091	0.000	0.909	22	**0.002**	1.000
NQ	0.012	0.042	0.946	167	**0.008**	0.345
2000						
MC	0.967	0.028	0.006	180	0.057	0.264
DM	1.000	0.000	0.000	118	1.000	NA
HS	1.000	0.000	0.000	146	1.000	NA
MT	1.000	0.000	0.000	30	1.000	NA
WB	0.973	0.027	0.000	111	0.986	0.000
LS	0.846	0.154	0.000	26	0.983	−0.083
HB	0.915	0.085	0.000	59	0.916	−0.049
PB	0.875	0.094	0.031	32	0.151	0.347
MP	0.935	0.056	0.008	248	**0.031**	0.194
MM	0.786	0.000	0.214	14	**0.001**	1.000
PT	0.061	0.041	0.898	98	**<0.001**	0.728
TV	0.167	0.083	0.750	60	**<0.001**	0.747
NQ	0.129	0.179	0.692	263	**<0.001**	0.477
PQ	0.341	0.244	0.415	41	**<0.001**	0.509

We can think of 3 possible explanations for the high frequency of *Mytilus edulis*-specific alleles in spat settling within the hybrid zone: (1) most of the spat immigrated from the pure *M. edulis* population located east of Start Point; (2) most of the spat immigrated from other pure or nearly pure *M. edulis* populations in the area; or (3) selection due to intrinsic differences in reproduction among genotypes or due to survival in the larval stage favors those with *M. edulis* alleles.

The first possibility is easily tested using the data presented in this and other papers. If most of the larvae settling within the hybrid zone originated from the *Mytilus edulis* population located east of Start Point there are a number of patterns that should be observed. First, the frequency of *M. edulis*-specifc alleles should decrease with distance from Start Point. While this pattern is observed to some extent, the allele frequencies observed in primary spat settling in the westernmost regions of the hybrid zone still require that most of the larvae originated from a pure *M. edulis* source population which is located >150 km to the east. Previous estimates of mussel larval dispersal distances in this region and others suggest that this is highly unlikely (McQuaid & Phillips 2000, Gilg & Hilbish 2003b, Becker et al. 2007), as do the oceanographic current patterns in the region which show that Start Point is a significant barrier to movement of neutral particles from both the east and the west (Gilg & Hilbish 2003a,b). Second, spat settling within the hybrid zone should show temporal variation in allele frequencies correlating to the observed reproductive patterns. Settlement at hybrid zone sites in all years corresponds well to the reproductive cycle of the hybrid zone population in 2000, and both settlement and reproduction are somewhat intermediate to the 2 pure populations. There are often settlements at hybrid zone sites that occur several weeks after the last significant settlement is observed at *M. edulis* sites east of Start Point. Therefore, if most of the larvae settling within the hybrid zone originated from east of Start Point, we would expect higher frequencies of *M. edulis*-specific alleles in spat collected early in the summer and lower frequencies in those collected after settlement has concluded in mussel populations east of Start Point. However, the allele frequencies of spat collected throughout the summer do not typically show temporal variation of any kind, and sites that show temporal variation do not fit the predicted pattern.

While the idea of larvae settling in the hybrid zone originating from other pure or nearly pure *Mytilus edulis* populations in the region is intriguing, the evidence of potential sources is severely lacking. Populations of mussels found within estuaries that are within the borders of the hybrid zone have high frequencies of *M. edulis*-specific alleles that are not significantly different from

those observed in the spat from hybrid zone sites (Hilbish et al. 2003). But in order for these estuaries to be the source of nearly all of the larvae settling within the hybrid zone, the populations would have to be reproductively active in the late spring and early summer months. Samples from the 2 largest estuaries within the hybrid zone, the Tamar and the Fal, did not show a build up of mature gametes in the late spring followed by a decline in GVF (data not shown). This suggests that their reproductive schedules are much different from those observed in coastal populations of the hybrid zone and much different from when larval settlement actually occurs. A potential source population would also have to be numerically superior to the hybrid zone population in order to effectively swamp out the genetic signature of the local adults. This is certainly not true of the estuarine populations and no significant sub-tidal populations are known from this region (Richardson 2005).

Therefore, it appears safe to conclude that the elevated *Mytilus edulis*-specific allele frequency observed in spat settling within the hybrid zone is not due to immigration from a *M. edulis* source population. This would suggest that the larvae settling within the hybrid zone are retained, but the allele frequencies are being modified from what the adult population would be expected to produce. Specifically, the frequency of *M. edulis*-specific alleles is increasing drastically which suggests selection of some form.

Interestingly, selection favoring *Mytilus edulis* alleles in the larval stage within the hybrid zone would be in direct opposition to the selective patterns observed in the adults. Adult mussel populations within the hybrid zone typically show patterns of selection favoring individuals with *M. galloprovincialis*-specific alleles (Skibinski 1983, Hilbish et al. 2002). There is some evidence that genotype-specific differences in mussel movement within a bed and differences in shape and attachment strength may result in the higher mortality of *M. edulis*-like adults within the hybrid zone (Schneider et al. 2005). Since the elevation of *M. edulis*-specific alleles observed in the present study occurs prior to settlement it is obvious that a different selective factor is involved. There are several possibilities that could be tested. One possibility is that fertilization success is not equivalent in all hybrid crosses. For example, if backcrosses to *M. edulis* are more successful than most other hybrid crosses the frequency of *M. edulis*-specific alleles would increase. Differences in survival during development are also possible, and this could take a variety of forms. If development is more successful in situations where *M. galloprovincialis* alleles at certain loci interact better in a predominantly *M. edulis* genetic background than do *M. edulis* alleles in a *M. galloprovincialis* background, then *M. edulis*-like individuals will tend to develop more often than *M. galloprovincialis*-like larvae. Genotype-specific predation differences or differential settlement success are other possibilities. Unfortunately, no data is currently available to test these hypotheses.

The one population that appears to consistently contain significant proportions of immigrants is the *Mytilus galloprovincialis* population. Individual settlement events in all 3 years contain significantly higher frequencies of *M. edulis*-specific alleles than can be produced by the local adult population suggesting they originated from the hybrid zone. In 1999 these immigrants are effectively swamped out by local larvae when data are pooled, but in both 1998 and 2000 the immigrants are still apparent in pooled data. This signature is most apparent in 2000 when the patterns of larval settlement differ the most from previous years. In both 1998 and 1999 settlement begins at *M. galloprovincialis* sites in August whereas in 2000 settlement begins more than 1 mo earlier. This early settlement in 2000 also appears to be out of line with the gamete volume fraction data for that year. Given the timing of settlement in 2000 and the patterns of reproduction of the 3 populations, we would predict that earlier settlements should contain high proportions of immigrants from the hybrid zone. This is definitely the case in 2000 where early settlements often had *M. edulis*-specific allele frequencies of >0.3. Significantly higher frequencies of *M. edulis*-specific alleles were also observed in the earlier settlement events at *M. galloprovincialis* sites in 1998, especially when data were pooled across sites. Significant deviations from Hardy-Weinberg equilibrium due to a lack of heterozygotes are also suggestive of a Wahlund effect due to population mixing. The consistency of these patterns with predictions based on the timing of reproduction suggests that the allele frequency patterns observed in primary spat from *M. galloprovincialis* sites are due to an influx of larvae from the hybrid zone.

The patterns of migration into the *Mytilus galloprovincialis* population suggest that migration events are sporadic and of differing intensities. In every year there are certain sites that appear to receive migrants while others do not. The same is true from a temporal perspective in that not all sites receive significant numbers of migrants on the same date. The 2-population admixture model using allele frequencies of pooled primary spat collected from *M. galloprovincialis* sites in the same year suggests that spat from these sites typically contain larger proportions of migrants than do the *M. edulis* sites. Assuming the hybrid zone contributes spat with a 0.71 *M. edulis*-specific allele frequency (Gilg & Hilbish 2003a) gives migrant proportions of 0.048, 0.000 and 0.246 in 1998, 1999 and 2000, respectively. When individual samples are considered the proportion of migrants can be as high as 0.435 (NQ 29 June 2000) or as low as 0.000 (in non-

significant settlements) where sample sizes are large enough to be reliable.

The observed settlement and allele frequency patterns seem consistent with the idea of pods of larvae from a common source dispersing and eventually settling out together. In 4 of 6 cases, multiple sites in the *Mytilus galloprovincialis* population contained primary spat with elevated *M. edulis*-specific allele frequencies on the same collection date. This pattern suggests that the pods of immigrating larvae can be large in size, potentially spanning 10s of km; alternatively, settlement patterns may result from smaller pods that consistently deliver larvae as they travel along the shore. Additionally, if conditions are right for delivery of larvae from the hybrid zone to a site within the *M. galloprovincialis* population then the proportion of migrants can be very high, as observed at several sites in 2000 and at TV in 1998. When conditions do not favor on-shore delivery of migrant larvae then settlers tend to be locally produced.

The patterns of larval import and retention are consistent with data generated from a hydrographic model in previous studies on this system (Gilg & Hilbish 2003a,b). Projections of this model show bidirectional movement of particles released from *Mytilus edulis* sites, but that these particles do not cross Start Point so they are effectively blocked from entering the hybrid zone (Fig. 1). Prevailing currents in the eastern portion of the hybrid zone move particles eastward; a small proportion of these particles cross Start Point and disperse into the *M. edulis* population, but most are advected offshore at Start Point. Currents in the western portion of the hybrid zone tend to move to the west and a fairly large proportion of particles disperse into the *M. galloprovincialis* population. However, many of the particles dispersing from the hybrid zone into the *M. galloprovincialis* population terminated well offshore suggesting that most hybrid larvae that disperse in the direction of the *M. galloprovincialis* population are likely lost, but certain conditions can allow for pods of larvae to disperse near shore. Particles released from *M. galloprovincialis* sites almost always move to the northeast along the coast of Cornwall.

It should also be noted that the present study, as well as previous work, provides no evidence for differences in settlement preferences between these 2 species and their hybrids. Within a region, allele frequencies of spat do not correspond to habitat type, such as open coast or embayment habitats. Instead they are typically consistent across sites within a region unless affected by immigration. Even in cases where spatial variation exists, like at some hybrid zone sites, both *Mytilus edulis*-specific and *M. galloprovincialis*-specific alleles are found in spat at all sites. Additionally, there are no differences in allele frequencies of primary and secondary spat collected from each site, suggesting that there is no genotype-specific pattern of movement after primary settlement. Previous work on this system has also shown that allele frequencies of spat collected within the hybrid zone are the same as those observed in adult populations in estuaries within the bounds of the hybrid zone (Hilbish et al. 2003), and that allele frequencies do not differ among spat collected at different tidal heights (Gilg & Hilbish 2000). Therefore, none of the observed patterns can be explained by differences in settlement preferences between the 2 species.

The results of the present study, therefore, suggest that the abrupt genetic edges between the hybrid zone and each of the 2 parental populations are likely maintained by different forces. The edge between the *Mytilus edulis* population and the hybrid zone appears to be primarily due to a lack of migration across the edge, since movement of hybrid larvae into the *M. edulis* population tends to be limited and evidence for migration of any mussel larvae from the *M. edulis* population into the hybrid zone is weak. This is likely due to a hydrodynamic barrier around Start Point that prevents significant movement of larvae in either direction and leads to nearly complete larval retention in both cases.

The opposite edge of the hybrid zone bordering the *Mytilus galloprovincialis* population, however, is often crossed by larvae originating from the hybrid zone. In order to maintain the low frequencies of *M. edulis*-specific alleles in this population the consistent influx of larvae must be countered by another force. It would appear that selection favoring individuals with *M. galloprovincialis*-like genotypes is necessary and would need to occur soon after settlement since even the smallest juvenile size classes at *M. galloprovincialis* sites have *M. edulis*-specific allele frequencies of ≤ 0.05 (Hilbish et al. 2002). On the other hand, the lack of evidence for movement of larvae from the *M. galloprovincialis* population into the hybrid zone again suggests the presence of a significant hydrodynamic barrier around Lands End. This barrier is consistent with the results of the previously described hydrographic model which shows currents consistently pushing northward along the coast of Cornwall (Gilg & Hilbish 2003b).

Caution should be taken, however, in some of the interpretation since the present study relied on the use of a single genetic marker. Factors such as selection can lead to incorrect estimates of dispersal rates. For example, in the present study we hypothesize that selection is favoring the production of higher *Mytilus edulis*-specific allele frequencies in larvae produced within the hybrid zone. If this is correct, then our estimates of dispersal rates from the hybrid zone into the adjacent pure populations will need to be adjusted. Assuming the hybrid zone produces larvae with a *M. edulis*-specific allele

frequency signature of 0.9 instead of 0.71 (see above) would lead to a higher estimate of dispersal rate from the hybrid zone into the *M. edulis* population and a lower estimate of dispersal into the *M. galloprovincialis* population. Larval import would still tend to be greater into the *M. galloprovincialis* population than into the *M. edulis* population but the specific numbers would change.

While the present study provides evidence for substantial larval retention in 2 of the 3 patches investigated, it is not effective at addressing questions of connectivity of sites within each patch. For example, even though there is little evidence supporting the import of larvae from either bordering parental population into the hybrid zone, there may be significant larval exchange among sites within the hybrid zone. Unfortunately, we are not currently able to address this question. Mussel populations within each of the 3 regions investigated are genetically similar to each other and have similar reproductive schedules. Therefore, it is impossible to discern the specific location of origin of spat collected from any site within the same patch using the current method. This leads to the possibility of open exchange of larvae over distances >100 km (even though most dispersal estimates are considerably less than that) within each of these patches, punctuated by sharp barriers to dispersal at key locations. Other techniques, such as elemental fingerprinting, may be more useful to address this question as long as location-specific elemental signatures differ at a finer scale than the genetic structure (Becker et al. 2007).

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LITERATURE CITED

Bayne BL (1965) Growth and delay of metamorphosis of the larvae of *Mytilus edulis* L. Ophelia 2:1–47

Becker BJ, Levin L, Fodrie FJ, McMillan PA (2007) Complex larval connectivity patterns among marine invertebrate populations. Proc Natl Acad Sci USA 104:3267–2372

Daguin C, Bonhomme F, Borsa P (2001) The zone of sympatry and hybridization of *Mytilus edulis* and *M. galloprovincialis*, as described by intron length polymorphism at locus *mac-1*. Heredity 86:342–254

Gilg MR, Hilbish TJ (2000) The relationship between allele frequency and tidal height in a blue mussel hybrid sone: a test of the differential settlement hypothesis. Mar Biol 137:371–378

Gilg MR, Hilbish TJ (2003a) Dispersal patterns of mussel larvae throughout a hybrid zone in Southwest England. Evolution 57:1061–1077

Gilg MR, Hilbish TJ (2003b) Geography of marine larval dispersal: Coupling genetics with fine–scale physical oceanography. Ecology 84:2989–2998

Hellberg ME, Burton RS, Neigel JE, Palumbi SR (2002) Genetic assessment of connectivity among marine populations. Bull Mar Sci 70:S273–290

Hilbish TJ, Carson EW, Plante JR, Weaver LA, Gilg MR (2002) Distribution of *Mytilus edulis*, *M. galloprovincialis*, and their hybrids in open-coast populations of mussels in southwest England. Mar Biol 140:137–142

Hilbish TJ, Timmons J, Agrawal V, Schneider K, Gilg MR (2003) Estuarine habitats protect hybrid mussels from selection. J Exp Mar Biol Ecol 292:177–186

Inoue K, Odo S, Noda T, Nakao S, Takeyama S, Yamaha E, Yamazaki F, Harayama S (1997) A possible hybrid zone in the *Mytilus edulis* complex in Japan revealed by PCR markers. Mar Biol 128:91–95

Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions. Integr Comp Biol 46:282–297

McQuaid CD, Phillips TE (2000) Limited wind-driven dispersal of intertidal mussel larvae: *in situ* evidence from the plankton and the spread of the invasive species *Mytilus galloprovincialis* in South Africa. Mar Ecol Prog Ser 201: 211–220

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics, 2nd edn. Sinauer Associates, Sunderland, MA

Palumbi SR (2001) The ecology of marine protected areas. In: Bertness M, Gaines SD, Hay ME (eds) Marine ecology: the new synthesis. Sinauer Associates, Sunderland, MA, p 509–530

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. Ecol Appl 13: S146–158

Rawson PD, Joyner KL, Meetze K, Hilbish TJ (1996) Evidence for intragenic recombination within a novel genetic marker that distinguishes mussels in the *Mytilus edulis* species complex. Heredity 77:599–607

Richardson K (2005) Application of surface ocean remote sensing to the characterization of the geographic patterns of benthic fauna in a temperate shelf sea. PhD thesis, University of Plymouth, Plymouth Marine Laboratory

Schneider KR, Wethey DS, Helmuth BST, Hilbish TJ (2005) Implications of movement behavior on mussel dislodgement: exogenous selection in a *Mytilus* spp. hybrid zone. Mar Biol 146:333–343

Secor CL, Day AJ, Hilbish TJ (2001) Factors influencing differential mortality within a marine mussel (*Mytilus* spp.) hybrid population in southwestern England: reproductive effort and parasitism. Mar Biol 138:731–739

Skibinski DOF (1983) Natural selection in hybrid mussel populations. In: Oxford GS, Rollinson D (eds) Protein polymorphism: adaptive and taxonomic significance. The Systematics Association. Academic Press, London, p 283–298

Sokal RR, Rohlf FJ (1981) Biometry. WH Freeman, New York

Strathmann RR (1985) Feeding and nonfeeding larval development and life-history evolution in marine invertebrates. Annu Rev Ecol Syst 16:339–361

Thorson G (1950) Reproduction and larval ecology of marine bottom invertebrates. Biol Rev 25:1–45

Veliz D, Duchesne P, Bourget E, Bernatchez L (2006) Stable genetic polymorphism in heterogeneous environments: balance between asymmetrical dispersal and selection in the acorn barnacle. J Evol Biol 19:589–599

Warner RR, Cowen RK (2002) Local retention of production in marine populations: evidence, mechanisms, and consequences. Bull Mar Sci 70:S245–S249

Wilhelm R, Hilbish TJ (1998) Assessment of natural selection in a hybrid population of mussels: evaluation of exogenous vs. endogenous selection models. Mar Biol 131:505–514

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