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SELECTION DURING LARVAL RECRUITMENT IN A BLUE MUSSEL HYBRID ZONE

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ABSTRACT

The processes of recruitment in marine bivalves and their larvae can be caused by process that occur before, during, or after these organisms settle. The mussel species *Mytilus edulis* and *M. galloprovincialis* form a hybrid zone located in southwest England. Individuals with hybrid genotypes settle in the *M. galloprovincialis* parental populations, but the hybrid zone does not appear to be expanding. Comparisons of allele and genotype frequencies of recently settled mussels from different age classes in the *M. galloprovincialis* population show a marked decrease in *M. edulis*-specific alleles and hybrid genotypes. These data demonstrate that there is likely selection acting against individuals with *M. edulis* alleles that settle in the *M. galloprovincialis* population.

Key words**:** Hybrid zone, *Mytilus edulis, M. galloprovincialis*, larval recruitment, marine mussels, England

Marine mussels have very complex life histories where in their larval stage they often develop in the plankton for several weeks to more than two months, depending on water temperature, salinity, and food availability (Bayne 1965). During this stage they are transported as passive particles with the ocean currents giving them the potential to disperse over long distances (Gilg and Hilbish 2003b). Even in the face of this dispersal, this occurrence has shown limited genetic differentiation over long distances (Slatkin 1981), but in other situations, this dispersal has shown relatively high levels of genetic differences over many spatial scales (Koehn et al. 1973, 1976; Johnson and Black 1984; Gosling and McGrath 1990; Watts et al. 1990). Studies have proposed that these different patterns of spatial genetic variation could be due to either post-settlement selection or disruption of dispersal by current or other geographical barriers (Gilg and Hilbish 2003c).

Hybrid zones are locations where two diagnostically distinct populations meet and interbreed producing offspring of mixed ancestry (Arnold 1997). These areas allow for tracking of different genotypes over space and time providing estimates of their rate of dispersal and of any selective processes that might be occurring over an organism's life history. The stability of a hybrid zone is partially determined by the patterns of dispersal throughout the zone of contact and the type of selection occurring in the region. Two species of blue mussels, *Mytilus edulis* and *M. galloprovincialis,* form a hybrid zone in southwest England along the coasts of Devon and Cornwall. In this region there are three genetically distinct populations of mussels including pure populations of both *M. edulis* and *M. galloprovincialis* bordering either side of a population of mussels with mixed ancestry that extends nearly 200 km (Hilbish et al. 2002). Within the hybrid zone, mussels with *M. galloprovincialis*-like genotypes are favored over those with *M. edulis*-like genotypes and mussels with intermediate genotypes have intermediate fitness (Skibinski, 1983; Wilhelm and Hilbish, 1998). From genetic analysis of recent settlers and hydrographic projections around southwest England, some larvae originating in the hybrid zone are known to settle in both of the bordering parental populations, but the parental populations do not move into the hybrid zone, demonstrating an asymmetrical dispersal pattern (Gilg and Hilbish 2003a). Even with this dispersal of hybrid larvae into the pure parental populations, the allele frequencies observed in the parental populations have remained stable and the borders of the hybrid zone have

remained stable for over twenty years (Skibinski 1983; Hilbish et al. 2002).

The question now arises of how the parental populations remain distinct in the face of this dispersal, even though gene flow should result in expansion of the hybrid zone. We hypothesize that habitat-specific selection favoring each of the pure parental populations is occurring in the regions that they occupy. Based on a decrease in non- parental alleles as older age classes are analyzed. For this study we will focus on the *M. galloprovincialis* population and should see a loss of *M. edulis* alleles as these individuals age.

METHODS

Collection and measurement

Recently settled mussel larvae (spat) and one year old juveniles (1YO) were collected between the years 1998-2000. Only sites located in the *M. galloprovincialis* parent population were analyzed including Portreath (PT), Trevaunance (TV), Newquay (NQ), and Port Quin (PQ) (Gilg and Hilbish 2003c). Each mussel was measured under a dissecting microscope using an ocular micrometer to determine its size. Individuals were separated into age classes based on these measurements and the date of their collection. Comparisons of size-frequency distributions on adjacent dates were used to determine growth rates (μm/day) for each site to establish a numerical standard of separating spat versus 1YO on dates where both classes were present (Fig. 1; Table 1). Age classes were determined by adding the maximum growth rate to the maximum size class of the first observed settlement for all dates afterward. For instance, past research has shown that settlement of spat occurs in August and when looking at settlement in the TV site during August 9, 1998, we see a marked increase of smaller settlers during that time (Figure 1). The average size of the individuals will then be calculated and would be used to determine what the approximate size of the same individual should be a week, month, etc. down the line.

Genetic Analysis and Comparisons

Individual mussels were then digested to extract the whole genomic DNA using a simple lysis buffer with Proteinase K (Gilg and Hilbish, 2000). Each individual 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. Those same samples were also genotyped with DAMP 1, which has a 10 bp insertion/ deletion polymorphism, to determine if a similar trend is apparent at another locus (Bierne 2002). Allele and genotype frequency changes within a cohort were measured by comparisons between the observed frequencies of spat and those of 1YO-juveniles from the same location collected the following year. Specifically, spat from 1998 were compared to 1YO's of 1999 and spat from 1999 were compared to 1YO's from 2000. A G-Test of independence was utilized to determine the statistical difference in allele and genotype frequencies between the age classes.

RESULTS

After the analysis of the growth rates, most of the sites showed a growth rate between 5 and 7 μm per day and the largest growth rate was 18μm per day. Overall, mussels that were classified as spat were <1mm in size and 1YO were ≥1mm in size. Looking at the Glu 5' comparisons, in all cases, spat in the year 1998 had *M. edulis* allele frequencies that were greater than the 1YO from 1999 (see Fig. 2). This allele frequency difference was significant at two of the *M. galloprovincialis* sites. TV had a $P<0.001$ and PQ had a $P<0.05$. PT was marginally significant, and had a $P<0.06$. A similar trend was observed at all other sites in the 1999 and 2000 cohort, but the allele frequencies were not significantly different (see Fig 2). Comparisons of spat genotype frequencies in the year 1998 to 1YO in 1999 show a significant decrease in the frequencies of both homozygous (EE) and heterozygous

(EG) genotypes with *M. edulis*- specific alleles, noting a P <0.001 (see Fig. 3). A similar trend could be seen in the second cohort, but the difference was not significant (see Fig. 3). Analysis of the TV site using the DAMP 1 locus demonstrated a significant difference between spat and 1YO for the 1998 and 1999 comparisons with a P <0.001 (see Fig. 4).

DISCUSSION

There appears to be selection against individuals with *M. edulis* specific alleles in the *M. galloprovincialis* parental population. The data shows consistently higher *M. edulis* allele frequencies and genotype frequencies in spat versus 1YO mussels in both cohorts, even though it is not always significant in each individual case. This decrease in *M. edulis* allele frequencies suggests that selection was acting against *M. edulis*-like larvae that settle in the *M. galloprovincialis* population. It appears that EE and EG genotypes are both at a disadvantage since their genotype frequencies decreased. It appears that selection removes most of the individuals with *M. edulis* alleles in the first year once they settle into the *M. galloprovincialis* population. Even though selection favoring *M. galloprovincialis*-like mussels is typically observed within the hybrid zone, the selection is not usually observed until the mussels are 3-4 years old (Wilhelm and Hilbish, 1998). Therefore, not only does it appear that selection occurs within the *M. galloprovincialis* populations, but it occurs much earlier than in the hybrid zone. Since most hybrid mussels that settle within the *M. galloprovincialis* population are dying prior to attaining reproductive age, this selection essentially prevents the hybrid zone from expanding and keeps the *M. galloprovincialis* population distinct. The allele frequencies of 1YO's are consistent with what is observed in adults at those sites (Hilbish et al., 2002).

Possible explanations for this selection suggest that the lower attachment rate of *M. edulis* compared to *M. galloprovincialis* could be a leading factor. Therefore, when *M. edulis* type individuals migrate to the *M. galloprovincialis* population, they have a higher dislodgement rate and hence a higher mortality rate (Schneider et al. 2005). Other possible explanations for this decrease in *M. edulis* allele frequency is that the settlement of all the *M. galloprovincialis* individuals is not complete and that further settlement occurs after the month of October. This is important to note since samples were not collected after the month of October for this study. If that were the case, and looking at the 1998-1999 cohort at significant sites, there would have to be a large number of settlement of *M. galloprovincialis* individuals occurring after the month of October to result in the allele frequencies seen in 1999. For instance, in the TV site that had a P <0.001, there would have to be 1,904 *M. galloprovincialis* alleles or 89% of the settlement occurring after the month of October to equal the *M. edulis* allele frequency of .0157 seen in 1YO of 1999. This would be from the original 230 *M. galloprovincialis* alleles that were already there at the beginning of settlement. For the PQ site, 443 or 71% of the *M. galloprovincialis* alleles need to be present after October to equal the 0.0221-drop in *M. edulis* frequency that originally had 178 *M. galloprovincialis* alleles. While for PT, there would have to be 346 or 60% of the *M. galloprovincialis* alleles added to the original 234 alleles. For this particular scenario, the number of *M. edulis* would not be varied, however in all cases the amount of new settlers would have to be great to offset the allele frequencies seen in 1999. This demonstrates that a majority of settlement does occur before October and that our predictions were valid. Processes of recruitment also seem to be apparent in another marine organism, the abalone (*Haliotis iris*). In the larval stage research has shown that their rate of settlement and growth is based on five species of coralline algae that these larvae decide to settle upon. All five species of algae that this study focused on were effective inducers of *H. iris* larval attachment however the selection of certain substrates would either increase or decrease

the rate of settlement and growth of these organisms (Roberts et al., 2004).

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FIGURE LEGEND

Figure 1: Size/ frequency distributions of recently settled mussels used in determination of growth rates.

Figure 2: Allele frequency comparisons between spat and one year (1 YO) mussels collected from the same site using Glu 5' marker.

Figure 3: Genotype frequencies of spat and 1YO mussels from two cohorts. Genotype frequencies were combined over all sites using Glu 5' marker.

Figure 4: Allele frequency comparisons between spat and 1 YO mussels collected from the same site using DAMP 1 marker.

TABLES

Table 1: Size class restrictions for different dates

Date	Spat	1 YO
	1998	
Pre-8/9	None	NA
Post-8/9	≤1000µm	NA
	1999	
Pre-8/31	-----	All
8/31	≤750µm	≥1000µm
9/13	≤1000µm	≥1100µm
9/26	≤1000µm	≥1300µm
Post-9/26	≤1000µm	-----
	2000	
Pre-7/3	NA	All
7/3	NA	≥900µm
7/20	NA	≥1050µm
7/31	NA	≥1050µm
8/15	NA	≥1350µm
Post-8/15	NA	-----

FIGURES

Figure 1

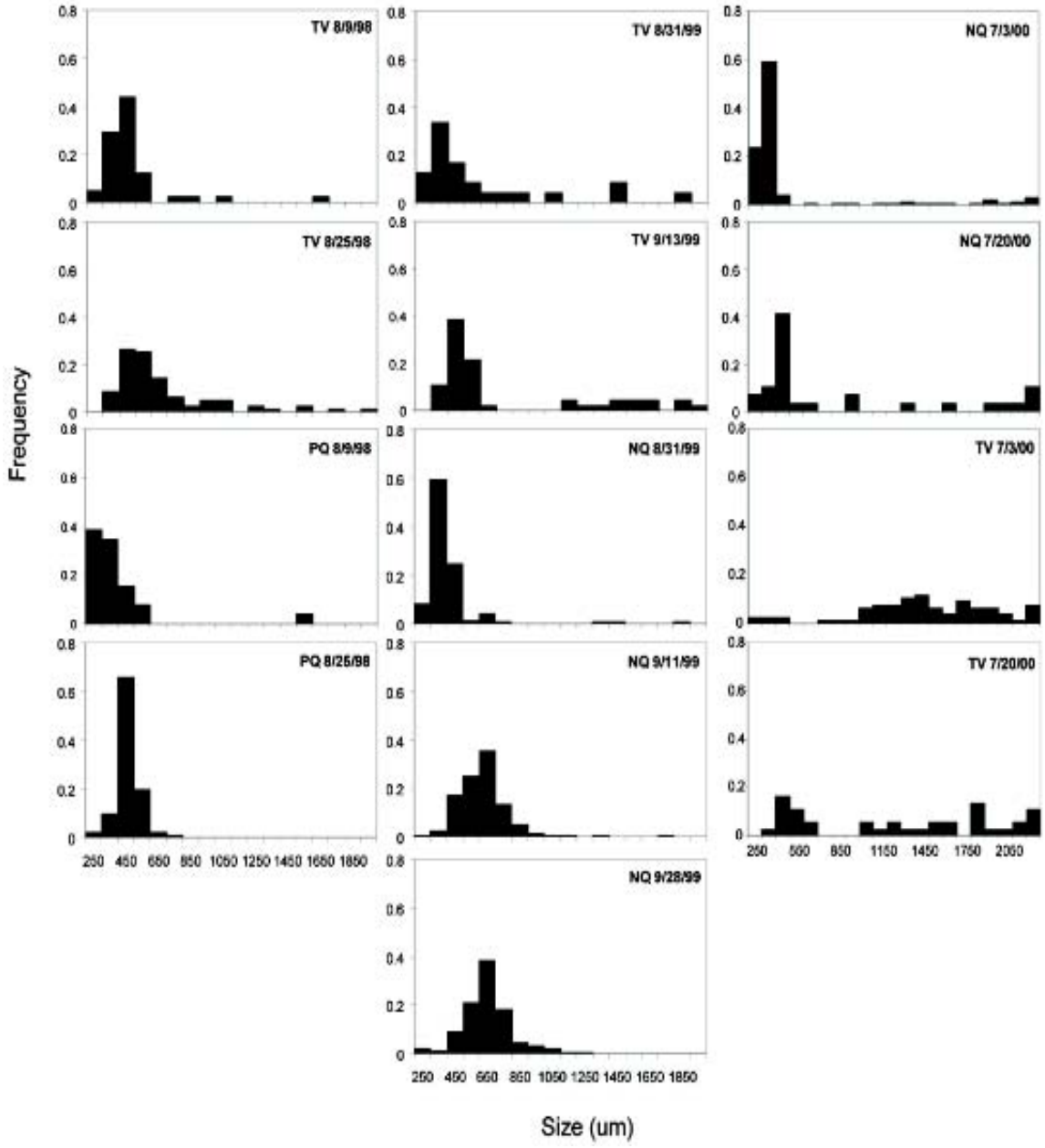

Figure 2

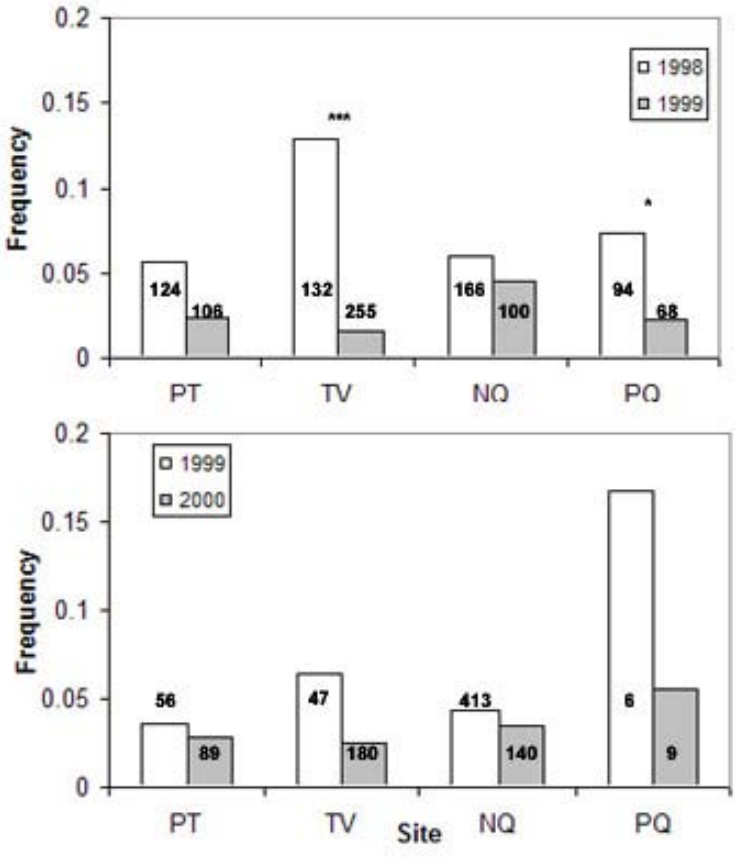

Figure 3

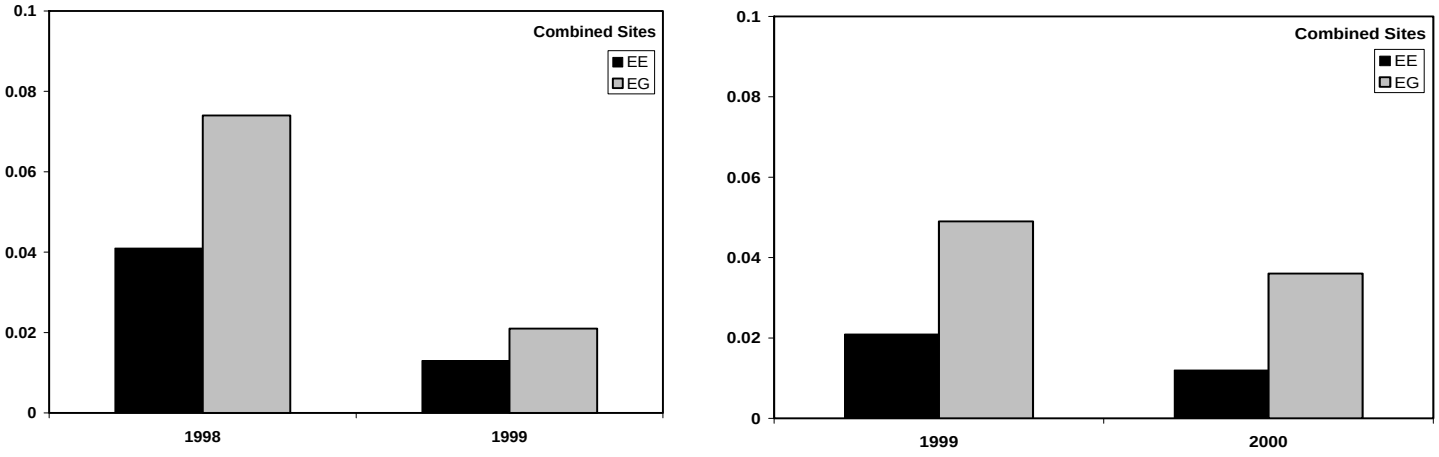
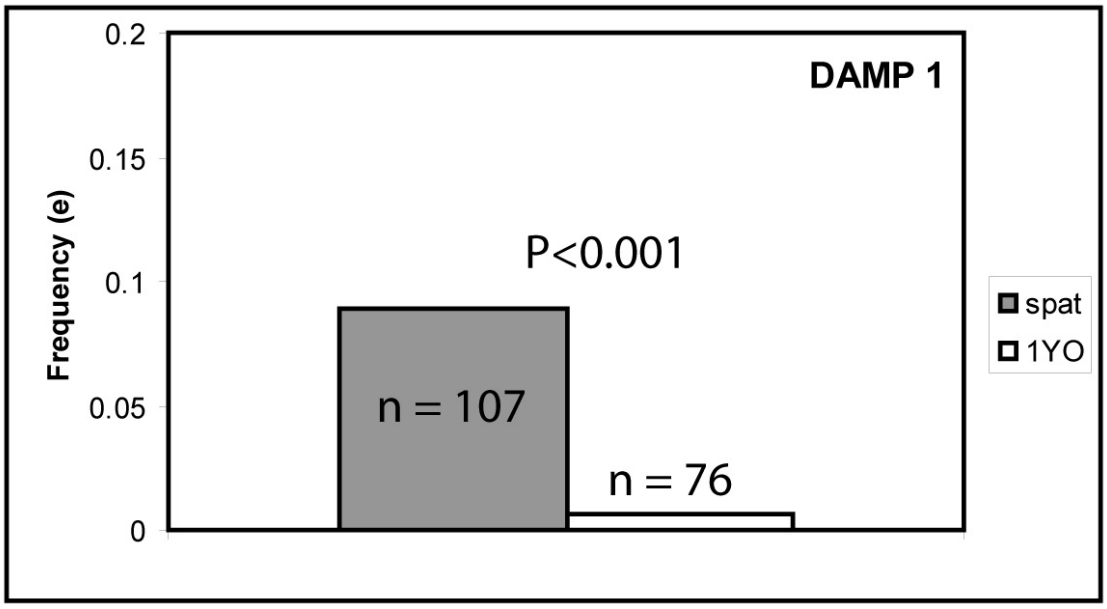
Figure 4