Cross-year comparison of gene diversity indices in *Mytilus galloprovincialis* samples from Corrubedo beach
(Impact on biological systems)

A. LLAVONA, T. LADO, A. SEOANE, A. P. DIZ, M. PÉREZ, and P. PRESA
Dep. Biochemistry, Genetics and Immunology, University of Vigo, Faculty of Biology, Genetics 36310 Vigo, Spain

ABSTRACT

Gene diversity levels were analysed in a mussel population of *M. galloprovincialis* sampled at an intertidal zone of Corrubedo beach, just after the Prestige spill (January 2003). Changes in gene diversity, putatively due to the spill, were estimated by comparing that affected sample with two other samples taken at the same site both, long before (year 2000) and long after (year 2005) the spill. The absence of significant shifts either in heterozygosity or in allelic richness between year samples, suggests that the spill oil has not significantly modified the genetic background of mussels from the Corrubedo intertidal zones sampled.

INTRODUCTION

It is of general concern that the genetic diversity of living resources determines, a) the production efficiency, due to the positive correlation between heterozygosity and fitness (especially high in molluscs), b) the adaptive potential of the species, and c) the genetic background which genetic improvement can progress on. The genetic erosion of a living resource is an irreversible threat due to the homogenization of genomes, and is caused by both, natural events (such as exotic invasions and environmental shifts) and artificial phenomena (such as intensive culturing, overexploitation, rarefaction of ecological chains, and anthropogenically-driven catastrophes) (Diz and Presa, 2003). The systematic monitoring of the genetic diversity of mussel populations allows for the detection of significant genetic changes, which are useful to maintain and improve the optimal genetic status of the species (Presa and Pérez, 2003). The affordability of appreciating gene diversity changes requires at least two priors: a) the availability of genetic data from mussel populations collected before the spill in the same site, and b) the availability of informative genetic tools to undertake the analyses with.

In this study we aimed to assess the putative impact of the Prestige spill-oil on the genetic diversity of a natural mussel population of *Mytilus galloprovincialis* from Corrubedo beach, one most affected sites. Genetic data have been recorded in years 2000 (long before spill), 2003 (just after the spill) and 2005 (long after the spill). DNA from fifty individuals from each three samples (Figure 1) was extracted using a modified phenol:chloroform protocol (Presa and Diz 2005, submitted). The genetic variation of microsatellite markers *Mgu1* and *Mgu2* (Presa et al., 2002) was PCR-amplified and the amplicons were electrophoresed in an ALFexpress II automatic sequencer. The parameters used to detect genetic shifts of allelic frequencies were: allele richness (*Rs*), observed heterozygosity (*Ho*), expected heterozygosity (H_S), and Wright's fixation indices (F_{IS} and F_{ST}). The computer programme Fstat 2.9.3.1 (Goudet 1995) was used to calculate those parameters. The null hypothesis of genetic homogeneity across years was tested through pairwise comparison tests (1,000 permutations).

Figure 1: Sample site of *Mytilus galloprovincialis* population.

RESULTS AND DISCUSSION

The genetic parameters measured on Corrubedo population across years are shown in figure 2. A large heterozygote deficit was observed in all three samples (*FIS* values, $P < 0.01$), indicating that this population might not be in Hardy-Weinberg equilibrium. However all year samples showed such heterozygote deficit, which is otherwise a common phenomenon of mussel populations not well understood. In our case, it could be partially due to null alleles segregating at the loci analysed or to a low sample size, that must be addressed in further studies.

Non-significant differentiation values were observed between years ($F_{ST} = 0.003$, $P = 0.402$), and the genetic variance within samples (0.600) was far larger than among samples (0.012). Allelic richness and expected heterozygosity are commonly used in managed populations to maximize gene diversity. Expected heterozygosity (*Hs*) and allele richness (*Rs*) were higher in years 2000 and 2003 than in the 2005 year sample (Figure 3), what could be due to a post-spill erosion effect. However, those differences were non-significant among years for any of those parameters ($F = 1.65$, $P = 0.081$, and $F = 1.35$, $P = 0.082$, respectively).

Sample-year	R_S	H_O	H_S	F_{IS}
2000	12.83	0.638	0.875	0.272
2003	13.13	0.457	0.893	0.489
2005	11.98	0.535	0.842	0.365
P-value	0.081	0.082	0.193	0.210

Figure 2: Genetic divergence between year samples from Corrubedo beach.

Expected heterozygosity fluctuates with gene frequencies and is less sensitive to the allelic composition than *Rs* (Figure 2). While heterozygosity is proportional to the genetic variance, and should be correlated with a negative effect of the spill, the threshold of genetic response over several generations is determined by the initial genetic composition and is independent of the allelic frequencies (James, 1971). Therefore it is commonly accepted that allele richness is more relevant to genetic management than allelic frequencies. In this sense, the absence of a significant shift of allelic richness (R_S) between year samples observed in this preliminary study, suggests that the spill did not significantly modify the genetic background of mussels from the Corrubedo site sampled. Analyses in progress, using larger sample sites and sizes, as well as more microsatellite loci, will provide a deeper insight into the across-year genetic variation of spill-affected mussel populations in Galicia. This genetic data would be helpful in decision-making process related to the assessment of the spill impact, and therefore in conservation acts aimed at maintaining the genetic diversity of the species (Diz and Presa, 2005).

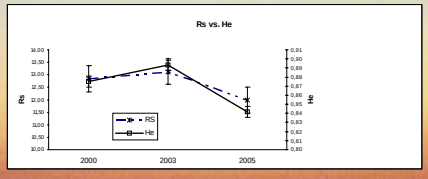

Figure 3: Comparison of allelic richness (R_S) and expected heterozygosity (H_e) of mussel populations from Corrubedo beach, sampled before, during, and after the spill.

REFERENCES

Diz A. P. and Presa P. (2005) Detection of first generation immigrants and introgression in *Mytilus* spp. using multilocus-based algorithms. *Submitted*.
Diz A. P. and Presa P. (2003) Homogenising effects of marine currents on the gene pool of *Mytilus galloprovincialis* from Northwest Iberian estuaries. *Thalassas: An International Journal of Marine Sciences*, 19 (2b): 101- 103.
Goudet J. (1995) Fstat (vers. 2.9.3), a computer program to calculate F-statistics. *Journal of Heredity* 86, 485-486.
James J. W. (1971) The founder effect and response to artificial selection. *Genetical Research* 12, 249-266.
Presa P. Perez M. and Diz A. P. (2002) Polymorphic microsatellite markers for blue mussels (Mytilus spp.). *Conservation Genetics*, 3(4): 441-443.
Presa P. and Pérez M. (2003) *Mytilus galloprovincialis* microsatellites to apply in authenticity testing of commercial produces. *In: Authenticity of species in meat and seafood products*. Ed. Ricardo I. Pérez Martin and Carmen G. Soleto. Gráficas Chapela, Vigo DLVG641-03, pp 237-244.

ACKNOWLEDGEMENTS

This work is funded by Xunta de Galicia through grant PGIDIT04RMA312002PR and by predoctoral grants from U. Vigo and MEC to A. Llavona and T. Lado respectively.