polymorphic, whereas the *L. truncatula* primer was monomorphic. Locus LAURMIC1 had 14 different alleles and locus LAURMIC2 had 18 alleles. A total of 79 individuals were analysed for each locus and the overall observed (H_o) and expected heterozygosity (H_l)[11] was calculated (Table 1) using Fstat, version 2.9.3.[12]

Although the applied enrichment procedure was specifically designed to enrich for microsatellites, the sequenced fragments often contained minisatellites, highly complex longer stretches of repetitive DNA.[13] Similar patterns of inadvertent minisatellite isolation have been observed in the snails *Bulinus obtusispira*[14], *Physa acuta* (P. Jarne, personal communication), *Buccinum undatum* and *Potamopyrgus antipodarum* (D. Weetman, personal communication) and the freshwater bivalve *Utterbackia imbecilis* (J. P. Curole, personal communication). Application of minisatellites is attractive as genotyping of individuals can be achieved on basic agarose gels, without the need of expensive and time-consuming labelling. However, minisatellite evolution is poorly understood (but see [13,15]) and may thus pose analytical difficulties. Moreover, the minisatellites we analysed were monomorphic, confirming theoretical predictions that minisatellites are less variable than microsatellites.[16]

In conclusion, we isolated two useful and highly polymorphic microsatellite loci for *L. auricularia*. The three minisatellite primer sets we developed did amplify, but the loci were monomorphic in our samples. Minisatellites may prove more useful in detecting variation between species.

Our attempt to use primers from the closely related species *L. truncatula* did not yield usable results as only one of the six primer pairs amplified and, unfortunately, this locus was monomorphic in our samples. This underscores the importance of species-specific development of genetic tools such as microsatellite primers in population genetics.

The authors would like to thank Katya Mamonova, Tanya Sitnikova, Hans Breeuwer, Dirk Erpenbeck, Peter Kuperus, Patrick Meirmans and Dmitry Sherbakov for technical assistance.

REFERENCES

1. JARNE, P. & LAGODA, P.J.L. 1996. *Trends Ecol. Evol.*, **11**: 424–429.
2. HUGHES, C.R. & QUELLER, D.C. 1993. *Mol. Ecol.*, **2**: 131–137.
3. STIFT, M., MICHEL, E., SITNIKOVA, T.YA., MAMONOVA, E. YA & SHERBAKOV, D. YU. 2003. *Hydrobiologia*, in press.
4. TROUVÉ, S., DEGEN, L., MEUNIER, C., TIRARD, C., HURTREZ-BOUSSES, S., DURAND, P., GUEGAN, J.F., GOUDET, J. & RENAUD, F. 2000. *Mol. Ecol.*, **9**: 1662–1664.
5. BARGUES, M.D. & MAS-COMA, S. 1997. *Mol. Biol. Evol.*, **14**: 569–577.
6. WINNEPENNINCKX, B., BACKELJAU, T. & DEWACHTER, R. 1993. *Trends Genet.*, **9**: 407–407.
7. ZANE, L., BARGELLONI, L. & PATARNELLO, T. 2002. *Mol. Ecol.*, **11**: 1–16.
8. KIJAS, J.M.H., FOWLER, J.C.S., GARBETT, C.A. & THOMAS, M.R. 1994. *Biotechniques*, **16**: 656–662.
9. SAMBROOK, J., FRITSCH, E. F. & MANIATIS, T. 1989. *Molecular cloning: a laboratory manual*, Edn 2. Cold Spring Harbor Laboratory Press, New York.
10. ROZEN, S. & KALETSKY, J. 1998. *Primer3*. Available at: http://www-genome.wi.mit.edu/genome_software/other/primer3.html.
11. NEI, M. 1973. Analysis of gene diversity in subdivided populations. *P. Natl Acad. Sci. USA*, **70**: 3321–3323.
12. GOUDET, J. 2001. *FSTAT version 2.9.3, a program to estimate and test gene diversities and fixation indices*. Available at http://www.unil.ch/izea/softwares/fstat.html.
13. ESTOUP, A. & ANGERS, B. 1998. In: *Advances in molecular ecology* (G.R. Carvalho, ed.), 55–79.
14. STOTHARD, J.R., BRÉMOND, P., ANDRIAMARO, L., SELLIN, B., SELLIN, E. & ROLLINSON, D. 2001. *Parasitology*, **123**: S261–S275.
15. TAYLOR, J.S. & BREDEN, F. 2000. *Genetics*, **155**: 1313–1320.
16. FLINT, J., BOND, J., REES, D.C., BOYCE, A.J., ROBERTS-THOMSON, J.M., EXCOFFIER, L., CLEGG, J.B., BEAUMONT, M.A., NICHOLS, R.A. & HARDING, R.M. 1999. *Hum. Genet.*, **105**: 567–576.

J. Moll. Stud. (2004) **70**: 96–98

Extinction risk and harbours as marine reserves?

J. M. Guerra-García[1], J. Corzo[1], F. Espinosa[1], D. Fa[2] and J. C. García-Gómez[1]

[1]*Laboratorio de Biología Marina, Departamento de Fisiología y Zoología, Facultad de Biología, Universidad de Sevilla, Avda Reina Mercedes 6, 41012 Sevilla, Spain and* [2]*The Gibraltar Museum, 18–20 Bomb House Lane, Gibraltar*

The mollusc *Patella ferruginea* Gmelin, 1791 is the most endangered marine invertebrate species on the list of the European Council Directive 92/43/EEC on the Conservation of Natural Habitat of Wild Fauna and Flora,[1] and is considered to be in danger of extinction.[2] Although its relative abundance in Palaeolithic and Neolithic deposits indicates an extensive former distribution in the Western Mediterranean, its range has progressively contracted.[3] These population regressions have been generally attributed to increasing pollution levels along the marine coastline and, especially, to human predation through the collection of specimens for food, fishing bait and decorative purposes, as this mollusc is one of the most attractive limpets of the Mediterranean. Today, the species has practically disappeared in the Iberian Peninsula and the North African coast of the Strait of Gibraltar. Strikingly, at the coast of Ceuta, and especially inside the harbour, we have found dense and stable populations of *Patella ferruginea* (Fig. 1).

The harbour of Ceuta is unusual from an environmental point of view, differing substantially from other conventional harbours. It is located between two bays connected by a channel, which increases the water movement and exchange, contributing to the maintenance of rich and diverse communities of marine invertebrates (Fig. 2).

After assessing the presence of *P. ferruginea* in Ceuta, a total of 70 stations were selected along the coast, inside the harbour and outside (North Bay and South Bay). The density of *P. ferruginea*

Correspondence: J. M. Guerra-García; e-mail:jmguerra@us.es

in each station was measured at low tide by counting the number of specimens found in a transect placed parallel to the coast with a length of 10 m and a width corresponding with the whole intertidal belt[4] (1.25 m on Ceuta's coast). A mean density of 11.33 individuals/10 m was measured inside the harbour, while outside the harbour the mean was 5.54 (North Bay) and 6.96 (South Bay), respectively. The values registered inside the harbour were significantly higher than those registered outside (one-way ANOVA, $F_{2,69} = 3.50$, $P < 0.05$) (Fig. 1). These values inside the harbour are even higher than the densities recorded in the Mediterranean relict populations of *P. ferruginea*, located in protected areas (7.9 ind/10m in Corsica[4] and 7 ind/10 m in Zembra Island, Tunisia[5]).

The unusual environmental structure of the harbour of Ceuta, together with the fact that people consider harbours as 'non-attractive' places to collect specimens for food and fishing, have contributed to the maintenance of high densities of *P. ferruginea*. Taking into account that *P. ferruginea* is considered a *K*-strategist species, with a low rate of growth and reproduction, and that it has been traditionally associated with high hydrodynamism and low levels of pollution,[3,5] the high densities of this mollusc reported inside a harbour is even more striking.

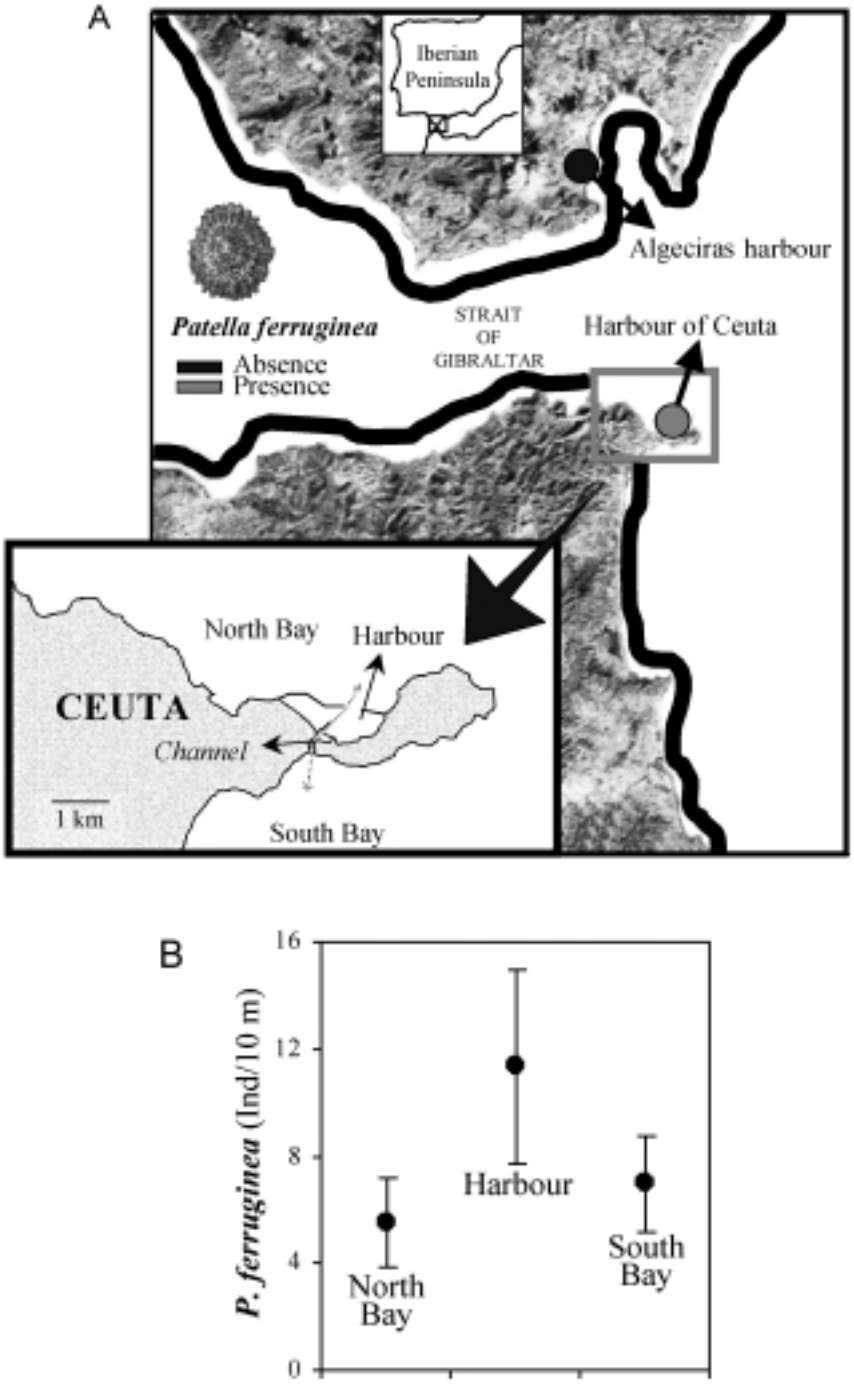

Figure 1. A. Location of Ceuta in North Africa. **B.** Density of *Patella ferruginea* (mean values ± standard deviation) in North Bay ($n = 33$), South Bay ($n = 29$) and the harbour ($n = 8$) of Ceuta.

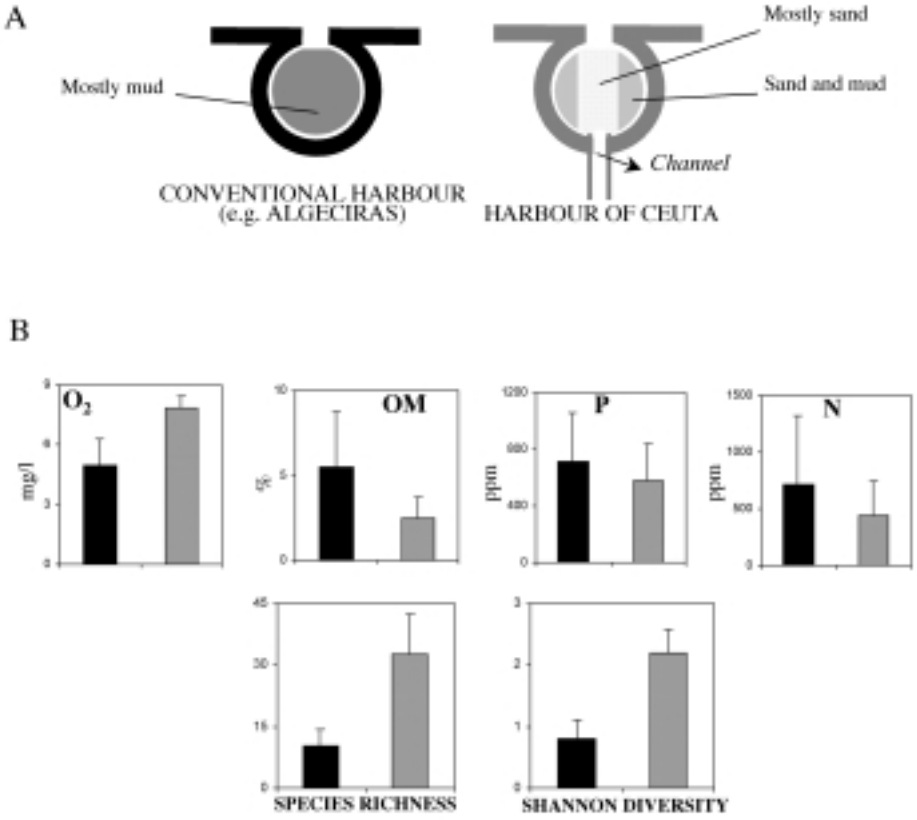

Figure 2. A. Schematic comparison between a conventional harbour and the harbour of Ceuta. **B**. Comparison of physicochemical and biological parameters between Algeciras harbour[7] (black) and the harbour of Ceuta[8] (dotted). Species richness and Shannon diversity were measured for sediment macrofaunal assemblages.Abbreviations: O_2, oxygen in water column; OM, organic matter in sediment; P, total phosphorus in sediment; N, total nitrogen in sediment.

Consequently, the design of the harbour of Ceuta, provided with a channel which increases the water renovation, should be taken into consideration for future civil engineering projects in order to reduce the negative impact of harbour building on marine environments. Furthermore, a harbour displaying adequate environmental features can offer useful monitoring and protection facilities (e.g. vigilance and installation of anti-pollution artificial barriers). This is particularly interesting in areas, like the Strait of Gibraltar, which have a high risk of environmental disaster because of intense maritime traffic.

REFERENCES

1. RAMOS, M.A. 1998. *J. Conch.*, **2**: 125–132.
2. BOE (Boletin Oficial del Estado), 1999. 22 junio 1999. No. 148: 23921–23922. Ministerio de la Presidencia.
3. CRETELLA, M., SCILLITANI, G., TOSCANO, F., TURELLA, P., PICARIELLO, O. & CATAUDO, A. 1994. *J. Moll. Stud.*, **60**: 9–17.
4. LABOREL-DEGUEN, F. & LABOREL, J. 1991. In: *Les espèces marines à protéger en Méditerranée* (C.F. Boudouresque, M. Avon & V. Gravez, eds), 119–128. GIS Posidonie Publishers, Marseille.
5. BOUDOURESQUE, C.F. & LABOREL-DEGUEN, F. 1986. In: *Le benthos marin d l'ile de Zembre (Parc National, Tunisie)* (C.F. Boudouresque, J.G. Harmelin & A. Jeudy de Grissac, eds), 105–110. GIS Posidonie Publishers, Marseille.
6. PORCHEDDU, A. & MILELLA, I. 1991. In: *Les espèces marines à protéger en Méditerranée* (C.F. Boudouresque, M. Avon & V. Gravez, eds), 105–117. GIS Posidonie Publishers, Marseille.
7. ESTACIO, F. J., GARCÍA-ADIEGO, E.M., FA, D.A., GARCÍA-GÓMEZ, J.C., DAZA, J.L., HORTAS, F. & GÓMEZ-ARIZA, J.L. 1997. *Mar. Pollut. Bull.*, **34**: 780–793.
8. GUERRA-GARCÍA, J.M. 2001. *Análisis integrado de las perturbaciones antropogénicas en sedimentos del Puerto de Ceuta. Efecto sobre las comunidades macrobentónicas e implicaciones ambientales.* PhD thesis, University of Seville.