



HAL
open science

Genetic diversity of two Portuguese populations of the pullet carpet shell , based on RAPD markers: contribution to a sustainable restocking program

Sandra Joaquim, Jorge Pereira, Alexandra Leitão, Domitília Matias, Raquel Chaves, Henrique Guedes-Pinto, Luís Chícharo, Miguel Gaspar

► To cite this version:

Sandra Joaquim, Jorge Pereira, Alexandra Leitão, Domitília Matias, Raquel Chaves, et al.. Genetic diversity of two Portuguese populations of the pullet carpet shell , based on RAPD markers: contribution to a sustainable restocking program. Helgoland Marine Research, 2009, 64 (4), pp.289-295. 10.1007/s10152-009-0182-7 . hal-00542556

HAL Id: hal-00542556

<https://hal.science/hal-00542556>

Submitted on 3 Dec 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Genetic diversity of two Portuguese populations of the pullet carpet shell *Venerupis***
2 ***senegalensis*, based on RAPD markers: contribution to a sustainable restocking program**

3
4 Sandra Joaquim^{1*}, Jorge Pereira², Alexandra Leitão¹, Domitília Matias¹, Raquel Chaves², Henrique
5 Guedes-Pinto², Luís Chícharo³, Miguel Gaspar¹

6
7 1. Instituto Nacional de Recursos Biológicos (INRB, I.P.)/ IPIMAR, Av. 5 de Outubro s/n, 8700-305
8 Olhão, Portugal.

9 2. Centro de Genética e Biotecnologia – CGB, Instituto de Biotecnologia e Bioengenharia – IBB,
10 Universidade de Trás-os-Montes e Alto Douro P-5000-911 Vila Real, Portugal

11 3. Universidade do Algarve, Centro de Ciências do Mar (CCMAR), Campus de Gambelas, P-8005-
12 139 Faro, Portugal.

13
14 *corresponding author: Instituto Nacional de Recursos Biológicos (INRB, I.P.)/ IPIMAR

15 Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal.

16 Telephone: +351 281 326951

17 FAX: +351 281 324028

18 E-mail: sandra@ipimar.pt

29 **Abstract**

30 The pullet carpet shell *Venerupis senegalensis* (= *V. pullastra*) is a commercially important species
31 in Portugal, Spain, France and Italy. In Portugal, this species was once abundant in the Ria
32 Formosa (southern Portugal). However, in the early 1980's its abundance declined dramatically due
33 to overfishing. In order to reverse this negative trend, the genetic sustainable management of the
34 wild stocks of *V. senegalensis* should be performed by promoting successful restocking actions and
35 the development of an aquaculture commercial production program of this species. In order to find
36 the best broodstock for aquaculture purposes and therefore minimize the deleterious effects of
37 hatchery practices, we analysed the genetic diversity of the natural population to be restocked (Ria
38 Formosa) but also of another potential genetically close population (Ria de Aveiro) by RAPD.
39 Similar and substantive percentage of polymorphic *loci*, effective number of alleles, Nei's gene
40 diversity and Shannon's diversity index was found within both populations. This high genetic
41 variability within populations suggests that they might have a gene pool with sufficient genetic
42 plasticity to support changes in the environmental conditions. Analyses of population genetic
43 structure also revealed a small genetic differentiation between the two populations. The high
44 genetic variability of the natural population to be restocked make it the preferential broodstock for
45 aquaculture purposes. However, the Ria de Aveiro population could also be a viable alternative,
46 due to its genetic plasticity and the genetic similarity of both populations. The results of this study
47 can be useful to the sustainable management of wild stocks as well as in promoting successful
48 restocking actions based on aquaculture production.

49

50 *Keywords:* Genetic diversity, Pullet carpet shell, RAPDs, sustainable management, *Venerupis*
51 *senegalensis*

52

53

54

55

56

57 **Introduction**

58

59 The pullet carpet shell *Venerupis senegalensis* (= *V. pullastra*) is an Atlantic–Mediterranean warm-
60 temperate species that inhabits sandy to muddy bottoms, usually from the low tide mark to a depth
61 of 40 m (Macedo et al. 1999). This species is commercially exploited in Portugal, Spain, France
62 and Italy. In Portugal, *V. senegalensis* occurs in estuaries and coastal lagoons. Presently, this
63 species is more abundant on the western coast, namely in the Ria de Aveiro coastal lagoon (Maia et
64 al. 2006). The pullet carpet shell was also, once abundant in the Ria Formosa lagoon (southern
65 Portugal). However, in the early 1980's the abundance of this species declined dramatically due to
66 overfishing (Massapina and Arrobas 1991). Despite the high commercial value of this species, the
67 lack of interest presented nowadays by local fishermen in harvesting this species lead us to suppose
68 that the population density in Ria Formosa has decreased below a threshold level, such that natural
69 recovery does not appear to be possible.

70

71 An active intervention seems, therefore, necessary to restore stocks to reproductive viability. In
72 order to do so and try to reverse this negative trend, a project to produce larvae and juveniles of *V.*
73 *senegalensis* has been implemented aiming to test different technical approaches to restock this
74 species in the Ria Formosa. This restock action can be performed in already existent bivalve-beds
75 of Ria Formosa. In this way, *V. senegalensis* can be valorised as an aquaculture product and as a
76 possible alternative to *Ruditapes decussatus*, the most important bivalve produced in this lagoon.
77 Hatchery practices may have deleterious effects on the genetic make-up of the released stock, and
78 consequently a deleterious effect on the wild population, such as the increasing genetic
79 homogeneity (Ward 2006). The decline of productivity and viability of the naturally spawning of
80 three species of wild Pacific salmonids when hybridised with hatchery produced species of lower
81 fitness (Reisenbichler and Rubin 1999) and the genetic homogenization of the pearl oyster stocks
82 by the impacts of transplants (Arnaud-Haond et al. 2004) are two examples of that.

83

84 Conscientious broodstock selection and husbandry could nonetheless minimise or eliminate this
85 negative effect, enhancing the creation of positive outcomes from restocking. Since wild
86 populations represent the primary source of genetic variability for aquaculture stocks (Alarcón et
87 al. 2004), the broodstock should be obtained from the natural population to be restocked or, if
88 polymorphism of this population is proven to be low and the population to be in genetic decline,
89 from the genetically close population. In order to develop sustainable aquaculture and
90 conscientious restocking programs, to ensure appropriate broodstock management programmes and
91 effective breeding programmes, it is of utmost importance to study the range of genetic variation,
92 genetic structure, diversification trend and other factors affecting genetic structure of the different
93 populations of this species.

94

95 To the author's best knowledge, no population genetic studies were performed until now in
96 populations of *V. senegalensis*. Only a study with ELISA and PCR-RFLP data was performed for
97 species identification (Fernández et al. 2002). The Random Amplified Polymorphic DNA (RAPD)
98 method has been frequently applied to reveal population-genetic variation, divergence and
99 biogeography (Schaal and Leverich 2001). This low cost technique, using short oligonucleotides of
100 arbitrary sequence for amplification of discrete regions of the genome, is simple, not requiring
101 much in up-front resources, no need for prior DNA sequence knowledge and represents the entire
102 genome (nuclear and mtDNA) (Williams et al. 1990; Star et al. 2003). In marine bivalves, RAPD
103 technique has been extensively used in population genetic studies, in oysters (e.g. Liu and Dai
104 1998), mussels (e.g. Star et al. 2003; Toro et al. 2004) and scallops (e.g. Heipel et al. 1998), and
105 proved to be an appropriate tool at the molecular level for the identification of the divergence
106 between populations or sibling species (Yu et al. 2004). In order to assess the most appropriate *V.*
107 *senegalensis* founder population to develop an aquaculture production program of this species and
108 for restocking purposes and since no information is currently available on Portuguese population
109 genetic structure of *V. senegalensis*, the Ria Formosa population (to be restocked) and the Ria de
110 Aveiro population were analysed by RAPD techniques.

111

112

113 **Materials and methods**

114 *Sample collection*

115 Samples of *V. senegalensis* were hand-collected from two locations in Portugal: Ria Formosa
116 (southern coast - 37°01'N; 07°49'W) and Ria de Aveiro (western coast - 40°42'N; 08°40'W)
117 (Figure1), both are shallow water mesotidal lagoons with semi-diurnal tidal regimes that constitute
118 the major hydrodynamic forcing (Nobre et al. 2005; Dias et al.2000). These lagoons, that distance
119 500 Km from each other, have several channels and a large intertidal area covered by sand, muddy
120 sand-flats and salt marshes (Falcão and Vale 1990; Picado et al. 2009). Ria Formosa has an
121 extension of 55 km and a maximum width of 6 km (Newton and Mudge 2003). The lagoon is
122 separated from the Atlantic Ocean by several barrier islands and two peninsulas. The tidal range
123 varies from 1.35 m on neap tides to 3 m on spring tides and the coefficient of renovation for the
124 lagoon is 3.2 for a spring tide and 1.0 for a neap tide. The freshwater inputs are almost negligible
125 and salinity remains close to 36 ‰ (Falcão and Vale 1990; Águas 1986). The Ria de Aveiro is 45
126 km long and 10 km wide, being connected to the Atlantic Ocean by only a narrow channel (Picado
127 et al. 2009) and the tidal amplitude is 0.6 m in neap tides and 3.2 m in spring tides (Dias et al.
128 2000). This lagoon has an important freshwater input coming from the Vouga and the Antuã rivers
129 (Moreira et al. 1993; Dias et al. 2000). These two ecosystems are currently used for clam
130 production and fish aquaculture ponds.

131

132 *Sample processing and DNA extraction*

133 The adductor muscles of 20 individuals (35-37 mm) of each location were dissected from fresh
134 specimens and immediately stored at 70% ethanol until DNA extraction. Genomic DNA was
135 extracted from approximately 15 mg of the muscle tissue following Pereira (2008) by using a
136 QuickGene DNA tissue kit and a Quickgene – 810 automatic nuclei acid isolation system (Fuji
137 Photo Film Co., Ltd, Life Science Products Division, Tokyo, Japan).

138

139 *RAPD amplification*

140 Since the reproducibility has been pointed out as the major RAPD's limitation (Harris 1999), a
141 series of optimization experiments was conducted, in which 20 decanucleotide RAPD primers
142 (Operon Technologies, Alameda, CA, USA) were screened in which 6 primers were selected on
143 their ability to amplify reproducible and easily scorable bands. The amplifications were performed
144 in a 25 μ l of reaction volume containing 1 \times *Taq* polymerase buffer (Tris-HCl 200 mmol/l pH 8.3,
145 KCl 500 mmol/l, 0.5% Triton-X 100), 2.0 mM MgCl₂, 100 μ M of each dNTPs (dATP, dCTP,
146 dGTP, dTTP) (Invitrogen, Life Sciences), 0.2 μ M of primer, 0.5 U *Taq* polymerase (Fermentas,
147 Canada), 50 ng of genomic DNA and water. Initial denaturation was for 3 min at 94°C, followed by
148 44 cycles of 1 min at 94°C, 1 min at the optimal annealing temperature (36°C), 2 min at 72°C and a
149 final extension step of 10 min at 72°C. The samples were finally cooled at 4°C. Amplification
150 products were analysed by electrophoresis on 2% agarose TBE (Tris borate EDTA) gels stained
151 with ethidium bromide and then visualized with ultraviolet light and image were saved in UVIDOC
152 (UVItec). A DNA ladder mix (Fermentas, Canada) was used as a molecular ruler. To avoid
153 inaccuracy in scoring due to differences in gels, two lines of control molecular ladder was included
154 in each set of samples for each gel.

155

156 *Data analysis*

157 Amplified fragments were scored as binary data, i.e. presence as 1 and absence as 0. The
158 differences in band intensities were not taken into account to avoid errors introduced by
159 competition among priming sites during the initial rounds of PCR (Bachmann 1997). The matrix of
160 RAPDs data was analysed on the basis of the following genetic parameters: number of
161 polymorphic *loci*, percentage of polymorphic *loci*, observed number of alleles (n_0), effective
162 number of alleles (n_e), Nei's genetic diversity (h), Shannon's information index (H), G_{ST} and the
163 effective migrants number by generation (N_m) (Wright 1951), using POPGENE program version
164 1.32 (Yeh et al. 1997; available at <http://www.ualberta.ca/~fyeh/>). TFPGA program version 1.3
165 (Miller 1997; available at <http://www.marksgeneticsoftware.net/tfpga.htm>) was also used to
166 calculate the Nei's (1978) genetic distance and fixation F_{st} value, over *loci*, based on Lynch and
167 Milligan's (1994) Taylor expansion estimate and assumed a Hardy-Weinberg equilibrium.

168 Significant levels were assessed by permutation of the alleles (multilocus genotypes) within
169 populations.

170

171 **Results**

172 The 6 decanucleotide RAPD primers generated a total of 222 reproducible and clear amplification
173 bands, with fragments ranging in molecular size from approximately 200 to 1500 bp. The bands
174 produced *per* primer ranged from 32 (OPE-02 primer) to 40 (OPE-08 and OPE-11) (Table 1), and
175 the average band *loci per* primer was 37.17. The percentage of polymorphic *loci* found was 86.55%
176 in the Ria Formosa *V. senegalensis* population and 87.44% in the Ria de Aveiro one. The number
177 of effective alleles *per* locus was 1.3545 and 1.3219, for Ria Formosa and Ria de Aveiro
178 populations, respectively. The values of genetic diversity and the Shannon's information index for
179 Ria Formosa ($h = 0.2276$; $H = 0.3608$) and for Ria de Aveiro ($h = 0.2105$; $H = 0.3394$) revealed a
180 high level of gene diversity in both populations and did not vary considerably between them,
181 (Table 2).

182

183 The Nei's (1978) values (0.064) revealed a small genetic distance between the two populations.
184 The value of the total genetic diversity (H_t) was 0.246 and the genetic diversity within populations
185 (H_s) was 0.2190. The G_{st} index value was 0.1097, meaning that most of the genetic variation was
186 found within populations (89.03%). Assuming Hardy-Weinberg equilibrium, F_{st} value (0,1659)
187 showed no evidence of genetic differentiation between populations. The genetic flow (N_m) between
188 the two populations was 4.057.

189

190

191

192

193

194

195

196 **Discussion**

197 Assessment of genetic variation is an important step towards the implementation of species
198 conservation strategies. Ward (2006) summarized that the high level of genetic variability in
199 invertebrates strongly depend on its life form, geographic range and larval dispersal mechanism. A
200 variety of genetic markers has been used to determine aquatic populations structure. These include
201 the use of allozyme, RAPD, restriction fragment length polymorphism (RFLP) and microsatellite
202 markers (Yan et al. 2005). RAPD markers have been widely used for estimating genetic diversity
203 and genetic structure in many species and have proved to be a powerful tool (Bussell 1999; Ouborg
204 et al. 1999; Chen et al. 2005). In our study, this methodology was efficient in obtaining information
205 on the population genetics of *V. senegalensis*.

206 Analysis of RAPD data from the 40 individuals revealed similar and substantive percentage of
207 polymorphic *loci*, effective number of alleles and Nei's gene diversity within *V. senegalensis*
208 populations. The values obtained in our study are slightly lower than the ones found for other
209 bivalves, such as in mussel (*Mytilus galloprovincialis*) populations in Southern California (Li Ma et
210 al. 2000) or in European populations of the razor clam *Ensis siliqua* (Fernández-Tajes et al. 2007),
211 but are similar to the one's obtained in another veneroid species (*R. decussatus*) populations in
212 Algarve coast (Southern Portugal) (Pereira 2008). Shannon's diversity index is well suited to the
213 analysis of RAPD data as it is relatively insensitive to the bias produced by failures to detect
214 heterozygous individuals (Dawson et al. 1995). The values obtained with this index confirmed the
215 high genetic variability in both populations. They were, however, lower than those observed in
216 populations of *R. decussatus* by Pereira (2008), which might be explained by the larger populations
217 size of that study. The high genetic variability found in both *V. senegalensis* populations suggests
218 that they have a gene pool with sufficient genetic plasticity to support changes in the environmental
219 conditions without endanger the species survival, even in the case of an overfished population like
220 the Ria Formosa one's. Indeed, the ability of a species to respond adaptively to environmental
221 changes and, therefore, to long term survival depends on the levels of genetic variability within
222 populations (Qian et al. 2001; Sofia et al. 2006). Fernández-Tajes et al. (2007) also reported a high

223 genetic variability for an *Ensis siliqua* Portuguese population which has suffered strong declines
224 due to overfishing.

225

226 The results of the population genetic structure of *V. senegalensis* analyses revealed a small genetic
227 differentiation between the two populations. Similar levels of genetic differentiation, using the
228 same methodology, have been recorded between populations of other bivalves (Star et al. 2003;
229 Casu et al. 2005; Fernández-Tajes et al. 2007; Pereira 2008). The main factors that might determine
230 the population genetic structure of bivalve species include mating and reproductive system, gene
231 flow (dispersal of larvae), genetic drift, environmental selection or adaptation, bottlenecks, founder
232 effects, among others (e.g. Bierne et al. 1998; Holmes et al. 2004). The high values of genetic
233 variability within populations and low levels of genetic variation among populations observed in
234 this study can be a consequence of the reproductive system of *V. senegalensis*. As in most marine
235 bivalves, it is characterised by high fecundity, large population sizes, external fertilization with
236 broadcast spawning, and extensive larval dispersal (Bierne et al. 1998). At a first view, the results
237 geared for the studied populations suggest a “canonical” model of population genetics known as
238 panmixia, without inbreeding, proposed for other bivalve populations (e.g. Bierne et al. 1998;
239 Fernández-Tajes et al. 2007). This model requires an intense gene flow (Nm) between populations
240 without geographic barriers in order to allow them to evolve together (Armbruster 1997, 1998;
241 Ward 2006). Traditionally, levels of $Nm > 1$ are thought to be sufficient to prevent population
242 genetic differentiation (Slatkin 1987). Thus, the number of migrants between the studied
243 populations ($Nm = 4.057$) may suggest that larval exchange could be responsible for the genetic
244 similarities between them. Fernández-Tajes et al. (2007) reported a gene flow of the same
245 magnitude among Galician populations of *E. siliqua* and they suggested that these populations
246 acted as a single interbreeding population and Pereira (2008) also obtained an $Nm = 2.9$ between
247 two populations of *R. decussatus* from Ria Formosa and from a near lagoon (Ria de Alvor).
248 However, some authors (Whitlock and McCauley 1999) believe that these results should be
249 interpreted carefully, since, Nm is calculated based on the F_{st} value and the genetic plasticity of
250 both populations (high genetic variability) can contribute to their similarity. *V. senegalensis* is an

251 actaeplanic species, i.e., with potential for dispersal (Ropes 1979; Havenhand 1995; Casu et al.
252 2005) and Ria Formosa and Ria de Aveiro, despite the fact that they are shallow lagoons systems,
253 they have a high tidal flow that allows a large water renovation of both lagoon systems and that
254 may promote larval drift to the coastal waters, could be a possible vector responsible for larvae
255 dispersal and consequent genetic similarity of the studied populations. Nevertheless, we believe
256 that the short larval life-spans of *V. senegalensis* that is of around 15 days, the 500 km that
257 geographically distanced Ria Formosa from Ria de Aveiro and other oceanic physical constraints
258 (e.g. variation in water temperature, salinity, predation, currents) can restrict the dispersal capacity
259 of larvae. These facts do not support the previous hypotheses of larval exchange between the two
260 *V. senegalensis* populations.

261

262 Nonetheless, natural dispersal is not always the operating transport mechanism (Levin 2006). The
263 genetic structure of natural populations can be significantly affected by anthropogenic activities
264 (Palumbi 2001; Simmons et al. 2006; Pereira 2008). Nowadays, there is no knowledge of market
265 exchanges involving *V. senegalensis* between the two studied sites owing to the overfishing of Ria
266 Formosa population but, an historical involvement of fishermen from Aveiro in Algarve fisheries
267 (Oliveira 1906; Garrido and Carvalho 2007) and the crescent importance of bivalve market in both
268 sites can be a possible vector to contribute to the transplantation of *V. senegalensis* between those
269 two lagoons and consequently to the genetic similarity between the two studied populations. This
270 hypothesis can lead us to suggest that the high levels of Nm between the two populations can result
271 from historically genotypic and allelic similarities between the individuals from these regions and
272 do not reflect the present day value of Nm . This hypothesis was already conjectured by Star et al.
273 (2003) for New Zeland *Perna canaliculus* populations.

274

275 Bell et al. 2005 have showed that the knowledge of spatial population structure is pivotal to
276 efficient management of marine invertebrate fisheries. According to Gaffney (2006), three primary
277 genetic concerns should be taken in consideration before implement shellfish restoration projects:
278 1) identification and use of the correct genetic material for producing hatchery lines; 2)

279 maintenance of genetic variability in hatchery stocks; 3) maintaining N_e in the wild population.
280 Given that *V. senegalensis* population of Ria Formosa still presents a high intrapopulation genetic
281 variability, it should be the one preferentially selected as broodstock for aquaculture purposes and
282 for subsequent restocking program. However, if the broodstock available from this population
283 reveals to be insufficient due to severe overfishing, Ria de Aveiro population could be a viable
284 alternative. Indeed, we have showed in this study that, by one hand genetic plasticity is high and by
285 other hand both population presented genetic similarity. Furthermore, when designing restocking
286 actions based on aquaculture production, we should nevertheless always try to ensure the
287 preservation of the genetic variability in the hatchery stocks and maintaining the effective size (N_e)
288 of the wild population to be restored. In addition to the favourable genetic factors, the similarities
289 between Ria Formosa and Ria de Aveiro in terms of habitat (both shallow water mesotidal lagoon
290 with a large intertidal area covered and with similar semi-diurnal tidal regimes, salinity and
291 sediment) can also positively influence the success of a restocking program. The results of this
292 study provided useful information on the genetic structure of two Portuguese populations of the
293 pullet carpet shell. This data can be useful to the sustainable management of wild stocks as well as
294 in promoting successful restocking actions based on aquaculture production. The protection of
295 genetic diversity in the Ria Formosa population should, however, always be a primary aim of any
296 restocking program to be performed.

297

298 **Acknowledgement**

299 We would like to thank the IPIMAR Aveiro staff and João Joaquim, for providing the Ria de
300 Aveiro and Ria Formosa clam samples, respectively. The authors also thank the Maritime Museum
301 of Ílhavo for the historical documents and to A. Good and J. White for English editing. This
302 study was funded by the Promopesca (Interreg IIIA) project.

303

304 **References**

305 Águas MPN (1986) Simulação da circulação hidrodinâmica na Ria Formosa. In Os sistemas
306 Lagunares do Algarve. Seminário comemorativo do dia mundial do ambiente (pp. 78–90). Faro:
307 Universidade do Algarve, (5–7 Junho 1985)

308 Alarcón JA, Magoulas A, Georgakopoulos T, et al (2004) Genetic comparison of wild and
309 cultivated European populations of the Gilthead Sea bream (*Sparus aurata*). *Aquaculture* 230:65-
310 80

311 Armbruster G (1997) Genetische verarmung aufgrund von populationseinbrüchen: Eine analyse bei
312 der seltenen landschneckenart *Cochlicopa ninens* (Gallenstein, 1848). *Nature Landscape* 2:444-446

313 Armbruster G (1998) Bei einer verbreiteten landschnecke *Cochlicopa lubrica* (O. F. Müller), wird
314 die frequenz von molekularem ph_notypen durch selbstbefruchtung und habitatspezifische
315 selektion beeinflusst. *Laufener Seminarbeiträge 2/98 der Bayerischen Akademie für Naturschutz*
316 *und landschaftspflege (ANL)* 39-49

317 Arnaud-Haond S, Vonau V, Bonhomme F, et al (2004) Spatio-temporal variation in the genetic
318 composition of wild populations of pearl oyster (*Pinctada margaritifera cumingii*) in French
319 Polynesia following 10 years of juvenile translocation. *Mol Ecol* 13:2001–2007

320 Bachmann K (1997) Nuclear DNA markers in plant biosystematic research. *Opera Bot* 132:137-
321 148

322 Bell JD, Rothlisberg PC, Munro JL, et al (2005) Restocking and stock enhancement of marine
323 invertebrate fisheries. *Advances in Marine Biology*, 49: 1–370

324 Bierne N, Launey S, Naciri-Graven Y, et al (1998) Early effect of inbreeding as revealed by
325 microsatellite analyses on *Ostrea edulis* larvae. *Genetics* 148:1893–1906

326 Bussell JD (1999) The distribution of random amplified polymorphic DNA (RAPD) diversity
327 amongst populations of *Isotoma petraea* (Lobeliaceae). *Mol Ecol* 8:775-789

328 Casu M, Maltagliati F, Cossu P, et al (2005) Fine-grained spatial genetic structure in the bivalve
329 *Gemma gemma* from Maine and Virginia (USA), as revealed by inter-simple sequence repeat
330 markers. *J Exp Mar Biol Ecol* 325:46-54

331 Chen SL, Xia T, Chen SY, et al (2005) RAPD profiling in detecting genetic variation in endemic
332 *Coelonema* (Brassicaceae) of Qinghai-Tibet Plateau of China. *Biochemical Genetics* 43:189 - 201

333 Dawson IK, Simons AJ, Waugh R, et al (1995) Diversity and genetic differentiation among
334 subpopulations of *Gliricidia sepium* revealed by PCR-based assays. *Heredity* 74:10-18

335 Dias JM, Lopes JF, Dekeyser I (2000) Tidal propagation in Ria de Aveiro lagoon, Portugal. *Phys*
336 *Chem Earth (B)* 25:369–374

337 Falcão M, Vale C (1990) Study of the Ria Formosa ecosystem: bentic nutrient remineralization and
338 tidal variability of nutrients in the water. *Hydrobiologia* 207, 137–146

339 Fernández A, García T, Asensio L, et al (2002) Identification of the clam species *Ruditapes*
340 *decussatus* (Grooved Carpet Shell), *Venerupis rhomboides* (Yellow Carpet Shell) and *Venerupis*
341 *pullastra* (Pullet Carpet Shell) by ELISA. *Food Agricult Immunol* 14:65–71

342 Fernández-Tajes J, Gaspar M, Martínez-Patiño D, et al (2007) Genetic variation of the razor clam
343 *Ensis siliqua* (Jeffreys, 1875) along the European coast based on random amplified polymorphic
344 DNA markers. *Aquac Res* 38:1205-1212

345 Gaffney PM (2006) The role of genetics in shellfish restoration. *Aquat. Living Resour.* 19, 277-282

346 Garrido A, Carvalho M (2007) A diáspora dos Ílhavos, Museu Marítimo de Ílhavo. Editora Ílhavo

347 Harris SA (1999) RAPDs in systematics – a useful methodology? In : Hollingsworth PM, Bateman
348 RM, Gornall RJ (ed) *Molecular Systematics and Plant Evolution.*, Taylor and Francis, London, pp
349 211-228

350 Havenhand H (1995) Evolutionary ecology of larval types. In: McEdward L (ed) *Ecology of*
351 *marine invertebrate larvae.* CRC Press, Boca Raton, FL, pp 79-122

352 Heipel DA, Bishop JDD, Brand AR, et al (1998) Population genetic differentiation of the great
353 scallop *Pecten maximus* in western Britain investigated by randomly amplified polymorphic DNA.
354 *Mar Ecol Prog Ser* 162:163-171

355 Holmes SP, Dekker R, Williams ID (2004) Population dynamics and genetic differentiation in the
356 bivalve mollusc *Abra tenuis*: aplanic dispersal. *Mar Ecol Prog Ser* 268:131-140

357 Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions.
358 *Int Comp Biol* 46(3):282–297

359 Li Ma X, Cowles DL, Carter RL (2000) Effect of pollution on genetic diversity in the bay mussel
360 *Mytilus galloprovincialis* and the acorn barnacle *Balanus glandula*. *Mar Environ Res* 50:559-563

361 Liu B, Dai J (1998) Studies on genetic diversity in oysters *Crassostrea*. J. Fish. China 22(3):193–
362 198

363 Lynch M, Milligan B (1994) Analysis of population genetic structure with RAPD markers. Mol
364 Ecol 3:91–99

365 Macedo MCC, Macedo MIC, Borges JP (1999) Conchas Marinhas de Portugal. Editorial Verbo,
366 Lisbon

367 Maia F, Sobral MP, Gaspar M (2006) Ciclo reprodutivo e primeira maturação de *Solen marginatus*
368 e *Venerupis pullastra* na Ria de Aveiro. Bases científicas para a gestão destes recursos. Relat.
369 Cient. Téc. IPIMAR, 30

370 Massapina C, Arrobas I (1991) A cultura de moluscos bivalves na Ria Formosa: estado actual e
371 perspectivas, 1º Simpósio NOPROT, Faro

372 Miller MP (1997) Tools for Population Genetic Analyses (TFPGA) 1.3: a Windows Program for
373 the Analysis of Allozyme and Molecular Population Genetic Data. Computer software distributed
374 by author

375 Moreira MH, Queiroga H, Machado MM, et al (1993) Environmental gradients in a southern
376 estuarine system: Ria de Aveiro, Portugal, Implications for soft bottom macrofauna colonization.
377 Neth J Aquat Ecol 27:465–482

378 Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of
379 individuals. Genetics 89:583-590

380 Newton A, Mudge SM (2003) Temperature and salinity regimes in a shallow, mesotidal lagoon, the
381 Ria Formosa, Portugal. Estuar. Coast. Shelf. Sci. 57 : 73–85

382 Nobre AM, Ferreira JG, Newton A, et al (2005) Management of coastal eutrophication: integration
383 of field data, ecosystem- scale simulations and screening models. J. Mar. Sys. 56, 375–390

384 Oliveira FXd'A (1906) Monografia do Concelho de Olhão da Restauração. Algarve em Foco
385 Editora

386 Ouborg NJ, Piquot Y, van Groenendael JM (1999) Population genetics, molecular markers and the
387 study of dispersal in plants. J Ecol 87:551-568

388 Palumbi SR (2001) Humans as the world's greatest evolutionary force. *Science* 293:1786–1790

389 Pereira J (2008) Perfis de RAPD-PCR em populações de *Ruditapes decussatus* (Linnaeus, 1758)

390 do sul de Portugal: Avaliação da diversidade genética. Master thesis

391 Picado A, Dias JM, Fortunato A (2009) Effect of flooding the salt pans in the Ria de Aveiro. *J*

392 *Coast R SI56*: 1395-1399

393 Qian W, Ge S, Hong DY (2001) Genetic variation within and among populations of a wild rice

394 *Oryza granulata* from China detected by RAPD and ISSR markers. *Theor Appl Genet* 102:440-449

395 Reisenbichler RR, Rubin SP (1999) Genetic changes from artificial propagation of Pacific salmon

396 affect the productivity and viability of supplemented populations. *ICES J. Mar. Sci.* 56:459–466

397 Ropes JW (1979) Biology and distribution of surf clams (*Spisula solidissima*) and ocean quahogs

398 (*Arctica islandica*) off the northwestern coast of the United States. *Proc Northeast Clam Industries:*

399 *management for the future.* University of Massachusetts and Massachusetts Institute of

400 Technology, Sea Grant Program SP 112:47–66

401 Schaal BA, Leverich WJ (2001) Plant population biology and systematics. *Taxon* 50:357–373

402 Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* 236:187-

403 792

404 Simmons M, Mickett K, Kucuktas H, et al (2006) Comparison of domestic and wild channel catfish

405 (*Ictalurus punctatus*) populations provides no evidence for genetic impact. *Aquaculture* 252:133–

406 146

407 Sofia SH, Silva CRM, Galindo BA, et al (2006) Population genetic structure of *Astyanax*

408 *scabripinnis* (Teleostei, Characidae) from an urban stream. *Hydrobiologia* 553:245 - 254

409 Star B, Apte S, Gardner JPA (2003) Genetic structuring among populations of the greenshell

410 mussel *Perna canaliculus* revealed by analysis of randomly amplified polymorphic DNA. *Mar*

411 *Ecol Prog Ser* 249:171-182

412 Toro JE, Ojeda JA, Vergara AM (2004) The genetic structure of *Mytilus chilensis* (Hupe 1854)

413 populations along the Chilean coast based on RAPDs analysis. *Aquac Res* 35: 1466-1471

414 Ward RD (2006) The importance of identifying spatial population structure in restocking and stock

415 enhancement programmes. *Fish. Res.* 80:9–18

416 Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: F_{ST} not equal
417 to $1/(4Nm+1)$. *Heredity* 82:117-125

418 Williams JGK, Kubelik AR, Livak KJ, et al (1990) DNA polymorphisms amplified by arbitrary
419 primers are useful as genetic markers. *Nucl. Acids Res.* 18:6531– 6535

420 Wright S (1951) The genetic structure of populations. *Annals of Eugenics* 15:323-354

421 Yan J, Liu S, Sun Y, et al (2005) RAPD and microsatellite analysis of diploid gynogens from
422 allotetraploid hybrids of red crucian carp (*Carassius auratus*) x common carp (*Cyprinus carpio*).
423 *Aquaculture* 243:49-60

424 Yeh FC, Yang RC, Boyle TBJ, et al (1997) POPGENE, the User-Friendly Shareware for
425 Population Genetic Analysis. Molecular Biology and Biotechnology Center, University of Alberta,
426 Edmonton, AB, Canada

427 Yu X, Mao X, Wang M, et al (2004) Genetic heterogeneity analysis and RAPD marker detection
428 among four forms of *Atrina pectinata* Linnaeus. *J Shellfish Res* 3 (1):165-172

429
430
431
432
433
434

435 Figure 1 - Ria Formosa and Ria de Aveiro locations.
436

437

438 Table 1 - Primers selected for RAPD analyses
439

Primer	Sequence (5'-3')	%GC	Tm	No of score bands
OPE-02	GGTGCGGGAA	70	36	32
OPE-03	CCAGATGCAC	60	36	35
OPE-06	AAGACCCCTC	60	36	39
OPE-08	TCACCACGGT	60	36	40
OPE-11	GAGTCTCAGG	60	36	40
OPE-16	GGTGACTGTG	60	36	37

440
441
442

Table 2 – Genetic variability at the two *Venerupis senegalensis* populations detected by RAPD

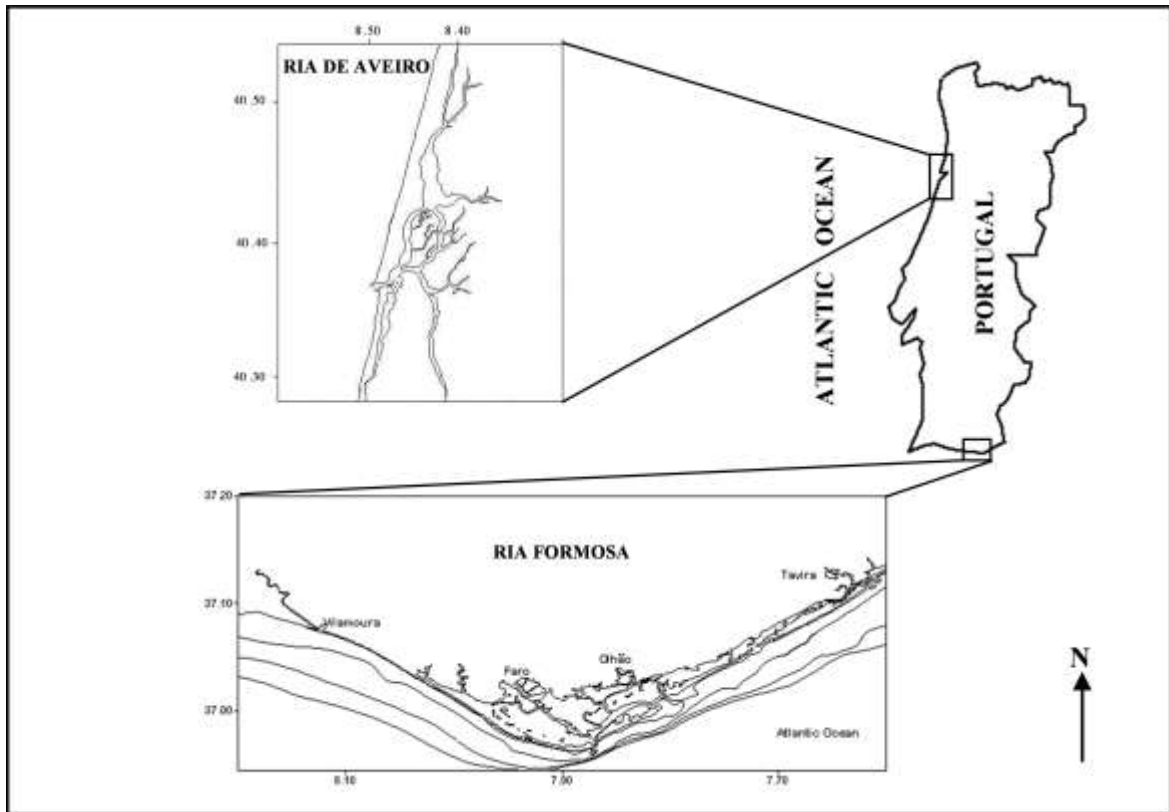
443

Population	Polymorphic loci (%)	Observed number of alleles (n_o)	Effective number of alleles (n_e)	Nei's gene diversity (h)	Shannon's information index (H)
Ria Formosa	86.55	1.8655	1.3545	0.2276	0.3608
Ria de Aveiro	87.44	1.8744	1.3219	0.2105	0.3394

444

445

446



447