Conservation Genetics
2010, Volume 11, Number 5, Pages 1899-1910
http://dx.doi.org/10.1007/s10592-010-0081-0

Strategies for the retention of high genetic variability in European flat oyster (*Ostrea edulis*) restoration programmes

Delphine Lallias[a, b, *], Pierre Boudry[c], Sylvie Lapègue[d], Jon W King[b],

Andy R Beaumont[a, b]

[a] School of Ocean Sciences, College of Natural Sciences, Bangor University, Menai Bridge, Anglesey, LL59 5AB, United Kingdom
[b] Centre for Applied Marine Sciences, Marine Science Laboratories, Menai Bridge, Anglesey, LL59 5AB, United Kingdom
[c] Ifremer, UMR M100 Physiologie et Ecophysiologie des Mollusques Marins, BP 70, 29280 Plouzané, France
[d] Ifremer, Laboratoire Génétique et Pathologie, Ronce-les-bains, 17390 La Tremblade, France

*: Corresponding author : Delphine Lallias, email address : d.lallias@bangor.ac.uk

Abstract:

The native European flat oyster Ostrea edulis is listed in the OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic (species and habitat protection) and in the UK Biodiversity Action Plan. Once extremely abundant in the 19th century, European stocks of O. edulis have declined during the 20th century to rare, small, localised populations due to overexploitation, habitat degradation and, most recently, the parasitic disease bonamiosis. Selective breeding programmes for resistance to bonamiosis have been initiated in France and Ireland. High genetic diversity and bonamiosis-resistance would be important features of any sustainable restoration programmes for O. edulis.

Oysters were sampled across Europe from four hatchery sources, four pond-cultured sources and four wild, but managed fisheries and were genotyped at five microsatellite loci. Hatchery-produced populations from small numbers of broodstock showed a significant loss of genetic diversity relative to wild populations and pedigree reconstruction revealed that they were each composed of a single large full-sib family and several small full-sib families. This extremely low effective population size highlights the variance in reproductive success among the potential breeders. Pond-cultured oysters were intermediate in genetic diversity and effective population size between hatchery and wild populations. Controlled hatchery production allows the development of bonamiosis-resistant strains, but at the expense of genetic diversity. Large scale pond culture on the other hand can provide a good level of genetic diversity. A mixture of these two approaches is required to ensure a healthy and sustainable restoration programme for O. edulis in Europe.

Keywords: Restoration programme, Ostrea edulis, Genetic variation, Pedigree reconstruction

1. Introduction

An increasing number of exploited marine species are threatened through overharvesting, habitat loss or degradation, and / or diseases (Gaffney 2006) and, as a consequence, restoration programmes are being developed. Any restoration programme should be coupled with habitat restoration / rehabilitation (McCay et al. 2003) and should attempt to restore populations that have the highest possible genetic diversity. Without sufficient genetic diversity and suitably restored habitat the long term sustainability of restored populations remains at risk.

There are three main strategies for molluscan shellfish population restoration attempts: adult transplant, juvenile seeding and larval release. Shellfish restoration projects generally focus on the second of these strategies and involve the release of hatchery-produced progenies for restocking and enhancing stocks of natural populations (Gaffney 2006; Laing et al. 2006). Gaffney (2006) reviewed the three major genetic concerns relevant to shellfish restoration projects: i) identifying source broodstock (after population genetics study at the geographical distribution scale of the species), ii) maintaining the maximum genetic variability possible and reducing inbreeding of hatchery stocks used for restoration purpose and iii) assessing the potential impact on the effective population size in wild populations of restocking with hatchery-propagated spat. Restocking with genetically improved hatchery strains could potentially have positive effects on wild populations particularly in terms of growth rate and disease resistance (Gaffney 2006). Several strategies were tested in Florida to restore and enhance populations of hard clam *Mercenaria* spp. (Arnold et al. 2002) and bay scallop *Argopecten irradians* (Arnold et al. 2005), following habitat degradation and loss. Hatchery-produced bay scallops were deployed in cages, but no significant contribution

77 from the restoration stock to the wild population could be detected with mitochondrial
78 DNA markers (Wilbur et al. 2005). On the other hand trials of bay scallop larval release
79 produced an increase in the scallops in the larval enclosure relative to the control
80 enclosure (Arnold 2008).

81 Oyster larvae settle out of the plankton and cement themselves to hard substrates,
82 preferentially other oyster shells. This natural process, when not disturbed by a fishery,
83 leads to oyster reefs that can support high biodiversity in their associated community.
84 The importance of the restoration of oyster reef habitat has been emphasised by Mann
85 (2000) and considerable investment has been made into the restoration of reefs of the
86 eastern cupped oyster, *Crassostrea virginica*, in Chesapeake Bay, USA (Brumbaugh et
87 al. 2000). In two separate studies, four million (Milbury et al. 2004) and 0.75 million
88 (Hare et al. 2006) *C. virginica* hatchery-produced spat with improved disease tolerance
89 were planted out in 1997 and 2002 respectively on natural oyster beds in different areas
90 of the Chesapeake Bay. The hatchery stocks could be distinguished from the wild
91 oysters by diagnostic single nucleotide polymorphisms (SNPs) in their mitochondrial
92 DNA, but in neither attempt was a high enhancement success detected (Milbury et al.
93 2004; Hare et al. 2006). Such studies on attempts at bivalve restoration from hatchery
94 production demonstrate the importance of an evidential basis to conservation outcomes
95 (Pullin and Knight 2009).

96 The native European flat oyster *Ostrea edulis* is listed in the OSPAR Convention
97 for the Protection of the Marine Environment of the North-East Atlantic (species and
98 habitat protection) and in the UK Biodiversity Action Plan and Laing et al. (2005)
99 carried out a feasibility study for its restoration.

100 *O. edulis* is a sessile, filter-feeding bivalve mollusc with a distribution ranging
101 from Norway to Morocco in the Atlantic Ocean, in the Mediterranean Sea and

102 extending into the Black Sea. This species was once of huge economic importance in
103 Europe but there were massive declines in abundance in the late 1800s and early 1900s
104 probably due to overfishing, habitat deterioration and unidentified diseases. Following a
105 plateau of low level fisheries in Europe into the 1960s a further drastic decline in oyster
106 numbers occurred due to two parasitic diseases, marteiliosis (*Marteilia refringens*),
107 mainly affecting estuarine populations, and the more serious bonamiosis (*Bonamia*
108 *ostreae*) that causes heavy mortalities in both intertidal and subtidal areas.
109 Selective breeding programmes for resistance to bonamiosis have been initiated
110 in France (Naciri-Graven et al. 1998) and Ireland (Culloty et al. 2004) and have
111 demonstrated an important potential for genetic gain through selective breeding.
112 Additionally, significant differences in growth, mortality and susceptibility to
113 bonamiosis and other diseases were observed between geographic origins and between
114 families (da Silva et al. 2005). More recently, quantitative trait loci (QTLs) linked to
115 bonamiosis resistance have been mapped (Lallias et al. 2009) and the search for
116 candidate genes involved in the resistance to bonamiosis has been initiated (Morga et al.
117 Unpublished results). Such studies highlight the possibilities of restoring flat oyster
118 populations by restocking with hatchery-produced bonamiosis-resistant seed.
119 Before implementing such restoration programmes in *O. edulis*, it is important to
120 assess the potential impact of hatchery-propagated stocks on the genetic variability and
121 the effective population size of wild populations (Gaffney 2006). Several studies have
122 reported the loss of genetic diversity and a reduced effective population size in hatchery
123 populations of shellfish, particularly oysters (Gosling 1982; Hedgecock and Sly 1990;
124 Gaffney et al. 1992; Hedgecock et al. 1992; Saavedra and Guerra 1996; Saavedra 1997;
125 Launey et al. 2001; Boudry et al. 2002; Sobolewska and Beaumont 2005; Appleyard
126 and Ward 2006; Taris et al. 2007; Lind et al. 2009). So, while hatchery production is an

127 effective method to produce large numbers of seed for restoration, and controlled
128 conditions allow the development of disease resistant strains, its major drawback is the
129 loss of genetic diversity.

130 The earliest method for artificial oyster production was the use of large ponds
131 that were seeded with hundreds of adult oysters and the resultant spat collected on shells
132 or tiles placed in the pond. Ponds were originally crude un-lined basins 2-3 m deep and
133 up to 10 hectares in area. Natural ponds or "polls" were exploited in parts of Norway
134 that enabled enhanced spat production by making use of the greenhouse effect of a layer
135 of surface fresh water (Kirkland et al. 1983). Ponds fell out of favour in the 1960s when
136 hatchery culture proved to be more controlled and more reliable, but recently smaller
137 replicated butyl-lined ponds have been revived and have proved to be a reliable source
138 of oyster spat production (Gathorne-Hardy and Hugh-Jones 2004; Laing et al. 2005).
139 However, there are no published data on the potential loss of genetic diversity inherent
140 in pond production.

141 Traditionally, natural recruitment from wild populations was encouraged by the
142 laying of tiles or shells to collect oyster spat. Young oysters were then scraped from the
143 tiles and re-seeded into the fishery. This enhancement of natural recruitment by
144 providing abundant settlement substrate would be expected to maintain existing genetic
145 variability.

146 In the present study we have used *O. edulis* supplied from hatchery culture, pond
147 culture and from natural recruitment to compare the genetic diversity at highly variable
148 microsatellite loci generated by these three types of seed sources. Such information is
149 critical to any proposed restoration programme for this species in Europe.

150

151 **2. Materials and methods**

152

153 *2.1. Sampling*

154

155 Twelve *O. edulis* populations were sampled (Figure 1). Between 30 and 48
156 oysters per population were analysed.
157 The wild or managed populations were sampled in in Loch Ryan (Scotland,
158 WLR) in 2006; in Quiberon (France, WQ) and Grevelingen (the Netherlands, WGr) in
159 2007; and in Ria Formosa (Portugal, WRF) in 2008. Quiberon, a large bay in southern
160 Brittany, is a major site of flat oyster spat collection and on-growing. Grevelingen is a
161 landlocked population, in a shallow (2-10 m) marine lake. Over-exploitation of its
162 stocks lead to successive importations of foreign spat (MacKenzie et al. 1997). Since
163 1964, no commercial production and no foreign importations have been reported for
164 Grevelingen oysters (Drinkwaard 1999). Ria Formosa is a shallow coastal lagoon
165 located in Algarve (southern Portugal). The clam *Ruditapes decussatus* and the cupped
166 oysters *Crassostrea gigas* and *Crassostrea angulata* are farmed in this site (Chícharo
167 and Chícharo 2001) but flat oysters are not sufficiently abundant for commercial
168 exploitation (Batista, Personal communication). The Loch Ryan population represents a
169 long-managed wild stock with regular natural spatfall and is the largest fishery for
170 native oysters in Scotland (Hugh-Jones 2003). Oysters from France, Holland or Essex
171 were laid in Loch Ryan in 1880s and in 1960s (Beaumont et al. 2006).
172 The pond-produced populations were sampled in Boemlo (Norway, PBo),
173 Vaagstranda (Norway, PVa), Venø (Denmark, PVe) in 2007 and in Rossmore (Ireland,
174 PRo) in 1999. In Norway two types of ponds (heliothermic polls) are traditionally used
175 for cultivation (Kirkland et al. 1983; MacKenzie et al. 1997). The “breed-polls”, 5-10
176 m deep, 1-5 ha in area, exhibit strong salinity stratification, have restricted water

177 exchange with the outside fjord (tidal exchanges controlled by a gate) and are used for
178 spawning and collecting spat. The "spat-polls", larger (up to 20 m deep and 40 ha in
179 area), exchange water with the outside fjord and are used as fattening grounds
180 (MacKenzie et al. 1997). The Boemlo population (Boemlo Skjell Ltd) was sampled
181 from a "breed-poll" of 2 ha, 5-6 m deep, containing around 2 500 oysters, the
182 broodstock originating in the 1980s from a mixture of different populations around
183 Norway (Magnesen, Personal communication). The Vaagstranda population (Arctic
184 Oysters Ltd) was sampled from a "spat-poll" of 40 ha, 10-12 m deep, containing
185 100 000 oysters which originated from an introduction from Holland in 1930
186 (Magnesen, Personal communication). The Danish Venø population was produced in
187 2004 in outdoor ponds (about 0.07 ha, 1.7 m deep) containing 400 oysters originated
188 from Limfjord (Nissum Bredning) (Ommaney, Personal communication). The
189 Rossmore population (Cork Harbour) was produced in the context of selective breeding
190 programme for resistance to bonamiosis: oysters were bred in spatting ponds from older
191 oysters that survived the epizooty or were resistant to *B. ostreae* and that have been
192 selectively bred for several generations (Sobolewska and Beaumont 2005). The
193 Rossmore pond production was based on 22 butyl-lined ponds (0.04 ha, 2 m deep).
194 These ponds were stocked with up to 700-800 oysters (Laing et al. 2005; Gathorne-
195 Hardy and Hugh-Jones 2004).
196 The hatchery-produced populations were derived either from a commercial
197 hatchery (Seasalter Shellfish Ltd, Whitstable, England) or from a research hatchery
198 (Ifremer, Argenton, France). The Loch Kishorn population (Scotland, HLK), sampled in
199 2000, originated from Seasalter hatchery (original seed) (Sobolewska and Beaumont
200 2005). The Orkney population (Scotland, HO), sampled in 2000, was three generations
201 removed from original Seasalter seed (Sobolewska and Beaumont 2005). The HBOR

202 population resulted from the mixing of two spawning events (11/06/2007 and
203 27/06/2007) from 58 *Bonamia ostreae*-resistant broodstock originating from the French
204 selective breeding programme (Naciri-Graven et al. 1998). The HMED population
205 resulted from the mixing of two spawning events (09/06/2007 and 22/06/2007) from 95
206 wild Mediterranean oysters from Thau lagoon.
207
208 *2.2. DNA extraction and amplification of microsatellite loci*
209
210 Genomic DNA was extracted from gill tissue, using a standard chloroform /
211 isoamylalcohol method (Sambrook et al. 1989) and purified with the Wizard DNA
212 Clean Up System (Promega). DNA quantification was performed using a
213 spectrophotometer (BioPhotometer, Eppendorf). Five microsatellite markers were
214 amplified following the authors' instructions: *Oedu*J12, *Oedu*T5 (Launey et al. 2002);
215 *Oedu*.B11 (Naciri et al. 1995); *Oedu*.HA7 (Sobolewska et al. 2001) and Oe3/44
216 (Morgan et al. 2000). *Oedu*T5 and *Oedu*.HA7 are distributed in the same linkage group,
217 *Oedu*J12, *Oedu*.B11 and Oe3/44 being distributed on three different linkage groups
218 (Lallias et al. 2007).
219
220 *2.3. Genetic analysis*
221
222 Genetic diversity within each of the twelve populations was measured as the
223 number of alleles per locus (N_a), the observed heterozygosity (H_o) and unbiased
224 expected heterozygosity (H_e) (Nei 1978) under Hardy-Weinberg equilibrium. Allelic
225 richness (A) (correcting frequency for unequal sample sizes) (El Mousadik and Petit
226 1996) was estimated per locus and per sample. All analyses were performed with

FSTAT ver. 2.9 (Goudet 1995). Allelic richness and expected heterozygosity were compared between the three groups (wild, pond, hatchery) using a one-way ANOVA marker by marker followed by Tukey's pairwise comparisons, or a Kruskall-Wallis test when variances were not equal (PAST software, Hammer et al. 2001). Wright's (1931) F-statistics were computed according to Weir and Cockerham's (1984) estimators, using FSTAT. Deviations from Hardy-Weinberg equilibrium (F_{is}) were computed in each sampled population and genetic differentiation between populations was estimated using Wright's fixation index F_{st}. The significance of departure from zero of F_{is} (or F_{st}) values was assessed by randomizing alleles within samples (or genotypes among samples), based on 2 000 randomizations and after Bonferroni adjustment.

2.4. Linkage disequilibrium analysis

Linkage disequilibrium was assessed by permutation tests (1 000 permutations) with GENETIX 4.1 software (Belkhir et al. 1996-2001), for each pair of markers in each population.

2.5. Estimation of effective breeding sizes

Effective breeding sizes (N_b) were estimated for the 12 populations sampled using three different methods. The heterozygote excess method was implemented in Colony v2.0 (Wang 2009). The linkage disequilibrium method (Hill 1981) was implemented in LDNE program (Waples and Do 2008). For this method, the lowest allele frequency used (P_{crit} value) was 0.02, as recommended by Waples and Do (2009). Sibship-based estimates were obtained using Colony v2.0 (Wang 2009), assuming a

polygamous breeding system for males and females, and using the full likelihood model with medium precision and no prior information. Also, Spearman rank correlations (r_s) were calculated between N_b estimates and allelic richness.

2.6. Pedigree reconstruction analysis

Pedigree reconstruction on the four hatchery-produced populations was performed with PEDIGREE 2.2 (Herbinger et al. 2006), which partitions individuals into family groups (full-sibs (FS) or half-sibs (HS)) based on molecular marker data in the absence of parental information. Four parameters are chosen by the user: number of iterations of the Markov Chain, full-sib constraint (to choose between a full-sib partition and a kin group partition), temperature of the Markov Chain and weight (W) used in computing the partition score.

The four hatchery datasets were analysed with the FS partition algorithm in order to detect the presence of FS families. To generate the best (with the highest score) full-sibs partition with W=1, we performed four runs with 1 million iterations and temperature of 10, followed by four runs with 1 million iterations and temperature of 30 and used the best FS partition W=1 as a start-up partition file to check that no better partition could be found. This procedure was repeated with W=5 and W=10. The three best FS partitions obtained with an increasing weight were then compared with the COMPARE function of PEDIGREE 2.2.

3. Results

3.1. Linkage disequilibrium

No significant linkage disequilibrium was observed for the four wild populations and PVa. Significant linkage disequilibrium was observed for PBo (1 out of 10 pairs), PRo (5 out of 10 pairs), PVe and HO (6 out of 10 pairs), HBOR (8 out of 10 pairs), HLK and HMED (all 10 pairs of loci).

3.2. Comparison of genetic diversity between wild, pond and hatchery populations

Allelic richness ranged from 6.14 (HBOR) to 19.08 (WLR) (Table 1.). Mean allelic richness (averaged over 5 loci and 4 populations) was 7.57 for the hatchery-derived populations, 14.07 for the pond-produced populations and 18.43 for the wild populations. One-way ANOVA performed for each marker revealed that, at each locus, hatchery-derived populations exhibited a significantly lower mean allelic richness than the pond-derived and the wild populations ($p<0.05$). For three out of five loci (*Oedu*.B11, *Oedu*.HA7 and *Oedu*T5), pond-derived populations exhibited a significantly lower ($p<0.05$) allelic richness than the wild populations. Mean observed heterozygosity varied between 0.756 (PBo) and 0.856 (HBOR) and mean expected heterozygosity ranged from 0.680 (HMED) to 0.915 (WLR) (Table 1). Mean expected heterozygosity (averaged over 5 loci and 4 populations) was 0.743, 0.857 and 0.900 for hatchery-produced, pond-produced, and wild populations respectively. For four out of five loci (all but Oe3/44), one-way ANOVA revealed that hatchery derived populations exhibited significantly lower expected heterozygosities than pond derived populations and wild populations ($p<0.05$ for each pairwise comparison).

Wright's (1965) F_{is} calculated for all five loci in each population showed significant overall heterozygote deficiencies for four populations (WQ, WGr, WLR and

PBo) after Bonferroni adjustment ($p<0.05$), mainly due to locus *Oedu*.B11 (Table 1). Null alleles were suspected at that locus. After the removal of locus *Oedu*.B11 from the analyses, no significant deficiencies of heterozygotes were observed. Significant heterozygote excesses were observed for two of the hatchery populations (HBOR and HMED) ($p<0.05$ after Bonferroni adjustment) (Table 1) for all markers. Finally, the four hatchery populations exhibited a significant overall excess of heterozygotes ($p<0.05$) after the removal of locus *Oedu*.B11 (Table 1).

3.3. Genetic differentiation among populations

F_{st} values for pairwise comparison among populations from *O. edulis* are given in Table 2. Among the wild populations, F_{st} values were low, ranging from 0.006 to 0.044. Only the Grevelingen population was significantly differentiated from the Quiberon and the Ria Formosa populations. Pond and hatchery-produced populations were significantly differentiated from each other and from the wild populations (F_{st} values ranging from 0.006 to 0.254). HMED was the population exhibiting the highest F_{st} values (from 0.126 to 0.254) in the pairwise comparison with the other populations.

3.4. Effective breeding sizes

N_b estimates for the 12 *O. edulis* populations are shown in Table 3. The heterozygote excess method only generated N_b estimates for two hatchery populations, HBOR (7) and HMED (7), without 95% confidence intervals. With the linkage disequilibrium (LD) method, very high effective breeding sizes were obtained for the four wild populations and two Norwegian pond-produced populations (PBo and PVa)

(from 138 [74-536] to ∞ [448-∞]). Estimated N_b were far smaller for PVe: 33 [26-45] and PRo: 49 [37-70]. For the hatchery-produced populations, relatively small N_b were reported for HO: 19 [14-25]; and very small N_b for HBOR: 6 [3-10], HLK: 4 [3-8] and HMED: 2 [2-3]. By contrast, the sibship assignment (SA) - based method produced N_b estimates and finite 95% confidence intervals for the 12 sampled populations. Both LD and SA methods gave similar N_b estimates for six populations: the two small pond-produced and the four hatchery-produced populations. However, for the two large pond-produced and the four wild populations, the SA method produced far smaller N_b estimates than the LD method. Figure 2 shows significant positive correlations between the two N_b estimates and allelic richness in the 12 flat oyster populations (r_s (LD) = 0.869, $P < 0.001$; r_s (SA) = 0.931, $P < 0.001$).

3.5. Pedigree reconstruction analysis

For the HMED population, the best FS partition (score 3924.03, W=5) identified 8 groups (Figure 3). We were able to reconstruct the parental genotypes of the large FS family (30 offspring) and calculate segregation distortion amongst the progeny (Table 4).

The best FS partition (score 1576.25, W=5) revealed 8 groups in the HBOR population (Figure 3). The parental genotypes of the two largest FS families could be reconstructed and were identical at four out of five loci. Differences between them occurred only at the *Oedu*.B11 locus. The parental *Oedu*.B11 genotypes of the first family were 122/122 x 122/166 but were 128/128 x 128/166 for the second family. Four different genotypes were present among the progeny of the two first families: 128/166, 128/128, 122/166 and 122/122. Therefore, this was compatible with the segregation of a

352 null allele (Table 5). We concluded that the two largest FS families (18 and 12 offspring
353 respectively) in this HBOR population were in fact a single FS family of 30 offspring
354 (Figure 3, Table 4).
355 For the HLK population, the best FS partition (score 1796.43; W=10) identified
356 7 groups (Figure 3). After reconstructing the parental genotypes of the largest FS family
357 containing 14 offspring, it was noted that a separate group of four offspring could be
358 placed within that FS family if an *Oedu*.B11 null allele was present in one parent (Table
359 5). Therefore, we concluded that the largest FS family in the HLK sample consisted of
360 18 offspring (Figure 3, Table 4).
361 Finally, the HO population revealed (score 3759.14, W=5) 16 groups (Figure 3).
362 By changing W from 5 to 10 some groups with one or two offspring merged with other
363 small FS groups. Parental genotypes could not be reconstructed for any FS family.
364 Therefore the HO population appeared to be composed of several small FS families, in
365 contrast to the HMED, HBOR and HLK populations.
366
367 **4. Discussion**
368
369 *4.1. Loss of genetic diversity*
370
371 Our results highlight the loss of genetic diversity in hatchery populations in
372 comparison with pond-produced and wild populations, both in terms of a reduction in
373 allelic richness and in the expected heterozygosity. Pond-produced populations were
374 quite effective in maintaining the genetic diversity but small scale ponds still showed a
375 significant reduction in allelic richness relative to wild populations. These results are
376 not surprising because reduced genetic diversity has been reported in hatchery or

377 aquaculture populations of finfish (Bouza et al. 1997; Lundrigan et al. 2005; Liu et al.
378 2005; Machado-Schiaffino et al. 2007), algae (Guillemin et al. 2008) and shellfish
379 (Gaffney et al. 1992; Hedgecock et al. 1992; Saavedra 1997; Taris et al. 2006; Hara and
380 Sekino 2007; Lind et al. 2009). However, we are able to demonstrate that alternative
381 managed reproduction methods using large scale ponds can retain high genetic diversity
382 in the flat oyster and that this information is of significant value for restoration of this
383 species.
384
385 *4.2. High variance in reproductive success and small effective breeding size*
386
387 N_b estimates were obtained based on different methods (Table 3). As reported by
388 Beebee (2009) and Wang (2009), the heterozygote excess method performed very badly
389 and gave meaningful estimates for only 2 (out of 12) populations. The linkage
390 disequilibrium (LD) and sibship assignment (SA) methods gave consistent N_b estimates
391 for six populations (the four hatchery and the two small pond populations), bolstering
392 confidence in our results. However, the SA method gave far smaller N_b estimates
393 (below 70), compared with the LD method (between 138 and infinity), for the two large
394 pond and the four wild populations. The low number of markers used in this study, as
395 well as a sample size much smaller than the actual effective population size could
396 explain the discrepancies in the N_b estimates. Indeed, in such a situation, the SA method
397 becomes biased and yields confidence intervals that are too narrow (Wang 2009). Also,
398 the SA method assumes sampling from a single cohort; violation of this assumption
399 could have an impact on N_b estimates. The LD-based N_b estimates, although less precise,
400 seem therefore to give more realistic estimates in our study. Despite the slight
401 discrepancies in the N_b estimates, allelic richness analyses and N_b estimates gave

consistent results: significant positive correlations between LD-based or SA-based N_b estimates and allelic richness were observed (Figure 2). This is in agreement with the neutral theory that predicts a positive, albeit nonlinear, relationship between ln N_e and genetic diversity (Soulé 1976). Such a linear correlation has previously been shown by Beebee (2009) in toad populations.

Our results revealed that the four hatchery populations had much reduced effective breeding sizes compared with wild and pond populations (Table 3). Also, the occurrence of heterozygote excesses in the hatchery populations (Table 1) could be a consequence of low N_b (Luikart and Cornuet 1999). HLK and HO populations both originated from Seasalter hatchery, the HLK oysters being first generation while the HO oysters are 3 generations removed from Seasalter imports. Interestingly HO was associated with a higher N_b than HLK. Following a hatchery bottleneck, the linkage disequilibrium is expected to decay over time (assuming random mating of hatchery-produced oysters after the bottleneck), with a consequent increase in N_b estimate. However, hatchery bottlenecks are likely to vary in their strength even within the same hatchery. Therefore, it would be best to make temporal comparisons of N_b estimates to assess the within-hatchery variance, before drawing conclusions about the long term consequences of hatchery practices.

The negative impacts on genetic diversity of hatchery practices such as mass spawning, culling of slow-growing larvae (Taris et al. 2006) or communal rearing of different families have been widely documented. In particular, the impact in terms of a reduced N_b has been previously reported for many shellfish species (e.g. Hedgecock et al. 1992), and oysters in particular: the pearl oyster *Pinctada maxima* (Lind et al. 2009); the cupped oysters *Crassostrea gigas* and *C. virginica* (e.g. Hedgecock and Sly 1990; Gaffney et al. 1992; Appleyard and Ward 2006) and the flat oyster *O. edulis* (e.g.

427 Saavedra and Guerra 1996; Saavedra 1997; Launey et al. 2001). Some remediation of
428 the problem can be achieved by pooling the progeny of multiple spawning events
429 (Gaffney et al. 1992) or by performing controlled spawnings (Lind et al. 2009).
430 However, flat oysters are sequential hermaphrodites and an individual's sex at any time
431 cannot be determined non-destructively. A further complication is that males produce
432 spermatozeugmata and females brood their eggs and early larvae up to approximately
433 10 days after fertilization (O'Foighil 1989) adding to the difficulty of making controlled
434 multiple crosses or mass matings. We know that two hatchery populations used in our
435 study (HBOR and HMED) involved the pooling of two spawning events but they still
436 had a very low effective breeding size (below 6 or 12 according to the LD and SA
437 methods respectively). Even at the small pond scale (Venø, Rossmore), where 400-800
438 broodstock were used, there was a reduced N_b although more genetic diversity was
439 retained than the hatchery-produced populations. Therefore, simply increasing the
440 number of breeders does not necessarily increase the effective breeding size. It is only
441 when we move to the Norwegian ponds of larger dimensions that LD-based N_b
442 approaches that of the wild populations. In such ponds, the oyster population remains
443 undisturbed from year to year, allowing spatial dynamics such as clumping to develop
444 among breeders, which may affect N_b. This does not happen in small ponds (such as
445 PRo) because they are emptied and cleaned each year.
446 Pedigree reconstruction of the four hatchery-produced populations revealed that
447 three of those populations (HMED, HBOR, and HLK) were composed of a large full-sib
448 family and a few small full-sib families. Pedigree reconstruction and N_b estimation gave
449 congruent results: HMED is the hatchery population with the lowest N_b and with the
450 highest skew in family contribution, while HO is the hatchery population with the
451 highest N_b and the highest number of contributing families as well as a more even

452 contribution of families (Table 3, Figure 3). This also strongly highlights the variance in
453 reproductive success among the potential breeders, something that has also been
454 demonstrated for salmonids (Herbinger et al. 2006). High relatedness among progenies,
455 as demonstrated by our pedigree reconstruction analyses, could have serious
456 implications for the long-term management of hatchery stocks, rapidly leading to
457 inbreeding depression (Bierne et al. 1998; Naciri-Graven et al. 2000; Taris et al. 2007)
458 due to a high genetic load in oysters (Launey and Hedgecock 2001). Recent studies
459 reported high variance of reproductive success in oysters both in the wild (Li and
460 Hedgecock 1998; Hedgecock et al. 2007) and under aquaculture conditions (Hedgecock
461 et al. 1992; Boudry et al. 2002; Taris et al. 2007; Lallias et al. Unpublished results).
462 Variance in reproductive success seems a fundamental factor influencing the N_b.
463 Therefore, further studies should be implemented to improve hatchery practices, in
464 order to reduce variance in family contributions.
465
466 *4.3. Implications for restoration*
467
468 Hatchery-produced populations of oysters are associated with a significant loss
469 of allelic diversity and heterozygosity. Moreover, almost no genetic differentiation was
470 observed across the wild populations (as shown by Launey et al. 2002; Sobolewska and
471 Beaumont 2005; Beaumont et al. 2006), whereas hatchery practices led the hatchery-
472 produced populations to be highly differentiated from the wild populations (Table 2).
473 Unless hatcheries significantly change their methods of production, the restoration of
474 wild populations of *O. edulis* by hatchery-produced stocks could be detrimental to the
475 conservation of oysters and the long-term sustainability of their fisheries. Further
476 studies are needed to estimate census numbers and N_e of European oyster populations to

477 better estimate the potential impacts and benefits of supportive breeding (Ryman and
478 Laikre 1991; Gaffney 2006). There is little sense in mounting a major restoration
479 programme for flat oysters if it will not lead to increased population sizes of wide
480 genetic diversity. Genetic diversity is essential for long term sustainability. Large scale
481 pond-production systems could represent a valuable alternative to hatcheries for
482 restocking flat oyster populations because they seem more efficient in the maintaining
483 of genetic diversity.

484 Restoration of flat oyster populations in Europe is complicated by the existence
485 of bonamiosis which can cause very high mortalities in flat oyster populations. At the
486 present time, some areas in northern Europe are bonamiosis-free while the disease is
487 common elsewhere. Restoration of oysters in bonamiosis-free regions could therefore
488 take advantage of the potential high genetic diversity provided by large scale pond
489 production. In bonamiosis areas the better strategy might be to attempt restoration using
490 bonamiosis-resistant strains from hatchery (Naciri-Graven et al. 1998) or small pond-
491 culture (Culloty et al. 2004) but to also to improve hatchery and small pond production
492 methods to gain the highest genetic diversity possible.

493

494 **Acknowledgements**

495

496 We would like to thank Thorolf Magnesen for supplying the two Norwegian oyster
497 populations, Alexandra Leitão for the Ria Formosa population, and René Robert for the
498 French hatchery HBOR and HMED samples. We thank two anonymous reviewers for
499 useful comments and suggestions. This research has been financed by the European
500 Regional Development Fund INTERREG IIIB Priority C, project 201 – AAAG2
501 (Atlantic Arc Aquaculture Group 2).

502

503 **References**

504

505 Appleyard SA, Ward RD (2006) Genetic diversity and effective population size in mass
506 selection lines of Pacific oyster (*Crassostrea gigas*). Aquaculture 254:148-159.
507 Arnold WS (2008) Application of Larval Release for Restocking and Stock
508 Enhancement of Coastal Marine Bivalve Populations. Rev Fish Sci 16:65 - 71.
509 Arnold WS, Marelli DC, Parker M, Hoffman P, Frischer M, Scarpa J (2002) Enhancing
510 hard clam (*Mercenaria* spp.) population density in the Indian River Lagoon, Florida:
511 a comparison of strategies to maintain the commercial fishery. J Shellfish Res
512 21:659-672.
513 Arnold WS, Blake NJ, Harrison MM, Marelli DC, Parker ML, Peters SC, Sweat DE
514 (2005) Restoration of bay scallop (*Argopecten irradians* (Lamarck)) populations in
515 Florida coastal waters: planting techniques and the growth, mortality and
516 reproductive development of planted scallops. J Shellfish Res 24:883-904.
517 Beaumont AR, Trebano Garcia M, Honig S, Low P (2006) Genetics of Scottish
518 populations of the native oyster, *Ostrea edulis*: gene flow, human intervention and
519 conservation. Aquat Living Resour 19:389-402.
520 Beebee TJC (2009) A comparison of single-sample effective size estimators using
521 empirical toad (*Bufo calamita*) population data: genetic compensation and
522 population size-genetic diversity correlations. Mol Ecol 18:4790-4797.
523 Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996-2001) GENETIX 4.02,
524 logiciel sous Windows TM pour la génétique des populations. Montpellier, France :
525 Laboratoire Génome, Populations, Interactions, CNRS UMR 5000, Université de
526 Montpellier II.

527 Bierne N, Launey S, Naciri-Graven Y, Bonhomme F (1998) Early effect of inbreeding
528 as revealed by microsatellite analyses on *Ostrea edulis* larvae. Genetics 148:1893-
529 1906.
530 Boudry P, Collet B, Cornette F, Hervouet V, Bonhomme F (2002) High variance in
531 reproductive success of the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed
532 by microsatellite-based parentage analysis of multifactorial crosses. Aquaculture
533 204:283-296.
534 Bouza C, Sanchez L, Martinez P (1997) Gene diversity analysis in natural populations
535 and cultured stocks of turbot (*Scophthalmus maximus* L.). Anim Genet 28:28-36.
536 Brumbaugh RD, Sorabella LA, Garcia CO, Goldsborough WJ, Wesson JA (2000)
537 Making a case for community-based oyster restoration: An example from Hampton
538 Roads, Virginia, U.S.A. J Shellfish Res 19:467-472.
539 Chícharo L, Chícharo MA (2001) Effects of environmental conditions on planktonic
540 abundances, benthic recruitment and growth rates of the bivalve mollusc *Ruditapes*
541 *decussatus* in a Portuguese coastal lagoon. Fish Res 53:235-250.
542 Culloty SC, Cronin MA, Mulcahy MF (2004) Potential resistance of a number of
543 populations of the oyster *Ostrea edulis* to the parasite *Bonamia ostreae*. Aquaculture
544 237:41-58.
545 da Silva PM, Fuentes J, Villalba A (2005) Growth, mortality and disease susceptibility
546 of oyster *Ostrea edulis* families obtained from brood stocks of different
547 geographical origins, through on-growing in the Ría de Arousa (Galicia, NW Spain).
548 Mar Biol 147:965-977.
549 Drinkwaard A (1999) Introductions and developments of oysters in the North Sea area:
550 A review. Helgol Meeresunters 52:301-308.

551 El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness
552 among populations of the argan tree [*Argania spinosa* (L.) Skeels] endemic to
553 Morocco. Theor Appl Genet 92:832-839.
554 Foighil DO (1989) Role of spermatozeugmata in the spawning ecology of the brooding
555 oyster *Ostrea edulis*. Gamete Res 24:219-228.
556 Gaffney PM (2006) The role of genetics in shellfish restoration. Aquat Living Resour
557 19:277-282.
558 Gaffney PM, Davis CV, Hawes RO (1992) Assessment of drift and selection in
559 hatchery populations of oysters (*Crassostrea virginica*). Aquaculture 105:1-20.
560 Gathorne-Hardy A, Hugh-Jones T (2004) Spat collection in native oyster ponds.
561 Shellfish News 17:6-9.
562 Gosling EM (1982) Genetic variability in hatchery-produced Pacific oysters
563 (*Crassostrea gigas* Thunberg). Aquaculture 26:273-287.
564 Goudet J (1995) FSTAT (vers. 1.2): a computer program to calculate F-statistics. J
565 Hered 86:485-486.
566 Guillemin ML, Faugeron S, Destombe C, Viard F, Correa JA, Valero M (2008) Genetic
567 variation in wild and cultivated populations of the haploid-diploid red alga
568 *Gracilaria chilensis*: how farming practices favor asexual reproduction and
569 heterozygosity. Evolution 62:1500-1519.
570 Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Palaeontological Statistics software
571 package for education and data analysis. Palaeontologia Electronica 4, 9 pp.
572 Hara M, Sekino M (2007) Genetic Differences Between Hatchery Stocks and Natural
573 Populations in Pacific Abalone (*Haliotis discus*) Estimated Using Microsatellite
574 DNA Markers. Mar Biotech 9:74-81.

575 Hare MP, Allen SK, Bloomer P, Camara MD, Carnegie RB, Murfree J, Luckenbach M,
576 Meritt D, Morrison C, Paynter K, Reece KS, Rose CG (2006) A genetic test for
577 recruitment enhancement in Chesapeake Bay oysters, *Crassostrea virginica*, after
578 population supplementation with a disease tolerant strain. Conserv Genet 7:717-734.
579 Hedgecock D, Sly F (1990) Genetic drift and effective population sizes of hatchery-
580 propagated stocks of the Pacific oyster, *Crassostrea gigas*. Aquaculture 88:21-38.
581 Hedgecock D, Chow V, Waples RS (1992) Effective population numbers of shellfish
582 broodstocks estimated from temporal variance in allelic frequencies. Aquaculture
583 108:215-232.
584 Hedgecock D, Launey S, Pudovkin AI, Naciri Y, Lapègue S, Bonhomme F (2007)
585 Small effective number of parents (N_b) inferred for a naturally spawned cohort of
586 juvenile European flat oysters *Ostrea edulis*. Mar Biol 150:1173-1182.
587 Herbinger CM, O'Reilly PT, Verspoor E (2006) Unravelling first-generation pedigrees
588 in wild endangered salmon populations using molecular genetic markers. Mol Ecol
589 15:2261-2275.
590 Hill WG (1981) Estimation of effective population size from data on linkage
591 disequilibrium. Genet Res 38:209-216.
592 Hugh-Jones T (2003) The Loch Ryan native oyster fishery. Shellfish News 15:17-18.
593 Kirkland DW, Platt Bradbury J, Dean WE (1983) The heliothermic lake - a direct
594 method of collecting and storing solar energy. Arch Hydrobiol 65:1-60.
595 Laing I, Walker P, Areal F (2005) A feasibility study of the native oyster (*Ostrea edulis*)
596 stock regeneration in the United Kingdom (CARD Project Report FC1016).
597 Available via DIALOG.
598 http://www.defra.gov.uk/science/project_data/DocumentLibrary/FC1016/FC1016_2
599 543_FRP.pdf.

600 Laing I, Walker P, Areal F (2006) Return of the native - is European oyster (*Ostrea*
601 *edulis*) stock restoration in the UK feasible? Aquat Living Resour 19:283-287.
602 Lallias D, Beaumont AR, Haley CS, Boudry P, Heurtebise S, Lapègue S (2007) A first-
603 generation genetic linkage map of the European flat oyster *Ostrea edulis* (L.) based
604 on AFLP and microsatellite markers. Anim Genet 38:560-568.
605 Lallias D, Gomez-Raya L, Haley C, Arzul I, Heurtebise S, Beaumont A, Boudry P,
606 Lapègue S (2009) Combining Two-Stage Testing and Interval Mapping Strategies
607 to Detect QTL for Resistance to Bonamiosis in the European Flat Oyster *Ostrea*
608 *edulis*. Mar Biotechnol 11:570-584.
609 Launey S, Hedgecock D (2001) High genetic load in the Pacific oyster *Crassostrea*
610 *gigas*. Genetics 159:255-265.
611 Launey S, Barre M, Gerard A, Naciri-Graven Y (2001) Population bottleneck and
612 effective size in *Bonamia ostreae*-resistant populations of *Ostrea edulis* as inferred
613 by microsatellite markers. Genet Res 78:259-270.
614 Launey S, Ledu C, Boudry P, Bonhomme F, Naciri-Graven Y (2002) Geographic
615 structure in the European flat oyster (*Ostrea edulis* L.) as revealed by microsatellite
616 polymorphism. J Hered 93:331-351.
617 Li G, Hedgecock D (1998) Genetic heterogeneity, detected by PCR-SSCP, among
618 samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of
619 large variance in reproductive success. Can J Fish Aquat Sci 55:1025-1033.
620 Lind CE, Evans BS, Knauer J, Taylor JJU, Jerry DR (2009) Decreased genetic diversity
621 and a reduced effective population size in cultured silver-lipped pearl oysters
622 (*Pinctada maxima*). Aquaculture 286:12-19.
623 Liu Y, Chen S, Li B (2005) Assessing the genetic structure of three Japanese flounder
624 (*Paralichthys olivaceus*) stocks by microsatellite markers. Aquaculture 243:103-111.

625 Luikart G, Cornuet JM (1999) Estimating the effective number of breeders from
626 heterozygote excess in progeny. Genetics 151:1211-1216.
627 Lundrigan TA, Reist JD, Ferguson MM (2005) Microsatellite genetic variation within
628 and among Arctic charr (*Salvelinus alpinus*) from aquaculture and natural
629 populations in North America. Aquaculture 244:63-75.
630 Machado-Schiaffino G, Dopico E, Garcia-Vazquez E (2007) Genetic variation losses in
631 Atlantic salmon stocks created for supportive breeding. Aquaculture 264:59-65.
632 MacKenzie CL, Burrel VG, Rosefield A, Hobart WL (1997) The history, present
633 condition, and future of the molluscan fisheries of north and central America and
634 Europe. National Marine Fisheries Service, Washington, DC.
635 Mann R (2000) Restoring the oyster reef communities in the Chesapeake bay: a
636 commentary. J Shellfish Res 19:335-339.
637 McCay DPF, Peterson CH, DeAlteris JT, Catena J (2003) Restoration that targets
638 function as opposed to structure: replacing lost bivalve production and filtration.
639 Mar Ecol Prog Ser 264:197-212.
640 Milbury C, Meritt D, Newell R, Gaffney P (2004) Mitochondrial DNA markers allow
641 monitoring of oyster stock enhancement in the Chesapeake Bay. Mar Biol 145:351-
642 359.
643 Morgan TS, Rogers AD, Iyengar A (2000) Novel microsatellite markers for the
644 European oyster *Ostrea edulis*. Mol Ecol 9:495-497.
645 Naciri-Graven Y, Martin A-G, Baud J-P, Renault T, Gerard A (1998) Selecting the flat
646 oyster *Ostrea edulis* (L.) for survival when infected with the parasite *Bonamia*
647 *ostreae*. J Exp Mar Biol Ecol 224:91-107.

Naciri-Graven Y, Launey S, Lebayon N, Gerard A, Baud JP (2000) Influence of parentage upon growth in *Ostrea edulis*: evidence for inbreeding depression. Genet Res 76:159-168.

Naciri Y, Vigouroux Y, Dallas J, Desmarais E, Delsert C, Bonhomme F (1995) Identification and inheritance of (GA/TC)n and (AC/GT)n repeats in the European flat oyster *Ostrea edulis* (L.). Mol Mar Biol Biotechnol 4:83-89.

Nei M (1978) Estimation of Average Heterozygosity and Genetic Distance from a Small Number of Individuals. Genetics 89:583-590.

Pullin AS, Knight TM (2009) Doing more good than harm - Building an evidence-base for conservation and environmental management. Biological Conservation 142:931-934.

Ryman N, Laikre L (1991) Effects of supportive breeding on the genetically effective population size. Conservation Biology 5:325-329.

Saavedra C (1997) Low effective sizes in hatchery populations of the European oyster (*Ostrea edulis*): Implications for the management of genetic resources. J Shellfish Res 16:441-446.

Saavedra C, Guerra A (1996) Allozyme heterozygosity, founder effect and fitness traits in a cultivated population of the European oyster, *Ostrea edulis*. Aquaculture 139:203-224.

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbour, New York.

Sobolewska H, Beaumont AR (2005) Genetic variation at microsatellite loci in northern populations of the European flat oyster (*Ostrea edulis*). J Mar Biol Assoc UK 85:955-960.

672 Sobolewska H, Beaumont AR, Hamilton A (2001) Dinucleotide microsatellites isolated
673 from the European flat oyster, *Ostrea edulis*. Mol Ecol Notes 1:79-80.
674 Soulé ME (1976) Allozyme variation, its determinants in space and time. In: Molecular
675 Evolution (ed. Ayala FJ), pp. 46-59. Sinauer Associates, Sunderland, Massachusetts.
676 Taris N, Batista FM, Boudry P (2007) Evidence of response to unintentional selection
677 for faster development and inbreeding depression in *Crassostrea gigas* larvae.
678 Aquaculture 272:S69-S79.
679 Taris N, Ernande B, McCombie H, Boudry P (2006) Phenotypic and genetic
680 consequences of size selection at the larval stage in the Pacific oyster (*Crassostrea*
681 *gigas*). J Exp Mar Biol Ecol 333:147-158.
682 Wang J (2009) A new method for estimating effective population sizes from a single
683 sample of multilocus genotypes. Mol Ecol 18:2148-2164.
684 Waples RS, Do C (2008) LDNE: a program for estimating effective population size
685 from data on linkage disequilibrium. Mol Ecol Resour 8:753-756.
686 Waples RS, Do C (2009) Linkage disequilibrium estimates of contemporary N_e using
687 highly variable genetic markers: a largely untapped resource for applied
688 conservation and evolution. Evol Appl doi:10.1111/j.1752-4571.2009.00104.x.
689 Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population
690 structure. Evolution 38:1358-1370.
691 Wilbur AE, Seyoum S, Bert TM, Arnold WS (2005) A genetic assessment of bay
692 scallop (*Argopecten irradians*) restoration efforts in Florida's Gulf of Mexico
693 Coastal Waters (USA). Conserv Genet 6:111-122.
694 Wright S (1931) Evolution in Mendelian Populations. Genetics 16:97-159.

Table 1. Genetic diversity summary statistics in twelve populations of *Ostrea edulis*. N_a: number of alleles, *A*: allelic richness, H_o: observed heterozygosity, H_e: expected heterozygosity. Significant ($p < 0.05$ after Bonferroni adjustment) F_{is} values in bold. For population abbreviations see Figure 1.

Locus		**Wild populations**				**Pond-produced stocks**				**Hatchery-produced stocks**			
		WQ	WGr	WLR	WRF	PBo	PVa	PVe	PRo	HO	HLK	HBOR	HMED
***Oedu*J12**	N_a	21	22	20	18	13	15	16	22	13	7	8	10
	A	18.66	18.20	17.70	17.65	11.72	13.16	14.00	18.34	11.97	6.70	6.78	8.81
	H_o	0.875	0.854	0.875	0.867	0.729	0.854	0.813	0.875	0.979	0.718	0.900	0.975
	H_e	0.918	0.879	0.925	0.922	0.860	0.875	0.847	0.912	0.885	0.757	0.712	0.729
	F_{is}	0.047	0.028	0.055	0.061	0.153	0.024	0.042	0.041	-0.107	0.052	-0.269	**-0.344**
***Oedu*.B11**	N_a	32	23	32	27	17	20	21	19	10	7	5	11
	A	26.16	20.89	26.81	27.00	15.54	18.03	18.16	16.40	9.09	6.71	4.94	9.36
	H_o	0.500	0.733	0.644	0.714	0.553	0.689	0.739	0.638	0.583	0.641	0.405	0.725
	H_e	0.956	0.947	0.960	0.970	0.912	0.875	0.927	0.899	0.808	0.810	0.687	0.633
	F_{is}	**0.480**	**0.228**	**0.332**	**0.267**	**0.396**	**0.215**	**0.205**	**0.292**	**0.280**	0.211	**0.414**	-0.148

***Oedu*.HA7**	N_a	20	22	21	20	16	17	20	18	11	7	9	12
	A	17.45	18.48	18.73	19.46	14.64	14.90	15.83	15.93	9.54	6.44	8.05	10.28
	H_o	0.979	0.937	0.937	0.967	0.937	0.896	0.958	0.875	0.896	0.872	1.000	0.875
	H_e	0.935	0.919	0.932	0.933	0.906	0.866	0.885	0.909	0.805	0.780	0.792	0.773
	F_{is}	-0.048	-0.020	-0.005	-0.036	-0.035	-0.034	-0.083	0.038	-0.113	-0.120	**-0.267**	-0.134
Oe3/44	N_a	12	21	16	9	8	11	11	15	5	6	4	7
	A	10.08	16.14	12.82	8.99	7.03	9.24	9.28	11.26	4.99	5.44	3.91	5.99
	H_o	0.687	0.792	0.854	0.800	0.687	0.646	0.681	0.875	0.667	0.769	0.975	0.975
	H_e	0.644	0.862	0.825	0.768	0.692	0.638	0.741	0.808	0.658	0.752	0.637	0.595
	F_{is}	-0.069	0.082	-0.035	-0.042	0.007	-0.012	0.082	-0.084	-0.013	-0.023	**-0.542**	**-0.652**
***Oedu*T5**	N_a	22	19	23	19	15	17	18	17	9	7	8	12
	A	18.46	16.98	19.33	18.59	13.48	13.97	15.53	15.02	8.34	6.98	7.00	10.06
	H_o	0.896	0.917	0.937	0.900	0.875	0.917	0.979	0.812	0.917	1.000	1.000	0.600
	H_e	0.901	0.929	0.930	0.935	0.885	0.907	0.921	0.887	0.843	0.826	0.716	0.672
	F_{is}	0.006	0.013	-0.008	0.038	0.012	-0.011	-0.064	0.085	-0.088	-0.214	**-0.404**	0.109

Overall	N_a	21.4	21.4	22.4	18.6	13.8	16.0	17.2	18.2	9.6	6.8	6.8	10.4
	A	18.16	18.14	19.08	18.34	12.48	13.86	14.56	15.39	8.79	6.45	6.14	8.90
	H_o	0.788	0.847	0.850	0.850	0.756	0.800	0.834	0.815	0.808	0.800	0.856	0.830
	H_e	0.871	0.907	0.915	0.906	0.851	0.832	0.864	0.883	0.800	0.785	0.709	0.680
	F_{is}	**0.097**	**0.067**	**0.072**	0.063	**0.112**	0.039	0.036	0.078	-0.011	-0.019	**-0.211**	**-0.223**
Overall	N_a	18.8	21.0	20.0	16.5	13.0	15.0	16.3	18.0	9.5	6.8	7.3	10.3
(without	A	16.16	17.45	17.15	16.17	11.72	12.82	13.66	15.14	8.71	6.39	6.44	8.79
***Oedu*.B11)**	H_o	0.859	0.875	0.901	0.884	0.807	0.828	0.858	0.859	0.865	0.840	0.969	0.856
	H_e	0.849	0.897	0.903	0.890	0.836	0.822	0.849	0.879	0.798	0.779	0.714	0.692
	F_{is}	-0.012	0.025	0.003	0.007	0.035	-0.008	-0.016	0.023	**-0.084**	**-0.079**	**-0.363**	**-0.241**

Table 2. Weir & Cockerham's (1984) estimator of F_{st} calculated for each pair of population, based on five microsatellite markers. Significant ($p < 0.05$ after Bonferroni adjustment) F_{st} values in bold. For population abbreviations see Figure 1.

	WQ	WGr	WLR	WRF	PBo	PVa	PVe	PRo	HO	HLK	HBOR
WQ	-										
WGr	**0.044**	-									
WLR	0.031	0.006	-								
WRF	0.006	**0.035**	0.024	-							
PBo	**0.076**	**0.035**	**0.033**	**0.063**	-						
PVa	**0.089**	**0.038**	**0.036**	**0.080**	0.006	-					
PVe	**0.072**	**0.010**	**0.021**	**0.059**	**0.036**	**0.040**	-				
PRo	**0.036**	**0.032**	**0.019**	**0.021**	**0.061**	**0.071**	**0.057**	-			
HO	**0.097**	**0.071**	**0.061**	**0.096**	**0.102**	**0.108**	**0.087**	**0.083**	-		
HLK	**0.109**	**0.083**	**0.069**	**0.108**	**0.106**	**0.114**	**0.105**	**0.107**	**0.095**	-	
HBOR	**0.095**	**0.132**	**0.118**	**0.100**	**0.172**	**0.172**	**0.167**	**0.123**	**0.168**	**0.212**	-
HMED	**0.142**	**0.149**	**0.129**	**0.126**	**0.146**	**0.160**	**0.158**	**0.141**	**0.206**	**0.217**	**0.254**

Table 3. Estimates of effective breeding sizes for *O. edulis* populations. H excess: heterozygote excess method implemented in Colony v2.0 (Wang 2009); LD: linkage disequilibrium method using LDNE program (Waples and Do 2008); Sibship: sibship-based estimates using Colony v2.0 (Wang 2009). 95% confidence intervals are given in brackets. n: sample size; N_b: effective breeding size; ∞: infinity.

Population (n)	Effective breeding size estimates (N_b)			Type
	H excess	LD	Sibship	
WQ (48)	∞	1130 [209-∞]	54 [34-88]	Wild
WGr (48)	∞	13652 [246-∞]	57 [37-92]	
WLR (48)	∞	∞ [448-∞]	64 [41-104]	
WRF (30)	∞	382 [80-∞]	47 [28-85]	
PBo (48)	∞	138 [74-536]	38 [24-64]	Pond
PVa (48)	∞	429 [129-∞]	40 [26-65]	
PVe (48)	∞	33 [26-45]	24 [13-46]	
PRo (48)	∞	49 [37-70]	38 [23-64]	
HO (48)	∞	19 [14-25]	18 [10-38]	Hatchery
HLK (39)	∞	4 [3-8]	10 [5-26]	
HBOR (40)	7	6 [3-10]	12 [6-28]	
HMED (40)	7	2 [2-3]	9 [4-23]	

Table 4. Reconstructed genotypes of parents of large full-sibs families identified in the four hatchery-produced populations, using the program PEDIGREE 2.2. n: number of offspring in the full-sibs group; null: null allele segregating in the family; Chi²: chi-squared value for the Mendelian segregation test in the progeny (significant $p<0.05$ values in bold).

Population	FS group (n)	Marker	Reconstructed genotype	Chi²
HMED	1 (30)	*Oedu*J12	238/238 x 228/250	0.00
		Oedu.B11	136/136 x 136/148	**8.53**
		Oedu.HA7	154/172 x 172/182	6.80
		Oe3/44	185/185 x 213/213	0.00
		*Oedu*T5	118/138 x 118/138	1.20
HBOR	1 (30)	*Oedu*J12	230/234 x 226/230	7.78
		Oedu.B11	122/128 x 166/null	1.73
		Oedu.HA7	164/178 x 172/186	2.44
		Oe3/44	185/185 x 205/215	**5.56**
		*Oedu*T5	134/134 x 126/144	0.00
HLK	1 (18)	*Oedu*J12	236/244 x 244/248	2.44
		Oedu.B11	142/null x 134/148	0.67
		Oedu.HA7	162/172 x 174/178	4.22
		Oe3/44	205/217 x 209/217	1.11
		*Oedu*T5	124/134 x 138/146	2.00

Table 5. Reconstruction of parental genotypes at locus *Oedu*.B11, compatible with the segregation of a null allele. 1: output of the software PEDIGREE 2.2 (Herbinger et al. 2006); 2: interpretation of the results based on the existence of null alleles at the locus.

HBOR	1		**Group 1 (18 offspring)**	**Group 2 (12 offspring)**
		Parents genotypes	122/122 x 122/166	128/128 x 128/166
			↓	↓
		Progeny genotypes	122/166, 122/122	128/166, 128/128
	2		**Pooled family (30 offspring)**	
		Parents genotypes	122/128 x 166/null	
			↓	
		Progeny genotypes	122/166, 128/166, 122/null, 128/null	
HLK	1		**Group 1 (10 offspring)**	**Group 2 (4 offspring)**
		Parents genotypes	134/148 x 142/148	134/134 x 134/134
			↓	↓
		Progeny genotypes	134/142, 142/148, 148/148	134/134
	2		**Pooled family (14 offspring)**	
		Parents genotypes	134/148 x 142/null	
			↓	
		Progeny genotypes	134/142, 142/148, 134/null, 148/null	

Fig. 1 Sample sites of *Ostrea edulis*. WQ: Quiberon (Brittany, France), WLR: Loch Ryan (Scotland), WRF: Ria Formosa (Portugal), WGr: Grevelingen (the Netherlands); PRo: Rossmore (Ireland), PVe: Venø, Struer (Denmark), PBo: Boemlo (Norway), PVa: Vaagstranda (Norway); HLK (Loch Kishorn, UK, commercial hatchery); HO (Orkney, UK, commercial hatchery); HBOR (Brittany, France, research hatchery) and HMED (Mediterranean, research hatchery, France)

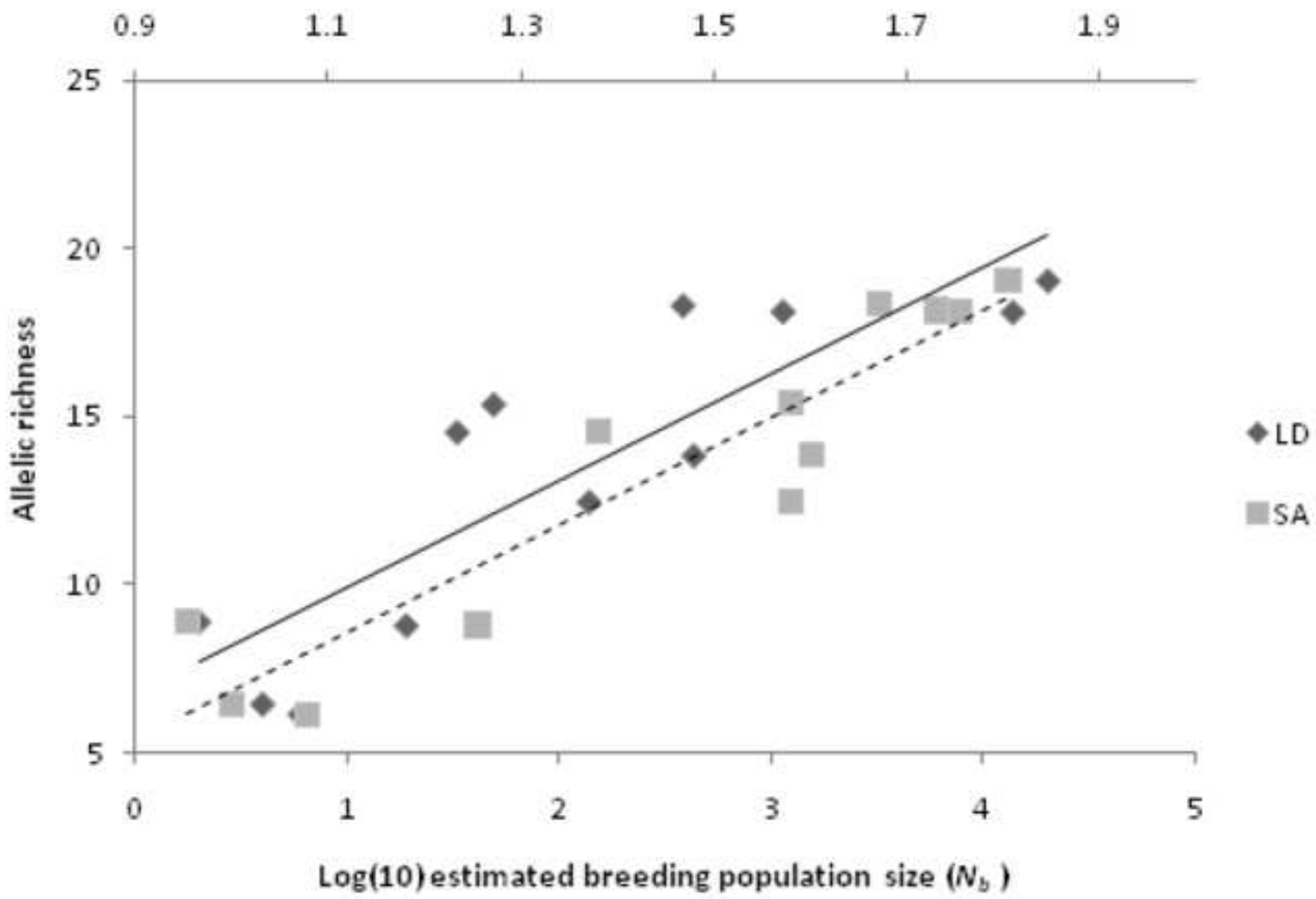
Fig. 2 N_b estimates and allelic richness for 12 *O. edulis* populations. LD: N_b estimates based on the linkage disequilibrium method. SA: N_b estimates based on the sibship assignment method. Upper *X* axis for SA estimates, lower *X* axis for LD estimates. Straight line: regression for LD estimates of N_b; dotted line: regression for SA estimates of N_b.

Fig. 3 Distributions of individuals from the four hatchery-produced populations into full-sib (FS) groups using the program PEDIGREE 2.2 (Herbinger et al. 2006). Each vertical bar represents a FS group. The output of the program has been modified for the populations HBOR and HLK due to evidence of null alleles (see 3.5. and Table 5)

line figure

Click here to download high resolution image

line figure
Click here to download high resolution image



line figure

Click here to download high resolution image