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Microsatellites and multiplex PCRs for assessing aquaculture practices of the grooved carpet shell *Ruditapes decussatus* in Spain

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ABSTRACT

Supplementation aquaculture is intended to reinforce harvestable abundances of viable, naturally reproducing populations. The grooved carpet shell *Ruditapes decussatus* is one of the most important shellfish species in northern Spain (Asturias and Galicia), and their wild populations are annually supplemented using seeds produced in hatcheries. The current genetic status of these populations and a genetic evaluation of the consequences of the supplementation campaigns are lacking due to the absence of useful genetic markers that allow these kinds of studies. In this work, twelve variable microsatellite markers (mean $H_E = 0.663$) and two useful multiplex PCRs are reported for *R. decussatus*. Different genetic characteristics were found between wild clams from Asturias and Galicia. Moreover, the seeds obtained in hatcheries for supplementation campaigns did not represent the wild gene pools well. Reductions of effective breeding numbers relative to the actual number of breeders were as large as 65%, due to unequal parental contributions and family variances. Finally, in an experimental supplementation programme conducted in a Galician population (Cambados), we report that the genetic status of the studied population changed significantly from one year to the next ($F_{ST} = 0.011$ $P < 0.05$) and we found what could be hatchery-produced seed (15%) in the wild restocked population. The accuracy of this estimate was evaluated using simulation procedures and we found less than 3% of type I error and values of 8–11% of type II error for three situations under analysis (32%, 10% and 1% of sampled true parent–offspring pairs) when using 95% as the threshold limit for parentage assignations. This work demonstrates the importance of temporal evaluations of the genetic status of supplemented and unsupplemented wild populations and indicates the need for changes in the protocols used for hatchery seed production for restocking purposes. A successful supplementation campaign can decrease genetic variance, and thus probably damage, the genetic status of wild populations.

1. Introduction

Fishery enhancement aquaculture, or supplementation aquaculture, is intended to reinforce harvestable population abundances of viable, naturally reproducing populations (Utter and Epifanio, 2002). Usually, a fraction of the wild parents (or their offspring) is brought into captivity for reproduction or preferential survival, and the offspring are released into the natural habitat where they mix with wild conspecifics, although no exogenous genes are introduced (Ryman and Laikre, 1991). The target populations are often a resource that is highly valued by local communities, and the idea of assisting the wild population to support harvest pressures is rapidly accepted and funded. Concerns arise when potential impacts on recipient populations and aquatic communities outweigh any production benefits (Bert et al., 2007). The ideal expected result for a supplementation programme should fulfil the "enhancement function" but not compromise the future (not damage the evolutionary potential) of the target populations. Ryman and Laikre (1991) first brought this issue to attention and argued that in supplementation aquaculture some part of the overall population is favoured (in terms of survival). Favouring part of the population will result in an increase in family variances that consequently diminish the genetically effective population sizes (N_e) of the supported populations. Supplementation programmes can thus involve serious alterations in genetic diversity and decreases in fitness of the target populations (Bert et al., 2007).

Utter and Epifanio (2002) reviewed supportive breeding practices for supplementation of wild marine species populations and found only one case (the red drum *Sciaenops ocellatus*) out of eight studied that seemed to fulfil (to some degree) the goals of a supplementation programme. More recently, Araki and Schmid (2010) reviewed the scientific literature on this subject from the past 50 years and suggested

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that scientific data supporting the positive effects of hatchery stocks on stock enhancement are largely missing. In shellfish, several studies trying to discern the best way/strategies for affording restoration programmes have been published recently (e.g., Lallias et al., 2010a). One of the key questions in supportive breeding practices is how large the contribution of the hatchery stock is to the breeding pool of wild individuals. It is supposed that the size of this contribution can be assessed using microsatellite markers (Araki and Schmid, 2010). Microsatellites are short, tandem-repeated sequences that are usually hypervariable and have proven to be valuable for research in different areas (Chistiakov et al., 2006). Microsatellites possess some drawbacks, such as null alleles or possible selection (e.g., Borrell et al., 2004; Nielsen et al., 2006); however, microsatellites combine the advantages of codominance, high polymorphism and multiple independently segregating loci. The use of microsatellites could describe population processes better than a single gene (Palsboll et al., 2007). Microsatellites are also useful tools for management strategies in aquacultured species because microsatellites help to determine parentage assignments and rates of inbreeding (Borrell et al., 2007, 2008; Herlin et al., 2008; Li et al., 2009; Lind et al., 2009; Navarro et al., 2008). Microsatellite-based parentage assignments have been proposed as a useful tool for the evaluation of reproductive fitness in natural settings, which is key for stock enhancement by hatchery-based stocking (Araki and Schmid, 2010; Araki et al., 2009; Boudry et al., 2002; Hedgecock et al., 2007; Lallias et al., 2010b). However, while parentage analyses within hatcheries have been undertaken with success (see above), parentage analysis in natural populations presents a valuable yet unique challenge because of the large numbers of pairwise comparisons, marker set limitations and the few true parent–offspring pairs sampled. These limitations can result in the incorrect assignment of false parent–offspring pairs that share alleles across multi-locus genotypes just by chance, biassing estimates of hatcheries' gene pools contribution to wild stocks. The use of strict exclusions, statistical thresholds and high numbers and quality of the markers have been proposed as the solution for undertaking with success this relevant task (Christie, 2010; Ford and Williamson, 2010; Harrison et al., 2013).

The grooved carpet shell *Ruditapes decussatus* is distributed from southern and western England to the Iberian Peninsula and into the Mediterranean. *R. decussatus* is also present in southern to western Morocco and Senegal, West Africa (Poppe and Goto, 1991). *R. decussatus* is one of the most important shellfish species in Spain where fishing and consumption have been recorded since ancient times (e.g., 16th century). Between the years 1950 and 1990, global fishery production varied from 2000 to 4000 tons; however, in the early 1990s, 17,000 tons were captured in a single year (FAO, 2012a). The catch values plummeted in 1994 to only 3000 tons, and through 2010, the average annual catch did not reach 2000 tons worldwide (FAO, 2012a). Besides heavy fishing, clams have declined because of increases in pollution and growth in seaports and urban areas, thereby degrading their habitat. *R. decussatus* aquaculture, an activity that began in the 1980s, has produced an annual average of 4000 tons in the past 15 years, mainly from only a few countries. Portugal, Italy, France and Spain are currently the main producers. Global production appears to be declining; in 2010, aquaculture production reached only 2000 tons (FAO, 2012b). In Spain, clam fisheries are enhanced by supplementation of seeds from wild breeders that are induced to spawn in hatchery facilities, and then the seeds are released into the harvest areas. Curiously, a comprehensive evaluation of the success of this practice to truly enhance the exploited wild populations has not been conducted. Capture numbers by year are strictly controlled in all areas and are documented, but no other evaluations have been performed.

Despite the importance of the analysis of genetic variability and population structure in managing exploited populations, the genetic status of *R. decussatus* populations in Spain is poorly studied. Available genetic markers to perform genetic studies in *R. decussatus* include some allozymes (e.g., Borsa et al., 1994), RAPDs (Pereira et al., 2011), introns (Cordero et al., 2008; Gharbi et al., 2010), and mitochondrial loci (Gharbi et al., 2010), which are often characterised by a moderate level of polymorphism and/or low reproducibility. This work reports the identification of the first panel of microsatellite markers in *R. decussatus*, the development of multiplex PCR for quick and cheap genetic analyses within the species and a first evaluation of their utility for population analyses and parentage studies that could help improve supplementation strategies used in *R. decussatus* stock enhancement programmes.

2. Materials and methods

2.1. Samples

A total of 348 individuals from wild populations in two northern Spain regions (Asturias and Galicia) were collected (Fig. 1). In Asturias, two sampling points were studied: Villaviciosa (46 individuals collected in April 2009; WVil09) and Eo (46 individuals, April 2009; WEo-09). In Galicia, samples from an experimental small area (approximately 4000 m^2) located inside the Cambados's clams bed (2,000,000 m^2), were collected in three consecutive years and were studied: samples from March 2009 (56 individuals; WCab-09), August 2010 (82 individuals taken just before the supplementation campaign conducted in 2010; WCab-10) and June 2011 (118 individuals taken in the supplemented area in 2010 with seeds from 2009; WCab-11).

In addition, breeders extracted from those wild populations but long adapted to culture conditions in 2 hatcheries were induced to spawn. Samples from these hatchery broodstocks (n = 152) were analysed genetically. We did not count samples from all the breeders used for producing annual seeds within the hatcheries due to logistical limitations (Villaviciosa June 2009: 19 out of 100 breeders (19%) (BVil-09); Eo June 2009: 46 out of 150 breeders (31%) (BEo-09); and Cambados:

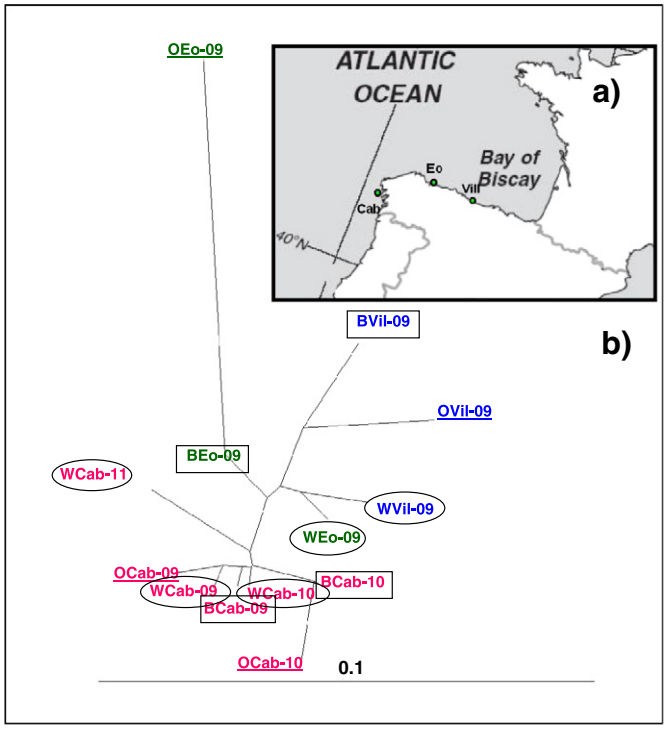

Fig. 1. a) Sampling locations in Spain and codes for the samples of *R. decussatus* analysed in this work using 12 microsatellite loci. Cab: Cambados-Galicia, Eo: Eo-Asturias, Vill: Villaviciosa-Asturias. Numbers refer to the year of sampling. b) Neighbour-Joining tree showing the unbiased Genetic Distance *D* (Nei, 1978) among *R. decussatus* samples analysed in this work. Samples from wild populations appear in circles, samples from breeders inside rectangles and samples from offspring are underlined. W: Wild, B: Breeders, O: Offspring.