# RESTOCKING OF AN ENDANGERED MARINE FISH: GENETIC MONITORING OF BREEDING PRACTICES FOR *EPINEPHELUS MARGINATUS*

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This study is part of a broader program focused on breeding, farming and restocking of dusky grouper (*Epinephelus marginatus*) in the south-western coast of Sicily. The dusky grouper is an endangered marine fish enlisted in the IUCN red list for threatened species, as a taxon which is currently facing a very high risk of extinction in the wild.

Genetic variation at six microsatellite loci was assayed to estimate relatedness and effective population size within captive broodstocks, to assess genetic variability of broodstocks and F1 juveniles in comparison to natural populations, to investigate genetic relationship among hatchery and wild specimens inhabiting the surroundings of the target area, and to provide molecular tags.

Individual DNA fingerprints were obtained for each specimen (4 males and 22 females) belonging to two captive broodstocks constituted of wild adult founders. F1 progeny individuals from preliminary experimental trials were analysed and an additional 450 virtual offspring from the same broodstocks were computer-generated, given the actual breeding schemes which took place in the hatchery. Genotype profiles from 3 wild populations were also analysed. Simulations and effective matings were used to examine the potential of this set of markers in assigning parentage to dusky grouper progeny, revealing high method reliability (combined exclusion probability = 0.996).

No relatedness within captive broostocks was found (Figure 1), and a potentially high number of effective breeders ($N_b$ = 86.9; CI 95% = 46.0-451.2) was registered. However very few breeders (n = 7), showing a positive degree of relatedness (r= 0.0297) among each other, were discovered to have spawned successfully. Genetic variability parameters appeared reduced for broodstocks and F1 juveniles, but not significantly (P>>0.05). Genetic differentiation was detected among

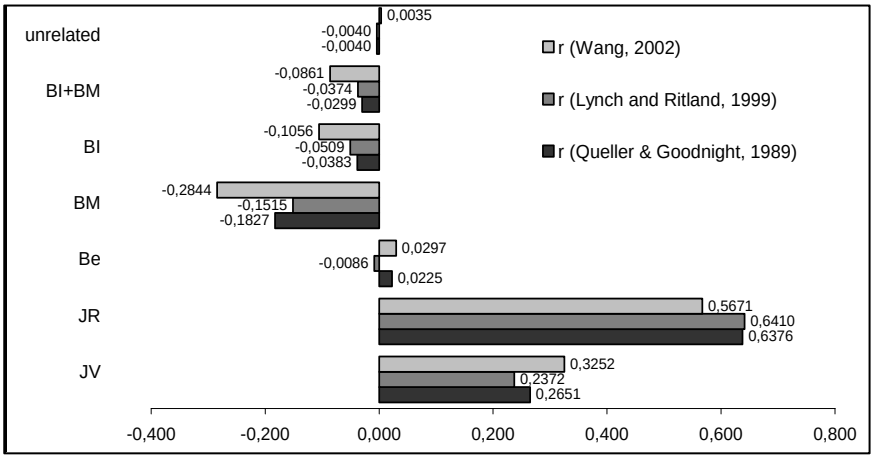


Figure 1. Relatedness estimates among *E. marginatus* individuals within hatchery samples. Unrelated = unrelated control individuals simulated trough HYBRIDLAB; BI+BM = merged sample of broodstocks BI and BM; BI and BM= captive broodstocks; Be = pool of breeders which successfully spawned.; JR = F1 progeny individuals from preliminary experimental trials; JV = virtual offspring computer-generated

released juveniles and wild samples collected 75Km away from the target area. However, the pattern of genetic distances (Figure 2) from other wild populations justifies the choice of these broodstocks as the best alternative donor stocks.

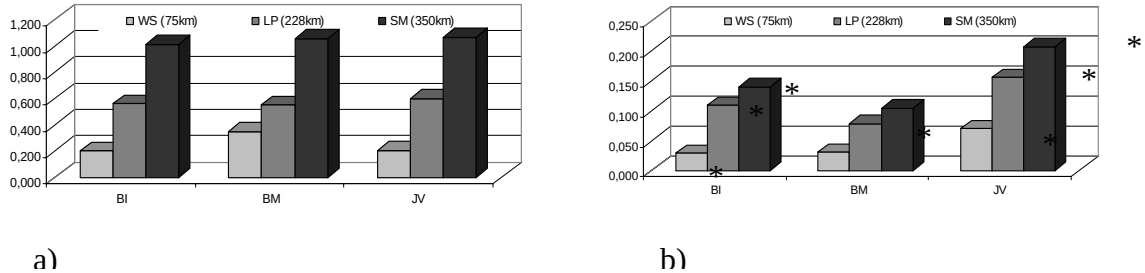


Figure 2. a) Genetic distances (D, Nei, 1972) and b) $F_{ST}$ (Weir & Cockerham, 1984) among hatchery samples and some wild populations. BI and BM= captive broodstocks; JV = F1 juveniles; WS, LP and SM = wild populations. Significant pairwise differentiations after genotypic Fisher's exact text (Goudet et al., 1996) are marked with *

Our results show that dusky groupers captive broodstocks meet the genetic criteria required for sustainable stock enhancement. Nevertheless, even if a strict minimal kinship criterion cannot be easily applied because of the complex mating behaviour and some biological constraints in captive propagation of this species, special attention should be given to husbandry practices and the resulting breeding combinations, to achieve long term conservation goals.