GENETIC POPULATION STRUCTURE OF THE ENDANGERED DUSKY GROUPER, *EPINEPHELUS MARGINATUS*, IN THE MALTESE ARCHIPELAGO AS REVEALED BY 14 MICROSATELLITE MARKERS.

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Abstract
The objective of this study is to describe the genetic population structure of the endangered marine fish, *Epinephelus marginatus*, in the Maltese archipelago for the purpose of localized conservation management planning. Genetic population structure was determined by examining 89 individuals collected within Malta's Fisheries Management Zone with 14 microsatellite markers. Results of genetic clustering by admixture analysis found one continuous population (K = 1) inhabiting the Maltese archipelago. Further genetic analysis describes a population decreasing in size ($Theta_{(mean)}$ = 2.2), which has gone through a significant size reduction in the past ($M_{(mean)}$ = 0.41) and shows signs of inbreeding (F_{IS} = 0.10, P > 0.001). Results suggest that management should be designed to reflect the archipelago as a single conservation unit.

Keywords: Conservation, Genetics, Teleostei, Islands, South-Central Mediterranean

Introduction
The dusky grouper, *Epinephelus marginatus* (Teleostei: Serranidae), is an endangered marine fish considered to be facing a very high risk of extinction in the wild. Due to concerns of population decline throughout their geographic range, the IUCN has currently listed them as endangered (EN) A2d [1]. Within Malta's Fisheries Management Zone (FMZ), catch landings data (kg) show, over a 62 year period between 1947 and 2009 a decrease in catch landings of over 90% [2]. Their native range includes the Mediterranean Sea, the eastern Atlantic Ocean along the west and south coasts of Africa around the cape to Mozambique as well as Brazil. Characterized by high site fidelity and protracted development to sexual maturity, this reef-associated protogynous hermaphorodite can usually be found in depths up to 50 meters off rocky coastal shores [3]. Localized population structure parameters include pelagic larval dispersal influenced by surface current direction during spawning season along with limited juvenile stage movement. Within Malta, the archipelago is surrounded by a 100 m bathymetry shelf. The predominant surface current direction, during the spawning months of June through August, is from the tip of the archipelago in north-west Gozo toward south-east Malta [4]. The purpose of this research is to describe and define the status of the Maltese population of *E. marginatus* in order to best develop a comprehensive conservation management and monitoring strategy.

Materials and Methods
A total of 89 *E. marginatus* specimens were sampled from the Maltese archipelago FMZ between 2007-2009. Fourteen polymorphic microsatellite markers were used in this study to explicate the population structure. Individuals were genotyped using a Roche 454 Genome Sequencer FLX. Genetic clusters were inferred using multilocus genotype admixture analysis with the software Structure 2.3.4 [5] while molecular and diversity indices and F-statistics were calculated using the software Arlequin 3.5 [6].

Results
A total of 227 alleles were observed within 14 polymorphic loci. Based on multilocus admixture analysis of 89 individuals a single genetic cluster (K = 1) was inferred from the genotypic data. Within group analysis reveals genotypic disequilibrium in 31 loci pair. Mean observed heterozygosity was H_O = 0.68 and the expected heterozygosity was H_E = 0.76. Rare breeding size males (T ≥ 85 cm) were present in all groups with a significantly higher incidence of large size individuals (T ≥ 70 cm) observed, at the tip of the archipelago, in north-west Gozo. Positive Theta (Hom) values were read as an indication of a population decreasing in size ($Theta_{(mean)}$ = 2.2). The Garza-Williams index, for detection of population reduction, interpreted M values less than 0.7 to be indicative of a recent population size reduction and anything less than 0.43 as specific to remnant populations and of a significant size reduction in the past ($M_{(mean)}$ = 0.41). In addition, a global F_{IS} test within the FMZ accepted the null hypothesis of an inbred population ($F_{IS(global)}$ = 0.01, P > 0.001).

Discussion
The Maltese islands are inhabited by single congruent population of *E. marginatus*, likely due to biogeographical isolation due to depth range and larval retention patterns. The 90% decline of catch landing within the FMZ over the last half century is consistent with the results of Theta(Hom), M and F_{IS}, which collectively point to a population which has gone through a significant size reduction in the past, is trending downward and consequently shows indications of moderate inbreeding. In addition, north-west Gozo has been identified as a possible spawning site and area of conservation interest due to the relatively high concentration of large size individuals. Average south-eastern surface currents originating from the northern tip of Gozo during spawning season as a mechanism for pelagic larval dispersal is a probable mode for genetic homogenization within the Malta population. We theorize Gozitan individuals may be an important source population to the rest of the archipelago. Therefore, we recommend management and pilot monitoring efforts initially focus on the northern Gozitan individuals.

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