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<sup>1</sup>I.C.R.A.M., Palermo, Italia.  
<sup>2</sup>COISPA, Bari, Italia.GENETIC VARIATION OF DUSKY GROUPER  
(*EPINEPHELUS MARGINATUS*, LOWE 1834)VARIAZIONE GENETICA DELLA CERNIA BRUNA  
(*EPINEPHELUS MARGINATUS*, LOWE 1834)

## Abstract

In the present work, we report data on genetic variation of *Epinephelus marginatus* (Serranidae) samples from Ustica island (MPA), Lampedusa and Azores islands (not MPA). Genetic variation has been studied using cytochrome b gene comparing the three Ustica reserve areas (A, B and C) and Lampedusa and Azores islands. In Ustica analysis, C area was much more different than A and B areas; among three islands, Ustica resulted the most variable population and the most different. These results could be provide useful information for management and conservation program.

**Key-words:** *Epinephelus marginatus*, Serranidae, cytochrome b sequences.

## Introduction

Dusky grouper is one of the most important coastal species, often considered flag species of marine protected areas (MPAs) in Mediterranean sea. Because of the strong fishery effort, dusky grouper populations showed a serious decrease in number of individuals; this has led to list it as endangered species in ECNC. High density of dusky groupers can be encountered only in MPA where commercial and recreational fishery effort is very low or completely absent. The behavioural (e.g. site fidelity and spawning aggregation) and biological characteristics (protogynous hermaphrodite, with a long life cycle) render this species more vulnerable where fishery effort is high. In the present work, we report data on genetic variation of *Epinephelus marginatus* samples from Ustica island (MPA), Lampedusa and Azores islands (not MPA). The islands have been chosen because of their geographical position and their different protection. Genetic variation has been estimated within the Ustica sample comparing three areas with different level of protection (A, B and C) and between samples from protected and no protected areas by analysing sequence variation of a 440 bp cytochrome b gene fragment.

## Materials and methods

Specimens of dusky grouper were collected from Ustica island (A, fully protected, B and C areas) Lampedusa, and Azores. A small piece of caudal fin for each specimen was preserved in ethanol (70-90%) or frozen at -20 °C. Genomic DNA was extracted using DNeasy Tissue Kit (QIAGEN). A portion of mtDNA gene, cytochrome b was amplified using 28For and 34Rev primers (Gilles *et al.*, 2000). PCR was carried out in a Perkin Elmer Cetus Thermal cycler in a 100 µl

solution containing 1 ng genomic DNA, 0.2 µM each dNTPs, 0.1 µM each primer, 10 mM buffer 10×, 1.5 mM MgCl<sub>2</sub> and 2.5 units of Perkin Elmer Taq polymerase. PCR products were purified and sequenced on ABI prism automated sequencer. Sequences were aligned using the alignment software Clustal W (Thompson *et al.*, 1994). Data analysis were conducted using the software DNAsp (Rozas & Rozas, 1997) Mega (Kumar *et al.*, 2000) e Arlequin (Excoffier *et al.*, 1992).

## Results

**Genetic variation in Ustica sample.** The haplotypic variation of the cytochrome b gene fragment in the three areas was shown in Tab. 1. The maximum value of Tamura-Nei genetic distance is 0.082 between area A and area C and the minimum value is 0.007 between A and B. Analysis of molecular variance (AMOVA) shows that the highest percentage of difference is within population;  $\Phi_{st}$  value is significant ( $\Phi_{st}=0.055$ ;  $p<0.05$ ) showing a genetic heterogeneity among areas analysed.

Tab. 1 - Sequence variability in the 440 bp portion of cytochrome b gene of dusky grouper specimens in area A, B and C from Ustica.

Variabilità delle sequenze di 440 bp del citocromo b di esemplari di cernia bruna nelle zone A, B e C dell'isola di Ustica.

	N° specimens	Haplotype Number	Unique Haplotype	Polimorphic sites	Haplotypic Diversity	Nucleotidic Diversity
A	12	9	6	10	0.939	0.007
B	10	6	3	10	0.867	0.007
C	6	4	2	6	0.900	0.007

**Genetic variation among Ustica, Lampedusa and Azores.** Sequence variability of the areas analysed is shown in Tab. 2. The maximum value of Tamura-Nei genetic distance is 0.022 between Ustica and Azores and the minimum value is 0.008 between Lampedusa and Azores. Analysis of molecular variance (AMOVA) shows that the highest percentage of difference is within population;  $\Phi_{st}$  value is significant ( $\Phi_{st}=0.13$ ;  $p<0.05$ ) showing a genetic heterogeneity among areas analysed.

Tab. 2 - Sequence variability in the 440 bp portion of cytochrome b gene of dusky grouper specimens from Ustica, Lampedusa and Azores.

Variabilità delle sequenze di 440 bp del citocromo b di esemplari di cernia bruna nelle zone A, B e C provenienti da Ustica, Lampedusa e Azzorre.

	N° specimens	Haplotype Number	Unique Haplotype	Polimorphic sites	Haplotypic Diversity	Nucleotidic Diversity
Ustica	28	16	11	15	0.886	0.007
Lampedusa	18	7	5	9	0.691	0.006
Azzorre	11	6	3	6	0.833	0.005

### Conclusions

The data reported show a heterogeneous genetic structure either in Ustica or in three islands comparisons. In the first case, area C results different from areas A and B which result homogeneous genetically; it is probable that fishery effort in the area C could affect genetic variability. In the second case, Ustica population results different from Lampedusa and Azores. This genetic heterogeneity could depend on different level of protection, Lampedusa and Azores are not MPA. These data could be helpful in management and conservation programme even if additional data on other marine reserves could be useful to evaluate the genetic heterogeneity of this species.

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### SULLA LUNGHEZZA DI PRIMA MATURITÀ DI *TRACHYRHYNCHUS TRACHYRHYNCHUS* (RISSO, 1810) (OSTEICHTHYES, MACROURIDAE) NEL BASSO ADRIATICO

### ON THE LENGTH AT FIRST MATURITY OF *TRACHYRHYNCHUS* *TRACHYRHYNCHUS* (RISSO, 1810) (OSTEICHTHYES, MACROURIDAE) IN THE SOUTHERN ADRIATIC

#### Abstract

In the present paper an estimation of the length at first maturity of *Trachyrhynchus trachyrhynchus* (*Osteichthyes*, *Macrouridae*) in the Adriatic Sea is reported. Specimens were caught during experimental surveys carried out in 2003, within the framework of the deep-fishery research project carried out at Southern Adriatic bathyal bottoms (700-1200 m). On the basis of maturity ogive the relevant length at maturity of females, in terms of Pre-Anal length (PAL) were 16.7 cm, 18.3 cm and 20.0 cm for PAL<sub>25%</sub>, PAL<sub>50%</sub> and PAL<sub>75%</sub>, respectively.

**Key-words:** *Osteichthyes*, *Macrouridae*, *Trachyrhynchus trachyrhynchus*, sexual maturity, Adriatic Sea.

#### Introduzione

*Trachyrhynchus trachyrhynchus* (Risso, 1810) è una specie bento-nettonica a prevalente distribuzione batiale, presente nel bacino del Mediterraneo e nell'adiacente Atlantico orientale (Cohen *et al.*, 1990; Whitehead *et al.*, 1986; Fisher *et al.*, 1987).

Sebbene gli aspetti generali della biologia della specie siano stati già affrontati in letteratura, rimangono alcune lacune su aspetti di dettaglio quali l'insorgere della maturità sessuale (Motais, 1960; Tortonese, 1970; Crespo *et al.*, 1976; Ibanez, 1977; Relini Orsi e Wurtz, 1979; Massuti *et al.*, 1995; D'Onghia *et al.*, 1996; D'Onghia *et al.*, 1999; Marano *et al.*, 2001). A riguardo, le informazioni disponibili sugli aspetti legati alla riproduzione sono, seppure menzionate, talvolta non approfondite nel dettaglio (Motais, 1960; Relini Orsi e Wurtz, 1979; Massuti *et al.*, 1995; D'Onghia *et al.*, 1996; D'Onghia *et al.*, 1999).

Obiettivo di questo lavoro, pertanto, è lo studio e l'identificazione della lunghezza di prima maturità della specie nel Basso Adriatico.

#### Materiali e metodi

Gli individui di *T. trachyrhynchus* esaminati sono stati raccolti durante due campagne sperimentali di pesca a strascico (una primaverile ed una autunnale) svolte nell'Adriatico Meridionale durante l'anno 2003, alle batimetrie comprese tra 700 e 1200 m. Gli esemplari sono stati misurati (PAL, lunghezza pre-anale in cm) e, utilizzando la scala riportata da Nikolsky (1963), sono stati attribuiti gli stadi maturativi tramite esame macroscopico delle gonadi.

Il numero di esemplari esaminati e le attribuzioni degli stadi maturativi hanno permesso di calcolare le percentuali di maturità per taglia, considerando individui "maturi" quelli caratterizzati da stadi compresi tra IV e VI.