GENETIC VARIATION OF DUSKY GROOPER (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 Epinephalus marginatus (Serranidae) samples from Ustica island (Mipa), 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 these 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: Epinephalus 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 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 hermaphroditic, 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 Epinephalus 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 24Rev 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 10X, 1.5 mM MgCl₂ 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; F_S value is significant (F_{TS}=0.055; p<0.05) showing a genetic heterogeneity among areas analysed.

<table>
<thead>
<tr>
<th>Nº specimens</th>
<th>Haplotype Number</th>
<th>Unique Haplotype sites</th>
<th>Polymorphic Diversity</th>
<th>Haplotypic Nucleotide Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12</td>
<td>9</td>
<td>6</td>
<td>0.839</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>0.867</td>
</tr>
<tr>
<td>C</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0.900</td>
</tr>
</tbody>
</table>

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; F_S value is significant (F_{TS}=0.13; p<0.05) showing a genetic heterogeneity among areas analysed.

<table>
<thead>
<tr>
<th>Nº specimens</th>
<th>Haplotype Number</th>
<th>Unique Haplotype sites</th>
<th>Polymorphic Diversity</th>
<th>Haplotypic Nucleotide Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ustica</td>
<td>28</td>
<td>16</td>
<td>11</td>
<td>0.846</td>
</tr>
<tr>
<td>Lampedusa</td>
<td>18</td>
<td>7</td>
<td>5</td>
<td>0.891</td>
</tr>
<tr>
<td>Azores</td>
<td>11</td>
<td>6</td>
<td>3</td>
<td>0.833</td>
</tr>
</tbody>
</table>
Conclusions

The data reported show a heterogeneous genetic structure either in Usitca 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, Usitca 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.

References


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Materiali e metodi

Gli individui di T. trachyrhynchos esaminati sono stati raccolti durante due campagne sperimentali di pesca a trascoso (una primaverile ed una autunnale) svolte nell’Adriatico Meridionale durante l’anno 2003, alle batimetriche 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.