# BIO-FUNCTIONAL DIVERSITY OF *CYMODOCEA NODOSA* AS BIOINDICATOR FOR ENVIRONMENTAL QUALITY IN THE LAKES OF CIRCEO NATIONAL PARK (ITALY)

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**Abstract**

In this research we evaluated the bio-functional diversity of *Cymodocea nodosa* populations by RAPD technique in two of the lakes of Circeo National Park (Caprolace and Fogliano). The ecological structure of the meadows was followed during the year 2004: physico-chemical parameters were measured in conjunction with seagrass phenological parameters A high degree of genotypic homogeneity of the seagrass, confirmed by statistical analysis (NT-SYS Cluster), was observed in association to a high vegetative continued growth via rhizome extention. In this investigation we confirm that a high nutrient concentration, in water and sediment, determines a high density and biomass values of meadow. Other environmental factors, such as temperature, salinity and light, are important in controlling seasonal biomass and abundance of *C. nodosa* in these lakes. Finally different modes of reproduction, such as continued growth, via rhizome extension, into new locations and sexual offspring (seedling) recruitment, may contribute to low differentiation in the seagrass *C. nodosa* that can be considered as biological indicators for environmental quality in lagoon ecosystems.

*Keywords : Bio-indicators, Biodiversity, Phytobenthos, Lagoons.*

Experimental

Cyanobacteria were grown in axenic laboratory conditions (tab.1) and subsequently DNA extraction was carried out (MagnaRack, Invitrogen SpA, Italy). Biodiversity was detected by PCR (Polymerase Chain Reaction) technique using RAPD markers (Random Amplified Polymorphic DNA). PCR was applied: 2 min 94 °C, 40 cycles of 30 s at 37 °C (annealing), 2 min at 72 °C (synthesis). The amplification products were separated by gel electrophoresis (agarose 1.4%) after ethidium bromide staining. Eight primers were used: BY11 (5'-ATCCACTGCA-3'); BY12 (5'-GGTCGCAGGC-3'); BY15 (5'-CTCACCGTCC-3'); DN4 (5'-GTCGTGCTAT-3'); DN5 (5'-CCGACGGCAA,-3'); DN6 (5'-TGGACCGGTG-3'); UB24 (5'-GGGTGAACCG-3'); UB28 (5'-GCTGGGCCGA-3'). UPGMA Cluster analysis (similarity index) was carried out by NT-SYS software [1].

To analyse fatty acid, the samples were methilated and detected by gas chromatography using C19:0 as internal standard [2, 3].

Discussion of results

UPGMA phenogram (fig.1) revealed the pattern of genetic distance, showing two different groups comprising the order of *Nostocales* and *Oscillatoriales*. This analysis confirms the endemism of *Nostoc* genus in the Antarctic and Mediterranean regions, revealing its phylogenetic distribution in relation to geographical position. Data relating to the matrix of similarity [1] revealed an average similarity of 15,37 % between the six populations of cyanobacteria analized; the average value of similarity of 27,04 % appears for *Nostoc* Mediterranean and two *Nostoc* Antarctic. Matrix correlation (r) obtained by Mantel test gave a value of 0.95, revealing a good statistical result. Genomic fingerprinting, revealed by PCR technique, gave several molecular fragments of varying sizes, ranging from 0.1 to 8.0 Kb.

Tab. 1. Morphology of *C. nodosa* plants from the four study sites during the spring season

| Stations | shoot n° (each plant) | leaf n° (each shoot) | leaf length mean (cm) | leaf width (cm) |
|---|---|---|---|---|
| ST. 1 | 2,2 ± 0,84 | 5,27 ± 0,90 | 25,8 ± 13,47 | 2-5 |
| ST. 2 | 3,2 ± 2,17 | 4,6 ± 1,5 | 19,9 ± 11,5 | 2-5 |
| ST. 5 | 1,8 ± 1,3 | 4,1 ± 0,93 | 31,2 ± 10,9 | 2-5 |
| ST. 8 | 1,8 ± 0,84 | 3,67 ± 0,5 | 24,58 ± 10,94 | 2-5 |

The distribution of molecular fragments for each species analysed indicates that the majority of the molecular fragments are in the range of 251-1000 Kb, especially in the *Nostoc* genus. The genetic variability we encountered was correlated to the fatty acid production of the different strains. The physiological features of the strains showed a high degree of specificity regarding their content of fatty acids. We observed that the largest percentage of fatty acid produced by all the strains was the 16:0 (palmitic) and 18: 2 n6 c (linoleic); whilst the 17:0 ante (IUPAC) was produced in large amounts by the *Leptolyngbya* instead of *Nostoc*. The 18:3 n3 (linolenic) showed an opposite trend with the highest percentage of production by Antarctic *Nostoc* rather than *Leptolyngbya* genera. *Plectonema* showed an intermediate concentration in percentage of production regarding this fatty acid. This analysis revealed that the amount of some fatty acids is sometimes correlated to the genus, as seen in linoleic and alpha-linolenic acids that were abundant in *Nostoc* species. The RAPD-PCR amplification revealed that *Nostoc* genera produced the greatest number and weight of molecular fragments. Molecular analysis together with morphological and physiological study, is confirmed to be a valid tool in determining a first screening of the different cyanobacteria, evidencing an ample range of molecular weight between 0.1 to 8.0 Kb. These results evidence the high capacity of cyanobacteria to adapt physiologically to different environments, producing secondary biologically active molecules which are useful for human health as well as for environmental monitoring in an evolutionary scheme.

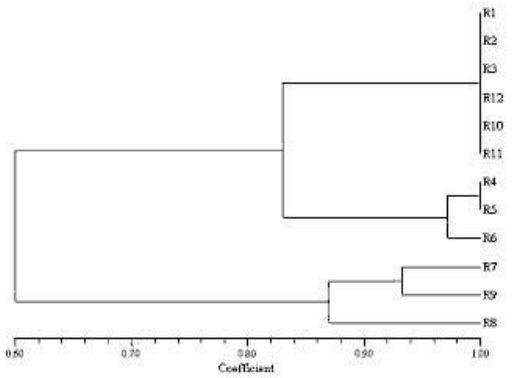


Fig. 1. UPGMA phenogram of RAPD genetic distance of *C. nodosa* populations at. Caprolace (St1= r1, r2, r3; St2= r4, r5, r6; St5= r7, r8, r9) and Fogliano (St8= r10, r11, r12)

## References

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