INVESTIGATION OF GENOMIC POLYMORPHISM IN *POSIDONIA OCEANICA* PLANTS COLLECTED IN DIFFERENT AREAS OF MEDITERRANEAN SEA USING RAPD MARKERS

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The monoic *Posidonia oceanica* (L.) Delile is a marine phanerogame endemic of Mediterranean sea which has a multifunctional role in the coastal ecosystem (BOUDOURESQUE *et al.*, 1984). During these last years the progressive reduction of *Posidonia* meadows claimed the attention toward the recovery of this marine phanerogame by means of experimental transplantation of different populations. (MEINESZ *et al.*, 1993). It is well known that vegetative reproduction appears to be the principal mode of proliferation for this species (MEINESZ and LEFEVRE, 1984), and it is correlated with environmental parameters (depth, light and temperature). However the sexual reproduction remains the principal way to create and to preserve genetic variability. With the aim of better knowing the genomic polymorphism in *P. oceanica*, we performed a study using molecular markers such as RAPDs (Random Amplified Polymorphic DNA) (WILLIAMS *et al.*, 1990). During may-november 1993, several plants of *P. oceanica* were collected from 7 different geografical areas of the Mediterranean sea : Giannutri (GR), Costa dell'Argentario (GR), Scoglio dell'Argentarola (GR), Civitavecchia (RM), Ponza (LT), Marina di Camerota (SA) and La Valette (Malta). After collection by SCUBA diving, individual plants were washed in distillated water and stored in liquid nitrogen at -80°C. Subsequently, all extraction steps of genomic DNA were carried out following the protocol reported by DELLAPORTA *et al.*, (1983). The PCR conditions used were similar to those described by ECHT *et al.*, (1992) with some modifications involving reaction buffer and temperature ramps. Amplification reactions were carried out in a thermal cycler (Perkin Elmer/Cetus), using 8 different oligonucleotide primers. The sequences (5'-3') of the primers are as follows : (DN4) GTGGTGCTAT; (DN5) CCGACGGCAA; (DN6) TGGACCGGTG; (BY11) ATCCACTGCA; (BY12) GGTCGCAGGC; (BY13) CCTTGACGCA; (BY14) GGACCCTTAC (BY15) CTCACCGTCC. The amplification products were separated by gel electrophoresis (Agarose 1.4%) and photographs (Polaroid 667) were taken under U.V. light illumination after ethidium bromide staining. The RAPD assay was able to generate informative genomic fingerprints of the *Posidonia* plants. The detected product sizes ranged from 0.25 to 1.95 Kb, while the number of amplification products varied from 2 to 12 (on average 5.6) for plant. The frequency distribution concerning the total number of amplification products detected with all of the primers is shown in figure 1. Most of DNA segments amplified from the *Posidonia* genomic DNAs were 0.26 to 1.50 Kb in length. This histogram also emphasized the different ability of primers to find homologous binding sites among *P. oceanica* templates. On the whole, primer DN5 produced complex electrophoretic banding patterns characterized by the largest number of amplification products and by the widest range of product size (Fig. 2). In addition, this primer resulted the best in discriminating *P. oceanica* plants and, therefore, in detecting genomic polymorphisms. The analysis of electrophoretic profiles allowed the identification of conserved and individual specific amplification products. In particular, primer DN4 amplified several genomic fragments which resulted population conserved (excepted for two products which were absent in plants P11 and P17 collected in Civitavecchia coast) (Fig. 3). One primer out of eight (BY14) was not able to generate scorable bands while a couple of primers (BY11 and BY13) supplied little informations. In conclusion, an appropriate choice of the oligonucleotide primers and the investigation of a larger number of plants would give a reliable estimation of the level of genomic polymorphism within and between *P. oceanica* populations. The results above reported confirm that RAPD markers represent a valuable tool for investigations such as phylogenetic analysis and that they could be used for monitoring the diffusion of single genotypes after transplantation programs.

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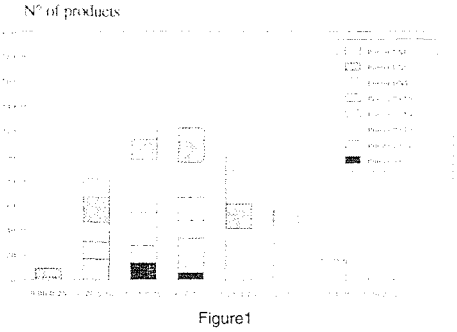

Figure1

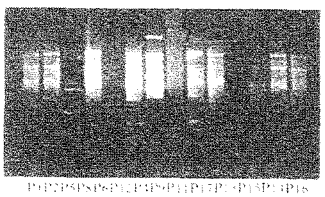

Figure 2

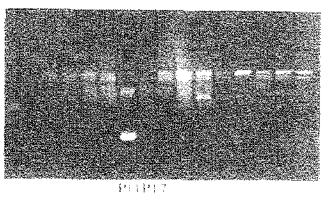

Figure 3

CRUSTACEA DECAPODA ASSEMBLAGE OF THE WESTERN POMO PIT. I - SPECIES COMPOSITION

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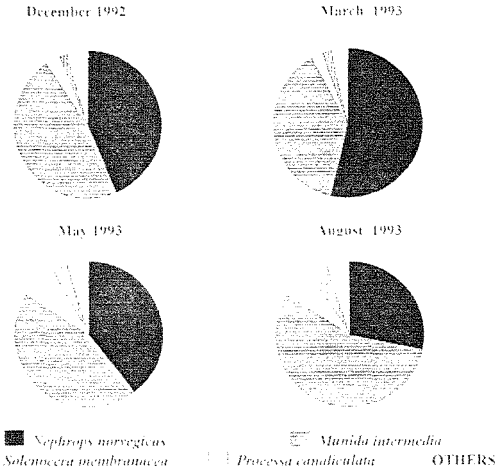
The IRPEM, in the last twenty years, has extensively studied fishery resources and oceanography of the Western basin of the Pomo pit, a depression with a maximum depth of 256 m. The Pomo (Jabuka) pit is the main Nephrops ground in the Central Adriatic; moreover it is a nursery ground for hake (*Merluccius merluccius*). From December 1992 to April 1994, during a comparative study of different Mediterranean and Scottish Nephrops grounds, the area was sampled with an experimental unimesh prawn trawl with cod-end meshes of 12 mm stretch. Fish and decapod crustaceans made the bulk of the trawl catch. At least once per season catches obtained around noon and midnight were sorted on the deck for commercial species and the residual bycatch was frozen at sea and subsequently sorted in the laboratory. The seasonal quantitative composition of the Decapod assemblage has been estimated from the highest value of biomass per swept area obtained for each species, either in day time or night time. Diel change in vulnerability of different species has been estimated according to FROGLIA & GRAMITTO (1986). A total of 26 species of Decapod crustaceans have been identified, compared to 17 species listed for the same area by FROGLIA in 1976.



In the following table species are listed in systematic order and classified according to their habitat.

(P = pelagic, EB = epibenthic, BB = benthic making its own burrow, DB = benthic dwelling into sediments at least in some day period) and their diel vulnerability to trawl gear (N = highest catches at night, D = highest catches at day, I = without a clear diel pattern in catches). Species marked with an * were found only once with one or few individuals.

SPECIES	RANK number	RANK weight	Habitus	Vulnerab
Parapenaeus longirostris	15	11	EB	I
Solenocera membranacea	4	3	DB	N
* Sergestes arcticus	25	25	P	
* Pasiphaea sivado	23	20	P	
Alpheus glaber	11	14	BB	N
Processa canaliculata	5	4	DB	N
Processa nouveli	9	12	DB	N
Chlorotocus crassicornis	8	7	EB	N
Pandalina profunda	7	6	EB	N
Plesionika antigai	10	15	EB	
Plesionika heterocarpus	19	8	EB	D
* Plesionika martia	25	24	EB	
Aegeon lacazei	17	17	DB	
Philocheras echinulatus	16	9	DB	N
Pontophilus spinosus	7	5	DB	I
Nephrops norvegicus	3	2	BB	D
Calocaris macandreae	14	16	BB	N
*Callianassa subterranea	26	26	BB	
Jaxea nocturna	21	21	BB	
* Pagurus excavatus	24	23	EB	
Munida intermedia	1	1	EB	I
Macropodia longipes	20	20	EB	
Liocarcinus depurator	12	13	DB	I
Macropipus tuberculatus	13	13	DB	N
Goneplax rhomboides	18	18	BB	
Monodaeus cfr. couchii	19	19	DB*	

The decapod assemblage was dominated all the year round by *Nephrops norvegicus* and *Munida intermedia*, accompained by *Solenocera membranacea* and *Processa canaliculata*. The latter two species being vulnerable mostly at night. All the other species never made more than 5% by weight of the total decapod catch. The assemblage includes species characteristic of muddy bottoms of the circalitoral and epibathyal levels. Several of them are known to make burrows in sediments (ATKINSON, 1986) and their importance may be somewhat underestimated from trawl sampling. Thus *Calocaris macandreae* was observed only with single specimens in the trawl catches, but its density, estimated from 90 grab samples taken in the area in 1992 and 1993 (FROGLIA unpublished), had to be around 1 individual / 1.5 square metre.

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