

Genetic structure of natural and cultured populations of the oyster (*Ostrea edulis* L.) in the Adriatic Sea

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*Starch gel electrophoresis was used to determine the genetic structure, as well as population differentiation in and among natural and cultured populations of the European flat oyster (*Ostrea edulis*, L.) from the eastern coast of the Adriatic Sea.*

From three natural and three cultured populations, seven enzyme loci were analysed: 6-Phosphogluconate dehydrogenase (6Pgd), Isocitrate dehydrogenase (Idh), Glucosephosphate isomerase (Pgi) and Peptidase (-a, -b, -c, -d).

*None of the populations revealed significant deviations from the expected HARDY-WEINBERG equilibrium frequencies of genotypes, which propose that mating was random within each population. The number of percent of polymorphic loci per population, mean heterozygosity and effective number allele per locus indicate that the levels of genetic variations *O.edulis* populations at the eastern coast of the Adriatic Sea are higher in comparison to other oyster populations from the Mediterranean and Atlantic beds.*

There is no heterozygote deficiency within populations, nor differentiations in gene frequencies between natural and cultured populations.

Key words: Genetic structure, *Ostrea edulis*, L., allozymes, shellfish genetics

INTRODUCTION

The development of aquaculture and intensifying of different shellfish species culture, such as the European flat oyster *Ostrea edulis* L. could cause a depletion of their natural genetic variability. Hatchery production makes breeding possible and in some cases development of new and genetically "improved" strains essentially different from their natural populations. At the same time, breeding leads to loss of genetic diversity and inbreeding depression may exert potentially serious negative effects

on the natural populations (WADA, 1986; GAFFNEY and SCOTT, 1984; PAYNTER and DIMICHELE, 1990; HEDGECOCK and SLY, 1990). Thus, it is important to protect the natural populations of fish and shellfish.

Genetic studies using starch gel electrophoresis of enzymes are widely applied in a shellfish population genetics. This technique uses allozymes as indicators of genetic variability. It may also be used to determine species or subspecies status of different populations, as well as the distribution of certain alleles and rate of migration among populations.

Many studies of enzyme loci variation have been undertaken on important commercial species, such as European flat oyster (*Ostrea edulis* L., family *Ostreidae*). However, most of these have been performed on the Atlantic populations, which have been very much affected by human activities such as overfishing (WILKINS and MATHERS, 1973; MAGENNIS *et al.*, 1983; LE PENNEC *et al.*, 1985; BLANC *et al.*, 1985; JAZIRI, 1990; SAAVEDRA *et al.*, 1987, 1993). Also, uncontrolled restocking of beds by the competitive species (*Crassostrea gigas*, L.) and infestation by parasites such as *Bonamina* sp., *Marteilia* and *Hexamitha* sp., makes that *Ostrea edulis* has almost disappeared from some areas of Europe (McARDLE, *et al.*, 1991; VAN BANNING, 1991; MONTES *et al.*, 1991; FIGUERAS, 1991).

There is almost no data on the genetic structure of natural and cultured populations of the European flat oyster from the eastern coast of the Adriatic Sea. The purpose of this study was to assess genetic structure of the natural and cultured populations, and possible differentiation at varying geographical distances.

MATERIAL AND METHODS

Ostrea edulis is a sequential hermaphroditic species, which can be found in subtidal habitats along the Atlantic coast, from Norway to Morocco and from the Mediterranean to the Black Sea (WILBUR and YONGE, 1964). In consideration of optimal ecological conditions the *O.edulis* species inhabits in the shallow coastal water on the 10 m depth, searching for the hard bottom with a higher salinity. The main nutrition of this species is plankton, while it's spawning occurs on the temperature between 12 and 16°C. Fertilisation occurs inside the pallial cavity of the female with subsequent brooding of the larvae (YONGE, 1960). After a brooding period of 8 to 10 days, follows a pelagic larval phase, which reduced compared to that of other oyster species (BUROKER, 1985; KORRINGA, 1940).

Although, along the eastern coast of the Adriatic Sea, many areas are suitable with the great conditions for oysters, its natural extent is very limited. The Mali Ston Bay has been inhabited with *O.edulis*, as well as in the bay of Pula and its surroundings. Although, density of these inhabitants was low that they do not have an economical character. Therefore, we need to take into account only artificial farms.

Collection of samples

Thirty one-year old oysters were collected from the natural populations in the Soca Cove (1), Kaštela Bay (3), Rovinj (5) and from cultured populations in the Bistrina Cove (2), Marina Bay (4) and Lim Channel (6) (Fig. 1.). All the samplings were performed in April 1994.

The anterior adductor muscle and part of the digestive gland were removed from each oyster and stored at -80°C until analysis. The frozen tissue was homogenised in distilled water and centrifuged. The supernatant was used as an enzyme source for electrophoretic analysis.

Electrophoretic technique

Starch gel electrophoresis and staining of enzymes were derived from PASTEUR *et al.* (1987). A total of seven enzymes were assayed. Loci, known to give the best discrimination between the natural and cultured populations of *Ostrea edulis* were chosen for this study (Table 1). The loci, were Phosphogluconate dehydrogenase (6Pgd; EC 1.1.1.44.), Isocitrate dehydrogenase (NADP+) (*Idh*; EC 1.1.1.42.), Glucosephosphate isomerase (*Pgi*; EC 5.3.1.9.), Peptidases (a,b,c) (*Pep a,b,c*; EC 3.3.22.) and Peptidase d (*Pepd*; EC 3.4.13.9.). The nomenclature of enzymes and their coding genes were determined by giving a number to each zone of activity to its corresponding locus, lower numbers referring to less anodal mobility zones.

Table 1. Enzyme assayed, loci coded for particular enzymes, tissue and buffer systems used for the electrophoresis

Enzyme	Locus	Tissue	Buffer System*
Phosphogluconate dehydrogenase	<i>6Pgd-2</i>	digestive gland	1
Isocitrate dehydrogenase	<i>Idh-2</i>	digestive gland	1
Glucosephosphate isomerase	<i>Pgi-2</i>	digestive gland	1
Peptidase A	<i>Pep-a</i>	muscle	1
Peptidase B	<i>Pep-b</i>	muscle	1
Peptidase C	<i>Pep-c</i>	digestive gland	2
Peptidase D	<i>Pep-d</i>	digestive gland	3

*Buffer system 1: Tris-citrate pH 8.0; 2. Tris-citrate pH 6.7; 3. Phosphate-citrate pH 6.3

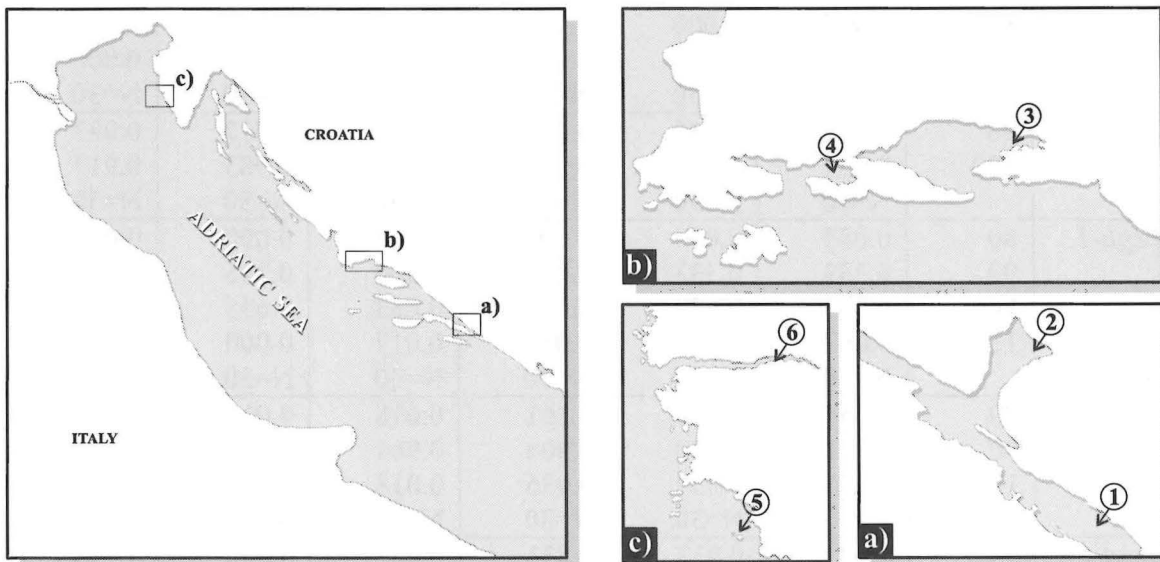


Fig.1. Sampling sites of *Ostrea edulis* populations on the eastern coast of the Adriatic Sea

Statistical data analysis

Genotypes were scored directly from the gels. A chi-square test was used for testing statistical significance of deviations of the genotypic frequencies from the HARDY-WEINBERG proportions (SOKAL and ROHLF, 1969).

The allele frequencies, the proportion of polymorphic loci ($P_{0.95}$ criterion), the mean average of alleles per locus (A), effective num-

ber of alleles (A_e), and mean heterozygosity ($\bar{H} \pm S.E.$) were calculated for each population (FERGUSON, 1980).

Levels of population structuring were quantified for all loci with WEIR and COCKERHAM (1984) estimators of F-statistics. F-statistics are a set of tools devised by WRIGHT (1965) to partition heterozygote deficit within and among population component. F_{IS} measures the

heterozygote deficit within populations (samples) (hypothesis that $F_{IS}=0$), F_{ST} represents deviations due to differences in gene frequencies between populations (hypothesis that $F_{ST}=0$), and F_{IT} is a deviation in the total population (whole set of samples).

The program package F-STAT (GOUDET, 1994) was used for the estimation of F-statistics, and for the analyses related to genetic parameters GENEPOP 1.0 (RYMOND and ROUSSET, 1994).

RESULTS AND DISCUSSION

In the populations from the Bistrina Cove and Marina Bay, the locus *6Pgd-2* is monomorphic, as well as the locus *Idh-2* in the populations from Bistrina Cove, Rovinj and Lim Channel (Table 2). The alleles *6pgd-2*⁹⁰, *pepb-1*⁸⁰ and *pgi*¹⁰⁰ were found only in the natural population from Soca Cove (Table 2). This could be due to these alleles being rarely and just accidentally found in one of the populations.

Table 2. *Ostrea edulis*. Allele frequencies of the samples from natural and cultured populations collected along the eastern coast of the Adriatic Sea

Locus	Alleles	SC* ¹	BC* ²	KB* ¹	MB* ²	RO* ¹	LC* ²
<i>6Pgd-2</i>	70	0.017	0.000	0.017	0.000	0.034	0.033
	80	0.967	1.000	0.983	1.000	0.966	0.967
	90	0.017	0.000	0.000	0.000	0.000	0.000
		N=30	N=30	N=30	N=30	N=30	N=30
<i>Pepa-2</i>	90	0.069	0.050	0.067	0.033	0.017	0.083
	100	0.931	0.950	0.933	0.967	0.983	0.917
		N=30	N=30	N=30	N=30	N=30	N=30
<i>Pepb-1</i>	80	0.033	0.000	0.000	0.000	0.000	0.000
	90	0.333	0.333	0.345	0.300	0.345	0.500
	100	0.567	0.650	0.603	0.683	0.655	0.500
	110	0.067	0.017	0.052	0.017	0.000	0.000
		N=30	N=30	N=30	N=30	N=30	N=30
<i>Pepc-1</i>	80	0.054	0.050	0.161	0.018	0.019	0.018
	90	0.911	0.900	0.804	0.964	0.852	0.964
	100	0.036	0.050	0.036	0.018	0.130	0.018
		N=30	N=30	N=30	N=30	N=30	N=30
<i>Pepd-2</i>	90	0.933	0.933	0.833	0.967	0.983	0.950
	100	0.067	0.067	0.117	0.033	0.017	0.050
		N=30	N=30	N=30	N=30	N=30	N=30
<i>Idh-2</i>	80	0.033	0.000	0.000	0.033	0.000	0.000
	90	0.950	1.000	0.983	0.967	1.000	1.000
	100	0.017	0.000	0.017	0.000	0.000	0.000
		N=30	N=30	N=30	N=30	N=30	N=30
<i>Pgi</i>	80	0.050	0.083	0.117		0.068	0.033
	90	0.933	0.917	0.833	-	0.931	0.967
	100	0.017	0.000	0.000		0.000	0.000
		N=30	N=30	N=30		N=30	N=30

*Population: ¹ natural populations-SC-Soca Cove; KB-Kaštela Bay; RO-Rovinj;

²cultured populations-BC-Bistrina Cove; MB-Marina Bay; LC-Lim Channel;

The observed number of genotypes for the six polymorphic loci is given in Table 3. Conformity with the HARDY-WEINBERG expectations was tested by the Chi-square test. No significant deviations from the expected HARDY-WEINBERG equilibrium frequencies of genotypes were found at the population of

Soca Cove ($\chi^2 = 11.04$, d.f.=39 $p < 0.999$), Bistrina Cove ($\chi^2 = 3.42$ d.f.=24 $p < 1.00$), Kaštela Bay ($\chi^2 = 5.53$ d.f.=33 $p < 1.00$), Marina Bay ($\chi^2 = 8.34$ d.f.=24 $p < 0.99$), Rovinj ($\chi^2 = 1.16$ d.f.=23 $p < 1.00$) and Lim Channel ($\chi^2 = 4.09$ d.f.=23 $p < 0.99$). There is no significant deviations from the expected HARDY-

Table 3. Observed number of genotypes in natural (SC-Soca Cove; KB-Kaštela Bay; RO-Rovinj) and cultured (BC-Bistrina Cove; MB-Marina Bay; LC-Lim Channel) populations of *O. edulis* from the eastern coast of the Adriatic Sea

LOCUS/ POPULATION	GENOTYPES													
	1/1	1/2	2/2	1/3	2/3	3/3	2/4	3/4	4/4	2/5	3/5	4/5	5/5	
6pgd-2	SC ¹	0	1	28	0	1	0	-	-	-	-	-	-	
	BC ²	30	-	-	-	-	-	-	-	-	-	-	-	
	KB ¹	0	1	29	-	-	-	-	-	-	-	-	-	
	MB ²	30	-	-	-	-	-	-	-	-	-	-	-	
	RO ¹	0	2	27	-	-	-	-	-	-	-	-	-	
	LC ²	0	2	28	-	-	-	-	-	-	-	-	-	
pepa-2	SC ¹	-	-	-	-	0	-	4	25	-	-	-	-	
	BC ²	-	-	-	-	0	-	3	27	-	-	-	-	
	KB ¹	-	-	-	-	0	-	4	26	-	-	-	-	
	MB ²	-	-	-	-	0	-	2	28	-	-	-	-	
	RO ¹	-	-	-	-	0	-	1	28	-	-	-	-	
	LC ²	-	-	-	-	1	-	3	26	-	-	-	-	
pepb-1	SC ¹	-	-	0	-	0	5	1	10	10	1	0	3	
	BC ²	-	-	0	-	0	4	0	11	14	0	1	0	
	KB ¹	-	-	0	-	0	4	0	11	11	0	1	2	
	MB ²	-	-	0	-	0	6	0	6	17	0	0	1	
	RO ¹	-	-	0	-	0	3	0	14	12	-	-	-	
	LC ²	-	-	0	-	0	8	0	13	8	-	-	-	
pepc-1	SC ¹	-	-	0	-	3	23	0	2	0	-	-	-	
	BC ²	-	-	0	-	3	24	0	3	0	-	-	-	
	KB ¹	-	-	2	-	5	19	0	2	0	-	-	-	
	MB ²	-	-	0	-	1	26	0	1	0	-	-	-	
	RO ¹	-	-	0	-	1	19	0	7	0	-	-	-	
	LC ²	-	-	0	-	1	6	0	1	0	-	-	-	
pepd-2	SC ¹	-	-	-	-	-	26	-	4	0	-	-	-	
	BC ²	-	-	-	-	-	26	-	4	0	-	-	-	
	KB ¹	-	-	-	-	-	24	-	5	1	-	-	-	
	MB ²	-	-	-	-	-	28	-	2	0	-	-	-	
	RO ¹	-	-	-	-	-	28	-	1	0	-	-	-	
	LC ²	-	-	-	-	-	27	-	3	0	-	-	-	
idh-2	SC ¹	-	-	0	-	2	27	0	1	0	-	-	-	
	BC ²	-	-	30	-	-	-	-	-	-	-	-	-	
	KB ¹	-	-	0	-	0	29	0	1	0	-	-	-	
	MB ²	-	-	0	-	2	28	-	-	-	-	-	-	
	RO ¹	-	-	30	-	-	-	-	-	-	-	-	-	
	LC ²	-	-	30	-	-	-	-	-	-	-	-	-	
pgi	SC ¹	-	-	0	-	3	26	0	1	0	-	-	-	
	BC ²	-	-	0	-	5	25	-	-	-	-	-	-	
	KB ¹	-	-	0	-	7	23	-	-	-	-	-	-	
	MB ²	unreadable												
	RO ¹	-	-	0	-	4	25	-	-	-	-	-	-	
	LC ²	-	-	0	-	2	28	-	-	-	-	-	-	

¹=natural population

²=cultured population

WEINBERG equilibrium frequencies of genotypes between all natural and cultured populations ($\chi^2=33.69$, d.f.150, $P<1.00$), as well. These results point to the fact that mating was random within each population.

The results of genetic variability in seven loci (including monomorphic one) of six *O.edulis* populations are summarised in Table 4.

In natural populations the highest average heterozygosity 0.23 ± 0.06 was recorded for the Kaštela Bay population, followed by the population from Soca Cove with an average heterozygosity of 0.16 ± 0.06 and Rovinj population with the lowest one, 0.13 ± 0.05 . Mean of the average heterozygosity of all natural populations studied is 0.17.

For seven polymorphic loci (including monomorphic one) the average number of alleles per locus was 2.0 in the natural population from Rovinj, 2.28 for the Kaštela Bay population and the highest for the Soca Cove population, 2.85. The number of effective alleles per locus in natural populations ranged from 1.21 to 1.37. The highest level of polymorphism ($P_{0.95}$) of natural populations was detected from the Soca Cove population, 85.7, followed by the Kaštela Bay population with 71.4 and the lowest polymorphism level ($P_{0.95}$) in the population of Rovinj, 42.9.

The lowest average heterozygosity in the cultured populations was recorded from the Marina Bay population 0.11 ± 0.06 (Table 4), somewhat higher from the Lim Channel population 0.13 ± 0.06 , and the highest from the Bistrina Cove population, 0.14 ± 0.06 . The mean of the average heterozygosity in these populations is 0.13. The average number of alleles per locus was 2.16 in the Marina Bay population, up to 2.0 in the Bistrina and Lim Channel populations. The number of effective alleles was 1.17 in the Marina Bay population, 1.21 in the Lim Channel population and 1.22 in the Bistrina population. The level of polymorphism of loci ($P_{0.95}$) was lower in the populations of an oyster from the Marina Bay and Lim Channel, with a mean of 16.6 for the Marina Bay, 42.8 for the Lim Channel and highest for the Bistrina population, 71.5 (Table 4).

Ordinary measures of genetic variability, such as the proportion of polymorphic loci ($P_{0.95}$) and the average number of alleles per locus (A), rarely reveal significant differences between natural and cultured populations of marine organisms (GOSLING, 1982; DILLON and MANZI, 1987). The number of P , \bar{H} and A_e indicate that levels of genetic variations *O.edulis* populations at the eastern coast of the Adriatic Sea are higher in comparison to other oyster populations (JOHANNESSON *et al.*, 1989).

Table 4. *Ostrea edulis*. Summary of genetic variability in natural and cultured populations from the eastern coast of the Adriatic Sea

	SC*	BC*	KB*	MB*	RO*	LC*
No.loci studied	7	7	7	6	7	7
Average no. allele per locus (A)	2.85	2.0	2.28	2.16	2.0	2.0
Effective no. of allele per locus (A_e)	1.30	1.22	1.37	1.17	1.21	1.21
Mean heterozygosity ($\bar{H}\pm S.E.$)	0.16 ± 0.06	0.14 ± 0.06	0.23 ± 0.06	0.11 ± 0.06	0.13 ± 0.05	0.13 ± 0.06
Percent polymorphic loci per population ($P_{0.95}$)	85.7	71.5	71.4	16.6	42.9	42.8

*Populations (SC-Soca Cove; BC- Bistrina Cove; KB-Kaštela Bay; MB-Marina Bay; RO-Rovinj; LC-Lim Channel)

Many studies have been performed on *Ostrea edulis* populations from the Atlantic and Mediterranean coast and these showed the geographical variations in gene frequencies (BUROKER, 1982; MAGENNIS *et al.*, 1983; JOHANNESSON *et al.*, 1989; SAAVEDRA *et al.*, 1987). SAAVEDRA *et al.* (1993) analysed cultured populations of the oyster, *Ostrea edulis* from the ponds in Venice. They found average heterozygosity of 0.080, the percent of polymorphism $P_{(0.95)}$ 27.3, and the average number of alleles per locus 1.91. In the ponds in Athens heterozygosity was 0.079, the level of polymorphism $P_{(0.95)}$ 18.2, and the average number of alleles per locus was 2.0.

The total level of heterozygosity of three oyster (*O. edulis* L.), populations from the Ireland was 0.14 (WILKINS and MATHERS, 1974). SAAVEDRA *et al.* (1987) also analysed three natural populations in the northwest of Spain, and established 11 monomorphic and six polymorphic loci - $P_{(0.99)}$ criterion. Analysing three natural populations of *Ostrea edulis* L. in Spain, SAAVEDRA *et al.* (1987) found the values of heterozygosity to range from 0.115 - 0.118, the percent of polymorphism $P_{(0.95)}$ = 30 and the average number of alleles per locus 1.70-1.80.

A population analysed in Maine (USA), descending from the oysters imported from Oosterschelde (Netherlands) around 1940, showed 22 monomorphic and seven polymorphic loci (0.95 criterion), with the average heterozygosity of 0.09 (BUROKER, 1982). BLANC *et al.* (1985) compared five Atlantic to two Mediterranean populations of *Ostrea edulis* L. from France and determined seven monomorphic and five highly polymorphic loci, whereas in seven other loci *Pgm*, *Mdh-2*, *Est-2*, *Aat-1*, *Lap-3* and *Lap-4* the heterozygosity ranged from 0.11 to 0.063.

The population of *Ostrea edulis* L. has a considerably lower mean genetic variability compared to other oyster species (BUROKER *et al.*, 1979a,b; BUROKER, 1982, 1983; HEDGECOCK and OKAZAKI, 1984).

Some studies reported no significant differences in genetic parameters (heterozygosity, polymorphism level and the average number of alleles per locus) between natural and cultured populations of bivalves (ALLENDORF and UTTER, 1979; GOSLING, 1982; DILLON and MANZI, 1987). This may point to the fact that controlled breeding of a species can be sufficient to prevent a deficiency of genetic variabilities and eventual occurrence of inbreeding. However, such conclusion is rather premature, since genetic changes, particularly in heterozygotes, may appear in cultured species not earlier than after several generations in isolation.

The deviations from the HARDY-WEINBERG expectations in natural and cultured oyster populations from the Adriatic Sea were estimated also by means of F- statistics. There is no significant heterozygote deficiency within populations, F_{IS} values for each locus overall populations are small (Fig.2). There is no differentiation in gene frequencies between populations, all F_{ST} values are low (Fig.3).

F_{ST} values for all natural populations of *O. edulis* from the eastern coast of the Adriatic Sea were 0.000 ± 0.010 , which shows that there is no differences in gene frequencies between these populations, and values of F_{IT} 0.038 ± 0.024 and F_{IS} 0.038 ± 0.026 (Fig. 4).

In the cultured populations there is no heterozygote deficit between populations with F_{IS} 0.172 ± 0.117 (Fig. 4) and F_{ST} 0.014 ± 0.013 , whereas F_{IT} was 0.185 ± 0.129 . Analysing all natural and cultured populations of *O. edulis* L. from the eastern coast of the Adriatic Sea, as a single population, F_{IS} value was obtained to be 0.092 ± 0.062 (Fig. 4), F_{ST} 0.005 ± 0.005 and F_{IT} 0.096 ± 0.059 .

According to these results there is no heterozygote deficiencies from the HARDY-WEINBERG expectations within and among populations. Also, there are no significant differences in gene frequencies between natural and cultured populations of *Ostrea edulis* in the eastern coast of the Adriatic Sea.

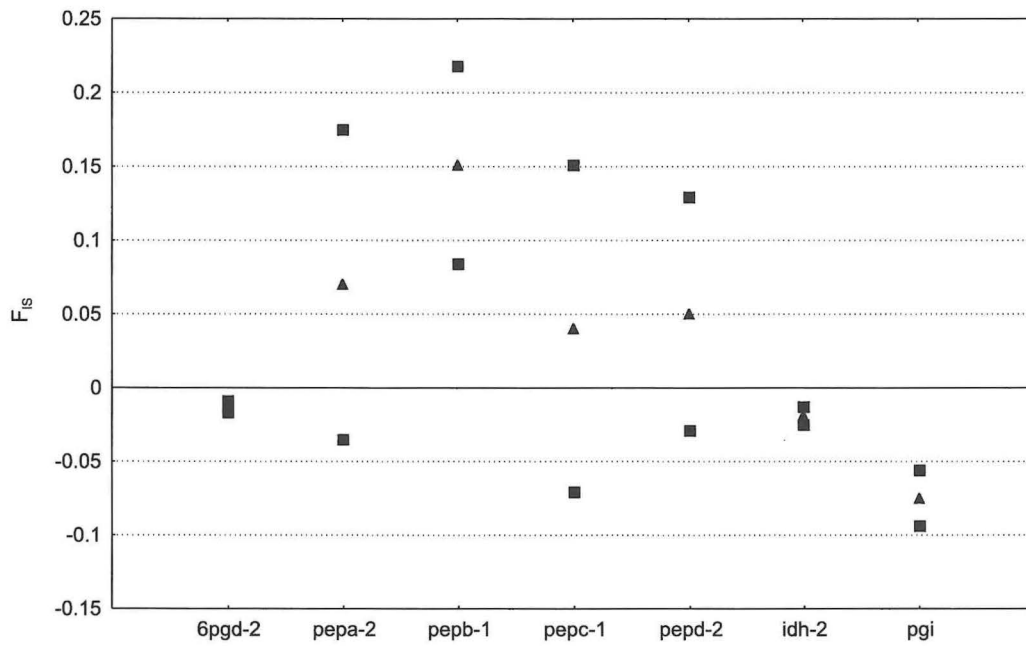


Fig.2. *Ostrea edulis* F_{IS} ($\pm S.D.$) values for each locus in the whole set of oyster populations in the eastern coast of the Adriatic Sea

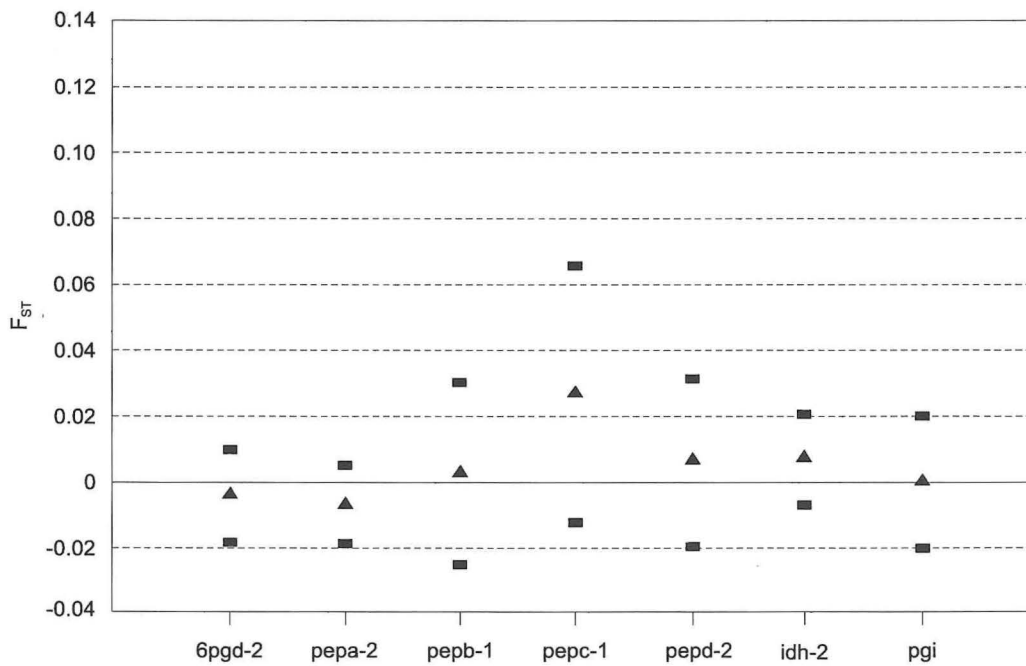


Fig.3. *Ostrea edulis* F_{ST} ($\pm SD$) values for each locus in the whole set of populations in the eastern coast of the Adriatic Sea

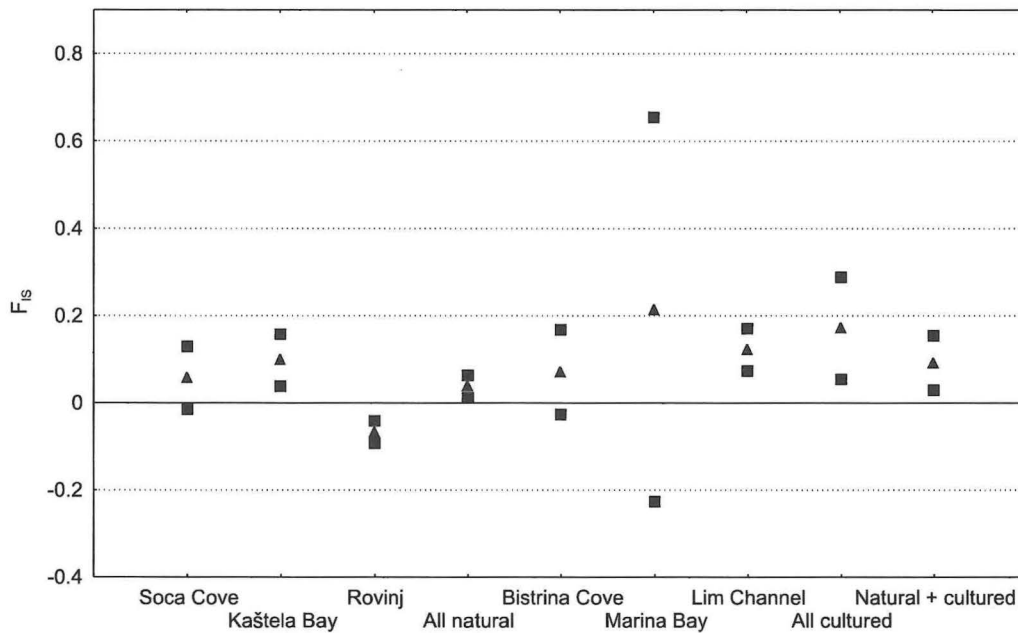


Fig.4. *Ostrea edulis* L. F_{IS} values ($\pm S.D.$) for the natural and cultured populations in the eastern coast of the Adriatic Sea

CONCLUSIONS

The results of the present work indicate that, the mating was random within the *Ostrea edulis* populations from the eastern coast of the Adriatic Sea. The levels of the genetic variations in the all oyster populations are higher in comparison to other Mediterranean and Atlantic populations. Also, there is no heterozygote deficiency within and among populations and no differentiations in gene frequencies between natural and cultured populations.

ACKNOWLEDGEMENTS

I would like to thank Dr. François RENAUD and Dr. Thierry de MEEÛS from the Laboratory of Compared Parasitology, University of Montpellier II, Montpellier, who helped with the experimental samples processing, as well as statistical data processing. This work was supported by the IFREMER (Institut Français de Recherche par l'Exploitation de la Mer), Paris, France.

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Accepted: 21 July 1999

Genetska struktura prirodnih i uzgojnih populacija kamenice (*Ostrea edulis* L.) u Jadranu

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SAŽETAK

Tehnikom škrobne gel elektroforeze određivala se genetska struktura, a isto tako i genetske različitosti između prirodnih i uzgojnih populacija europske plosnate kamenice (*Ostrea edulis* L.) s istočne obale Jadranskog mora.

Analizirano je sedam enzimatskih sistema iz tri prirodne i tri uzgojne populacije i to: 6-Fosfoglukonat dehidrogenaza (6Pgd), Izocitrat dehidrogenaza (Idh), Glukozafosfat izomeraza (Pgi) i Peptidaza (-a, -b, -c, -d).

Nijedna populacija nije pokazala signifikantno odstupanje od očekivane HARDY-WEINBERGove ravnoteže frekvencije genotipova, što ukazuje na slučajno križanje unutar svake populacije. Postotak polimorfnihih lokusa po populaciji, srednjak heterozigotnosti i efektivan broj alela po lokusu ukazuje da je stupanj genetskih različitosti populacija *O. edulis* na istočnoj obali Jadrana viši u odnosu na druge mediteranske i atlantske populacije.

Nema smanjenja broja heterozigota unutar populacija niti različitosti u frekvencijama gena između prirodnih i uzgojnih populacija *O. edulis* na istočnoj obali Jadrana.

