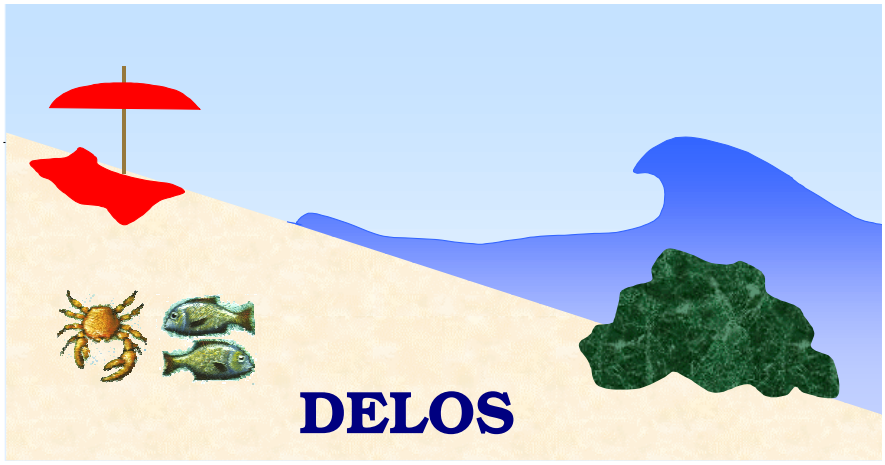
EU Fifth Framework Programme 1998-2002
Energy, Environment and Sustainable Development

Environmental Design of Low Crested Coastal Defence Structures



Deliverable 40

Draft Version

Meta-population model as a function of large-scale distribution of breakwaters

Contract n°EVK3-CT-2000-00041

EU Fifth Framework Programme 1998-2002
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D.40 Assessment of positive and negative corridor effects on species dispersal Preliminary version

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Introduction

Deployment of artificial rocky substrata along sandy shores to prevent coastal erosion causes dramatic changes in the composition and structure of benthic assemblages (Bacchiocchi & Airoldi 2003). The new substratum is rapidly colonised by algal and animal species, which did not previously occur in the area. Patterns of colonisation depend on the biological features of the species (Glasby, 1999; Davis et al 2002). The first colonisers are usually organisms with a long period of spawning and long living planktonic larvae, able to colonise habitats over a wide geographic range. Species with limited dispersal ability follow, and the stepping stone model (Rousset, 1997), a one dimensional dispersal model best describes their spread. If the environmental conditions are favourable, the latter spread from a source population (e.g. natural rocky shores) along the shores where new substrata have been deployed (Glasby & Connell, 1999). The analyses of the genetic structure of species having different life histories and dispersal ability could contribute to the understanding of the impact of artificial rocky shores on genetic structuring and genetic biodiversity in coastal habitats. Artificial substrata could increase gene flow among formerly isolated populations, leading to the genetic homogenisation of the species aver large geographic scales and to the loss of locally adapted genotypes.

The overall aim of the WP 3.4 is to asses large scale effects of breakwater spatial arrangement, including the analyses of the effects on the genetic structuring of marine species. The first step in understanding these effects is to compare populations from natural and artificial substrata and to assess the influence of the spatial arrangement of the breakwaters (i.e. distance from natural reefs and other artificial structures) on the population genetic structure of the target species. These studies have been done at 2 study sites: the Adriatic coast of Italy and the Spanish coast.

The target species for the Adriatic Sea is the mollusk *Patella caerulea* (Fig. 1), it has been selected based on the results of Deliverable 29 (Relationships between breakwater spatial arrangement and large scale species distribution). The limpet *Patella caerulea* shows major variability in abundance along the North Adriatic coast and, based on the literature is known to have limited dispersal ability (Dodd, 1957; Hawkins personal communication). Moreover, limpets play a major ecological role in controlling the dynamics of the epibenthic assemblages on rocky shores (Hawkins & Hartnoll, 1983). The study covered about 700 km of shoreline, characterized by the presence of extensive defence schemes. The aims of the study were: (1) to compare the genetic structure of populations from artificial and nearby natural substrata and (2) to quantify the genetic structuring among *Patella caerulea*

Fig. 1 - *Patella caerulea*

populations to test for potential corridor effects due to the deployment of the breakwaters. Sampling was done following a hierarchical design. The scales covered ranged from 10s of meters (distance among breakwaters) to 100s of kilometres (distance among locations).

Along the Spanish cost the study has been focused on the blenny fish *Tripterygion delaisi* (fig 2). The blackfaced blenny, *Tripterygion delaisi sp.* (Cadenat & Blache 1971) is a common occupier of Mediterranean and Atlantic natural and artificial rocky shores and it is one of the most abundant fishes in these habitats (Corbera *et al.* 1996). Two subspecies have been identified: *T. d. xanthosoma* in the Mediterranean Sea, and *T. d. delaisi* along the Atlantic coast from south England to Senegal and Azores, Madeira and the Canary Islands (Zanders 1986). Morphological differences between them are limited, and can be determined only by analysing large samples (Wirtz, 1980). This species has limited dispersal ability and could represent a good model for investigating the effects of the introduction of artificial habitats on dispersal and genetic structure of populations. Although larvae spend 17 days in the plankton (Raventos & Macpherson 2001), they do not disperse more than 100m offshore (Sabatés *et al.* in press). Furthermore, adult fishes show high territorial fidelity (Heymer 1977) and migratory movements have not been described for this species.

Fig 2: *Tripterygion delaisi*

In the present study microsatellite loci have been isolated from an enriched genomic library and 10 loci have been selected for use in the analysis of the population genetic structure of *Tripterygion delaisi* throughout its distribution area.

Adriatic coast of Italy

Methods

Genetic variability of *P. caerulea* was analysed along approximately 700 km of coast. Samples were collected at 5 locations: Trieste, Cesenatico, Gabicce, Ancona and Ortona (Fig. 3). At 4 locations samples were collected on both natural reefs and artificial structures, while at Cesenatico only limpets on artificial structures were sampled, due to the lack of natural reefs. At each location about 60 specimens were collected from each of the 2 areas on artificial substrata and, if present, from 2 areas on natural reefs. The genetic structure of *P.*

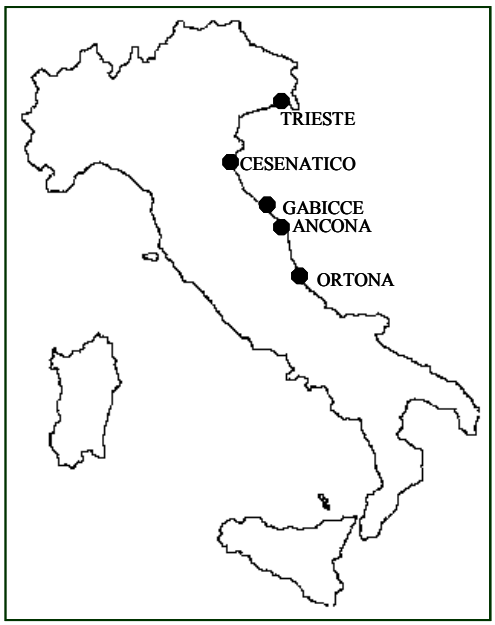


Fig. 3 Map of the sampling area with location of the sampling stations

caerulea was analysed using both biochemical (allozymes) and molecular (microsatellites) markers. Protocols for the biochemical markers have been tested and set up. After a preliminary screening we identified 13 suitable enzymatic systems encoding for 15 loci (Tab. 1). Four of these enzymes resulted to be monomorphic in all the analysed populations. Microsatellite loci for *P. caerulea* were neither available in literature nor in Gene Bank: isolation and characterisation of microsatellites markers for this species were done and are still in progress.

Abbreviation	Enzyme	E.C. number
AK	adenylate Kinase	2.7.4.3
ALD	aldolase	4.1.2.13
αGPD	glycerol-3-phospate dehydrogenase	1.1.1.8
G3PDH	glyceraldehyde 3 phosp. Dehydrogenase	1.2.1.12
HK	hexokinase	2.7.1.1
IDH1, IDH2	isocitrate dehydrogenase	1.1.1.42
MDH	malate dehydrogenase	1.1.1.37
ME1, ME2	malic enzyme	1.1.1.40
MPI	mannose-6-phosphate isomerase	5.3.1.8
PGD	phosphogluconate dehydrogenase	1.1.1.44
PGI	glucose-6-phosphate isomerase	5.3.1.9
PGM	phosphoglucomutase	2.7.5.1
SDH	sorbitol dehydrogenase	1.1.1.14

Tab. 1 – Allozymic markers optimised for the analysis of the genetic structure of *Patella caerulea*.

Results and discussion

A pilot study which applied a small scale sampling design was done in Cesenatico to test if genetic patterns of populations of *P. caerulea* differ among breakwaters within a single location. Variability within samples taken from a single artificial structure (within 100s meters) was investigated by collecting samples from the sheltered and the exposed sides and from the northern and the southern ends of a single breakwater (a total of 240 specimens were collected on each structure). Genetic variation between 2 breakwaters was compared to estimate the variability within a set of artificial structures (1 to 10s kms distance). Genetic structure of samples collected at Cesenatico using the small scale sampling design were analysed by allozyme electrophoresis.

No statistically significant differences were found between samples collected on the internal and external side of the defence structure, or between samples collected on the northern and the southern ends (Tab. 2). ANOVA to test differences in the heterozigosity values of the 8 samples showed statistically significant differences between the two breakwaters (Tab. 3).

The sampling design for the Adriatic coast of Italy was planned based on these results. Collection of samples at the 5 localities was completed between June 2002 and June 2003. Genetic structure of all samples was analysed by allozyme electrophoresis on 15 loci. Data analysis revealed that genetic variability of *P. caerulea* populations in the study area is quite low. Values of mean observed heterozygosity ranged from H_{obs} =

		Mean unbiased expected Heterozigosity	Mean observed Heterozigosity	P(0.95)	P(0.99)	Mean no. of alleles per locus
Breakwater 1	Site 1	0,0663 (0,1011)	0,0352 (0,0429)	0,1000	0,7000	2,1
	Site 2	0,0716 (0,0774)	0,0459 (0,0472)	0,3000	0,7000	2,2
	Site 3	0,0761 (0,0635)	0,0486 (0,0465)	0,4000	0,8000	2,3
	Site 4	0,0767 (0,0990)	0,0484 (0,0690)	0,2000	0,6000	2,1
Breakwater 2	Site 5	0,0769 (0,0864)	0,0553 (0,0632)	0,3000	0,6000	2,2
	Site 6	0,0741 (0,0793)	0,0566 (0,0683)	0,4000	0,7000	2,2
	Site 7	0,0933 (0,0922)	0,0611 (0,0786)	0,5000	0,7000	2,1
	Site 8	0,1106 (0,0869)	0,0715 (0,0656)	0,6000	0,9000	2,5

Tab. 2 - Genetic variability of the 8 samples of *P. caerulea* collected at Cesenatico. Values refer to the 15 loci analysed standard errors are in parentheses.

Anova	df	MS	F	p
Breakwaters	1	0.0006	11.70	*
RES	6	0.0000		
TOT	7			
C = 0.5736	p > 0.005			

Tab. 3 – ANOVA on observed heterozygosity values of *P. caerulea* populations to test for differences between breakwater 1 and 2 at Cesenatico.

0.022 (S.E. 0.0407) to H_{obs} = 0.079 (S.E. 0.021), percentage of polymorphic loci (P_{95}) ranged from 0.20 to 0.50 (Tab. 4). All the surveyed populations showed a heterozygote deficiency. Significant deviations from Hardy-Weinberg equilibrium were also confirmed by F_{is} values, resulting in high significant values for all the loci except AK and PGI (Tab. 5).

Nei's genetic distance between populations was extremely low and did not reflect effective geographic distances. F_{st} values did not show significant differences between populations for any enzymatic system but AK. No significant differences were found between samples from different substrates or between samples from different sites.

Microsatellites are highly variable and they represent a more sensitive tool to assess intra-specific genetic differentiation at small spatial scales (Chambers & MacAvoy, 2000). A genomic library enriched with the biotinylated probe $(CA)_{12}$ was created following the protocol FIASCO (Zane *et al*., 2002). Twenty-one primer pairs were tested and four of these could be reliably scored. PAT11, PAT15, and PAT38 resulted to be polymorphic, whereas PAT27 was monomorphic (Tab. 6). In order to find further possible microsatellite loci a second genomic library has been created and development and optimisation of further microsatellite loci for *P. caerulea* are in progress.

Population	Mean sample size	Mean number of alleles per locus	Percentage of polymorphic loci	Mean observed heterozygosity	Mean expected heterozigosity
SIST ART	55.6 (1.8)	2.0 (0.3)	30.0	0.062 (0.027)	0.086 (0.033)
SIST NAT	55.4 (2.7)	2.2 (0.3)	40.0	0.060 (0.019)	0.084 (0.027)
MIRA ART	57.0 (2.2)	2.4 (0.4)	20.0	0.042 (0.018)	0.068 (0.023)
MIRA NAT	55.6 (1.0)	2.3 (0.4)	30.0	0.040 (0.018)	0.071 (0.023)
CES N	178.9 (3.7)	2.7 (0.3)	20.0	0.044 (0.015)	0.073 (0.025)
CES S	189.2 (4.4)	2.9 (0.3)	30.0	0.060 (0.020)	0.088 (0.025)
GAB ART	58.7 (1.0)	2.0 (0.3)	40.0	0.045 (0.021)	0.080 (0.033)
GAB NAT	55.5 (2.1)	2.0 (0.3)	30.0	0.059 (0.023)	0.070 (0.024)
VALL ART	57.6 (2.3)	2.1 (0.2)	40.0	0.069 (0.032)	0.086 (0.030)
VALL NAT	53.9 (2.1)	1.9 (0.3)	30.0	0.050 (0.034)	0.077 (0.038)
P NOVO ART	53.6 (3.2)	1.8 (0.2)	10.0	0.049 (0.026)	0.047 (0.024)
P NOVO NAT	47.2 (1.6)	1.9 (0.2)	10.0	0.041 (0.014)	0.043 (0.017)
SIROLO ART	45.5 (2.6)	1.7 (0.3)	10.0	0.031 (0.014)	0.037 (0.017)
SIROLO NAT	35.0 (0.6)	1.7 (0.3)	30.0	0.022 (0.013)	0.052 (0.022)
ORT ART	52.7 (3.0)	2.8 (0.3)	50.0	0.079 (0.021)	0.109 (0.027)
ORT NAT	49.2 (2.0)	2.3 (0.3)	20.0	0.043 (0.015)	0.073 (0.021)
S.VITO ART	43.2 (1.9)	2.6 (0.3)	40.0	0.069 (0.019)	0.088 (0.023)
S.VITO NAT	47.5 (3.8)	2.3 (0.4)	40.0	0.051 (0.018)	0.078 (0.024)

Tab. 4 - Genetic variability of *P. caerulea* samples collected at 9 sites along the Northern Adriatic coast. Values refer to the 15 loci analysed standard errors are in parentheses.

	F_{IS}	F_{IT}	F_{ST}
PGM	0.5719 (0.0615) ***	0.5746 (0.0613) ***	0.0063 (0.0040)
ME1	0.6677 (0.1290) ***	0.6672 (0.1293) ***	-0.0017 (0.0018)
ME2	0.9218 (0.0857) ***	0.9221 (0.0853) ***	0.0037 (0.0041)
G3PDH	0.4403 (0.2546) ***	0.4384 (0.2537) ***	-0.0025 (0.0013)
IDH1	1.0000 (0.0000) ***	1.0000 (0.0000) ***	-0.0115 (0.0026)
IDH2	0.4813 (0.1163) ***	0.4810 (0.1161) ***	-0.0004 (0.0025)
PGD	0.3185 (0.0461) ***	0.3192 (0.0456) ***	0.0011 (0.0024)
MPI	0.2971 (0.0648) ***	0.2986 (0.0633) ***	0.0023 (0.0033)
PGI	0.0123 (0.0314)	0.0119 (0.0307)	-0.0004 (0.0021)
AK	0.0324 (0.0456) ***	0.0400 (0.0446) ***	0.0079 (0.0063) *
All loci	0.3073 (0.0693) ***	0.3090 (0.0690) ***	0.0024 (0.0012) *

Tab. 5 – Summary of Wright's *F*-statistics for the samples of *P. caerulea* collected along the Northern Adriatic coast . (*) P<0.05, (***) P<0.001.

A preliminary screening of two samples collected at Cesenatico and one sample collected at Trieste was done using the polymorphic loci PAT11, PAT15, and PAT38. After a preliminary screening, genetic patterns of the three samples resulted to be quite similar. Low values of Nei's genetic distances between the three populations were found and the observed pattern reflects geographical distances among collections (Tab. 6). The F_{ST} values, including the mean value, were not significant, suggesting that limited differentiation occurs among the investigated populations. Mean F_{IS} value was significant, showing a general heterozygote deficiency.

LOCUS	REPEAT SEQ	T°C a	N° BASES	PRODUCT SIZE
PAT11	$(AC)_3GC(AC)_7$	58 58	20 20	156
PAT15	$(TG)_{12}(TATG)_4(TG)_{1\,2}$	58 56	20 20	328
PAT38	$(AG)_{18}(AC)_6$	59 54	22 17	211

Tab. 6 – Microsatellite loci isolated for *P. caerulea*.

Southern coast of Spain

Methods

Genomic DNA has been isolated using QIAamp® DNA minikit columns (Qiagen) from two specimens of *Tripterygion delaisi xanthosoma* collected at Blanes (42°02'N; 03°13'E, NW Mediterranean), a microsatellite enriched partial genomic library was constructed, using this genomic DNA mix, following the protocol FIASCO (Zane *et al.* 2002) but with two biotinylated probes ($(CA)_{15}$ and $(GA)_{15}$). Positive clones were detected following the protocol described in Estoup & Turgeon (1996) (detailed protocol available at http://www.inapg.inra.fr/dsa/microsat/microsat.htm).

Approximately 1500 colonies were screened for microsatellites using a mixture of two probes ($(CA)_{15}$ and $(GA)_{15}$), yielding 216 positively hybridising clones. All positive clones were stored in glycerol at –80ºC (Estoup & Turgeon 1996). 51 positive clones (Tab. 7) were sequenced. 38 of these 51 clones contained microsatellite sequences and a sufficiently long flank sequence, and 19 sets of primers were designed using the Primer3 web-based software package (Rosen & Skaletsky 1996), available at http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi.

Clon	Insert (bps)	Repeat seq.	kind	PDP
1515	375	(TG)31(CG)3(TG)3	C	NO
1528	328	(AC)15	P	YES
1545	204	(CT)6TT(CT)7CCTT(CT)2/(TC)4GC(TC)3	I	YES
2209	194	(AG)20	P	YES
2212	319	(AC)41	P	YES
2304	210	(GA)20	P	YES
2311	322	(AC)4AA(AC)6	I	YES
2315	303	(GA)14A4(GA)8/(AG)14	I	NO
2317	ns	(CA)4CG(CA)10	I	NO
2327	306	(TG)5/(CA)7	C	YES
2330	450	(TC)13	P	YES
2332	295	(CA)33	P	NO
2342	ns	(GT)25	P	YES
2345	302	(GA)5CA(GA)5AA(GA)7/(GA)17	I	YES
2346	258	(GT)21	P	YES
2401	285	(AG)8G(AG)2	I	NO

2424	295	(CA)45	P	YES
2429	352	(CA)12	P	YES
2431	187	(AG)29	P	NO
2440	197	(AG)12	P	YES
2508	473	several (GT)3	…	…
2546	323	(CA)4CT(CA)4CTCA(TA)2(CA)18CG(CA)3	I	YES
2730	176	(CA)9/(CA)5(TA)2(CA)2TA(CA)3	I	YES
2745	261	(AC)23	P	NO
2801	250	(CA)16	I	YES
2812	261	(TG)8	P	YES
2813	423	(CA)26	P	YES
2825	231	(CA)13	P	NO
2827	280	(CA)7	P	NO
2830	ns	(TG)9	P	YES
2834(B)	431	(CA)4CG(CA)2CG(CA)4	I	YES
2837(B)	431	(CA)4CG(CA)2CG(CA)4	I	YES
2843(A)	255	(CA)6	P	YES
2902	273	(AC)31	P	YES
2909	351	(CAT)8/(CA)10	C	YES
2915	234	(CA)6TG(CA)5	I	YES
2922	350	(CA)7CG(CA)2CG(CA)15GA(CA)3	I	YES
2928	221	(AC)5(AT)2(AC)11	I	YES
2929	408	(GA)8/(CA)5(TACA)3/(AC)15/C11GC7	C	YES
2933	ns	several GTs	…	…
2937	256	(CA)6GA(CA)7	I	YES
2941	166	(TG)8CT(TG)6(AG)9	C	YES
2947	ns	(CA)11CG(CA)32	I	NO
3016	262	(CA)13C(CA)3[C(CA)6]2	I	YES
3036	251	(TG)10	P	YES
3102	184	(CA)12	P	YES
3124	336	(AC)4AT(AC)3TAC	I	YES
3131	ns	(CA)21	P	YES
3217	ns	(TG)7CG(TG)16	I	YES
3221	ns	(AC)15	P	YES
3223(A)	255	(CA)6	P	YES

Tab. 7 - Clones that have been screened. A and B: pairs of repeat sequences. Ns: number of inserts not defined. Primer design probability (PDP): C: compost microsatellite, I: imperfect microsatellite, P: perfect microsatellite.

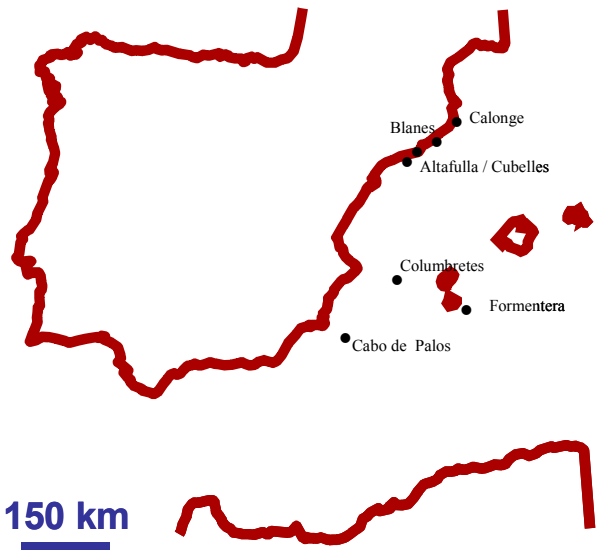
Of the 19 sets of primers designed, ten functional primer pairs (Table 7) were obtained. The other nine loci failed to amplify. Primers were optimised using the conditions described herein. The genomic DNA for genotyping was prepared using a rapid BIO RAD (Chelex 100 resin) extraction protocol (Estoup *et al.* 1996). Polymerase chain reactions (PCR) were carried out in 20µL reactions, containing 1X reaction buffer, 2mM $MgCl_2$, 250µM dNTPs, 0.25µM of each primer, 1U Taq polymerase

(Genotek) and 20-30ng genomic DNA. PCR was performed in a Primus 96 plus (MWGAG Biotech), and cycle parameters consisted of a first denaturation step at 95ºC for 5 min, followed by 35 cycles of 1 min at 95ºC, 1 min at annealing temperature (Tab. 8), and 1min at 72ºC, and a final extension at 72ºC for 7 min. The amplified loci were sequenced in an automatic sequencer ABI 3700 (Applied Biosystems).

Locus	Ta (ºC)	[Cl2Mg]	Fluorochrom	NA	Amin	Amax
Td1	57	2	NED	7	168	184
Td2	57	2	NED	8	402	440
Td3	57	2	HEX	6	124	138
Td4	55	2	HEX	10	219	257
Td6	55	2	6-fam	8	117	147
Td7	55	2	HEX	4	107	117
Td8	55	2	HEX	6	323	356
Td9	55	2	6-fam	6	294	323
Td10	55	2	6-fam	12	134	212
Td11	55	2	NED	4	281	301

Tab. 8 - Selected microsatellite loci.

Genetic structure of *Tripterygion delaisi sp.* samples form the Mediterranean Sea and the Atlantic Ocean has been investigated using 10 microsatellite loci, to assess the dispersal features of this species. Four populations sampled at different geographic distances have already been analysed (Columbretes Is. - Castellon, Spain 39˚53.9'N; 0˚41.2E, NW Mediterranean; Cape of Palos-Murcia, Spain 37˚37.9'N; 0°41.9W, NW Mediterranean; Hierro-Canary Is., Spain, 27˚38.0'N; 17°59.0W, E Atlantic; Horta-Azores Is., Portugal 38˚31'N; 28˚43' Mid-E Atlantic), the analysis of two further populations (Blanes, Spain 42°02'N; 03°13'E, NW Mediterranean; Formentera, Spain 38˚44'N; 1˚25.1' NW Mediterranean) is in progress. In order to compare levels of genetic differentiation between populations living on natural rocky shores separated by distances similar to those separating LCS, two additional subpopulations have been sampled in the Cape of Palos and Blanes. The sampling sites are rocky outcrops separated by hundreds of meters of sandy shores.



Results and discussion

We've found a large genetic differentiation between all pairs of populations, where levels of differentiation increase with the geographical distance. These results confirm

the hypothesis of two clearly differentiated subspecies living in the Atlantic and Mediterranean, respectively. The Straight of Gibraltar seems to represent a relevant barrier to the dispersal of this species. Differentiation within Atlantic and Mediterranean populations is limited.

Values of Fst between all pair of populations:

	Hierro	Columb.	Palos
Columb.	0.2207		
Palos	0.2144	0.0302	
Azores	0.0648	0.2087	0.1966

Only two of the investigated loci show a linkage disequilibrium (Td8 vs. Td11) in all populations. Populations from Columbretes and from Cape of Palos are not in Hardy-Weinberg equilibrium, while Hierro and Azores populations do not deviate from it (Tab. 9).

Hierro

LOCUS	P-val	S.E.	Fis W&C	Fis R&H
td01	0.7915	0.0220	-0.198	-0.075
td02	0.6044	0.0238	-0.041	-0.046
td03	1	0.0000	-0.067	-0.039
td04	0.5951	0.0284	+0.090	+0.032
td06	0.1996	0.0327	+0.062	+0.005
td07	0.3609	0.0195	+0.073	+0.158
td08	0.6004	0.0220	+0.079	+0.078
td09	1	0.0000	-0.115	-0.117
td10	0.9489	0.0123	-0.038	+0.026
td11	-			

Columbretes

LOCUS	P-val	S.E	Fis W&C	Fis R&H
td01	0.6051	0.0265	-0.001	+0.020
td02	0.7597	0.0196	-0.157	-0.064
td03	0.0000	0.0000	+0.482	+0.405
td04	0.5059	0.0403	+0.022	+0.043
td06	0.6419	0.0429	-0.067	-0.019
td07	0.9342	0.0035	-0.147	-0.107
td08	0.0441	0.0194	+0.189	+0.074
td09	0.0228	0.0073	+0.274	+0.158
td10	0.9949	0.0051	-0.017	-0.013
td11	0.3350	0.0262	+0.020	-0.034

Cabo de Palos

LOCUS	P-val	S.E	Fis W&C	Fis R&H
td01	0.4371	0.0306	-0.070	-0.039
td02	0.8504	0.0146	-0.113	-0.054
td03	0.0000	0.0000	+0.409	+0.206
td04	0.0022	0.0019	+0.149	-0.001

td06	0.9992	0.0008	-0.031	-0.020
td07	0.4455	0.0127	-0.204	-0.083
td08	0.0000	0.0000	+0.302	+0.207
td09	0.0153	0.0037	+0.356	+0.154
td10	0.8437	0.0289	-0.027	-0.017
td11	0.2198	0.0187	-0.002	+0.033

Azores

LOCUS	P-val	S.E	Fis W&C	Fis R&H
td01	0.9447	0.0066	-0.014	-0.028
td02	0.2883	0.0213	+0.053	-0.005
td03	1	0.0000	-0.040	-0.041
td04	0.5464	0.0172	-0.009	+0.020
td06	-			
td07	0.9392	0.0100	-0.057	-0.026
td08	0.7382	0.0116	+0.073	+0.023
td09	0.0612	0.0014	-0.405	-0.410
td10	0.0463	0.0083	+0.172	+0.204
td11	-			

Tab. 9 - Test for the Hardy - Weinberg equilibrium (ns: $p > 0.05$, *: $0.01 < p < 0.05$, **: $p < 0.01$)

Although only data from Cape of Palos have been analysed (collection of further data are in progress), the present results suggest that subpopulations separated by a distance of 100-200 m are not in Hardy-Weinberg equilibrium, whereas individuals from the same site (sampled on the same rocky shore) are in Hardy-Weinberg equilibrium at all loci. These differences suggest that, in *T. delaisi,* small geographic barriers (100-200 m) play a certain role in the isolation of the populations.

Limited larval dispersal and lifestiles of adults of *T. delaisi*, which stay on the rocky bottoms and can't move over sandy areas, could explain the genetic differentiation observed between populations. Deviations form the Hardy-Weinberg equilibrium between subpopulations from the same locality suggest a certain degree of isolation at a small geographic scale. Higher levels of differentiation were measured among localities separated by thousands of kilometres. LCS situated very close to areas where populations of *T. delaisi* live, can easily be colonised (e.g. Galonge LCS), whereas other LCS located several miles from areas inhabited by this species are not easily colonised (e.g. Cubelles and Altafulla LCS). These hypotheses will be tested using the data obtained from the populations, which are currently being analysed. Further investigations, using the serranidae fish *Serranus cabrilla* (Linnaeus, 1758) as a target species, are in progress . This species has a greater dispersal ability in comparison to *T. delaisi*. Its larvae spend 3 to 4 weeks in the plankton (Raventos & Macpherson 2001), while adult fishes show high territorial fidelity. It is therefore an interesting model to compare with *T. delaisi sp.*, because of differences in their dispersal capabilities. Ten microsatellites have been isolated and selected from an enriched genomic library that will be used to analyse the genetic population structure of *S. cabrilla* throughout its

distribution area (We have followed the same methods described for *T. delaisi sp.*). The analyses of two populations, one from Blanes and one from Formentera, is in progress.

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