Results

1. Pattern of recolonization

1.1. Trophic condition

Concentration of nutrients differed between enriched and non enriched area constantly in time. Mean nitrogen compounds concentrations are the following: NH_4^+, 2.16 ±0,8 µm/l in enriched plots *vs* 0,46 ± 0,4 µm/l in not enriched plots; NO_2, 0,09 ±0,03 µm/l in enriched plots and 0,08 ±0,03 µm/l in not enriched plots; NO_3^-, 3,25 ±0,5 µm/l and 2,24 ±1,5 µm/l; the total N 5,49 ±1,02 µm/l and 2,80 ±1,03 µm/l. Conversely, as far as phosphorous compounds, the concentration of PO_4^{3-} and of the total P didn't show relevant differences (mean concentration 0,18 ± 0,06 µm/l for PO_4^{3-} in enriched plots *vs* 0,19 ± 0,05). Also chlorophyll *a* concentration didn't show differences between treatments across time. Concentration values ranged between a minimum of 0,14 µg/l (September 2007 in control areas) and a maximum of 0,27 µg/l (January 2008, both in control area and in enriched ones).

1.2. Assemblage structure

A total of 46 taxa were recognised, 26 of them at species level (Appendix 1), 21 algae and 25 invertebrates. High values of cover of encrusting red algae (ECR), typical of barren assemblages were found at all sampling dates and in all plots. In the first sampling period (Time 1 and 2, so after one and two month from the beginning of the experiment), however, the peryphyton of microalgae and unicellular algae, and subsequently of dark filamentous algae (DFA) contributes with mean percentage cover of sometimes exceeding the 50% of the plots. Among the invertebrates, Porifera, including the dominant species *Cliona viridis* showed an high cover, followed by the clam *Gastrochaena dubia*, the madrepores *Balanophyllia europea* and *Caryophylla smithi* and tunicates belonging to the family of Didemnidae.

A total of 17 functional groups were obtained from the original matrix (Appendix 2) Functional groupings of algae are based on anatomical and morphological characteristics (Steneck and Dethier 1994) that often corresponds to ecological

characteristics: “encrusting calcified algae” are those with prostrate thallus; “sub turf algae” are small then 5 cm, primary space holders forming limited vertical height turf on substrate, and could be ephemeral (in this case were called “sub turf ephemeral algae”), or with a calcified thallus (“sub turf calcified algae”); “turf algae”: algae forming more then 5 cm high turf on the substrate, characterized by a more or less or non calcified thallus (“turf non calcified algae”). Invertebrates were grouped as “boring”, “massive”, “encrusting”, “small”, “unitary” suspension\filter feeders, and “colonial predators”(see for details appendix).

1.2.1. Univariate analyses

The analysis of variance (ANOVA) carried out on the number of taxa and on the cover within the plot in order to test the effects of treatments in time shows a significant interaction for the term Plot(T × E × G) ($F_{36, 648}$=5.35, P=0.006; $F_{36, 648}$= 2.59, P=0.007). This is a very common outcome possibly reflecting processes operating at small spatial scale (centimeters – meters). Significant differences were found also for the term T × E × G ($F_{8,36}$=2.3, P=0.0422; $F_{8,36}$=4.93, P=0.0002), suggesting that differences among treatments are not consistent in time. In other words, both the number of taxa and the cover by algae and invertebrates varies among different treatments and these differences vary not consistently in time. Conversely, for the number of functional groups, there is an effect of grazing changing with nutrient enrichment, consistently in time (E × G, $F_{1,36}$= 4.5, P= 0.0008). These patterns are graphically represented in Fig 1.2.1a.

Tab 1.2.1a Results of the ANOVA conducted on the number of taxa (a), total cover (b) and number of functional groups, *p<0.05, **p<0.01, ***p<0.001

	df	Number of Taxa		Cover		Number of functional groups	
Source		**MS**	***F***	**MS**	***F***	**MS**	***F***
Time = T	**8**	56.4316		112.7526	26.86	1.2389	4.09
Enrichment = E	**1**	119.2347		2.221	0.53	2.3277	7.68
Grazing = G	**1**	567.1125		590.227	140.61	17.0764	56.33 ***
T x E	**8**	24.6316		18.8294	4.49	0.3032	5.52 ***
T x G	**8**	59.0344	4.61***	15.9282	3.79**	0.5951	1.96
E x G	**1**	26.8347	2.1	12.632	3.01	1.3628	4.5 ***
T x E x G	**8**	29.4191	2.3*	20.6779	4.93 ***	0.3554	1.17
Plot (T x E x G) = P	**36**	12.8069	5.35 ***	4.1977	2.59 ***	0.5121	1.69
RES	**648**	2.3949		1.6226		0.055	
TOT	**719**						
Transformation		none		Sqrt(X+1)		Sqrt(X+1)	
Cochran's C		C = 0.0465 ns		C = 0.0598 **		C= 0.0438 ns	

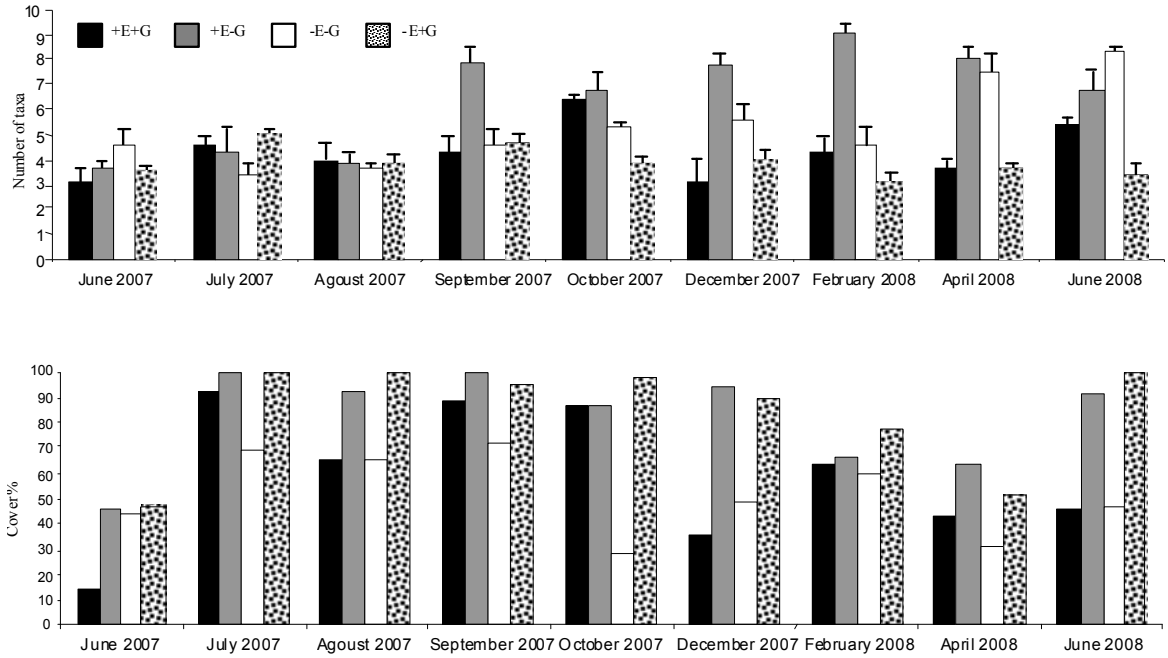

Fig.1.2.1a Mean of number of taxa and percentage cover in each combination of treatments in time. Black rectangle = Enrichment and Grazing, Grey rectangle = Enrichment no Grazing, White rectangle = no Enrichment no Grazing, White rectangle with black spot = controls.

As we can see in the representation above, the number of taxa in control plots (white rectangle with black spots), where grazing pressure and nutrient availability are not modified, didn't change persisting at low value. Percentage of cover of the rocky surface are conversely high, but it is mostly due to encrusting algae and boring invertebrates, tipical of barren community.

1.2.2. Multivariate analyses

PERMANOVA (Table 1.2.2a) conducted to assess potential differences in the structure of assemblages subjected to different treatments, indicates significant differences between plots, P (T × F × G). As already stressed, this is possibly due to the large variability caused by small scale processes (from competition to substratum heterogeneity).

The interaction of the two factors E × G was found significant, suggesting that the effect of nutrient enrichment changes in presence or absence of grazers. The estimation of the components of variance showed that the effect of grazing has an overwhelming importance in determining the differences observed. The *a posteriori* pair wise comparisons on the interaction term E × G (Tab 1.2.2b) revealed that the

factor enrichment strongly changes on the basis of the presence of grazers. The results were graphical represented in the nMDS (Fig 1.2.2a) of plot centroids.

Tab 1.2.2a PERMANOVA based on the Bray-Curtis dissimilarities on not trasformed abundance data from 46 variables. Each test was based on 4999 permutations of appropriate units. The term used for the denominator mean square is given in the column MSdenom. The appropriate permutable units are indicated by the denominator mean square in each case and are shown in the final column (see Anderson and ter Braak 2003 for details).

Source	*df*	SS	MS	*F*	P	MSdenom
Time = T	8	3.94E+05	49194	8.7049	0.0002	P (T x E x G)
Enrichment = E	1	12098	12098	2.1408	0.0788	P (T x E x G)
Grazing = G	1	1.94E+05	1.94E+05	34.366	0.0002	P (T x E x G)
T x E	8	90267	11283	1.9966	0.0094	P (T x E x G)
T x G	8	1.24E+05	15466	2.7367	0.0002	P (T x E x G)
E x G	1	17470	17470	3.0914	0.022	P (T x E x G)
T x E x G	8	46972	5871.5	1.039	0.4212	P (T x E x G)
Plot (T x E x G) = P	36	2.03E+05	5651.3	6.4317	0.0002	RES
RES	648	5.69E+05	878.66			
TOT	719	1.65E+06				

Tab 1.2.2b Pair wise comparisons conducted for the term E x G for pairs of levels of factor E and than for factor G.

	t	P
Within level '+G' of factor 'Grazing'	3,5341	0,0002
Within level '+E' of factor 'Enrichment'	2,5441	0,0032

Fig 1.2.2a Non-metric multidimensional scaling ordinations (nMDS plots) on the basis of the Bray-Curtis dissimilarity measure of centroids of each plot.
Black triangles = enriched and grazed plots, grey triangles = enriched no grazed plots, circle = no enriched no grazed plots, star = control areas so no enriched, grazed plots.

The SIMPER test (Tab 1.2.2c) revealed that turf forming algae are the principle responsible of dissimilarity between plots enriched without the effect of grazing and plots where grazers were not manipulated.

Tab 1.2.2c Summary of SIMPER analysis on taxa contributing to percentage dissimilarities between each treatments

	Treatments comparisons					
	+E+G			+E-G		-E+G
Taxa	+E-G	-E+G	-E-G	-E+G	-E-G	-E-G
Turf forming algae	**27.94**	25.1	**29.9**	26.54	**29.8**	29.3
Cliona viridis	12.95	17	13.9	13.25	14	13.8
ECR (Encrusting Calcified Rhodophytes)		14.3		10.94		10.7

PERMANOVA conducted on functional groups (Tab 1.2.2d), put in evidence significant differences between treatments in time ($F_{8,36}$=1.92, P<0,05). *A posteriori* test on the term Time × Enrichment × Grazing (Tab 1.2.2e) revealed that differences after one, four and twelve months depend on the factor Enrichment, while after two, six and ten by the factor Grazing as shown in the nMDS of plot centroids. In particular at the end of the 2007 (nMDS c,d) the recolonization in enriched plots, where grazing pressure has been removed is evident. Similar pattern were observed in spring of 2008 (nMDS e). After one year of experiment (nMDS f) difference become again evident between plots grazed and non grazed.

Tab 1.2.2d PERMANOVA based on the Bray-Curtis dissimilarities (no transformation) of the multivariate data of all functional groups identified

Source	*df*	SS	MS	*F*	P	MSdenom
Time = T	8	2.90E+05	36254	9.7459	0.0002	A (T x E x G)
Enrichment = E	1	21118	21118	5.6769	0.0042	A (T x E x G)
Grazing = G	1	1.92E+05	1.92E+05	51.55	0.0002	A (T x E x G)
T x E	8	54287	6785.9	1.8242	0.0236	A (T x E x G)
T x G	8	1.09E+05	13669	3.6745	0.0002	A (T x E x G)
E x G	1	10105	10105	2.7163	0.0488	A (T x E x G)
T x E x G	8	57031	7128.8	1.9164	0.0192	A (T x E x G)
Area (T x E x G) = A	36	1.34E+05	3720	4.1692	0.0002	RES
RES	648	5.78E+05	892.25			
TOT	719	1.45E+06				

Tab 1.2.2e Summary of the *a posteriori* test on the PERMANOVA output. Here only the significant test P (p<0,01) are reported (Monte Carlo –MC- asymptotic P values were used given the small number of unique permutations). See values of time 1, 2, 4, 5, 7, 8 in the following nMDS.

Pair wise comparisons for the term 'TxExG'					
For pairs of levels of factor 'Enrichment'			For pairs of levels of factor 'Grazing'		
	t	P(MC)		t	P(MC)
Time 1. +G	3.6952	0.018	Time 1. - E	3.807	0.0189
			Time 2. + E	3.1485	0.0226
			Time 2. - E	2.6113	0.0278
Time 4. + G	5.3635	0.0014	Time 4. - E	8.9327	0.0004
			Time 5. + E	4.2659	0.002
			Time 5. - E	2.4621	0.0465
			Time 7. + E	3.6692	0.0034
			Time 7. - E	2.8999	0.0116
Time 8. -G	2.5867	0.026	Time 8. + E	9.2454	0.0006
			Time 8. - E	4.1731	0.0118

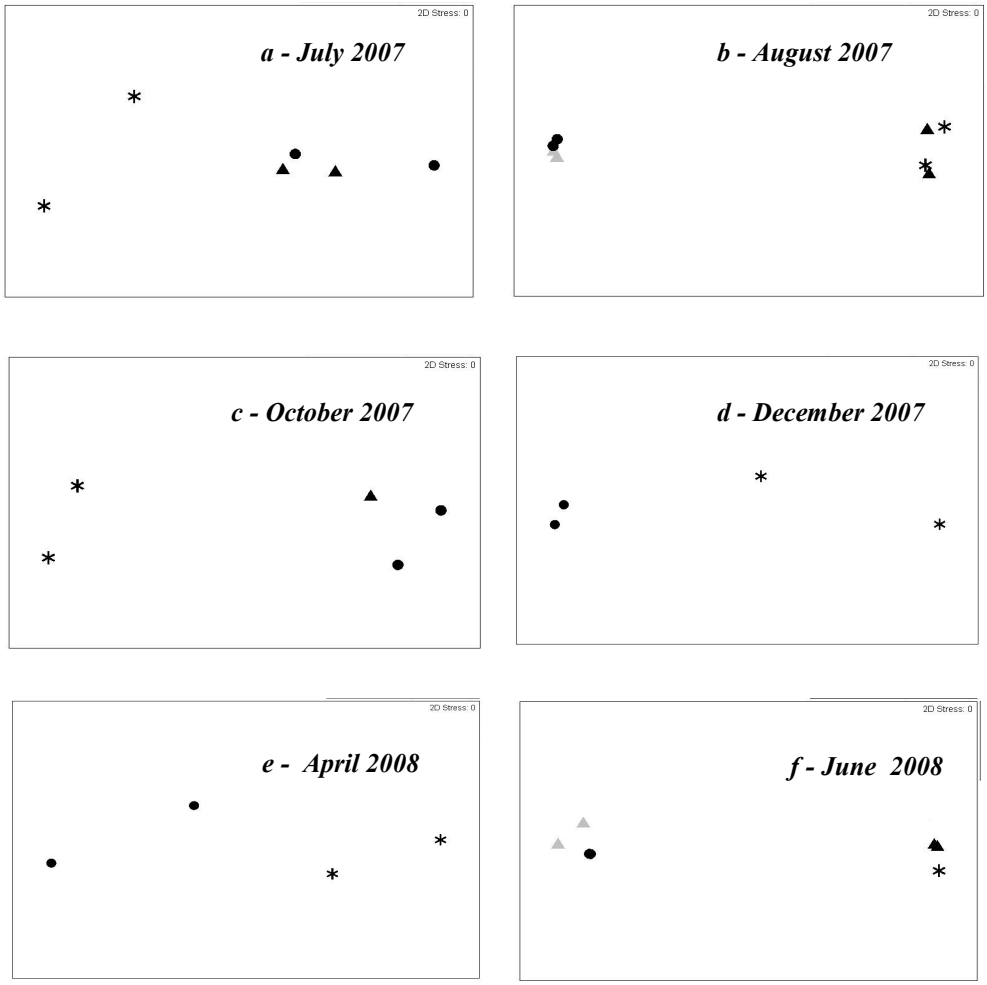

Fig 1.2.2b Non-metric multidimensional scaling ordinations (nMDS plots) on the basis of the Bray-Curtis dissimilarity measure of centroids of each plot. a = Time 1, b= Time 2, c = Time 4, d= Time 5, e = Time 7, f = Time 8. Black triangles = enriched and grazed areas, grey triangles = enriched no grazed areas, circle = no enriched no grazed areas, star = control areas, grazed areas.

The principal groups responsible of major changes revealed by the dissimilarity percentage in the SIMPER analysis (Tab 1.2.2f), are compared across treatments in Fig. 1.2.2 c. The figure reports results from sampling times that the analyses revealed informative in showing recolonization pattern of the benthic assemblages. For the graphical representation of cover, also the percentage of the variable bare rock (BR) was considered.

Tab 1.2.2f Summary of SIMPER analysis on taxa contributing to percentage dissimilarities between each treatments. STEA = Sub Turf Ephemeral Algae, BSFF = Boring Suspension/Filter Feeders, ECA = Encrusting Calcified Algae, MA = Mucillagenous Algae, TnCA = Turf non Calcified Algae, TCA = Turf Calcified Algae

TIME 1

	+E+G		-E+G
Functional group	-E+G	-E-G	-E-G
STEA	59.88	47.58	57.77
BSFF	20.7	21.93	18.71
ECA	17.06	20.33	17.39

TIME 2

		+E+G		+E-G		-E+G
Functional group	+E-G	-E+G	-E-G	-E+G	-E-G	-E-G
STEA	45.35	45.74	39.68	45.28	38.97	39.68
BSFF	18.03	28.74	18.16	18.04	26.01	17.77
MA	17.55		24.44	17.28	26.8	24.31
ECA	15.05	23.05	13.58	16.43		15.15

TIME 4

	+E+G		-E+G
Functional group	-E+G	-E-G	-E-G
STEA	68.95	58.31	79.17
BSFF	16.1	17.55	11.45
ECA		10.59	

TIME 5

		+E+G		+E-G	
Functional group	+E-G	-E+G	-E+G	-E+G	-E-G
STEA	62.71	36.19	68.01	59.15	46.11
BSFF	16.55	42.69	19.45	19.33	22.61
ECA	7.25	18.9			10.7
TnCA	6.82				

TIME 7

		+E+G		+E-G		-E+G
Functional group	+E-G	-E+G	-E-G	-E+G	-E-G	-E-G
ECA	23.69	50.81	30.2	15.52	19.11	19.24
BSFF	19.5	45.02	29.63	19.49	25.18	31.59
TnCA	18.93			21.62	16.59	10.91
STEA	18.54		15.89	21.15	16.13	19.31
TCA	14.61				15.67	

TIME 8						
Functional group	+E-G	+E+G -E+G	-E-G	+E-G -E+G	-E-G	-E+G -E-G
STEA	54.38		60.3	53.64	34.83	60.88
ECA	19.44	47.6	19.19	14.73		14.45
TCA	11.56			11.53	18.15	
BSFF	10.11	47.35	9.8	15.72	28.05	13.92
STA						4.64

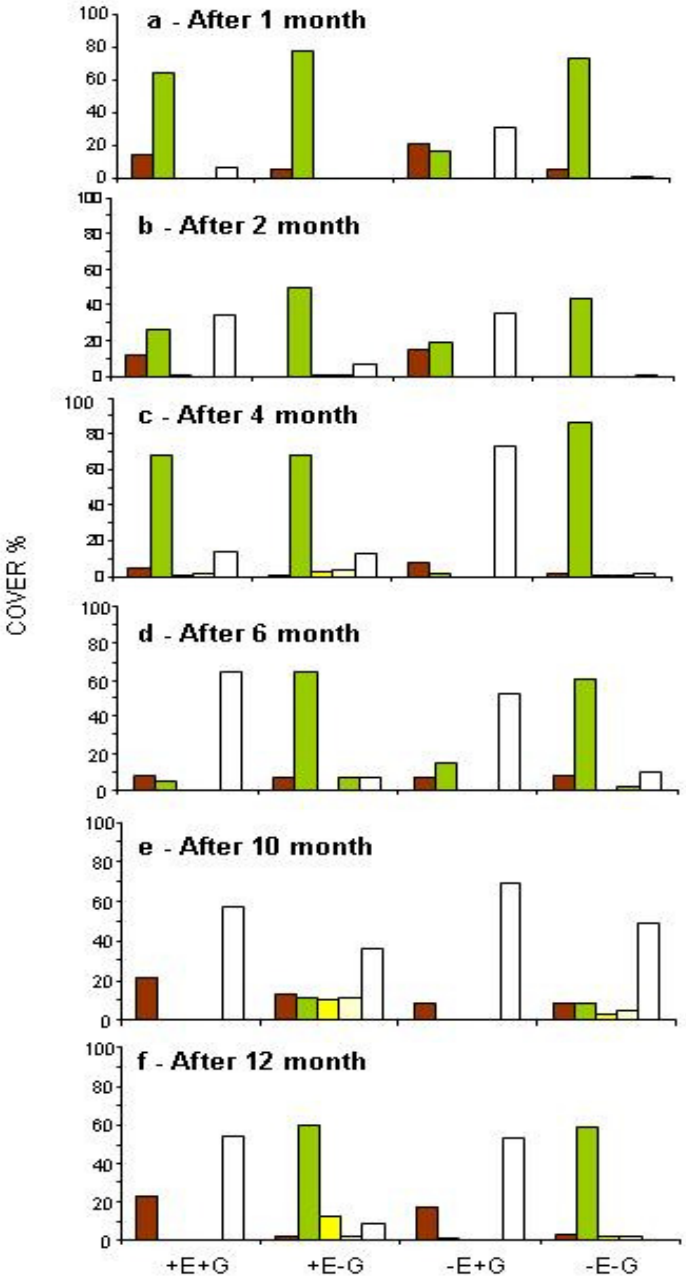

Fig 1.2.4. Mean coverage of principle functional group across treatment and time. Green rectangle= STEA, Brown rectangle = ECA, Yellows rectangle = TCA, Pink rectangle = TnCA, White rectangle = Bared rock.

One month after the beginning of the experiment, in plots with nutrient enrichment and without grazing pressure, ephemeral algae (STEA Sub Turf Ephemeral Algae) covered almost completely the total rocky surface, reducing the percentage of bare rock. This pattern persists for four months, when grazers drastically reduce the coverage of turf forming species, where nutrients availability was not modified. During the spring of the 2008 cover of turf is reduced to very low values. Turf calcified algae (TCA like *Padina pavonica*) and turf non calcified algae (TnCA) like *Dyctiota dichotoma* and *Laurencia* spp. become dominant. These are species typically characterizing initial colonization patterns of macroalgal community of rocky habitats.