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Research Article

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Subtidal littering: Indirect effects on soft substratum macrofauna?

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Abstract

Changes in macrofauna community structure, abundance and species richness were examined both before and one year after the deployment of plastic and glass bottles at littered (litter density: 16 items / 100 m^2) and non-littered (control) surfaces at three unimpacted coastal areas of the western Saronikos Gulf (Greece). In parallel, LOI% at the adjacent sediments and changes in the composition of feeding types of the megaepifauna that colonized the litter were examined across treatments. Significant changes in macrofauna community structure were demonstrated between before and after littering. At only one of the sites was there detected a significant difference in macrofauna community structure between control and littered plots after littering. This difference was linked with a significant increase in the abundance of opportunistic polychaete species and LOI% levels in the sediment surface due to the entrapment of macrophytal debris within the littered surface. The study did not show a consistent direct response of macroinfauna community to litter and the associated megafauna. Unlike the megafauna attracted by litter items, soft-substratum macrofauna is less responsive to the addition of novel hard substrates in adjacent sediments. Alternatively, it could be that the impact of littering with small items triggers a macrofauna response detectable in the long-run.

Keywords: Macrofauna; Community structure; Marine litter; Coastal zone; Saronikos Gulf; Mediterranean Sea.

Introduction

Marine litter, also known as marine debris, is any man-made, solid waste material that enters the marine environment, along shorelines, coastal waters, estuaries and oceans throughout the world. Marine litter originates either from land sources, e.g. beach users, municipal landfills located near the coast, sewage treatment and port installations, land run-off, or from maritime sources, e.g. ships or offshore installations (COE & ROGERS, 1997). Objects ranging from household and industrial containers, packaging material, medical wastes and discarded fishing line all qualify as marine debris. The main types of marine litter impacts include potential health risks to inhabitants of coastal areas, economic losses to fisheries and tourism (NASH, 1992; WILLIAMS *et al.,* 1993), substantial damage to coral reefs (DONOHUE *et al.,* 2001; CHIAPPONE *et al.,* 2005) and reduced fitness and increased mortality to vertebrate pelagic wildlife through entanglement and intestinal tract blockage when ingested (LAIST, 1997). Special attention is also required to the potential threat posed by the dispersal of alien species encrusted or attached on freely drifting debris in receiving environments (WINSTON *et al.,* 1997; BARNES, 2002). Finally, there is rising concern about the impacts on benthic ecosystems as the seafloor from the intertidal and shallow sublittoral to outer shelf, slope and abyssal depths has been identified as an important sink for marine debris (GOLDBERG, 1997; BACKHURST & COLE, 2000).

Plastic litter makes up to 80 % of seabed debris, followed by metal and glass (RYAN & MOLONEY, 1990; GABRIELIDES *et al.,* 1991; KANEHIRO *et al.,* 1995; STEFATOS *et al.,* 1999; DERRAIK, 2002). The accumulation of plastic litter on the seafloor can inhibit ventilation of the sediments thus resulting in hypoxia or anoxia (GOLDBERG, 1997, UNEPUTTY & EVANS, 1997). Benthic plastic debris may also provide solid attachment for species that would not usually occur there (RYAN & MOLONEY, 1990; MINCHIN, 1996), thus acting as novel hard substrata, often in areas that are otherwise sandy or muddy (CHAPMAN & CLYNICK, 2006). Indeed, waste material, such as tyres and car bodies, has been used to build artificial reefs, often specifically to attract fish and turn a 'sand desert' to a 'rich in habitats' benthic environment (DAVIS *et al.,* 1982; BOHNSACK & SUTHERLAND, 1985; GROSSMAN *et al.,* 1997; SVANE & PETERSEN, 2001). Therefore, the impacts caused by large marine litter items are expected to bear great similarities to other man-made structures placed at the bottom of the sea, including artificial reefs. These impacts include alterations of wave field and current patterns, thus causing scour and changes in sediment grain size and texture (MIZUTANI *et al.,* 2000; GUIRAL *et al.,* 1995) and entrapment of algae and other organic material. These, along with the activities and deaths of reef-associated organisms, can result in organic enrichment (WILDING, 2006) and modification of granulometry caused by the introduction of shell fragments that derive from fouling biota (DAVIS *et al.,* 1982; AMBROSE & ANDERSON, 1990; BAROS *et al.,* 2001). Finally, predators attracted by the structures may forage on plants and animals that live in adjacent sediments (DAVIS *et al.,* 1982; BAROS *et al.,* 2001, FABI *et al.,* 2002).

Smaller items such as plastic, metal and glass containers comprise a large part of benthic litter near urban centres (STEFATOS *et al.,* 1999; BLACKHURST & COLE, 2000; MOORE & ALLEN, 2000). There is little documented information about the potential use of this form of waste as a habitat for coastal communities and its potential impact on adjacent soft bottom communities (CHAPMAN & CLYNICK, 2006). In a recent manipulative study on the effect of benthic litter on biological communities in unimpacted shallow soft bottom areas in the Saronikos Gulf (Eastern Mediterranean), KATSANEVAKIS *et al.* (2007) demonstrated an enhancement in the abundance and diversity of megafauna attached to discarded bottles, either because the litter provided refuge or reproduction sites for mobile species or because hard-substratum sessile species had the opportunity to settle on the provided substrata. In parallel to megafauna, macrofauna abundance and species composition were collected from the adjacent sediments. The aim of the present study was to investigate the effect of littering on benthic macrofauna in these recently formed small litter reefs and explore several litter-associated causes of impact, including changes in organic matter content of adjacent sediments as well as changes in the presence of epifaunal predators.

Material and Methods

The study was conducted from June 2005 to June 2006 in the Saronikos Gulf, an area of 2600 km^2 and a maximum depth 450 m that receives in its northeast part the urban effluents of Athens, untreated until 1995. Surface water temperatures range from 11°C in the winter up to 30°C in the summer. The circulation of water masses within the gulf have been reported to depend strongly on the local wind while the prevailing currents are from the northeast to the southwest, continuing anticyclonically in the deeper layer of the west sub-basin (NCMR, 2001).

The experiment took place at three coves, unimpacted in terms of littering and organic pollution, in the western Saronikos Gulf, i.e. Amoni (A), Frangolimano (F), and Lychnari (L) (Fig. 1). The study sites were away from urban centres and were characterized by bare sandy substrates, varying from medium to very fine sand (KATSANEVAKIS *et al.,* 2007). At each cove, two 100 m^2 plots (10 m X 10 m), 50 m apart were defined on the seafloor with nylon line, at similar depths (16-20 m). On one of the surfaces, 16 items of litter comprising 12 plastic bottles and 4 glass jars were placed uniformly after the first sampling, while the other surface remained 'clean' (i.e. control). Detailed information

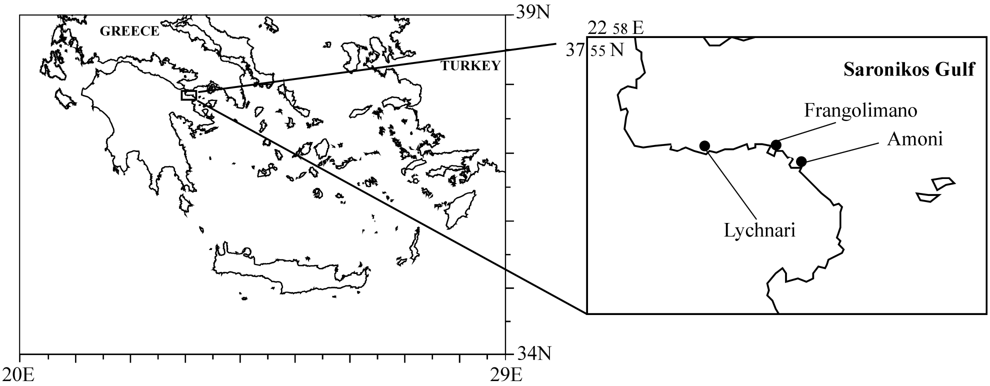

Fig. 1: Map of the experimental sites.

on the sampling sites and the sampling design is given by KATSANEVAKIS *et al.* (2007).

At each site, the sampling design included two factors: (a) 'Plot', fixed with two levels: littered plots containing litter and control plots (denoted with C) devoid of litter and (b) 'Time' of sampling, fixed and orthogonal to Plot with two levels: before the deployment of litter items in June 2005, hereafter denoted with 1, and after the deployment of litter items in June 2006, hereafter denoted with 2. The time-lag of a year was proved by in situ visual observations to be appropriate for the establishment of a litter-associated megafauna. Five replicate diver-held undisturbed cores (internal diameter: 10 cm, penetration depth: 20 cm, core sampling area: 78.5 cm^2) were taken within each plot at each site to assess macrofauna community changes between before and after littering, and between control and littered surfaces at each site. In conjunction with macrofauna sampling, five undisturbed sediment cores were taken for the estimation of organic matter content. Samples were taken at random within the plots.

Organic matter content of the sediment was determined as loss on ignition (LOI), i.e. the difference between the dry weight (60˚C, 24h) of the sediment and the residue left after combustion at 450˚C for 3h (PARKER, 1983).

The sediment from each replicate was sieved through a 0.5 mm mesh and the residue was immediately fixed in 4% formaldehyde. The macrofauna organisms retained were sorted, stored in 70% ethanol and then enumerated and identified to species level. The species from all major taxa were classified to functional groups according to their food acquisition mode, be it surface (S) or subsurface (B) deposit feeders, suspension feeders (F), omnivorous (O) and carnivorous (C) feeders. The information used in this classification was based on the ecological literature for families, genera or species (WOODIN, 1976; FAUCHALD & JUMARS, 1979; RUPPERT *et al.,* 2004; KAMERMANS, 1994; LEVINTON, 1982) as well as in specific systematic keys for the Mediterranean Sea. The species were also classified in three groups with respect to their time of appearance in the succession process, temporal and spatial persistence as well as rates of population growth and decline, following RHOADS *et al.* (1978) i.e (a) Group 1 or first order opportunistic species, (b) Group 2 or second order opportunistic species and (c) Group 3 or equilibrium species. Additional information for the present classification was taken from the review by SIMBOURA & ZENETOS (2002) assuming that similar species are eliminated, or significantly reduced, following any major environmental disturbance.

Megafauna were censused by divers before and after littering at the littered and the control plots (KATSANEVAKIS *et al.,* 2007). In the present study, the species censused by KATSANEVAKIS *et al.* (2007), were classified to major taxa, i.e. fish, bivalves, gastropods, crustacea, cephalopods and miscellanea, including bryozoans, tunicates, sponges and cnidarians and feeding and motility types. Feeding types included predators, suspension and deposit feeders whereas motility types comprised partially motile species, i.e. those restricted within a plot, highly motile – schooling species, i.e. capable of moving further away from the plot, sedentary and cryptic species. The information given for feeding and motility classification was taken by WHITEHEAD *et al.* (1986) for fish fauna and by RUPPERT *et al.* (2004) for invertebrate megafauna.

All analyses were completed at the level of replicate unit (n=5) at each station. Macrofauna species diversity for each sample and the associated evenness component J' were calculated applying the (log2) Shannon-Wiener diversity index (H') (SHANNON & WEAVER, 1963). Total community variables, i.e. abundance / 100 cm^2 and numbers of species per corer and LOI, were compared according to the two-factor sampling design using ANOVA, preceded by Cochran's test for homogeneity of variances and followed by a posteriori Student–Newman–Keuls (SNK) tests. Abundance and species numbers per macrofauna major taxon were also tested by ANOVA. The Pearson product moment correlation coefficients were also calculated between abundance and species numbers, and the LOI levels.

The response of the macrofauna community to the two-factor mensurative sampling design was examined using permutational analysis of variance (PERMANOVA, ANDERSON, 2001) followed by analysis of multivariate dispersion to test for homogeneity of dispersions among plots and times (PERMDISP, ANDERSON, 2006). In the present factorial design, the evidence for an impact (littering effect) at each site appears as a significant 'time' by 'plot' interaction (GREEN, 1979). The tests were based on 9999 unrestricted random permutations of the raw data.

Non-metric multidimensional scaling (MDS, KRUSKAL & WISH, 1978) was carried out to visualize multivariate patterns in macrofauna species data among treatments at each site. The effect of the species on the observed ordination of treatments was visualized with projection biplots in which the vectors represent the Pearson correlation relationship between macrofauna species and the MDS axes. All multivariate analyses were obtained using Primer 6 for Windows (CLARKE & GORLEY, 2006) and Permanova+ for Primer (ANDERSON & GORLEY, 2007) and used Bray-Curtis dissimilarities that were calculated between all pairs of range-standardized observations.

Results

Average LOI varied from 2.29 (1AC) to 4.82% (1LC) in spring 2005 and from 3.2 (2AC) to 6.67% (2L) in spring 2006. Significant interactions between Plot and Time were only detected at L site ($F_{1, 16}$ = 40.3) where LOI% levels at plot 2L were higher that at plots 1L, 1LC and 2LC. Seagrass leaf detritus derived from *Posidonia oceanica* or *Cymodocea nodosa* patches at site L, was observed during visual censing one year after littering only within the littered plot.

A total of 248 macrofauna species was recorded from the three sites, 167 were present at site A, 139 at site F, and 159 at site L. Overall, 29% of the species found in this study were common between site A and F and F and L whereas sites A and L shared 24% of the species. 67% of the species were different between before and after littering while 41% of the species were common between littered and control plots. At each site and plot 15 to 20% of species were represented by 1 individual. The average values of total abundance, species richness and diversity indices at each site and plot are given in Table 1. The two most dominant species comprised more than 20% of total abundance at all treatments. A significant Plot

Table 1

Average percentage contribution to total abundance of the species that were important in describing differences and similarities among sites, plots and sampling times. Functional classification is given. Trophic mode (T): S = surface deposit feeder; B = subsurface deposit feeder; F = suspension feeder; O = omnivorous; C = carnivorous. Colonization pattern (sensu Rhoads et al., 1978): Group 1 (G1) = first order opportunists; Group 2 (G2) = second order opportunists; Group 3 (G3) = equilibrium species. A: Amoni, F: Frangolimano, L: Lychnari.

	Functional classification	June 2005 Littered			Control				June 2006 Littered			Control			
Species	**T CP**	**1A**	**1F**	**1L**	**1AC**	**1FC**	**1LC**		**2A**	**2F**	**2L**	**2AC**	**2FC**	**2LC**	
Loripes lacteus	S G2or 3 25	14	9	12	14	4		16	16	1	34	21	<1		
Eunice vittata	O G2 or 3 9	12	22	10	15	18		2	7	5	1	9	6		
Aricidea cerrutii	B G3	9	5	2	9	5	3		5	3	7	5	4	7	
Chone duneri	F G3	6	12	2	2	6	1		5	1	<1	1	4	1	
Protodorvillea kefersteini	O G2	5	1	2	4	2	1		3	6	13	5	5	4	
Tellina cf *compressa*	S G3		4	< 1	2	2	1	2		-	<1	<1	2	1	1
Aricidea catherinae	B G3	2	0	1	1	1	3		2	1	1	1	2	2	
Micronephthys mariae	O G2 or 3 2	1	1	5	3	1		7	7	2	6	6	2		
Pseudoleiocapitella fauveli	B G2	2	4	3	1	4	1		1	3	4	<1	1	4	
Lumbrineris gracilis	O G3	2	3	2	3	5	8		3	2	2	4	3	4	
Marphysa belli	O G3	1	5	2	0	1	1		1	<1	<1	<1	0	3	
Paradoneis lyra	S G 1 or 2 1	5	5	6	7	5		9	14	10	7	4	4		
Aricidea capensis bansei	B G3		1	1	1	2	4	1		4	9	7	5	3	<1
Sigambra tentaculata	C G3	< 1	3	1	1	1	3		1	3	7	2	5	5	
Mastobranchus trinchesi	B G2		-	2	1	3	2	1		<1	<1	1.1	1	0.7	0.5
Prionospio ehlersi	S G2		-	2	1	2	5	-		<1	<1	0.9	<1	0.5	1
Aonides oxycephala	S G2 or 3 -	-	1	-	-	2		-	-	0.6	-	-	1.5		

(continued)

Table 1 (continued)

Species	Functional classification T	CP	June 2005 Littered 1A	1F	1L	Control 1AC	1FC	1LC	June 2006 Littered 2A	2F	2L	Control 2AC	2FC	LC
Amphitritides kuehlemanni	S	G3	-	-	5	-	-	1	-	-	<1	-	-	<1
Microdeutopus gryllotalpa	F	G2	-	1	2	-	-	-	1	1	5	<1	1	8
Apseudes latreilli	S	G2	-	< 1	1	-	-	<1	1	<1	6	-	1	16
Total abundance / 100 cm^2			113	112	113	127	150	88	124	134	227	130	108	102
Number of species per core			27	30	33	32	27	33	36	29	39	32	31	31
Shanon-Wiener H' (log2)			3.8	4.1	4.3	4.4	4.1	4.5	4.5	4	4.3	4	4.2	4.4
Pielou eveness J'			0.8	0.8	0.9	0.9	0.9	0.9	0.9	0.8	0.8	0.8	0.9	0.9

X Time interaction was detected at site L ($F_{1, 16}$ = 10.56, $p < 0.05$). SNK tests revealed that abundance at plot 2L was significantly higher that at plots 1L and 2LC. No significant Plot X Time interaction was found at any of the sites with respect to species richness. Overall, abundance and species diversity were correlated ($r = 0.59$, $p < 0.05$) although strong correlations ($r > 0.75$, $p < 0.05$) were only detected at site L in both sampling times and at all sites after the deployment of bottles at the littered plots.

The contribution of macrofauna major taxa to total abundance and species numbers is shown in Figure 2. Polychaetes contributed to total abundance(61 - 84%) and species richness (66 – 79%) to a high degree, followed by Molluscs, which accounted for 2 (2L) to 42% (2AC) of total abundance and 6 to 16% of total numbers of species. Numbers of Crustacean individuals comprised less than 8% at all sites except for site L whereas Crustacean species contributed from 13 to 16%. Echinoderms, exclusively represented by *Amphiura chiajei*, and the group of miscellanea taxa generally accounted for less than 0.6 and 3% of numbers of individuals, respectively. Significant Plot X Time interactions were detected at site L for Polychaete abundance ($F_{1, 16}$ = 19.12, p<0.05, SNK tests: 2L>2LC and 2L>1L) and for Molluscan abundance ($F_{1, 16}$ = 5.12, p<0.05, SNK tests: 1L>1LC and 1L>2L). Polychaete, crustacean and miscellanea abundances were highly correlated to their corresponding species numbers ($0.71 < r < 0.77$, $p < 0.05$).

The species *Loripes lacteus* and *Eunice vittata* accounted for 20 to 30% of the fauna at all sites and plots before littering, except for plot 1LC where *L. lacteus* was replaced by *Lumbrineris gracilis* (Table 1).

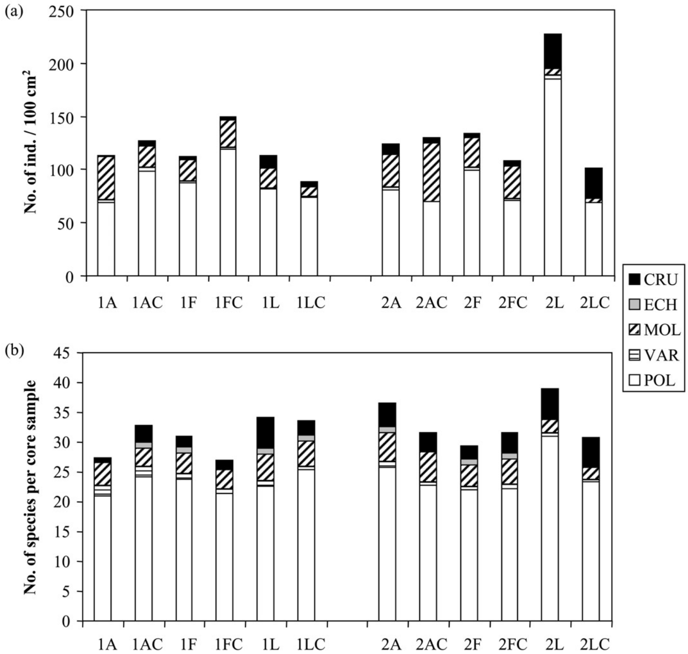

Fig. 2: Contribution of macroinfauna major taxa to (a) total abundance /100 cm^{-2} and (b) total species richness per core sample at littered plots (1A, 1F, 1L) and control (1AC, 1FC, 1LC) plots in June 2005 and at littered (2A, 2F, 2L) and control (2AC, 2FC, 2LC) plots in June 2006.

After littering, *Paradoneis lyra* and *L. lacteus* contributed from 25 to 30 % to total abundance at sites A and F whereas *Protodorvillea kefersteini* and *P. lyra* at plot 2L and *Apseudes latreilli* and *Microdeutopus gryllotalpa* at plot 2LC comprised 24% of total abundance (Table 1). As shown in Table 1, despite the change in the rank of dominance between sampling times and plots, the majority of the most abundant species exhibited ubiquitous distribution along the study area, with considerable densities even when their percentages to total abundance was low. Among the species that were abundant at all sites, times and plots are *Prionospio ehlersi, Mastobranchus trinchesi, Aricidea catherinae* and *L. gracilis.*

A significant relationship ($0.4 < r < 0.5$, $p < 0.05$) between abundance and densities of dominant species such as *P. kefersteini, P. lyra, Pseudoleiocapitella fauveli* and *Aricidea cerrutii* and LOI% was detected at site L. This is in accordance with the observation that significantly higher levels of LOI% at plot 2L coincided with highest abundance (Fig. 3) and high dominance of species known to display a second order opportunistic pattern of colonization (Table 1).

The difference in the abundance of litter-associated megafauna major taxa, feeding and motility types between before and one year after littering is given in Table 2. The greatest changes were exhibited by miscellanea, mostly sedentary and filter feeding megafauna. On the other hand, fish and Crustacea, mostly predatory

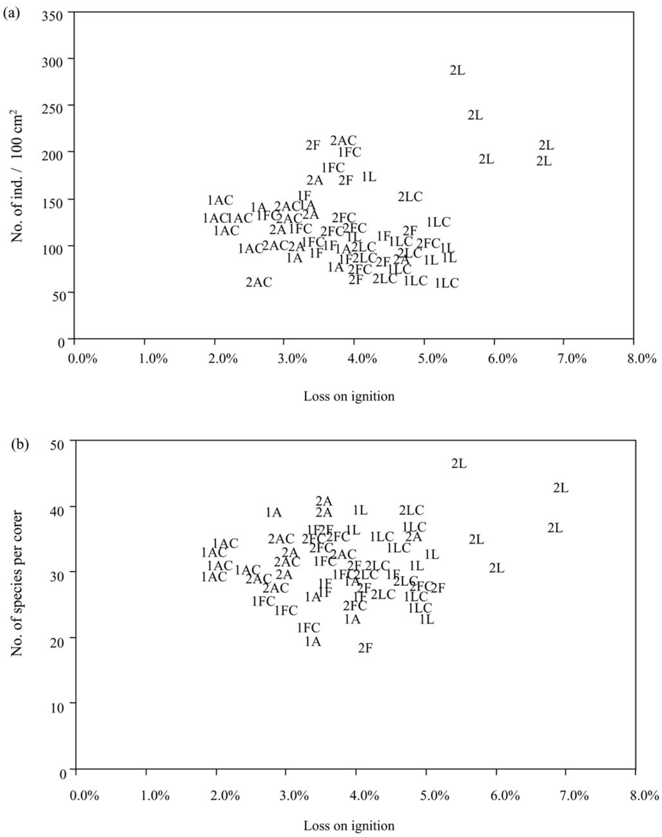

Fig. 3: Relationship of (a) total number of individuals /100 cm^{-2} and (b) total number of species per core sample with LOI% at littered plots (1A, 1F, 1L) and control (1AC, 1FC, 1LC) plots in June 2005 and at littered (2A, 2F, 2L) and control (2AC, 2FC, 2LC) plots in June 2006.

and cryptic, increased after littering at sites A and F, although not exclusively at littered plots. At site L there was a substantial increase in crustaceans and gastropods, mainly represented by predatory, cryptic and partially motile species. Summarising, predatory and partially motile gastropods and crustaceans mainly increased at site L whereas fish and cryptic fauna mainly increased at sites A and F.

Permanova detected significant Plot X Time interaction only at site L (Table 3). Permdisp did not reveal any heterogeneity in multivariate dispersion among the treatments. However, when dispersions across times and plots were tested at each site, there was detected significantly higher dispersion before littering at site A and at the control plots at site F.

The MDS ordination plots of stations supported the results of permanova by revealing a great overlap in macrofauna community structure among treatments at each site A and F (Fig. 4). At site L there was observed an effect of littering on macrofauna community, with plot 2L replicates clustering separately from plot 2LC, in agreement with the Permanova

Table 2
Differences in the abundance (ind. /100 m^2) of megafauna organisms between before and twelve months after the start-up of the manipulative experiment at littered and control plots at all sites. A, F and L: Amoni, Frangolimano and Lychnari littered plots in June 2006. AC, FC and LC: Amoni, Frangolimano and Lychnari control plots in June 2006.

(a) Taxonomic classification

Station	Bivalves	Cephalopods	Crustacea	Fish	Gastropods	Misc
A	7	0	3	23	2	217
F	1	-1	4	48	1	225
L	1	0	40	-6	112	229
AC	0	0	0	36	0	37
FC	1	0	0	3	0	2
LC	1	-1	-2	-1	-1	10

(b) Feeding type

Station	Predatory	Suspension feeders	Deposit feeders
A	25	224	3
F	47	226	4
L	106	229	41
AC	36	37	0
FC	3	3	0
LC	3	12	-2

(c) Motility pattern

Station	Partially motile	Highly motile	Sedentary	Cryptic
A	12	-2	217	25
F	5	0	226	47
L	153	1	228	-6
AC	0	0	37	36
FC	1	-1	2	4
LC	-3	-1	11	-1

tests. The biplots of Figure 4 clearly show that only at L there is a consistent association of group 2 colonizers such as *P. lyra* and *P. kefersteini* with the 2L plot.

Discussion

Extensive ecosystem monitoring studies have been undertaken in the Saronikos Gulf, focusing on the examination of the impact by the Athens metropolitan area before and after the operation of the wastewater Treatment Plant (NCMR, 1999; 2001). The western Saronikos Gulf is less affected by land-based polluting activities and it is generally characterized by deeper waters, steeper slopes and an oligotrophic euphotic zone (SCOULLOS *et al.,* 2007). However, the zoobenthic assemblages in the lower circalittoral zone of this part of the gulf are indicative of moderate pollution due to the increased

Table 3
Testing the effect of plots and sampling time and their interactions on macrofauna communities at the three study sites using permutational analysis of variance (permanova) and dispersion (permdisp). df: degrees of freedom, perm: permutation, ns: non significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. BRAY – CURTIS distance measure was used.

(a) PERMANOVA tests							
Source of variation	**df**	**Amoni Pseudo-F**	**P(perm)**	**Frangolimano Pseudo-F**	**P(perm)**	**Lychnari Pseudo-F**	**P(perm)**
Time (Ti)	1	3.334	**	4.304	***	7.904	***
Plot (Pl)	1	1.492	ns	0.976	ns	2.127	*
TixPl	1	1.812	ns	1.206	ns	2.257	*
Resisual	16						
Total	19						
(b) PERMDISP tests							
Group factor		**Amoni** $F_{1, 18}$	**P(perm)**	**Frangolimano** $F_{1, 18}$	**P(perm)**	**Lychnari** $F_{1, 18}$	**P(perm)**
Time (Ti)		7.777	*	0.784	ns	0.296	ns
Plot (Pl)		1.838	ns	10.345	**	0.002	ns

organic load and nutrients transported by the prevailing westward currents from the inner Saronikos Gulf (NCMR, 1999).

On the other hand, the sandy infralittoral benthic communities of the western part were examined for the first time during the course of this study. The species composition at the three study sites, A, F and L, indicated the co-existence of taxa found at a variety of infralittoral and circalittoral biocoenoses as described by PERES (1967). These biocoenoses include the 'coastal detritic' community (DC) and the communities of sands or muddy sands in shallow areas protected against wave action and currents (SRPV and SVMC, respectively), and other sublittoral communities described at unpolluted heterogeneous sediments in coastal areas of the western and central Mediterranean Sea (DRAGO & ALBERTELLI, 1978; SIMONINI *et al.,* 2004; COSENTINO & GIACOBBE, 2006). This resemblance is consistent with the observation that the sediments across the study area varied from fine to medium sands and remained highly heterogeneous, as shown by the poor sorting coefficient of grain size (KATSANEVAKIS *et al.,* 2007), thus allowing for a great variety of species, with no specific preference in grain size, to settle and establish sizeable populations. This finding is in agreement with the high temporal and spatial dispersion at sites A and F.

Marine litter densities on the sea floor of the Saronikos Gulf range from 0.4 to 25 items / 100 m^2, higher along the northeast coastline (KATSANEVAKIS & KATSAROU, 2004). This is the first study with respect to the impact of littering on the infauna in the Saronikos Gulf. The hypothesis of indirect impact of seafloor littering upon macrofauna community

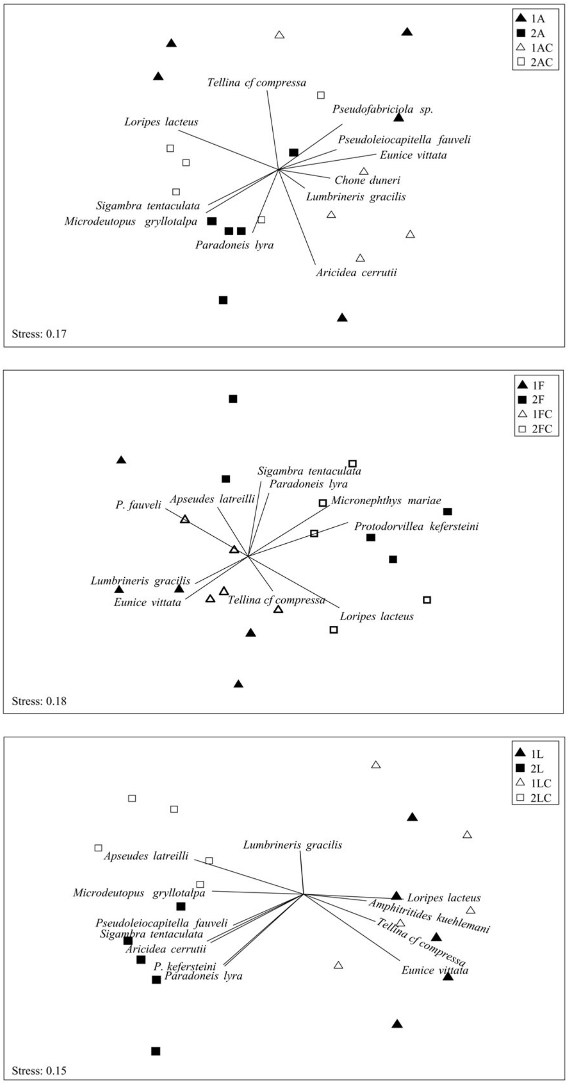

Fig. 4: Euclidean MDS biplot showing the Pearson correlation coefficients of macrofauna species densities with MDS axes at each site. Non-metric multidimensional scaling was applied on the range standardized densities of macrofauna species using BRAY-CURTIS similarities. At each biplot prefixes 1 and 2 for each sample code denote June 2005 and June 2006, respectively, while the suffix C denotes control plot. A: Amoni, F: Frangolimano, L: Lychnari.

structure due to attraction of predatory organisms, previously absent or at significantly lower densities, stated by KATSANEVAKIS *et al.* (2007), predicts that macroinfauna abundance and species composition will be substantially limited within the littered plots. By contrast, an increase from June 2005 to June 2006 by

106 individuals / 100 m^2 in predatory megafauna at site L coincided with a significant density increase in macrofauna, especially polychaetes and crustacea. These taxa are included in the prey of many infralittoral schooling and cryptic fish and decapods (CASTRIOTA *et al.,* 2005; RELLINI *et al.,* 2002) as well as of the predatory muricid gastropod Hexaplex trunculus (MORTON *et al.,* 2007), which was one of the most abundant predators at the littered plots (KATSANEVAKIS *et al.,* 2007). Conversely, there was observed a significant reduction of macrofaunal molluscs at this site, which may indicate that bivalves may have belonged among the preferred prey of the predatory colonizers of litter. However, molluscs simultaneously increased, not only at the corresponding control plot (2LC) but also at the littered and control plots at sites A and F, indicating that changes in the density of molluscs cannot be always associated with predators attracted by litter. Finally, the noteworthy filter feeding community that was established using the litter items as substrates is unlikely to have negatively interacted with macroinfauna in the adjacent sediments, even though a lot of soft-bottom species found, including those that dominated the infauna, follow pelagic larval development (RUPPERT *et al.,* 2004).

Regarding organic matter (OM) levels at the surface sediments of the study sites, these were elevated for sandy bottoms. OM values after littering were on average 1.2 times higher than before at both littered and clean surfaces. However, only at site L did these levels exceed 5% at the littered plots. As pointed out by PEARSON & ROSENBERG (1978) disturbances associated with OM enrichment and the resulting hypoxic conditions in the sediment leave carnivorous and generally predatory macrofauna species unaffected, while they affect tolerant subsurface and surface deposit feeders positively. Conversely, suspension feeders and species typical of later stages of succession in soft-bottom communities are gradually eliminated, thus resulting in low diversity and high dominance values. At the study area filter feeding, omnivory-carnivory and surface and subsurface deposit feeding were well represented among the most abundant species, implying high trophic complexity. Therefore, in terms of trophic structure, the macrofauna community in the study area resembled other sandy sublittoral communities of the Mediterranean Sea not directly influenced by eutrophication, exhibiting a wide range of trophic ethological habits (GAMBI & GIANGRANDE, 1985; CARDELL *et al.,* 1999; SIMONINI *et al.,* 2004).

However, species not affected by alterations and species known to take advantage under conditions of environmental stress by means of having a short life-span, rapid growth and many generations throughout the year, were also present. These belonged mainly to polychaetes and crustaceans, and exhibited, as expected, considerable variations. Despite the high dominance levels of the two most abundant species (10 to 25% before and 15 to 35% after littering), these comprised group 3 colonizers before littering. On the other hand, after littering the contribution and the ranking of the group 2 colonizers such as *P. kefersteini, A. latreilli, P. lyra* and *M. gryllotalpa* increased at the littered plots and especially at site L. Simultaneously, *L. lacteus,* a species typical of later stages of succession, remained the most dominant species at sites A and F while other bivalves that reach high abundances in disturbed sediments, such *Corbula gibba, Thyasira flexuosa, Mysella bidentata* had a very low density throughout the study. Although the group 2 colonizers were represented by elevated numbers at littered and control plots in both sampling times, their enhancement after littering at littered plots, especially at site L, raises questions as to whether this is an indication of stress triggered by littering.

Since organic matter levels at sites A and F were not indicative of enrichment but significantly higher LOI% levels at site L after littering coincided with dense macrophyte detrital cover and the predominance of second order opportunistic species, it is reasonable to assume that the macrofauna community was impacted by a reduction in sediment oxygenation caused by the entrapment and subsequent accumulation of seagrass detritus. Nevertheless, the accumulation started taking place only two months before the end of the manipulative experiment, i.e. June 2006, thus indicating that changes detected in this study could be the first stages of a gradual organic enrichment process. It is noteworthy that comparable LOI% values were recorded in the sediments adjacent to artificial reefs that trapped macroalgal phytodetritus at their periphery (WILDING, 2006). The accumulation of phytodetrital material for longer periods is expected to cause effects similar to that of localized organic enrichment (GRAY *et al.,* 2002). As the phytodetrital mats significantly reduce water movement over the sediment, the bottom water in the mats is often stagnant, resulting in a permanently anoxic sediment surface. It is possible that the marked increase of *P. kefersteini* at L littered plots, where there was a pronounced accumulation of seagrass debris, is an indication of the hypoxia caused by the slow decomposition rates of seagrass leaves. Apart from the nature of the deposited material, the extent of the impact of the accumulation of phytodetritus is proportional to a number of factors, including decreases in current velocity around the litter (WILDING, 2006). Given the small volume of the litter items in the present study, impacts related to changes of hydrodynamic regime around the marine litter may take longer than a year (i.e. the duration of the manipulative experiment of the present study) to be detectable. The entrapment of phytodetritus and subsequent changes in sediment oxygenation around artificial reefs is a side-effect of littering on macrofauna of adjacent sediments, as the entrapment of phytodetritus was facilitated by the deployment of litter and requires a longer monitoring for an adequate evaluation of the impact on soft-bottom macrozoobenthos.

It has been estimated that up to 70% of the marine litter that enters the sea ends up on the sea bed (UNEP, 2005). The studies indicating that discarded waste material, in the form of pieces of metal, glass, plastic, tyre and wood does provide usable habitat for subtidal invertebrates and fish either in areas where natural habitat is lost or even in non-polluted areas are gradually increasing (CHAPMAN & CLYNICK, 2006; KATSANEVAKIS *et al.,* 2007). The lack of significant differences between littered and control plots at sites A and F, preclude any generalization about the effect of littering on macrofauna. Logistic restrictions did not allow for several sampling times before and several sampling times after littering and adequate within site replication of plots, so as to estimate the range of natural fluctuations of macrofauna populations in the area and eliminate effects of short-term temporal variation. However, the present study demonstrated the increase of second order opportunistic macrofauna species adjacent to marine litter sediments in the presence of seagrasses trapped within the littered plots. Further manipulative studies, with extensive temporal replication at a variety of receiving environments with a variety of waste materials are required so as to evaluate the full array of impacts of marine litter at sublittoral areas.

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