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**Effect of marine litter on the benthic megafauna of coastal soft bottoms: a
manipulative field experiment**

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Abstract

The effect of litter on the abundance and community structure of soft-bottom epibenthic megafauna was investigated in three coves of the Saronikos Gulf (Aegean Sea). At each site, two surfaces were defined on the sea-bottom. One of the surfaces was uniformly littered with debris (16 items per 100 m²), while the other remained 'clean' and acted as control. Benthic megafauna was censused with SCUBA diving, once before the littering episode and then monthly for one year. Both total abundance and the number of species showed an increasing trend in the impacted surfaces, either because the litter provided refuge or reproduction sites for mobile species or because hard-substratum sessile species had the opportunity to settle on provided surfaces. A marked gradual deviation in the community structure of the impacted surface from the control and a clear successional pattern of change in the community composition of the impacted surfaces were demonstrated.

Keywords: benthic megafauna, coastal ecology, Greece, marine debris, litter, plastics

Introduction

Marine debris, defined as any manufactured or processed solid material that enters the marine environment, is a greatly underestimated component of marine pollution. Although there are various types of debris, plastics (synthetic organic polymers) make up most of the marine litter worldwide (Derraik, 2002). The threat of marine debris to the marine environment has been ignored for a long time and only in the last decades has it received serious attention. The entanglement of marine species, especially turtles (Carr, 1987; Mascarenhas et al., 2004), fish (Sazima et al., 2002), mammals (Shaughnessy, 1980; Beck and Barros, 1991; Arnould and Croxall, 1995; Boren et al., 2006) and birds (Arnould and Croxall, 1995) has been frequently described as a serious mortality factor. Marine species may ingest plastic particles (mainly plastic pellets used by the industry for plastic manufacturing), presumably mistaking them for prey. Ingestion of debris often has many harmful effects such as a worsening physical condition (Spear et al., 1995), diminished food stimulus (Ryan, 1988), blockage of gastric enzyme secretion, lowered steroid hormone levels, delayed ovulation and reproductive failure (Azzarello and van-Vleet, 1987), internal injury and death following blockage of the intestinal tract (Ryan, 1988; Beck and Barros, 1991). Polychlorinated biphenyls (PCB's) and other contaminants enter marine food chains (mainly through ingested plastics) with yet unknown, but potentially very negative effects (Ryan et al., 1988; Bjorndal et al., 1994). Drift debris can increase the distribution range of certain marine organisms and introduce species into an environment where they were previously absent (Winston, 1982; Barnes, 2002; Barnes and Milner, 2005). Barnes (2002) estimated that human litter more than doubles the rafting opportunities for biota, assisting the dispersal of 'alien' species. Derraik (2002) has recently reviewed the threats to the marine environment from plastic pollution and stated the need for further research into the topic.

Litter may concentrate on the seafloor reaching very high densities. In shallow coastal areas of Greece the density of marine debris ranged from 0 to 25.1 items per 100 m², with a mean density of 1.5 items per 100 m² (Katsanevakis & Katsarou, 2004). These high marine debris densities have a potential effect on the structure of benthic communities by altering the characteristics of the local biotope, but such an

investigation has not been conducted as yet. So far, investigations have focused mainly on the direct effects of marine debris on the physiology or mortality of certain species. In the present study, a field experiment was carried out to investigate the effect of marine pollution with litter on the abundance, biodiversity, and community structure of benthic megafauna in soft-bottom areas.

Materials & Methods

In each of three coves of the Saronikos Gulf (Amoni, Frangolimano, and Lychnari) (Fig. 1), two square 100-m² surfaces (10 m x 10 m), 50 m apart, were defined on the seafloor with nylon line, at similar depths (16 - 20 m) (Fig. 2). In all areas, the bottom was soft with no vegetation.

To characterize the sediment type in each surface, three 50-ml samples of the surface sediment (upper 5 cm) were taken from each transect. Particle size analysis and classification of the samples was carried out according to Buchanan (1984) and for each sample the median diameter $Md\phi$ and the quartile deviation $QD\phi$ were calculated, as measures of the central tendency and the degree of scatter of the granule size frequencies respectively.

On one of the surfaces at each site, 16 items of litter (12 plastic bottles and 4 glass jars) were placed uniformly (Fig. 2), while the other surface remained 'clean' and acted as a control area. We chose to use rather inert materials (and not e.g. metallic items that might be corroded and enter easier the trophic web), and mostly plastics as they dominate in the composition of marine debris (Katsanevakis & Katsarou, 2004). The experimental areas were not affected by waves or strong currents and thus the litter items were simply placed on the bottom and there was no need to fix them in some way. The litter items were all maintained in their position until the end of the experiment. Apart from the square outline, 5 strips (2 m x 10 m) were defined inside each square to facilitate the visual census of the surface (Fig. 2). The benthic megafauna was quantitatively censused, during SCUBA dives, on all surfaces, once before littering the surfaces (June 2005) and then monthly for one year, till June 2006. Species identification was done *in situ* whenever possible or else individuals were collected by hand or using small aquarium nets and identified in the laboratory; the number of collected individuals was kept minimum to avoid significant disturbance due to sampling.

The time-series of the difference in total abundance ($\Delta N = N_{\text{impact}} - N_{\text{control}}$) and total number of species ($\Delta S = S_{\text{impact}} - S_{\text{control}}$) between impacted and control surfaces were modeled based on the Information Theory approach (Burnham & Anderson, 2002).

According to the information theory approach, data analysis is assumed to be the integrated process of *a priori* specification of a set of candidate models (based on the science of the problem), model selection based on the principle of parsimony, and the estimation of parameters and their precision. The principle of parsimony implies the selection of a model with the smallest possible number of parameters for adequate representation of the data (a bias versus variance tradeoff) and is expressed by Akaike's Information Criterion or AIC (Akaike, 1973), summarized in the formula $AIC = -2\ln(\mathcal{L}(\hat{\theta} | \text{data})) + 2K$, where $\ln(\mathcal{L}(\hat{\theta} | \text{data}))$ is the numerical value of the log-likelihood (natural logarithm) at its maximum point and $\hat{\theta}$ is the vector of the model's estimated parameters. Furthermore, rather than estimating parameters from the 'best'

model only, parameters can be estimated from several or even all the models considered. This procedure is termed multi-model inference (MMI) and has several theoretical and practical advantages (Burnham and Anderson, 2002).

In this study, 7 models were fitted to ΔN and ΔS time-series (with non-linear least squares, using Marquardt's algorithm): the constant model (g_1), the linear model (g_2), the parabolic model (g_3), the exponential model (g_4), the von Bertalanffy growth model (g_5), the Gompertz growth model (g_6), and the logistic model (g_7) (Table 1). Model g_1 assumes that there is no temporal change in ΔN or ΔS , g_2 assumes a linear temporal trend, g_3 and g_4 assume a non-linear temporal trend with no asymptote that is described by a 2nd degree polynomial or an exponential curve respectively, while models g_5 , g_6 , and g_7 assume a non-linear temporal trend with an asymptotic value Y_∞ ($Y = \Delta N$ or ΔS).

The small-sample, bias-corrected form AIC_c (Hurvich and Tsai, 1989) of the AIC (Akaike, 1973; Burnham and Anderson, 2002) was used for model selection.

Specifically, $AIC_c = AIC + \frac{2k(k+1)}{n-k-1}$, where for least squares $AIC = n \ln(\hat{\sigma}^2) + 2k$,

$\hat{\sigma}^2 = \frac{RSS}{n}$, RSS is the residual sum of squares, n the number of observations, and k

is the total number of estimated regression parameters including σ^2 (i.e. k is the number of parameters in the model equation plus 1). Normally distributed deviations with constant variance were assumed. The model with the smallest AIC_c value ($AIC_{c,min}$) was selected as the 'best' among the models tested. The AIC_c differences $\Delta_i = AIC_{c,i} - AIC_{c,min}$ were computed over all candidate models g_i . According to Burnham and Anderson (2002), models with $\Delta_i > 10$ have essentially no support and might be omitted from further consideration, models with $\Delta_i < 2$ have substantial support, while there is considerably less support for models with $4 < \Delta_i < 7$. To quantify the plausibility of each model, given the data and the set of six models, the

'Akaike weight' w_i of each model was calculated, where $w_i = \frac{\exp(-0.5\Delta_i)}{\sum_{j=1}^6 \exp(-0.5\Delta_j)}$. The

'Akaike weight' was considered as the weight of evidence in favor of model i being the actual best model of the available set of models (Akaike, 1983; Buckland et al., 1997; Burnham and Anderson, 2002). Akaike weights may be interpreted as a posterior probability distribution over the model set. To obtain more robust inferences, the final results were based on model-averaging the response variable using Akaike weights, rather than simply on the 'best' model (Burnham & Anderson, 2002).

Non-metric multidimensional scaling (MDS) was also performed on benthic community data to investigate before/after impact differences in epibenthic megafauna at each site. Similarity matrices were based on Bray-Curtis similarity coefficients of square-root transformed data (Clarke and Warwick, 2001). PRIMER software (version 5.1.2) was used for MDS analysis.

Results

The sediment (Table 2) of the experimental surfaces can be characterized as sandy, varying from medium to very fine sand. On all surfaces the sediment was poorly sorted indicating low wave or current energy at all sites.

At all three sites both total abundance and number of species exhibited an increasing trend on the impacted surfaces (Table 3). The best model for ΔN on all three experimental surfaces was the logistic model (g_7). The second best model was the Gompertz equation (g_6), which was substantially supported by the Amoni data but considerably less supported than the logistic equation at the other two sites (Table 4). No other model had substantial support. The ‘no impact’ assumption (model g_1) had essentially no support by the data, and thus this hypothesis was falsified. The ‘average’ models for ΔN at the three sites were calculated based on Akaike weights (Fig. 3).

The best model for ΔS was g_2 (positive linear trend) for Amoni and Frangolimano, and Gompertz (as well as logistic) for Lychnari; other models also had substantial support (Table 5). As for ΔN , the ‘no impact’ assumption (model g_1) had essentially no support by the data. The ‘average’ models for ΔS at the three sites were calculated based on Akaike weights (Fig. 4).

Normal probability plots of the residuals of the average models gave fairly straight lines indicating no substantial deviation from normality. In addition, residuals showed no trend, curve or other systematic variation and there was no evident deviation from the constant variance assumption except from the ΔN time series in Amoni, where there were increased absolute residuals during the last two months.

In all three coves, MDS plots demonstrated a marked gradual deviation of the impacted surface from the control and a clear successional pattern of change in the community composition of the impacted surfaces (Fig. 5). The initial community structure (before impact) was quite similar (small distances between C_0 and S_0) but the dissimilarity gradually increased substantially (larger distances between C_{12} and S_{12}).

Species responded differently to marine pollution with litter (Fig. 6). Three main types of response were observed during the one-year period that followed littering:

(1) “Increasing positive effect”: The presence of litter items seemed to favor the abundance of some species, e.g. the gastropod *Hexaplex trunculus*, the hermit crab *Cestopagurus timidus*, the ascidian *Ciona intestinalis*, and the sponge *Chondrilla nucula* (Fig. 6a, b, c, d, respectively). The time lag between littering and the initiation of any increase in abundance varied among species, e.g. it was ~ 1 month for *C. timidus*, ~ 3 months for *C. intestinalis* and *H. trunculus*, and ~ 6 months for *C. nucula*. In some cases a plateau seemed to be reached, as in *C. intestinalis*, while in other cases no apparent limit of abundance increase was observed.

(2) “A positive event”: On the impacted surfaces, some species, such as the barnacle *Chthamalus stellatus* (Fig. 6e) and the fish *Serranus hepatus* (Fig. 6f), exhibited a temporary increase in abundance, which then gradually diminished.

(3) “No effect”: The presence of litter items had no effect on the abundance of some species, e.g. in *Alicia mirabilis* (Fig. 6g), an actinarian with high mobility in relation to other soft-bottom sea anemones, and *Gobius geniporus* (Fig. 6h), a benthic fish with low mobility that may use the same shelter for long periods of time.

Discussion

The marine debris density used in this study (16 items per 100 m²) was in the upper scale of the range of densities found in coastal areas of Greece (0 – 25.1 items per 100 m²; Katsanevakis and Katsarou, 2004). Much higher marine debris densities have been reported elsewhere. In five shallow coastal sites of the Caribbean island of

Curaçao, off public beaches, marine debris densities varied between 19.8 and 66.0 items per 100 m² with a mean of 36.5 items per 100 m² (Nagelkerken et al., 2001). In five shallow coastal sites of the Kawau island in northeastern New Zealand, marine debris items ranged between ~20 and ~400 items per 100 m² (Backhurst and Cole, 2000). In such areas with much higher marine debris concentration than that of the experimental surfaces of the present study, the effect of marine pollution with litter on the benthic megafauna would probably be much more intense.

Both the total abundance and the number of species showed an upward trend on the impacted surfaces (in relation to the control), mainly because of two reasons: 1) hard-substratum sessile species had the opportunity to colonize the litter surfaces, thus increasing both the number of species and total abundance, and 2) the litter provided refuge for mobile species (fishes, crustaceans, sea-urchins, octopuses etc.) either by direct use of their cavities or by digging in the sediment beneath them.

Among the species that exhibited an 'increasing positive effect', *H. trunculus* and *C. timidus* used the cavities of the litter items for refuge; 65% of *H. trunculus* and 61% of *C. timidus* recorded during this survey were found inside the cavities or in direct contact with litter items. Furthermore, *H. trunculus* used litter to attach large egg masses. During May and June 2006 (t = 11 and 12) many reproductive aggregations of the species were observed on impacted surfaces (enumerating up to 50 individuals each) and many egg masses were found attached on litter; that was the main reason for the sharp increase of *H. trunculus* abundance at that period (Fig. 6a). The sessile *C. intestinalis* and *C. nucula* used litter for attachment, the former exclusively on the litter cavities, while the latter both on internal and external surfaces. The plateau reached in *C. intestinalis* increase of abundance may be partly attributed to intraspecific competition for resources (i.e. settlement substrate) as in many cases the litter cavities were packed with ascidians leaving no empty space.

The initiation of a 'positive event' was related to a settlement episode or to the arrival of a new cohort, while the end of the event was due to mortality or migration to other areas. *C. stellatus* individuals settled on the litter surfaces during winter and did not survive more than five months. All *C. stellatus* individuals had been predated by the end of the experiment; *H. trunculus* was observed more than once to predate on *C. stellatus*. The new cohort of *S. hepatus* (0+ age class) arrived on the experimental surfaces during July-August 2005 and soon showed preference for the littered surfaces, as the juvenile fishes used the litter for refuge. Nevertheless, total abundance declined continuously and became nearly zero by June 2006; the reason for this decline might be mortality or/and migration or/and interspecific competition for litter cavities as most of them were gradually filled with the ascidian *C. intestinalis* leaving no empty space.

The large increase of *G. geniporus* abundance at t = 11 was rather incidental and not related to litter; at that time, the new cohort (0+ age class) appeared and as young *G. geniporus* move in schools (contrary to adults that were observed to be solitary) the accidental presence of large-numbered schools on two of the impacted surfaces caused an apparent peak in abundance, which was not related to litter.

A positive effect of litter on some species abundance has been shown before, especially on soft bottoms where both hard surfaces for settlement and natural dens for shelters are lacking. *Octopus vulgaris* used litter as den with a frequency of 38.7 % on soft bottoms of Greek coastal areas and enrichment experiments with artificial dens (plastic pots) increased local density significantly (Katsanevakis and Verriopoulos, 2004). Beer bottles allowed *Octopus rubescens* to utilize the sand/mud habitat in areas where natural dens were limited (Anderson et al., 1999).

This study, however, demonstrated not only that the abundance of some species increased because of littering but that there was also a marked and gradual increase in total abundance and number of species as well as a substantial change in the megafauna community structure. New relationships were established in the modified communities. Intraspecific (e.g. in *Ciona intestinalis*) and interspecific (e.g. between *C. intestinalis* and other species) competition for hard substratum and shelter was observed; the plateau in the total abundance time series of all three sites (Fig. 3) is indicative of approaching the carrying capacity of the new microhabitat provided by the litter items. New predator-prey interactions occurred, e.g. between *Hexaplex trunculus* and *Chthamalus stellatus*.

Sandy and muddy bottoms are generally low in productivity and total biomass in relation to rocky areas, coral reefs or seagrass meadows. A naive interpretation of the results of this study would be that littering is 'beneficial' for soft substrate habitats, because both total abundance and number of species were increased. Such an interpretation, however, would ignore the long-term effects of this ecological change on benthic communities. Littering may act as the means for the invasion of many hard-substratum species to soft bottoms. Indigenous soft bottom species might be displaced due to competition or predation by invading species and the extent of such an impact is yet unpredictable. As marine littering has become a global and large-scale issue, many populations of soft-bottom species might be greatly affected and even driven to local extinction, especially small populations of species with limited geographical distribution. Sandy and muddy bottoms are also important habitats for maintaining marine biodiversity. Any activity that globally modifies those habitats and changes their communities may not be considered 'positive' in any sense and is certainly against the policy of habitat and biodiversity conservation and sustainability.

Furthermore, the long-term effect of littering on endobenthic communities is unknown and has to be investigated. Virnstein (1977), conducted manipulative field experiments, in which the blue crab and two species of bottom-feeding fishes were either excluded from or confined to small areas using wire mesh cage experiments. In that work, the significance of large motile predators in controlling the distribution, abundance, and structure of the macrobenthic invertebrate infauna was demonstrated and it was concluded that infaunal population sizes were limited by predation and not by food or space. Thus, the substantial increase in the abundance of large benthic predators found in this study, e.g. of the gastropod *Hexaplex trunculus* or the fish *Serranus hepatus*, is expected to affect the infauna structure significantly.

The indirect effect of marine debris on the benthic community structure is expected to become a stronger issue in the future, as marine debris concentration on the seafloor continuously increases. The versatility of plastics, which have only existed for just over a century, has led to a great increase in their use over the last decades. Those important properties (light, strong, durable, and low-cost) that make plastics suitable for the manufacture of a very wide range of products are also the reasons why plastics are a serious hazard to the marine environment (Derraik, 2002), where they may persist for centuries and accumulate in large densities. Effort by all states to reduce marine littering should be intensified in the near future and more research is needed to evaluate the effect of marine littering on other habitats (e.g. coral reefs, seagrass beds, deep bottoms) and for other constituents of marine fauna (e.g. endobenthic communities).

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Figure Captions

Fig. 1: Map of the experimental sites.

Fig. 2: The experimental setup of each of the three sites (not in scale). Black dots represent the litter items. The five strips of each experimental surface, defined to facilitate the visual census, are also drawn.

Fig. 3: The ΔN time series and the corresponding ‘average’ models, according to the Akaike weights of the model set.

Fig. 4: The ΔS time series and the corresponding ‘average’ models, according to the Akaike weights of the model set.

Fig. 5: MDS plots of epi-megabenthic community data at each of the three experimental sites. C stand for the control sites and S for the impacted surfaces. Indexes represent the months passed after impact (C0 and S0 represent the community structure before impact).

Fig. 6: Time series of average abundance (among the three sites of the survey) of selected species in impacted and control surfaces as well as the difference in average abundance between impacted and control surfaces.

Table 1: The seven candidate models used to describe the time series of the difference in total abundance ($Y = \Delta N$) and in total number of species ($Y = \Delta S$) between impacted and control surfaces. k is the total number of estimated regression parameters (including σ^2).

Set of Candidate Models			k
g_1	Constant	$Y = a_0$	2
g_2	Linear	$Y = a_0 + a_1 t$	3
g_3	Quadratic	$Y = a_0 + a_1 t + a_2 t^2$	4
g_4	Exponential	$Y = Ae^{rt}$	3
g_5	von Bertalanffy	$Y = Y_\infty (1 - e^{-r_1(t-t_1)})$	4
g_6	Gompertz	$Y = Y_\infty \exp(-\lambda e^{-r_2 t})$	4
g_7	Logistic	$Y = Y_\infty (1 + e^{-r_3(t-t_3)})^{-1}$	4

Table 2: Particle size analysis results and classification of median particle diameter (Md ϕ) and quartile deviation (QD ϕ)

	Amoni		Frangolimano		Lychnari	
	Md ϕ	QD ϕ	Md ϕ	QD ϕ	Md ϕ	QD ϕ
Impacted Surface	2.98 ± 0.16	1.07 ± 0.08	3.05 ± 0.05	1.04 ± 0.08	2.16 ± 0.11	1.49 ± 0.08
	fine sand	poorly sorted	very fine sand	poorly sorted	fine sand	poorly sorted
Control	1.86 ± 0.05	1.01 ± 0.02	3.14 ± 0.04	1.11 ± 0.06	3.29 ± 0.23	1.04 ± 0.09
	medium sand	poorly sorted	very fine sand	poorly sorted	very fine sand	poorly sorted

mean \pm stand. deviation

Table 3: The time series of total abundance (number of individuals per 100m²) and number of species in the experimental surfaces (0: before impact, 1-12: months after litter addition, n.a.: non available).

Time (months):		0	1	2	3	4	5	6	7	8	9	10	11	12
		Total Abundance												
Amoni	Impacted Surface	9	24	32	27	43	38	43	133	158	n.a.	305	351	261
	Control	7	36	37	15	16	13	12	18	20	n.a.	85	67	80
Frangolimano	Impacted Surface	14	31	43	32	31	48	56	91	140	213	321	341	292
	Control	9	19	26	2	5	5	7	6	10	10	14	15	15
Lychnari	Impacted Surface	26	39	83	43	73	44	73	83	164	264	353	329	402
	Control	21	44	35	22	27	11	12	16	15	14	26	14	27
		Number of Species												
Amoni	Impacted Surface	2	7	6	7	8	12	12	14	16	n.a.	18	17	15
	Control	3	6	6	5	7	4	6	7	10	n.a.	7	9	6
Frangolimano	Impacted Surface	8	7	7	8	7	11	13	17	16	17	17	20	19
	Control	3	7	6	2	3	4	4	5	6	5	5	6	5
Lychnari	Impacted Surface	6	7	9	6	10	8	11	13	14	14	21	16	16
	Control	7	7	6	6	9	9	8	9	6	8	8	6	9

Table 4: Modelling the time-series of the difference in total abundance between the impacted surface and the control ($\Delta N = N_{\text{impact}} - N_{\text{control}}$). For each site and for each candidate model of the set, AIC_c , Akaike differences Δ_i , and Akaike weights w_i are given. The 95% confidence set of models is given bolded. Model definition as in Table 1.

Model	Sites								
	Amoni			Frangolimano			Lychnari		
	AIC_c	Δ_i	w_i	AIC_c	Δ_i	w_i	AIC_c	Δ_i	w_i
g_1	148.9	24.3	0.0%	165.6	35.8	0.0%	169.0	38.5	0.0%
g_2	130.9	6.3	2.6%	145.7	15.9	0.0%	148.8	18.3	0.0%
g_3	133.0	8.4	0.9%	140.4	10.6	0.4%	138.3	7.8	1.7%
g_4	133.4	8.8	0.8%	141.3	11.5	0.3%	139.1	8.6	1.2%
g_5	135.7	11.0	0.2%	150.1	20.3	0.0%	153.1	22.7	0.0%
g_6	125.8	1.1	34.5%	134.5	4.7	8.6%	134.4	4.0	11.8%
g_7	124.6	0.0	61.0%	129.8	0.0	90.7%	130.5	0.0	85.3%

Table 5: Modelling the time-series of the difference in the total number of species between the impacted surface and the control ($\Delta S = S_{\text{impact}} - S_{\text{control}}$). For each site and for each candidate model of the set, AIC_c , Akaike differences Δ_i , and Akaike weights w_i are given. The 95% confidence set of models is given bolded. Model definition as in Table 1.

Model	Sites								
	Amoni			Frangolimano			Lychnari		
	AIC_c	Δ_i	w_i	AIC_c	Δ_i	w_i	AIC_c	Δ_i	w_i
g_1	71.7	15.7	0.0%	81.4	20.8	0.0%	79.9	14.5	0.0%
g_2	56.1	0.0	42.0%	60.6	0.0	47.3%	67.6	2.1	13.6%
g_3	58.9	2.8	10.2%	64.9	4.3	5.4%	71.4	6.0	2.0%
g_4	62.0	6.0	2.1%	64.2	3.6	7.8%	69.9	4.4	4.3%
g_5	59.2	3.1	8.8%	64.9	4.3	5.4%	71.9	6.5	1.6%
g_6	57.5	1.4	20.8%	63.0	2.5	13.8%	65.4	0.0	39.4%
g_7	58.0	1.9	16.0%	62.3	1.7	20.3%	65.5	0.0	39.1%

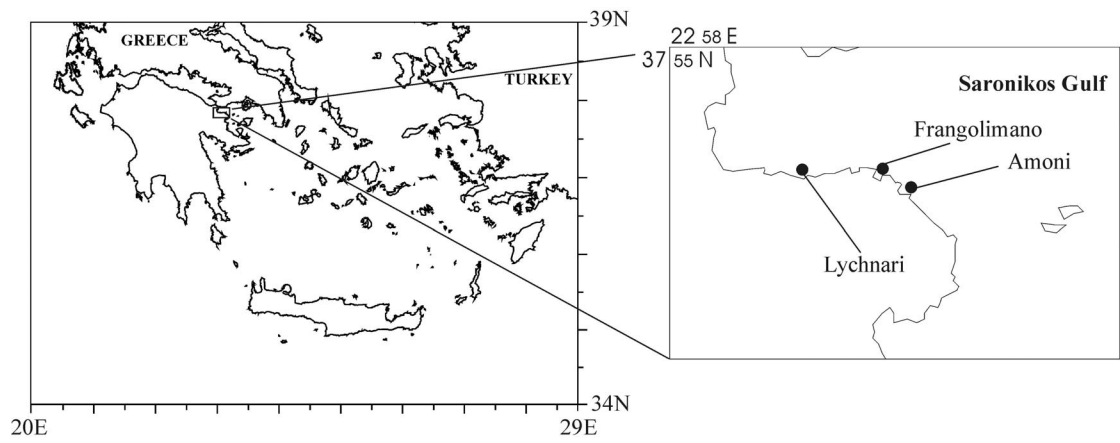


Fig. 1

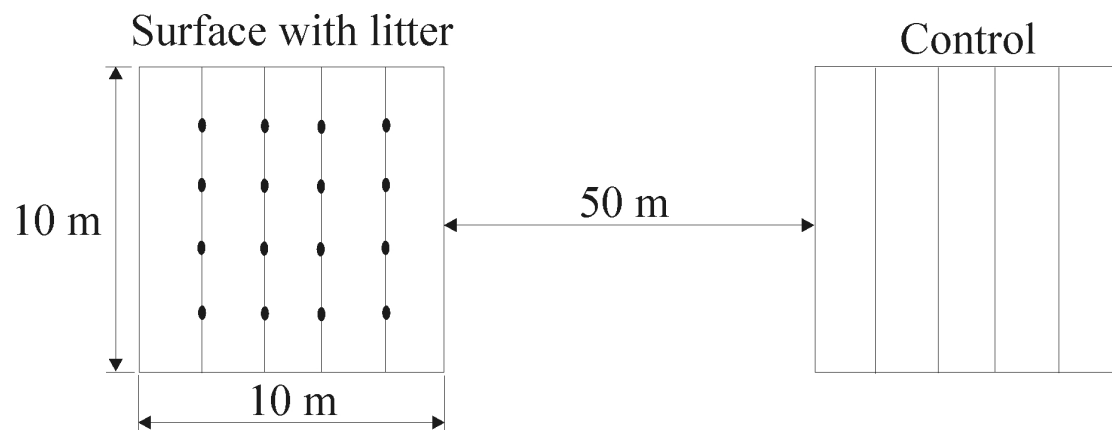


Fig. 2

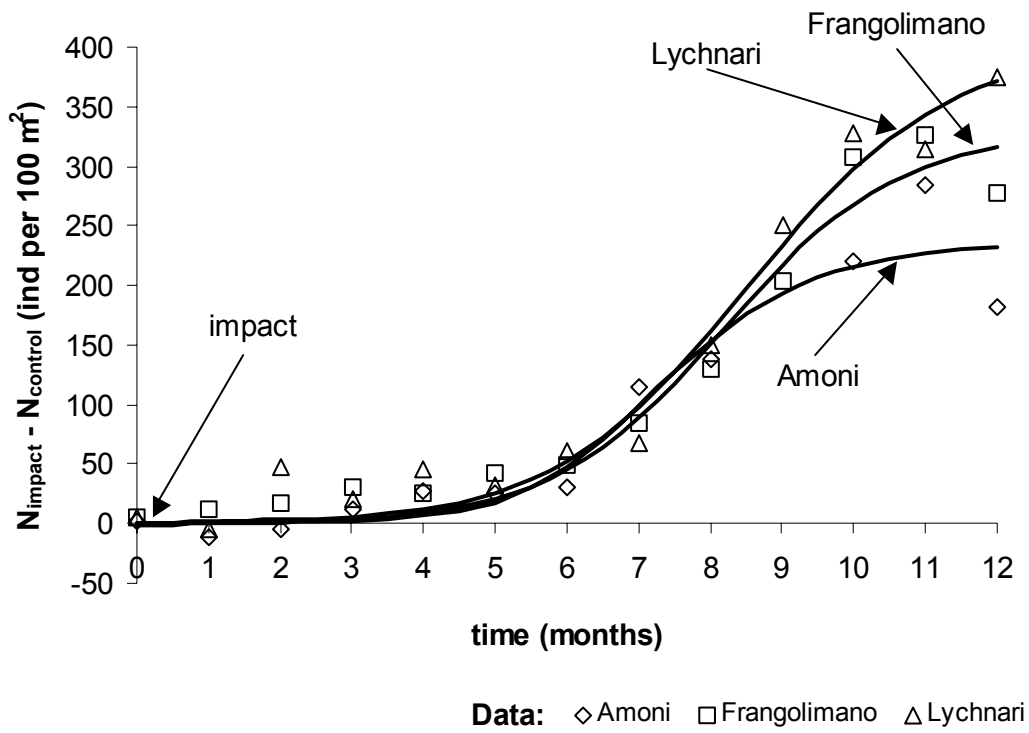


Fig. 3

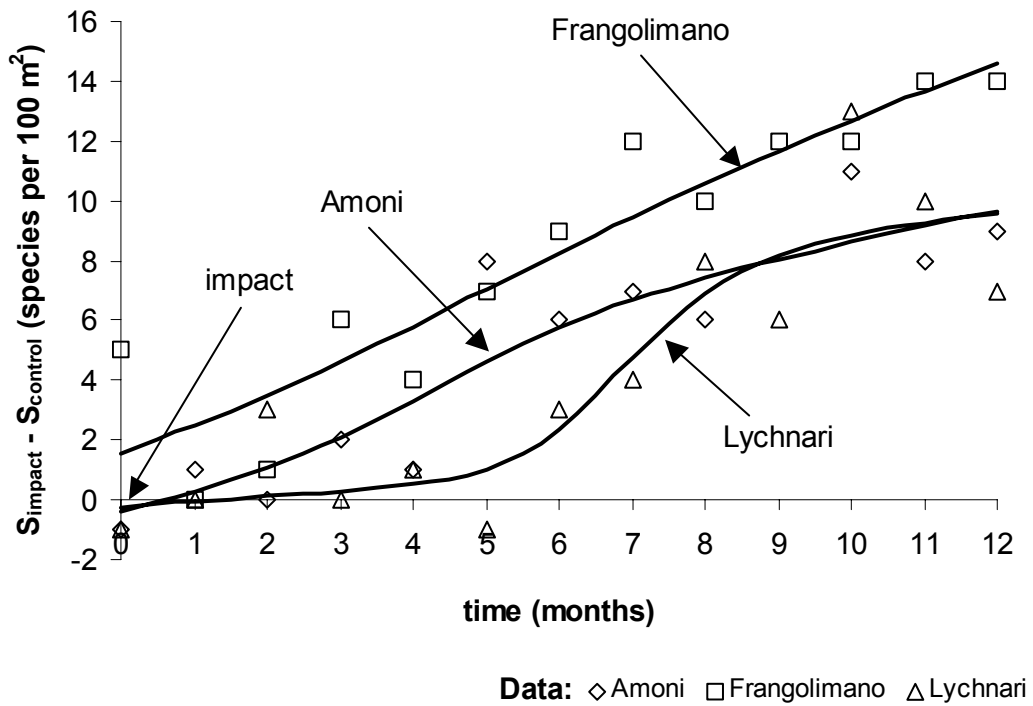


Fig. 4

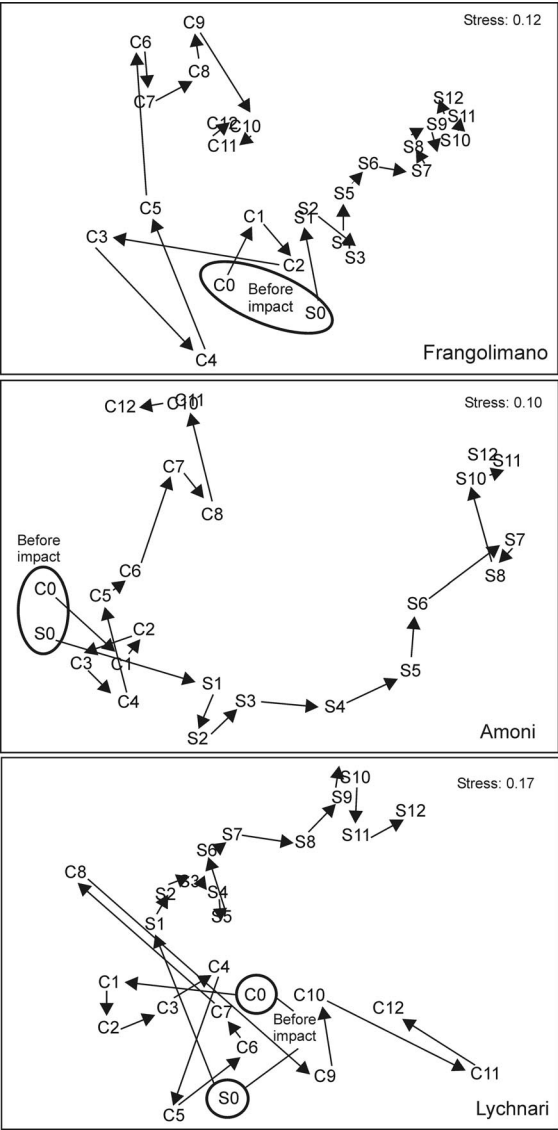


Fig. 5

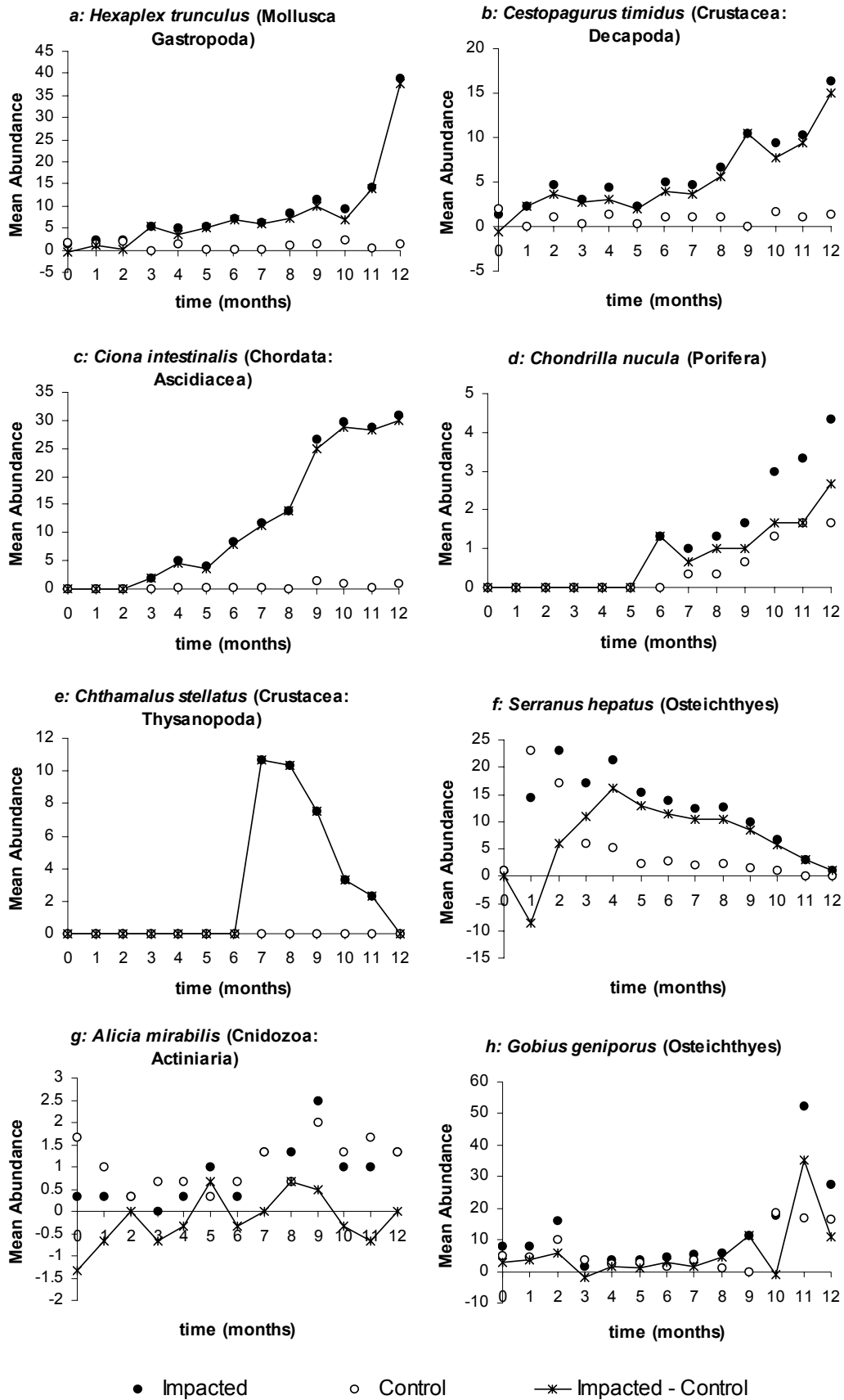


Fig. 6