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Small-scale experimental contamination with diesel oil does not affect the recolonization of *Sargassum* (Fucales) fronds by vagile macrofauna

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ABSTRACT. Coastal regions are subject to various forms of environmental impacts, such as spills of crude oil and associated products, with a wide range of effects on benthic biodiversity. This study characterized the patterns of recolonization of the macrofauna associated with the brown alga *Sargassum cymosum*(C. Agardh), on fronds contaminated by diesel oil in a small-scale field experiment. We collected 40 fronds of *S. cymosum* from an algal bed in southeastern Brazil and defaunated each frond by immersion in fresh water. Half of the fronds were then immersed in seawater (control group) and the other half in a mixture of 50% diesel oil and 50% seawater (impacted group). The test fronds were returned to the algal bed, and natural recolonization took place over a period of 12 days. Samples of the vagile macrofauna were taken randomly at three-day intervals over the course of the recolonization period. No significant differences in the densities of most taxa were found between the impact treatment (IG) and control treatment (CG). At the end of the recolonization period (day 12), the faunal composition of the treated fronds was very similar to the natural conditions, indicating a high rate of community recovery and suggesting that benthic associations can be rather resilient to diesel-oil impacts on a small scale.

KEY WORDS. Colonization; marine macrofauna; phytal.

The phytal community associated with marine macrophytes is highly diverse in composition, and in the characteristics of the species that constitute it (Jacobucci & Leite 2002). The phytal supports large faunal densities and species richness (Schneider & Mann 1991), because of the large number of microhabitats offered (Walthers & Wethey 1996) and the interactions among macrophytes, epiphytic algae and invertebrates, functioning on many different spatial and temporal scales (Jerkanoff *et al*. 1996, Duffy & Hay 2000).

Polluting agents such as petroleum and its derivatives have various impacts on biodiversity, that is, through physical, environmental or toxic effects (Kennish 1997). The degree of impact on a marine ecosystem depends on the concentration of the pollutant, the period of exposure to the polluting agent and the sensitivity or resistance of the organisms to these agents (Gin *et al*. 2001).

In Brazil, the majority of spills in marine environments occur along the southeast coast (Etkin 1998). This region is particularly vulnerable to pollution by crude oil and its derivatives, due to the intensive transport of this product and the operations of the DTCS (Duto e Terminais Centro Sul), the largest terminal dealing with petroleum and derivatives in the country. According to the CETESB (São Paulo environmental agency) database, CADEQ (Register of Chemical Emergencies) received reports of 421 leakages between 1978 and September 2010 along the northern coast of the state of São Paulo, mainly because of accidents with maritime transport and oil-line breaks.

Although the environmental consequences of large oil spills resulting from accidents involving oil tankers and transporters garner the most publicity, the discharge of industrial residues and even smaller, daily leakages from routine activities such as transportation and fishing may also cause considerable environmental damage (Pacheco & Santos 2001).

The effects of oil on biodiversity have been investigated after accidental spills. These investigations basically explain biological patterns after the disturbance, or compare affected and unaffected areas (Sanders *et al*. 1980) by means of estimaties of mortality and resistance of the communities (Gesteira & Dauvin 2000). Such events are by their nature unpredictable, and therefore, experimental control and establishment of cause-and-effect relationships between the impact and the biological responses are unfeasible.

The preferred fuel used by most maritime transporters and port or dock areas is diesel oil. Leakages, large or small,

from small boats to large ships and tankers are routine. These impacts can be potentially harmful to marine ecosystems if they continue over medium or long periods of time.

The dynamic and structural analysis of macrobenthic invertebrate associations has been a reference for programs monitoring the effects of pollutants (Warwick 1986). Some controlled experiments, simulating spillages and their effects compared with areas that have not been affected across many different coastal environments, have been carried out (Hyland *et al*. 1990, Faraco & Lana 2003). However, no studies have examined the effect of diesel oil on the recolonization process and succession in macrobenthic phytal communities.

The present study assessed the pattern of recolonization and succession of the macrofauna associated with fronds of *Sargassum* after experimental defaunation, and investigated the impacts of temporary exposure of *Sargassum* fronds to diesel pollution in a small-scale experiment.

MATERIAL AND METHODS

The study was carried out at Lázaro Beach on the coast of the Ubatuba municipality (23°31'S, 45°08'W), near Fortaleza Bay on the northern coast of state of São Paulo, Brazil (Fig. 1). This beach is surrounded by the mountains of the Serra do Mar state park, but is located in an important touristic area of Ubatuba.

Figure 1. Location of the study area, Lázaro Beach (23°31'S, 45°08'W), Ubatuba, state of São Paulo, Brazil.

The rocky shore, where the study was carried out, slopes moderately from the shoreline and contains dense algal beds dominated by *Sargassum cymosum* (C. Agardh), being exposed to moderate wave action (Széchy & Paula 1997).

In some Brazilian coastal areas, *Sargassum* species are very abundant (Eston & Bussab 1990), and can represent more than 80% of the benthic communities along the São Paulo and Rio de Janeiro coasts (Paula & Oliveira-Filho 1980, Széchy & Paula 2000).

To characterize the vagile macrofauna of *Sargassum cymosum* (natural baseline condition – NC), 20 fronds were randomly collected from the algal beds along the shore. The sampling technique involved free-diving, involving each of the fronds in an individual cloth bag with a 2-mm mesh size, and removing the frond from the substrate with a spatula.

In the laboratory, each frond was placed in a separate dish with a solution of 4% formaldehyde in sea water. The fronds were then rinsed four times to remove the macrofauna. According to Taylor & Cole (1994), this process can remove up to 99% of the vagile macrofauna from the frond. The solution was filtered through a 2-mm cloth mesh, to retain the organisms, which were then placed in 70% ethanol for further quantification and identification to major taxonomic groups. The dry weight of *Sargassum* fronds was obtained after drying at 60°C for 48 hours.

For the colonization experiment, 40 fronds of *S. cymosum* of similar size were collected and defaunated by means of three rinses in fresh water for three minutes. Holmlund *et al*. (1990) confirmed that this technique removes between 93 and 97% of the amphipods, without detrimental effects to the algae. The 40 defaunated fronds were then divided into two groups: 20 fronds were immersed in dishes of seawater for 12 hours (control group – CG), and 20 fronds were immersed in dishes containing a 50% mixture of seawater and diesel oil (obtained from commercial gas stations) for the same period (impacted group – IG). The latter treatment was designed to simulate the effects of temporary exposure to diesel oil, as might be experienced after a leak from a boat engine.

The algae in the CG group were marked with yellow cloth tape, and other in the IG group were marked with red cloth tape on the main branch, both were attached to a lead fishing sinker with monofilament fishing lines, on the appressoruim of the frond, to avoid wave entrainment. The fronds was then reintroduced randomly onto the algal bed at Lázaro Beach, in direct contact with other *Sargassum* fronds.

Four replicas of each group were removed at intervals of 1 (D1), 3 (D3), 6 (D6), 9 (D9) and 12 (D12) days following transplantation. The samples were randomly removed from *Sargassum* bed and transferred to the laboratory for analysis. The same procedures as described above were used to assess the composition of the associated vagile macrofauna and obtain the dry weight of the fronds.

Since the fronds varied in size and the abundance of macrofauna on a frond is dependent on the surface area of the frond available for colonization, it was necessary to control for size. The density of organisms was then expressed as the number of individuals per gram of *Sargassum* dry weight.

Three types of comparisons were performed. First, a one-way ANOVA was used to compare the final density of the taxonomic groups in the two treatments at the end of the experiment (D12) with the densities obtained from the baseline study (NC). Second, we assessed whether the pattern of

recolonization varied between the control (CG) and impacted (IG) groups by comparing the densities of the most abundant taxa of different taxonomic groups at the different time periods after the fronds were returned to the algal bed, using a 2-factor ANOVA with 'time' (D1 to D12) and 'treatments' (CG and IG) as fixed factors. Finally, the community structure of the fronds during the process of recolonization was compared, using Non-metric Multi-dimensional Scaling (MDS) and Bray-Curtis similarity index (Clarke & Warwick 2001). The groups on the ordination diagram should reflect any underlying processes structuring the community. In this case, the similarity between samples is approximately proportionate to the distances between them on the ordination plot. The calculated stress value indicates the difficulty of representing the multidimensional community in a two-dimensional figure. Analysis of similarities (ANOSIM) was carried out to test for differences between treatments and days of recolonization.

All variables were checked for normality (Shapiro-Wilk, $p > 0.05$) and homogeneity of variances (Levene's test, $p > 0.05$), before parametric tests were performed. Variables that did not meet the assumption of normality were log (x+1) transformed. When the one-way ANOVA and 2-factor ANOVA indicated statistical differences, the Tukey (HSD) post-hoc test was applied for pairwise comparisons.

RESULTS

Faunal composition

Of a total of 15,481 recorded individuals from five phyla, 10,951 were counted in the baseline study (NC), 2,330 in the control (CG) and 2,200 in the impacted (IG) treatments. Mollusca, Arthropoda, and Crustacea were the most numerous taxa, especially the isopods and amphipods, and in this last group, mostly from the suborder Gammaridea. Decapod and tanaid crustaceans were present in lower abundances in all three treatments. The least abundant taxa in all cases were Turbellaria, Pycnogonida and Ophiuroidea, as indicated in Table I.

Because of the dominance of Gammaridea, samples were identified and quantified to the family level (Tab. II). The Hyalidae and Ampithoidae were the most abundant families. Corophiidae showed the lowest abundances in all treatments.

Comparison of densities

The one-way ANOVA indicated no differences in the density of organisms for most taxa analyzed after 12 days of recolonization, comparing the control group (CG), the impacted group (IG) and the natural baseline condition (NC) ($p > 0.05$) (Figs 2 and 3). The exceptions included the order Decapoda (Fig. 2), which showed a higher density (ind/g) in the NC than in the

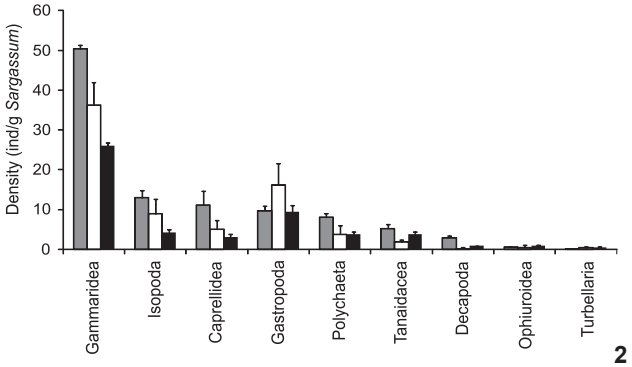
Table I. Major taxa recorded on *Sargassum* fronds, and their total and relative (%) abundance in natural conditions (day 0), control and impacted groups (days 1-12).

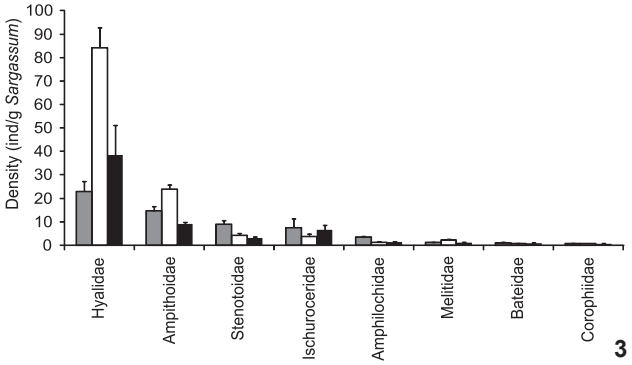
Taxon	Natural conditions (105.25 g of algae)		Control group (46.26 g of algae)		Impacted group (55.85 g of algae)	
	Total	%	Total	%	Total	%
Platyhelminthes						
Turbellaria	23	0.2	2	0.1	5	0.2
Annelida						
Polychaeta	787	7.2	87	3.7	120	5.5
Mollusca						
Gastropoda	1003	9.2	241	10.3	292	13.3
Arthropoda						
Crustacea						
Amphipoda						
Caprellidea	1013	9.3	159	6.8	105	4.8
Gammaridea	5984	54.6	1606	68.9	1417	64.4
Isopoda	1253	11.4	118	5.1	94	4.3
Tanaidacea	480	4.4	66	2.8	72	3.3
Decapoda	328	3.0	35	1.5	60	2.7
Chelicerata						
Pycnogonida	27	0.3	4	0.2	11	0.5
Echinodermata						
Ophiuroidea	53	0.5	12	0.5	24	1.1
Total	10,951	100.0	2,330	100.0	2,200	100.0

Table II. Gammaridean families recorded, and their total and relative (%) abundance in natural conditions (day 0), control group and impacted group (days 1-12).

Taxon	Natural conditions (105.25 g of algae)		Control group (46.26 g of algae)		Impacted group (55.85 g of algae)	
	Total	%	Total	%	Total	%
Amphilochidae	340	5.7	29	1.8	20	1.4
Ampithoidae	1420	23.7	321	20.0	245	17.3
Bateidae	94	1.6	28	1.7	17	1.2
Corophiidae	72	1.2	8	0.5	11	0.8
Hyalidae	2332	39.0	1066	66.4	929	65.6
Ischyroceridae	697	11.6	63	3.9	91	6.4
Melitidae	113	1.9	23	1.4	22	1.6
Stenothoidae	916	15.3	68	4.2	82	5.8
Total	5,644	100.0	1,577	100.0	1,397	100.0

IG (F_{24} = 4.4026, $p < 0.05$) and in the CG (F_{24} = 11.7879, $p < 0.01$); and the order Isopoda, which showed a lower density in the IG than in the CG (F_{24} = 4.7684, $p < 0.05$) (Fig. 2). Of the gammaridean families, the test showed a lower density in the NC than in the CG for Hyalidae (F_{24} = 3.5726, $p < 0.05$) (Fig. 3).





Figures 2-3. Mean density (± SE) of the most abundant taxa (2) and of the gammaridean families (3) in the natural conditions before the treatment (gray), control group (white) and the impacted group (black), after 12 days of treatment. * indicates significant differences among treatments within the taxon.

Comparison of the control and impacted treatments throughout each colonization period

According to the 2-factor ANOVA, the density of organisms was significantly higher for Isopoda, Gammaridea Ampithoidae and Hyalidae ($p < 0.05$) in the control group (CG), than in the impacted group (IG) (Tab. III, Figs 4-21).

Comparing the periods of the experiment, most taxa showed significant differences between the periods of recolonization. For the most abundant taxa analyzed, two different responses were observed between the periods of colonization: 1) The lack of significant differences in density throughout the recolonization process (Decapoda and Caprellidae); 2) Significant differences between the first half and the second half of the process, in some cases with an overlap between the intermediate periods (Tab. III, Figs 4-21).

According to the Non-metric Multidimensional Scaling (MDS) plot, the ordination was similar, without evident differences in the recolonization process between the control and impacted groups (ANOSIM, $p = 0.401$). In relation to the recolonization periods, the ANOSIM results showed a highly significant difference ($p < 0.0001$), with samples from later periods separated from the initial periods. In this case, a possible difference was observed between the first half (D1, D3 and D6) and the second half of the experiment (D9 and D12) (Fig. 22).

DISCUSSION

The major finding of this study is that the short-term exposure of *Sargassum cymosum* fronds to diesel oil, for the small spatial scale adopted here, did not appear to influence the recolonization pattern of vagile macrofauna. The apparent lack of impact of exposure to diesel is probably the result of two factors. First, the physiology of some algae, such as *Sargassum*, may mitigate the effects of short-term exposure to toxic chemicals such as diesel oil. This is because *Sargassum* and many other algae, are covered by a thick gelatinous coat that is continually

Table III. Results of 2-factor ANOVA and post-hoc Fisher (LSD) test results of density variance between the control group (CG) and impacted group (IG) at different periods of recolonization (D1 = day 1, D3 = day 3, 6 = day 6, 9 = day 9, 12 = day 12, * = not significant), on the density of the most abundant taxa on Sargassum fronds.

Source of variation	d.f.	MS	F	p	
Gastropoda					
Treatments	1	0.033020	0.48920	0.490036	*
Periods	4	0.423440	6.27420	0.000980	D1 D3 D6 D9 D12
Treatment x Periods	4	0.037740	0.55920	0.694058	*
Isopoda					
Treatments	1	0.492798	9.13020	0.005324	CG > IG
Periods	4	0.688498	12.75590	0.000005	D1 D3 D6 D9 D12
Treatment x Periods	4	0.009183	0.17010	0.951828	*
Tanaidacea					
Treatments	1	0.160025	3.07664	0.090365	*
Periods	4	0.264882	5.09263	0.003271	D1 D3 D6 D9 D12
Treatment x Periods	4	0.085269	1.63939	0.192170	*
Decapoda					
Treatments	1	0.051542	0.99066	0.328105	*
Periods	4	0.032391	0.62257	0.650225	*
Treatment x Periods	4	0.009458	0.18179	0.945920	*
Polychaeta					
Treatments	1	0.057037	1.08330	0.306872	*
Periods	4	0.766474	14.55710	0.000002	D1 D3 D6 D12 D9
Treatment x Periods	4	0.005993	0.11380	0.976591	*
Caprellidae					
Treatments	1	0.367774	3.87079	0.059112	*
Periods	4	0.115444	1.21504	0.326641	*
Treatment x Periods	4	0.017862	0.18800	0.942678	*
Gammaridea					
Treatments	1	0.890220	7.78860	0.009360	CG>IG
Periods	4	0.877900	7.68080	0.000261	D1 D3 D6 D9 D12
Treatment x Periods	4	0.143000	1.25110	0.312413	*
Ampithoidae					
Treatments	1	0.652140	11.73290	0.001913	CG>IG
Periods	4	0.649560	11.68640	0.000010	D1 D3 D6 D9 D12
Treatment x Periods	4	0.049400	0.88880	0.483498	*
Hyalidae					
Treatments	1	0.927750	5.74000	0.023509	CG>IG
Periods	4	0.708850	4.38570	0.007048	D3 D1 D6 D9 D12
Treatment x Periods	4	0.241110	1.49170	0.231430	*
Ischyroceridae					
Treatments	1	0.058678	0.73928	0.397194	*
Periods	4	0.369608	4.65670	0.005229	D1 D3 D6 D9 D12
Treatment x Periods	4	0.058551	0.73769	0.574184	*
Melitidae					
Treatments	1	0.025335	0.99516	0.327029	*
Periods	4	0.136599	5.36564	0.002455	D1 D3 D6 D9 D12
Treatment x Periods	4	0.015342	0.60262	0.663909	*
Stenothoidae					
Treatments	1	0.002286	0.05380	0.818196	*
Periods	4	0.247737	5.83450	0.001518	D1 D3 D6 D9 D12
Treatment x Periods	4	0.025962	0.61140	0.657857	*

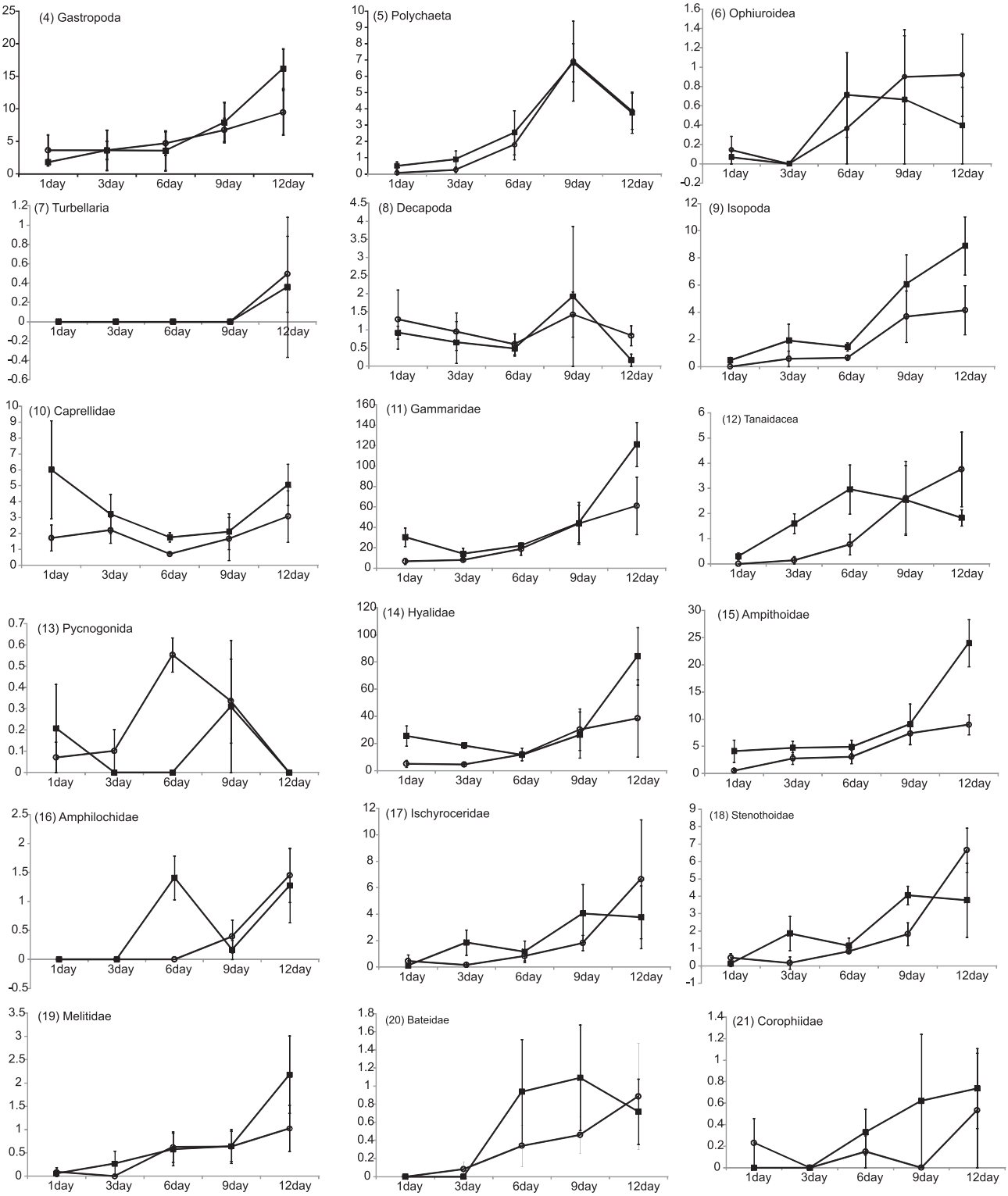


Figure 4-21. Mean density (± SE) of the major taxonomic groups (4-13) and the gammaridean families (14-21), during the recolonization process of *Sargassum* fronds (■ control group; ○ impacted group).

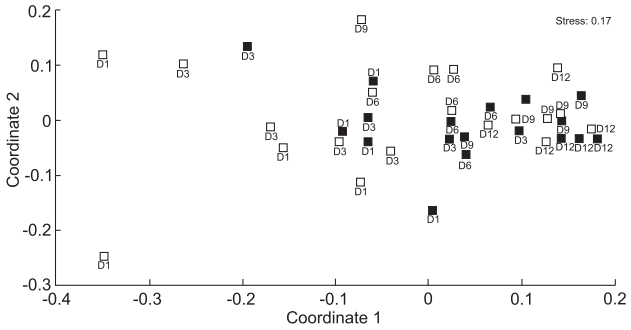


Figure 22. Non-metric Multidimensional scaling (MDS) ordination of Bray-Curtis similarity matrix of density data (number of individuals per gram of *Sargassum*) in different treatments and periods of recolonization (■ control group; □ impacted group/ D1 = day 1, D3 = day 3, D6 = day 6, D9 = day 9, D12 = day 12).

sloughing off (Filion-Myklebust & Norton 1981). Thus, if the toxic oil molecules do not penetrate beyond the surface of the alga (as might occur during a temporary exposure) they may be quickly dispersed into the surrounding environment.

Secondly, the scale of exposure can strongly influence the pattern of recolonization. Large oil spills may defaunate huge areas of the sea bed, requiring recolonization from distant subpopulations. In these cases, some taxa may take months or even years to recover to a near-original status. In contrast, small-scale studies with areas of highly localized defaunation, as simulated in the current experiment where fronds of *Sargassum* from both treatments (CG and IG) were reintroduced onto intact algal banks, may favor a rapid rate of recolonization and recovery by the original fauna. This was noted by Ritchie (1995), who examined the effects of crude oil spills, and their derivatives, on different types of shoreline, and Faraco & Lana (2003) who observed the rapid response of polychaetes in peripheral oil spill areas in natural and defaunated subtropical mangrove sediments. In the present study, the similarity in densities of associated fauna, 12 days after treatment, to those in the natural conditions (NC) indicate a rapid rate of recolonization and recovery.

The recolonization and recovery of densities can be explained by active migration of the vagile macrofauna, which can aid in recolonization of the habitat by individuals from unaffected adjacent areas. Small-scale dispersal by the mobile fauna is one of the mechanisms maintaining the stability of phytal communities with respect to composition and abundance, due to rapid emigration and immigration rates (Martin-Smith 1994), with more movement among nearby macrophytes (Taylor 1998).

In this study, the fronds which were treated as part of the experiment were reintroduced in the *Sargassum* bed, and possible migrations from peripheral areas could have been responsible for the recolonization and recovery of population densities. Analysis of natural disturbances in a subtropical tidal creek suggested that the speed of recolonization in unconsolidated substrates is more dependent on the mobility of the species involved (Netto & Lana 1994), than on their tolerance to oil or other variables.

A factor that may have affected the process of recolonization in this study is migration by foraging, in which herbivores, mainly amphipods, may be attracted to decomposing filamentous algae, as observed by Edgar (1992). However, the *Sargassum* fronds collected in the present study showed no visible signs of deterioration.

According to Schratzberger *et al.* (2003), the recovery of macrobenthic communities also depends on the patterns of recruitment of the species and their tolerance to toxic compounds, in which local environmental conditions can accelerate or slow the decontamination process. Environmental parameters, such as hydrodynamism, are important to the structure of the local communities, and can minimize or amplify the effects caused by oil spills. Species from more sheltered environments may be more sensitive to such disturbances (Tuck *et al.* 1998, Hawkins *et al.* 2002), compared to organisms in more dynamic surroundings (Le Hir & Hily 2002, Dernie *et al.* 2003), so areas with more intense hydrodynamics have a tendency to disperse oil, thereby reducing the impact on the environment or even making it imperceptible. Although the local hydrodynamics was not measured in this study, previous studies (Széchy & Paula 1997, Cunha *et al.* 2008) attested that the sampling site is exposed to moderate wave action. Consequently, the diesel oil on the fronds could have been easily removed soon after the experiment, which would explain the lack of differing results between the CG and the IG during each recolonization stage, for the majority of the groups evaluated.

Based on the results obtained, the general trend of the taxonomic groups counted during the treatments (CG and IG) and in the natural conditions group (NC) was very similar, dominated by Gammaridea and its families Hyalidae and Ampithoidae, characteristic inhabitants of the *Sargassum* fronds in the Ubatuba region (Jacobucci *et al.* 2009). Marine macrophyte faunas are often dominated by gammarideans, crustaceans that may select areas with more availability of food and refuges against predators and unfavorable conditions (Buschmann 1990). On the other hand, similar, relatively low numbers of turbellarians, pycnogonids and ophiuroids in the treatments and natural conditions, were also detected in other *Sargassum* studies in this region (Jacobucci & Leite 2002), and these groups occurred in low abundance on the *Sargassum* fronds before the treatment.

In Isopoda, the suborder Gammaridea and the gammaridean families Hyalidae and Ampithoidae showed significant differences in the initial recolonization stages between the two treatments, CG and IG. These results suggest a possible sensitivity of these groups to impact, even during this small-scale, short-term experiment. Other studies have also indicated that members of Amphipoda are sensitive to the presence of hydrocarbons (Gesteira & Dauvin 2000) and metals (Roberts *et al.* 2006).

Significant differences between the CG and IG groups in the recolonization by polychaetes were not detected in the present study. This result was expected, considering that previous studies have indicated that sediment-dwelling polychaetes are resistant to high level of hydrocarbons (Gesteira & Dauvin 2000). Some species are even able to benefit from the presence of the contaminant (Hutchings 1998).

Experimental studies and evaluations of disturbances during real events, or in experiments carried out on larger scales, have demonstrated that the dynamics of a benthic community may or may not be influenced by oil spills (Gascon & Travis 1992, Ruth *et al*. 1994, Thrush *et al*. 1996). Kingston *et al*. (1995) did not observe changes in the benthic community structure after the Braer spill. However, one study carried out in New York, USA, by Lytle & Peckarsky (2001) found that a community of freshwater invertebrates was potentially affected after a diesel-oil spill, with high mortality rates and low resilience.

The lack of significant differences between the treatments demonstrates that the rate of recolonization was not affected by the presence of diesel oil after the defaunating process. We also conclude that a diesel-oil spill on the spatial scale used here would have no effect on the recolonization of the *Sargassum* fronds.

Although major differences were not identified in the recolonization pattern of vagile macrofauna, we cannot rule out the possibility that the oil had an influence at lower taxonomic levels. The high taxonomic diversity of some major groups evaluated in the present study, such as Amphipoda, Isopoda and Gastropoda, may have masked effects at the genus and species levels. The experimental contamination may have created a mutual compensation, where some species may have been favored and some impaired by it.

The restoration or recovery of macrofauna can be very different after a real oil or petroleum-based products spillage, but it should be noted that this response can be extremely variable, depending on which communities are involved, together with the physical and environmental conditions, and of course, the scale of the impact.

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