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Experimental Study of Oil Impact and Its Consequences upon Benthic Macrofauna in the Estuary of Patos Lagoon (RS-Brazil)

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ABSTRACT

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Petroleum, a fossil fuel of great signification for world economy, represents a serious environmental problem, due to its frequent introduction, not only because of its large-scale transportation but also its broad industrial use. The present study describes the effects of oil upon benthic macroinvertebrates through a simulation of an oil spill. The study was developed along 5 months in a shallow-water portion of the estuary of Patos Lagoon (RS-Brazil). Evidence of detrimental oil effect was found upon the crustaceans *Sinelobus stanfordi*, *Kupellonura sp*., *Sphaeromopsis mourei* and *Kalliapseudes schubartii*. The polychaetes *Laeonereis acuta*, *Nephtys fluviatilis* and *Heteromastus similes* did not suffer oil influence.

ADDITIONAL INDEX WORDS: *Oil spill,estuary, faunal tolerance.*

INTRODUCTION

Estuarine regions are particularly susceptible to oil-spill impacts, due to accidental discharges caused by inappropriate handling of oil tankers, oil pipes during loading and unloading operations, and oil transportation within port areas and its surroundings (HYLAND *et al.*, 1990). Oil may remain in an estuarine marsh area for years, making it one of the most vulnerable environments to oil discharge (GUNDLACH and HAYES, 1978). All over the world, coastal and oceanic environmental assessment and monitoring have been performed in order to attend demands from the oil industry itself or pressure from society (FARRINGTON, 1989). The analysis of benthic macroinvertebrate associations structure and dynamics has been highly useful for programs monitoring pollutant effects (WARWICK, 1986). According to LANA (1994), such organisms, which live within or upon the substrate, more precisely reflect the pre-sampling environmental conditions when compared to forms living in the water column. Their relative sedentary nature, long life cycles, and the existence of species that might be considered biological indicators of impacted environments make benthic organisms particularly appropriate for impact assessment and monitoring work in coastal areas, whether with descriptive or experimental approaches. Thus, effects of oil impacts have been studied upon benthic macroinvertebrates in the field and the laboratory (SCPMEU, 1985). The sensitivity of vegetated and non-vegetated intertidal plain benthic organisms to oil impacts has caught the interest of a number of researchers (KINGSTON *et al.*, 1995; FITZPATRICK *et al.*, 2000; GESTERA and DALVIN, 2000). Most analyses of macrobenthos are performed after accidental discharges, basically consisting in a description of the biological patterns observed after such disturbances. Field experimental manipulation is one of the most appropriate tools not only for establishing causal relations between polluting agents and biological variability, but also for decreasing the low signal/noise relationship inherent to biological variations in monitoring programs (SANDERS *et al.*, 1980).

Petroleum has been identified as one of the most serious contaminants in the oceans (WARWICK and CLARKE, 1991). The region where the present study was conducted houses the port of Rio Grande, which, in spite of not being characterized by a large operational volume in the oil industry, has some companies related to the oil and gas sector among the components of its surrounding industrial matrix. Such companies operate refining, storage and transportation of oil products. On top of it there also is the risk involved in fueling docked ships and the pipes connected to industries and ships.

Within such context, the present study aims at assessing oil effects upon the benthic macroinvertebrate association structure and the survival of such organisms in an estuarine embayment.

METHODS

The experiment was set up in a shallow embayment in Patos Lagoon so as to simulate an oil discharge. The chosen environment was a shallow-water spot, with depth varying from 0 to 1m. This region (32º01´325´´S and 52º 05´528´´W) is located by the margin of Cavalos Island, east of Pólvora Island, in the city of Rio Grande, RS-Brazil, having to its north the Ponta da Marambaia (Marinheiros' Island) and to its south the Old Port of Rio Grande (Figure 1).

The experiment lasted 5 months, being developed from March to August 2001, with samplings at pre-established time intervals.

The experiment was acute and non-cumulative, with simulation of a single impact at the selected areas. Refined oil was used, being inserted in the environment without suffering any previous weathering process. According to USEPA (2001) the oil used is classified as being predominantly composed of low-weight hydrocarbons, which spread upon the water surface.

At this study area, 18 1-m^2-squares were placed 1 meter apart from one another. They were identified with wood sticks, holding a cloth strip for oil contention. 1.5 liter of oil was added in each square, remaining in place for 4 hours. The oil was then removed along with the cloth strip. An adjacent area with similar features was used as control. In each area (control and impacted), 3 squares were randomly assigned for benthos sampling, with 3 replicates for each square. The same process was simultaneously conducted at the control area, numbering a total of 108 benthic macrofauna samples at the end of the experiment (54 at the impacted and 54 at the control area). Altogether, six samplings were conducted, being performed at (1d); (8d); (14d); (52d); (114d); (147d) days after the beginning of the experiment (April 3rd, 2001).

Following this experiment, an organism "survival test" to the discharged oil was conducted. In order to distinguish live and dead organisms, on the same day of oil discharging, three biological samples were collected at the impacted area to be compared to three other samples from the control area,

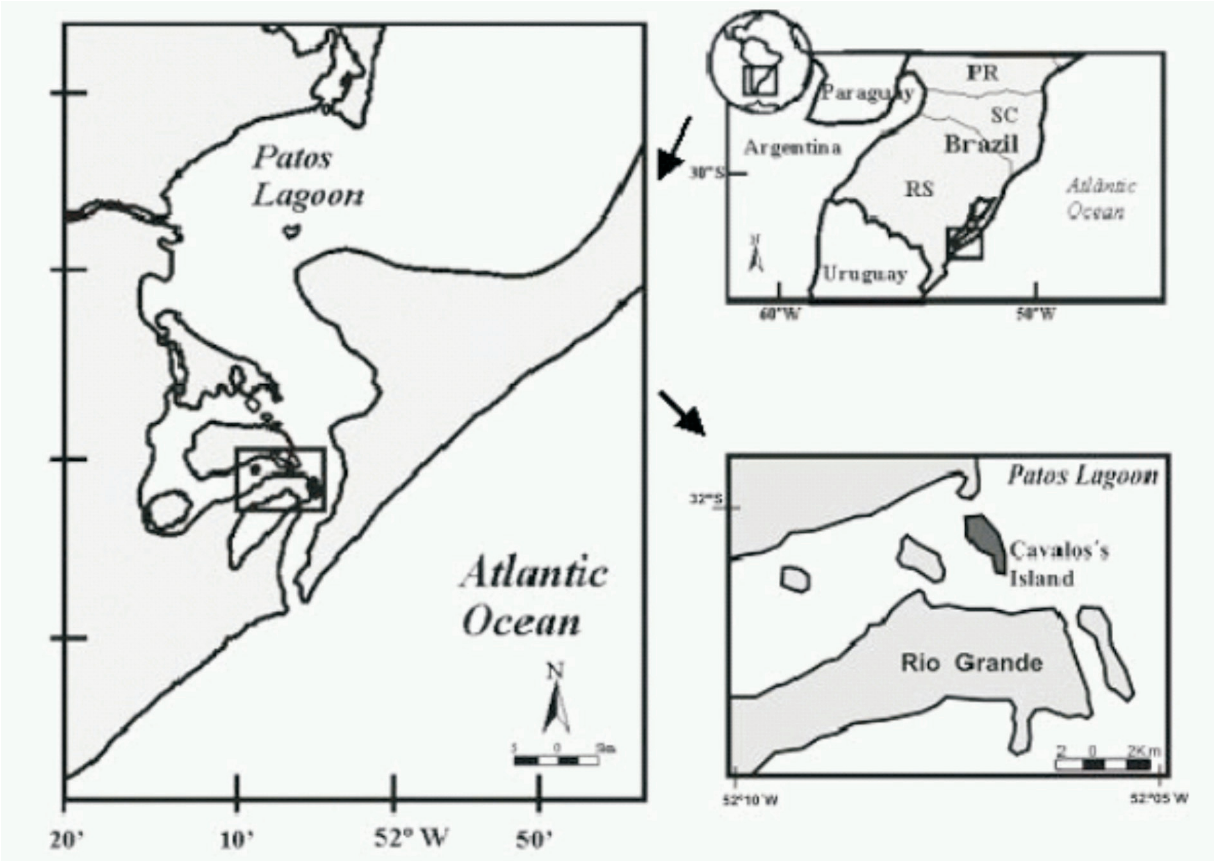

Figure 1. Study area.

accounting for 6 samples. Those samples were collected 4 hours after oil discharging and taken "in natura" to the lab.

For obtaining benthic macrofauna, a 10-cm PVC tube was used (area 0.008 m^2), burying it 15 cm in the sediment. After collecting, the samples were sieved through a 0.3-mm 'nylon' screen, this size being recommended for assuring retention of a higher number of juvenile macrofauna specimens (BEMVENUTI, 1992). After the process, the organisms were fixed with 5% formalin and soon taken to the lab for later analysis.

For the "survival test", the same technique was used for obtaining material, but the organisms were not fixed with 5% formalin, being taken to the lab "in natura" for future analysis.

Abiotic values of water and air temperature (ºC), salinity, potential hydrogen (pH) and redox potential (Eh) were collected. These were measured at 2, 10 and 15 cm of depth. For granulometric samples, sediment samples were collected with a 10-cm PVC extractor tube, buried 10-cm-deep into the substrate, divided in two 5-cm strata. Sand, silt and clay proportions in the sediments were determined by sieving of coarse material (SUGUIO, 1973). For sediment organic matter content, the same sample collected for granulometry was used, with method proposed by WALKLEY and BLACK (1934).

In the laboratory, in order to separate macrofauna from sediment, samples were sieved through a 0.3-mm nylon screen. Organisms were identified to the least possible taxon, with the help of stereomicroscope. After sorting and identification, their density was calculated as number of ind./m². Then, they were preserved in plastic bottles in 70% ethanol, and identified by taxon. The same process was performed with organisms used for the survival test.

For sediment analysis, the software SYSGRAM® was used. Changes in benthic macroinvertebrate associations structure were assessed through univariate and multivariate statistical methods (CLARKE and WARWICK, 1994). In order to identify possible significant differences between control and impacted regions, regarding benthic macrofauna mean densities, the data were submitted to Variance Analysis tests (ANOVA) performed with the help of the software Statistica®. The level of significance adopted for mean contrast was 5%. Mean contrast was performed through Tukey Contrast Test. Among univariate analysis, the Shannon-Wiener (H') diversity index was used, which integrates species number and abundance in the association (CLARK, 1997). Also used was the Pielou evenness index (J), which varies from 0 to 1 and provides a measure of uniformity in the relative species representation in number of individuals for a given sample (ACIESP, 1987).

For multivariate analyses, techniques of classification (CLUSTER) and ordination (MDS Multi-dimensional Scaling Ordination) was carried out. Both group samples according to the similarity criterion. For these analyses, a data matrix was calculated using density values from Bray-Curtis similarity index (ROMESBURG, 1984). For data transformation, log (x+1) was used.

For the survival test, data were submitted to variance analysis tests (ANOVA), with a level of significance for mean contrast of 5%.

Statistical analyses were performed using the software Primer 5.0 computer package (Plymouth Marine Laboratory).

RESULTS

Environmental Variables

Water temperature oscillated between 13 and 25.3 ºC, and air temperature between 11.4 and 27 ºC, reflecting the estival conditions at which the experiment was developed. Salinity varied between 0 and 6. Potential hydrogen (pH) values did not vary along the experiment, remaining between 6.71 (114d sampling 20-cm stratum) and 7.40 (52d sampling 20-cm stratum) at the impacted area. There was also no variation at the control area, remaining between 6.66 (8d sampling 20-cm stratum) and 7.93 (14d sampling 2-cm stratum). During the experiment, the redox potential (Eh) in the 2-cm sediment stratum always presented positive values, at the control and well as the impacted area. In the 10-cm stratum at the control

Table 1. *Composition and mean density (ind./m²) of species found along the experiment at control (C) and impacted (I) areas.*

Sampling	1st day		8th day		14th day		52nd day		114th day		147th day	
	AREAS											
Species	C	I	C	I	C	I	C	I	C	I	C	I
CRUSTACEA												
Kalliapseudes schubartii	18625	15889	16125	21458	17347	19583	16388	19472	2333	8361	1277	5055
Sinelobus stanfordi	28	0	25	0	0	194	139	222	0	222	0	1000
Kupellonura sp.	347	0	83	83	250	111	97	97	27	27	41	139
Callinects sapidus	0	0	0	14	0	0	0	0	0	0	0	0
Diastilis sympterigiae	0	0	0	0	0	0	0	0	0	0	14	14
Sphaeromopsis mourei	97	0	14	28	250	41	83	55	55	41	69	42
POLYCHAETA												
Laeonereis acuta	500	1138	875	1000	402	902	777	1000	236	388	236	292
Nephtys fluviatilis	194	292	375	180	69	277	361	389	361	541	208	528
Heteromastus similis	152	139	333	236	305	361	208	569	375	597	333	611
Sigambra grubei	0	14	0	0	0	0	0	0	0	0	0	0
MOLLUSCA												
Heleobia australis	152	14	0	0	41	0	28	0	0	0	69	28

area, an oscillation between oxidizing and reducing was verified along the experiment. In the 20-cm stratum, the sediment only showed to be oxidizing in the 1d-sampling at the impacted area. Granulometric analysis results revealed little variation in mean values between the first and the last day of the experiment. There was a predominance of fine to very-fine sand. Coarse to very-coarse sand values were only found on the first day sampled (1d), at the control area, in the 10-cm stratum. Organic matter content values were similar in the first and last sampling days (1d and 147d).

Biological Variables

During the work, a total of 11 species were found. Among those, 9 species were found at the control area and 10 species at the impacted area (Table 1). During the whole of the sampled period, 1,637,000 ind./m², being 724,500 ind./m² found at the control area (44.26%) and 912,500 ind./m² at the impacted area (55.26%). The crustacean *Kalliapseudes schubartii* was the dominant species, representing 89.56% of organisms found at the control area and 88.42% at the impacted area. The polychaete *Laeonereis acuta* represented 3.76% at the control area and 4.6% at the impacted one.

On the first two samplings (1d and 8d), the control area presented a little higher diversities than the impacted area. On day 14d, diversity at the impacted area was the least from the whole of the sampling period (0.33). On the following sampling, a decrease in diversity was verified at the control area (0,35) along with an increase at the impacted area (0.41). On the next samplings, successive species diversity increases followed along the experiment, culminating in a maximum on the last sampling day (147d), with 1.14 at the impacted area and 1.12 at the control area.

The smallest evenness indexes were found in samplings 1d (C=0.26; I= 0.29) and 14d (C=0.22; I=0.25). On the other hand, the highest indexes were seen in samplings 114d, with 0.67 and 0.42, respectively in the control and impacted area.

Through Ordination Analysis, the formation of two groups was observed at a level of similarity over 80% (Figure 2). The first group (GROUP A) comprehended virtually every area sampled during the experiment. The impacted and control areas presented a high similarity between one another (except the ones from sampling 1d). This group (A) was characterized by presenting a higher number of species than group B. Group B, in its turn, only integrated the first sampled day (1d, impacted area), thus being different (ANOSIM) from its correlate (1d, control area) in group A. In spite of having presented similar densities to group A, what differentiated this group was its smaller composition and number of species.

In the survival test (Table 2), the specific composition of the benthic macroinvertebrate association showed 11 species, among which 5 species of Crustacea, 4 of Polychaeta, and 2 of Mollusca. The impacted area presented 8 species (1 of Mollusca; 4 of Polychaeta and 3 of Crustacea). The total dead organism density at the control area was 10,625 ind./m², whereas the impacted area had 18,875 dead ind./m². Live organisms presented densities of 60,500 ind./m² at the control area and 46,000 ind./m² at the impacted one. Out of the total organisms found, the dominant species was the crustacean *Kalliapseudes schubartii* representing 3% at the control area and 47% at the impacted one. This test presented a significant

Table 2. *Alive and death organisms found in the control and impacted areas (density/m²).*

	control		impacted	
Species	alive	death	alive	death
MOLLUSCA				
Erodona mactroides	125	0	0	0
Heleobia australis	375	0	500	0
POLYCHAETA				
Heteromastus similis	100	0	750	0
Laeonereis acuta	1375	0	3125	125
Nephtys fluviatilis	625	0	375	0
Neanthes sp.	0	0	250	0
CRUSTACEA				
Diastilis sympterigiae	125	0	0	0
Kalliapseudes schubartii	55375	10625	40250	18375
Kupellonura sp.	625	0	0	125
Sphaeromopsis mourei	375	0	625	250
Sinelobus stanfordi	500	0	125	0

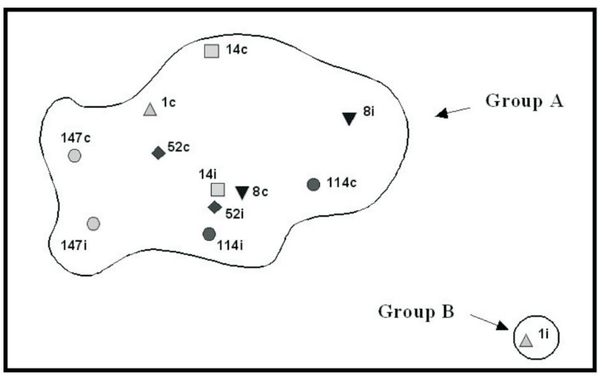

Figure 2. Multi-dimensional Scaling Ordination (MDS) Bray Curtis Similarity, where C= control area; I= Impacted area. Cluster separation according to similarity among species number, composition, mean density and diversity.

difference in organism survival between control and impacted areas, indicating mortality of organisms at the oil-impacted area in relation to the control area ($p<0.05$)

DISCUSSION

During the period of the experiment, the abiotic variables (salinity, temperature, pH, Eh, granulometry and organic matter content) did not show significant changes, that is, they were not influenced by the oil.

According to the results analyzed, it is may be concluded that there possibly was an effect of oil upon benthic macroinvertebrates, particularly the crustacean *Kalliapseudes schubartii* (mortality), and also upon the crustacean species *Sinelobus stanfordi*, *Kuppelonura sp.* and *Sphaeromopsis mourei* (escape).

Multivariate analysis showed the formation of two groups. The first group (Group A) virtually presented every sampling point from Group B, except for sampling 1i (1st sampling day, impacted region). By using ANOSIM, such difference was confirmed between the groups. The difference is related to species composition and density in the groups. In the following samplings, no significant difference was verified between control and impacted areas.

By analyzing only the area showing difference (sampling 1i), the absence of 3 species in the impacted area can be noted, when compared to the control area. They are the crustaceans *Sinelobus stanfordi*, *Kupellonura sp.* and *Sphaeromopsis mourei*. The way of life of those species in the superficial sediment layers may have contributed for their initial exclusion due to oil presence. The species *S. stanfordi* build superficial tubes and are small, slow organisms (BEMVENUTI, 1997). On the other hand, the species *Kupellonura sp*. is adapted to live in burrows in shallow muddy and sandy bottoms (RUPERT and BARNES, 1996), the same happening to the species *Sphaeromopsis mourei*, another superficial burrower (LOYOLA and SILVA, 1999). Several works have demonstrated that crustaceans are sensitive to organic contamination. ANGONESI (2000) did not find crustaceans in areas near urban sewage discharge, and related such finding to the sensitivity of the organisms to environmental stress, as their presence increased with the distance from the effluent discharge source. ROSA-FILHO (2001), studying the structure of benthic invertebrate associations subject to environmental contamination in the estuary of Patos Lagoon, found low density of crustaceans in places contaminated by metal and organic carbon, coming to agree with STARK (1998), who noted that crustaceans were more significantly abundant at uncontaminated places. PEARSON and ROSEMBERG (1978) also pointed to crustaceans as the most sensitive group to organic enrichment.

Such hypotheses raised in regard to the initial impact of oil upon macrobenthos on the first sampling day may be further supported by the survival test performed on the day of oil impact. The test also presented a significant difference in survival of the crustacean *Kalliapseudes schubartii* between impacted and control areas, indicating organism mortality due to oil at the impacted area in contrast to the control area ($p<0.05$).

Among the organisms which did not show significant changes between control and impacted areas are the group of estuarine polychaetes, *Laeonereis acuta*, *Nephtys fluviatilis* and *Heteromastus similis*. According to the analyzed data, such organisms suffer no influence from oil. Such tolerance had also been found by GESTEIRA and DAUVIN (2000), who verified that polychaetes presented resistance to oil spills and only amphipod species presented negative responses to the impact. The same happened during a great oil spill by the Norwegian coast, where no significant damage was found for the macrobenthos (KINGSTON *et al.*, 1995). On the other hand, FITZPATRICK *et al.* (2000), assessing the impact of tar balls, verified noticeable damaging effects upon macrobenthos. In other spills, total extinction of all animal life was verified, better studied in Amoco Cadiz (CABIOCH *et al.*, 1980), West Falmouth (SANDERS *et al.*,1980) and Toney Canyon (SOUTHWARD and SOUTHWARD, 1978).

Parallel to the oil effect, it is highly important to verify space benthic macroinvertebrate species variability (MORRISEY *et al.*, 1992) as an explanation for the differences found in those regions.

CONCLUSIONS

In view of such different responses from macrobenthos to oil, and based on the results that were presented and their analysis, it is necessary to be cautious before asserting the actual effect of oil upon a given species or community. In order to do that, it is necessary to have a thorough knowledge of the qualitative and quantitative structure of the benthic macrofauna for precisely identifying the effect of oil. Additionally, petroleum oils describe a great amount of natural substances based on hydrocarbons and refined petroleum products, each of which with a different chemical composition. As a result, each kind of crude or refined oil has distinct chemical and physical features which affect the way of spreading, oil degradation, and the damage they may cause to marine and human life (USEPA, 2001). Other important factors for verifying the magnitude of oil impacts upon estuarine organisms, according to CLARK (1997), would be a history of biota exposition to pollutants, season, environmental stress associated to temperature fluctuations and type of the affected habitat.

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