Monitoring Coastal *Río de la Plata* Oil Spillage

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Informative Abstract

On January 15, 1999, 250 m^3 of Hydra oil, affected 15 km of shoreline of Magdalena Municipality, *Río de la Plata* coast, Argentina, after the C/V Sea Paraná struck T/V Estrella Pampeana. Clean up efforts were performed, aided by natural processes to reduced mobile oil after the spill. From the 4th to 36th months 7 oil polluted sites were chosen for longer term assessment of recovery and 2 unoiled sites as controls. There followed monthly degradation of hydrocarbons, ecological and limnological monitoring.

Ecological monitoring evaluated the degree and recovery rate of environments and performance of cleaning up procedures. Manual cutting (in the areas where it was done), showed significant impacts during few months on wildlife habitats due to changes in vegetation structure and damage of plant root system by walking while cutting. The performance of different clean up procedures was studied along ecological recovering over time, focusing on the recovery by the bulrush and *Zizaniopsis bonariensis*. Based on a statistical design, the recovery of communities was compared after the following treatments (unintentionally applied): 1. oiled but not cleaned, 2. oiled and clear-cut, 3. oiled with weathered oil remaining on the surface and no cleaning, and (3) controls not oiled. The periodical follow up of the community structure (plants and birds) showed a slow recovery of clear-cut areas. All other sites showed, no community differences with controls, while the clear-cut areas were in middle successional stages (2002) and almost complete (2003).

Objectives of limnological study were to consider the quantity of plankton, larvae and juvenile fish in creeks. Objective for fish populations was to determine whether they had suffered impoverishment. No dead fish were reported. High number of fish larvae and juveniles estimated during warm periods within the studied area would indicate a normal fish reproduction, and sufficient food supply. Objectives for plankton were to know the structure and dynamics of communities from different studied habitats and compare with those of unoiled areas. Quantitative studies have revealed a diatom- and rotifer- dominated plankton community. Differences among sampling sites are not significant, as phyto and zooplankton structure and dynamics concerns.

Objectives of total hydrocarbons monitoring (biogenic + oil), were to evaluate residual hydrocarbons on a monthly basis, in sediments and periodically in water, bulrushes and clams. Oil concentrations decreased over time in all sites until the control sites were reached. There was a close correlation between the near shore geomorphology and the persistence of intertidal oil. Lowest HCs concentrations were found in the sandy sites. Coarse sediments were not a significant sink for petroleum hydrocarbons 1 year after the spill. During the summer of 2000, the oil that had penetrated the sand was liquid, resulting in re-oiling of the surface after flushing periods. Rather, the HCs appeared to be sorbed onto the fine sediment sites. Considering treatment strategies for oil spill affecting temperate beaches, we concluded that natural processes in tidally flushed sediments with great volumes of water, are enough to dissipate and degrade crude oil, returning beaches to the pre-spill conditions before 3 years.

1. Introduction to Hydrocarbons Monitoring

The Magdalena oil spill was released on January 15, 1999. Shell CAPSA with the assistance and collaboration of our University, ITOPF and specialized consultants, defined a Shoreline Response and Treatment Plan (PRYT) with particular objectives for each sector, depending on its characteristics, amount of spilled oil, crude condition and ecological and human use factors. The PRYT Monitoring began on April 15, 1999, with a main objective: trends evaluation in the oiled beaches recovery. No oil spill resembles one another and this is an unique case to perform a follow up of the response of wetlands and *Rio de la Plata* littoral, to the oil and clean up activities. The work was designed to provide scientific basis for deciding the treatment of coastline, to describe the evolution and identify mechanisms that contribute to oil degradation and understand natural cleaning processes.

Objectives of Hydrocarbons (HCs) Monitoring

Observations and sampling was designed: 1. To diagnose the beginning of HCs analysis. 2. To perform the follow up and recuperation of flora and fauna in different environments. 3. To verify oil degradation in order to reach HCs baseline of not oiled sites. Rio de la Plata receives HCs from sewerage (Buenos Aires, Berazategui, La Plata, Ensenada, Berisso, Magdalena, etc.) and industrial effluents (shipyard, steel industry, oil refinery, petrochemicals, etc.). 4. To evaluate the pertinence of recommendations and predictions in the treatments. 5. To describe tendencies in the weathered and re-oiling of sediments.

Total HCs Monitoring. The monitoring of total HCs concentrations in water, sediments, bulrush and clams from 7 principal affected sites: Alberdi (ABE), Atalaya (AT), Juncal 1 (J1), Juncal 2 (J2), Gauchito (GA), Ricardo (RI) y Alborada (ABO) and 2 reference not oiled sites: El Destino (ED) and La Balandra (LB), downstream and upstream, respectively, is part of an integral biologic, geochemical and chemical study. Observations and samplings were done monthly since Apr/99 to Oct/00 and thereafter to Apr/01 each 2 months. Last campaign was in Feb-Mar/02.

Sediments. One form to follow the evolution of a polluted environment is by its sedimentary register. Poluents deposited on sediments give an integrated view of the water column, at least the time space when water is covering them. Sediments were used to follow the evolution of oiled sites with water and biota analyses.

Sediments Without Visible Weathered Oil. The quantitative analysis of HCs in sediments began when no mobile oil was observed (Apr/99). Since then, monitoring of HCs ended when HCs concentrations fell as low as those of reference sites.

Sediments with Weathered Oil. After the oil spill, weathered oil remained spread in aleatory and patchy form, of around 0.5 to 1 m^2, in layers of a few mm. Thus, crude oil reached around 10 m inland and 18 km of coastline was affected. Analysis of visible weathered oil began in J2, GA and RI in Apr/00.

Re-oiling. The resurgence of oil trapped in the subsurface by lixiviation or trampling could be because of the washing that diminish as a consequence of weak and quiet high tides, summer heat, vegetal growth with roots generation, soil movements, particle size distribution, ground water rise, etc.

Flora and Fauna: The bulrush *(Schoenoplectus californicus)* was chosen due to its perennial character, abundance, widely distributed and it incorporates and bioconcentrates HCs, contributing to the bioremediation of oil spill detoxifying sediments, water or air, to turn into innocuous metabolites or blocking them. Mollusks are useful to monitoring pollution because of their wide distribution, abundance, sessile behavior in adults, ease of collection and ability to accumulate HCs. The Asiatic clam *Corbicula fluminea* was chosen.

Materials and Methods

Sampling, Transport and Storage.
Samples of sediments with and without weathered oil, bulrush, clams and water were collected in each campaign, monthly, along 3 years in 7 oiled and 2 unaffected sites.

A) Sediments Without Visible Weathered Oil. Sediments were aleatorily sampled when visible oil disappeared from oiled sites. Sediment observation and sampling were done from Apr/99 until HCs concentration fell to those of reference sites. Samples were collected with hand-held stainless steel coring tubes (length 50 cm, diameter 2,4-4 cm). Finally, sediment observation and sampling were repeated in Feb-Mar/02 in all sites and in those with weathered oil in past years. Multicorer extraction of sediments consisted of 6-7 successive take-up around 8 cm depth, each 2 m approximately, in transects of 5-10 m. Corers were covered with aluminum (Al) foil washed with hexane-dichloromethane (DCM) 50:50, and kept at -20ºC until analysis.

B) Sediments With Visible Weathered Oil. Sampling, etc. as above mentioned. Sediments with weathered oil had a patchy distribution, occasionally dissimulated in the vegetation. Because it is not abundant, the probability of enter in an aleatory sampling is low. Thus, this oil was systematically sampled, looking for it.

C) Bulrush *(Schoenoplectus californicus)*. Stems were cut with stainless steel knife, multifold, wrapped in Al foil previously washed with hexane-DCM 50:50, transported and kept at -20ºC, until analysis.

D) Clams *(Corbicula fluminea)*. Clams were sampled, wrapped in Al foil washed with hexane-

DCM 50:50. Clams were transported and kept at -20ºC until analysis. Soft tissues were pooled and homogenized.

E) Water. Samples were collected with one gallon amber bottles opened at around 15 cm depth. Each sample of 4 l from reference sites and 2 l from GA was extracted *in situ* with HCs free hexane. Hexane extracts were transported and stored in flasks to -20ºC.

Other analysis(UNEP 1992)
Sediments and bulrush were dried overnight to room temperature. A subsample of sediment was used to determine humidity drying at 105ºC to constant weight. The organic matter (OM)(ignition loss 6 hs at 550ºC) and hexane extractable organic matter (HEOM), were determined in sediments of all campaigns depending their values on the quantity of oil in sediments. Besides, other factors that affect the weathered oil in beaches like particle size distribution, oil location either above or under tides, rainy seasons, presence and type of vegetation in the area, were considered. In the first campaign testing pits were done in J1 and 2, in order to understand the behavior in natural and oil spill sediments throughout depth.

Extraction, Lipids Clean up, Fractionation and Analysis (UNEP 1992)
Sediment subsamples (20 g) were mixed with anhydrous Na_2SO_4 to extract humidity. Bulrush were cut in pieces, quartered and 5 g (wet wt) were grinded and homogenized with anhydrous Na_2SO_4. Wet soft tissues of 25 clams were grinded. A subsample of around 12 g was homogenized with anhydrous Na_2SO_4. Sediments, bulrush and clams were Soxhlet extracted for 12 hs with hexane:DCM, 50:50. HEOM was determined in an aliquot of extract. In all cases, extracts were concentrated and purified by adsorption chromatography in silica : alumina column, activated by heat and partially deactivated with distilled water (5% w/w). Two fractions were obtained eluting with hexane (aliphatic) and hexane:DCM (50:50)(no saturated and PAHs).

The lipids clean up was standardized with subsample loaded with mixture of aliphatic and aromatic HCs. The first fraction (saturated HCs) was analyzed by gas liquid chromatography with flame ionization detector (GLC-FID, H&P 5890 Serie II, fused silica capillary column, J&W Sci, DB-1, 30 m long, 0.32 mm i.d. and 0.25 µm film thickness) in a splitless mode. Data were processed in a H&P Chemstation 3365. The first calibration and detector linearity determination were done injecting quantitative solutions (n-alkanes at 4 concentrations). Aliphatic HCs were quantitated against a set of standard solutions containing n-alkanes (n-C_{10}-n-C_{32}). UCM (unresolved complex mixture) was quantitated in each sample using the average response factor of n-alkanes in the range of retention time. PAHs determinations were carried out by GLC-mass spectrometry (Metcalfe et al., 1997).

Quality control for all samples included a blank and a full analysis of a spiked sample with a mixture of aliphatic and aromatic HCs, also used to standardize the clean up column. The standard solution included phytane, pristane and n-octadequene as surrogate. Recovery was 68.5±12.8% in the range n-C_{10}-n-C_{32}. Detection limit for n-alkane in sediments was 20 ppb (ng/g, dry wt) and for vegetal and animal tissues 20 ppb (ng/g, wet wt). All determinations were by duplicates (Janiot et al., 2003).

Results

Sites were put together following these criteria: Not affected by the oil spill: ED and LB; the most affected sites by the oil spill and similar particle size distribution: J2, GA and RI; and similar affectation degree and OM content: ABE, AT, J1 and ABO.

Reference Sites: ED and LB.

1. Sediment samples showed low values of OM and HEOM, LB: 0.3-1.7% and 0.1-0.5 mg/g, respectively and ED: 0.9-1.1% and 0.04-0.5 mg/g, respectively, and therefore high values of sand (almost 95% of the mineral fraction) and low values of clay & silt (Table 1).

2. After 3 years of sampling, total HCs sediments were between 0.6-8.5 ppm (LB) and < Quantitation Limit (QL): 3.2 ppm (ED). The n-alkanes were in the HCs range C_{11}-C_{34} and concentrations below 2.7 ppm (LB) and 2.0 ppm (ED). UCM values were below 7.7 ppm (LB) and 1.2 ppm (ED) indicating few cyclic components, naphtenic type, of petrogenic origin. Phenanthrenes+anthracenes were the greatest components in sediments of both sites (May/99) with 86.9% of total PAHs equivalent to 0.64 ppm, LB and 1.31 ppm, ED (Fig. 1).

3. Total HCs in water did not exceed 1 ppb in both sites with UCM absence (Fig. 2).
4. In bulrush, total HCs from both sites oscillated between 16-46 ppm (LB) and 11-45 ppm (ED)(Fig. 3). The n-alkanes, range C_{11}-C_{40}, showed concentrations below 22 ppm (LB) and 30 ppm (ED). UCM was below 24 ppm (LB) and 15 ppm (ED).

5. Total HCs in clams from ED ranged between 210-510 ppm, with size range between 20.5-23 mm, respectively (Fig. 4). The n-alkanes, oscillated in the HCs range C_{14}-C_{34} and concentrations between 38-94 ppm. UCM values from 168 to 419 ppm.

Oiled Sites: J2, GA and RI.

1. Total HCs in testing pit of J2 reached the highest value (473 ppm) in the surface layer 0-10 cm depth, with OM 4.7% and HEOM 6.0 mg/g. In the horizon 10-26 cm the concentration decreased to 3.6 ppm and the OM and HEOM to 2.0% and 0.2 mg/g, respectively. Finally, in the deeper layer 26-30 cm, OM and HEOM lowered to 1.0 % and 0.4 mg/g, respectively and HCs were < QL.

2. Surface sediments presented high values of OM and HEOM in the 3 sites in comparison with the rest and oscillated in 0.8-4.7% and 0.3-6 mg/g, respectively (Table 1). These sediments are rich in clay (10-20% of mineral fraction) and variable values of silt and sand (Table 2). J2, showed low silt % and high of sand while the GA sediments had high silt %. Sediments from RI showed silt and sand values between the other two. Total HCs in sediments without weathered oil from J2, lowered from 473 ppm (Apr/99) to 19.2 ppm (Jan/00)(Fig. 5). This value remained low until Feb/00 when 27.4 ppm was registered, probably because of weathered oil processes. In the summer-autumn/00 the values in GA and RI did not exceed 10 ppm (Fig. 4) and remained low until Jun/00, last sampling without weathered oil. The n-alkanes found in J2 were in the range of C_{11}-C_{34} showing concentrations of 71 ppm (Apr/99) that lowered to 3.2 ppm (summer/00). The alkanes in GA and RI were below 2 ppm (autumn/00). The UCM showed values below 7 to 8 ppm in RI and GA, respectively (autumn/00)(Fig. 5). PAHs values from sediments from J2 were 1.74 ppm with 81.4% for phenanthrenes+anthracenes and 8.3% chrysene (Fig. 1). The predominance of these compounds indicate that they are the most refractory to degradation.

Nevertheless, total PAHs concentrations, were lightly higher than reference sites.

3. Sampling sediments with weathered oil began in Apr/00 (J2); Jun/00 (RI) and Jul/00 (GA). They covered around 1.35% of oiled beaches in the 2000. The HCs in sediments from J2 lowered from 21475 ppm (May/00) to 7427 ppm (Feb/01) and 157 ppm (Feb-Mar/02)(Fig 5). The sites were controlled in their recovery, but their treatment was discouraged because it would cause more damage than the oil. In the penultimate campaign, the weathered oil sites were covered with vegetation without weathered oil and samples were not taken. Nevertheless, in Feb-Mar/02, the 3 sites were sampled again. GA and RI also showed high values (spring-summer/00) fell to 890 ppm (Feb/01) and 94 ppm (Feb-Mar/02); 658 ppm (Apr/01) and 29 ppm (Feb-Mar/02), respectively (Fig. 5). RI was the last site with visible weathered oil. The decrease of total HCs was determined by a fell in n-alkanes and UCM. The n-alkanes were in the range C_{11}-C_{34} reaching to C_{40}.

4. Total HCs in water from GA was 39 ug/l (Sept/99) and lowered to 1 ug/l (Apr/01, following the decreasing tendency of the other matrixes (Fig. 2).

5. Bulrush stems from J2 were stained with oil in Apr/99 (total HCs 47336 ppm). Oil vanished in May/99 and total HCs fell to 789 ppm and in Jun/00 to 40 ppm. The total HCs from bulrush in GA and RI lowered to 60 and 123 ppm, respectively, in Jun/00. Thus, bulrush sampling was stopped until Feb-Mar/02 when it was done again. The ratio UCM/alkanes showed higher values in bulrush from GA, RI and J2 than in reference sites. The PAHs from bulrush in J2, reached 2.39 ppm corresponding 41.5% to phenanthrenes+ anthracenes and 39.1% to benzo (a) and (k) fluoranthene (Fig. 6 7).

Oiled Sites: ABO, J1, AT and ABE.

1. Total HCs testing pit of J1 reached the highest value (1190 ppm) in the layer between 11-26 cm, with 6.1% of OM and 14.6 mg/g of HEOM. In the surface horizon 0-11 cm, the concentration was 950 ppm and the OM and HEOM were 2.9% and 26.8 mg/g, respectively. Finally in the deeper layer, 26-38 cm the OM and HEOM fell to 1.3% and 0.4 mg/g, respectively and the total HCs level reached 17 ppm.

2. Surface sediments of the 4 sites presented low OM values and lightly higher than reference sites: 0.7-2.9%. Likewise, HEOM values oscillated between 0.06-0.8 mg/g, but April and May/99, which showed 26.8 and 25.0 mg/g, respectively (Table 1). These sediments had low % of clay and silt (fines and coarse) and high (more than 86 %) of sand (Table 2). The sediments of J1, AT and ABE have never had visible weathered oil.

3. Re-oiling was not observed in J1, site of around 10000 m^2, since the Apr/01 campaign.

4. Total HCs in sediments without weathered oil from J1 were 982 ppm (Apr/99) reaching values <QL in Feb-Mar/02 (Fig. X). In ABE total HCs lowered rapidly from 89 ppm (Jul/99) to <QL (Feb-Mar/02). ABO showed low concentrations with 139 ppm (May/99) and fell notably to <QL (Feb-Mar/02)(Fig. 2). Since Jun/99 HCs values in sediments from AT were low 9.6 ppm and as a consequence AT was not analyzed until Feb-Mar/02, when it showed 1.6 ppm (Fig. 4). Alkanes values from ABE and J1 were in Spring/00 <1 ppm. Sediments from both sites in

addition to ABO, once ended sampling, showed UCM concentrations <QL, meaning almost total recovery of the sites. The PAHs concentration in sediments from J1 (May/99) were similar to that from J2. Percentage distributions of PAHs in sediments from J1 and J2 showed <5% of those with low molecular weight and high volatility: naphtalene, acenaphthylene, acenaphtene, fluorene and phenanthrene+anthracene. This indicated that an important loss of PAHs by evaporation had occurred during the first months after the oil spill. The phenantrenes+anthracenes in sediments from J1 and J2 reached 72.1 and 81.4%, respectively. Total HCs values in sediments with weathered oil were only observed in ABO (Aug/00), with values of 16205 ppm.

5. Total HCs values found in bulrush of J1 were 440 ppm (Apr/99), 17 ppm (Jun/00) and 74 ppm in summer/02. Bulrush from ABO fell from 1753 ppm (May/99) to 42 ppm (Feb-Mar/02). In ABE, bulrush fell from 460 ppm (Aug/99) to 59 ppm (Feb-Mar/02) while in bulrush from AT lowered from 330 ppm (Jun/99) to 54 ppm (Feb-Mar/02)(Fig. 3). Alkanes concentrations in bulrush from these sites lowered to values <32 ppm while UCM below 42 ppm (Feb-Mar/02).

6. Clams from J1 showed total HCs values between 182-400 ppm along the months since the beginning of Monitoring, corresponding to a mean size range of 24-36.3 mm (Fig. 4). A dominance of UCM over the alkanes was observed.

Discussion

Oil HCs are hydrophobic compounds and when they are released into a water body they are adsorbed onto organic surface complexes from suspended particulate materials on fine bottom sediments in aquatic ecosystems (Voudrias & Smith, 1986)(Owens et al., 2003).

Total HC concentrations in water from GA reached similar values to reference sites (LB and ED) in Apr/01. The environmental fate of HCs depends on their physicochemical properties: volatility, water solubility and lipophilicity, as well as sediment properties: OM content and particle size distribution. Immediately after spilled oil, evaporation and dissolution are the more important mechanisms for removing low molecular fractions. In general a lost greater than half of the light oil spilled, occurred before it reached the coast. Another processes will follow for weathered oil remotion: biodegradation, metabolization by microorganisms (bacteria, yeast & fungi) and macrophytes, surface photooxidation in water and sediments.

After monitoring along three years 2 phenomena undergone by remnant oil were verified: 1st The weathered oil covering an area of, approximately, 4 m^2 and 2nd Re-oiling covering an area of 0.25 m^2at the end of 2001. Both phenomena were not observed in 2002.

The weathered oil occurs when oil adsorbed onto surface organic complex and fines sediments, undergoes biotransformation (microbial degradation) and physicochemical processes (volatilization), river runoff, tidal flushing and during storm periods, leaching and oil absorption by macrophytes and animal biota, etc. High OM and clay and low sand contents, were found in surface sediments from J2, GA and RI with respect to the other sites. Concomitantly, the higher hydrophobic HC concentration, were found in these sites. Moreover, due to the high adsorption capacity of the surface sediments, little lixiviation was observed. During Apr/00, the weathered oil showed only a thin layer covering an area of 0.5-1m^2 (1.4 % coastline). Weathered oil patches were aleatory spread although were preferentially found in J2, GA, RI and ABO sites. An scarce or null vegetation growths, was found onto these patches. As a consequence of several weathered processes above mentioned, patches disappeared throughout monitoring progress, being Feb/01 the last time that they were observed in J2, GA and RI, with total HC concentration ranging between 29-157 ppm, dry weight. The presence of weathered patches in these sites, after long time, could be justified by the high OM content in these sediments, leading to a less degradation of HCs.

The 2nd phenomenon observed was the re-oiling. Oil that had penetrated in sediments as a result of leaching and trampling on, during the summer, was present in liquid and mobile state. Thus, ground water pushing up and heating of sediments, during absence of high tides, resulted in re-oiling of the surface. It was observed in J1, GA y ABO, sites rich in sand and HCs were not absorbed in the surface. In relation to the extent of oiled beaches the re-oiling cases were scarce. They happened sporadically at a rate of 1-2 drops by site and campaign in 1999, 00 and 01. They were not observed in 2002.

After 3 years of the oil spill, total HCs concentrations found, are within literature ranges for: Rio de la Plata , surface and intertidal sediments: 0.06-240 ppm (aliphatics), 0.05-17 ppm (aromatics) (Colombo et al., 1989). Australia, NW coast, mesolittoral sediments: 0.015-0.050 ppm (aliphatics), 0.002-0.005 ppm (aromatics) (Pendolley, 1992)

One of the sources of HCs in sediments could be the oil, but also sewage and soil runoff. Biogenic sources include algae, aquatic animals, other microorganisms and land vegetation. Odd alkanes in the range C_{15}-C_{21} were described as important components of algae. The most important by quantity in vascular plants, like bulrush, were alkanes of C_{23}-C_{31}, preferably odd. The HCs levels in sediments in almost all the sites, since Jun/99, were in the range C_{23}-C_{31}, indicating the important contribution of land vegetation to the HCs load of sediments. This natural transport of HCs would be the consequence of an important soil runoff and river level oscillations (rainy SE winds, low tide-high tide) carrying undertow, masking oil HCs.

Macrophytes are known to accumulate oil HCs in theirs tissues. In bulrush, stems are exposed to HCs from water column and atmosphere, while from bottom sediments throughout roots. Once absorbed, oil HCs can be degradated together with theirs own biogenic HCs. So, all HCs can be degradated by the macrophyte. As a consequence, bulrush degradated oil HCs. During maximum growth season, they do it at a rate of 2 cm/day. It allows to understand the speed of the process that caused fast disappearance of HCs in affected sites. So, sampling was stopped in Jun/00 until it was reinitiated in Feb-Mar/02. The results of bulrush HCs analyses of the last campaign reflect also the presence of biogenic HCs in all sites.

Mollusks incorporate HCs by food and water filtered by gills. In addition to pollution events, the factors that determine concentration of HCs in clams are: age/size, available food, sex, gonad maturation and spawn. These factors would be responsible of the lack of a define trend in HCs levels found in clams from J1 through time, as well as the no significant differences in HCs levels between clams collected from oil spill site and reference site. Experimental research have demonstrated that invertebrates are sensitives to oil HCs, mainly PAHs compounds. Benthic communities may be altered by HCs concentrations <50 ppm in sediments, although they depend on compound toxicity (Kingston, 1992). After the oil spill, sudden changes occurred in oil composition, thus, the weathered oil that reached the bottom was less toxic because the most toxic compounds (benzene, naphthalene) evaporated easily and dissolved in water and did not induce significative changes in biota (Lee et al., 1980). Therefore, clams from J1 have survived to the oil spill, to the Treatment Plan and were useful to Monitoring along 3 years.

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		LB	ABE	AT	J1	J2	GA	RI	ABO	ED
NW	**OM**	0.3-1.7	1.4-1.8	1.3-4.3	1.2-2.9	0.9-1.3**	0.8-2.0	0.9-1.2	0.7-1.3	0.9-1.1
	HEOM	0.1-0.5	0.1-0.7	0.1-0.3	0.1-0.8*	0.4-0.9**	0.4-0.6	0.3-0.6	0.1-0.6	0.1-0.5
W	**OM**					2.5-30.3	2.9-11.4	1.7-10.5		
	EHOM					1.1-76.8	0.8-70.9	0.2-131		

Table 1: Ranges of organic matter (OM) percentage and hexane extracted organic matter (HEOM), expressed as mg/g, in sediments with and without weathered oil over time.
LB: La Balandra, ABE: Alberdi, AT: Atalaya, J1: Juncal 1, J2: Juncal 2, GA: Gauchito, RI: Ricardo, ABO: Alborada, ED: El Destino. NW: non weathered, W: weathered.
*: April and May/99, 26.0 mg/g (maximum value)
**: April/99, 4.7 % and 6mg/g (maximum value)

Sampling Sites	Particle size distribution (%)			
	Clay	Silt		Sand
		Fine	Coarse	

LB	2.0	1.8	0.4	95.8
ABE	4.9	5.8	3.4	85.9
J1	4.1	1.9	3.7	90.3
J2	10.6	6.6	5.3	77.5
GA	19.2	17.8	15.9	47.1
RI	13.4	13.4	10.3	62.9
ABO	4.2	3.4	4.1	88.3
ED	3.7	0.8	0.7	94.8

Table 2: Particle size distribution (%) of sediments from sampling sites.
LB: La Balandra, ABE: Alberdi, J1: Juncal 1, J2: Juncal 2, GA: Gauchito, RI: Ricardo, ABO: Alborada, ED: El Destino.

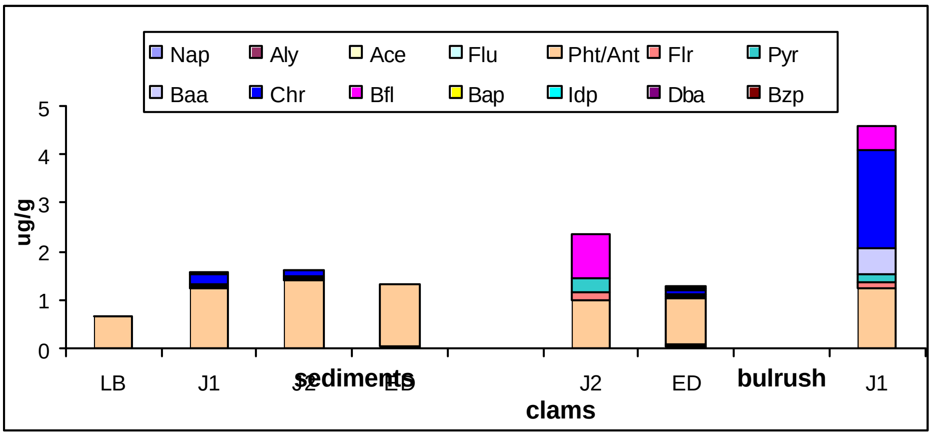

Fig. 1

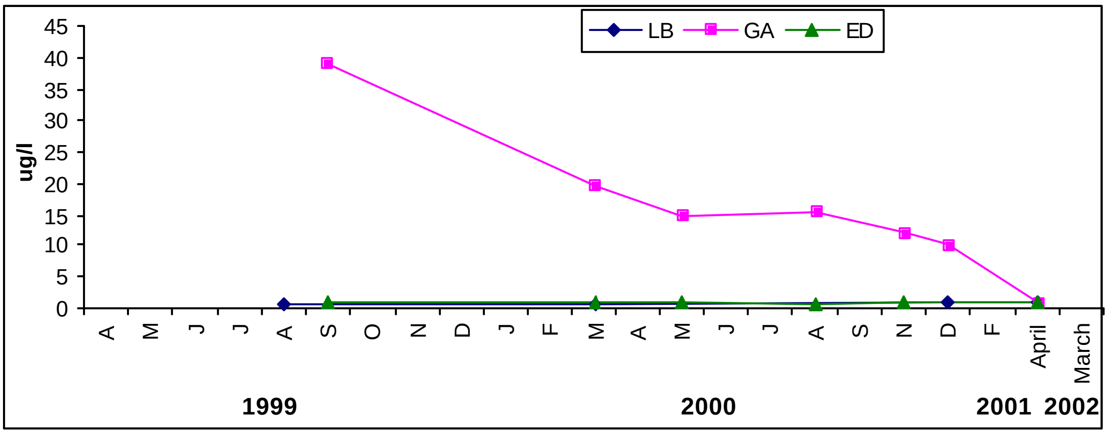

Fig. 2

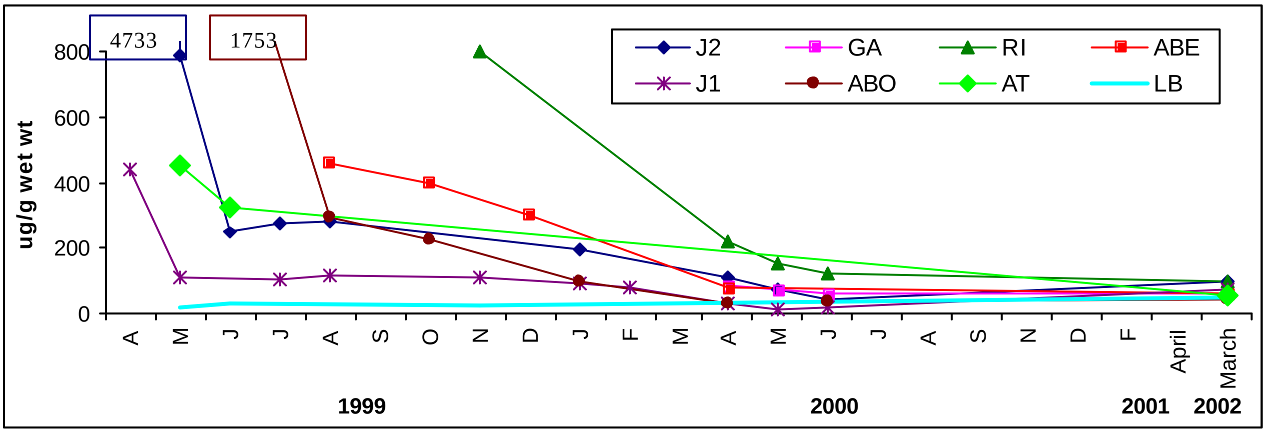

Fig. 3

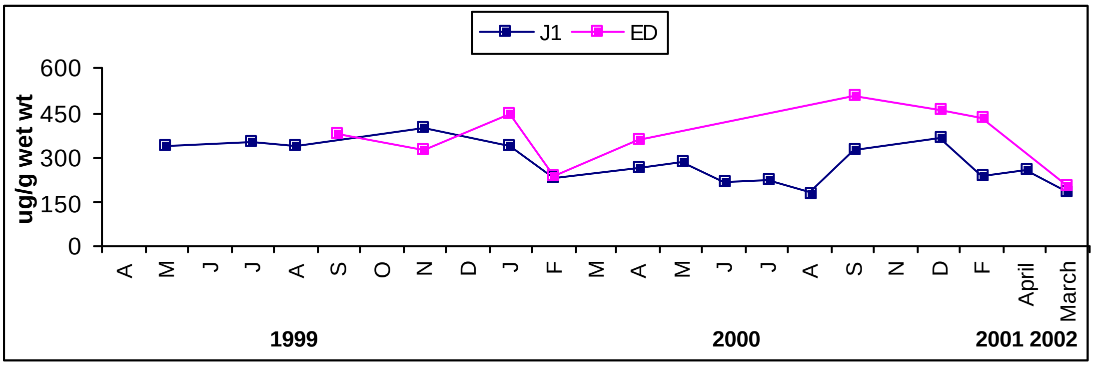

Fig. 4

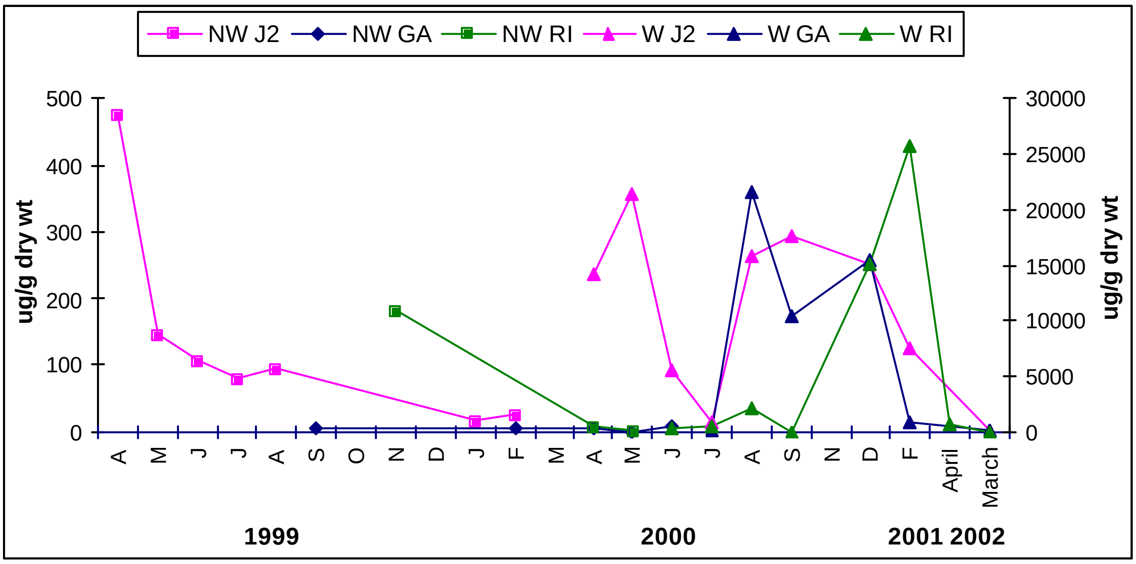

Fig. 5

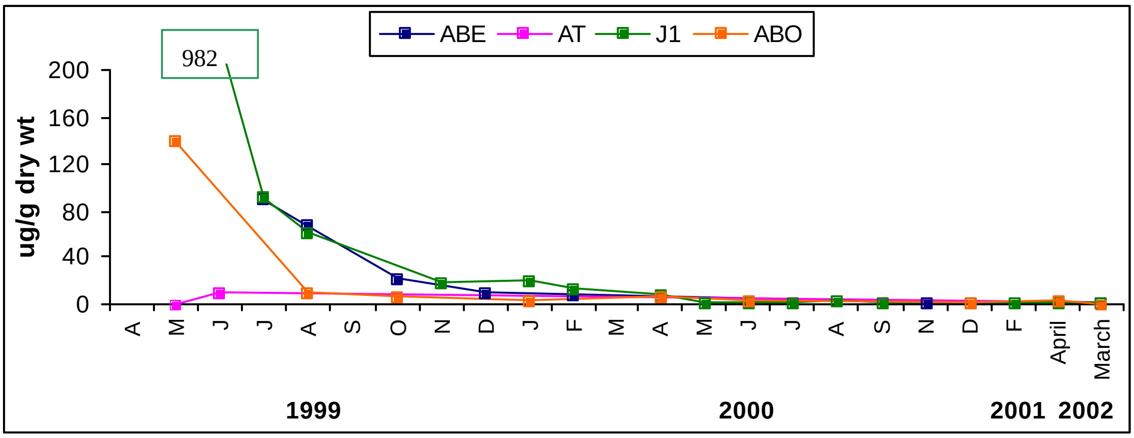

Fig. 6

Figure 1: Polynuclear aromatic hydrocarbons in sediments (ug/g dry wt), bulrush and clams (ug/g wet wt) from La Balandra (LB), Juncal 1 (J1), Juncal 2 (J2), and El Destino (ED) sampling sites.
Nap: naphthalene, Aly: acenapfthylene, Ace: acenapthene, Flu: fluorene, Pht/Ant: phenanthrene/anthracene, Flr: fluoranthene, Pyr: pyrene, Baa: benz[a]anthracene, Chr: chrysene, Bfl: benzo[b]- and benzo[k]fluoranthenes, Bap: benzo[a]pyrene, Idp: Indeno[123-cd]pyrene, Dba: dibenz[ah]anthracene, Bzp: benzo[ghi]perylene.

Figure 2: Total hydrocarbons concentrations (ug/l) in water samples.

Figure 3: Total hydrocarbons concentrations (ug/g wet wt) in bulrush samples from Juncal 2 (J2), Gauchito

(GA), Ricardo (RI), Alberdi (ABE), Juncal 1 (J1), Alborada (ABO) and La Balandra (LB), as reference site.

Figure 4: Total hydrocarbons concentrations (ug/g wet wt) in whole soft tissues of clams from juncal 1 (J1) and El Destino (ED) sites.

Figure 5: Total hydrocarbons concentrations (ug/g dry wt) in sediments samples from Juncal 2 (J2), Gauchito (GA) and Ricardo (RI) sites.
NW: non weathered oil (left axis), W: weathered oil (right axis).

Figure 6: Total hydrocarbons concentrations (ug/g dry wt) in sediments samples from Alberdi (ABE), Atalaya (AT), Juncal 1 (J1) and Alborada (ABO) sites under non weathered oil conditions.

2. Phyto, Zoo and Ichthyoplankton Monitoring

Introduction

Relatively little is known about HC contamination effects upon plankton community. The scarce information available results heterogeneous, and it is based mainly on laboratory experiments, mesocosm enclosures, and less frequently, on field works (Davenport, 1982). The *in situ* observation of the HC contamination effects on plankters results difficult due precisely to the natural fluctuations they suffer spatially and temporally (Michel, 1975; I.T.O.P.F., 1991).

The plankton community from *Río de la Plata* estuary has been subjected to discrete studies, in terms of time and space. Fish inhabiting this estuary correspond to freshwater, marine, and exclusively estuarine species. But at the inner part of *Río de la Plata* estuary, there is a typically freshwater fish fauna, though marine fish sometimes can entry (Menni, 1983). The aim of the limnological monitoring was to evaluate the viability of plankton, fish larvae and juvenile fish populations in canals and creeks following the oil spill, given that these areas are known to be important fish nursery.

The specific objective for fish populations was to determine if they have suffered impoverishment after the oil spill, by means of direct observation on field and the estimation of fish larval and juvenile abundances. The specific objectives for plankton were to know the structure and dynamics of phytoplankton and zooplankton communities from different ecosystems and to compare their behaviours in different studied areas with those of unoiled areas to the South (ED) and North (LB).

Materials And Methods

Ichthyological samplings were bimonthly planified for the period Dec/00-Apr/01 in GA Creek and Canal 1 of ABO within the studied area around Magdalena, and in LB Creek (to the North) and Juan Blanco and Morales creeks (to the South) as control areas (Table 1).
Trawler fishing (2 mm mesh net) along the coastline was made, given that littoral zone of canals and creeks represents a suitable habitat for fish larvae and juveniles to refuge. Captures from the coast to mid-waters were added, when the littoral zone was also covered with free-floating plants. The number of meters trawled in each fishing was variable, according to coastline development of sampling sites, and the water level reached at sampling moment.

Phyto- and zooplankton samples were monthly collected between May/99 and Sep/00 and bimonthly between Nov/00 and Apr/01 in 6 sites (4 included within the studied areas and 2 selected as controls): ED (southern control); ABO (canal 1); GA Creek; J2; J1, and LB (northern control). Qualitative and quantitative subsurface plankton samples were taken in each sampling site, using a 35 µm mesh net, by means of filtering 50 l of water per sample, except in ED, J1 and J2, where 100 l of water were filtered for zooplankton (APHA, 1998; Macluf *et al.*, 1998). Temperature (armoured thermometer), depth, transparency (Secchi disk), pH (Hanna waterproof pH meter) and salinity (Hand Refractometer REF211 model) were measured *in situ* at each site. Qualitative plankton samples were carried alive to laboratory for taxonomic determination of the plankters. Quantitative samples were collected always by triplicate and fixed with Transeau (APHA, 1998). Counts were performed in Sedgwick-Rafter chamber (José de Paggi and Paggi, 1995). Species abundances were expressed as number of individuals per liter. Diversity was estimated by Margalef index (Margalef, 1967). Trophic status for the six sampling sites was determined by means of different indices: simple Nygaard index (Nygaard, 1955; Brook, 1965; Hall and Smol, 1999), complex Nygaard index (Nygaard, 1949), Sladecek index (Rocha and Guntzel, 1998), and Karabin index (Ravera, 1996), that introduce the relationship among phyto- and zooplankton species numbers and/or among their densities.

Results

Five hundred and one specimens of fish belonging to 21 species, 10 families, and 6 different orders were captured. Among them, *Prochilodus platensis* ("sábalo") and *Odontesthes* spp. ("silverside") are species of great commercial value and local consumption, respectively. The 18% of the specimens captured were larvae, the 87.5% of them corresponding to two species of the genus *Odontesthes*. The more abundant and diversified captures were obtained in Morales Creek (one of the two southern control sites), though with a low percentage of fingerlings (3.5%) not belonging to *Odontesthes* spp. In Juan Blanco Creek (the other southern control site) no fingerlings were collected. On the contrary, in LB (the northern control site) few fish specimens were captured, the 73% of them being larvae of *Odontesthes* spp. Within the studied area, few specimens of three species of "tetras" (Characidae) were captured in GA Creek, but no fingerlings. Instead, during Dec/00 sampling, the 93% of the capture obtained in Canal 1 of ABO was constituted by fish larvae of *Odontesthes* spp. ("silverside").

The number of phytoplankton and zooplankton species registered during May/99-Apr/01 monitoring was greater in the studied sites (164 phytoplankton and 175 zooplankton species) than in control sites (144 phytoplankton and 137 zooplankton species), but the relative proportion of the different groups of plankters was similar at both ecosystem types. Central diatoms constituted the more abundant group, while the pennate diatoms were the more diversified. In the zooplankton, the rotifers were the more abundant group as well as the more diversified. Higher phytoplankton densities were observed during Nov/99-Apr/00 period in the six sampling sites. The highest peak of blue-green algae occurred in Canal 1 of ABO during the 1999/2000 summer. On the contrary, zooplankton densities were higher in the two control areas. Rotifers registered the greatest peak during the warmer period of 1999.

Environmental Parameters

The pH values ranged from neutral to slightly alkaline, with an average value of 7.6 and 7.5 at ED and LB control sites, respectively; and 7.3 in ABO, 7.5 in GA Creek; 7.6 at J1 and J2. Minima values of 6.8 were measured in different sampling periods in the affected and in the control sites. Maximum value of 9.2 was registered at GA Creek in Sep/99. The mean water transparency value was greater in ABO, with 0.18 m of Secchi disk; following 0.16 m in GA Creek; 0.14 m in LB; 0.13 m in J1; 0.08 m in J2, and 0.05 m in ED. In five samplings the Secchi disk registered values of zero at coastal waters from ED. The salinity values were zero in most samples. The maximum value of 0.2 per thousand was measured in ABO and ED in Aug/99; and in J1, J2, and ABO in Jul/00. Depth was very changeable in all the sampling sites, according to the high and low tides. Samples were always taken when depth was no lesser than 0.40 m, in order to avoid bottom disturbance and the removal of non-planktonic organisms.

Discussion

The naked-eye field observation of a high number of fish larvae and juveniles during 1999-2000 warm period in creeks and canals, within the oiled area around Magdalena, in addition to the estimation of a great number of fish larvae, corresponding to *Odontesthes* spp. in Canal 1 ABO, would indicate a normal fish reproduction, and a appropriate food supply for their larvae following the oil spill.

Quantitative studies have revealed a diatom- and rotifer- dominated plankton community.
All sampling stations represent mesotrophic-eutrophic systems, being La Balandra (the northern control site) the more eutrophic one, possibly due to the influence of the more intensive antropic activities nearby. Differences among sampling sites are not significant, as phytoplankton and zooplankton structure and dynamics concerns.

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Table 1. Ichthyological samplings*

Species	Site	Date	Trawl	Nºfish
Callichthys callichthys	Morales Creek	April/01	16	4
Cichlasoma facetum	Morales Creek	April/01	16	1
Cnesterodon decemmaculatus	La Alborada C.	Dec/00	22	1
	Juan Blanco C.	April/01	24	3
	Juan Blanco C.	Feb./01	34	28
	Morales C.	Dec/00	30	36
	Morales C.	April/01	16	2
Corydoras microps	Morales C.	Dec./00	30	32
	Morales C.	April/01	16	173
	Morales C.	April/01	16	173
Hoplias malabaricus (larvae)	Morales C.	Feb./01	16	4
	Morales C.	April/01	16	3
Loricarichthys anus	La Balandra C.	Dec./00	76	1
	Juan Blanco C.	Dec./00	47.5	8
	Juan Blanco C.	Feb./01	34	1
	Morales C.	April/01	16	1
Odontesthes sp.1 (larvae)	La Alborada C.	Dec./00	22	65
	La Balandra C.	Dec./00	76	6
Odontesthes sp.2 (larvae)	La Balandra C.	Dec./00	76	5
Oligosarcus jenynsi	Juan Blanco C.	Dec./00	47.5	1
Pimelodus sp. (larvae)	Morales C.	Dec./00	30	1
Prochilodus platensis (larvae)	Morales C.	April/01	16	1
Rhamdia quelen (larvae)	Morales C.	Dec./00	30	1
Symbranchus marmoratus (larvae)	Morales C.	April/01	16	2
Tetragonopterinae sp.1	La Alborada C.	Dec./00	22	1
	Juan Blanco C.	Dec./00	47.5	8
Tetragonoperinae sp.2	Gauchito C.	April/01	65	3
	La Alborada C.	Dec./00	22	3
	La Balandra C.	April/01	30	3
	Juan Blanco C.	Dec./00	47.5	3
	Juan Blanco C.	April/01	24	5
	Morales C.	Feb./01	16	6
Tetratonopterinae sp.3	Gauchito C.	Dec./00	75	1

	Juan Blanco C.	Dec./00	47.5	3
	Morales C.	Dec./00	30	59
Tetragonopterinae sp.4	Gauchito C.	Dec./00	75	2
	Juan Blanco C.	Dec./00	47.5	1
Tetragonopterinae sp.5	Morales C.	Dec./00	30	1
Tetragonopterinae sp.6	Juan Blanco C.	Feb./01	34	4
	Morales C.	Feb./01	16	1
Tetragonopterinae sp.7	Juan Blanco C.	Feb./01	34	3
Tetragonopterinae sp.8	Morales C.	Feb./01	16	5
	Morales C.	April/01	16	9

* Fish species, site, date, trawler fishing (meters), and total number of specimens.

3. Ecological Efficiency of Different Cleaning Strategies After an Oil Spill at Coastal *Río de la Plata* Estuary

Introduction

Responses to oil spill in coastal wetland are often highly controversial. A clean-up method commonly used in marshes is manual cutting (Zengel and Michel 1996). There are several examples concerning cutting of *Spartina*-dominated salt marshes, and the general result shows that it should be restricted to areas where oil may persist, significant impacts to wildlife are likely, and less destructive clean-up techniques have proven insufficient (Zengel and Michel 1996). There are only few examples on brackish or tidal freshwater marshes dominated by *Scirpus spp.*, *Typha spp.* and their results show no differences between cutting and not cutting even under heavy oiling. However, often cutting itself is not the problem, instead the physical disturbance to plant roots and sediments due to the cutting action is the factor that poses negative effects.

The coastal strip encompasses the shores of Northeast Buenos Aires province and the Southwest Uruguayan coast of the *Río de la Plata*. Inland, on the Argentine side, there are grassy plains ending in a steep riverbank. In the lowlands, at some distance from the shore, exists a halophytic steppe of *Distichlis spicata* and *Distichlis scoparia* that is progressively replaced by frequently flooded places abounding in vegetation composed mainly by *Scirpus giganteus* or *Eryngium eburneum*. Close to the riverside there are areas where the typical vegetation is a marsh dominated by *Zizaniopsis bonariensis*, and sandy beaches frequently flooded by daily tides, with low diversity communities dominated by the Giant Bulrush *Schoenoplectus californicus*.

The main purpose of this section was to evaluate the ecological effects of the spill, and also the performance of the different clean up procedures used after this event. In this case we focused on marshes dominated by the giant bulrush *Schoenoplectus californicus*, and others dominated by the bulrush *Zizaniopsis bonariensis*.

Material and Methods.

Study site and general comparison: The general sampling design included comparisons between sites located upstream of the oiled areas (LB), the oiled sites (J1 and J2) and downstream clean sites (ED). This design was performed to avoid the confounded effects of the estuarine gradient when choosing control sites.

Effect on the Giant Bulrush (*Schoenoplectus californicus*) population and community: The area most affected by oil was the exposed sandy beach dominated by the giant bulrush *S. californicus*. In this area, trampling of oil was not detected, so this possibility was not evaluated. Within the oiled area the treatments evaluated were:(1) not oiled, (2) weathered surface (a layer of dry oil on the surface, but without penetrating into the sediment) and (3) areas with trampled oil due to human activities during clear cutting. In the case of birds we also used areas located upstream and downstream as controls. In these areas, we set 5 fixed sampling sites (1 x 1 m areas demarcated with wood sticks) randomly located within similar type of environment. From these areas we evaluated (a) density, (b) number of dead individuals, (c) number of new individuals, (d) inflorescences, (e) altitude, (f) biomass, and (g) plant diversity. Numbers were expressed per m^{-2}. ANOVAs and t-test were used to evaluate the null hypothesis of no difference between areas of different level of oiling (Zar 1984). The following variables were measured in each treatment: Mortality and recruitment index: In the fixed sampling sites, we monthly evaluated the number of new (less than 10 cm high) and dead shoot. These numbers were expressed per m^{-2}. ANOVA and t-test were used independently to evaluate the null hypothesis of no differences between treatments. Sexual reproduction: Changes in sexual reproduction may be a response of mainly asexual plants to disturbances. To evaluate this indicator we estimated the number of inflorescences from individual plants randomly selected from each treatment. An ANOVA test was used to evaluate the null hypothesis of no difference between treatments. Plant altitude: The altitude of 10 plants randomly selected from each 1 m^2 sampling site was estimated. ANOVA were then used to evaluate the null hypothesis of no difference between treatments (Zar 1984). Species were identified following Lahitte and Hurrell (1997, 1999) and Cabrera and Zardini (1979).

Effect on birds: Birds are always important and probably one of the most important conservation issues in several ecosystems around the world. They have aesthetic value and they area also well known indicators of habitat integrity (or recovery after an environmental impact). Due to these characteristics, birds were sampled in the different environments to evaluate their habitat use. To evaluate if birds use areas affected by the spill, at least 3 replicates of each of the areas above described were selected. In each area censuses of birds were performed at low tide by an observer using a 10x70 binoculars to identify species (following Narosky and Yzurieta 1993) and counting individuals per species. Sampling was based on a scan sampling and transects (following Green 1974) depending on the habitat type. In each census, we recorded if individuals were feeding or resting. Censuses were performed in 3 main areas, 2 controls (LB and ED) and 1 oil spill area (Balneario Magdalena). In each site, we selected 3 main habitats: streams, river coasts and marshes. The streams are represented for different habitats as marshes, exotic and native trees, and open waters on the stream. The river coasts are influenced for periodic tides and are represented for open waters; swallow waters, beaches, outer and inner marshes, and short grass prairies in the high part. For the sampling we selected strip transect as the best method to census birds in these three type of habitats. The censuses were made in the first 4 hours after the sunrise, and 3 hours before sunset. In river coasts transects of 700 m (20 min) were made walking parallel to the coast and censing all birds observed from short grass prairie up to 150 m into the water. We recorded which type of the habitat in the transect use each birds. In beaches, we compared the effect of tide level on the birds. We also compared coasts with extended marshes and others with dispersed ones. The transects in the marshes were of 250 x 30 m and were parallel to the coast in the border of the marsh, given the difficult to walk into the marsh. In oiled areas (Balneario Magdalena), we compared also between 2 different cleaning strategies. In one of them, the marsh was totally removed and, in the other the marsh were not affected.

Results

Effect on the Giant Bulrush (*Schoenoplectus californicus*) community: We analyzed weathered oil, trapped and controls. Density biomass, and plant high showed an increasing trend until Mar/00. Then density and biomass decreased until the end of the studied period. The plant height continues increasing to reach the higher values by the end of the studied period. By Mar/00, the density and biomass of oil trapped treatments decreased, and maintained lower values during the remaining of the studied period. Considering biomass and plant altitude, differences between treatments were significant since Mar/00. The comparison of plant altitude between controls and weathered sites were always significant, while after Dec/00 the lowest significant values were in oil trampled treatments. There were no clear trends in plant density. Weathered sites were the treatments that took longer to reach density values similar to the control sites. Mortality rate was stable along the studied period, but showing significantly higher values in the weathered sites by Mar/00 and until Jan/01. However, density did not show important changes due to the higher increase in recruitment of new shoots. The flowering season of *Schoenoplectus californicus* spanned between September and March, with very low density of inflorescences during autumn and increasing in winter. Weathered sites in general showed significantly higher values during the 2nd reproductive season.

Comparing the community structure between weathered areas and controls (given that in trapped areas the only species was *S. californicus*) the most important species was *Eleocharis montana* in controls and weathered sites (where *Juncus pallescens* was also important), while there was very low coverture in oil entrapped sites, with dominance of *Bidens laevis* and *Sagittaria montevidensis*. The highest richness appeared in weathered sites, followed by control sites. Most species showed covertures lower than 5%. Most of the species found here belong to other communities, such as *Eryngium pandanifolium*, *Bidens laevis*, *Senecio bonariensis, Sphillanthes stolonifera, Apium sellowianum, Pluchea sagitalis* and *Trifolium repens*. By the end of the study period, there was an increase in density of *Z. bonariensis* in trapped areas but without important changes in the community physiognomy.

Effect on birds: There were a total of 80 bird species recorded. The 3 habitats showed different amount of species (streams with = 21 species, river coast = 47 species, and coastal marsh = 53 species. Most species are permanently residents, with only 6 summer residents, 3 summer visitants and 2 winter visitants. The 69% of the species recorded are zoophagous, 23.5% omnivores, 3.7% granivores, 2.5% nectarivores and 1.2% herbivores. The two main habitats, river coast and marsh shared 22 species of birds. Respect to the abundance the 27.5% were occasional species, 15% rare species, 45% common and 12.5% abundant. In the coast, the proportions of the different status of abundance were: occasional species 10.5%, uncommon 15%, common 53.2% and abundant 21.3%. In the marsh, the proportions of the abundance status were: occasional species 28.3%, uncommon 9.4%, common 49.1% and abundant 13.2%. The frequency between habitats was different (X^2 = 35.6, p<0.0001) because of the great number of occasional species in marshes. In the coast, the groups more representatives were gulls (2 species) and herons (3 species). In the marsh the main groups were Furnarids (4 species), Tyranids (3 species), Embericids (5 species) and Icterids (2 species). It was not detected evidence of a gradient of bird species in the 3 sites of study along this part of the river coast. In this way the data of the 2 control sites (ED and LB) were joined in a unified control site.

Comparing the species composition between control and treatments of the external marsh and exposed intertidal there was 51% of shared species (20 of 41 species). There was no difference in species or individuals between controls and impacted areas in any of the sampling periods (Man-Whitney Test, P>0.05). Similarly, there were no differences for species that only used beaches and shallow water, and also for zoophagous species (the ones that can be most seriously affected) from these sites (Man-Whitney Test, P>0.05).

Comparing the species composition of the internal marshes between control and oiled areas, they shared 24 of a total of 53 species (45%). Most of the species (91%) are commons species associated to marsh areas. There were no differences in total number of species between control and oiled sites in any of the studied periods (Man-Whitney Test, P>0.05). The same pattern was found when comparing only species associated with marsh and the coastal forest and in species that feed only on vertebrates (the ones most likely to be affected) in the marsh area (Man-Whitney Test, P>0.05).

Comparing areas with different cleaning strategies within the oil spill area (clear cut and untouched areas), there was clear increase in similarity (Jaccard Index) along time, reaching very similar values by the end of the study period. These changes are associated to the recovery of the vegetation. The number of species and individuals per species was not significant between areas cleaned and areas untouched (Man-Whitney Test, P>0.05). However the maximum values were always in control areas.

Discussion

The results show that always in areas with weathered oil on the sediment the plant community tend to reach similar values to control sites mainly in terms of density, recruitment and biomass. In this area the community switched from a monoculture stand (only Great Bulrush) to a new community that incorporated *Bidens laevis* and *Sagittaria montevidensis*. Always, area with trapped oil in the sediment showed a much slower recovery, staying as a monoculture stand dominated by Great Bulrushes. The weathered sediments also retarded the community recovery, but decreased erosion rate generating stable sediments. The community that developed in this treatment generally reached high coverage at faster rate. Most species that grew in all the sites were those that have asexual reproduction. Thus, species such as *S. stolonifera* and *Cynodon sp*. colonized at a very high rate. Similarly, species with ryzomas such as *E. montana*, *Hydrocotyle bonariensis* and *S. californicus* were not very affected by the weathered sediments, being then the species that when generating new shoots broke the oiled sedimentary surface.

Our results also show that birds were not highly affected by the oil spill. In this study we examined the habitat use by birds after the oil spill as an indirect tool to evaluate the effect of the spill. The results obtained from 8 months after the oil spill until 3 years later do not show evidences of effect of the spill on birds in any of the studied parameters. Within the oiled areas the sampling addressed to evaluate the effect of the different cleaning strategies showing that vegetation removal exhibited the highest effects on the bird community. The bird community evolved following the recovery of vegetation. At the beginning, the dominance of the great bulrush showed bird species clearly associated to this species. At the end of the studied period, the plant community has largely recovered in terms of structure, and thus the associated bird community also recovered. Indeed, at the end of the studied period the bird community was very similar to the nearby areas.

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