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PRELIMINARY EVALUATION OF THE USE OF PHOSPHOGYPSUM FOR REEF SUBSTRATE. II. A STUDY OF THE EFFECTS OF PHOSPHOGYPSUM EXPOSURE ON DIVERSITY AND BIOMASS OF AQUATIC ORGANISMS

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The effects of cement consolidated phosphogypsum (PG) on marine organisms was investigated under natural conditions in four 1000 m^2 estuarine ponds. Two ponds were seeded with 160 kg of PG arranged in aggregations of blocks and two ponds received similar mass of sand/cement blocks. Meiofauna were sampled quarterly and PG did not affect total meiofauna or major taxa (nematodes and copepods) density. Abundant species of copepods either were slightly increased in ponds with PG or were inconsistently affected.

All ponds were drained after one year. Three species of macroinvertebrates and 15 species of fishes were collected. Diversity indices showed modest but inconsistent variation among ponds. Only Pond 1 (control) and Pond 4 (experimental) had similar species abundances and all ponds showed unique distributions of biomasses among species. Thus, no differences in community structure attributable to the presence of PG could be detected among benthic invertebrates, natant invertebrates, or fishes.

Keywords: Phosphogypsum; community structure; oysters; meiofauna; impoundments; artificial reefs

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INTRODUCTION

The fertilizer industry in the southeastern United States annually produces vast quantities of phosphogypsum (PG), a solid by-product of phosphoric acid production. Although largely composed of calcium sulphate dihydrate, PG also contains low levels of various radionuclides and heavy metals. Due to these contaminants, the disposal of PG is a problem which has resulted in on site stockpiling as the only alternative. The use of PG as road aggregate and as a substitute for sand in the manufacture of building materials, among other proposals, has experienced modest success but done little to decrease on site PG inventories. We proposed the use of cement consolidated PG as a material for the construction of artificial reefs in marine environments as a means of utilizing PG while minimizing public exposure to its hazardous contaminants.

In a preliminary trial of PG as a material for construction of artificial reefs, the authors attached PG/cement experimental blocks and sand/cement control blocks to an oil platform in the Gulf of Mexico off Louisiana and inspected them for meio- and macrobenthic organisms after 60 days. Among those blocks that remained intact, no differences in the densitites of amphipods, barnacles and total meiofauna were found between treatments. More impressive were the numbers of organisms attached to both the control and experimental blocks. Amphipods were found in abundances of 4,000–6,000 per 10 cm diameter by 10 cm length cylindrical block.

Subsequently, we undertook two projects to examine PG effects on benthic and natant invertebrate/vertebrate communities. The first project was the bioaccumulation study reported in this proceedings (Nieland *et al.*, 1998). The goal of the project reported here was to determine if PG reefs could develop healthy, diverse aquatic communities; support the growth of meiofauna, crabs, shrimp and in particular oysters; and enhance the populations of desirable forage and gamefish species. Oysters are a particularly criticial source of natural reef material in Louisiana. Not only do they comprise an important economic industry in the region, but also they serve as a critical habitat for a number of organisms important to the estuarine food web and related recreational and commercial species (Day *et al.*, 1987). In the process of settling out of the water column and beginning a sedentary life, oyster larvae require hard substrate. This life history strategy assures that oyster larvae will settle in areas where other oysters have successfully attached and grown. It is also known that oyster larvae will preferentially select substrates high in calcium. Based on its chemical constituents, we would expect cement consolidated PG to be an ideal substrate for oyster settlement. However, it is necessary to investigate thoroughly the effect of associated PG contaminants, such as radionuclides and heavy metals, on settlement and continued growth of the oysters and associated organisms such as meiofauna and macrofauna.

Meiofauna are benthic organisms, < 1 mm in body length, which are frequently used as a test group in laboratory and field studies. Because they are in intimate contact with the sediment throughout their entire life history (no dispersing stage larvae) and because they are sensitive to sediment contamination (Coull and Chandler, 1992; Lotufo, 1997), meiofauna were identified as a group of organisms potentially impacted by PG. Densities of total meiofauna in estuaries average from $0.5-20 \times 10^6\,m^{-2}$ (McCall and Fleeger, 1993). Previous studies suggest that colonization, especially by copepods, into azoic sediment is rapid (Chandler and Fleeger, 1983; Palmer, 1988), and that densities in small experimental units reach background levels in 1–2 days (Sun and Fleeger, 1994). Thus, meiofauna were expected to be good colonists in experimental ponds. Meiofauna are also a criticial component to estuarine food webs (Coull, 1990). Juvenile fish and shellfish utilize meiofauna from sediments and near bottom water (McCall and Fleeger, 1995), and undoubtably serve as prey for a variety of gamefish and invertebrate species common in the south Louisiana marshes.

To investigate further the possible effects of cement consolidated PG on marine aquatic organisms under natural conditions, we established our study in four $1000\,m^2$ ponds (two replicate control ponds, two replicate experimental ponds) located on the coast of Louisiana. Our objectives were to determine the influences of PG on both the colonization, abundance, diversity and distribution of meiofaunal organisms and the diversity and biomass of macroinvertebrates and fishes.

METHODS

Four 1000 m^2 ponds (mean depth ~ 1 m) on the premises of the Louisiana Department of Wildlife and Fisheries' Lyle S. St Amant Marine Laboratory (approximately 70 km south of New Orleans, Louisiana) were used for the study. Two of the ponds (PG1 and PG2) were seeded with 576 cylindrical (5 cm diameter × 10 cm length) blocks of cement consolidated PG (70% : 30%, PG : cement) arranged standing on end in six equidistantly spaced "reefs" (about one meter square) of 96 blocks each (Fig. 1). The number of PG blocks in each pond was constrained by our United States Environmental Protection Agency (EPA) hazardous materials permit which allowed only 320 kg of PG on site. The two remaining ponds (Control 1 (C1) and Control 2 (C2)) were similarly seeded with 576 identical blocks of cement consolidated sand of like proportions. A ten horsepower submersible pump was used to deliver ambient sea water to the ponds in a flow-through manner beginning 2 August 1994. All four ponds received similar continuous flow rates of approximately 750 l min^{-1} and were

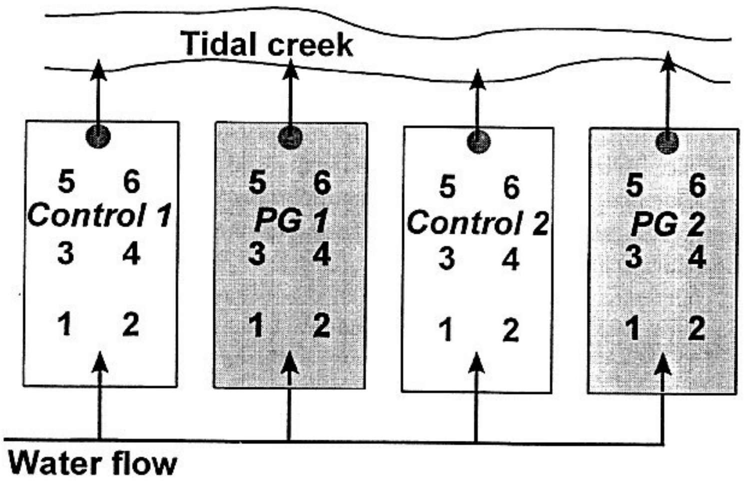

FIGURE 1 Layout of control ponds (Control 1 and Control 2) and experimental ponds (PG1 and PG2) with approximate positioning of PG/cement block reefs and sand/cement block reefs (numbers 1–6).

allowed to become naturally stocked with eggs and larvae of a variety of native marine organisms.

Sediment samples were collected quarterly (September and December 1994 and March and June 1995) from all ponds to quantify the abundance of meiofauna. From each pond, a total of 12 sediment cores (2.61 cm inner diameter) were collected on each sampling date; a core was taken directly among each of the six aggregations of blocks (later called reefs) and a paired core was taken away from each reef, equidistant from adjacent reefs. Cores were taken to 2 cm in depth and fixed in the field with buffered formalin and stained with rose bengal. In the laboratory, the sediments were washed through 500 and 63 μm sieves, and the material retained on the 63 μm sieve was examined under a stereo-dissection microscope. All metazoans were identified and enumerated to major taxon, and meiobenthic copepods were removed and later identified to species. A total of 192 cores were examined.

Sediment samples were taken from each pond during the June meiofauna collection to determine sediment type and heavy metal concentration. Samples, approximately 1.5 l of sediment taken to about 2 cm in depth, were collected equidistant from block aggregations and placed in sealable plastic bags. Bags were stored at −20°C until analysis was conducted. Granulometry measurements were conducted by wet sieving. Sediments were sieved through a 0.063 mm screen, oven dried (at 60°C) and weighed. Percent silt and clay of the total was calculated. Sediment organic carbon (after acidification with 1 N hydrochloric acid to remove inorganic carbonate) was measured in duplicate on a Perkin Elmer (Norwalk, CT) 2400 CHN Elemental Analyzer. Sediment trace metal concentrations were quantified without replication from one location within each pond. Metal concentrations were measured with the Inductively Coupled Argon Plasma Emissions Spectroscopy (ICAP) system and were expressed as parts per million (ppm) wet weight. Radium analysis was conducted at the LSU Nuclear Science Centre with the following standard techniques: Radium in the digested samples was co-precipitated with a barium carrier in sulphate. Extracted radium was allowed to come into equilibrium with radon daughters which were measured by quantification of alpha particle emission in a photomultiplier. All radium concentrations are given as pCi per gram sediment.

Statistical analysis of meiofauna populations was conducted by a univariate split-plot analysis of variance (ANOVA). Treatment effects associated with reef type (ponds containing PG or cement control blocks), position (cores taken among reefs or away from reefs) and collection date were examined using this whole-plot analysis of the univariate split plot conducted separately on total meiofauna, abundant major taxa and copepod species. Repeated measures multivariate analysis of variance (MANOVA) was then used to examine specific date, species or major taxon variation as well as interactions related to the presence of PG. If the MANOVA revealed significant interaction terms associated with PG, univariate analysis of all taxa on each date was performed. *A posteriori* tests were performed as a Tukey's Studentized Range test. Species diversity was examined for the meiobenthic copepod community from samples collected among reefs (with the greatest opportunity for contact with PG) using the Shannon index of diversity ($\log_e$) and Pielou's evenness index. Copepod numbers were averaged among replicates within a pond for this calculation. The copepod community within reefs was also analyzed by non-metric multi-dimensional scaling (MDS) and Analysis of Similarities (ANOSIM) as suggested by Clarke (1993). All 15 species were used, as was the Bray-Curtis Similarity Index, for each collection date-pond combination. ANOSIM is designed to detect differences in community similarity, and was first calculated as a one-way analysis comparing variation among the four ponds (to determine among pond differences in species composition). Large among pond differences precluded pooling of samples to test for treatment effects, and a two way nested ANOSIM was conducted to test for PG effects.

At the end of one year (8 August 1995), the ponds were drained, seined (6 mm mesh) and dip netted to harvest the natant macrofauna of each and all blocks were removed for assessment of oyster growth. The harvest of each pond was sorted to species and each species lot was enumerated and weighed.

Representative individuals from each species found in each pond were frozen for future elemental analyses. The Shannon diversity index (the maximum diversity if all species are present in equal abundance), Pielou's evenness index, and the Simpson diversity were all calculated for each pond (Krebs, 1972). Also, Komolgorov-Smirnov tests of goodness of fit were also applied to both the proportions of species present and the species biomass distributions among ponds (Sokal and Rohlf, 1969). Due to the difficulty in effectively and efficiently harvesting some smaller species of fishes (gobies, blennies, and sheepshead minnows), these were only recorded as present and were not included in comparisons among ponds.

RESULTS

Granulometry among the four ponds differed slightly. C2 had the lowest (4.6%) and PG2 had the highest (12.9%) silt and clay content; C1 and PG1 were very similar (9.0 and 9.2%, respectively). Organic carbon content was low in all ponds but especially so in control ponds, means were 0.64 and 0.28%, respectively, in C1 and C2 and values in PG1 and PG2 (1.09 and 1.34%, respectively) were only slightly higher. Sediment trace metal (copper, zinc, cadmium, lead, chromium, and nickel) concentrations were generally low in all control ponds, but slightly higher in experimental ponds with PG (Tab. I). For example, copper averaged 7.2 ppm in control ponds and 16.1 ppm in PG enriched ponds. On the other hand, lead was not detected in ponds with PG, but was present at relatively low levels in controls. Radioactivity was quite low in control ponds (0.3 and 0.15 $\mathrm{pCi\,g^{-1}}$ in C1 and C2), but higher in ponds containing PG (1.6 and 1.4 $\mathrm{pCi\,g^{-1}}$ in PG1 and PG2).

Total meiofauna ranged from lowest densities in December (ca. $0.37 \times 10^6\,\mathrm{m^{-2}}$) to highest in March (over $2 \times 10^6\,\mathrm{m^{-2}}$) (Fig. 2). Nematodes were the most abundant taxon, averaging 83% of the total

TABLE I Concentrations (ppm) of copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), chromium (Cr), and nickel (Ni) in sediment samples from control (C1 and C2) and experimental (PG1 and PG2) ponds

	Concentrations (ppm)					
Pond	*Cu*	*Zn*	*Cd*	*Pb*	*Cr*	*Ni*
C1	7.4	30.5	0.0	1.3	9.4	7.3
C2	6.9	25.1	0.0	1.4	9.3	7.3
PG1	11.6	47.4	0.0	0.0	11.1	9.3
PG2	20.6	90.1	1.0	0.0	17.9	13.8

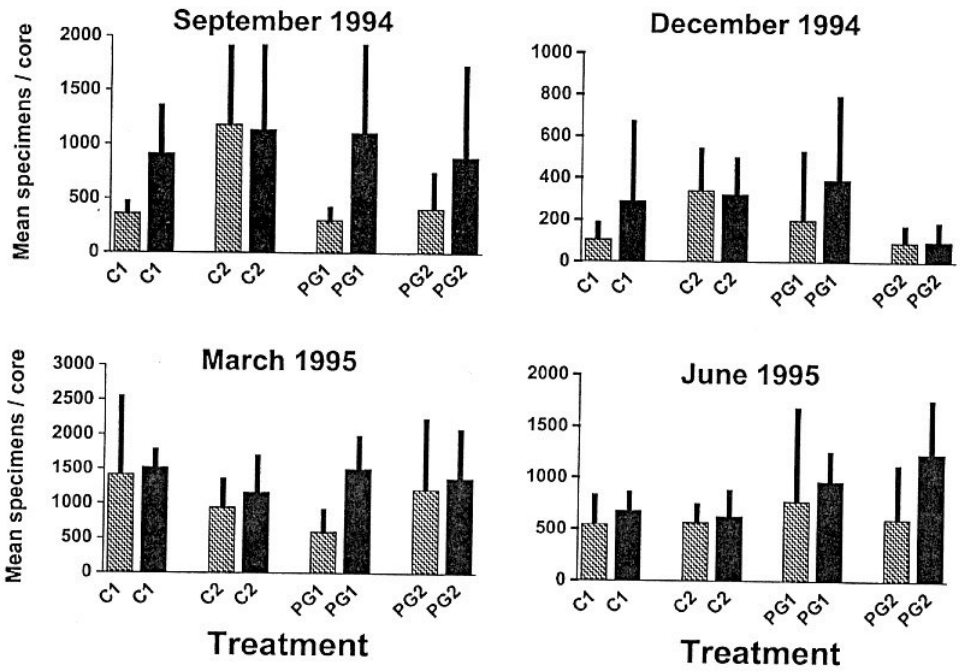

FIGURE 2 Mean total meiofauna abundances per sediment core from within block reefs (black bars) and between block reefs (hatched bars). Narrow black bars indicate plus one standard error of the mean. To convert density values (number per core) to area (number 10 cm^{-2}), multiply by 1.87. Multiply this number by 1000 to convert to density m^{-2}.

meiofauna. Trends in the seasonal abundance of nematodes mimicked that of total meiofauna. Copepods were the second most abundant taxon, comprising 7% of the total. Copepods were highest in September and March and lowest in December and June. Copepod nauplii (larval stages of benthic copepods) and ostracods were low in abundance, each comprising < 10% of the total. Polychaetes (mostly juveniles) and rare taxa (*e.g.*, tanaids and amphipods) were also found, but were considered to be too low in abundance to examine statistically.

Across all collections, three copepod species comprised the majority of the meiobenthic copepod assemblage; *Cletocamptus deitersi* (Richard) and *Coullana* sp. are harpacticoid copepods and *Halicyclops coulli* Herbst is a cyclopoid copepod. A total of 15 species (epibenthic and infaunal in lifestyle) were identified from collections. *Cletocamptus deitersi* proved to be a very good colonizer (but found on all collections) and was the most abundant species overall. It was very abundant in September and March samples, averaging over $10\,\mathrm{cm}^{-2}$; densities in December and June were lower ($< 2\,\mathrm{cm}^{-2}$). *Coullana* sp. (second in abundance) also was a good colonist, and densities, although lower, followed a very similar trend compared to *C. deitersi*. Density ranged from < 0.5 to $> 2\,\mathrm{cm}^{-2}$. *Halicyclops coulli* was present in all collections but was most abundant in June, reaching about $2\,\mathrm{cm}^{-2}$. The copepod fauna was similar in September and December but quite different from March and June. During the September and December collections, *C. deitersi*, *Coullana* sp. and *H. coulli* comprised over 85% of the copepods collected. In March and June, collections became more diverse and these three species comprised only about 55% of the fauna. Two other species, *Onychocamptus mohammed* and *Paronchocamptus huntsmani*, reached high abundance only in March but were not analyzed statistically due to low numbers on other collection dates. Rare species include the harpacticoid copepods *Enhydrosoma* sp., *Harpacticus* sp. *Nitocra lacustris*, *Schizopera knabeni* and unidentified species in the families Laophontidae and Ectinosomatidae.

Examination of abundance trends and the results of statistical analysis suggest that PG did not cause consistent or long-term effects on the density of the major taxa of meiofauna. Overall, meiofaunal densities in ponds with cement (control) blocks were similar to densities in ponds containing PG blocks. Variation among replicate ponds was very high, especially in collections taken 9 – 12 mo after initiation of the experiment, making it more difficult to identify PG effects. Split-plot ANOVA examined PG treatment and core position effects. For total meiofauna and the major taxa of nematodes, copepods, copepod nauplii and ostracods, no treatment effects associated with the presence of PG were found. Core position (cores taken among blocks or between blocks) was significant for all major taxa (and total meiofauna) tested, but in no instance was the PG by position interaction term significant. The lack of position by PG treatment interactions suggests that samples taken from inside the reefs were no more likely to be influenced by PG than those taken away from reefs. Generally, higher total meiofaunal and major taxon abundances were found in samples taken away from reefs. Repeated-measures MANOVA revealed that significant differences among taxa and dates occurred for all groups, but no interaction terms with PG treatment were significant.

Statistical results for copepod species (*Cletocamptus deitersi*, *Coullana* sp. and *Halicyclops coulli*) were quite different from those for major taxa and are more difficult to interpret. Split-plot ANOVA reveals a significant PG treatment effect ($P = 0.001$) on copepod species, but position and treatment by position interaction terms were not significant. Repeated measures MANOVA suggests that species and date variation were significant, and most interaction terms, even three- and four-way interactions, with PG were significant, making biological interpretation problematic at best (Tab. II). Examination of species trends shows that the effect of the PG treatment differed over time. Based on univariate tests, PG significantly influenced the density of *C. deitersi* in September, December and March; *Coullana* sp. and *Halicyclops coulli* were significantly influenced by PG in March (Tab. III). Means for *C. deitersi* in ponds with PG were higher in September and December but lower in March, compared to control ponds. In March, means for *Coullana* sp. and *H. coulli* were higher in PG than control ponds. Thus, when effects occurred, they were complex and variable over space and time and there was no pattern of a reduction in density in ponds with PG.

Species diversity of meiobenthic copepods in the experimental ponds was very similar to mudflats and subtidal zones throughout south

TABLE II Repeated measures MANOVA results for time, treatment, and position effects on the density of meiofaunal major taxa (MT; nematodes, copepods, copepod nauplii, and ostracods) and copepod species (CS; *Coullana* sp., *C. deitersi*, and *H. coulli*). Treatment refers to ponds with phosphogypsum or control (cement) reefs; position to samples taken among or between reefs; time to collection date; taxon to major taxon or species

Manova test criteria	*MT*	*CS*
Taxon	**0.0001**	**0.0001**
Taxon*Tmt	0.0772	**0.0148**
Taxon*Pos	0.1530	**0.0013**
Taxon*Tmt*Pos	0.5683	**0.0199**
Time	**0.0001**	**0.0001**
Time*Tmt	0.3542	**0.0058**
Time*Pos	**0.0001**	**0.0001**
Time*Tmt*Pos	0.1298	0.1325
Taxon*Time	**0.0001**	**0.0001**
Taxon*Time*Tmt	0.0832	**0.0036**
Taxon*Time*Pos	0.0599	**0.0001**
Taxon*Time*Tmt*Pos	0.9561	**0.0153**

TABLE III Univariate ANOVA performed on copepod species of interest on each collection date. Abbreviations as follows: Cou = *Coullana* sp., Cle = *C. deitersi*, Hal = *H. coulli*. Date variables as follows: SE = September 1994, DC = December 1994, MA = March 1995, JU = June 1995. Treatment (Tmt) refers to ponds with phosphogypsum or control (cement) reefs; position (P) to samples taken among or between reefs

Variable	*P(Tmt)*	*P(Pos)*	*P(Tmt * Pos)*
SE Cou	0.339	**0.007**	0.105
SE Cle	**0.001**	**0.009**	0.165
SE Hal	0.556	0.194	0.743
DC Cou	0.127	**0.001**	0.462
DC Cle	**0.011**	**0.037**	**0.021**
DC Hal	0.500	**0.047**	0.673
MA Cou	**0.023**	**0.007**	0.175
MA Cle	**0.002**	0.421	0.141
MA Hal	**0.003**	**0.001**	**0.015**
JU Cou	0.078	0.148	0.148
JU Cle	0.123	0.202	0.845
JU Hal	0.255	**0.001**	0.438

Louisiana (see Fleeger, 1985). When ponds were examined separately, species richness (measured as the number of copepod species) of collections taken among blocks ranged from 3–13, and the Shannon index ranged from 0.684–1.80 (Tab. IV). Evenness ranged from 0.494–0.804. Diversity tended to be slightly higher during the spring and summer collections and slightly lower in fall and winter. Variation

TABLE IV Diversity values for the copepod assemblage as calculated by the Shannon's and Pielou's evenness indices. Site designation refers to ponds C1, C2, PG1 and PG2 and to the month of collection (SE for September, DC for December, MA for March and JU for June)

Site	*Species number*	*Shannon index*	*Evenness index*
C1-SE	4	0.88	0.636
C2-SE	7	0.96	0.496
PG1-SE	6	1.13	0.631
PG2-SE	4	0.68	0.494
C1-DC	6	1.23	0.684
C2-DC	5	0.80	0.498
PG1-DC	5	1.29	0.804
PG2-DC	3	0.69	0.631
C1-MA	13	1.80	0.703
C2-MA	10	1.79	0.776
PG1-MA	9	1.71	0.777
PG2-MA	8	1.23	0.592
C1-JU	9	1.43	0.650
C2-JU	6	1.20	0.669
PG1-JU	5	1.15	0.717
PG2-JU	4	0.85	0.614

among replicate ponds was at least equal to, if not greater than, variation associated with treatment, and no pattern of diversity variation could be attributed to the presence of PG. Analysis of Similarities (ANOSIM) of copepod species from cores taken within aggregations of blocks documents this high among-pond variation (significant differences among replicate ponds, $P < 0.05$, were found in each collection except December), and data from replicate ponds could not be pooled. Two-way ANOSIM detected no treatment effect; however, the power of this test with two treatments is very low (Clarke, 1993). MDS plots (not shown) revealed no consistent trend in variation among collections that could suggest a community response to PG.

A total of three species of macroinvertebrates (American oyster *Crassostrea virginica*, white shrimp *Penaeus setiferus*, and blue crab *Callinectes sapidus*) and 15 species of fishes were collected from the four ponds (Tab. V). Mean numbers of species (and biomass) for the two experimental ponds and the two control ponds were 10.5 (32.35 kg) and 14 (32.41 kg), respectively. Blue crab were abundant (and quite large) in all four ponds and comprised 14 – 30% of the numbers and 22 – 40% of the biomass. White shrimp were abundant only in C2.

The fish species silver perch, *Bairdiella chrysoura*, spotted sea trout, *Cynoscion nebulosus*, spot, *Leiostomus xanthurus*, gobies (Clinidae), and blennies (Blenniidae) were also particularly abundant in all. The former three species (all members of the drum family Sciaenidae) were 20–60% of the individuals and 21–54% of the biomass.

Diversity indices (Tab. VI) calculated from proportional abundances of species present showed modest variation. Indices for C1 and PG2 are virtually identical. Differences between these and the remaining two ponds appear to be driven by the numerical dominance of spotted sea trout in PG2 and of white shrimp in C2.

TABLE V Numbers and biomasses of species harvested from phosphogypsum enriched (PG1 and PG2) and control ponds (C1 and C2), 7 – 10 August 1995

Species	*C1* *(number, mass (kg))*	*PG 2* *(number, mass (kg))*	*C 2* *(number, mass (kg))*	*PG 2* *(number, mass (kg))*
American oyster	Present	Present	Present	Present
Blue crab	85, 11.260	26, 7.935	68, 9.800	59, 8.765
White shrimp	4, trace	6, 0.020	265, 6.180	19, 0.440
Gulf menhaden	1, 0.020		7, 0.130	2, 0.040
Gulf killifish			7, 0.255	
Inland silverside			1, 0.005	
Sheepshead	1, 0.050			
Atlantic spadefish			1, 0.285	
Pinfish	23, 2.320	2,0.250	9, 0.710	25, 2.740
Silver perch	41, 1.730	18, 0.950	62, 1.880	39, 1.480
Spotted sea trout	36, 6.135	104, 10.450	6, 1.400	29, 4.955
Spot	73, 8.805	10, 1.255	31, 1.965	73, 9.170
Atlantic croaker	1, 0.160			
Black drum	8, 1.125		30, 2.150	3, 0.545
Red drum	2, 1.290	2, 1.360		1, 0.690
Striped mullet	11, 7.170	27, 13.650		
Total	286, 40.065	221, 35.870	487, 24.760	250, 28.825

TABLE VI Diversity indices based on proportional abundances of macroinvertebrate and fish species present. Ponds C1 and C2 are controls (seeded with sand : cement blocks), ponds PG1 and PG2 are experimentals (seeded with PG : cement blocks). H = Shannon-Wiener Diversity, $-(p_i)(\log_2 p_i)$; H_{max} = Maximum diversity if all species equal in abundance, $\log_2$ S; E = Pielou's evenness index, H/H_{max}; D = Simpson Diversity, $1-(p_i)^2$

Index	*C1*	*PG1*	*C2*	*PG 2*
H	2.65	2.13	2.14	2.57
H_{max}	3.58	3	3.46	3.17
E	0.74	0.71	0.62	0.81
D	0.80	0.70	0.66	0.81

Komolgorov-Smirnov comparisons of the distributions of proportional species biomasses (Fig. 3A) and proportional species occurrences (Fig. 3B) among ponds revealed all four ponds to be quite dissimilar. Only C1 and PG2 showed similar distributions of species abundances and all ponds showed unique distributions of biomasses among species.

Oyster growth was evident and abundant on both the PG : cement and the sand : cement blocks in all ponds; all exposed block surfaces were blanketed with a dense growth of these organisms. In many cases the oysters had formed a crown which measured 15 – 20 cm in diameter on the top of the blocks. Again, no evident differences in oyster growth were noted between the experimental and control ponds.

DISCUSSION

Meiofaunal density, taxonomic composition, and species diversity in the experimental ponds were quite representative of surrounding mudflats and subtidal environments (Fleeger, 1985; McCall and Fleeger, 1993). In muddy sediments, nematodes typically comprise 85 – 90%, and copepods 3 – 5%, of the total meiofauna (Coull, 1988), and the copepod species present in the experimental ponds are well studied and found in high densities throughout coastal Louisiana (Carman *et al.*, 1997; Sun and Fleeger, 1994; Chandler and Fleeger, 1987). Densities of total meiofauna in the ponds were well within the range found in Louisiana marshes (averages from $0.5 - 20 \times 10^6\,\mathrm{m}^{-2}$, McCall and Fleeger, 1993). Previous studies in the area suggest that colonization, especially by copepods, into azoic sediment is rapid (Chandler and Fleeger, 1983; Palmer, 1988), and that densities in small experimental units reach background levels in 1 – 2 days (Sun and Fleeger, 1994). Life history traits of the abundant species (*Cletocamptus deitersi*, *Coullana* sp. and *Halicyclops coulli*) favour rapid colonization through the water column (Sun and Fleeger, 1994) and high population growth rates. Thus, by our first collection three months after initiation, there was sufficient time to colonize ponds and foster population growth, and densities in all ponds were high.

Phosphogypsum did not affect the density of total meiofauna or the major taxa of meiofauna. Furthermore, samples taken directly among

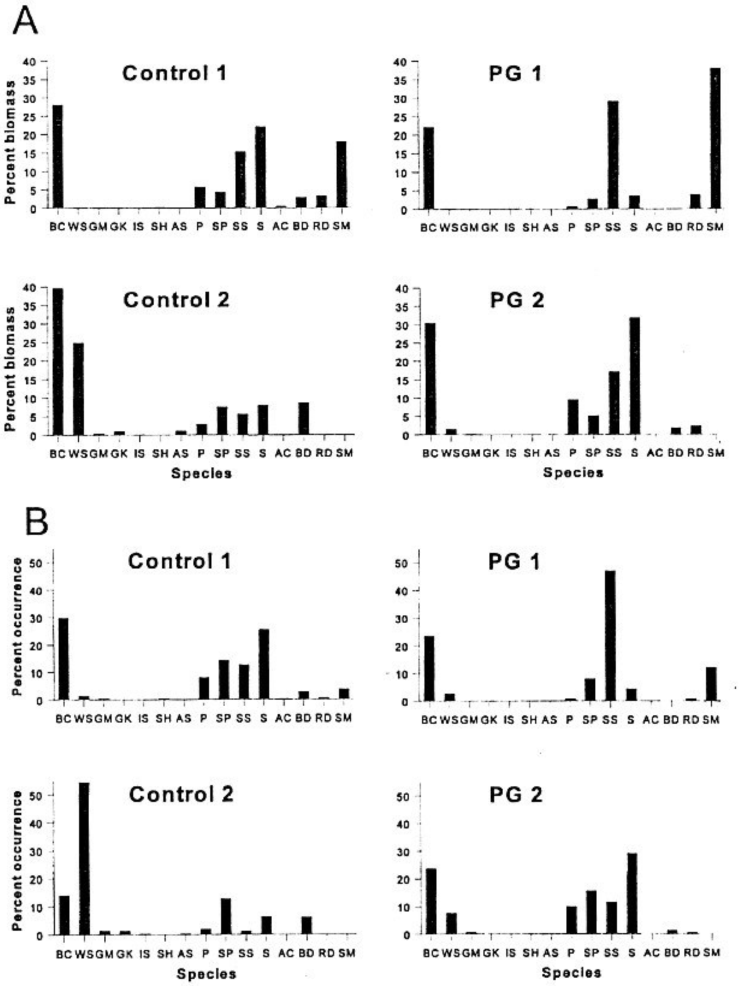

FIGURE 3 Percent biomasses (A) and percent occurrences (B) of two species of macroinvertebrates and 12 species of fishes in control ponds 1 and 3 and experimental ponds 2 and 4. Abbreviations are: BC = blue crab *Callinectes sapidus*, WS = white shrimp *Penaeus setiferus*, GM = gulf menhaden *Brevoortia patronus*, GK = gulf killifish *Fundulus grandis*, IS = inland silverside *Menidia beryllina*, SH = sheepshead *Archosargus probatocephalus*, AS = Atlantic spadefish *Chaetodipterus faber*, P = pinfish *Lagodon rhomboides*, SP = silver perch *Bairdiella chrysoura*, SS = spotted sea trout *Cynoscion nebulosus*, S = spot *Leiostomus xanthurus*, AC = Atlantic croaker *Micropogonias undulatus*, BD = black drum *Pogonias cromis*, RD = red drum *Sciaenops ocellatus*, and SM = striped mullet *Mugil cephalus*.

blocks of PG were no more likely to experience effects than those taken within ponds with PG but distant from the blocks (no position by PG by position interactions were found). Variation among replicate ponds was high, but differences in density associated with collection dates and major taxa were detectable statistically. Studies have shown that the effects of sediment contaminants are unlikely to be expressed at the level of meiofaunal phyla (Warwick, 1988), and comparisons for effects should be made at lower taxonomic levels.

Phosphogypsum effects on copepod species density were detected for the three most abundant species, but effects were either such that density was higher in ponds with PG or that effects differed among collections (*i.e.*, *C. deitersi* increased in ponds with PG on two collections but decreased on another). Furthermore, significant three and four way ANOVA interaction terms preclude interpretation of main effects on copepod species. Analysis of the copepod species diversity and community similarity revealed that variation among ponds was too high to permit identification of effects of PG (although the power of such tests with only two treatment levels is very low, Clarke, 1993). Certainly no consistent reduction in density or diversity in ponds with PG was observed. *C. deitersi* has been found to be very abundant in field sites and microcosms contaminated with polynuclear aromatic hydrocarbons (Delaune *et al.*, 1984; Carman *et al.*, 1997), but is also present in high numbers at locations that are physically disturbed (*e.g.*, low oxygen or high salinity stress) without contaminants (Dexter, 1995; Fleeger, personal observation). Members of the genus *Cletocamptus* are quite tolerant of physical stress (Vopel *et al.*, 1996).

Trace metal effects on meiofauna have been examined in the field and laboratory. Field and microcosm studies have shown that metals can influence meiofaunal communities by reducing densities, altering community composition and reducing diversity, but at concentrations much higher than that found in PG blocks used in our study (Austen *et al.*, 1994; Somerfield *et al.*, 1994; Millward and Grant 1995) and laboratory studies have found effects of metals on meiofauna but also at relatively high levels (Coull and Chandler, 1992). Long *et al.* (1995) examined the minimum levels of sediment-sorbed trace metals at which biological effects can be detected. Values for six trace metals found in the ponds were generally low. Long *et al.* (1995) established effects range low criteria for these trace metals. In no instance did values in ponds exceed these criteria suggesting few toxic effects to benthic organisms. To the best of our knowledge, no field studies have been conducted on the effects of radium on meiobenthic communities.

The prodigious growth of oysters on the surfaces of all exposed blocks in all four ponds (reefs 5 and 6 in C1 were silted over during the course of the experiment and had only a few deal shells) was most impressive. Such was their abundance that no attempt to quantify and compare their growth among ponds was made. However, no apparent differences in both the quantity and quality of oyster growth between the PG and the sand blocks were discerned from visual inspection. The intimate association of the oysters and the PG blocks appears to have had no apparent effect on settlement, survival, and growth.

White shrimp were found in large numbers only in C2; this is strongly suspected not to be the result of constraints on the growth of shrimp populations imposed by the presence of either PG or sand blocks. As white shrimp are scavengers that glean food particles from the substrate, they assuredly would have come in contact with, and perhaps taken refuge among, both the PG and the sand blocks. The experimental ponds PG1 and PG2 and control pond C1 remained barren of flora throughout the course of the experiment. For reasons unknown to us, C2 developed a lush stand of submersed widgeon grass (*Ruppia maritima*). Pond C2 also had the smallest population of spotted sea trout, a notorious predator of shrimp. The refugia provided by the widgeon grass and the lack of predators undoubtedly combined to produce conditions favourable for the maintenance of a large population of white shrimp in C2.

Another scavenging macrocrustacean, the blue crab, would also have been expected to come in contact with the blocks and would presumably have experienced any possible detrimental effects of the presence of PG. Mean percent occurrence of blue crab in the two pond groups are equivalent (22% in control ponds, 23% in experimental ponds); however, the two control ponds did in fact show a larger overall mean percent biomass. The exceptional amount of forage and habitat provided by the widgeon grass in C2 again likely contributed to the greater biomass of blue crab found in this pond.

No clear differences in the distributions of fish species occurrence or biomass could be discerned. Spot, silver perch, and spotted sea trout clearly dominated the fish faunas in all four ponds with significant contributions by striped mullet, *Mugil cephalus*, pinfish, *Lagodon rhomboides*, and black drum, *Pogonias cromis*, in C1, by striped mullet in PG2, by pinfish and black drum in C2, and by pinfish in PG2. The many hundreds, perhaps thousands, of sheepshead minnows, *Cyprinodon variegatus*, found in C2 (and not quantified due to the difficulty in collecting all of them) undoubtedly had a regulating effect on the fish population due to interspecific competition with the larval and juvenile stages of other species.

Overt differences between the mean numbers of all species found in the experimental and control ponds is the result of the enrichment of the control pond faunas by five fish species which were represented by single individuals. If these five species are omitted from consideration, the mean number of species in the experimental ponds becomes 11.5 species, a number much more in line with the 10.5 species found in the experimental ponds. No clear trends of differences between the control and experimental pond faunas, as compared with the various diversity indices and the Komolgorov-Smirnov goodness of fit statistic, further indicate no deleterious effects from the PG blocks.

An obvious concern is that any possible deleterious impacts of the application of only 160 kg of PG to each of the two experimental ponds may be insufficient to produce any observable effects. However, the experimental design here could be expected to produce results comparable to those of a much larger scale project. The arrangement of the PG blocks into discrete reefs undoubtedly created PG exposure gradients, particularly within the sediment and, thus, to the meiobenthic community. Oyster growth within all four ponds was confined exclusively to the control and PG blocks, yet no overt differences between treatments in colonization and growth of these sessile organisms were observed. Further, as the water in each pond was replaced on average once every day, leachates from the PG blocks would have a greater residence time in the ponds than in an open water situation where constant tides and currents would remove same from the system. Considering the results of both the laboratory experiment (Nieland *et al.*, 1998) and the field experiment presented here, it is impossible to identify any detrimental effects on meiobenthic organisms, macroinvertebrates, or fishes from either trophic or environmental exposure to PG.

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