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BACKGROUND: Polycyclic aromatic hydrocarbons (PAH) are a highly toxic group of compounds present in crude oil and many petroleum products. Estuarine salt marshes are critical marine habitat because of their high productivity and importance as nursery grounds for many commercially important species. They are susceptible to chronic and/or catastrophic inputs of petroleum hydrocarbons, such as PAH, because of the physical and geochemical character of salt marshes. Seldom, however, are studies of biological effects able to predict subtle, long-lasting ecological effects, especially in regards to functional interactions among organisms and trophic levels. We report here a microcosm approach to study the impacts of PAH on a benthic food web within a Louisiana salt marsh. Our primary focus involves the meiofaunal component of the benthos, with emphasis on their trophic relationships with microorganisms (food) and juvenile fish (predators).

OBJECTIVES: Our goal was to determine the influence of sublethal exposure to sediment-bound PAH on the following aspects of a salt-marsh, benthic food web: (1) the physiological condition, activity, and abundance of sedimentary microorganisms (bacteria and microalgae), (2) the physiological condition of meiobenthic copepods; (3) the grazing rate of meiobenthic copepods on sedimentary microorganisms, (4) the meiofaunal community structure; and, (5) predation by juvenile spot (*Leiostomus xanthurus*) on meiofauna.

DESCRIPTION: *Microcosm experiment*: The microcosm experiment portion of this study was carried out at the LUMCON Marine Education Center in Cocodrie, LA. The benthic community used in the microcosm experiment was obtained from a shallow creek-fed pond in the Terrebone Bay estuary (29°15'N:91°21'W) adjacent to the LUMCON facility. Microcosms consisted of intact sediment samples that were collected by gently pushing 15.2 cm i.d. PVC pipe into marsh mud exposed at low tide. A base was placed on the microcosm, which was then transferred to the LUMCON facility and placed in one of four wet tables. Windows were cut in the side of microcosms and covered with Nitex mesh (62-um) to allow exchange of water (but not meiofauna). Microcosms were placed in wet tables and irrigated with water pumped directly from the marsh near the LUMCON facility and illuminated with banks of fluorescent lights. Microcosms were treated with "high" doses of PAH-contaminated sediment by adding a volume of contaminated sediment (from Port Fourchon) sufficient to cover the entire surface of the microcosm with a 1-mm-thick layer of sediment. "Medium" and "low" doses were obtained by mixing Port Fourchon sediment with "uncontaminated" sediment from Lake Champagne. Uncontaminated sediment only was added to an additional set of microcosms and served as an "application control", i.e., a control for the process of adding contaminants to microcosms. Finally, a set of microcosms served as unmanipulated controls (no sediment added). The experimental design was 4 x 7 x 5 factorial design, with `wet tables' (as blocks), `PAH concentration', and `exposure time' as factors. Sediment microcosms were treated with three concentrations of PAH (low, medium, and high) and compared to two types of control sediments (described above). Four replicate microcosms (one from each wet table) of each of the five treatment levels (20 total microcosms) were harvested at each of seven dates (0, 1, 3, 7, 14, 21, and 28 days). Thus, a total of 140 microcosms was used in this experiment.

Microcosms were harvested by collecting core samples for the following measurements: PAH concentration, copepod lipid-storage material, copepod grazing on microbes, bacterial abundance, bacterial activity and physiological condition, microalgal abundance, microalgal activity and physiological activity.

Fish predation experiment: Juvenile spot (between 35 and 60 mm standard length) were collected from the western end of Bay Champagne, and transported to our laboratory at LSU. Feeding trials were conducted using azoic sediment collected near Cocodrie, LA. Contaminated sediment (21 ppm total PAH) from Pass Fourchon was mixed with azoic Cocodrie sediment to create intermediate levels of PAH, and meiofauna of known densities were added.

All feeding trials were videotaped in a quiet room with constant light. Each feeding trial was observed and taped using an 8 mm video-camcorder and later viewed with a SVHS video-cassette recorder. Filming continued for 30 min or until the water became too murky to record. Such abbreviated trials were abandoned and not analyzed. Fish from successful trials were immediately removed from the aquarium and measured for standard length (SL). The complete gut of each spot was removed and placed in 10% buffered formalin with Rose Bengal for later gut-content analysis.

Fish behaviors were observed and quantified from video tapes. The frequency of behaviors over the 30 min was recorded and statistically analyzed. Behaviors included a feeding strike, defined as fish taking a bite of sediment and/or detritus into mouth and processing it in buccal cavity, and processing time. Other behaviors commonly observed, but not quantified, included searching, escape, and resting or hiding.

Three separate fish-predation experiments were conducted. The first ("Frequency Experiment") was designed to determine how PAH affects spot feeding intensity and behavior. A spot was placed in an aquarium with dishes containing meiofauna and sediment with a single PAH concentration. Three different concentrations of PAH were used in separate aquaria. The second experiment ("Preference Experiment") was designed to determine if spot has a preference for feeding in sediments with or without PAH within the same aquarium. The third experiment ("Selection Experiment") tested the ability of spot to detect and exploit high-density patches of meiofauna in sediments contaminated with PAH. Spot has been shown to feed preferentially on high-density patches of meiofauna, but the effect of PAH on this important behavior is unknown.

SIGNIFICANT CONCLUSIONS: From the microcosm experiment, we concluded that PAH had a relatively minor influence on the salt marsh benthic food web. Significant effects were observed with regard to microalgal activity and physiological condition, and meiofaunal community composition. No effects were manifested in the grazing activity or physiological condition of meiobenthic copepods. We suggest that the microbial/meiofaunal community of this Louisiana salt marsh may have adapted to chronic inputs of hydrocarbons over the past few decades. Juvenile fish (spot) did not alter their feeding behavior in response to sediments contaminated with PAH. This failure to recognize and avoid contaminated sediments could be harmful to spot since PAH at the concentrations used here are known to be harmful to them.

STUDY RESULTS: No significant effects of PAH were observed for the following: (1)copepod lipid-storage material, (2) copepod grazing, (3) algal synthesis of neutral lipids, (4) sedimentary chlorophyll *a*, (4) bacterial phospholipid synthesis, (5) bacterial physiological condition, (6) bacterial abundance, (7) copepod abundance, (8) copepod nauplii total abundance, or (9) total meiofaunal abundance.

Significant effects of PAH were observed for the following: (1) algal synthesis of phospholipid, (2) algal physiological condition, (3) nematode abundance, (4) the nematode/copepod ratio of abundance, and (5) the nauplius/copepod ratio of abundance.

There was a significant influence of "time" on all parameters except nematode abundance. This presumably was a result of deteriorating conditions in microcosms. Nevertheless, the statistical design of this experiment allowed us to distinguish between any possible containment effects from effects that were the result of PAH contamination.

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