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Toxicity of Texan Petroleum Well Brine to the Sheepshead Minnow (*Cyprinodon variegatus*), a Common Estuarine Fish

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Petroleum (crude oil and natural gas) is of major economic importance in Texas. In 1979 almost 200,000 wells were producing in Texas and more than 16,000 new wells were drilled. About 23% of the natural gas and 12% of the crude oil production of Texas comes from the counties near the Gulf of Mexico (Fig. 1.). Exploration and production are expected to increase in the immediate future because of a recent federal decision to decontrol domestic crude oil prices, the high price of oil in the world market, and the U.S. goal of energy independence.

In petroleum-producing geologic formations in much of the southeastern and southcentral U.S., enormous reservoirs of salt brine are associated with the petroleum. During production, the petroleum and brine are brought to the surface together, where they are separated before the petroleum is transported to refineries. Before 1971, Texas allowed disposal of petroleum brine directly into surface waters, which resulted in extreme dysfunctioning of many rivers and streams (SPEARS 1971). Under current Texas regulations, brine from inland wells must be injected into disposal wells or stored in surface pits and allowed to evaporate. However, more than 2000 exceptions have been issued to oil companies in Texas that allow direct disposal of brine into surface waters unless it can be shown that environmental damage will result. Brine produced from offshore wells is released directly into the surrounding estuarine or marine environment. Galveston Bay, the largest estuary on the Texas Gulf Coast receives more than 67,250 barrels (1 barrel = 42 gallons) of petroleum brine a day (W. C. Steed, Railroad Commission of Texas, pers. comm.).

The chemical constituents and salinity of the brine varies with the geologic formation containing the oil and with the efficiency of the oil/water separator. In addition to inorganic salts, brine contains organic hydrocarbons derived from petroleum. ARMSTRONG et al. (1979) found more than 25 mg/l hydrocarbons in brine waters discharged into Galveston Bay. Some of these hydrocarbons, especially the lighter fractions such as naphthalene, are extremely toxic to aquatic organisms (ARMSTRONG et al. 1977).

The effects of brine disposal at offshore separators on marine benthos have been evaluated in several studies. LUND (1957) found that exposure of oysters to 3% brine for 96 h did not affect "clearance rate." However, HEFFERNAN et al. (1971)

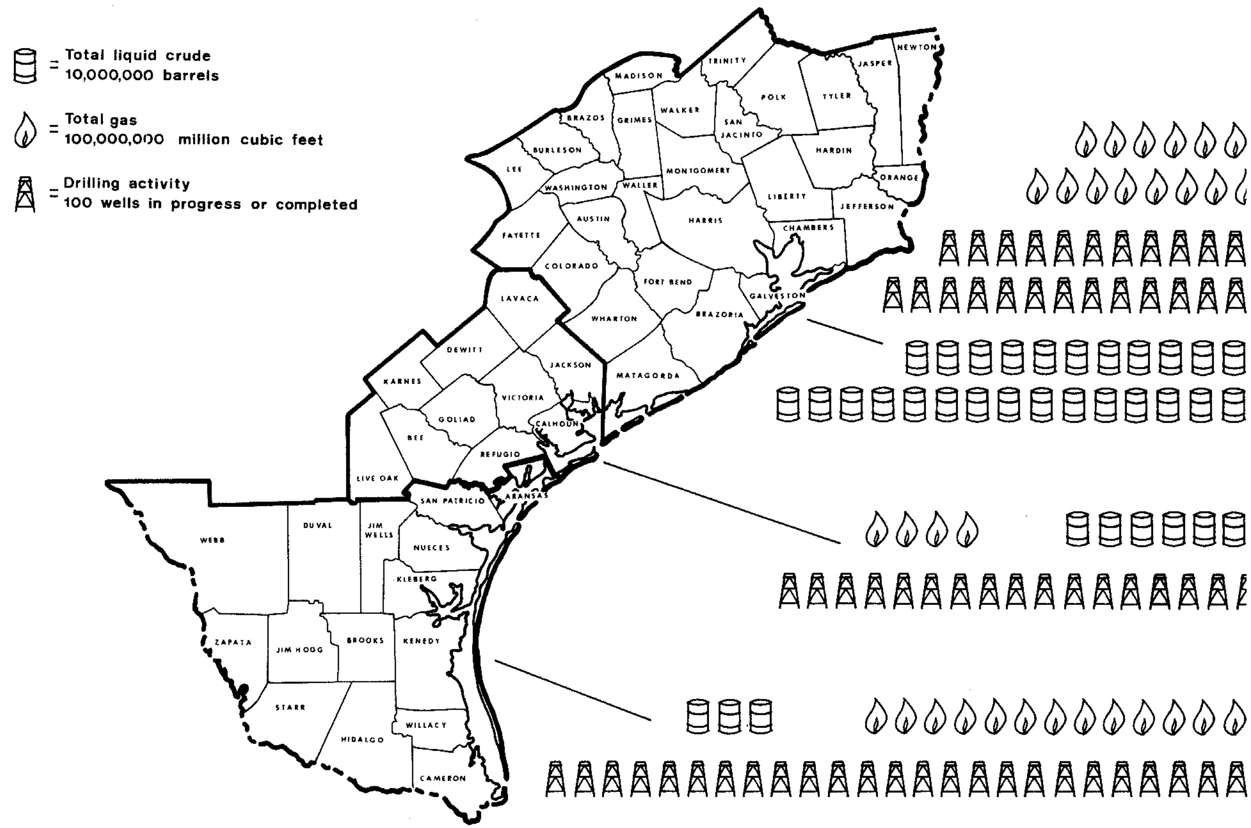

Fig. 1. Petroleum production and well drilling activity for three groups of coastal counties of Texas (RAILROAD COMMISSION OF TEXAS 1980).

found that a 40% solution of brine was toxic to white shrimp (*Panaeus setiferus*), brown shrimp (*P. aztecus*), and blue crab (*Callinectes sapidus*) larvae. MACKIN (1971, 1973) reported no effect or only a localized reduction in population levels of marine benthos in Trinity Bay, Texas after exposure to petroleum brine. Variable results were found in a series of research projects in Timbalier Bay, Louisiana. Some investigators reported that the density of invertebrates was much lower at oil platforms than at control stations, but others could find no reductions in benthic standing crop (work reviewed by ARMSTRONG et al. 1977).

Texas bays and estuaries are important sites of oil and gas exploration and production, but these same areas are also used by economically important shellfishes and finfishes. Because of the importance of Texas bays and estuaries to fish production and the certainty of increasing levels of oil production, we conducted this study to determine the acute toxicity of brines associated with petroleum.

MATERIALS AND METHODS

Petroleum brine, pumped from 2000-3000 feet (Frio Formation) and from 5000-6000 feet (Miocene), was collected from a settling tank at an American Petroleum Institute separator in Aransas County near the Texas Gulf Coast and transported to the Texas Parks and Wildlife Department Marine Laboratory, Rockport, Texas, where it was stored before being used in static acute toxicity studies.

Sheepshead minnows (*Cyprinodon variegatus*) were collected from coastal marshes near Rockport, Aransas County, Texas with dip nets or a seine. Pinfish (*Lagodon rhomboides*), Atlantic croaker (*Micropogonias undulatus*), white mullet (*Mugil curema*), and brown and white shrimp were caught near the Gulf Intracoastal Waterway with a shrimp trawl. The tests were conducted in 57-L Pyrex bioassay jars, by standard methods (COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS 1975). All test animals were held in Aransas Bay water at the laboratory for at least 48 hours before they were used in the tests. Any lot in which more than 10% of the animals died was discarded. All tests were conducted at $22 \pm 2^{o}C$. Test water was analyzed for calcium, magnesium, and sulfates by standard procedures of the AMERICAN PETROLEUM INSTITUTE (1965). Dissolved oxygen, salinity, and pH were also measured.

Before starting the toxicity tests, we determined the salinity tolerance of sheepshead minnows. Deionized water and synthetic seasalts were mixed to provide salinities ranging from 20 to 100 0/00 in 10 0/00 increments. Sheepshead minnows were exposed to this water and a LC50 value was determined. Initial range finding tests were conducted with petroleum brine diluted with deionized water. Concentrations ranged from 0 to 100% brine in 20% increments. After 100% survival and mortality limits were established, we conducted a second series of tests using 5 0/00 increments to establish the 96 h LC50 for petroleum brine. A third series of tests was conducted in which Aransas Bay seawater was used to dilute the brine. In another test, Aransas Bay water was allowed to evaporate until the salinity matched our calculated LC50 for the petroleum brine. Test organisms were then exposed to this water. In each test 20 fish were used and each test was replicated. A control with normal seawater was conducted with each series.

We calculated LC50 values by using a computerized probit analysis program at the University of Missouri Computer Center. One 18-L brine sample was analyzed by gas chromatography (GC) to quantify the organic components in the brine.

RESULTS AND DISCUSSION

The complete series of tests, which included tolerance testing with artificial seasalts, and with petroleum brine in two different dilution waters, were conducted only with sheepshead minnows. However, less detailed studies of exposure of five other species (three fishes and two shrimps) to petroleum brine diluted with seawater yielded results similar to those obtained with sheepshead minnows. Although all of the data are shown in Table 1 for comparison, our further discussion of the results is limited to data for sheepshead minnows.

The 96 h LC50 for sheepshead minnows exposed to artificial seasalt was 66 (62-69) 0/00 (Table 1). Some sheepshead minnows survived salinities as high as 80 0/00. The initial reaction of the fish was a twofold to threefold increase in the respiration rate over that observed in control fish. Before death, moribund fish in salinities above 70 0/00 moved to the surface and exhibited a loss of equilibrium.

The 96 h LC50 for petroleum brine diluted with deionized water was 35 0/00. This salinity occurred when the test water consisted of about 55% brine. For brine diluted with seawater the LC50 was 52 (37-55) 0/00 or 60% dilution. The behavior of moribund sheepshead minnows in these two experiments was similar. Within 15 min. after the exposure had begun, mucus began sloughing from the fish and equilibrium was lost. Shortly before death, the fish swam erratically to the surface and many underwent tetany, dying with their opercles flared and mouths open. The behavior of the other three species of finfish was similar.

In tests in which bay water was evaporated to the same salinity as the test brine concentrations, all sheepshead minnows survived.

Analysis of the test water showed that concentrations of calcium and manganese were much higher and the concentration of sulfate was lower in petroleum brine diluted with either deionized water or seawater than in normal seawater. GC analyses of the brine showed that it contained 15.5 mg/l of total aliphatic and 2.4 mg/l of total aromatic hydrocarbons. The GC pattern is typical of the water soluble fraction of Texas crude oil (P. L. Parker, Univ. Texas Marine Science Inst., pers. comm.).

Sheepshead minnows are hardy in terms of salinity tolerance

Table 1. Acute toxicity (96 h LC50 and 95% confidence interval) of petroleum well brine to common estuarine organisms. Percent dilution of brine at the LC50 and the calculated hydrocarbon concentation at the LC50 are shown.

Species	Natural salinity range (0/00)	Test Media and LC50 0/00				
		Artificial seasalt	Brine diluted with seawater	Brine diluted with deionized water	Percent Brine	Hydrocarbon concentration (mg/l)
Sheepshead minnow	1.8-142	66(62-69)	52(37-55)	35(33-39)	60(20-65)	11(4-12)
Pinfish	0.1-74	71	51	37	50	9
Atlantic croaker	2-75		46		35	6
White mullet	15-34		37		50	9
Brown shrimp	1-60		37		50	9
White shrimp	1-45		36		50	9

and have been collected from areas with salinity as high as 142 0/00 (GUNTHER 1967). In the present study fish were able to survive up to 80 0/00, or more than twice the normal salinity of the ocean. However, 54 0/00 was the highest salinity at which any fish survived in petroleum brine diluted with seawater. Thus the toxicity of the petroleum brine may be due to a component other than salinity--possibly the petroleum hydrocarbons in the brine, the ionic imbalance of the brine or to synergistic combination of the two.

Petroleum brine differs from normal seawater in the relative concentration of various ions. Seawater was low in calcium and magnesium and high in sulfate compared with the petroleum brine. Fish tissues were not analyzed in this study, but MATTHIESSEN and BRAFIELD (1977) found that elevated calcium levels increased the uptake of dissolved zinc in threespine sticklebacks (*Gasterosteus aculeatus*). The high concentration of calcium in the brine could have similarly affected organisms in this study.

The brine contained 17.9 mg/l of the water soluble fraction of crude oil. Previous studies have shown that the water soluble fraction can cause a variety of effects in fish, including increased respiration rates (THOMAS and RICE 1975); increased liver weight to body weight (YARBROUGH et al. 1976); and at petroleum hydrocarbon concentrations of 1.1 mg/l, changes in developmental time, pathological changes in fish tissues and death (ERNST et al. 1977). Even at the lowest dilution (20%) we calculated that the petroleum brine contained about 4 mg/l of hydrocarbons. Some of the lighter hydrocarbon components, such as naphthalene, are rapidly lost from crude oil (WINTERS and PARKER 1977) but analyses by others has shown that napthalenes are concentrated in the sediments (ARMSTRONG et al. 1979).

The results of these tests show that in the laboratory, petroleum brines are acutely toxic to sheepshead minnows when diluted about 40% with seawater to a salinity of 52 0/00. Petroleum brine mixed 50% with seawater to a salinity of 37 0/00 was acutely toxic to white and brown shrimp. The toxicity of petroleum brine in a natural situation is determined by the amount of brine being discharged, the efficiency of the brine/oil separator, the volume of the receiving water, and the rate at which mixing and exchange take place. Bays and estuaries along the Texas coast are isolated from the Gulf of Mexico by a nearly continuous barrier island and water exchange with the Gulf is slow. Upstream, dams have been constructed in all of the major rivers entering the Gulf, and freshwater inflow is reduced even further during drought years. Reductions in the freshwater inflow to Texas estuaries or increases in brine discharge will increase the probability of toxic conditions being created in some estuarine systems. Preservation of the continued high productivity of these valuable biological resources will require that brine discharges and estuarine conditions be closely monitored to insure that salinity and hydrocarbon concentrations do not reach toxic levels.

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