AN EXPERIMENTAL OIL SPILL: THE DISTRIBUTION OF AROMATIC HYDROCARBONS IN THE WATER, SEDIMENT, AND ANIMAL TISSUES WITHIN A SHRIMP POND

Bruce A. Cox  
County Extension Marine Agent  
San Benito, Texas

Jack W. Anderson  
Department of Biology  
Texas A&M University  
College Station, Texas

and

Jack C. Parker  
Agricultural Extension Service  
Texas A&M University  
College Station, Texas

ABSTRACT

A common practice in the mariculture of shrimp on the Texas coast is the application of fuel oil on the surface of the pond. This thin oil layer serves to eliminate large aquatic insects which are predators of the small juvenile shrimp. Ordinarily, a common diesel fuel is used and it is removed from the pond after one day's treatment. In this experimental spill study, a high aromatic (#2 fuel oil was utilized in higher quantity than normal and the residue was not removed. Mortalities of juvenile shrimp (Peneaus setiferus) and other invertebrates associated with the pond were recorded over a period of 96 hours following the oil treatment. A peak in the level of mortality occurred 48 hours after the spill, coinciding with the peak in the concentration of naphthalenes (naphthalene, methyl-naphthalenes in the tissues of caged and free swimming organisms occurred at the point (48 hours) at which the concentration of these compounds was maximal in the water column. The concentration of naphthalenes in the sediment reached a peak approximately 12 days after the maximum peak in the tissues and water.

While the pond water and sediments studied contained measureable levels of naphthalenes, the shrimp and clams (Rangia cuneata) were shown to release a major portion of accumulated naphthalenes by day 96. Oysters and sediment measured at this same time interval showed levels between 3 and 6 parts per million (ppm) total naphthalenes. Shrimp, clams, and oysters taken to the laboratory 38 days after the spill released naphthalenes to background or near background levels. The data compiled in this study were compared to the findings of various field and laboratory investigations.

INTRODUCTION

The unpredictability of oil spills in the environment makes it difficult to commit financial resources, technical analytical services, and manpower to study the immediate biological and chemical changes which occur directly after a spill. To date, research which has monitored the fluxes of oil hydrocarbons between the water, sediments, and tissues of marine organisms directly after (within the first 96 hours) a spill is fragmentary.

Blumer and Sass [1] stimulated research in the area of petroleum hydrocarbon uptake and release when they reported that petroleum-contaminated oysters, Crassostrea virginica, placed in oil-free seawater for six months retained oil without change in composition and quantity. Lee et al. [2] investigated uptake and release of specific oil hydrocarbons by the marine mussel Mytilus edulis and they found that mussels rapidly accumulated selected oil hydrocarbons from seawater solutions. When placed in oil-free seawater, the mussels quickly discharged most of their contained petroleum compounds. Stegeman and Teal's work [3] with the oyster, Crassostrea virginica, indicates that oysters containing petroleum released the majority of oil hydrocarbons rapidly when placed in a running seawater system containing a low background of oil. Anderson [4] exposed oysters to a #2 fuel-oil-water-dispersion in the laboratory. Of the oil compounds in the exposure medium, naphthalenes—a general term referring to naphthalene (N), methyl-naphthalenes (MN), and dimethylnaphthalenes (DMN)—were primarily accumulated in the tissues of the oysters in high concentrations. Anderson [4] further reported that both saturated hydrocarbons and the naphthalenes were released to below detectable levels (less than 0.1 ppm) within 52 days. When brown shrimp, Peneaus aztecus, were exposed to a water-soluble fraction prepared from #2 fuel oil, they selectively accumulated alkyl-naphthalenes over triaromatics and n-paraffins, but these were depurated to background levels within 10 days [5]. Studies by Cox and Anderson [6] and Tatem and Anderson [7] on the crustaceans Peneaus aztecus and Palaemonetes pugio, respectively, have demonstrated the rapid accumulation and release of naphthalene and alkyl-naphthalenes.

The compounds N, MN, and DMN represent the major portion of the toxicity of the water-soluble fraction (WSF) prepared from a #2 fuel oil [5]. Because of the biological importance of naphthalenes, consideration of the distribution and concentration of naphthalenes in the environment after spills would be very meaningful.

In order to relate laboratory derived data to the response of organisms in the natural environment, a controlled field experiment was initiated. The opportunity for this experiment came when Dr. Jack Parker, a director of shrimp mariculture studies at Texas A&M University, San Benito, Texas, suggested the use of #2 fuel oil as an experimental spill. Parker's research [8] has monitored the fluxes of oil hydrocarbons between the water, sediments, and tissues of marine organisms directly after (within the first 96 hours) a spill is fragmentary.

607
University, added #2 fuel oil to a shrimp pond containing juvenile white shrimp, *Penaeus setiferus*, to control diving beetles which are predacious on young shrimp. This method of control is commonly used, but in this instance, the quantity of oil was increased, the fuel oil contained a higher amount of aromatics (38%), and the excess oil was not removed from the pond after treatment.

**Materials and Methods**

The day before #2 fuel oil was added to the experimental shrimp pond, a wire cage containing clams, *Rangia cuneata*, and oysters, *Crassostrea virginica*, and two cages each containing 20 white shrimp, *Penaeus setiferus*, were placed in the pond. These same three species were held in identical cages and placed in a randomly selected control shrimp pond to which oil was not added. The cages were constructed of 0.625 cm² galvanized mesh hardware cloth molded into a 28.1 x 55.8 x 8.91 cm rectangular shape. Cages containing the oysters and clams were supported about 5 cm above the bottom by two galvanized runners. Shrimp cages were placed directly onto the sediment. The locations of clam, oyster, and shrimp cages and of stations where water and sediment samples were taken are shown in figure 1. Water and sediment samples and tissues from oysters, clams, and shrimp were taken 24 hours prior to the addition of #2 fuel oil for the determination of background levels of petroleum-derived hydrocarbons.

At 8:00 A.M. on August 27, 1973, 113.4 liters of #2 fuel oil (API reference oil #3) was evenly layered over the 0.204 hectare (.504 acre) earth-bottomed experimental shrimp pond (1.2 m in depth) containing an estimated 2.786 x 10⁶ liters of seawater. This produced a calculated oil-to-water mixture of approximately 40 ppm.

Samples of live shrimp were taken for analyses of oil hydrocarbons in the tissues by trawling the perimeter of the pond with a hand-drawn bait trawl. During the times when oil was dispersed over the pond the trawl net containing shrimp was simply placed, while remaining under water, into a large plastic trash bag which was sealed and brought up through the slick. Moribund shrimp swimming aimlessly at the surface were collected by hand for analysis to compare their diaromatic hydrocarbon content with that of trawled shrimp.

When necessary, oysters and clams to be checked for oil hydrocarbon content were brought through an oil slick by placing them in a sealed jar which was submerged, uncapped, filled, and then sealed beneath and raised through the oil layer.

Water samples for determination of oil hydrocarbon concentrations were taken at the surface and at approximately two feet below the surface at stations 1 and 2. Surface samples were collected by holding the mouth of a 16-ounce glass bottle at the air-water interface and allowing the water to flow into the bottle. Water bottles were sealed with aluminum-lined caps. Water was sampled two feet beneath the oil, or water surface, by submerging a sealed bottle, then uncapping the bottle, and allowing it to fill with water. After filling, the bottle was recapped and brought through the oil slick.

Sediments for oil hydrocarbon analyses were obtained by scraping the top inch of mud into a 16-ounce wide-mouth glass jar which was unsealed and ressealed beneath the water. Excess water in the sediment-containing jar was then removed after samples were brought through the slick. Care was taken to collect sediments from previously unsampled areas.

After the spill, shrimp, clams, oysters, sediment, and water samples were collected at various time intervals in both experimental and control ponds. Nine months after the spill, 13-cm sediment cores were taken at stations 1, 2, and 3 with a 5.05-cm internal diameter core, and for each 2.5-cm interval the concentration of N, MN, and DMN was determined. All tissues and water samples were analyzed by UV spectrophotometry [8] for N, MN, and DMN. The same UV technique was used to determine the concentrations of naphthalenes in sediment by adding 10 ml spectrophotometric grade hexane and 1 g of Florisil to 3-7 g sediment. The hexane was then scanned from 190 to 240 nanometers (nm) for peaks at 221, 224, and 228. Concentrations of naphthalenes could then be analyzed by solving simultaneous equations. When concentrations of N, MN, and DMN versus time are graphically expressed using semilogarithmic scales, the origins should be interpreted as 0 points for time and concentrations of naphthalenes. Also, the concentrations of naphthalenes in tissues and sediment which are illustrated in either figures or tables have had background levels, determined from control samples (not exposed to oil), subtracted from them.

The percent mortalities of shrimp in the holding cages were recorded between 8:00 and 9:00 A.M. on days 1, 2, 3, and 4 after the spill and dead shrimp were removed from experimental and control pond cages at these times. Mortalities of shrimp outside of the holding cages and deaths of naturally occurring invertebrates were noted daily from 7:30 to 9:00 A.M.

Salinity, temperature dissolved O₂, pH, PO₄, alkalinity, CO₂, NH₄ and NO₃ were monitored in both the experimental and the control shrimp ponds one day before the spill, the day of the spill, and 1, 2, 3, and 4 days after the spill. All but PO₄ and alkalinity were measured at various intervals from 16 to 29 days after the spill. After the 29th day, salinity, temperature, pH, and dissolved O₂ were periodically determined.

**Results**

Number 2 fuel oil was spread over the shrimp pond in 30 minutes and it blew to one side 45 minutes later. During most of the first week, prevailing southwest winds contained the oil at the north end of the pond during the day, but at night, the wind generally subsided and allowed the oil to respread over the pond.

While oil was present at the surface of the water 4 days after the spill, none was seen at 14 days. When walking through the sediment...
along the edges of the pond at 14 days, small slicks of oil formed at the surface. Thirty-eight days past the spill, the water was very clear and blue-green algae were prevalent on the bottom of the pond. The algal mat was especially thick in areas along the west and north edges of the pond where #2 fuel oil was found most of the time. Lush growths of benthic blue-green algae were not observed in the control pond. Between 38 and 96 days, water was drained from the control pond and, therefore, this pond was eliminated from further study. Also, between 38 and 96 days, the exposure pond developed a slow leak and the water level was reduced from a depth of about 1.2 meters to approximately 0.6 meters. During the eight months after the spill, water was added to the pond raising the water level back to approximately 1.2 meters.

Deaths of various invertebrates associated with the experimental (oil-exposed) pond were observed and recorded (table 1). For all juvenile white shrimp, deaths outside of the holding cages took place mostly at night. Because refined estimates of the total number of shrimp present in the pond before the spill could not be made, data stating the percent of mortalities of shrimp outside of holding cages were not determined. The greatest mortality (4,979 dead) occurred during the night between 24 and 48 hours after the spill. A total of 5,802 dead shrimp were counted from 0 to 72 hours after the spill, but no mortalities were observed after 96 hours. On any given day, recent shrimp mortalities could be distinguished from shrimp mortalities of the previous day because the former were white, while the latter turned pink while decaying. When shrimp were stressed and close to death they swam aimlessly at the surface and did not attempt to escape when approached. Because of their random swimming, many moribund shrimp would eventually indirectly migrate to shore and die.

Great numbers of the fiddler crab, *Uca minax*, inhabited the banks around the perimeter of the pond. Their habit of passing back and forth across the air-water interface even when the water had oil floating on top did not result in any apparent mortality problems with these animals. Only three dead fiddler crabs were observed (table 1). The small white crab, *Sesarma cinerea*, was not very abundant in and around the experimental pond but, of those present, no mortalities were recorded. This crab also spends a major portion of its time out of the water. The small clam, *Mulinia lateralis*, was very abundant in the bottom mud of the experimental and control ponds. No mortality was observed during the first four days after the spill, but after two weeks, empty shells were observed in the experimental pond. Similar numbers of dead *Mulinia* were not observed in the control pond. Moribund and dead *Mysidopsis almyra* were noted at 24, 48, and 72 hours after the spill. *Mysidopsis* mortalities were not observed after 72 hours. Many water boatmen, *Corixidae*, were found floating in the oil while 5 diving beetles of the family hydrophilidae were found dead.

### Table 1. Observed mortality of selected invertebrates in the experimental shrimp pond

<table>
<thead>
<tr>
<th>Species</th>
<th>Mortalities observed but unable to estimate number.</th>
<th>No. alive before spill</th>
<th>No. alive 48 hr.</th>
<th>No. alive 72 hr.</th>
<th>No. alive 96 hr.</th>
<th>Total</th>
<th>Mortalities observed</th>
<th>Number of Mortalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penaeus setiferus</td>
<td>on shore and small walkway over the water</td>
<td>0</td>
<td>430</td>
<td>4,797</td>
<td>676</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sesarma cinerea</td>
<td>dead or dying at or beneath water surface; relatively abundant</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Uca minax (Fiddler crab)</td>
<td>all around the bank of the pond; abundant</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Mulinia lateralis (clam)</td>
<td>throughout the bottom of the pond; abundant</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Cornelia sp. (Corixidae)</td>
<td>floating dead at surface; relatively abundant</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>Hydrophilidae (diving beetles)</td>
<td>rare</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
</tbody>
</table>

The percent mortality of shrimp held in cages in the experimental pond was compared with those held in the control pond (table 2). By 96 hours, a significantly greater number of deaths had occurred among shrimp in cages in the experimental pond than in the control pond, and the main period of mortality for shrimp in the experimental holding cages was between 72 and 96 hours.

### Table 2. Percent mortalities of *Penaes setiferus* confined in holding cages in the experimental and control shrimp ponds

<table>
<thead>
<tr>
<th>Time Interval</th>
<th>Control</th>
<th>Experimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day before spill</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>spill day</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>24 hr. after</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>48 hr. after</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>72 hr. after</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>96 hr. after</td>
<td>20.75</td>
<td>75</td>
</tr>
</tbody>
</table>

The concentrations of N, MN, and DMN in the water, sediment, and tissues of white shrimp, clams, and oysters were determined by UV analysis at various time intervals after the spill (figure 2). The concentrations of N, MN, and DMN in subsurface water samples from station 2 reached a peak at 48 hours. From 8 through 7,104 hours (296 days), DMN was more concentrated in the water than either N or MN. At 4,344 hours (181 days), the concentrations of N, MN, and DMN had dropped below 0.01 ppm. Water samples taken at 7,104 hours after the spill still showed definite traces of naphthalene: a well-defined maximum absorption peak was seen at 221 nm.
The accumulation of naphthalenes in the sediment peaked at 14 days (336 hours) and seemed to lag behind the increase of N, MN, and DMN concentrations in the water (figure 2). While the concentration of the sum of these compounds in the water was decreasing, the concentration in the sediments was increasing. The magnification factors (mud-to-water ratio) for the sum of N, MN, and DMN at 336, 2,256 and 7,104 hours were 78, 378, and 88, respectively. These ratios are indicative of the lag in peak hydrocarbon levels.

The concentration of the sum of N, MN, and DMN reached a peak in the tissues of shrimp, clams, and oysters at 24, 48, and 72 hours, respectively (figure 2). As the sum of the concentrations of N, MN, and DMN in the water peaked, so did the concentrations in shrimp, clams, and oysters. The decrease of naphthalenes in these organisms seemed to reflect the decreases in the water more than those in the sediment. An exception is noted for oysters which maintained high levels of naphthalenes without drop from 96 to 2,256 hours, while the concentration in the water had fallen. At all sampling periods past 24 hours, DMN was more concentrated in the tissues of shrimp, clams, and oysters than either MN or N.

Cores were taken at 7,104 hours (296 days) after the spill to trace the movement of naphthalenes into the sediment. A 15.5 cm core was taken at stations 1, 2, and 3, and the amount of N, MN, and DMN was determined by UV analyses at various depths (figure 3). Each point, indicating the concentration of N, MN, or DMN, is the average of the concentrations found at a particular depth at each of the three stations. The concentrations of naphthalenes rapidly decreased from 2.54 to 7.62 cm beneath the water-sediment interface, then slowly decreased to 0 (background) at 15.24 cm. Absorbance peaks indicating N, MN, and DMN were not found in sediment samples taken 15.24 cm beneath the sediment-water interface.

Figure 3. The concentration of naphthalenes and alkynaphthalenes at various depths in the sediment of the pond 296 days after oil treatment.

In an attempt to explain the cause of high mortality of white shrimp in the experimental pond at 24 hours and 48 hours, a comparison was made between the concentration of N, MN, DMN, and trimethylnaphthalenes (TMN) compounds in the water of the experimental pond and those in the water of bioassay tests on this species. In table 3 the concentrations of these hydrocarbons in the pond water after 24 hours are compared with those present in the water-soluble fractions of #2 fuel oil used in laboratory toxicity tests. The TLm is that concentration which resulted in 50% mortality of the organisms in the 24-hour period. The shrimp used in the bioassay were from the control pond and were of approximately the same size as those in the experimental pond. The concentrations of N, MN, DMN, and TMN compounds in the water of the shrimp pond and the bioassay containers were determined by gas chromatographic and mass spectrophotometric analysis (GC-MS). The N, MN, DMN, and TMN concentrations were much higher in the bioassay solution producing the 24-hour TLm than in the water of the shrimp pond. It should be noted that the 24-hour water concentration in the pond was approximately the maximum level reached. When the concentrations of these compounds are totaled and compared, the concentration was 10.4 times higher in the bioassay water than in pond water.

The concentrations of N, MN, and DMN determined by UV analysis in the combined tissues of 5 fiddler crabs, Uca minax, and 3 wharf crabs, Sesarma cinerum, at 72 hours after the spill were compared with that in three combined samples of white shrimp collected at the same time (table 4). Although the crabs were not in the water nearly as long as the shrimp, the concentrations of the diaromatics in their tissues were very close to the levels found in the shrimp.

Table 4. Concentrations of naphthalene and alkynaphthalenes in the tissues of Penaeus setiferus, Uca minax, and Sesarma cinerum from the experimental shrimp pond. The tissue samples were taken 72 hours after #2 fuel oil was added to the shrimp pond.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Concentration (ppm) at 72 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Penaeus setiferus</td>
<td>0.45</td>
</tr>
<tr>
<td>Uca minax</td>
<td>0.75</td>
</tr>
<tr>
<td>Sesarma cinerum</td>
<td>0.93</td>
</tr>
</tbody>
</table>

* N = naphthalene; MN = methylnaphthalene; DMN = dimethylnaphthalene. TMN = trimethylnaphthalene.

Contaminated white shrimp, clams (Rangia cuneata), and oysters were taken from the experimental shrimp pond 38 days after the spill and placed in oil-free, charcoal-filtered seawater in the laboratory. Animals were periodically harvested for determination of the remaining sum of N, MN, and DMN by UV technique (figure 4). Molluscs were not fed during the depuration period, but the shrimp were fed a finely ground commercial fish food. The shrimp released their diaromatics within 240 hours (10 days) in the laboratory to previously established control (background) levels, while oysters depurated to background levels after 47 days in the laboratory.

1 These analyses were conducted by Dr. J. Scott Warner, Battelle Memorial Laboratories, Columbus, Ohio.
From laboratory and field studies by Cox [5] it would not appear that white shrimp significantly accumulate naphthalenes from manipulation or ingestion of contaminated sediments.

While a detailed study of the invertebrates associated with the experimental pond was not intended, the findings recorded were of interest. The death of insects was anticipated and was indeed one of the purposes of the oil treatment. The mortality exhibited by the small crustacean Mya smaragdina was reasonable in light of earlier laboratory studies which indicated that this species is more sensitive to petroleum hydrocarbons than white shrimp and the other crustaceans tested by Anderson et al. [9,10]. While a few of the shore crabs inhabiting the pond area were killed by the oil, nonstatistical observations indicate that the percentage mortality was low. The bivalves (Rangia and Crassostrea) used in the accumulation and release phases of the study all survived the exposure. The apparent mortality of a portion of the natural Mulinia lateralis population could not be quantitatively estimated for the lack of baseline data.

Perhaps the most interesting aspect of the findings is the scenario which can be constructed from the data on fluxes of naphthalenes between water, tissues, and sediments. As has been noted from numerous studies conducted in our laboratory, the fluxes of N, MN, and DMN from oil to water and water to tissue are very similar. While the fluxes of these individual compounds of the oil were recorded it is possible and logical to speak in terms of the activities of total naphthalenes derived from the #2 fuel oil. The concentration of total naphthalenes in the exposure pond water increased from a background level of about 12 ppb to 500 ppb 48 hours after oil treatment and slowly decreased to background levels by 296 days after the oil treatment. There was evidence that a portion of the contamination had reached a level of 12.5 cm below the water-sediment interface. It is not possible to discern whether the migration to lower levels was the result of capillary action of interstitial water or periodic resuspension of sediments during high winds. Blumer [11] has stated that sediments may retain oil for long periods, particularly if they are anaerobic. Further monitoring of the sediments in the exposure pond will provide more information on the stability of naphthalenes under specific environmental conditions.

Since man is very much concerned with the survival of commercial species and the possibility of long-term contamination of their tissues, the most significant portion of the scenario relates to these topics. We have already noted the significant mortality exhibited by the white shrimp exposed to the high initial concentration of hydrocarbons. From chemical and behavioral data, it seems likely that the presence of an oil slick on the pond over a period of several days greatly enhanced mortality. Those animals surviving the initial 4 days of the oil treatment accumulated the naphthalenes at rates which closely correlated with the appearance of these compounds in the water. As noted above the sediment contamination did not peak until approximately 12 days after the peaks in water and tissue content. As noted in several earlier reports (Lee et al. [2], Stegeman and Teal [3], and Anderson with several co-workers [6,7,10,12,14]) the petroleum hydrocarbons are accumulated by marine invertebrates from solution in water. The contribution to tissue contamination from sediments cannot be directly ascertained, but the decrease in tissue levels during the period in which sediment content was increasing seems to indicate that for these animals this is a very minor pathway. The exception to this general observation is the longer retention by oysters, which might be the result of renewed contamination from particulates.

While in the contaminated pond and during laboratory maintenance, all species were shown to release hydrocarbons from their
ACKNOWLEDGMENTS

This research was supported by contract No. 0520C from the American Petroleum Institute. We would like to express our appreciation to Mrs. Betty Foster and Mr. Scott Ward for their technical assistance in this investigation.

REFERENCES


