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# Project Summary

# Sublethal Effects of Number 2 Fuel Oil on Lobster Behavior and Chemoreception

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**The experiments described here are designed to determine the oil exposure levels at which lobsters show behavioral abnormalities and inappropriate responses. These levels were determined as 0.1 to 1.0 parts per million (ppm) of oil in water. The behavioral abnormalities can lead to lack of feeding and subsequent population decline; they occur at exposure levels below those levels that cause obvious loss of equilibrium and coordination (levels over 1 ppm), eventually leading to death of the organism.**

**Extensive control measurements were incorporated in the experimental design to ensure that the observed behavioral changes were due to oil exposure and not to natural variability. Rigorous chemical procedures determined the actual exposure levels in the lobster tanks. To understand the consequences of the behavioral abnormalities measured in these highly controlled and, thus, artificial laboratory experiments, the results must be interpreted in the context of the lobster's natural behavior and ecology. Such field studies and naturalistic observations are areas of active research in this laboratory.**

**In an attempt to explain the mechanisms by which the behavior deteriorates, two topics were examined: interference with normal smell and taste, and change of motivation. A combination of neurophysiological and behavioral experiments on chemoreception were designed to investigate these topics.**

*This Project Summary was developed by EPA's Environmental Research Laboratory, Narragansett, RI, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).*

## Introduction

Research on the effects of oil pollution on marine organisms is a matter of general concern because of increasing tanker transportation, offshore drilling and the companion risk of oil spills. High concentrations of petroleum hydrocarbons can be lethal to many marine species. Lower, sublethal concentrations may interfere with certain life processes such as mating and reproduction, feeding and growth, and defense against predation. Over time, reduced efficiency in these processes may decrease populations without directly killing individuals. Sublethal pollution may shift the ecological balance of affected areas, resulting in the eventual disappearance of desirable species (those beneficial to man) and, possibly, the proliferation of undesirable species. Such was the case after the well-studied 1969 West Falmouth, Massachusetts, spill of No. 2 fuel oil (Sanders *et al.*, 1980). The rich fish and lobster grounds of the North Atlantic may be directly affected.

One sublethal effect may be that oil can interfere with the chemical signals vital to marine life. Most of these organisms use chemical signals more than vision and hearing for feeding, hunting, scavenging, mating, habitat selection, migration, alarm and escape. Mounting evidence also indicates that chemical signals from the egg, in both plants and animals, attract sperm cells to it. Petroleum hydrocarbons interfere with this attraction causing decline of algal populations. In general, interference with chemical communication systems can be expected to have significant consequences, some obvious, some not. Thus, animals that show no signs of locomotor difficulties may still have sensing problems in feeding, finding mates, and escaping from predators. Detecting chemical stimuli has been the focus of several of our studies on pollution interference with normal lobster behavior.

Speculation of petroleum hydrocarbon interference with chemoreception has appeared frequently in the literature, starting with Blumer (1970). The compounds that animals use for communication and orientation have chemical features in common with compounds in petroleum, such as carbon skeleton, functional groups, volatility and solubility. The chemical look-alikes in oil may mimic or mask the reception of biologically important signals. Mimicked signals may cause "false alarms", with animals looking for food or mates, or avoiding predators, where there is none. Alternatively, if the chemical signals are masked, animals may miss opportunities to feed, mate or escape. Another possibility, less often mentioned, is that animals may receive two competing signals, such as an attracting signal from food and a repelling signal from oil In this case, while chemoreception processes may be normal, the animal would not be able to decide whether to feed or hide. Any hesitation might be critical since even slight delays in responding to food can put the animal at a disadvantage in competition with an unimpaired animal.

This study is the first documentation of the specific way in which oil interferes with chemoreception, using behavioral combined with neurophysiological analyses. These studies can provide a better insight into the potential consequences of oil pollution, as the physiological processes of chemoreception are probably similar in all animals. Also, interference with chemoreception or chemically mediated behavior is one of the most sensitive biological measures of low-level oil pollution.

The lobster, *Homarus americanus,* was chosen for the study of sublethal effects of low-level oil pollution for a number of reasons. The lobster lives in and on the floor of inshore and continental shelf waters, areas often affected by oil spills and chronic discharge. In some places, the lobster is the dominent benthic species, so that a decrease in the lobster population could have widespread ecological ramifications The lobster supports an important commercial and recreational fishery, and is a symbol of the region itself. Its decline would have significant socioeconomic consequences. Finally, extensive background data on the lobster already exists, along with oil and drilling mud toxicity studies on various life stages Several of these studies have been and are being conducted in our laboratory (Figure 1) which facilitates interpretation of results from controlled laboratory experiments

Lobsters use chemical cues from their environment to direct a number of vital behavioral responses, such as feeding, courtship and larval settlement Oil may alter many of these. For this study we selected bait localization, which is crucial to survival, is noticeably affected by oil, and is amenable to laboratory testing. In conjunction with behavioral observations, we studied the effects of oil on the two major chemoreceptor systems The antennular system (smell) is normally used to alert the lobster to the presence of food and to convey directional information for odor localization The dactyl chemoreceptor system (taste) is concerned with food evaluation and feeding This study, using the water-accommodated fraction (WAF) of No. 2 fuel oil, considers both distance chemoreception of the antennules and dactyls, and contact chemoreception of dactyls and maxillipeds.

The first purpose of these experiments is to determine the range of No 2 fuel oil exposures affecting the feeding behavior of lobsters without causing neuromuscular disturbance. The second goal is to examine the effects of chemoreceptors in animals in which sublethal behavioral abnormalities have been observed. This approach was used to determine whether the behavioral abnormalities result from oil-induced malfunction of the chemoreceptors, or from what we have called in other studies lack of "motivation" (Atema and Stein, 1974).

Toxicity studies such as this one must be carried out under well-controlled laboratory conditions to be able to document the specific effects of given concentrations and to compare different stages in the life cycle and different seasons. At the same time, the tests would have little relevance without an understanding of the animal's behavior in the natural environment. In order to develop a complete picture of the impact of oil pollution on lobster behavior, the following sequence of experiments should be undertaken: 1) nature studies to determine the general context in which the animal evolved and presently lives, and to determine its sensitivity to particular stresses of its environment; 2) laboratory studies to quantify promising behavioral measures; 3) detailed, rigorously controlled laboratory tests to collect a data base to generate a response model; and 4) field studies on the same behavioral effects to verify laboratory results. Field studies on the American lobster are currently underway. Since these studies are technically and physically difficult, an intermediate step has been successfully used which employs naturalistic large aquaria, where a small group of animals can be observed for extensive periods of time under semi-natural conditions (Atema *et al.,* 1979). Such basic information is needed to interpret whether, for instance, a given behavior results from oil exposure or rather from a combination of pre-molt aggression and lack of food

## Experimental Procedures

Lobsters were trapped locally in the Woods Hole, Massachusetts, area and fed herring or mussels while being laboratory acclimated. Animals which had molted 2 to 8 weeks prior to trapping were selected for the experiments to avoid effects of premolt behavior. Equal numbers of males and females were distributed between experimental and control groups. All lobsters were early adults measuring from 65 to 75 mm carapace length.

The experiments were conducted in a continuous flow-through dosing system with two head tanks (Figure 2). Seawater inflow to the experimental head tanks was 4 l/min. Oil was introduced at a fixed rate into a fast jet of seawater by a syringe pump causing rapid emulsification. From the head tank, the surface layer was skimmed off and discarded and the remaining oil-water mixture entered six individual 100-liter lobster tanks. This oil-water mixture is called the water accommodated fraction (WAF). The overflow from the individual tanks ran into a holding tank where

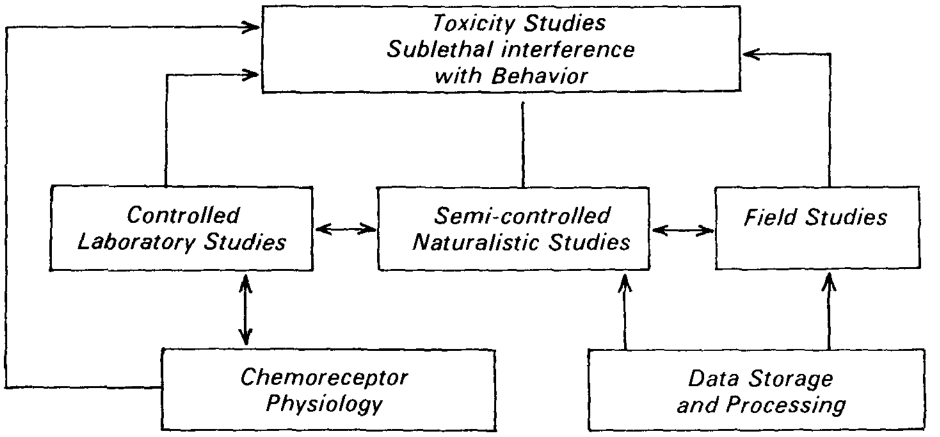


***Figure 1.*** *Relationship of projects: A multidisciplinary approach provides the context in which controlled laboratory and toxicity studies may be interpreted.*

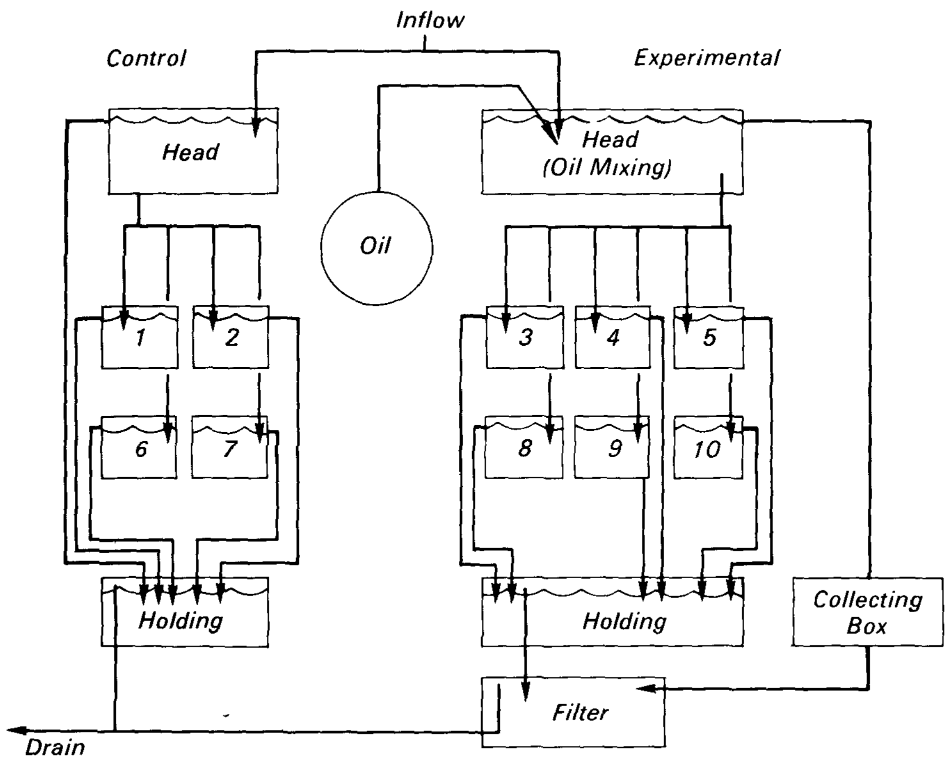


***Figure 2.*** *Flow-through oil dosing system.*

other lobsters were exposed to oil for the neurophysiological studies. The control head tank, which had an inflow of 2.6 l/min and no oil, supplied four 100-liter lobster tanks. Inflow to all individual lobster tanks was kept between 400 to 460 ml/min. These tanks had a front glass window for observation, a shelter and a pebble substrate. Experiments were carried out at temperatures ranging from 8° to 23°C in ambient, untreated, and unfiltered seawater. The oil, Exxon No. 2 fuel oil, was obtained from the U.S. Environmental Protection Agency (EPA), Environmental Research Laboratory, Narragansett, Rhode Island. All lobsters were acclimated to their individual tanks and experimental conditions for at least one week prior to starting the experiments.

Each experiment started with an additional five days in the tanks serving as an internal control for the individuals to be exposed to oil. The experimental lobsters were exposed to predetermined concentrations of oil for five days. The external control group was not exposed A third 5-day period after the tests allowed for measurement of possible recovery or persisting effects. The entire experiment lasted 15 days.

The behavioral series included nine tests. Three were performed at oil concentrations of approximately 0 1 ppm WAF, three at approximately 0.3 ppm, and one at 1.5 ppm. Two additional tests were done at 0.3 ppm, the first on lobsters whose antennules were being cut off during the course of the experiment, and the second, a month later on the same lobsters, now without the antennules

The feeding behavior of all lobsters was recorded twice daily, each day at the same times in the early morning and the late afternoon. After one minute of behavioral observation, food was lowered to them on a string alternately from the right and left corners of the tank Subsequent feeding behavior was measured by breaking down activity into five parts. ALERT was the first observable response to food; WAIT was the period from then until the lobster left its shelter; SEARCH was from that point to its grasping the food, or HIT; and finally it was noted if the animal actually did EAT the food. Lobsters were given 10 minutes to complete the sequence

An entirely separate series of neurophysiological tests was performed on lobster antennules to determine the effects of oil exposure on its chemoreceptors. For this experiment, the lateral flagellum of the antennule was cut off and placed in a small chamber with continuously flowing seawater. Test chemicals were injected into the seawater flow. Recordings were made by picking up a small nerve bundle with a platinum electrode The signal was amplified, displayed and recorded on conventional equipment. Experimental and control antennules were taken from animals during this experiment and also from animals in the flow-through holding tank.

The chemical stimuli consisted of five preparations. mussel juice, No. 2 fuel oil, mussel juice plus oil, artificial seawater and, again, mussel juice. The mussel juice was a mixture of homogenized mussel tissue and artificial seawater, used in concentrations of 1 to 3 ppm. Artificial seawater was the conventional MBL formula used to provide consistency. The concentration of oil was much higher (1 to 3 ppm) than in the behavioral tests because to measure chemoreceptor responses one often needs concentrations about ten times higher than those needed to see reactions in the live animal The sequence of stimuli provided an internal control for the test of oil effects on chemoreceptors. The mussel juice stimulus at the beginning and at the end showed whether the nerve bundles were still intact after the test series; the artificial seawater stimulus applied midway through the experiment showed whether they were reacting to mechanical water flow and chemically neutral stimuli.

Daily samples of water were taken from the lobster tanks. Infrared (IR) and ultraviolet (UV) spectroscopic and gas chromatographic (GC) analyses were performed to determine the concentrations and types of hydrocarbons present in both experimental and control lobster tanks. Salinity, pH, oxygen content and ammonia were monitored every other day during the first experiment.

## Results and Discussion

Results from the chemical analyses of lobster tank water show that experimental tanks received petroleum at the approximate rates intended, and that the oil cleared from the water quickly after inflow stopped. Neither control nor experimental tanks carried significant amounts of petroleum before the tests (Figure 3). The IR-observed background of about 0.05 ppm consisted mostly of non-petroleum lipids. Behavioral results did not show observable differences between experiments with fluctuating as compared with constant petroleum dosing. Other water quality criteria (pH, oxygen, ammonia, and salinity) remained at satisfactory levels. Thus, the added oil is shown to have been the primary variable in these experiments

The behavioral results fall into three groups which together bracket the sublethal levels of exposure. The 0.1 ppm level caused little observable effects during the oil exposure (days 6 to 10). The 1.5 ppm level resulted in gross neuromuscular defects which appeared after a few hours and lasted for several days after exposure had stopped. Five out of six such animals could hardly walk and twitched in cramped postures; they responded poorly or inappropriately to food. This level approaches lethal effects; in nature such animals would be helpless and could be described as "ecologically dead." This leaves the relatively narrow range of 0.1 to 1.0 ppm as the oil concentrations which cause sublethal effects. All experiments at the exposure level of 0.3 ppm showed lobsters less likely to look for food and eat after 6 to 24 hours of exposure to oil. They usually recovered after one day in clean water Behavioral changes were apparent in both the occurrence and duration of feeding behaviors. Some

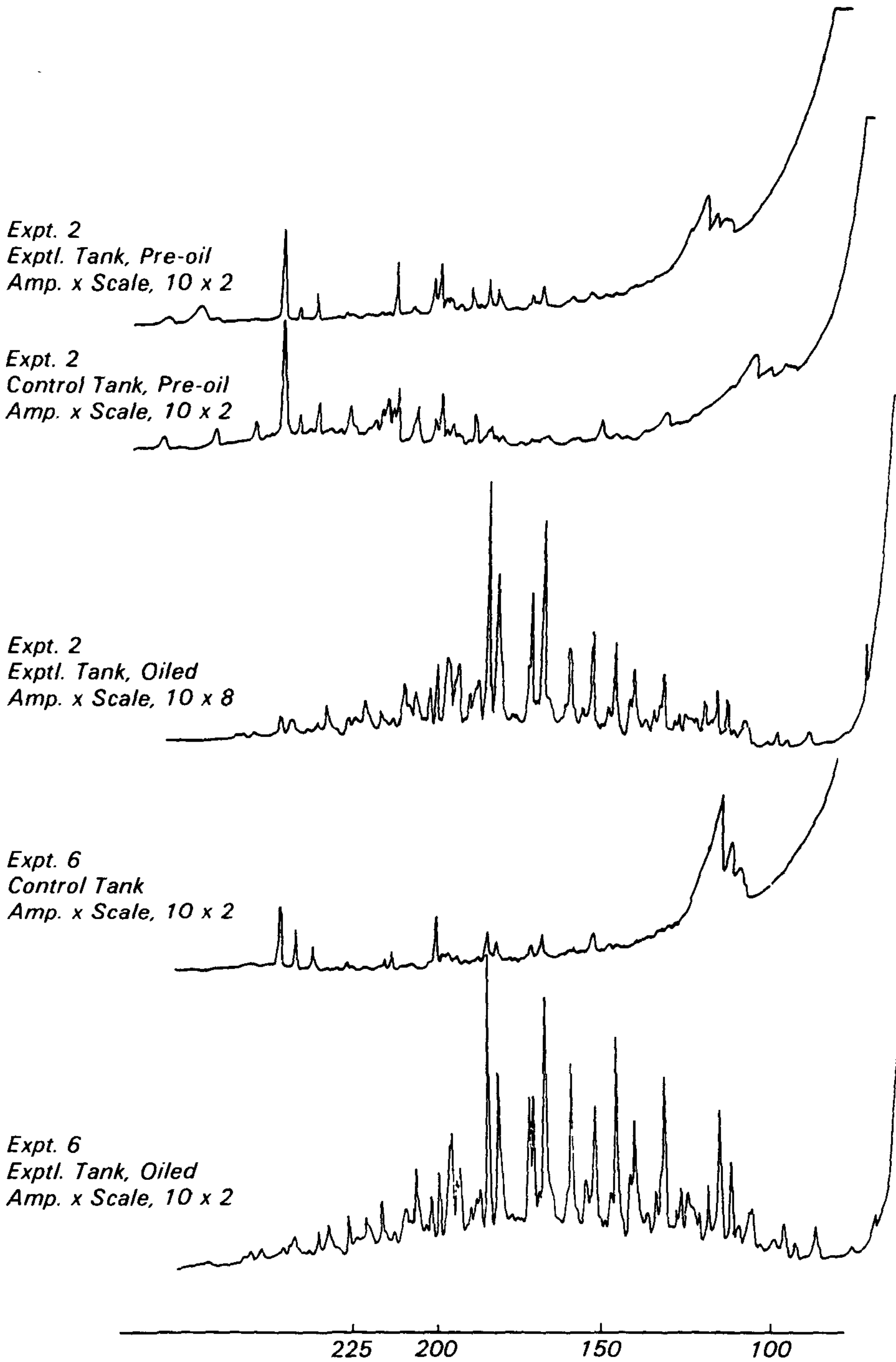


***Figure 3.*** *Gas chromatograms indicating lack of petroleum lipids in control tanks and in experimental tanks before oil was added, and the presence of typical Number 2 fuel oil peaks in experimental tanks during oil exposure at 0.1 ppm and 0.3 ppm.*

animals failed to ALERT to food; others SEARCHED, but did not find the food. The result was that most lobsters missed several feeding opportunities during the exposure period. Similar effects were observed at high and low temperatures. Lower temperature and lack of antennules did not change the effect of 0.3 ppm oil exposure on lobster feeding behavior.

The neurophysiological experiments on antennular chemoreceptors show that 1 ppm WAF No. 2 fuel oil is a chemical stimulus and interferes with the normal response to a common food odor, mussel juice (Figure 4). Because antennules of control and experimental lobsters react alike, chemoreceptor interference is probably not the only way in which petroleum exposure disrupts lobster behavior. Previous experiments (Atema, 1976) suggest that oil can serve as a chemical attractant, repellent and general neurotoxin, meaning that lobsters may be subjected to a mixture of chemical stimuli in the presence of food and petroleum. This may well cause sensory "confusion" and hence inappropriate responses. These could combine with general neurotoxic effects, which may render the animal unmotivated, uncoordinated and unresponsive.

Data from the behavioral studies support this hypothesis. If antennular chemoreceptors were the only mediators controlling feeding behavior, one would expect animals without antennules to react less to oil exposure than those with antennules. One experiment indicates otherwise, suggesting either that chemoreceptors from another part of the lobster, e.g., legs, take over the function of the antennular chemoreceptors, or that oil exposure affects the lobster's motivational state.

The inconsistent behavior of lobsters exposed to a critical concentration of oil argues against purely chemosensory interference, but for an effect on motivational state. On different days, exposed lobsters reacted differently to the presence of food. Those animals that one day alerted normally to the presence of food (an activity more likely under chemosensory control) also searched and found the food that day (activities more likely under motivational control), whereas those that did not show ALERT behavior also did not search and eat. The physiological basis for the observed lack of motivation is not known, but may be caused by general neurotoxicity of certain concentrations of petroleum. These effects appear quickly and obviously at higher exposure levels (>1 ppm of oil) when lobsters lose neuromuscular control.

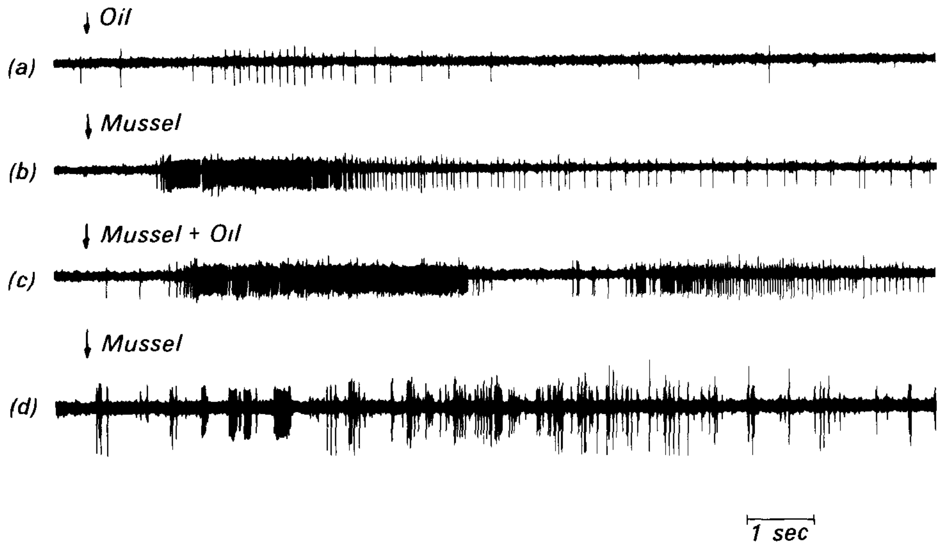


**Figure 4.** *Neurophysiological responses of antennular chemoreceptors to chemical stimuli.*

There apparently is a limited range (between 0.1 and 1 ppm) in which oil toxicity is sublethal for the American lobster. Clear effects on feeding behavior are measured consistently at 0.3 ppm. The ecological implication of impaired feeding behavior is a loss of survival fitness in competition with other animals.

## Conclusions and Recommendations

1) Five days of exposure to 0.3 ppm WAF No. 2 fuel oil caused consistent sublethal interference with feeding behavior of adult and subadult lobsters.
2) Feeding interference lasted for one day after 5-day exposure to 0.3 ppm.
3) Sublethal interference with lobster behavior was not observed at 0.1 ppm exposures, however, at 1 to 2 ppm exposures severe neuromuscular abnormalities appeared, leading within a few hours to cramped postures, spastic behavior and unresponsiveness, and eventually death.
4) Thirty hours of exposure to 1.5 ppm WAF No. 2 fuel oil caused cessation of feeding for over 6 days in most lobsters.
5) Lobsters smell the presence of 3 ppm fuel oil; furthermore, some of their chemoreceptors showed modified responses to food odors in the presence of 3 ppm fuel oil, indicating that oil interferes with their smelling food.
6) Feeding behavior interference may be caused partly by oil effects on chemoreception and by oil-induced changes in feeding motivation, i.e., hunger and/or fear.
7) Based on these results and a safety factor of 10, short-term exposure to water column levels of 0.01 ppm WAF No. 2 fuel oil may not interfere with feeding behavior of adult lobsters. However, further investigations involving larval stages and long-term exposures are recommended before safety levels for chronic petroleum pollution are firmly established. Specific behaviors such as hatching, molting, growth, feeding, phototaxis, settling and substrate selection should be studied.

## References


Atema, J. Sublethal effects of petroleum fractions on the behavior of the lobster, *Homarus americanus*, and the mud snail, *Nassarius obsoletus*. *In:* M. Wiley (ed.), *Estuarine Processes*, Vol I. *Uses, Stresses, and Adaptation to the Estuary*. Academic Press, Inc. New York, NY, pp 302-312, 1976.

Atema, J., S. Jacobson, E. Karnofsky, S. Oleszko-Szuts, and L. Stein. Pair formation in the lobster, *Homarus americanus:* behavioral development, pheromones, and mating. *Mar. Behav. Physiol.*, 6:277-296, 1979.

Atema, J. and L. Stein. Effects of crude oil on the feeding behavior of the lobster, *Homarus americanus*. *Environ. Pollut.*, *6*:77-86, 1974.

Blumer, M. Oil contamination and the living resources of the sea. FAO Technical Conference on Marine Pollution and Its Effects on Living Resources and Fishing. Rome, Italy, December 9-18, 1970.

Sanders, H.L., J.F. Grassle, G.R Hampson, L.S. Morse, S. Garner-Price, and C.C. Jones. Anatomy of an oil spill: long-term effects from the grounding of the barge Florida off West Falmouth, Massachusetts. *J. Mar. Res.*, *38*:265-280, 1980.

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*The complete report, entitled "Sublethal Effects of Number 2 Fuel Oil on Lobster Behavior and Chemoreception," (Order No. PB 82-192 444; Cost: $9.00, subject to change) will be available only from:*

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