

## Long-Term Effects of an Oil Spill on Populations of the Salt-Marsh Crab *Uca pugnax*

**Abstract.** A spill of fuel oil at West Falmouth, Massachusetts, in 1969, contaminated contiguous salt marshes with up to 6000 micrograms of oil per gram (ppm) of wet mud and affected local populations of *Uca pugnax*. Directly related to high-sediment oil content were reduced crab density, reduced ratio of females to males, reduced juvenile settlement, heavy overwinter mortality, incorporation of oil into body tissues, behavioral disorders such as locomotor impairment, and abnormal burrow construction. Concentrations of weathered fuel oil greater than 1000 ppm were directly toxic to adults, while those of 100 to 200 ppm were toxic to juveniles. Cumulative effects occurred at lower concentrations. Recovery of the marsh from this relatively small oil spill is still incomplete after 7 years.

Numerous laboratory and short-term field studies have been conducted on the effects of oil on marine and estuarine organisms (1, 2). In general these studies have been directed to revealing the immediate effects of oil and have not dealt with the consequences of long-term environmental contamination on organism behavior and population dynamics. Moore and Dwyer (3) emphasize the importance of indirect, environmental effects of oil that are significant at population and community levels.

A spill of fuel oil (No. 2 oil) in Buzzards Bay, Massachusetts, in September 1969 provided the opportunity to study the long-term effects of oil contamination (4). Population changes in subtidal benthic animals related to sediment oil content have been reported (5). We report here the results of a 7-year study that dealt with the long-term effects of oil contamination of salt-marsh sediments on the population dynamics and behavior of the fiddler crab *Uca pugnax* in Wild Harbor Marsh (West Falmouth, Massachusetts).

Fiddler crabs in the Wild Harbor estuary were exposed to oil in both sediments and water after the oil spill in 1969. Qualitative behavioral observations were made in the field immediately after the spill. Initial observations of crab densities indicated considerable reduction of populations in heavily oiled areas. In spring of 1972, we established stations in areas of the marsh where chemical analysis of sediments showed contamination with fuel oil. A control station was designated in the nearby uncontaminated Great Sippewissett Marsh (West Falmouth, Massachusetts). In 1973, we added another control station in Wild Harbor, where oil contamination was slight and the crab population was fairly large. Stations consisted of two sets of four quadrates, 0.25 m<sup>2</sup>, aligned horizontally along the creek banks where *Uca* densities are usually greatest (6). All stations were at the same average height above low water.

Methods used to estimate *U. pugnax* densities have been described (6). Briefly, the numbers of open crab burrows and their diameters were measured in each quadrate at all stations monthly from June to September in 1972 and 1973 and again in July 1976. Crab densities and size-class distributions were obtained from regressions relating burrow density to crab density and burrow diameter to the width of the crab carapace. These methods permitted long-term nondestructive sampling at field stations. Since juveniles often do not make their own burrows, they were sampled separately by collecting with soft forceps all crabs whose carapace width was less than 5 mm within 0.07-m<sup>2</sup> quadrants. These quadrants were taken in transects through each station in early August 1973, during the peak of juvenile settlement (6, 7). In February and early March 1974, a 0.125-m<sup>3</sup> block of marsh sediment was excavated from each station, sieved through a 1-mm screen, and all crabs were collected. The percentage of juveniles surviving winter dormancy was estimated by comparing the number of juveniles in excavated sediments with the number present in the same transects of the August census.

Behavioral effects of the oil on crabs were bioassayed in the laboratory by timing the crabs' escape responses from a standard stimulus (8). Separate groups of 15 crabs were acclimated to 15° and 25°C for 2 weeks. Individual response times were measured prior to and after 5 days of feeding on sediments to which No. 2 fuel oil was added at a concentration of 100 parts per million (ppm).

Crabs were collected live from the field for hydrocarbon analysis. Sediments were collected with piston covers, extruded onto aluminum foil, and cut to 5-cm lengths. Samples were frozen at -30°C until analysis. Apparatus and containers were rinsed with redistilled solvents before use. Procedures for hydrocarbon analysis and criteria for distinguishing petroleum from biogenic

hydrocarbons have been described (9).

Observations made immediately after the oil spill showed numerous dead and moribund *Uca* throughout the heavily oiled marsh areas. Many surviving crabs had moved away from the heavily oiled creek edges and had burrowed into the sandier sediments above the mean high-tide mark. Crabs remaining in the oiled areas immediately after the spill displayed aberrant locomotor and burrowing behavior. Many adults showed physiological disorders, such as increased molting and display of mating colors, although the time of year was inappropriate for these responses. The usually rapid escape response (10) was very slow, and many crabs made no attempt to escape when frightened. Survivors were lethargic and displayed loss of equilibrium. Locomotor impairment persisted for at least 4 years after the oil spill, but was only obvious in the field at temperatures near the lower limit of *Uca*'s activity range (about 13°C). Thus, the aberrant behavior was most visible in late May and September, just after and before winter dormancy.

Laboratory experiments corroborated field observations. The escape response times of crabs maintained at 15°C more than doubled in crabs ingesting oiled sediments (4.5 ± 0.5 seconds before and 10.2 ± 2.3 seconds after 5 days of feeding), while crabs kept at 25°C showed no significant difference in escape times after eating oiled sediments (2.2 ± 0.3 seconds before and 2.7 ± 0.9 seconds after). The symptoms recorded were similar to those produced in *Uca* experimentally fed on sediments containing sublethal concentrations of chlorinated hydrocarbon insecticides (8, 11). Similar locomotor effects have been reported for decapod crustaceans exposed to oil emulsions and other surfactants (12) in which a sequence of impaired activity, loss of equilibrium, immobilization, and death was observed with increasing concentrations of pollutants. The duration of this sequence was prolonged as concentrations decreased. Thus, immobilization and death can be expected from long-term exposures to sublethal concentrations of these lipid-soluble toxicants. In addition to direct toxic effects, slowed escape responses leave individual crabs more vulnerable to predation as compared to healthy animals (13).

Analysis of crab tissues showed that *Uca* at Wild Harbor incorporated oil hydrocarbons up to 280 ppm into body tissues. These concentrations persisted at least through 1973 (14), when we took our last samples. Oil is a complex mix-

ture of thousands of different hydrocarbons, having different toxicities. Low-molecular-weight paraffins have narcotic effects in high concentrations (3, 15) and may have caused the lethargy noted immediately after the spill and the slowed escape responses seen in the laboratory bioassays. However, the long-term behavioral disorders displayed by crabs in the field year after year may be the result of the accumulation of hydrocarbons (particularly substituted aromatics) in the central nervous system, resulting in interference with locomotor coordination (1).

Population data showed that *Uca* densities in Wild Harbor Marsh were reduced at all stations in which the sediment concentrations of petroleum hydrocarbons exceeded 200 ppm. Greatest reductions were seen at stations with the highest oil concentrations in the sediment (Fig. 1A). The population reductions persisted for at least 7 years before recovery began, as evidenced by recruitment into the first two year-classes (Fig. 2). Recovery at the most highly contaminated area (station IV) progressed slowly over the last 3 years, indicating that recruitment was inhibited as long as oil concentrations greater than 1000 ppm persisted in the sediments. Although increased densities and more normal size distributions were recorded at all other stations in 1976, the numbers remained depressed at concentrations above 500 ppm. At concentrations below 100 ppm of petroleum hydrocarbons in the sediments, recovery in crabs appears to have been complete (station V) (Fig. 2).

Qualitative observations made from

1970 to 1972 showed that substantial numbers of juvenile crabs settled out of the plankton at all stations each year. The highest densities were, however, observed at the less oiled stations. The quantitative juvenile census conducted in 1973 showed this same pattern with the higher densities at the less-oiled areas (Fig. 1B). The densities at the less-oiled areas (stations II and V) were within the range of juvenile densities reported for Sippewissett Marsh (16), but higher than the average. Even at the most heavily oiled areas (stations I and IV) there was substantial settlement. The decrease in juvenile numbers with increasing oil concentrations of sediments may have resulted from direct toxicity of the sediments to the juvenile crabs or may have been due to an indirect effect, such as alteration of the habitat by oil. The decreased densities could also have resulted from avoidance of the oiled sediments by the postlarvae, but this explanation would appear unlikely as postlarval lobsters do not avoid oiled substrates containing up to 1740 mg of oil per liter (17). Since juveniles require preexisting refuges, the lack of *Spartina* grass, mussels, and *Uca* burrows at the oiled stations could have contributed to reduced settlement.

Figure 2 shows data only for July, but these population reductions were representative of all months sampled. There was an increase in numbers observed in August and September in the Sippewissett control, and in all Wild Harbor stations in 1973 (resulting from an increase in the number of juveniles constructing burrows, after settlement in early Au-

gust), which can be used as a relative measure of recruitment. This seasonal increase in density did not occur at the oiled stations in 1972, in spite of substantial settlement; there was no increase in densities in June or July 1973, indicating that little recruitment occurred at any of the oiled stations. A fivefold increase in density at heavily oiled areas (stations I and IV) in August and September of 1973 showed that limited recruitment was beginning, perhaps for the first time since the 1969 spill.

Figure 1B shows that oil hydrocarbons at concentrations in excess of 200 ppm in sediments were toxic to overwintering juveniles. This toxicity would severely limit the potential recruitment of the juveniles into larger-sized classes and the subsequent recovery of the population that could be expected from the heavy settlements observed in 1973. Comparison of the size distributions at station V in 1973 with those at the Sippewissett control in 1972 (Fig. 2) showed that recruitment into the first- and second-year classes (1.2 to 11.2 mm) was below normal even at the least-oiled station. This probably resulted from the lowered overwintering success of Wild Harbor juveniles [81 percent versus 90 to 95 percent in Sippewissett (Fig. 1B) (16)] and emigration to the less densely populated oiled areas contiguous to station V. Immigration from high density, favorable areas into less dense areas attributable to antagonistic intraspecific interactions has been reported for several *Uca* species (6, 18).

Crabs at the moderately and heavily oiled stations constructed burrows that

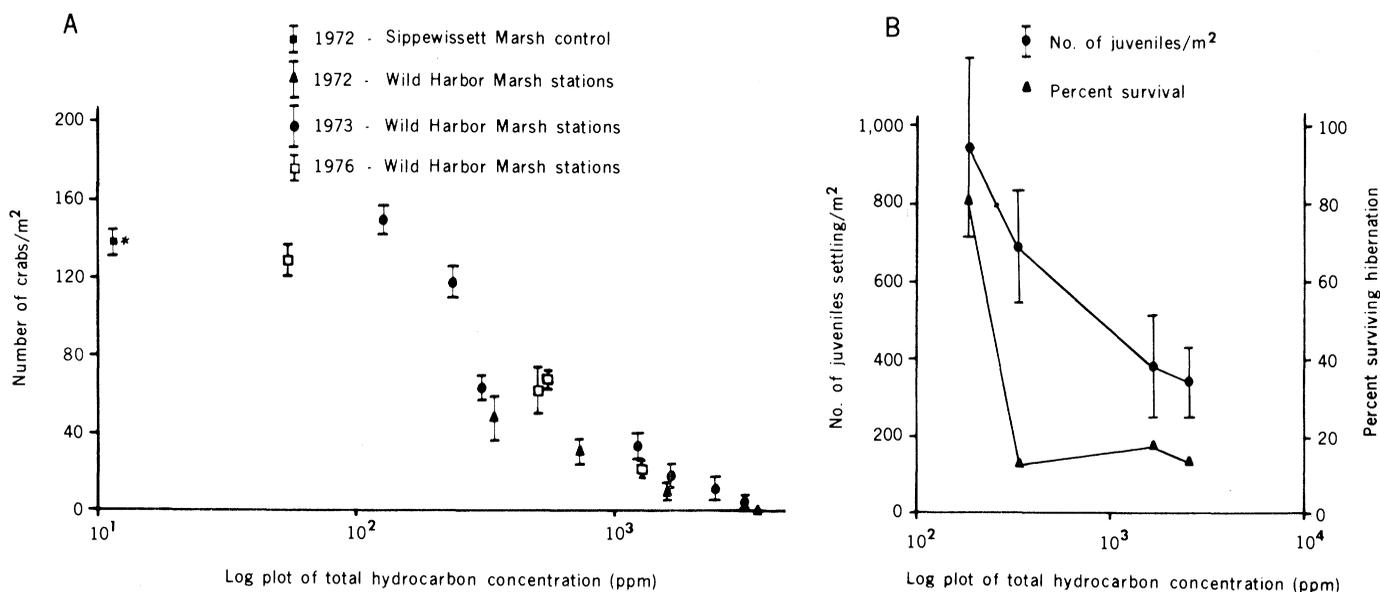


Fig. 1. (A) Log plot of total hydrocarbon concentrations in sediments versus crab density in salt-marsh stations; \*denotes all biogenic hydrocarbons. (B) Number of juveniles settling per square meter (August 1973) at Wild Harbor and percentage of those surviving hibernation versus parts per million of hydrocarbons in sediments. Values are mean and standard error ( $N = 4$ ).

were abnormal in-shape and seldom exceeded 10 cm in depth. Instead of ranging in profile from the normally steeply inclined to the vertical (19), the burrows in these oiled areas began steeply but rapidly became horizontal and even inclined upward. The burrow is important in crab survival, especially in winter, since crabs position themselves with depth to maintain a favorable temperature regime (19). Fifty percent of the burrows examined in the control marsh exceeded 10 cm in depth, and many were up to 50 cm in depth. Many immature crabs (less than 10 mm in carapace width) were observed to overwinter in these larger, deeper burrows.

The shallow burrows at stations I, II, and IV in Wild Harbor probably increased overwinter mortality. This abnormal burrowing behavior probably did not result from attempts to avoid increased oil concentrations [deep mud cores showed oil concentrations rapidly decreased with depth (9)] but probably resulted from locomotor impairment.

Several factors could have accounted for the observed effects of the con-

taminated sediments on the *Uca* populations. Uptake and concentration of hydrocarbons into crab tissues could have interfered with successful molting, as observed with other surfactants (12, 20) and oils (21). *Uca* molt in their burrows and remain in them until the carapace hardens. Thus, they would be exposed to hydrocarbons in the sediments at an extremely sensitive point in their development. Since juveniles molt every 5 to 14 days, they would be expected to suffer higher mortality than adults that only molt once a month or once a season (16). Direct exposure to the contaminated sediments under temperature stress in winter may have caused heavy overwinter mortality. Temperature and pollutants are known to have synergistic effects on the behavior and survival of fiddler crabs (22). Laboratory studies have shown that oil-water emulsions are more toxic than nondispersed oils (1) to marine animals. Perhaps the marsh sediments act like dispersing agents, increasing the rate of exposure of benthic and burrowing organisms. The fine-grained, organic-rich sediments act as reservoirs

retaining the oil (2, 4, 23). Since salt-marsh muds are anoxic below the surface, degradation of the oil is slowed, prolonging the toxic character of contaminated sediments.

Excavated samples showed a shift in the sex ratio in crabs surviving in oiled areas. Populations with 40 percent females are normal for *Uca pugnax* (24). In 1972, Sippewissett Marsh had  $40.8 \pm 2.4$  percent females, whereas oiled stations at Wild Harbor had only  $17.5 \pm 5.4$  percent females in the population. In 1973 the low-oil station V was up to  $43.5 \pm 5.3$  percent, and heavily oiled stations I, II, and V were up to  $28.4 \pm 4.7$  percent female *Uca*. The greatly reduced percentage of females at oiled stations may have resulted from differential immigration or differential mortality (or both) between males and females at these stations as a result of the oil contamination.

Reduction of fiddler crab populations by oil pollution is ecologically significant since *Uca* are important in the energy flow both within salt marshes (24, 25) and between marshes and coastal waters (16). The large reduction in numbers of females surviving in oiled areas would augment these energy losses because *U. pugnax* release large amounts of energy into coastal waters in the form of larvae (16).

We suggest that the toxicity of oiled sediments to juvenile crabs and the impairment of locomotor ability and other behavior in adults account for the persistent reduction in fiddler crab populations observed at Wild Harbor at least 7 years after the original oil spill. By comparing the petroleum hydrocarbon content of the sediments with the numbers of surviving crabs, we estimated levels of oil contamination that would affect *Uca* populations. Surface sediment concentrations greater than 1000 ppm with aromatic content greater than 20 percent (9) were directly toxic to adults. Concentrations greater than 100 to 200 ppm were toxic to overwintering juveniles. Where concentrations were below 100 ppm, recovery of the populations appears to be nearly complete. These estimates are based on the sediment content of highly weathered No. 2 fuel oil.

A large part of the oil concentrations reported here probably have limited toxicity to most organisms, and it is likely that only the aromatic fractions (mostly naphthalenes in this oil) were primarily responsible for the long-term toxic effects observed (1).

Whatever the mechanism of effect, it is now clear that, although oil spilled in certain environments may have rather

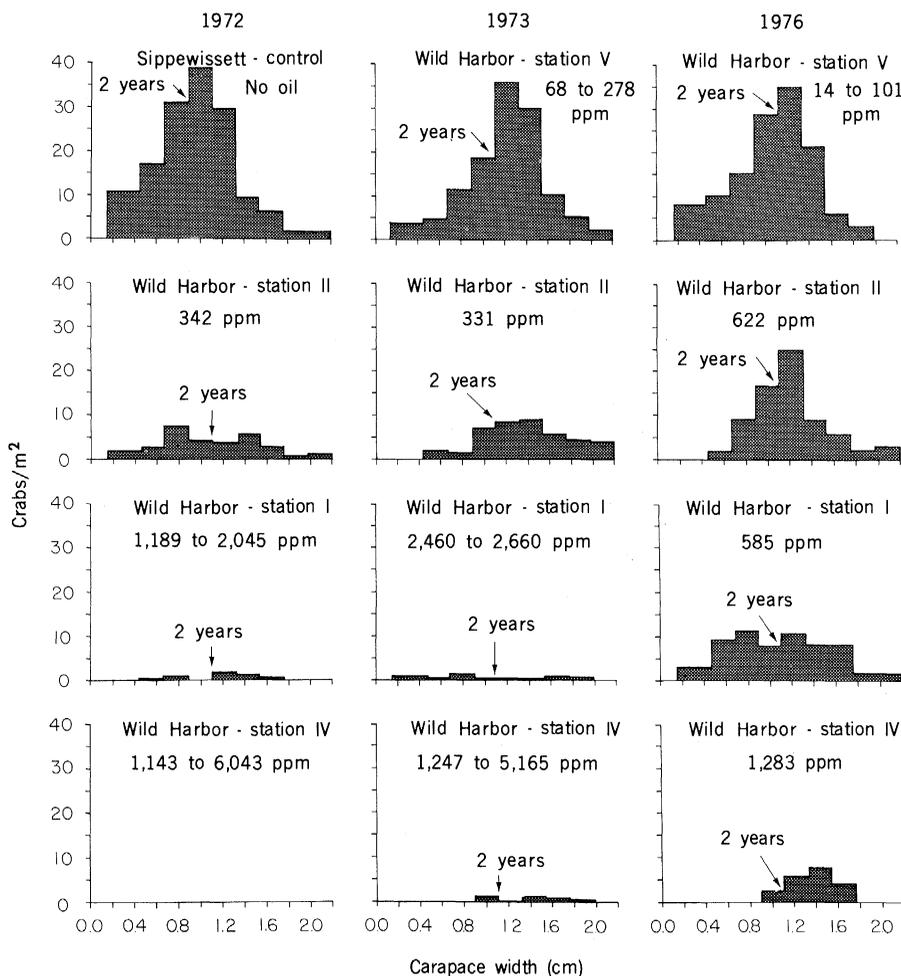


Fig. 2. Size-frequency distribution of *Uca pugnax* in salt-marsh plots after the 1969 oil spill at West Falmouth, Massachusetts, and concentration of petroleum hydrocarbons in surface sediments.

short-term effects on benthic organisms (3, 26), oil stranded in marshlands contaminates the sediments and becomes incorporated in the tissues of marine organisms affecting the survival and recovery of marine populations for many years. Recovery from even this small spill of fuel oil at West Falmouth is still incomplete after 7 years.

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## Ovarian Dynamics in Heliconiine Butterflies: Programmed Senescence versus Eternal Youth

**Abstract.** *New oocytes are generated throughout long lives in butterflies of the genus Heliconius, which as adults feed on amino acids from pollen. In Dryas julia, a related heliconiine that feeds only on nectar and is relatively short-lived, the original oocyte supply is eventually depleted. Such divergent ovarian dynamics in closely related organisms are significant in terms of both their evolutionary basis and their physiological controls.*

Reproduction and longevity in Lepidoptera are generally thought to be limited by nitrogenous reserves accumulated during larval feeding and stored in the fat body (1, 2). Gametes, which are largely proteinaceous (2), form at the expense of these reserves, and in female Lepidoptera the fat body is usually depleted as eggs mature (1, 3, 4). Although carbohydrates (and water) are often essential for complete utilization of fat body reserves and full realization of potential fecundity (3, 4), they cannot replace nitrogenous compounds consumed in egg formation. Therefore maximum egg production should correlate with the amount of

stored nitrogenous nutrient available at eclosion. In fact, fecundity in several moths is proportional to pupal weight, which is a measure of larval feeding (2). Further, in one butterfly (*Euphydryas editha*) the maximum number of eggs laid is close to the average number of both immature and mature oocytes present at emergence (5), and hence is evidently determined by then. Older female Lepidoptera of a variety of species, allowed to oviposit throughout life, die with very few potential oocytes left (6–8). Limited nutritional supply may therefore dictate a limited oocyte supply, with both being determined at adult eclosion

and simultaneously depleted during adult life.

Adults of the neotropical butterfly genus *Heliconius* are, however, not constrained by larval reserves, but extract amino acids from pollen, incorporate them rapidly into eggs, and live up to 6 months in nature (9, 10). Because these butterflies exploit a continual and theoretically unlimited source of dietary nitrogen, their oocyte supply may not necessarily be limited or determined by larval feeding. In fact, Gilbert (9) demonstrated that adult intake of amino acids has affected the reproductive pattern in *Heliconius*, since old females (4 to 6 months) do not differ from much younger individuals in either oviposition or the number of mature eggs present in dissected ovaries. We have extended these initial observations by monitoring both oviposition and ovarian composition as a function of age in individual *Heliconius*, primarily *H. charitonius*, and by comparing these parameters with those of individual *Dryas julia*, a related but shorter-lived heliconiine that does not collect pollen and hence may be more typical of the order (11). We show that pollen-fed *Heliconius* continuously produce new oocytes throughout lives of several months while both *Dryas* and *H. charitonius*, fed only carbohydrates, mature few if any more oocytes than are present at emergence and die at about 1 month of age with only residual ovaries. *Heliconius* offers the first case of long-term open-ended oogenesis reported for any lepidopteran and represents a convergence with ovarian dynamics of other insects whose reproduction depends on adult feeding (1, 2, 12).

Females were selected as pupae or as newly eclosed adults from populations originally collected in Costa Rica and Mexico and maintained in greenhouses (11). These were mated and reared individually, in 1.8 by 0.8 by 1 m net cages, with appropriate oviposition plants (vines of the family Passifloraceae) and either with or without flowering pollen plants [*Anguria umbrosa* (Cucurbitaceae)]. All were provided with 20 percent sucrose solution as artificial nectar (13). At least once a day, eggs were counted and removed, and the approximate amount of pollen collected was recorded. Butterflies were dissected at various ages or at death, the size and condition of the fat body was noted, and ovaries were removed and prepared as Feulgen-stained whole mounts (14).

Oviposition in *Heliconius* and *Dryas* begins 3 to 7 days after eclosion (15), and eggs are laid each day. In both genera, daily production of eggs fluctuates con-