USC Sea grant program

SUSCEPTIBILITY TO CRUDE OIL WITH RESPECT TO SIZE, SEASON AND GEOGRAPHIC LOCATION IN MYTILUS CALIFORNIANUS (BIVALVIA)

By

Robert Kanter

Allan Hancock Foundation
University of Southern California
Los Angeles, California 90007

USC-SG-4-74

SUSCEPTIBILITY TO CRUDE OIL WITH RESPECT TO SIZE, SEASON AND GEOGRAPHIC LOCATION IN *MYTILUS CALIFORNIANUS* (BIVALVIA)

By

Robert Kanter

Allan Hancock Foundation
University of Southern California
Los Angeles, California 90007

This work is a result of research sponsored by
NOAA Office of Sea Grant, Department of Commerce
under Grant #2-35227 to the University of Southern
California. The U.S. Government is authorized to
produce and distribute reprints for governmental
purposes notwithstanding any copyright notation that
may appear hereon.

USC-SG-4-74

Table of Contents

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Mytilus californianus Conrad from Pismo Beach, Coal Oil Point, Palos Verdes and Santa Catalina Island, California were exposed to Santa Barbara crude oil under laboratory conditions. The oil dispersed into two phases both of which contacted the mussels directly; an emulsified phase of small globules ($>0.45\mu$) and a soluble or partitioned phase ($<0.45\mu$). Six two-month studies spanning twelve months were conducted. The animals were maintained in a constant environment chamber at $15°C \pm 1°$. *Mytilus californianus* succumbed faster and in higher numbers in the $1x10^5$ppm concentration of crude oil than in the lower concentrations of $1x10^3$ppm and $1x10^4$ppm crude oil. Larger experimental animals from Pismo Beach, Coal Oil Point and Santa Catalina exhibited significantly higher mortalities than their smaller counterparts. The two most susceptible populations were from Pismo Beach and Santa Catalina Island. Each population had different months of highest mortalities, with no encompassing seasonal pattern for all the groups. No correlation between periods of highest mortality and spawnings was detected.

I. INTRODUCTION

The increase in offshore oil exploration and production as well as transportation in the last few years has brought with it a corresponding increase in the potential hazard of oil spillage. In the event of an offshore spill, depending on wind, tide and current conditions, oil will reach the intertidal area along the exposed coastlines first.

The mussel Mytilus californianus Conrad is a conspicuous and important member of the rocky intertidal community. The numerical dominance of this organism is reflected in the dense clumps, often several centimeters deep, carpeting the middle intertidal zone. The fact that Mytilus is a suspension feeding generalist, capable of efficient space utilization, probably accounts for its dominance (Paine 1966, Levinton 1972). Rao (1953) estimates that M. californianus pumps from 1-2 liters of water per hour through its mantle cavity, depending on the size of the individual and the water temperature. Food and other suspended or dissolved materials enter the mantle cavity on this feeding and respiratory current. This same water current can bring emulsified, or partitioned fractions of contaminating oil into the mantle cavity of mussels.

Mytilus californianus is itself a food source for many species of animals, including man. In addition to this, and possibly more important biologically is the extensive microcommunity supported within the masses of mussels. The byssus threads, which attach the mussel to its substrate, catch and trap tremendous amounts of detritus and sediment which provide both a home and food source for a wide range of marine invertebrates. The stability of this microcommunity is intimately tied to the well being of its sheltering mussel bed. An oil spill or some other major disturbance which destroys the mussel bed would also eliminate this entire association of organisms. This in turn could directly affect predator and other groups which are dependent on these organisms. Chan (1973) mentions his concern for the microcommunity harbored in the Duxbury reef mussel beds following the oil spill in San Francisco Bay.

Mytilus californianus is abundant in most areas throughout its geographic range (Alaska to Baja California). Thus different localities, both those exposed to chronic natural oil seepage and those not exposed, can be sampled throughout a year without unduly stressing individual populations. These ecological and practical considerations make Mytilus californianus an ideal choice for experimental study, with the resulting data having more than localized significance.

Generalizations about crude oil and its effects on marine organisms can be a hazardous practice since there are numerous physical and biological factors which may modify these effects. The massive mortalities of marine organisms, expected both by Chan (1973) in the San Francisco area and workers studying the aftermath of the 1969 Santa Barbara spill did not occur. Nicholson and Cimberg (1971), studying the Santa Barbara spill, suggest that mortality among intertidal animals e.g. _Chthamalus fissus_, was a result of physical effects, i.e. smothering and not chemical toxicity. Chan (1973) reports similar results for limpets and barnacles following the San Francisco spill. Straughan (1972) listed nine specific variables which can and do alter the effects of oil during a spill in nature. These included, 1) the type of oil spilled, 2) the dose of oil, 3) the weather conditions prevailing at the time of the spill, and 4) the biota of the affected area.

The purpose of the present experiments is to examine the effects of some of these variables, namely, size, geographic location, previous exposure to natural chronic oil pollution, and seasonality on the tolerance of _Mytilus californianus_ to Santa Barbara crude oil. Kanter _et al_ (1971) present a preliminary discussion of intraspecific and geographic factors influencing the "toxic" effects of crude oil on _M. californianus_.

There are a large number of uncontrollable variables operating in the field. The best way to determine the effect of the variables listed is to simplify conditions by performing laboratory experiments. This series of experiments will consider only long-term (two months) exposure to low levels of crude oil, i.e. 10% crude oil in seawater. Other research currently in progress considers factors such as short-term exposure to high levels of oil pollution, i.e. 100% crude oil, the effects on closely related species and natural chronic exposure to oil versus man-made pollution.

II MATERIALS AND METHODS

A. Collection of Mussels and Physiography of Collecting Sites

Mytilus californianus Conrad were collected from four localities along the California coast. Figure 1 and Table 1 show the location and coordinates of each collection site.

The aim of this study is to examine the effects of oil on physiologically different populations of mussels. Therefore the populations selected were from sites influenced by varying physical conditions. The sites included one area exposed to chronic oil pollution (Coal Oil Point) and three areas with minimal or no exposure to oil (Pismo Beach, Palos Verdes and Santa Catalina Island). One area was exposed to pollution from a large city (Palos Verdes); while the other three areas being relatively remote from any large population centers were not. In one area (Pismo Beach) the mussels are exposed to the cold water of the California current and at another locality (Santa Catalina Island), the animals are at least periodically exposed to the warm waters of the Davidson current.

Pismo Beach is north of the Santa Barbara Channel and free from the influence of any major population Center, e.g. Los Angeles. Coal Oil Point is a natural oil seep locality within the Santa Barbara Channel. The third locality Palos Verdes is south of the Santa Barbara Channel and within the influence of the metropolitan Los Angeles area. The Los Angeles city and county sewage outfall systems and the city's harbor entrance empty into the ocean close by (Figure 1). Santa Catalina Island is a non-mainland locality 40 km away from any major population center and south of the Santa Barbara Channel.

All experimental mussels were collected at low tide from the mid tidal level. After removal from the substrate the animals were immediately transported back to the laboratory. Care was taken not to unduly stress the animals, i.e. minimal aerial exposure time. In the laboratory the mussels were examined and the macro organisms attached to the shell and among the byssus threads removed. The mussels were then acclimated in aquaria with fresh filtered and aerated sea water for 24 hours at 15° ± 1°C.

The mussels from the Pismo Beach locality (Pismo) were collected from large clumps of animals attached to wooden pier pilings. Coal Oil Point (Coal Oil) mussels were collected off large boulders. Clumps of associated *Pollicipes polymerus* and *Anthopleura elegantissima* were also attached to this substrate. These three animals formed dense mats covering numerous boulders found at this site.

Palos Verdes mussels were collected from a large rock outcrop. These animals were in very dense, "carpet-like" mats dominated by mussels.

Santa Catalina Island (Catalina) mussels were collected from an exposed rock some distance offshore. This large rock outcrop (Bird Rock) is located outside of Isthmus Cove, Santa

Catalina Island. This population also formed dense mats dominated entirely by Mytilus.

B. Acquisition and Chemical Analysis of Crude Oil

The crude oil used in these experiments came from off-shore oil fields located on the Rincon trend, Santa Barbara California. Oil samples were obtained as needed from a shore-site facility. Gas chromatography was used to analyze the consistency between samples, and the composition of the crude oil. A "fingerprinting" technique was employed using a Perkin-Elmer gas chromatograph. The gas chromatographic conditions included dual flame ionization detectors with 10 ft. x 1/8 in. o.d. stainless steel columns packed with 10% OV-101 on Chromosorb-W, 80-100 mesh, acid washed, and dimethyldichloro-silane treated. The temperature of the injection port was 325°C, the manifold and detector temperature was 340°C. The column was programmed to 50-325°C at 5°C/min. with a Helium carrier gas.

When the crude oil was added to each experimental aquaria, three distinct phases were obtained. The first phase was represented by the bulk of the crude floating on the surface of the seawater. This phase is essentially the same as the whole crude oil. When the aquarium, containing oil and seawater, is aerated and kept in the environmental chamber the next two phases arise. One was an emulsion of tiny globules ($>0.45\mu$ diameter) suspended within the sea-water. The other was the soluble or partitioned crude oil fractions ($< 0.45\mu$ diameter) in the seawater.

Analysis of the latter two phases was carried out as follows. Three separatory funnels were set up inside an en-vironmental chamber. To each was added filtered seawater and crude oil to concentrations duplicating those described in materials and methods section C (Experimental Design) $1x10^3$ppm, $1x10^4$ppm and $1x10^5$ppm crude oil. Each mixture was vigorously aerated for 24 hours. At the end of this period the seawater phase was separated from the emulsified phase by the use of a Millipore filtration system. The emulsified phase was col-lected on 0.45μ (pore size) Millipore filters, the soluble (partitioned) phase was retained in the seawater filtrate. Both phases were triply extracted with 50 ml of pentane and then concentrated in a Rotoevaporator. Both phases were ana-lyzed by gas chromatography using the same "fingerprinting" technique as mentioned above, but using a Hewlitt-Packard model 700 gas chromatograph. The chromatographic conditions were similar to those above using flame ionization detectors with 10 ft. x 1/8 in. o.d. stainless steel columns packed with 10% OV-1 on Chromosorb-W, 80-100 mesh, acid washed and dimethyldichlorosilane treated. The temperature of the injection port was 300°C, the manifold and detector temperature was 390°C. However, the columns were programmed to 120° - 320°C at 5°C/min. The carrier gas used was 40 ml/min. of Helium.

C. Experimental Design

Eighty acclimated mussels representing a distinct size range (Table 2) were selected from each locality for each group of experiments. The eighty animals were divided into four groups of twenty animals and each group placed in a separate aquarium. For each locality; one aquarium contained 4,000 ml filtered seawater only (the control); one aquarium contained 4 ml oil plus 3,996 ml filtered seawater (1×10^3 ppm oil); one aquarium contained 40 ml oil plus 3,960 ml filtered seawater (1×10^4 ppm oil); and one aquarium contained 400 ml oil plus 3,600 ml filtered seawater (1×10^5 ppm oil). All aquaria were kept in a constant environment chamber at a temperature of 15° ± 1°C* with a photoperiod of 14 hours light and 10 hours darkness and vigorously and continuously aerated. Seawater and oil were changed at 48 hour intervals. Mortality was recorded at each change in terms of numbers and size class distribution. At the end of each experiment, the shell length of survivors was recorded. Healthy animals were those that produced new byssus threads. Smith (1968) used this same criteria in his toxicity studies of BP 1002 following the Torrey Canyon spill. An animal was judged to be dead when the posterior adductor muscle failed to close the gapping shell halves on stimulation by mechanical pressure.

A total of six experiments were completed in the period October 6, 1970 to December 21, 1971 with each experiment lasting approximately two months. One experiment conducted May 17, 1971 to July 14, 1971 was repeated May 17, 1972 to July 15, 1972 because of inexplicably high mortality in control groups.

---

* Environmental chamber broke-down Oct. 20, 1971
Apr. 9, 1971, Sept. 8, 1971, Sept. 30, 1971
Jun. 17, 1972

D. Data Analysis

The relationship between size and susceptibility to crude oil was examined by comparing the mean sizes of the surviving and dead experimental animals. This involved multiple comparisions, thus increasing the probability of a type I error (Siegel 1956 p. 160, Steel and Torrie 1960 p. 106). The lsd i.e. least significant difference (Steel and Torrie 1960 p. 106) test was employed to combat this problem. It is an overall test for the planned comparisons of paired means. Basically it is a students-t test using a pooled error variance. The level of significance was set at 0.05.

Control survivor and mortality size data were treated in a similar manner to determine the normal trend of mortality in each population.

III. RESULTS

A. Crude Oil Analysis

The crude oil used throughout the entire period of experiments was found to be a typical full range low paraffinic crude. The chromatogram shown in Figure 2 displays a wide carbon number distribution from C_2 to C_{35} with large peaks typical of n-paraffins. Due to solvent masking effects, compounds below C_{10} could not be determined accurately during analysis of the various phases, and therefore are not discussed in detail. All samples used in the experiments were found to be identical with only slight variations in the concentrations of the front end weathering products.

The crude oil was also found to be extremely similar to that spilled from platform "A" during the 1969 Santa Barbara oil spill. This similarity was ascertained by the superimposition of gas chromatograms of the two crude oils (Thomas J. Meyers personal communication). Hence the results of this study can be related to field observations following the Santa Barbara spill.

Coal Oil Point crude from the natural oil seep was also analyzed (Figure 3). This oil was found to differ distinctly from the crude used in this study. There was a definite absence of the n-paraffins and isoprenoids. Instead, a broad, unresolved envelope of compounds composed of aromatics, napthenes, and higher end asphaltic components were present.

During the initial period of weathering either in nature or under these laboratory conditions, the physical processes of evaporation, dissolution or mechanical dispersion are the primary mechanisms for change in the character of the crude. Under the experimental conditions of this study, the crude forms three distinct phases. The first comprises the bulk of the crude, and floats on the surface forming a surface film. This oil has the composition of the full range crude, differing only by slight evaporative volatile losses.

The next two phases result from physical dispersion and the combined effects of partitioning and solubility of crude oil in seawater. These two phases are perhaps the most important to this work because they act to bring various crude fractions into direct contact with the submerged mussels.

The emulsified phase ($>0.45\mu$ dia., Figure 4) is composed of small globules kept in suspension by the action of the aeration system. Boylan and Tripp (1971) found a similar phase appearing as they increased the turbulence of their stirring. I have personally observed this same sort of emulsion forming under natural conditions of wind and wave action. The emulsified phase displayed a composition very similar to the full range crude (Figures 2 and 4). Volatiles below C_{11} are absent, this loss is most likely due to the vigorous aeration of the aquaria, and/or solvent masking effects. A similar loss below the C_{12} range was found by Smith and MacIntyre (1971) in their artificial oil slick studies. In nature the crude oil spill is not a closed system, therefore, equilibration between gas and water phases never actually occurs. Instead, normal wave action and turbulence caused by wind and waves will be responsible for considerable loss of volatiles. In this respect, the experiments simulated natural loss of volatiles and low molecular weight compounds. Since volatility and solubility are directly related (McAuliffe, 1966), the fractions that remain contain the less soluble higher boiling point compounds. The gas chromatogram of the emulsified phase (Figure 4) shows the presence of the higher molecular weight n-paraffins, branched paraffins and small amounts of aromatics and napthenes. In addition, the end of the gas chromatogram where resolution has decreased shows the presence of some residuum asphalts and asphaltenes. The emulsified phase, because of its suspension, may be pumped into the mantle cavity of the mussel during respiration and feeding.

The third phase (Figures 5a,b,c) is composed of crude fractions that are either dissolved or partitioned into the seawater. These fractions, like the emulsified phase can enter into the mussels' cavity. They represent the fraction collected during filtration $< 0.45\mu$ diameter. This phase forms a distinct chromatographic envelope which includes the more soluble aromatics, i.e. mononuclear benzene-like derivatives, high molecular weight alkylbenzenes, and other polynuclear aromatics. However its chromatogram differed from the crude oil in lacking n-paraffin and isoprenoid peaks. This phase as with the emulsified phase, displays a lack of volatiles, this loss again associated with the aeration.

The concentration of this last ("soluble") phase was calculated for the various experimental aquaria. This was accomplished by simple disc integration of the area underneath the gas chromatogram curves. Although the original experimental concentrations were 1×10^{3}ppm, 1×10^{4}ppm, and 1×10^{5}ppm crude oil by volume, the actual concentrations were much less being 1×10^{-1}ppm, 5×10^{-1}ppm, and 11×10^{-1}ppm respectively.

B. Size Analysis

The results of the size analysis can be found in Table 2. No mortalities were recorded from the Palos Verdes control groups, and very few total mortalities were observed in the experimental groups from this locality. Analysis showed no significant size difference between the groups of survivors or mortalities. In addition the animals from this locality appeared in the field to be smaller than those from other localities. This observation was born out with mean sizes of experimental animals being considerably smaller than experimental animals from other localities (Table 2).

Mortality was significantly higher among larger animals than among smaller animals in experimental groups from Pismo Beach, Coal Oil Point, and Santa Catalina (Table 2). These differences in size were significant at $P < 0.001$, $P < 0.02$, and $P < 0.01$ respectively.

The Pismo Beach and Coal Oil Point control results (Table 2) indicate that there is no significant difference between the sizes of the living or dead animals. However, a significant ($P < 0.01$) difference exists between the sizes of the living and dead in the Catalina control groups. This difference is negative, indicating a normal trend of death in the larger animals from this population.

C. Survival at each locality with regard to season and concentration of crude oil

(1) Figures 6-9 contain the analyzed data for the experiments conducted from October 6, 1970 through December 1, 1970.

A single mortality was recorded from the Pismo Beach control group (Figure 6) while no mortalities were recorded for the $1x10^3$ppm and $1x10^4$ppm groups. During the first ten days of the experiment four deaths were recorded from the $1x10^5$ppm group.

A single mortality was recorded from the Coal Oil Point control group (Figure 7) while no mortalities were recorded for the $1x10^3$ppm and $1x10^4$ppm groups. During the first sixteen days of the experiment six deaths were recorded from the $1x10^5$ppm group.

No mortalities were recorded from the Palos Verdes control or $1x10^4$ppm groups (Figure 8) during the experimental period. The $1x10^3$ppm and $1x10^5$ppm groups suffered low mortalities of 3 and 2 animals respectively. These occurred during the first eight days of the experiment.

Inexplicably high mortalities were recorded for the Santa Catalina Is. control animals (Figure 9). Mortalities began on the eighteenth day and by the twentieth day deaths exceeded 50%. After twenty-four days only 4 animals remained alive and no further deaths were recorded. No mortalities were recorded from the $1x10^3$ppm and $1x10^4$ppm groups of animals. High mortalities were recorded for the $1x10^5$ppm group beginning on the sixteenth day and dropping to less than 50% survivorship by day twenty. Mortalities continued until the thirty-fourth day when only 2 animals remained alive.

(2) Figures 10-13 contain the analyzed data for the experiments conducted from December 30, 1970 through March 3, 1971.

The Pismo Beach control group suffered 2 mortalities, both of these occurring during the first twenty-one days (Figure 10). No mortalities and 1 mortality were recorded from the $1x10^3$ppm and $1x10^4$ppm groups of animals respectively. Two mortalities were recorded at the start of the experiments from the $1x10^5$ppm group.

No mortalities were recorded for the Coal Oil Point control animals (Figure 11). Deaths occurring near the end of the experiments resulted in low mortalities of 2 and 1 animals from the $1x10^3$ppm and $1x10^4$ppm groups respectively. Three deaths occurring at various times were recorded for the $1x10^5$ppm group.

No mortalities were recorded from the control or experimental groups of animals collected at Palos Verdes (Figure 12).

The Santa Catalina Is. experiments were conducted from January 11, 1971 through March 3, 1971. This delay resulted from collection problems. No mortalities were recorded from the control or experimental groups collected at this locality (Figure 13).

(3) Figure 14-17 contain the analyzed data for the experiments conducted from March 8, 1971 through April 30, 1971.

A single mortality was recorded from the Pismo Beach control group (Figure 14) while no mortalities occurred in the $1x10^3$ppm group of animals. Four mortalities were recorded from the $1x10^4$ppm group of animals, most of these occurring during the last two days of experiments. Dead animals were recorded from the $1x10^5$ppm group after two days of experiments. After six days, mortalities exceeded 50% and after two weeks, there were only 4 surviving animals. Two more deaths were recorded from this group in the last few days of experiments.

Very few deaths were recorded for either the control or experimental animals from Coal Oil Point for this experimental period (Figure 15). One mortality occurred in the control and $1x10^3$ppm groups while none occurred in the $1x10^4$ or $1x10^5$ppm groups of animals.

No mortalities were recorded from the control or $1x10^4$ppm groups of animals collected at Palos Verdes (Figure 16). Two mortalities, occurring at the end of the experimental period were recorded from the $1x10^3$ppm group. The $1x10^5$ppm group suffered 4 mortalities during the last four days of experiments.

No mortalities were recorded from the control or $1x10^4$ppm groups of animals collected at Santa Catalina Is. (Figure 17). Only 1 mortality was recorded from the $1x10^3$ppm group. High mortalities were recorded from the $1x10^5$ppm group of animals beginning at day two. Deaths exceeded the 50% level by day twenty and leveled off on the thirtieth day with 2 surviving animals.

(4) Figures 18-21 contain the results of the repeat experiments conducted from May 17, 1972 through July 15, 1972.

No mortalities were recorded from the control, $1x10^3$ppm, or $1x10^4$ppm groups of animals from Pismo Beach (Figure 18). The $1x10^5$ppm group suffered 5 mortalities occurring between day two and twenty-two.

No mortalities were recorded from the control, $1x10^3$ppm or $1x10^4$ppm groups of animals from Coal Oil Point (Figure 19). A single mortality was recorded from the $1x10^5$ppm group.

No mortalities were recorded from the control, $1x10^3$ppm or $1x10^4$ppm groups of animals collected at Palos Verdes (Figure 20). The $1x10^5$ppm group suffered 4 mortalities between fourtieth day and the end of the experimental period.

No mortalities were recorded from the control group collected at Santa Catalina Is. (Figure 21). No mortalities were recorded from the $1x10^3$ppm group until day twenty-two. A sharp increase in mortalities was noted at this time with deaths exceeding 50% by day twenty-eight and by day thirty there were no survivors. The $1x10^4$ppm group suffered heavy mortalities from the beginning of the experiments. Mortalities exceeded the 50% level by day eight and only 1 animal remained alive at day thirty. Mortalities began at day six in the $1x10^5$ppm group. Deaths exceeded the 50% level by day twenty-two, and deaths continued with no surviving animals past the thirtieth day.

(5) Figures 22-25 contain the results of the experiments conducted from August 10, 1971 through October 8, 1971.

Two mortalities were recorded at the beginning of the experiments for the Pismo Beach control animals (Figure 22). No mortalities were recorded from the $1x10^3$ppm group until day thirty-six. Beginning at day thirty-six high mortalities were recorded and by day forty-two deaths exceeded the 50% level. On day forty-eight mortalities ceased with 3 surviving animals. The $1x10^4$ppm group followed a similar pattern with the onset of mortality at day thirty-six. High mortalities were then recorded with deaths exceeding the 50% level by day fifty and leveling off by day fifty-two with 9 surviving animals. The $1x10^5$ppm group suffered the highest mortalities in the shortest time period. Mortalities began on day twenty-two with deaths reaching the 50% level by day twenty-six. There were no surviving individuals from this group by day forty-four.

During the first ten days 2 deaths occurred in the Coal Oil Point control animals (Figure 23). Low mortalities of 2 and 0 were recorded from the $1x10^3$ppm and $1x10^4$ppm groups of animals respectively. The $1x10^5$ppm suffered high mortalities beginning at day twenty-eight. Deaths reached the 50% level by day fifty and by the end of the experimental period only 8 animals remained alive.

No mortalities were recorded from the Palos Verdes control group (Figure 24). The $1x10^3$ppm and $1x10^4$ppm groups suffered 1 and 2 mortalities respectively. Only 3 deaths were recorded from the $1x10^5$ppm group of animals these occurring at various times throughout the experiment.

No mortalities were recorded from the Santa Catalina Is. control group (Figure 25). The $1x10^3$ppm and $1x10^5$ppm groups suffered low mortalities of 2 and 1 respectively. High mortalities were recorded for the $1x10^4$ppm group beginning on day twenty. Survivorship dropped to 15 by day twenty-two where it levels off with no further mortalities.

(6) Figures 26-29 contain the results of experiments conducted from October 20, 1971 through December 21, 1971.

Two mortalities were recorded from the Pismo Beach control group (Figure 26), while only 1 mortaltiy was observed from the $1x10^3$ppm group of animals. Relatively high suvivorship (18 out of 20) was noted for the $1x10^4$ppm group until day twenty-six. Four additional deaths between this time and the end of the experiments resulted in 14 survivors. No mortalities were recorded from the $1x10^5$ppm group until day twenty-eight. From this time through day thirty-four, mortalities increased resulting in 13 surviving animals.

No mortalities were recorded for either the control or $1x10^5$ppm groups collected from Coal Oil Point (Figure 27). A single mortality was recorded from the $1x10^3$ppm group and 2 deaths were observed in the $1x10^4$ppm group of animals.

No mortalities were recorded from the control or $1x10^5$ppm groups collected at Palos Verdes (Figure 28). The $1x10^3$ppm group suffered 3 mortalities which occurred between the fourteenth and twenty-sixth day. Two deaths were recorded from the $1x10^4$ppm group of animals.

Moderate mortalities (6 out of 20) were recorded from the Santa Catalina control animals (Figure 29). These deaths occurred between day ten and day twenty. The $1x10^3$ppm group suffered high mortalities beginning after day twelve. Deaths exceeded the 50% level by day sixteen, and continued so that there were only 5 survivors by day twenty, and no further mortalities after this. Four mortalities occurred in the $1x10^4$ ppm group between day eight and sixteen. High mortalities were recorded from the $1x10^5$ppm group. Deaths began on day eighteen and by the end of the experimental period only 50% of the animals remained alive.

D. Results of Observed Spawnings

Spawning occurred during several of the experiments. Only obvious spawns, involving several animals, were discernible within the oil and water mixtures in experimental aquaria. The recorded occurrences (Table 3) can be assumed to represent a minimal number of spawns. It is quite likely that individual spawns would pass undetected as the gametes are diluted and masked within the aquaria. There was no correlation between dates of spawning and periods of high mortality.

IV. DISCUSSION

A. Size

Mortality was related to the size of the mussel in three of the four geographic populations studied. Mortality was highest among the larger animals from the Pismo Beach, Coal Oil Point and Santa Catalina experimental populations. There was no relationship between animal size and mortality for animals collected at Palos Verdes. If more deaths had occurred among the Palos Verdes animals perhaps a susceptible size range would emerge.

These results may be attributed strictly to physiological differences between these geographically different populations. Experiments comparing physiological data for animals from each locality might help in interpreting these findings. Rao's (1953) observations supply the basis for a possible explanation for these results. He reports that for M. californianus maintained at minimal temperatures, pumping rates increase with wet weight until a certain peak point and then decline with increasing weight. In the present experiments, size data (length) is not directly comparable to Rao's wet weight. Figure 30 (D. Straughan, personal communication) shows an exponential increase in weight with a corresponding length increase for M. californianus from Ellwood Beach, California (Figure 1). Assuming this type of relationship for M. Californianus from other localities, the following explanation is advanced for the present findings. We can assume that the smaller animals (those that lived) were pumping less water through their mantle cavity than the larger animals (those that died). This is also true for the Palos Verdes animals which were not only the smallest animals overall but also the group suffering the lowest number of mortalities.

Pumping rate and water transport are related to feeding and respiration in M. californianus as well as other mollusks (Jørgenson, 1952, 1966). This would mean that oil components carried in the water would pass through the mantle cavity at a rate proportional to the pumping rate. The gas chromatographic results indicate that both the soluble (partitioned) fractions, and those fractions emulsified in the seawater could be, and probably were, pumped into the mantle cavity. Smith (1968) observed oil globules in the mantle cavity of seemingly healthy M. edulis during the Torey Canyon study. The emulsified fractions could be responsible for the deaths in experimental animals but more likely they would be voided as faeces or pseudofaeces. Lee et al (1972) reported discharge of contaminating petroleum hydrocarbons by M. edulis. The other possible fate for either of these phases is temporary or permanent retention in the tissues. Blumer et al (1970) found polluting petroleum hydrocarbons present in Aequipecten and Crassostrea virginica following the West Falmouth spill.

Pumping rate appears to be one important factor in explaining the contrasting results of size and mortality from crude oil. Radioactive tracer experiments similar to those conducted by Lee et al (1972) are a promising approach to solving the dilemma of which crude fractions and in what quantities are responsible for observed deaths.

B. Season and Location

The toxicity studies were conducted over a twelve month period. A seasonal pattern was anticipated which would provide a basis for predicting periods of highest susceptibility to crude oil effects. No general pattern was detected that encompassed all the groups tested. Instead each geographic population reacted in its own unique way.

Coal Oil Point and Palos Verdes animals were the two most tolerant groups tested. The consistently high survival in all concentrations during each experiment was proof of this. Only once did deaths ever exceed 50% and this was in Coal Oil Point $1x10^5$ppm oil during the experiments of August 10, 1971 to October 8, 1971. In the remaining experiments mortalities never exceeded 30% for either locality. The Coal Oil Point findings were consistent with the findings of Kanter et al (1971) for the lower concentrations, and with slight differences in the $1x10^5$ppm oil group. Chan et al (1973) testing the tolerance of M. californianus, from Duxbury reef, to Bunker C oil found similar high survival.

To explain the high resistance to the effects of crude oil in the Palos Verdes and Coal Oil Point animals, it is necessary to look at these individual geographic populations. Coal Oil Point is subjected daily to crude oil coming from the natural seeps found at this locality. Allen et al (1969) estimates from 11 to 160 barrels per day escape from the seeps. The portion of this reaching the intertidal animals varies with prevailing tides, currents and wind conditions. The animals settling and living here are subjected to this environmental stress. The result can be selection in the water column (of larvae) and/or on the substrate for more fit individuals. The individuals surviving may at least tolerate or acclimate to the persistent presence of oil. Whatever the specific reason, the fact remains that these animals are less sensitive to the toxic effects of crude oil than either Catalina or Pismo Beach groups. The high survival in Coal Oil Point animals adds further support to the low mortality findings of Nicholson and Cimberg (1971) in field studies of this same area following the Santa Barbara spill.

The explanation for the highly tolerant population of animals from Palos Verdes is open to even more speculation than that for Coal Oil Point animals. Emery (1960) cites the presence of several natural oil seeps in the vicinity of Palos Verdes. These seeps however, appear to be far enough offshore and infrequent enough in activity to have little if any significant effect on the intertidal animals. The proximity of the Los Angeles County and City sewage outfalls makes these likely sources of environmental stress. As with the natural seep oil of Coal Oil Point, the effluents of these outfalls could provide selective pressures for the establishment of resistent populations. Galloway (1972) reports exceptionally high concentrations of copper and other trace metals in the area surrounding the county and city outfalls. Undoubtedly further investigation will yield data on waste petroleum products also in this effluent. Again the exact factors operating to establish this tolerant population are not clear, and one can only speculate on the likely possibilities.

The animals from Santa Catalina and Pismo Beach suffered the greatest number of mortalities from the crude oil. The mortality figures varied not only with the month but also with the concentration of crude oil. During the months of highest mortalities deaths in general occurred sooner and were greater in number in the $1x10^5$ppm oil than any other concentration.

Santa Catalina animals show exceptionally high mortalities ($>50\%$) in all concentrations during the summer months of May and June. In addition the animals in $1x10^5$ppm oil suffered high mortalities during March, October, November and December.

Pismo Beach animals suffered high mortalities in all concentrations during the fall months of September and October. In addition there was high mortality in the $1x10^5$ppm oil group during the spring month of March.

The exact reasons for the high susceptability of these two populations is not known. However, these animals are from two localities with relatively "pollution free" environments. There are no natural oil seeps or major sewage effluents adjacent to either locality, which would provide the selective pressures presumably acting to establish the hardy Coal Oil Point and Palos Verdes populations.

During the months of highest mortalities for the various populations and concentrations of crude oil two general patterns of mortality emerged. In some instances, e.g. Figure 21 Santa Catalina Is. $1x10^5$ppm oil, mortalities began almost immediately at the outset of the experiment and continued until there were few or no surviving individuals. The entire population appeared to be susceptible to the crude oil. In most instances this pattern of mortality was associated with the groups exposed to the higher concentrations i.e. $1x10^5$ppm, of crude oil. This pattern would suggest the "die-off" was a result of acute toxic effects of certain crude oil fractions.

The second pattern of mortality is displayed by groups subjected to all the concentrations, and particularly the $1x10^5$ppm group, e.g. Figures 22 and 23. The trend shows high survival for approximately three to four weeks of exposure to the crude oil. This was followed by a period of rapid and continuous mortalities until few or no animals remained alive. One can only speculate on the explanation behind this pattern. A behavioral or physiological modification on the part of the mussel to avoid or minimize contact with the oil is likely. Support for this idea is supplied by observations made during the 48 hour changing of seawater and crude oil. When the fresh seawater was added the mussels would separate their shell halves and appear to be pumping water and respiring normally.

When the crude oil was added to the experimental aquaria the mussels were observed to immediately close their shells. How long they can remain closed or the time period for adductor fatigue is not known, nor is the effectiveness of this avoidance behavior. This can only be ascertained with further study. Whatever behavioral or physiological change does occur, it is nonetheless apparent that this group of animals can survive for three to four weeks time when subjected to chronic exposure to Santa Barbara crude oil.

Two patterns of survival arise as a result of moderate mortality among experimental animals. The first pattern appears after initial mortality of a few mussels at the beginning of the experiment (Figures 6,8,18). Following these initial deaths, there is a leveling off with survivors remaining alive until the end of the experimental period. The second pattern, not quite as common, is mortality of a few mussels after approximately three weeks, followed by no further deaths (Figure 21,25,28). These patterns of survival appear to be a result of acclimation by the animals. That is, a few initial deaths occur during critical periods, and the remaining organisms acclimate to tolerate the presence of oil and therefore survive.

Successful spawning was noted at various times throughout the experiments for all localities. No direct correlation was found to exist between mortality and periods of observed spawning. It has been reported by many authors (Whedon 1936) that spawning occurs year round with peak periods that vary from one location to another. The spawnings that occurred in these experiments appear to only incidently coincide with those reported in the literature. Spawning obviously is related to the physiological state of the animal. If the organism is in a ripe state when collected, handling or thermal shock may initiate spawning.

No explanation is offered at the present time to account for the freak high mortalities observed in the control group from Santa Catalina October 6, 1970 through December 1, 1970. However experiments are now in progress which are examining inconsistencies such as this. The possibility of heterogeneous mussel populations containing "healthy" and "unhealthy" individuals or "old age" classes is being seriously considered.

There was no general annual cycle of susceptibility to Santa Barbara crude oil in *M. californianus* from the four localities sampled. Perhaps a large scale study of a single locality spanning several years would yield a distinct pattern.

Straughan's (1972) list of factors which can influence changes in the environment following an oil spill should perhaps be modified. As a result of this study the "Biota" classification should be expanded. When considering the biota one must not only consider the species, but also intraspecies variations, such as those brought about by size, age, geographical and seasonal population differences.

V. ACKNOWLEDGMENTS

I would like to express my gratitude to the many people who assisted me both in field and laboratory work. Among these people I am particularly grateful to Tena Martella, Ronald Harris, William Jessee, and Marion Johnson. I am indebted to Dr. Dale Straughan for her continual encouragement and valuable criticisms during the development of my ideas. I wish to thank Dr. Thomas J. Meyers for his assistance with gas chromatographic analysis and subsequent valuable discussions. I would also like to thank Robert Smith for supplying his computer program and assistance in statistical analysis. I wish to thank Nicholas Condap for his assistance in computer programming of the survivorship figures. I would also like to thank Tom Gaines and Don Craggs of Union Oil for supplying crude oil during the entire experimental period. My thanks also to the Marineland of the Pacific for supplying filtered seawater.

Finally I would like to thank Dr. Ian Straughan, Dr. Richard Pieper, Dr. Kristian Fauchald and Dr. David Morafka who read and made valuable criticisms of a draft of this paper. The research was supported by Sea Grant #2-35227.

LIST OF FIGURES

LIST OF TABLES

Table 1. Sampling localities.

Locality	Coordinates
Pismo Beach	Long. 121°18'20" Lat. 35°3'30"
Coal Oil Point	Long. 119°52'45" Lat. 34°24'30"
Palos Verdes	Long. 118°21'40" Lat. 33°44'50"
Santa Catalina Island	Long. 118°30'00" Lat. 33°28'00"

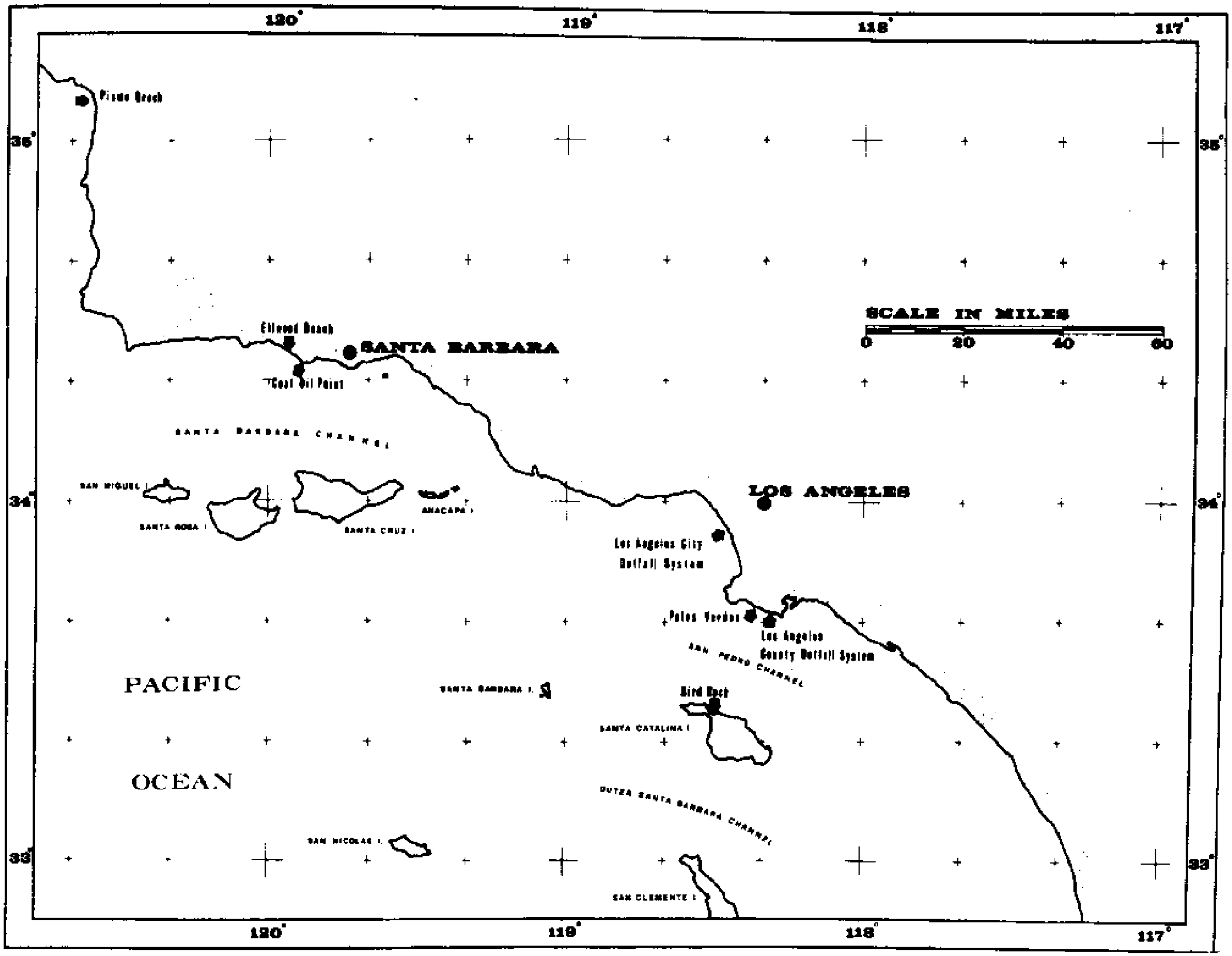

Figure 1: Chart of California coastline showing sampling localities and waste discharge sites.

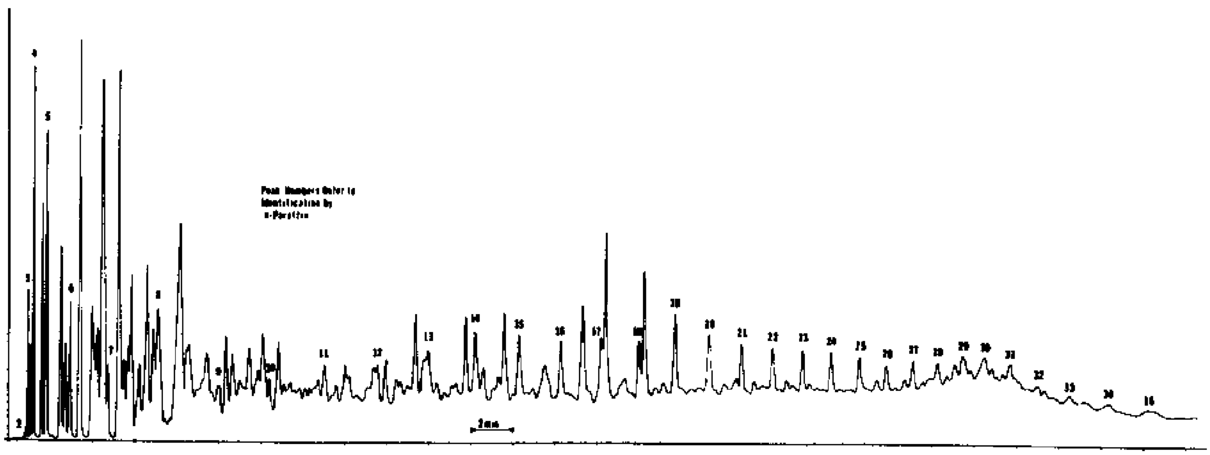

Figure 2: Gas chromatogram of Santa Barbara crude used in all experiments.

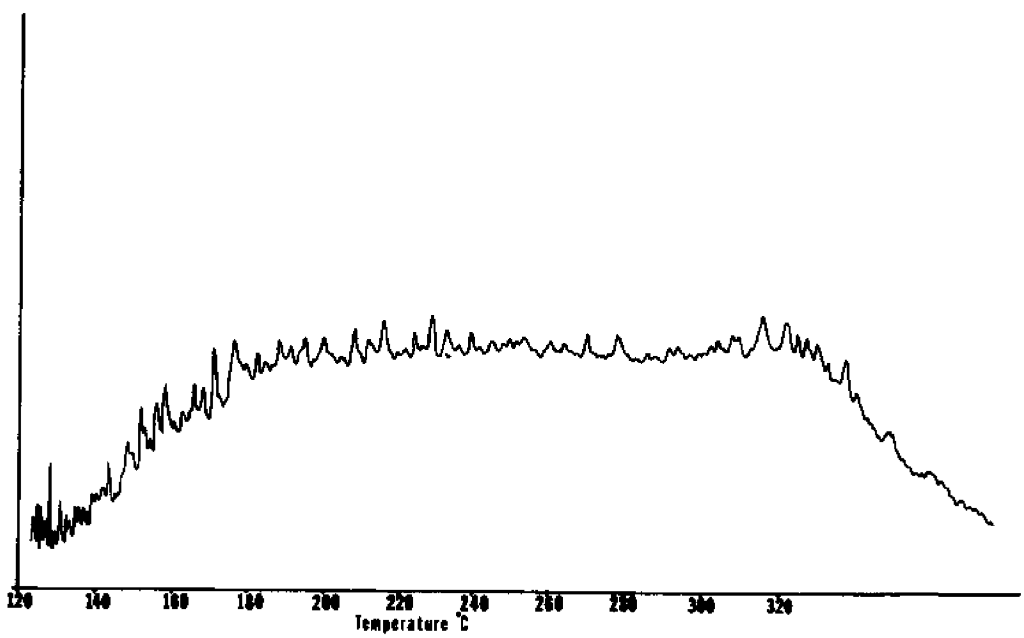

Figure 3: Gas chromatogram of Coal Oil Point natural oil seep crude.

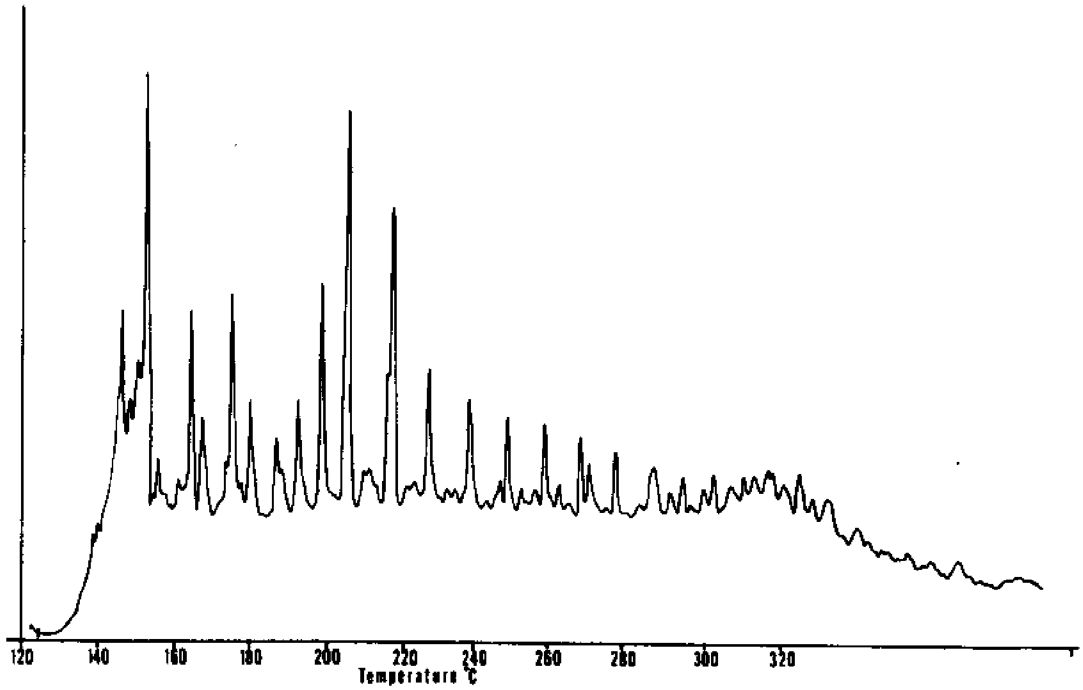

Figure 4: Gas chromatogram of emulsified phase (> 0.45 dia. of Santa Barbara crude oil.

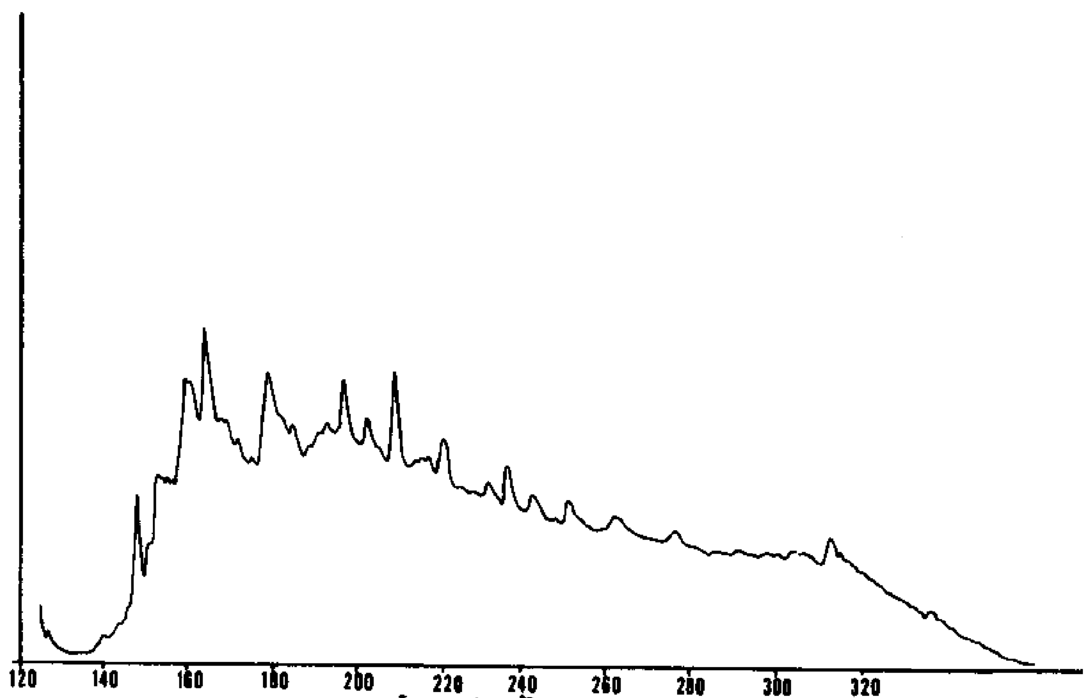

Figure 5a: Gas chromatogram of soluble or partitioned fractions of Santa Barbara crude from experimental concentration, $1x10^3$ppm oil.

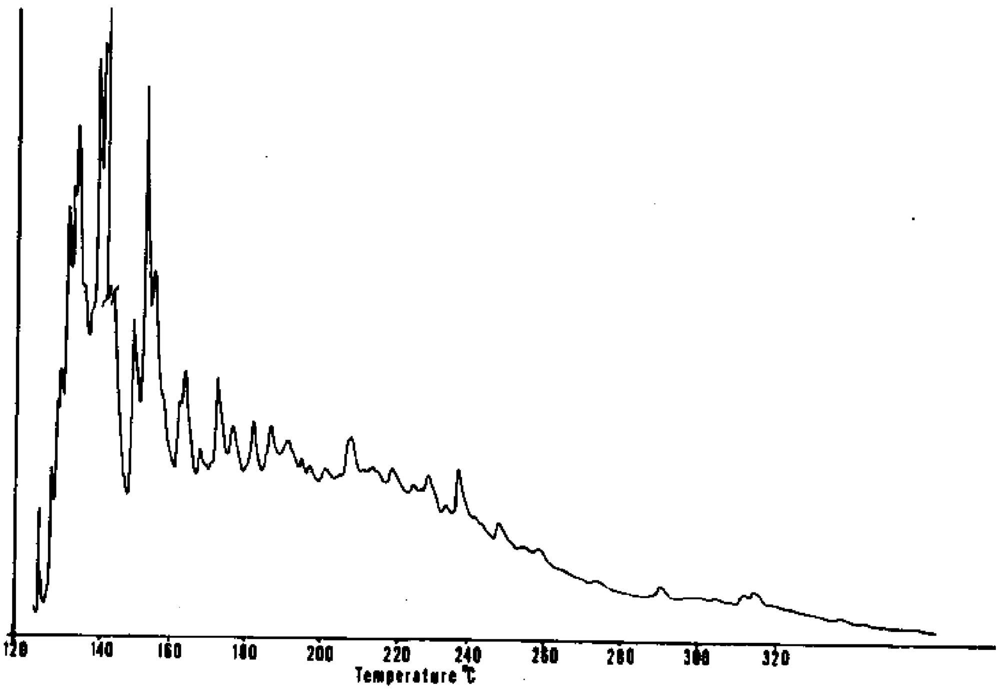

Figure 5b: Gas chromatogram of soluble or partitioned fractions of Santa Barbara crude from experimental concentration, $1x10^4$ppm oil.

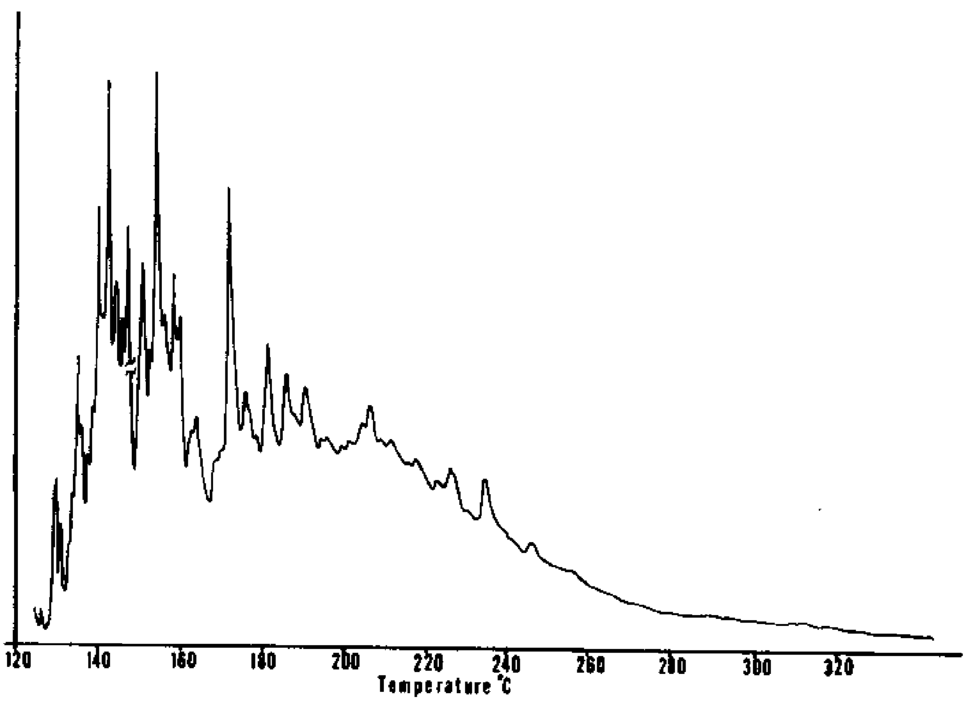

Figure 5c: Gas chromatogram of soluble or partitioned fractions of Santa Barbara crude from experimental concentration, $1x10^5$ppm oil.

Table 2. Results of size analysis, and experimental group size data.

	Location	Size range	After Exposure to Oil: Mean Size of Live	Mean Size of Dead	Difference: Mean Size of Live (-) Mean Size of Dead	df	Probability
Experimental Group	Pismo Beach	28-144mm	70mm	89mm	-19mm	∞	$P < 0.001$
	Coal Oil Pt.	24-145mm	79mm	89mm	-10mm	∞	$P < 0.05$
	Palos Verdes	22-107mm	54mm	57mm	- 3mm	∞	NS
	Catalina Is.	24-139mm	72mm	79mm	- 7mm	∞	$P < 0.01$
Control Group	Pismo Beach	28-144mm	72mm	79mm	7mm	118	NS
	Coal Oil Pt.	24-145mm	82mm	79mm	3mm	118	NS
	Palos Verdes	22-107mm	----	----	---	---	---
	Catalina Is.	24-139mm	71mm	88mm	-17mm	118	$P < 0.001$

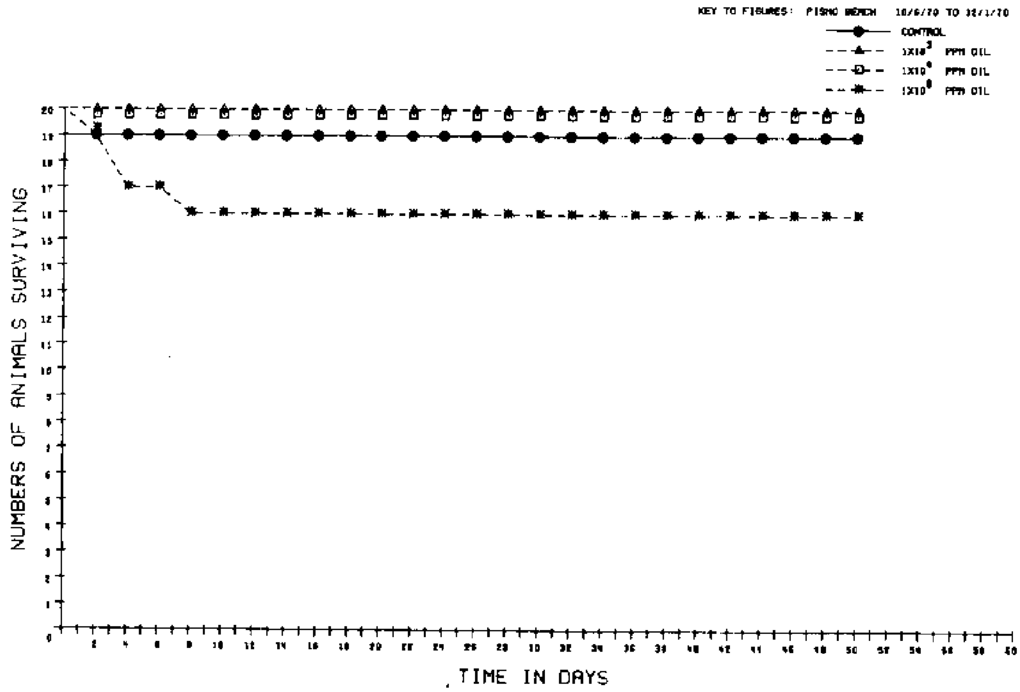

Figure 6: Survivorship curves for Pismo Beach animals Oct. 6, 1970 to Dec. 1, 1970.

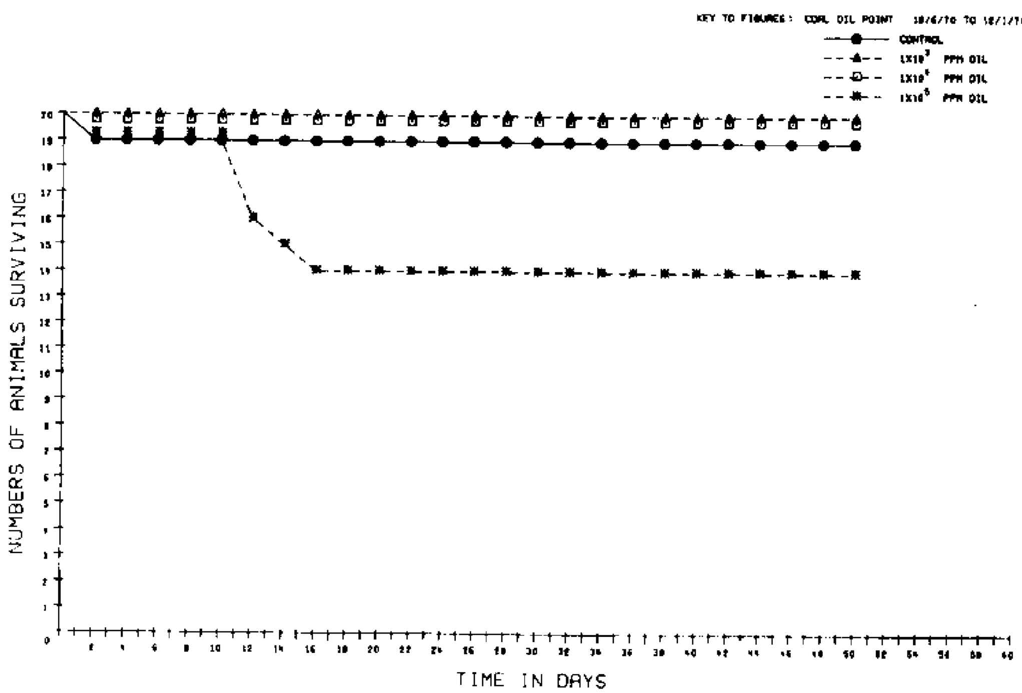

Figure 7: Survivorship curves for Coal Oil Point animals, Oct. 6, 1970 to Dec. 1, 1970.

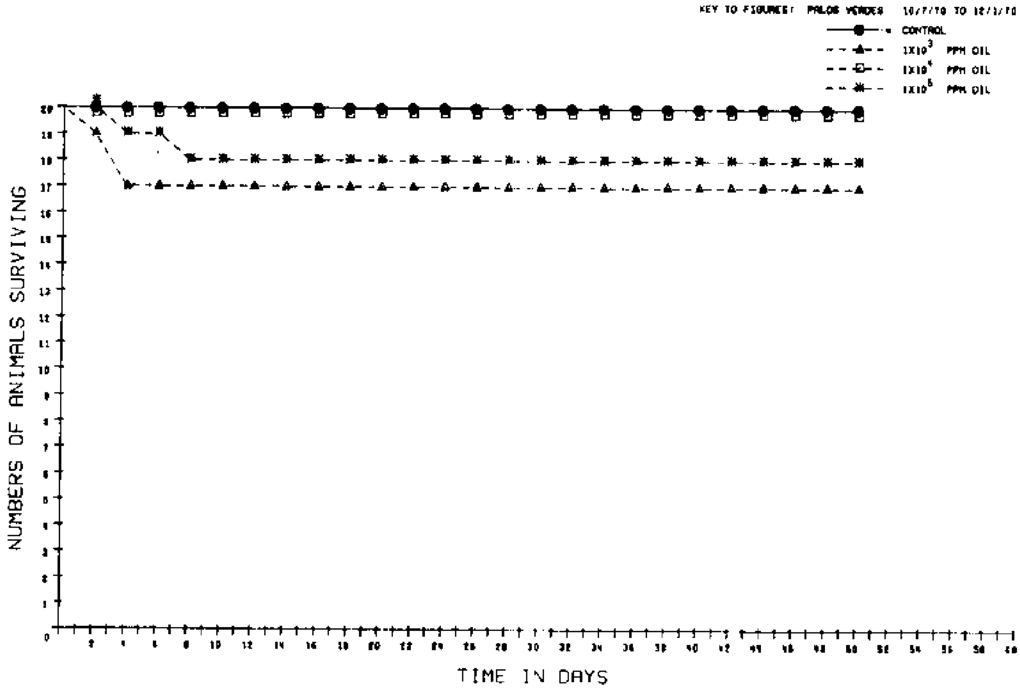

Figure 8: Survivorship curves for Palos Verdes animals Oct. 7, 1970 to Dec. 1, 1970.

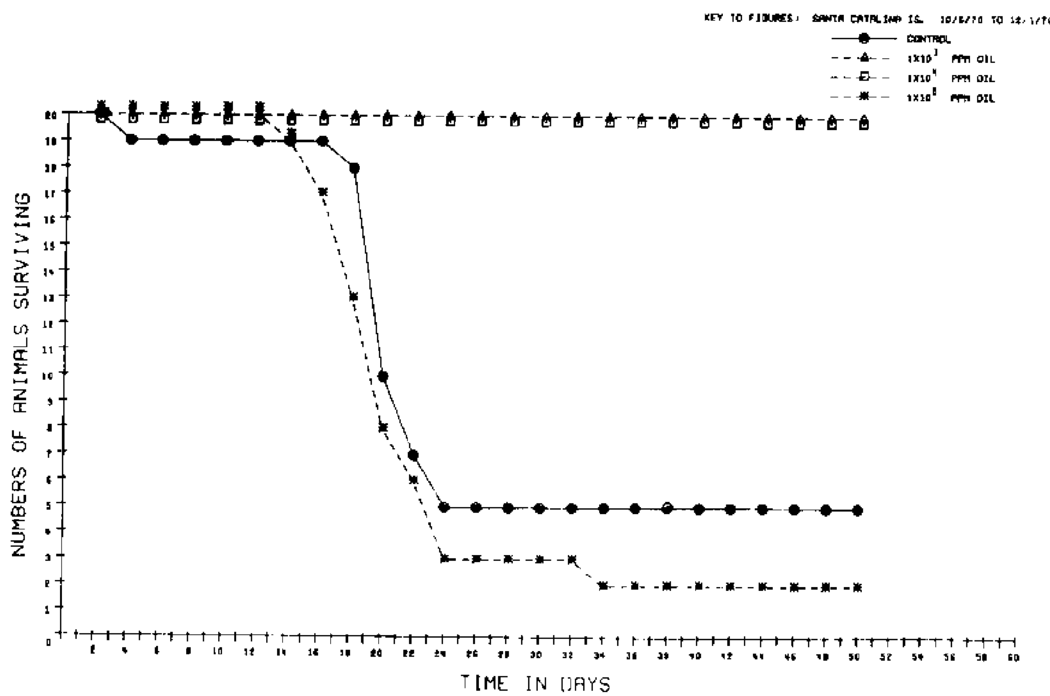

Figure 9: Survivorship curves for Santa Catalina Island animals, Oct. 6, 1970 to Dec. 1, 1970.

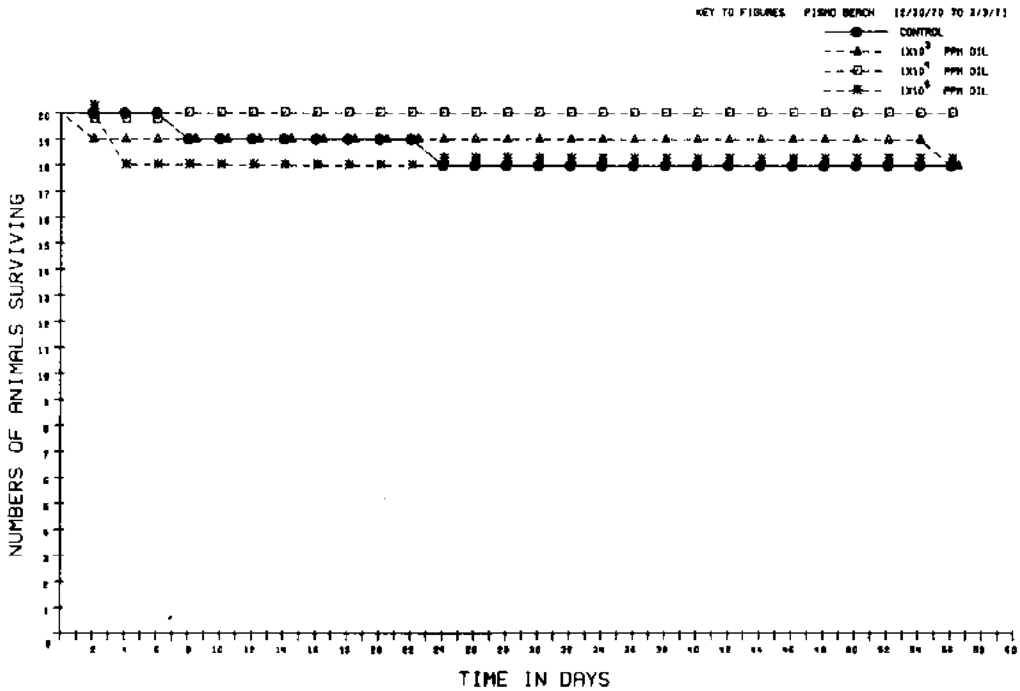

Figure 10: Survivorship curves for Pismo Beach animals Dec. 30, 1970 to Mar. 3, 1971.

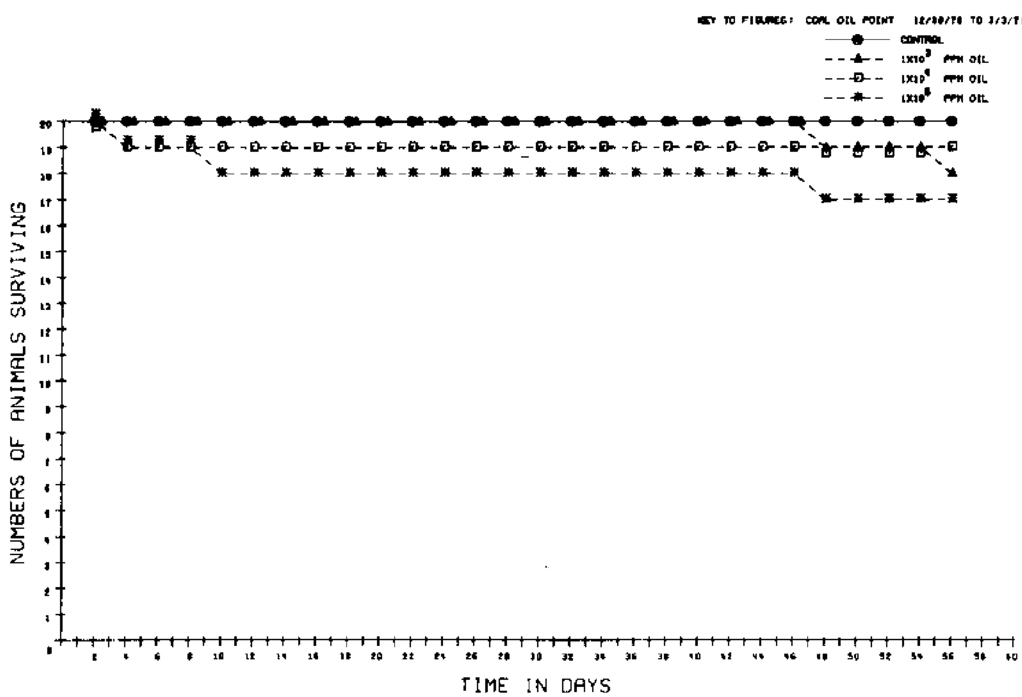

Figure 11: Survivorship curves for Coal Oil Point animals Dec. 30, 1970 to Mar. 3, 1971.

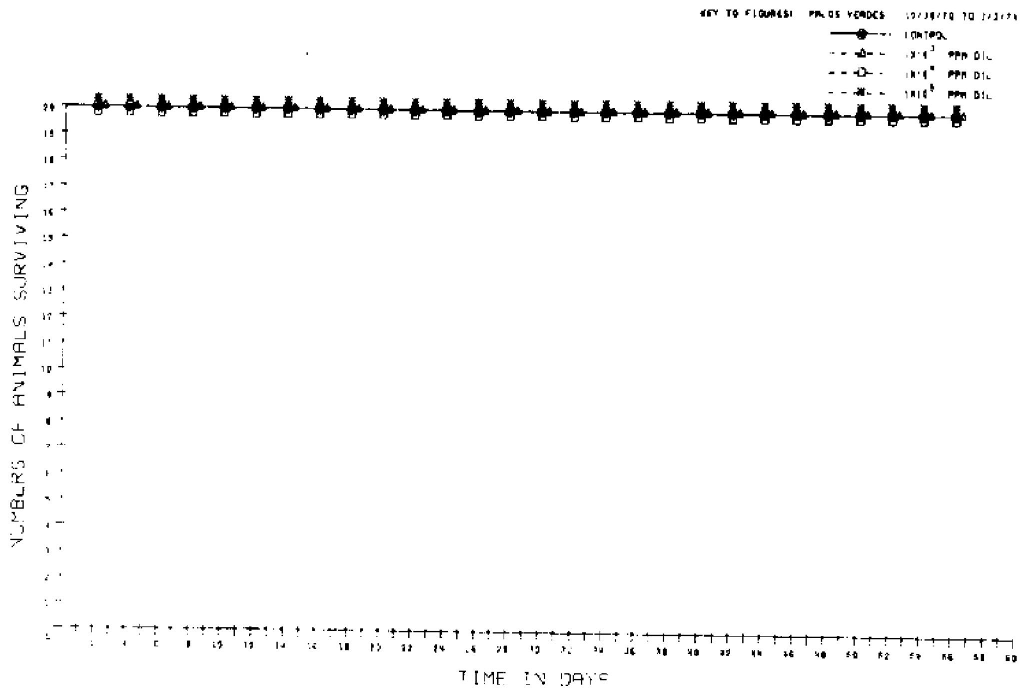

Figure 12: Survivorship curves for Palos Verdes animals Dec. 30, 1970 to Mar. 3, 1971.

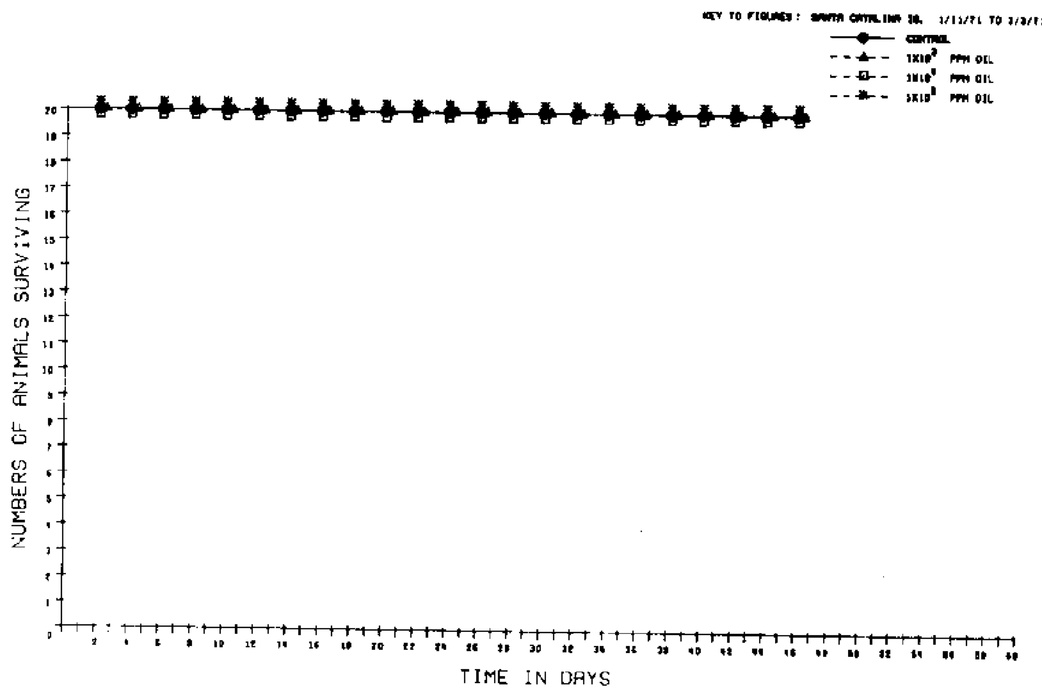

Figure 13: Survivorship curves for Santa Catalina Island animals, Jan. 1, 1971 to Mar. 3, 1971.

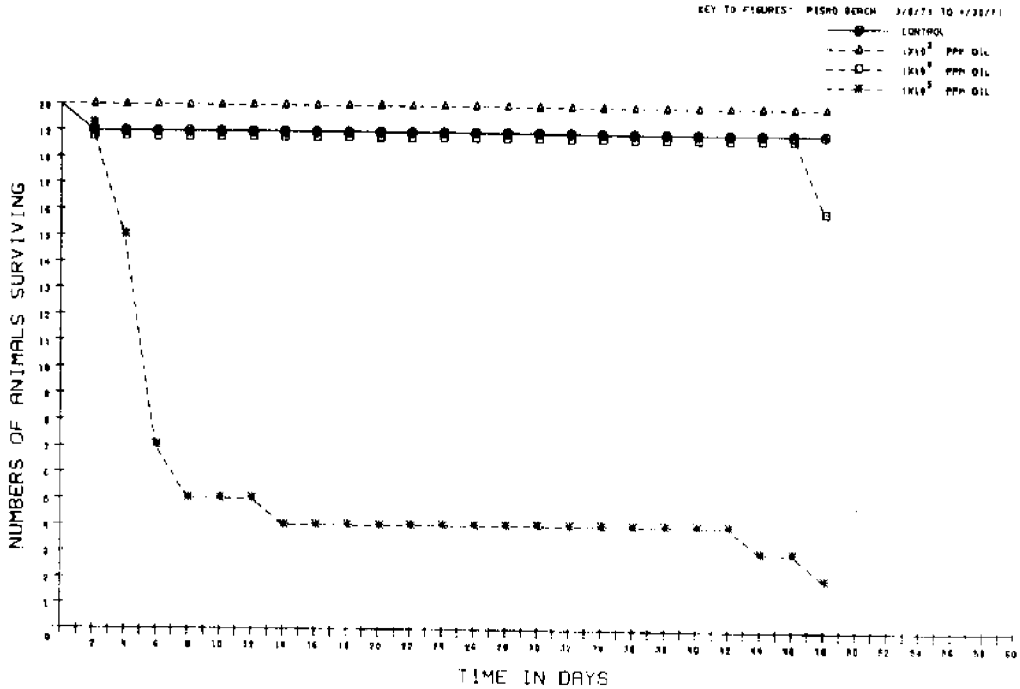

Figure 14: Survivorship curves for Pismo Beach animals Mar. 8, 1971 to Apr. 30, 1971.

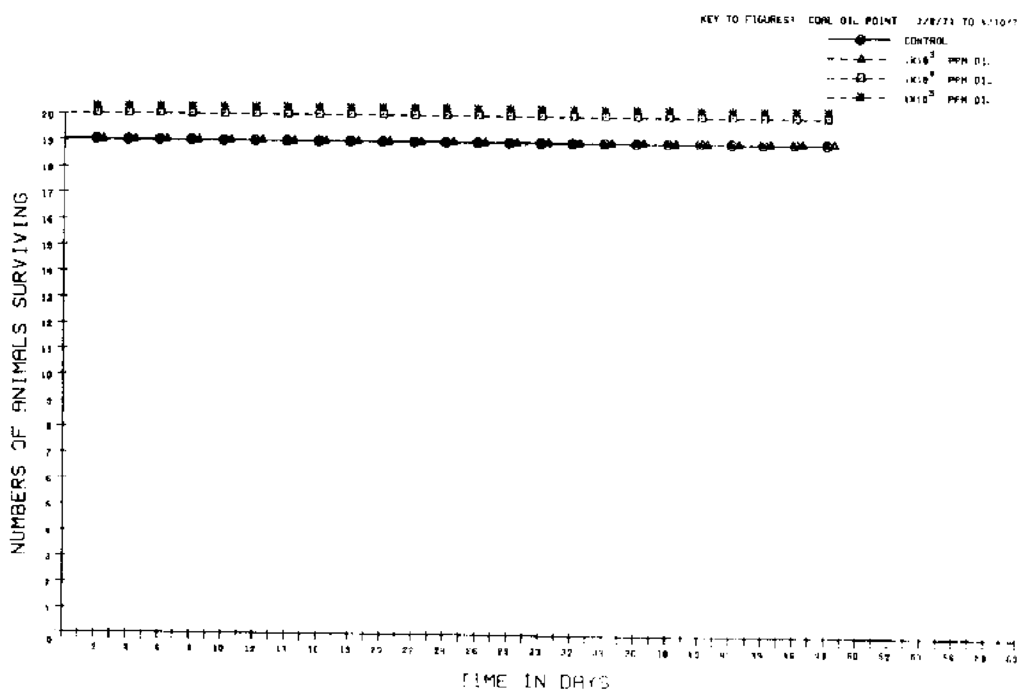

Figure 15: Survivorship curves for Coal Oil Point animals Mar. 8, 1971 to Apr. 30, 1971.

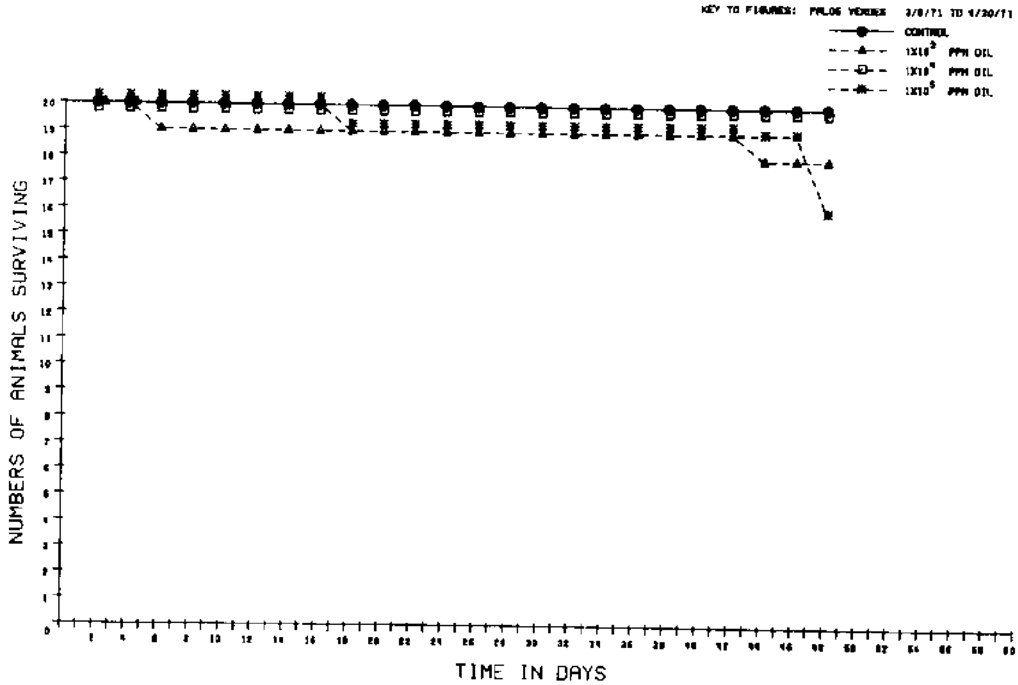

Figure 16: Survivorship curves for Palos Verdes animals Mar. 8, 1971 to Apr. 30, 1971.

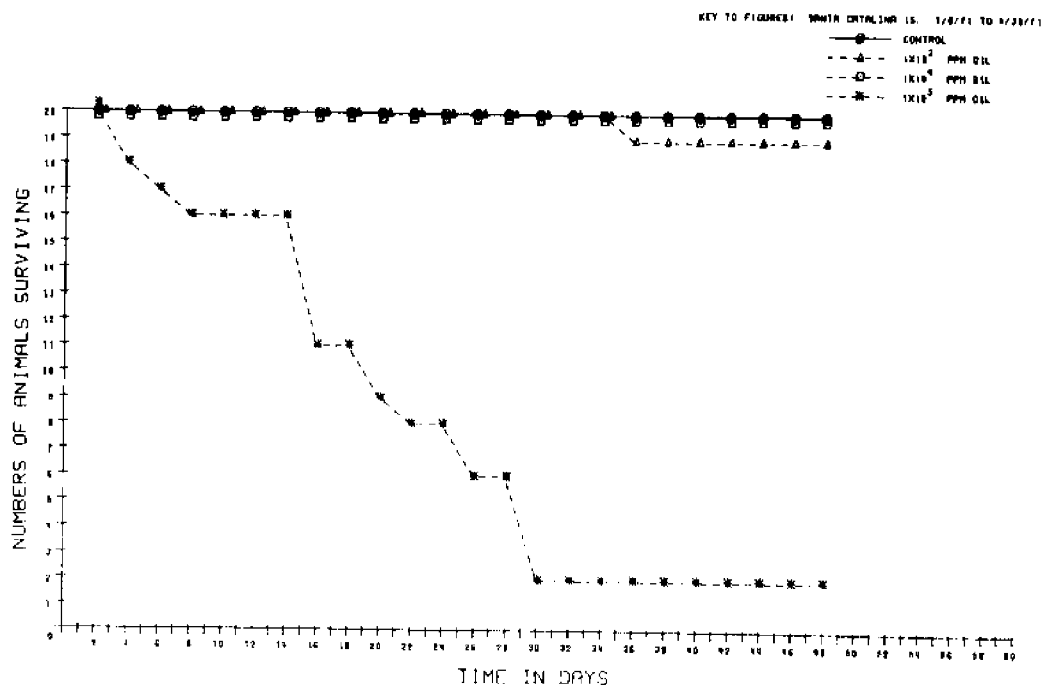

Figure 17: Survivorship curves for Santa Catalina animals Mar. 8, 1971 to Apr. 30, 1971.

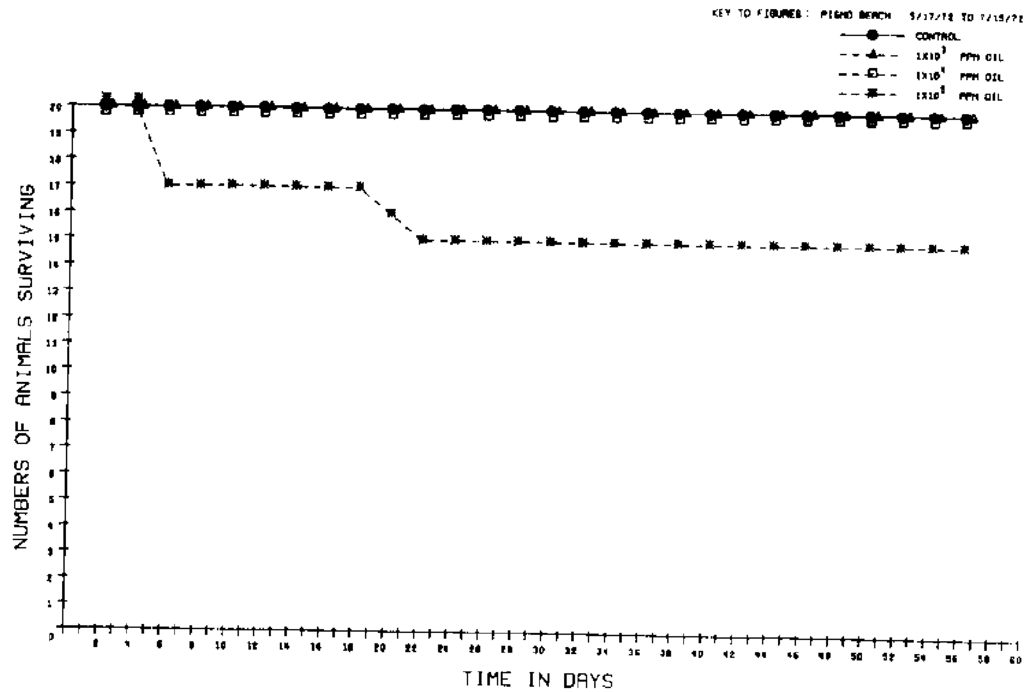

Figure 18: Survivorship curves for Pismo Beach animals May 17, 1972 to July 15, 1972.

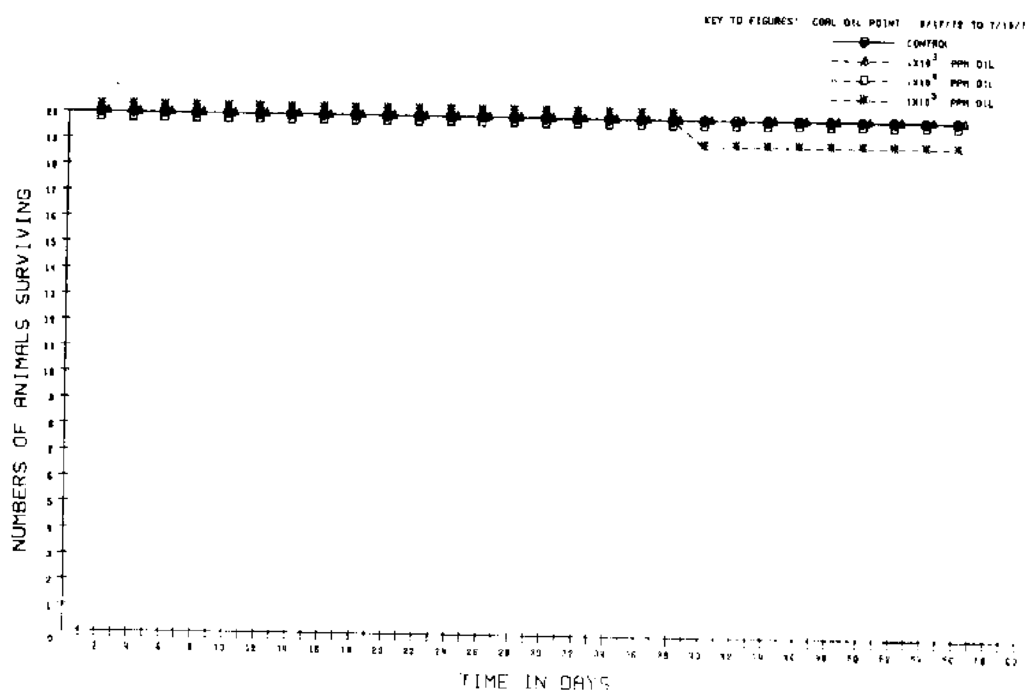

Figure 19: Survivorship curves for Coal Oil Point animals May 17, 1972 to July 15, 1972.

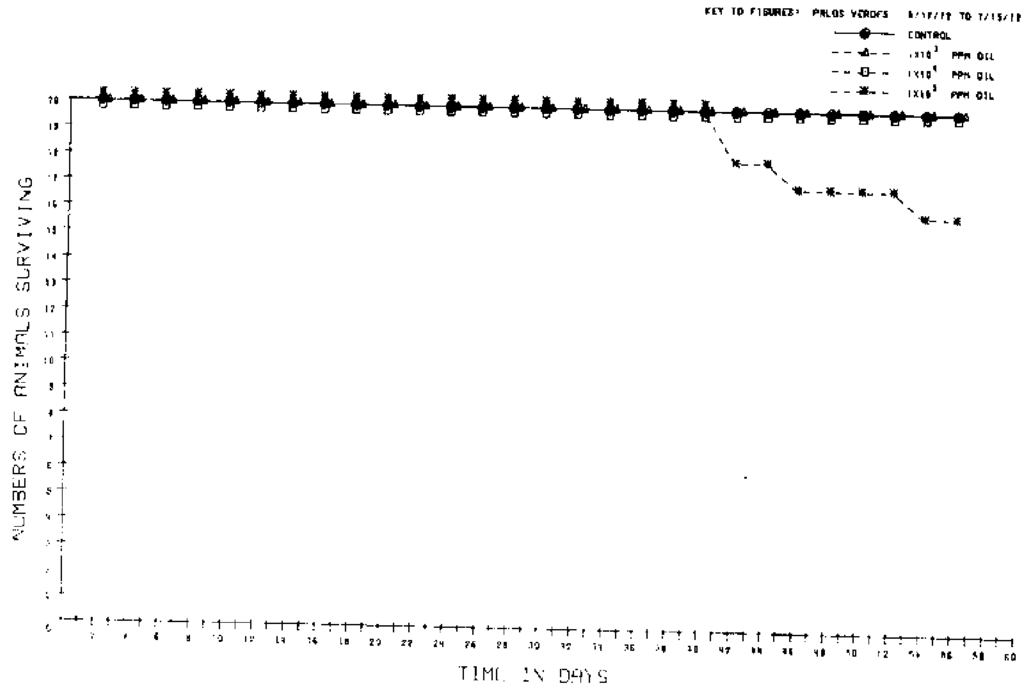

Figure 20: Survivorship curves for Palos Verdes animals May 17, 1972 to July 15, 1972.

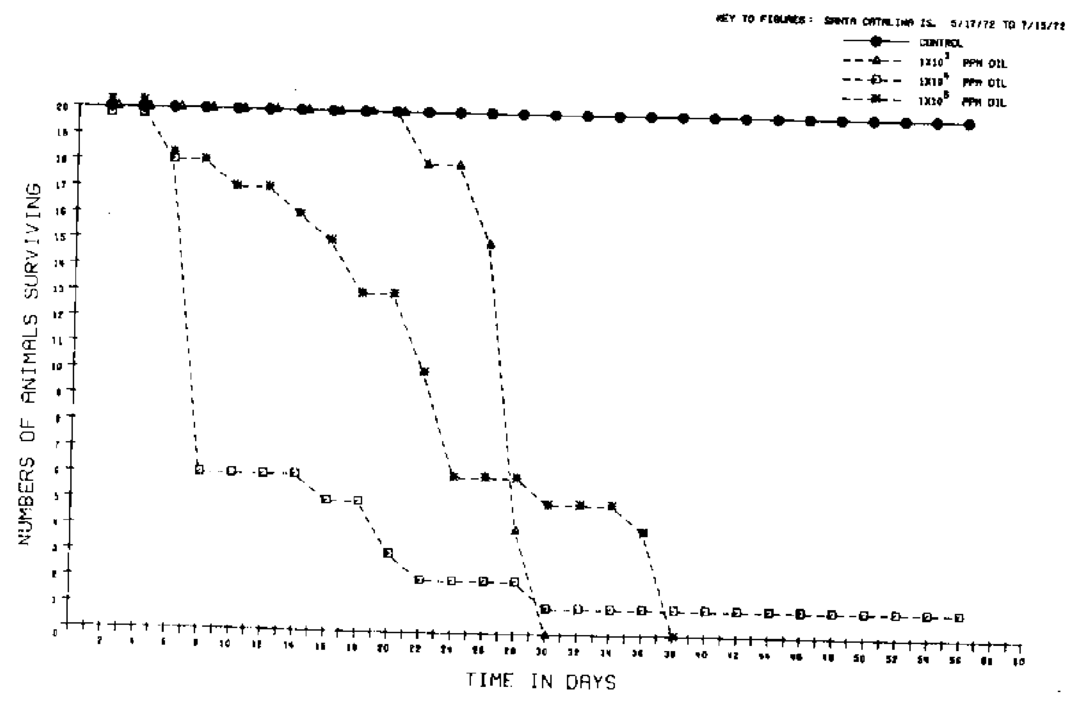

Figure 21: Survivorship curves for Santa Catalina Island animals, May 17, 1972 to July 15, 1972.

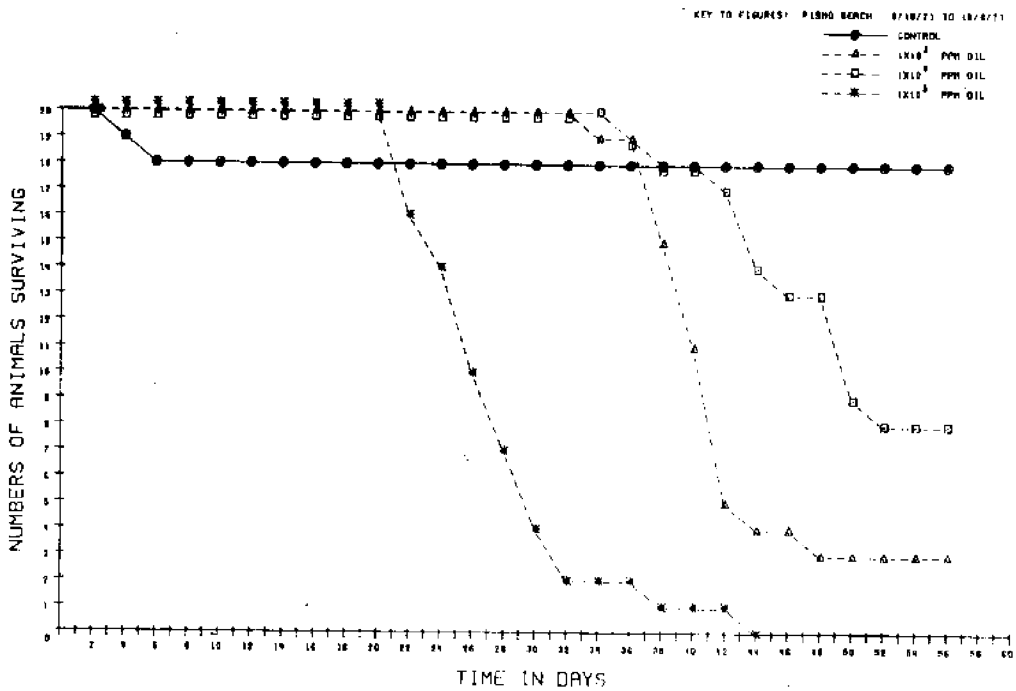

Figure 22: Survivorship curves for Pismo Beach animals Aug. 10, 1971 to Oct. 8, 1971.

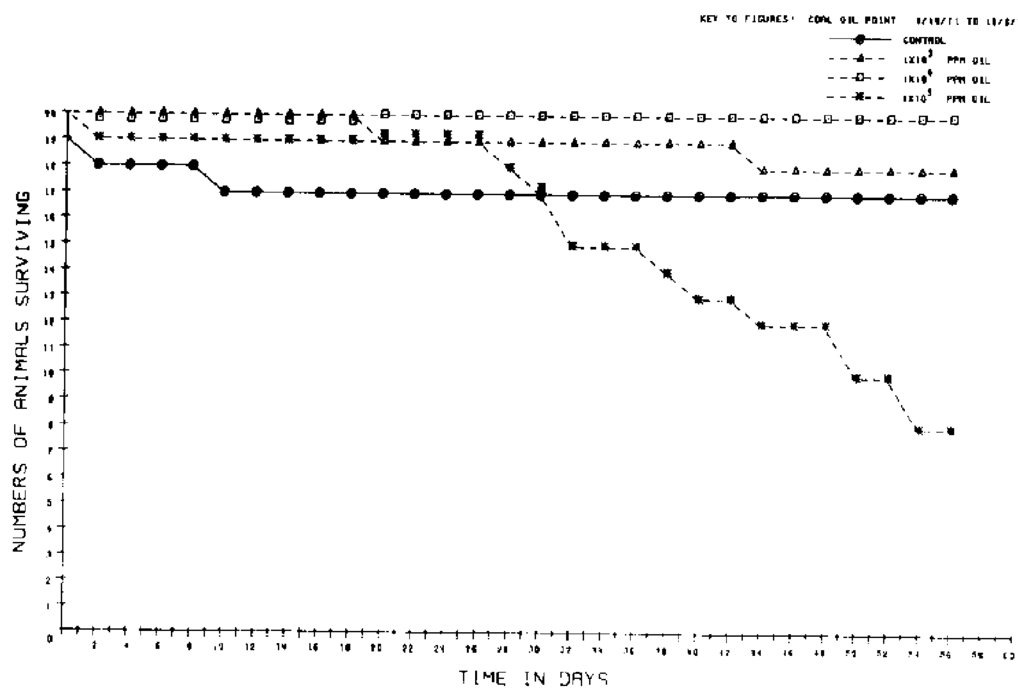

Figure 23: Survivorship curves for Coal Oil Point animals Aug. 10, 1971 to Oct. 8, 1971.

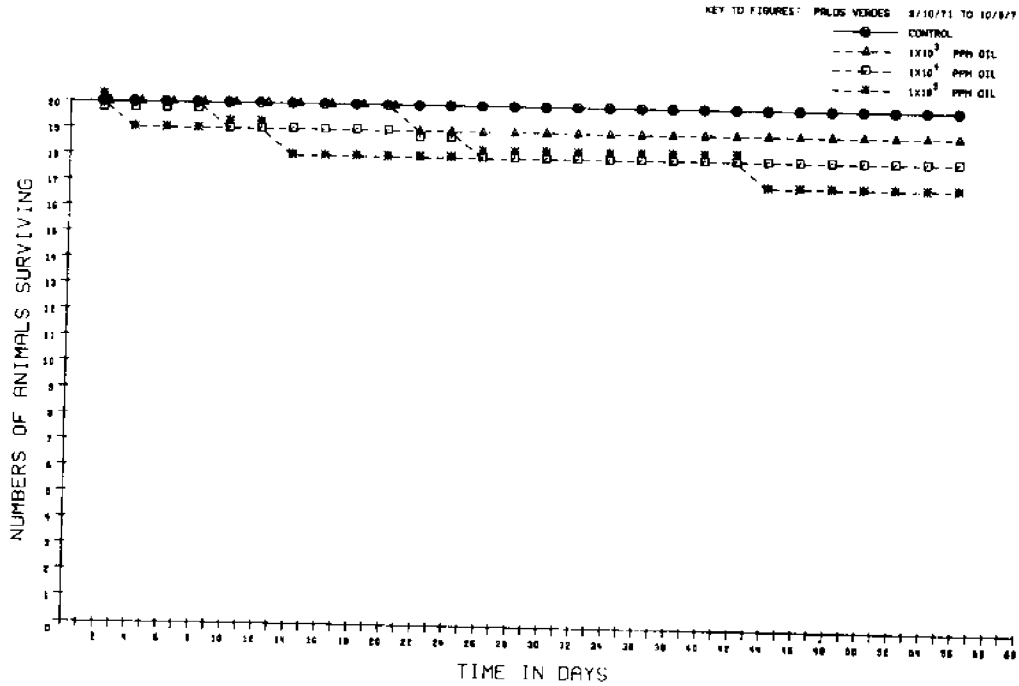

Figure 24: Survivorship curves for Palos Verdes animals Aug. 10, 1971 to Oct. 8, 1971.

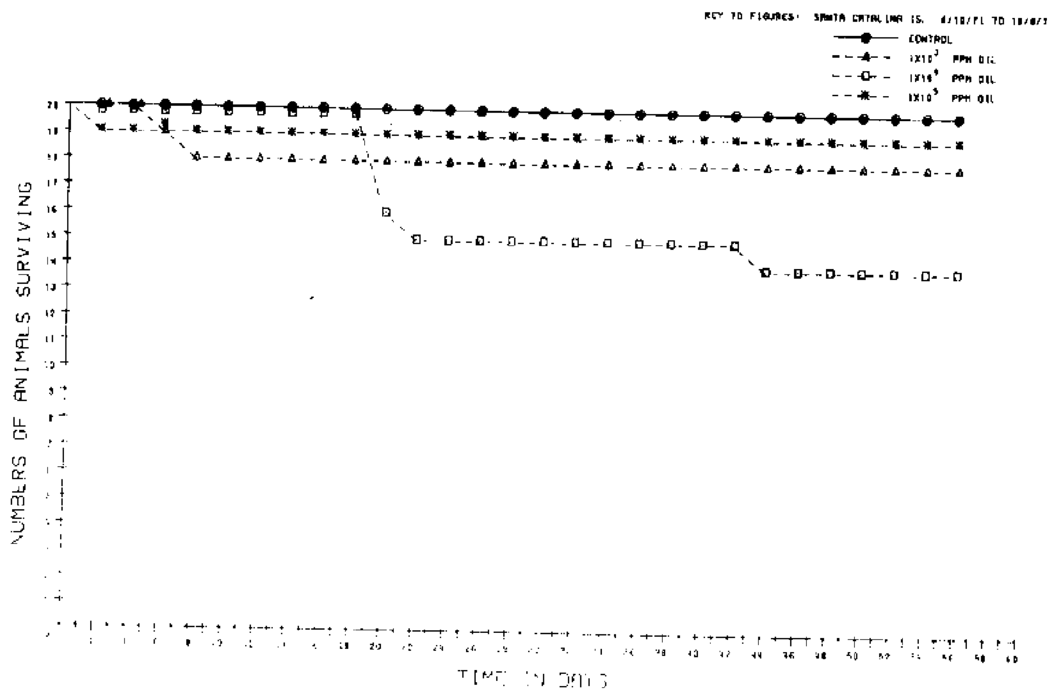

Figure 25: Survivorship curves for Santa Catalina Island Aug. 10, 1971 to Oct. 8, 1971.

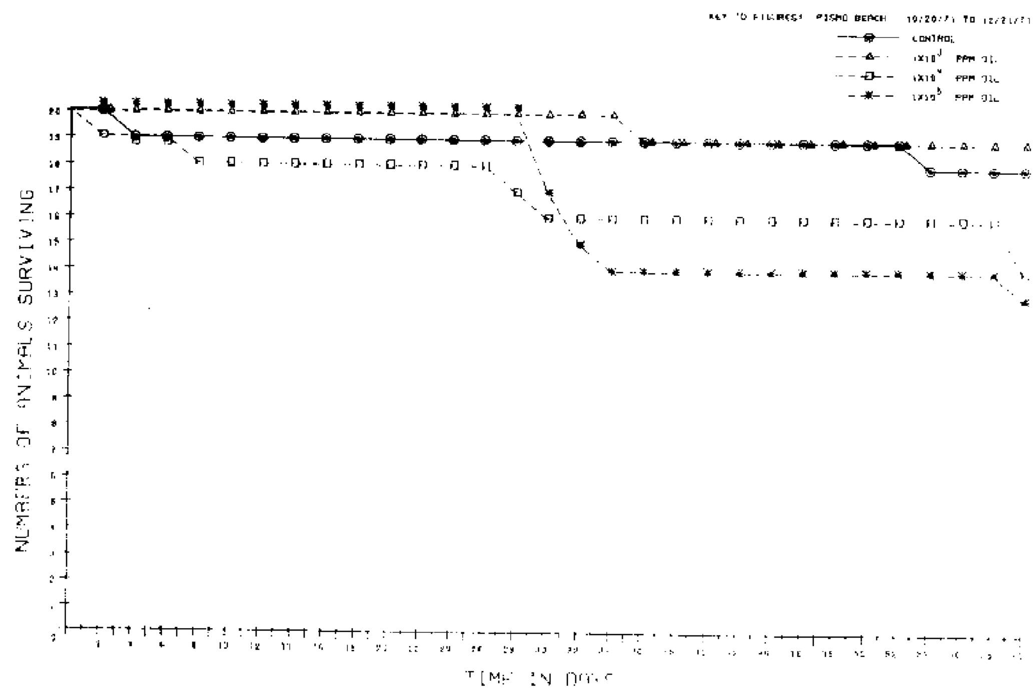

Figure 26: Survivorship curves for Pismo Beach animals Oct. 20, 1971 to Dec. 21, 1971.

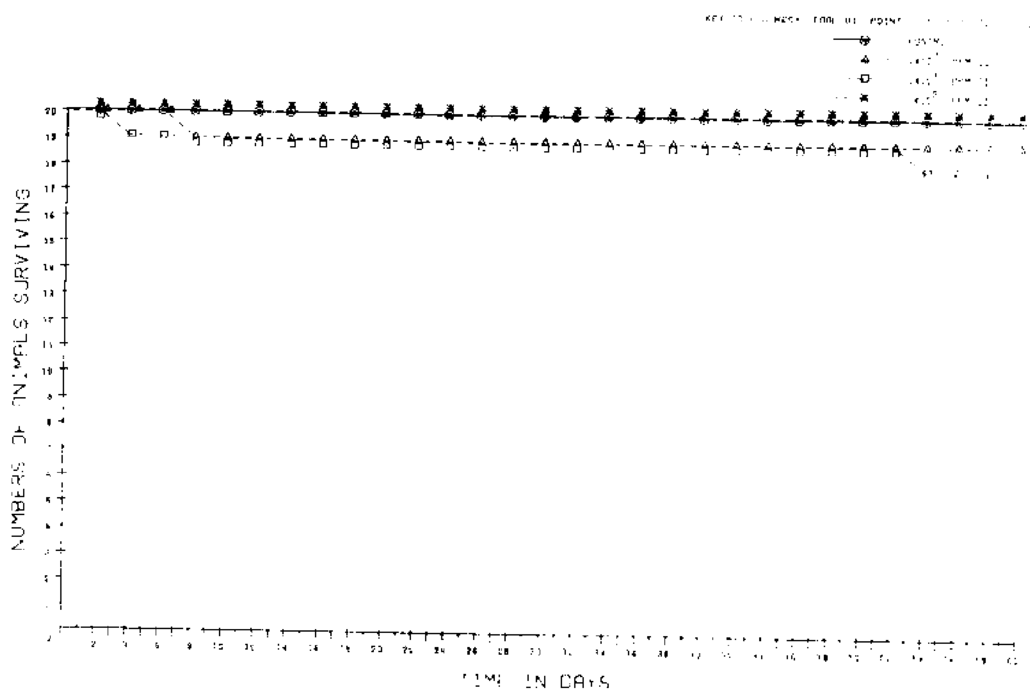

Figure 27: Survivorship curves for Coal Oil Point animals Oct. 20, 1971 to Dec. 21, 1971.

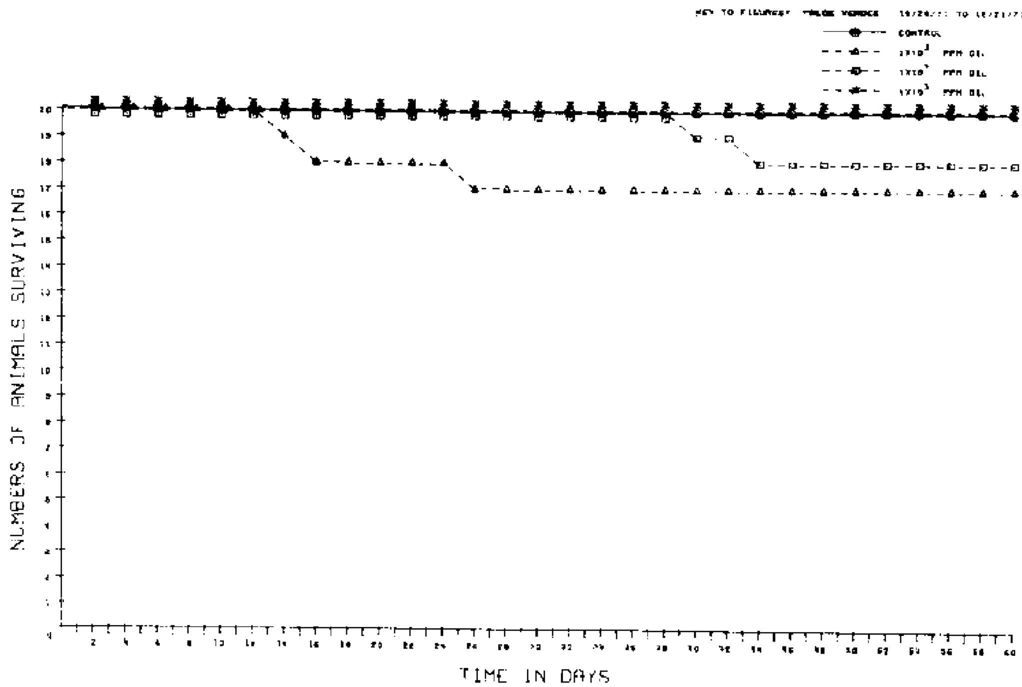

Figure 28: Survivorship curves for Palos Verdes animals Oct. 20, 1971 to Dec. 21, 1971.

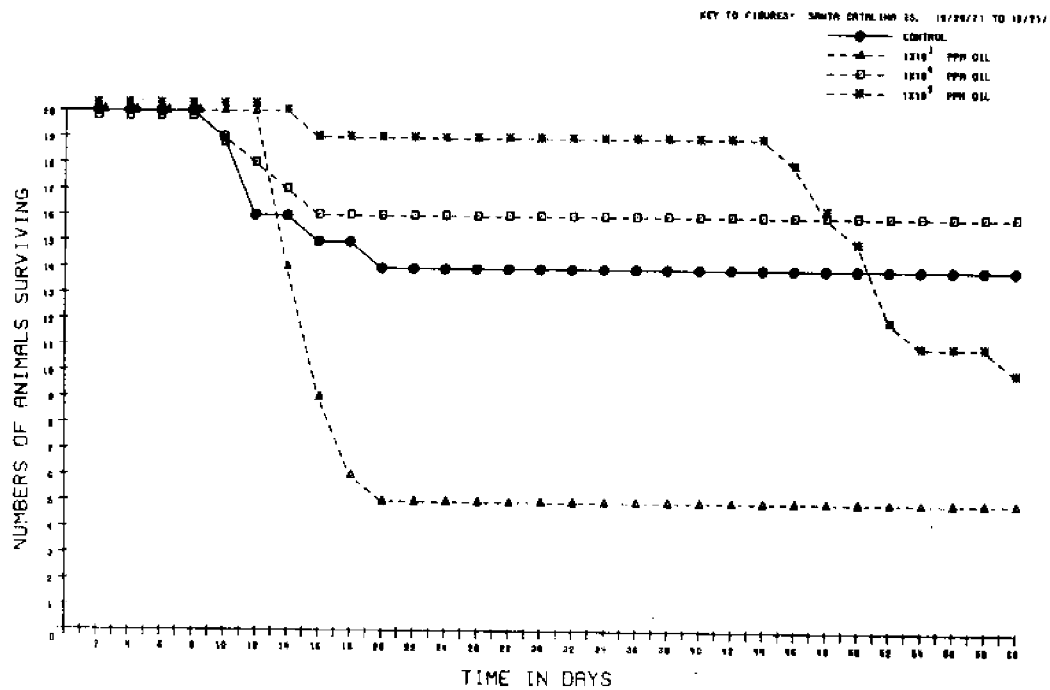

Figure 29: Survivorship curves for Santa Catalina Island animals, Oct. 20, 1971 to Dec. 21, 1971.

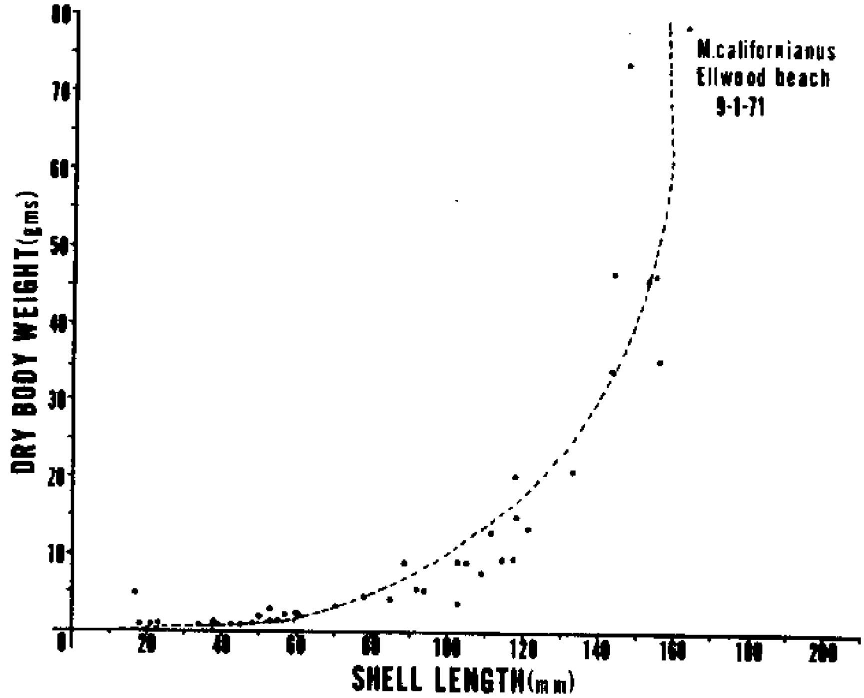

Figure 30: Relationship of size in length versus body weight in Mytilus californianus (from Dr. Dale Straughan, personal communication).

Table 3. Dates of detected spawnings.

Location	Dates
Pismo Beach	March 1, 1971; March 3, 1971
Coal Oil Point	February 12, 1971; March 3, 1971
Palos Verdes	October 9, 1970; March 10, 1971; October 20, 1971

LITERATURE CITED

Allen, A.A., Schlueter, R.S. 1969. Estimates of Surface Pollution, Resulting from Submarine Oil Seeps at Platform A and Coal Oil Point. Technical Memorandum 1230. General Research Corp., Santa Barbara, Calif.

Blumer, M., Souza, G. and Sass, J. 1970. Hydrocarbon Pollution of Edible Shellfish by an Oil Spill. Ref. No. 70-1. Woods Hole Oceanographic Institution. Unpublished Manuscript.

Boylan, D.B. and Tripp, B.W. 1971. Determination of Hydrocarbons in Seawater Extracts of Crude Oil and Crude Oil Fractions. Nature. 230:44-47.

Chan, G.L. 1973. A Study of the Effects of the San Francisco Oil Spill on Marine Organisms. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. API, EPA, USCG. Washington D.C.: 741-782.

Emery, K.O. 1960. The Sea Off Southern California. Pub. John Wiley & Sons Inc. New York. 320-321.

Galloway, J.N. 1972. Global Concepts of Environmental Contamination. Mar. Pol. Bull. 3(5):78-79.

Jørgensen, C.B. 1952. On the Relation Between Water Transport and Food Requirements in Some Marine Filter Feeding Invertebrates. Biol. Bull. 103:356-363.

_____ 1966. "Biology of Suspension Feeding". Pergamon Press.

Kanter, R.G., Straughan, D, Jessee, W.N. 1971. Effects of Exposure to Oil on Mytilus californianus from Different Localities. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. API, EPA, USCG. Washington D.C.:485-488.

Lee, R.F., Sauerheber, R., and Benson, A.A. 1972. Petroleum Hydrocarbons: Uptake and Discharge by the Marine Mussel Mytilus edulis. Sci. 177:344-346.

Levington, J. 1972. Stability and Trophic Structure in Deposit-Feeding and Suspension-Feeding Communities. Am. Nat.106 (950).

McAuliffe, C. 1966. Solubility in Water of Paraffin, Cycloparaffin, Olefin, Acetylene, Cycloolefin, and Aromatic Hydrocarbons. J. Phys., Chem. 7(4).

Nicholson, N.L. and Cimberg, R.L. 1971. The Santa Barbara Oil Spill of 1969: A Post-Spill Survey of the Rocky Intertidal. In Biological and Oceanographical Survey of the Santa Barbara Channel Oil Spill 1969-1970. Pub. Allan Hancock Fndn.:325-400.

Paine, K.P. 1953. Rate of Water Propulsion in Mytilus Californianus as a Function of Latitude. Biol. Bull. 104:171-181.

Siegel, S. 1956. Non Parametric Statistics for the Behavioral Sciences. Mc.Graw-Hill. Pub. New York. 159-161.

Smith, C.L. and MacIntyre, W.G. 1971. Initial Aging of Fuel Oil Films on Sea Water. In Proceedings of Joint Conference on Prevention and Control of Oil Spills. API, EPA, USCG. Washington D.C.: 457-462.

Smith, J.E. 1968. "Torrey Canyon Pollution and Marine Life". Cambridge at the Univ. Press: 133-134.

Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistics. McGraw-Hill Pub. New York. 106-107.

Straughan, D. 1972. Factors Causing Environmental Changes After an Oil Spill. J. Pet. Tech. Mar.: 250-254.

Whedon, W.F. 1936. Spawning Habits of the Mussel Mytilus californianus Conrad. Univ. Calif. Pub. Zool. 41(5): 35-44.