ACUTE TOXICITY OF NO. 6 FUEL OIL TO INTERTIDAL ORGANISMS IN THE LOWER YORK RIVER, VIRGINIA

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ABSTRACT

Seven estuarine intertidal species were tested for acute toxicity of the water soluble components of "Bunker C", No. 6 fuel oil (referred to as WSOC). The measurements of toxicity were based on observations of mortality, and were recorded as median tolerance limits (TL_{50}), i.e., those concentrations of WSOC which killed 50% of the sample populations over a 48 hour exposure period. The organisms tested were *Spiochaetopterus costarum oculatus* (Polychaeta), *Nereis succinea* (Polychaeta), *Nassarius obsoletus* (Gastropoda), *Modiolus demissus* (Bivalvia), *Gammarus mucronatus* (Amphipoda), *Edotea triloba* (Isopoda) and *Pagurus longicarpus* (Decapoda).

Of the seven species tested, only three were susceptible to acute toxicity. The pooled TL_{50} values for these affected species included concentrations of 8.42% (0.47 ppm) for *Gammarus mucronatus*, 9.75% (0.54 ppm) for *Pagurus longicarpus*, and 89.00% (4.92 ppm) for *Spiochaetopterus costarum oculatus*. Slope functions and 95% confidence limits of TL_{50} were calculated.

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INTRODUCTION

In recent years, an increase in population and per capita consumption of oil has resulted in a great increase in the supply and demand of petroleum and petroleum products. U. S. production of crude oil, crude refinery capacity, and stocks and transportation of oil and oil products have increased tremendously since 1961 (Bureau of Mines, 1962 and 1972). Furthermore, there has been an especially large demand for residual fuel oils in the United States, particularly along the East Coast. In 1971, the annual demand for residual fuel oils was estimated at 837,675,000 barrels, 75% of which occurred along the East Coast (Bureau of Mines, 1972).

Through deliberate disposal or unintentional losses during production, refining, transportation, or use, large volumes of oil inevitably find their way to the marine environment. A result of this has been a long history of oil pollution, extensively documented in the literature (see Nelson-Smith, 1971, 1973). North, et al. (1964) observed community changes following the _Tampico Maru wreck_ in 1957, which spilled 8000 metric tons of diesel fuel into a marine cove in Baja California. Diaz-Piferrer (1962) studied the damage to marine life caused by the wreck of the Argea Prima, July, 1961, which released 10,000 tons of crude oil into Guayanilla Harbour, Puerto Rico. Numerous authors have investigated the biological consequences of the Torrey Canyon disaster of March, 1967, which released 117,000 tons of crude oil into waters near Cornwall, England: (Drew, et al., 1967; Holme, 1967; O'Sullivan and Richardson, 1967; Bellamy, et al., 1967; Spooner, 1967; Carthy and Arthur, 1968; Smith, 1968; Ranwell, 1968; Simpson, 1968; Nelson-Smith, 1968 a; Tendron, 1968; Cowell, 1969). Blumer, et al. (1970 a, b), Hampson and Sanders (1969), and Burns and Teal (1971) have covered the biological effects of a No. 2 fuel oil spill which occurred during the Fall of 1969 in West Falmouth, Massachusetts. Many authors have reported the effects of the offshore drilling accident which occurred near Santa Barbara, California, during January, 1969: (Battelle-Northwest, 1967 and 1969; Holmes, 1969; Allen and Schlueter, 1969; Jones, et al., 1969; Straughan, 1971; Straughan and Abbott, 1971; Foster, et al., 1971 a, b; Kolpack, 1971). M. L. H. Thomas (1973) reported the biological effects of a Bunker C spill in the Chedabucto Bay, Nova Scotia, due to the wreck of the tanker Arrow, February, 1970. Alpine Geophysical Associates (1971) investigated the crude oil and natural gas leak which occurred at Chevron Oil Company wells in Louisiana, February through March, 1970. Lastly, Chan (1972) studied the biological consequences of a Bunker C spill in January, 1971, which released 840,000 gallons near San Francisco, California.

In addition to the field studies following specific spills, laboratory toxicity studies of various oils and their effects on aquatic plants and animals have been performed (see Nelson-Smith, 1971, 1973). Also, due to a history of oil spills which have occurred in Milford Haven, England, 1960-1968, the Orielton Oil Pollution Research Unit has performed numerous controlled experiments dealing with the ecological effects of oil and detergents on marine animal and plant life (Cowell, 1971). Furthermore, a number of toxicity studies with oil were reported during a conference on the prevention and control of oil spills, held in Washington, D. C., March 13 through 15, 1973.

An oil pollution incident occurring in an estuary may arouse considerable concern, since estuaries are considered the most potentially productive areas of the sea. Accordingly, the occurrence of an oil spill in the Chesapeake Bay area, which is a complex and biologically productive estuarine environment, could be disastrous economically as well as ecologically. Relevantly, there are large volumes of oil transported on estuarine waters. According to the U. S. Coast Guard (1971), during 1970, 78.8% of the 3,711 oil spills reported in the United States occurred in inland and coastal waters. Furthermore, during 1972, 384 spills were reported from tidal and coastal waters of Virginia, Maryland, and North Carolina (Fifth District Coast Guard, personal communication).

The York River estuary, in particular, is threatened by oil contamination, due to the large volume of oil transported through the area. The American Oil Company, for example, operates a refinery (located on the York River in Yorktown) with a crude running capacity of 38,000 barrels per day. All domestic and foreign crude enters the Yorktown refinery by vessel and 90% of the refinery's products, including heavy fuel oils, leave by vessel (American Oil Company, 1970). In addition to the refinery's activities, a major barge route for the transportation of fuel oils is located on the York River. There is also a great deal of commercial fishing and recreational activity in the area.

In May, 1971, an oil spill occurred in the York River. Approximately 44,000 gallons of a cracking residue, which had been thinned with a lighter distillate to the consistency of No. 6 fuel oil, leaked from a broken pipeline at the Amoco oil refinery in Yorktown, and then washed onto an intertidal beach in the nearby Guinea Marsh. Results of the field study following the spill indicated that the structure of the intertidal macrofaunal community was affected by the presence of the oil, since species diversity values were considerably lower in the spill area than in the two control areas (Bender, Duncan, and Hyland, in preparation). The results of the field study suggested a possible toxic effect of the residual fuel oil.

Due to the results of the Guinea Marsh oil spill and the potential threat of another spill in the area, the present study was performed.

The test organisms included _Spiochaetopterus costarum oculatus_ (Polychaeta), _Nereis succinea_ (Polychaeta), _Nassarius obsoletus_ (Gastropoda), _Modiolus demissus_ (Bivalvia), _Edotea triloba_ (Isopoda), _Gammarus mucronatus_ (Amphipoda), and _Pagurus longicarpus_ (Decapoda). Generally, the organisms were chosen because, based on numerical ranking analyses, they appeared to be York River dominants (J. Hyland, unpublished data; Marsh, 1970; Orth, 1971; Boesch, 1971), and secondly, each represented a major taxonomic division of York River intertidal macrofauna. _Modiolus demissus_ and _Pagurus longicarpus_ have not previously been ranked as community dominants, because the types of studies and sampling techniques involved have not allowed their capture. However, it was felt that both species were community dominants from York River intertidal zones, based on visual observations of their abundance along the beach.

"Bunker C" (No. 6 fuel oil) was chosen as the toxicant because of its close resemblance to the oil spilled in the Guinea Marsh and because of its extensive use as vessel, industrial, and utility fuels. It is not surprising that

50.7% of the spills reported to the U. S. Coast Guard during 1970 (U. S. Coast Guard, 1971) were of residual oils. Residual fuel oils are bottom fractions remaining from the distillation of crude oil, or blends of the bottom fractions with lighter distillates. "Bunker C" is a particular grade of residual fuel oil, designated as such according to certain limiting factors placed on several properties of the oil which are important in determining its performance in the specific type of burner in which it is used. According to ASTM specifications (1967), the properties of No. 6 fuel oil for which limitations are placed include flash point (150°F), water and sediment content (2.00% by volume), and Saybolt viscosity (Furol at 122°F, 45-300 sec).

The toxicity of the water soluble components of No. 6 fuel oil (WSOC), rather than the tarry residue, was assessed in this study. The water soluble components of crude oil have been considered the most immediately toxic to marine animals (Blumer, 1969, and 1970).

The purpose of the present study was to determine the relative tolerance limits of a number of types of intertidal organisms to the water soluble components of "Bunker C", No. 6 fuel oil (WSOC). The results will aid in predicting the toxic potential of No. 6 fuel oil to an intertidal community.

MATERIALS AND METHODS

Field

Test organisms were collected at low tide, during July through September, 1972, from locations along the lower York River. *Edotea triloba*, *Spiochaetopterus costarum oculatus*, *Gammarus mucronatus*, and *Nereis succinea* were collected by shovel from *Zostera marina* sediments at Gloucester Point. The contents of the shovel were sieved in the field through a 1.0 mm screen. *Spiochaetopterus* and *Nereis* were easily recognized and picked directly from the screen; however, collecting *Gammarus* and *Edotea* required additional handling. The remaining contents on the screen were placed in a bucket partially filled with river water and brought back to the laboratory, where a light was placed over the bucket. Soon, *Gammarus* and *Edotea* were attracted to the top of the bucket, where they were easily collected with an 8.0 mm diameter pipette and syringe. On one occasion, several *Nereis* were collected from a Capahosic beach (located approximately 19 km upriver from Gloucester Point). *Nassarius obsoletus* and *Pagurus longicarpus* were collected by hand from mud flats at Gloucester Point. All *Modiolus demissus* were collected by hand from *Spartina alterniflora* roots, on a beach located approximately 3 km upriver from Gloucester Point.

The No. 6 fuel oil used in the present study was purchased in 55 gal. drums from Curtis Oil Company, Lee Hall, Virginia. The fuel oil was believed, by the supplier, to have been derived from Aruban crude.

Laboratory

The test organisms were held in 8 in. (20.32 cm) diameter glass dishes through which unfiltered river water continuously flowed (Figure 1). The organisms were able to obtain particulate food from the unfiltered source of water.

The dosing apparatus was a closed continuous flow system, where peristaltic and proportioning pumps delivered various dilutions of an equilibrated oil and water solution (referred to as 100% WSOC) to corresponding 125 ml dosing flasks (sidearm distilling flasks) in which the test animals were placed (Figure 2). The amount of dilution occurring in each dosing flask was determined by a specific combination of flow rates from the 100% WSOC carboy and the artificial sea water (ASW) dilution carboy. Individual flow rates were dependent upon the diameter of the pump tubing which lay in the peristaltic and proportioning pumps. If possible, glass containers and tubing were used wherever they were needed to eliminate the possibility of the oil leaching away plastic components. Solvaflex pump tubing was used in the pump region to reduce leaching. Reserve carboys constantly siphoned into the distribution carboys in order to maintain constant pressure heads; constant pressure heads were required so that the pumps removed volumes of 100% WSOC and ASW from the distribution carboys at constant rates.

The equilibrated solutions of oil and water were prepared by mixing 50 ml of No. 6 fuel oil with 19.3 l of ASW (15.00 ppt), in a 19.6 l carboy. The solutions were mixed on a magnetic stirrer for 12 hours at approximately 21.40°C. It was suggested that a majority of the water soluble components of the fuel oil (WSOC) would go into solution by following this procedure (C. L. Smith, personal communication). An undiluted volume of the equilibrated oil and water solution was thus regarded as having a concentration of 100% WSOC.

A quantitative estimate of WSOC in the equilibrated solution of oil and water was made using infrared absorption spectroscopy (Frankenfeld, 1973). A sample of 650 ml was removed from the 100% WSOC carboy and shaken vigorously in 25 ml of CCl_4. Absorbance of the CCl_4 extract at 2930 cm^{-1} was measured as the height (in centimeters) of the transmittance peak, on a Beckman IR-5A infrared spectrophotometer. Peak heights were also recorded for known concentrations of No. 6 fuel oil, and an absorptivity curve was constructed by plotting the known concentrations of the oil against their corresponding peak heights. The total organic content of the original sample was then interpolated from the absorptivity curve.

The test organisms were subject to five concentrations of WSOC, 0% WSOC representing the control. The remaining concentrations were 0.73%, 9.35%, 50.00%, and 100.00%. Prior to each experiment, test organisms were acclimated to 28°C for 24 hours. The acclimation chamber was designed so that during the acclimation period the organisms were continuously subject to a flow of unfiltered river water from which they could obtain food. The average salinity of the water which flowed through the acclimation chamber was 16.1 ppt, and ranged from 12.3 to 17.9 ppt (Rivkin, unpublished data). Although the animals were able to obtain food during the acclimation period, they were not fed during the bioassays, since the addition of a solution of particulate food may have caused contamination in the dosing flasks. Ten individuals were placed in each of the five flasks, and mortality over a 48 hour period was recorded. Observations of mortality were used to calculate TL_{50} values for each species. Two experiments were performed for each species to express repeatability of results. Temperature, pH, dissolved oxygen, and salinity were controlled as much as possible; each was recorded daily throughout an experiment to explain any unexpected fluctuations. Experiments were performed July through September, 1972.

RESULTS

The total dissolved organic content of the equilibrated oil and water solution was 5.53 ppm. Frankenfeld (1973) previously obtained up to only 1.9 ppm water extractable organic compounds from a bunker fuel. The difference between the two values may have been the result of two different techniques of obtaining "maximum" solubility, since during the latter study solutions of oil and water were not mechanically agitated, but were instead left undisturbed for periods of up to two weeks. Furthermore, in the present study mechanical agitation of the oil and water solution tended to create an emulsion; and although the solution was left undisturbed until all of the visible oil appeared to float to the surface, emulsified droplets of oil could still have been present in the water column, increasing the total organic content of the withdrawn sample.

The accuracy of the apparatus in delivering the desired concentrations (percent dilutions) was tested using a salt solution of known salinity. While the apparatus delivered various dilutions of the salt solution to the dosing flasks, periodically the salinity of the discharge from each flask was obtained from a salinometer and converted to percent dilution of the original salt solution. These observed concentrations were then compared to the corresponding expected concentrations, resulting in an efficiency of 91.97% ± 6.31%. It must be noted that the above procedure for testing efficiency was dependent upon the accuracy of the salinometer in yielding precise salinities; thus, the calculated efficiency of the dosing apparatus may be a conservative figure.

The total flow rate through each dosing flask was approximately 7.0 ml/min, which represented 5.6% of the flask volume. Replacement occurred within 20 minutes. Sprague (1969) recommended a flow rate of 2-3 l/g/day for the normal respiration of fish. In the present study, flow rates gave approximately 10 l/day. In view of the fact that for all test species, except Nassarius obsoletus and Modiolus demissus, total animal weight within a flask was less than a gram, it can be concluded that the flow rates were adequate. Flow rates, 0.4-1.2 l/g/day, probably were not inadequate for Nassarius and Modiolus, or if they were the effects were insignificant, since generally neither species showed mortality.

Salinity, pH, temperature, and dissolved oxygen were maintained at ambient levels, in order to standardize the test procedures. The conditions of the bioassays did not appear to contribute to the toxic variability of the oil, since generally animals survived in the control flasks. However, it is possible that one or more of the conditions of the bioassays subjected the animals to enough stress so that a synergistic effect with the toxic properties of the oil could then have contributed to mortality. By using ASW, the possibility of hydrocarbon contamination from the field was eliminated. The salinity of the ASW was adjusted to approximately 15.00 ppt, which is representative of intertidal salinities in the lower York River (J. Lucy, unpublished data). The pH was approximately 7.75, which is representative of normal York River pH values (Jacobs, 1972). Temperature was held constant at 28°C, summer ambient intertidal temperature (J. Lucy, unpublished data), in the constant temperature bath. Dissolved oxygen was controlled as much as possible, by saturating the ASW with presurrized oxygen before the start of an experiment. An aerator was not used throughout an experiment since the steady stream of oxygen would accelerate the oxidation of oil components. Table 1 summarizes dissolved oxygen concentrations during all bioassays. The mean DO for all experiments was 6.60 mg/l. Generally, the DO values remained above 5.00 mg/l, and never exceeded 9.10 mg/l. None of the DO values reached critical levels. The lowest DO value was 3.53 mg/l, which was recorded from a flask in which there was no mortality. There was no evidence of a trend in changes in oxygen concentration with changes in concentrations of oil or with time.

TABLE 1

Summary of dissolved oxygen concentrations during 48-hr continuous flow bioassays on all test species; salinity = 15.00%, pH = 7.75; temperature = 28.0°C

		Replicate I				Replicate II			
		Days				Days			
Test Species	Concentration WSOC	1	2	3	Mean	1	2	3	Mean
Gammarus mucronatus	0.00	6.15	7.04	5.66	6.28	5.74	6.15	4.88	5.59
	0.73	6.30	5.30	5.21	5.60	6.71	7.40	5.63	6.58
	9.35	6.89	5.30	5.45	5.88	5.68	7.75	5.81	6.41
	50.00	6.15	5.05	4.95	5.38	6.93	6.33	5.59	6.28
	100.00	5.89	5.51	4.75	5.38	6.46	7.05	5.43	6.31
	Mean	6.28	5.64	5.20		6.30	6.94	5.47	
Pagurus longicarpus	0.00	7.77	6.19	7.18	7.05	7.38	6.36	6.15	6.63
	0.73	7.41	6.09	6.77	6.76	7.69	7.29	6.77	7.25
	9.35	6.94	6.39	6.93	6.75	7.59	6.77	5.64	6.67
	50.00	7.25	6.33	8.82	7.47	7.18	7.27	7.59	7.35
	100.00	7.31	4.87	5.02	5.73	7.69	6.97	5.30	6.65
	Mean	7.34	5.97	6.94		7.51	6.93	6.29	

TABLE 1 (Continued)

		Replicate I				Replicate II			
		Days				Days			
Test Species	Concentration WSOC	1	2	3	Mean	1	2	3	Mean
Edotea triloba	0.00	6.74	6.21	7.86	6.94	7.34	6.32	7.24	6.97
	0.73	5.48	6.80	7.28	6.52	7.74	7.50	6.44	7.23
	9.35	6.98	6.80	7.47	7.08	7.84	7.02	6.34	7.07
	50.00	6.29	7.11	6.87	6.76	7.78	7.32	7.62	7.57
	100.00	6.29	6.66	7.12	6.69	7.62	7.70	5.36	6.89
	Mean	6.36	6.72	7.32		7.66	7.17	6.60	
Nereis succinea	0.00	7.43	6.30	6.26	6.66	7.38	7.48	7.04	7.30
	0.73	7.09	6.78	6.70	6.86	7.48	7.50	7.34	7.44
	9.35	7.56	6.34	6.40	6.77	8.20	6.26	7.10	7.19
	50.00	7.41	5.50	5.28	6.06	7.90	7.06	7.02	7.33
	100.00	6.76	5.00	4.40	5.39	8.34	8.50	7.50	8.11
	Mean	7.25	5.98	5.81		7.86	7.36	7.20	

TABLE 1 (Continued)

Test Species	Concentration WSOC	Replicate I Days 1	Replicate I Days 2	Replicate I Days 3	Replicate I Mean	Replicate II Days 1	Replicate II Days 2	Replicate II Days 3	Replicate II Mean
<u>Modiolus</u> <u>demissus</u>	0.00	7.10	6.70	6.74	6.85	6.50	6.48	6.40	6.46
	0.73	7.44	6.28	6.74	6.82	7.70	7.82	6.40	7.31
	9.35	9.10	6.70	8.00	7.93	7.80	7.82	7.86	7.83
	50.00	7.40	7.50	6.24	7.05	7.26	7.18	7.00	7.15
	100.00	8.02	7.02	6.68	7.24	6.46	6.04	5.84	6.11
	Mean	7.81	6.84	6.88		7.14	7.07	6.70	
<u>Spiochaetopterus</u> <u>costarum</u> <u>oculatus</u>	0.00	6.30	7.25	5.92	6.49	6.77	6.00	6.61	6.46
	0.73	4.20	6.71	6.50	7.97	7.50	7.21	6.17	6.96
	9.35	7.20	7.12	6.31	9.53	7.48	6.64	6.08	6.73
	50.00	7.10	7.37	7.04	7.17	6.68	6.34	6.23	6.42
	100.00	7.70	6.71	5.38	6.60	6.56	4.88	6.68	6.04
	Mean	6.50	7.03	6.23		7.00	6.21	6.35	

TABLE 1 (Continued)

Replicate I

Test Species	Concentration WSOC	Days 1	Days 2	Days 3	Mean	Days 1	Days 2	Days 3	Mean
Nassarius obsoletus	0.00	6.81	6.32	4.69	5.94	6.17	6.70	5.48	6.12
	0.73	6.94	6.42	5.36	6.24	6.17	6.60	4.70	5.82
	9.35	7.38	6.93	5.41	6.57	6.10	6.54	5.28	5.97
	50.00	6.45	6.63	5.71	6.26	6.50	6.60	3.65	5.58
	100.00	7.41	6.93	4.08	6.14	5.43	5.48	3.53	4.81
	Mean	7.00	6.65	5.05		6.09	6.38	4.53	

Lack of food during the bioassays did not appear to contribute to the variable toxicity of the oil, since little or no mortality occurred in the controls.

Table 2 summarizes observations of mortality for all test species. In each replicate experiment, Nassarius obsoletus, Edotea triloba, Nereis succinea, and Modiolus demissus showed no significant mortality attributable to the presence of oil. Percent mortality from these experiments was plotted against concentration of WSOC on arithmetic graph paper (Figures 12-15). The few incidents of death of Nereis were primarily due to their cannibalistic activities. Cannibalism was reduced to a minimum by providing nylon tubular shelters which individuals occupied. Forty percent mortality of Edotea was observed on one occasion; however, no overall pattern of mortality was displayed. Gammarus mucronatus, Pagurus longicarpus, and Spiochaetopterus costarum oculatus all displayed patterns of mortality from which TL_{50} values could be determined.

Table 3 summarizes the TL_{50} values. A TL_{50} value represents that concentration of WSOC which kills 50% of the test population during a 48 hour exposure period. The TL_{50} values were obtained using graphic estimates from dose-response curves (Litchfield and Wilcoxin, 1949). Doses were plotted on a logarithmic scale and percent mortality on a probability scale, so that a straight probit line could be constructed and tested by chi^2 for goodness of fit. Probit lines and TL_{50} values appear in

TABLE 2

Summary of percent mortality for all test species

				Concentration		
Test Species	Replicate	0.00	0.73	9.35	50.00	100.00
Gammarus mucronatus	1	10	20	40	80	100
	2	0	20	20	80	100
	Pooled	5	20	30	80	100
Pagurus longicarpus	1	0	20	50	60	100
	2	0	20	60	80	100
	Pooled	0	20	55	70	100
Spiochaetopterus costarum oculatus	1	10	20	30	30	50
	2	10	20	30	30	60
	Pooled	10	20	30	30	55
Nassarius obsoletus	1	0	10	0	0	0
	2	0	0	0	0	0

TABLE 2 (Continued)

Test Species	Replicate			Concentration		
		0.00	0.73	9.35	50.00	100.00
Edotea triloba	1	0	10	0	0	0
	2	0	0	0	0	40
Nereis succinea	1	0	20	0	10	0
	2	0	0	10	20	0
Modiolus demissus	1	0	0	0	0	0
	2	0	0	0	0	0

TABLE 3

Summary of TL_{50} results expressed in percent concentration of water soluble oil components (WSOC)

Test Species	Replicate	TL_{50}	Slope Function	Confidence Limits on TL_{50}
Gammarus mucronatus	1	7.60	4.54	3.55 - 16.26
	2	9.20	5.28	2.04 - 41.40
	Pooled	8.42	6.29	2.11 - 33.68
Pagurus longicarpus	1	11.20	9.75	2.80 - 44.80
	2	8.20	16.72	2.00 - 33.62
	Pooled	9.75	11.09	4.24 - 22.43
Spiochaetopterus costarum oculatus	1	89.00	-	-
	2	89.00	-	-
	Pooled	89.00	-	-

Figures 3-11. The lines suggest expected toxicity of WSOC over a continuous range of concentrations. Arrows around observed points on the graphs indicate corrected estimates of 0 and 100% mortality, which were determined according to procedures adopted from Litchfield and Wilcoxin (1949). TL_{50} values included concentrations of 7.60% (0.42 ppm) and 9.20% (0.51 ppm) for Gammarus mucronatus, 8.20% (0.45 ppm) and 11.20% (0.62 ppm) for Pagurus longicarpus, and 89.00% (4.92 ppm) for Spiochaetopterus costarum oculatus.

The slope function, S, when raised to an exponent, represents a factor by which the TL_{50} value must be multiplied or divided to yield its upper and lower 95% confidence limits. S was calculated as:

$$S = \frac{TL_{84}/TL_{50} \pm TL_{50}/TL_{16}}{2}$$

Ninety-five percent confidence limits of the TL_{50} value were calculated as:

$$TL_{50} \text{ X } S^{2.77/\sqrt{N'}} = \text{upper limit}$$

$$TL_{50}/S^{2.77/\sqrt{N'}} = \text{lower limit}$$

where N' represents the total number of animals tested at those doses whose expected effects were between 16 and 84 percent (Litchfield and Wilcoxin, 1949). The slope function and 95% confidence limits ranged from 2.04% (0.11 ppm) to 41.40% (2.29 ppm) for Gammarus mucronatus and from 2.00% (0.11 ppm) to 44.80% (2.48 ppm) for Pagurus longicarpus. Slope functions and TL_{50} confidence limits could not be calculated for Spiochaetopterus costarum oculatus, since TL_{84} values could not be obtained. TL_{84} values were not obtained for Spiochaetopterus because the greatest concentration possible (100% WSOC), which represented maximum solubility of No. 6 fuel oil in water, never resulted in mortality greater than 60%. In its strictest statistical interpretation, not being able to place confidence limits on the TL_{50} value means that if a number of tests were performed in an identical manner, a certain portion of the tests may present results revealing no significant mortality attributable to the toxic properties of the oil.

A double-classification analysis of variance was performed on probit transformed observations of mortality for the three affected species (Table 4). At the 1% significance level, there were differences in mortality attributable to different concentrations of WSOC. A test for relative efficiency revealed that there was very little positive gain in blocking the data (removing any variations in mortality between replicate experiments from the error term), which indicated that for each species data from replicate experiments were homogeneous. In addition, for each species, the two replicate experiments were treated as paired observations, and T-tests were performed. The values of T were 0.2517 for Gammarus mucronatus, 0.2469 for Pagurus longicarpus, and 0.1546 for

TABLE 4

Analysis of variance, including test for relative efficiency of blocking, illustrating differences in toxicity to test species associated with concentrations (0.00%, 0.73%, 9.35%, 50.00%, and 100.00%) and replicates

Test Species	Source of Variation	df	ss	ms	F	Relative Efficiency
Gammarus mucronatus	R (Replicates)	1	0.1345	0.1345		1.0210%
	C (Concentration)	4	16.5329	4.1332	36.5446 *	
	Error	4	0.4525	0.1131		
	Total	9	17.1199			
Pagurus longicarpus	R (Replicates	1	0.1146	.1146		1.3749%
	C (Concentration)	4	14.9418	3.7354	142.5725 *	
	Error	4	0.1050	.0262		
	Total	9	15.1614			
Spiochaetopterus costarum oculatus	R (Replicates)	1	0.0063	0.0063		1.0000%
	C (Concentration)	4	2.1237	0.5309	84.2698 *	
	Error	4	0.0252	0.0063		
	Total	9	2.1552			

* indicates significant difference at 1% level.

Spiochaetopterus costarum oculatus. At the 1% significance level, the values of T also indicated that there were no differences in mortality between replicates.

In view of the lack of differences between the two replicates, the sample size was increased by pooling observations of mortality. Probit lines were constructed using the pooled percent mortalities. Pooled TL_{50} values were 8.42% (0.47 ppm) for Gammarus mucronatus, 9.75% (0.54 ppm) for Pagurus longicarpus, and 89.00% (4.92 ppm) for Spiochaetopteous costarum oculatus. The 95% confidence limits fell between 2.11% (0.12 ppm) and 33.68% (1.86 ppm) for Gammarus and 4.24% (0.23 ppm) and 22.43% (1.24 ppm) for Pagurus.

DISCUSSION

Acute laboratory bioassays have an advantage of providing a quick and easy method for establishing comparative toxicities. The results can be useful in predicting the toxic potential of an oil to several species of fauna representative of a community. The conclusions drawn from such a study can then be used to interpret effects observed in the field (see Wilson, 1973). However, the results of acute laboratory tests alone cannot be used in predicting the total ecological effects of an oil pollution incident. For example, an acute study in which TL_{50} values are computed would not include measures of sublethal effects on the test species or of ecological changes resulting from the disappearance of the more sensitive species. Furthermore, what information the acute laboratory tests do give may not be one hundred percent accurate, since additional variables having possible synergistic or antagonistic effects with the oil may be introduced when natural environmental conditions are not duplicated precisely in the laboratory. If one relied entirely on the information resulting from an acute laboratory study, he may inaccurately assess the effects of the oil on the natural biotic community. Therefore, before interpreting the results of an acute bioassay, its limitations should be well understood.

The laboratory results indicated that *Nassarius obsoletus*, *Modiolus demissus*, *Edotea triloba*, and *Nereis succinea* were completely tolerant of the immediately toxic fractions of No. 6 fuel oil. These species also did not reveal patterns of mortality following the Guinea Marsh oil spill (Bender, Duncan, and Hyland, in preparation). Resistance by these four species cannot be explained entirely. For example, it is not understood why *Edotea* was tolerant to the fuel oil, since the other crustaceans tested were, in fact, quite sensitive to the oil. Since *Edotea* is an ubiquitous species, perhaps it is generally tolerant to a wide range of environmental conditions. The tolerance to fuel oil displayed by *Nereis* seems to agree with previous data which lend to its recognition as a pollution indicator (Richardson, 1971).

The tolerance displayed by *Nassarius obsoletus* and *Modiolus demissus* may reflect a general resistance of molluscs to acute toxicity of No. 6 fuel oil. Accordingly, Mironov (1967) reported that heavy fuel had no toxic effect on *Bittium reticulatum*, *Rissoa euxinica*, or *Gibbula divaricata*. Furthermore, Nelson-Smith (1971) reported that weathered Arabian crude had no toxic effect on the mussel *Mytilus edulis*. In a summary of the effects of oils and dispersants on benthic fauna, Moore, et al. (1973) reported that gastropods appeared to be the most resistant species. Also, Scarratt, et al. (1970) reported that ingestion of Bunker C had no effect on *Littorina littorea*. One might expect the molluscs to resist immediate poisoning due to their ability to shut out the surrounding environment for rather long periods of time. Nevertheless, in the present study *Nassarius* and *Modiolus* were even observed with their siphons extended while subject to the most concentrated dose.

However, a statement like the one above which proclaims resistance by all molluscs to the toxic properties of No. 6 fuel oil may be too general, in view of the possibility of a few species being particularly tolerant. Also, it must not be forgotten that molluscs are susceptible to various other adverse effects (mentioned later). Indeed, Thomas (1973) reported that the softshell clam, *Mya arenaria*, was adversely affected by a Bunker C oil spill. Also, Scarrat, et al. (1970) reported that after the *Arrow* spill, *Modiolus modiolus* incorporated fractions of Bunker C in concentrations of 100-125 ug/gm. Furthermore, Gilfillan (1973) found that seawater extracts of crude oil reduced the amount of carbon available for growth and reproduction of *Modiolus demissus*.

The crustaceans, *Pagurus longicarpus* (hermit crab) and *Gammarus mucronatus* (amphipod), both revealed susceptibility to acute toxicity of No. 6 fuel oil. Of the species tested, these were the most sensitive. The mechanism of toxicity is not known, but the general susceptibility of crustaceans to abnormal environmental conditions is well known. Moore, et al. (1973) reported that of all benthic fauna the crustaceans are the most sensitive to various oils and dispersants. Perkins (1968) reported that the hermit crab, *Eupagurus bernhardus*, is sensitive to the dispersant, BP 1002, the 96 hour LD_{50} equalling 5 ppm. Smith (1968) reported that another hermit crab, *Diogenes pugilator*, is also sensitive to BP 1002, the 24 hour LD_{50} equalling 25 ppm. It has previously been shown that *Gammarus* (McCauley, 1966), the pink shrimp, *Pandalus montagui*, the brown shrimp, *Crangon crangon*, and the shore crab, *Carcinus maenas* (Portmann, 1969), and the barnacles, *Elminius modestus* (Spooner, 1968; Corner et al., 1968) and *Balanus balanoides* (Chipmann and Galtsoff, 1949) are sensitive to acute poisoning from various types of oils and dispersants.

While the literature indicates that crustaceans are generally susceptible to oils and dispersants, data concerning the effects of Bunker C, specifically, on various crustaceans are scarce. The results of the present study indicated that at least *Gammarus mucronatus* and *Pagurus longicarpus* are sensitive to Bunker C. On the other hand, Scarratt, et al. (1970) has reported that the lobster, *Homarus americanus*, is very resistant to Bunker C, since the laboratory determined, 96 hour LD_{50} was greater than 10,000 ppm. Perhaps the lobster's relatively large size, as opposed to that of the hermit crab and amphipod, contributed to its resistance to toxicity.

In the field, *Gammarus* did not reveal a definite pattern of mortality in response to the presence of oil.

Individuals generally did not appear at the oil-polluted site; however, occasionally, none appeared at control sites either. Had Gammarus been more abundant at control sites, an obvious reduction in the number of individuals at the oil-polluted site may have been revealed. Since the sampling technique used during the field study did not allow the capture of Pagurus longicarpus, a comparison between responses in the field and in the laboratory could not be made for this species.

The polychaete, Spiochaetopterus costarum oculatus, was also sensitive to No. 6 fuel oil, although the TL_{50} value was relatively high. As with the other affected species, the sensitivity of Spiochaetopterus to fuel oil cannot be explained. However, the relatively high TL_{50} value may be explained by the tubicolous habits of the animal. George (1971) reported that mucous secretions may have contributed to the resistance of Cirriformia tentaculata and Cirratulus cirratus to fuel oil. In the field, Spiochaetopterus showed the most drastic reduction in numbers due to the presence of oil. The field data slightly contradict the laboratory data, which reveal the relatively high TL_{50} value; however, other adverse effects of the oil may have contributed to the high mortality of this species in the field.

It is generally accepted that the acute toxicity of crude oil is due to certain water soluble components (Blumer, 1969 and 1970; Tarzwell, 1971). These components include the low boiling saturated hydrocarbons, olefinic hydrocarbons, low boiling aromatic hydrocarbons, non-hydrocarbon portions, phenolic compounds, quinolines, pyridines, and hydroxybenzoquinolines. The majority of the test species in the present study were tolerant to No. 6 fuel oil, probably because most of the lower boiling toxic fractions were distilled off during processing. The greatest percentage of compounds in No. 6 fuel oil have boiling points greater than 300°C. However, components of the lower boiling distillate fractions, used to thin the oil, may have contributed to its partial acute toxicity. Furthermore, some immediate toxicity may have been attributable to its relatively high sulfur content, which is sometimes as high as 5.26% (Kawahara, 1969).

A decrease in Gammarus, Pagurus, and Spiochaetopterus populations may have additional ecological effects, although none were presently measured. For example, Gammarus, being one of the first species to attack fresh detritus, is an important link between Spartina alterniflora detritus and carnivores (Sikora, Heard, and Dahlberg, 1972). Therefore, a decline in the Gammarus population may have a detrimental effect on the detritus food chain of a salt marsh. Spiochaetopterus probably plays an important role both in bioturbation and stabilization of the sediments. A decline in the Spiochaetopterus population may, then, cause a decrease in the vertical flow of nutrients and oxygen to lower depths in the sediments, and at the same time, allow a measurable degree of horizontal shifting of the sediments. Unstable, shifting sediments can cause a decrease in benthic diversity (Young and Rhoads, 1971). Furthermore, it has been noted that several commensal organisms, living on the shell occupied by the hermit crab, are dependent on the crab's feeding activities (Orton, 1927; Calder, 1971). Also, hermit crabs are probably important grazers on microflora. Therefore, a decline in the Pagurus population may seriously affect the population of its commensal organisms, and additionally, allow a flourishing crop of microflora to overpopulate the intertidal zone of a beach.

The present study was concerned strictly with acute toxicity of the water soluble components of No. 6 fuel oil (WSOC). However, as suggested earlier, No. 6 fuel oil may be responsible for various chronic, adverse effects not presently measured: reduction in pumping rates of molluscs (Galtsoff, et al., 1935; Lunz, 1950; Blumer, 1969; Tarzwell, 1971), tainting (Blumer, 1970), physical entanglement, suffocation, and dislodgment from the substrate (Nelson-Smith, 1968; Tarzwell, 1971), inducement of cancer, changes of chemotactic reactions essential to normal activities and development, repelling effects, and adverse effects on sensory organs, feeding mechanisms, enemy avoidance, and sexual attraction (Blumer, 1969; Tarzwell, 1971). Therefore, all the test organisms, including the ones which did not reveal susceptibility to acute toxicity, may be adversely affected by the fuel oil, and the results of these adverse effects may lead to further ecological consequences not mentioned above. For example, Modiolus demissus serves an important function of aiding in the cycling and retention of phosphorus in the marsh (Kuenzler, 1961; Odum, 1966). A reduction in the pumping rate of the mussel would have an effect on this biogeochemical process.

As an oil spill of appreciable size washes onto a beach, it is quite obvious that laboratory determined lethal concentrations of WSOC (0.47 to 4.92 ppm) could be reached, especially in intertidal pools and interstitial spaces within the sediment. However, in conclusion, it must be stated that, since only three out of the seven species presently tested were sensitive to acute toxicity, the toxic potential of No. 6 fuel oil (WSOC) to an entire intertidal community does not appear to be significant. At most, immediate poisoning from WSOC may explain mortality of only a few species within a community. This does not mean, though, that various adverse effects of the oil leading to additional mortality will not occur. Accordingly, a certain degree of the disruption of community structure within the Guinea Marsh oil-spill area was presumably due to various adverse effects on the fauna rather than to immediate poisoning.

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Figure 1

Holding system for test organisms

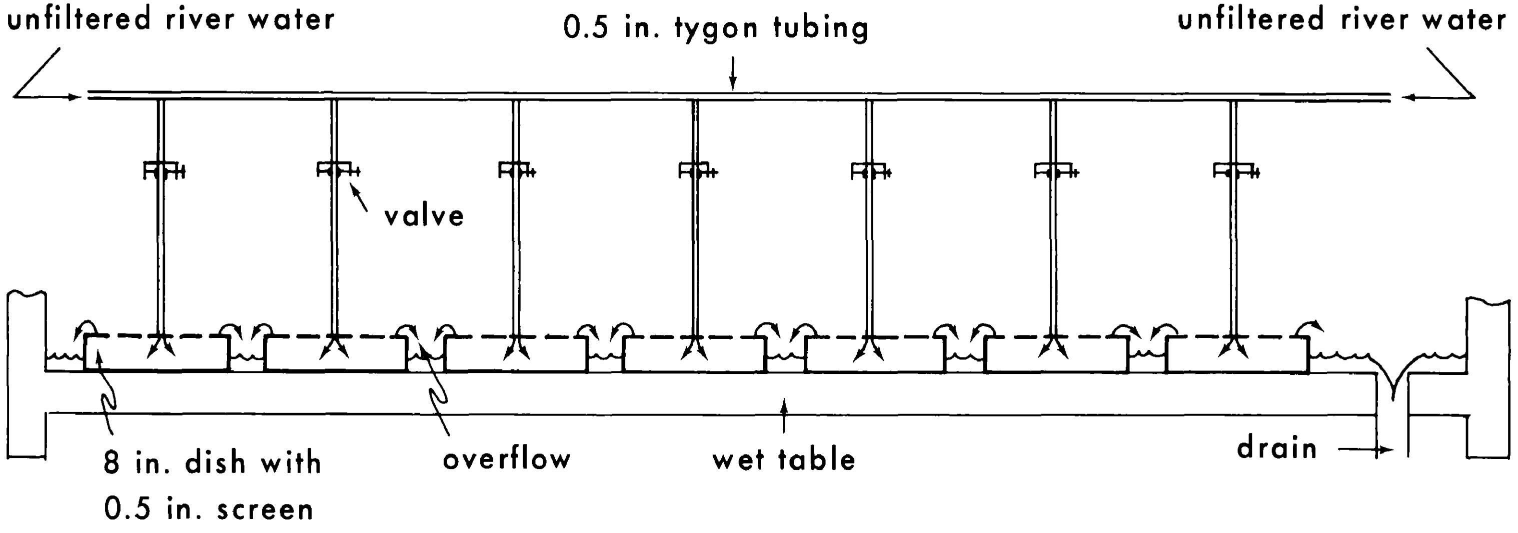


FIGURE 2

Continuous flow dosing apparatus

Identification Letter	Description
A	Glass oil reservoir
B	Nalgene ASW reservoir
C	Glass oil distribution carboy (with constant pressure head)
D	Nalgene ASW distribution carboy (with constant pressure head)
E	Glass distributing tubes
F	Solvaflex pump tubing
G	Proportioning pump
H	Peristaltic pump
I	Dosing flasks
J	Constant temperature bath
K	Heater
L	Discharge tubes

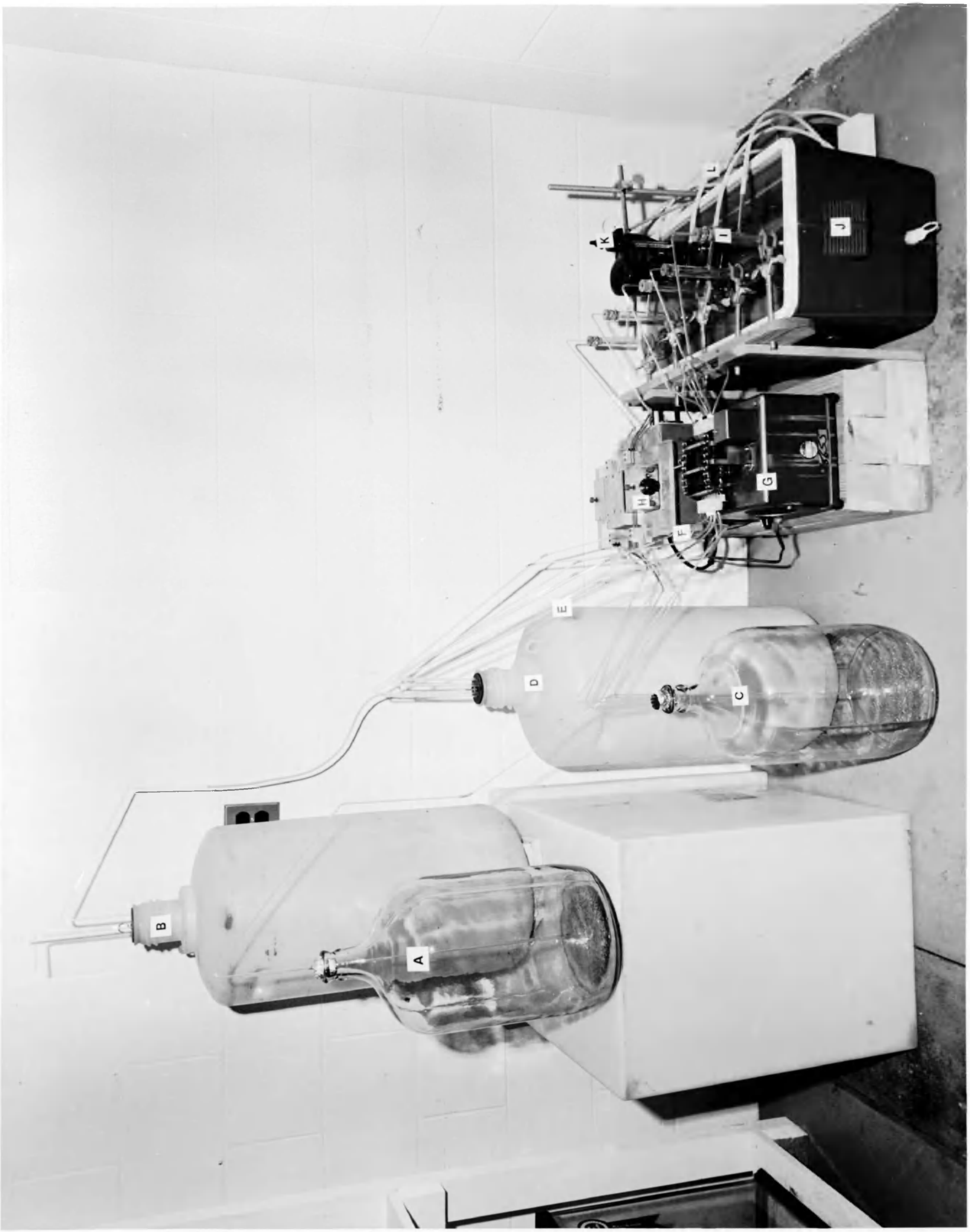
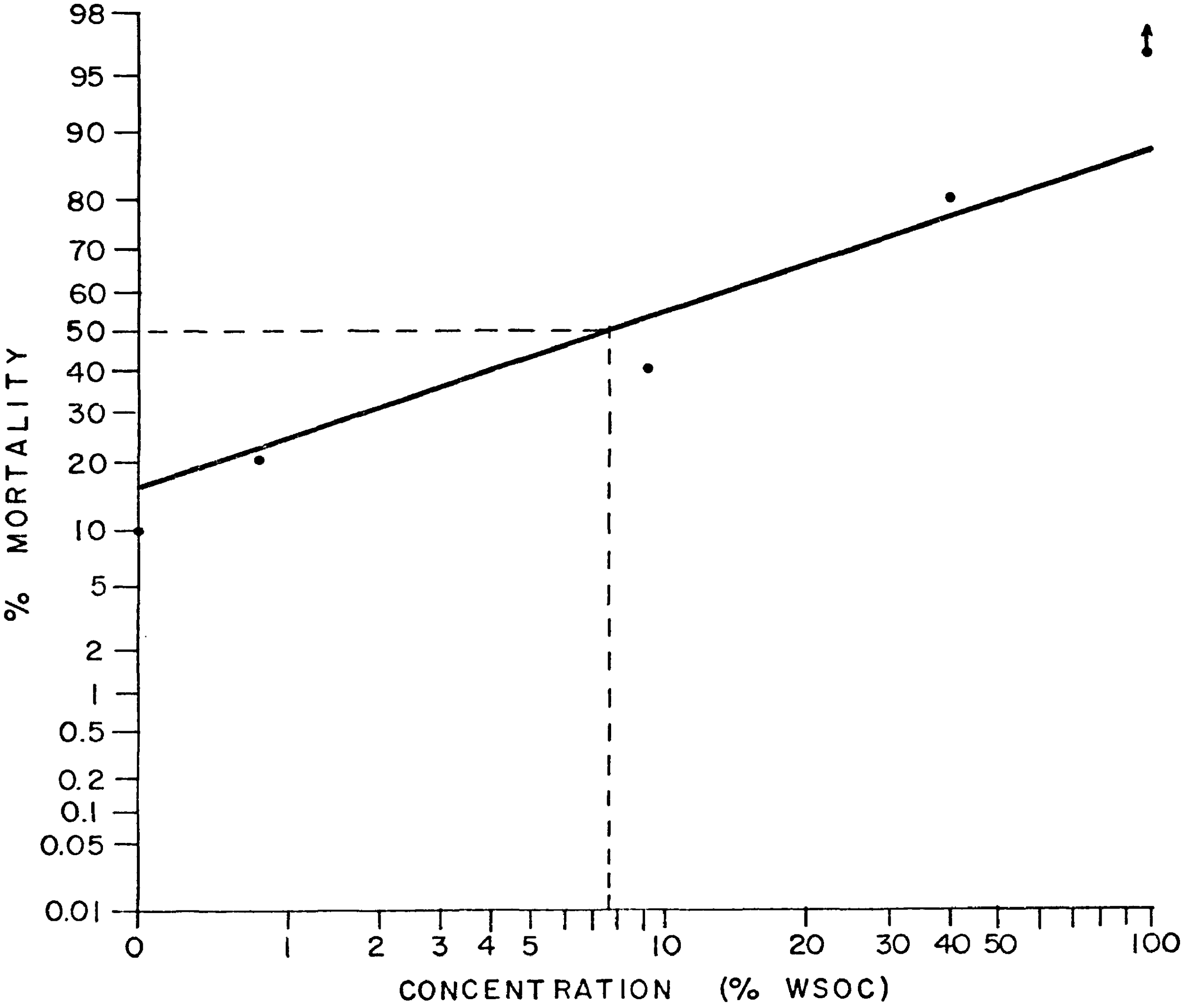

A
B
C
D
E
F
G
H
I
J
K
L

FIGURE 3

Relation between percentage deaths in 48 hours and WSOC concentration for _Gammarus_ _mucronatus_, (replicate 1).



98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
% MORTALITY
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 4

Relation between percentage deaths in 48 hours and WSOC concentration for *Gammarus mucronatus*, (replicate 2).

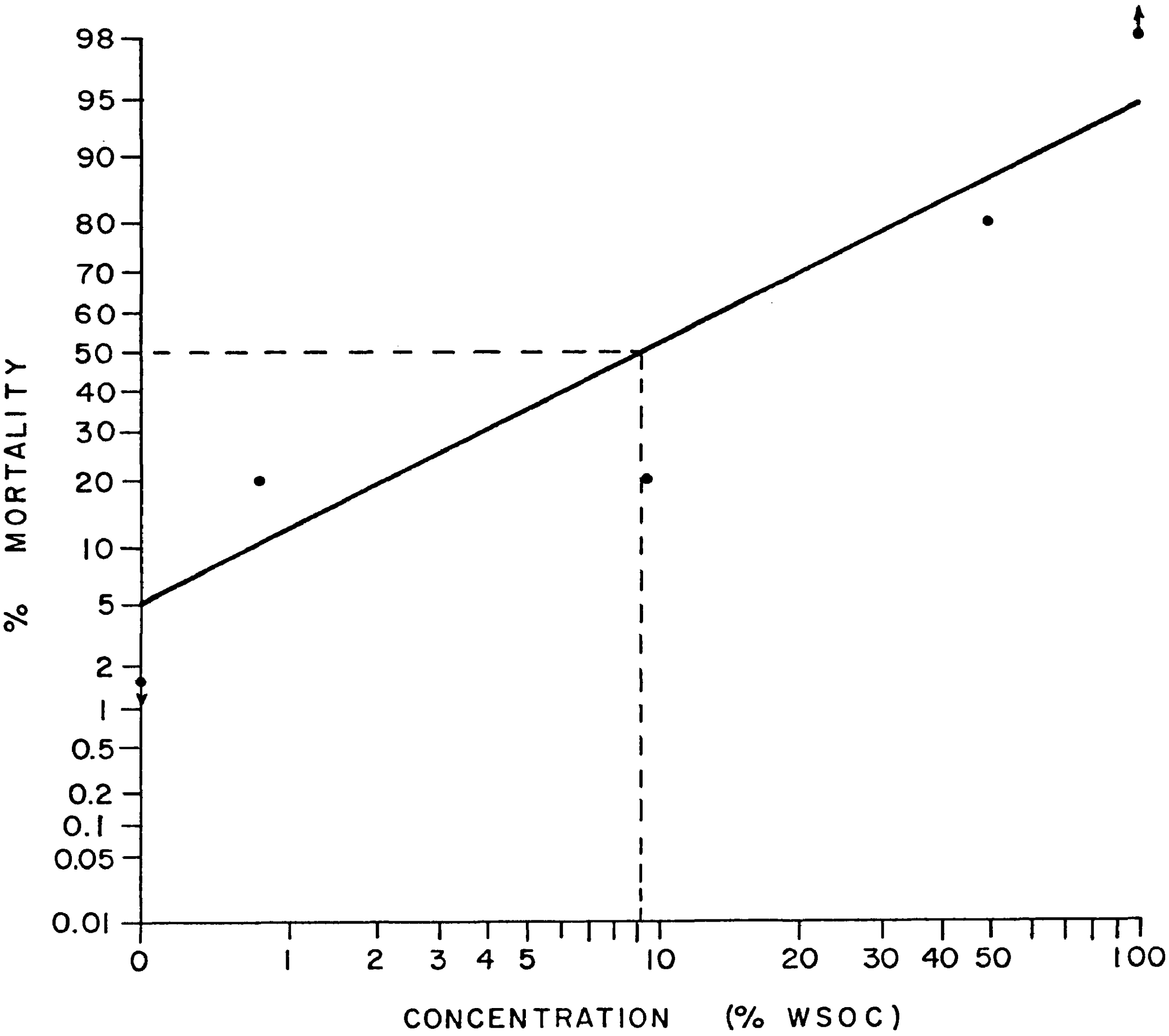
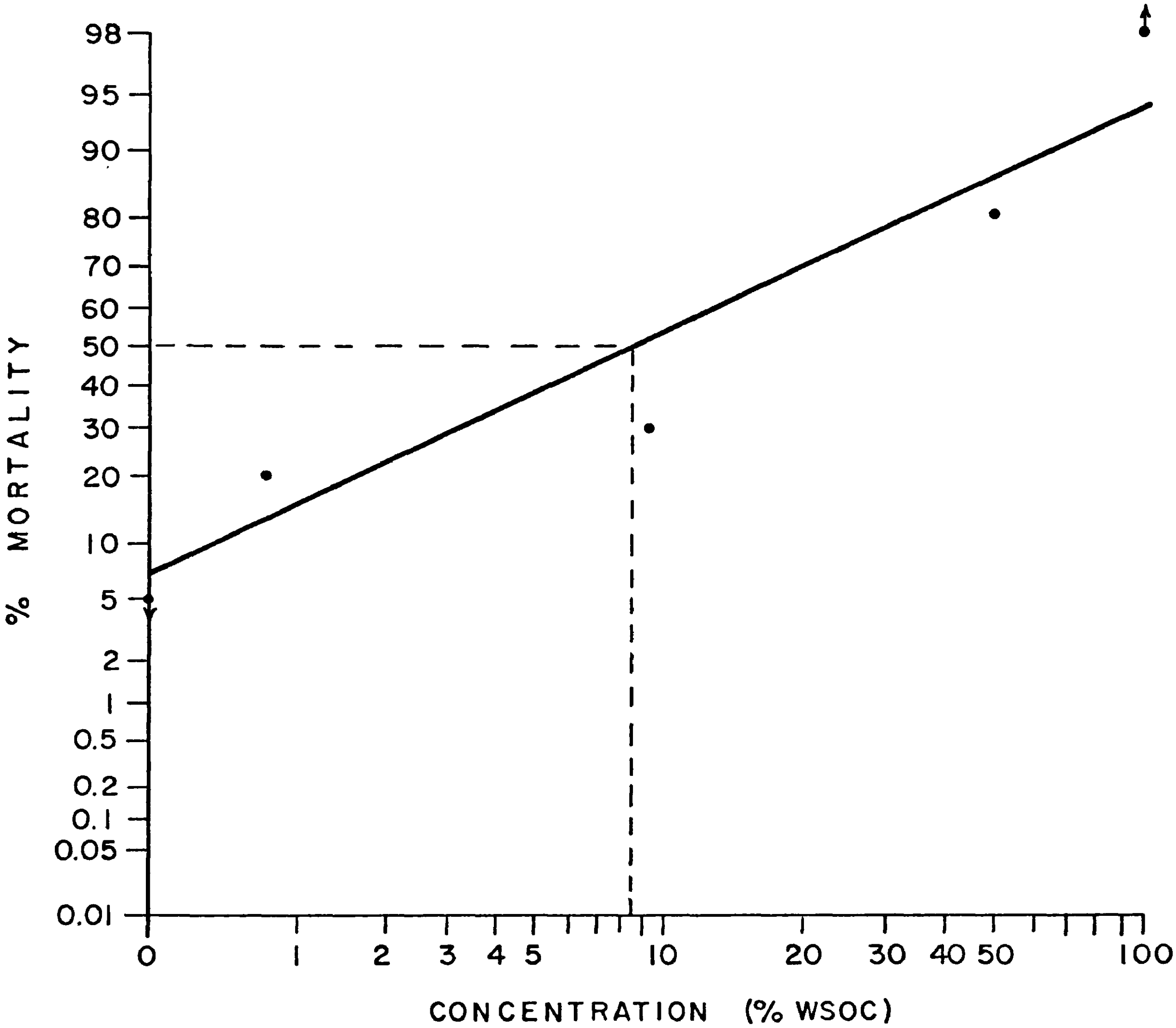

98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
% MORTALITY
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 5

Relation between percentage deaths in 48 hours and WSOC concentration for Gammarus mucronatus, (pooled observations).



98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
% MORTALITY
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 6

Relation between percentage deaths in 48 hours and WSOC concentration for *Pagurus longicarpus*, (replicate 1).

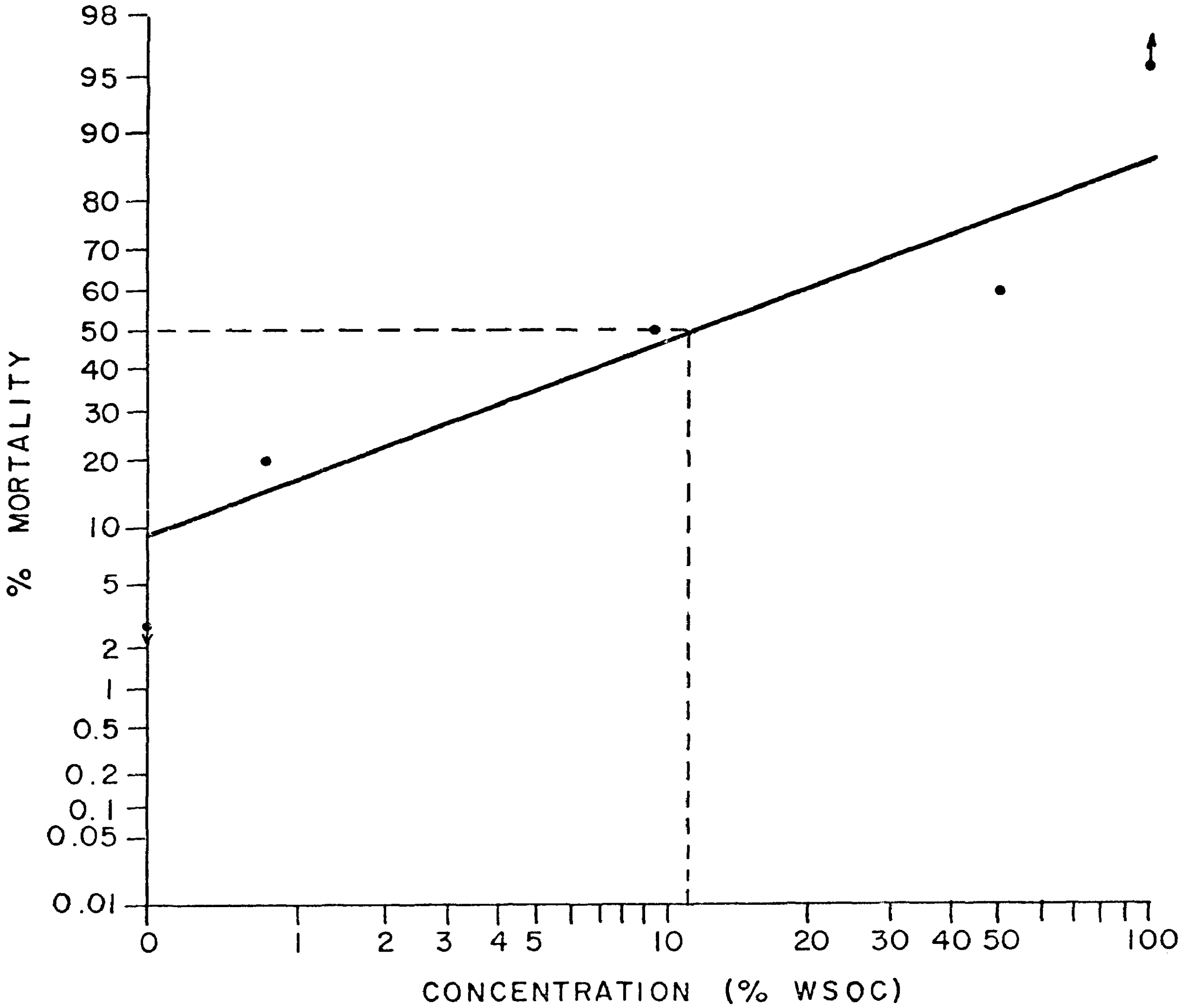
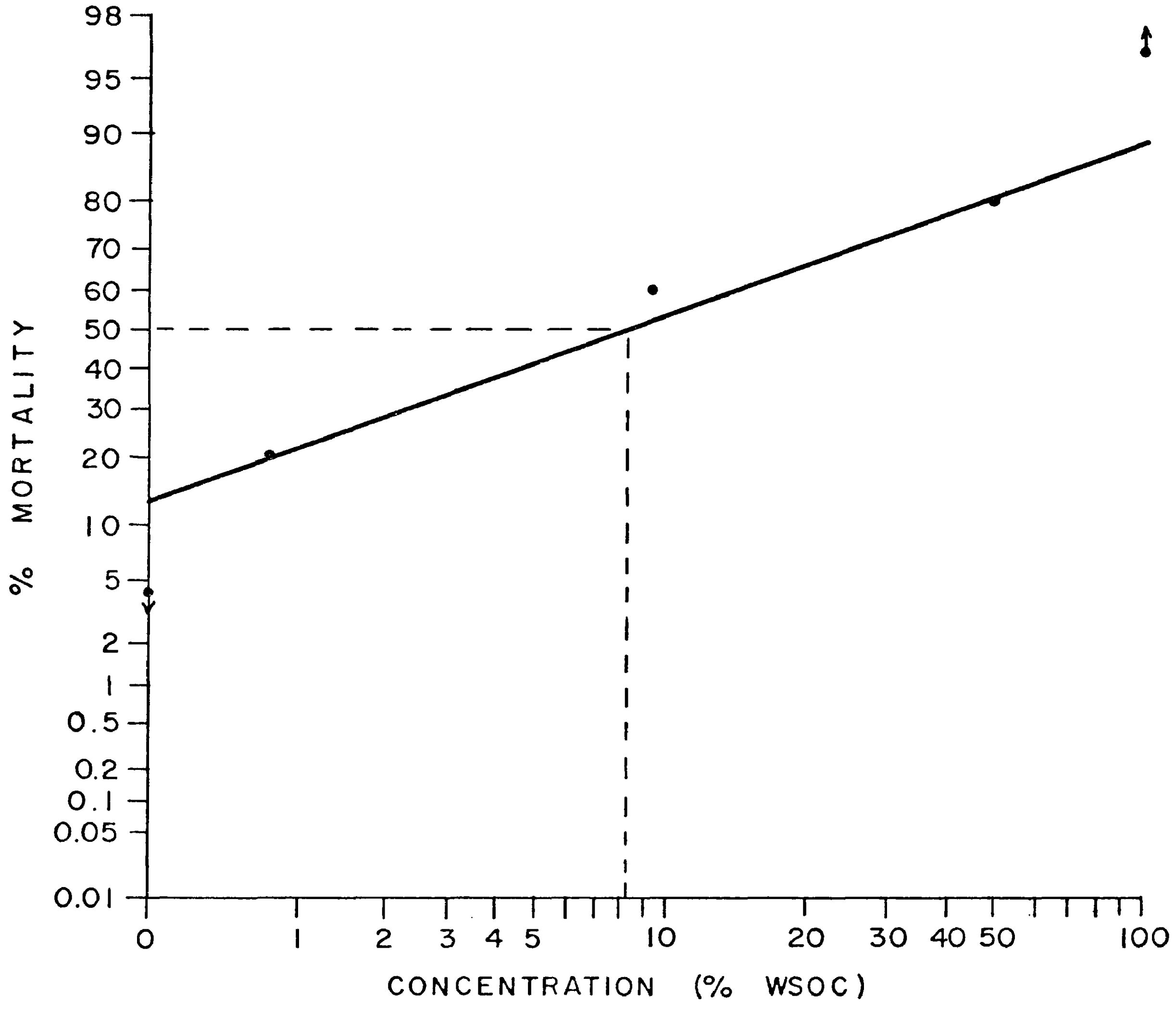

98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
% MORTALITY
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 7

Relation between percentage deaths in 48 hours and WSOC concentration for *Pagurus longicarpus*, (replicate 2).



98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
% MORTALITY
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 8

Relation between percentage deaths in 48 hours and WSOC concentration for *Pagurus longicarpus*, (pooled observations).

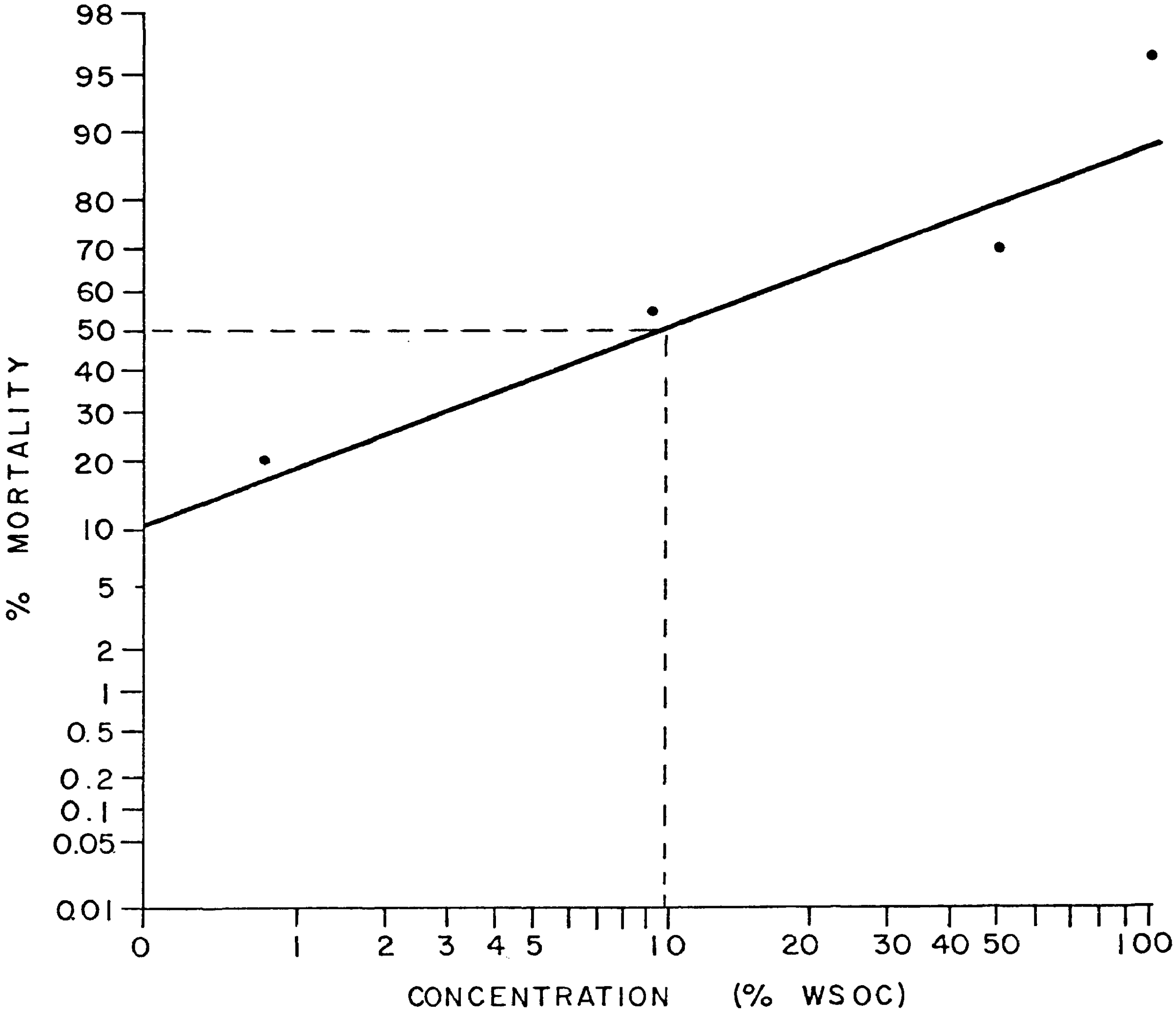
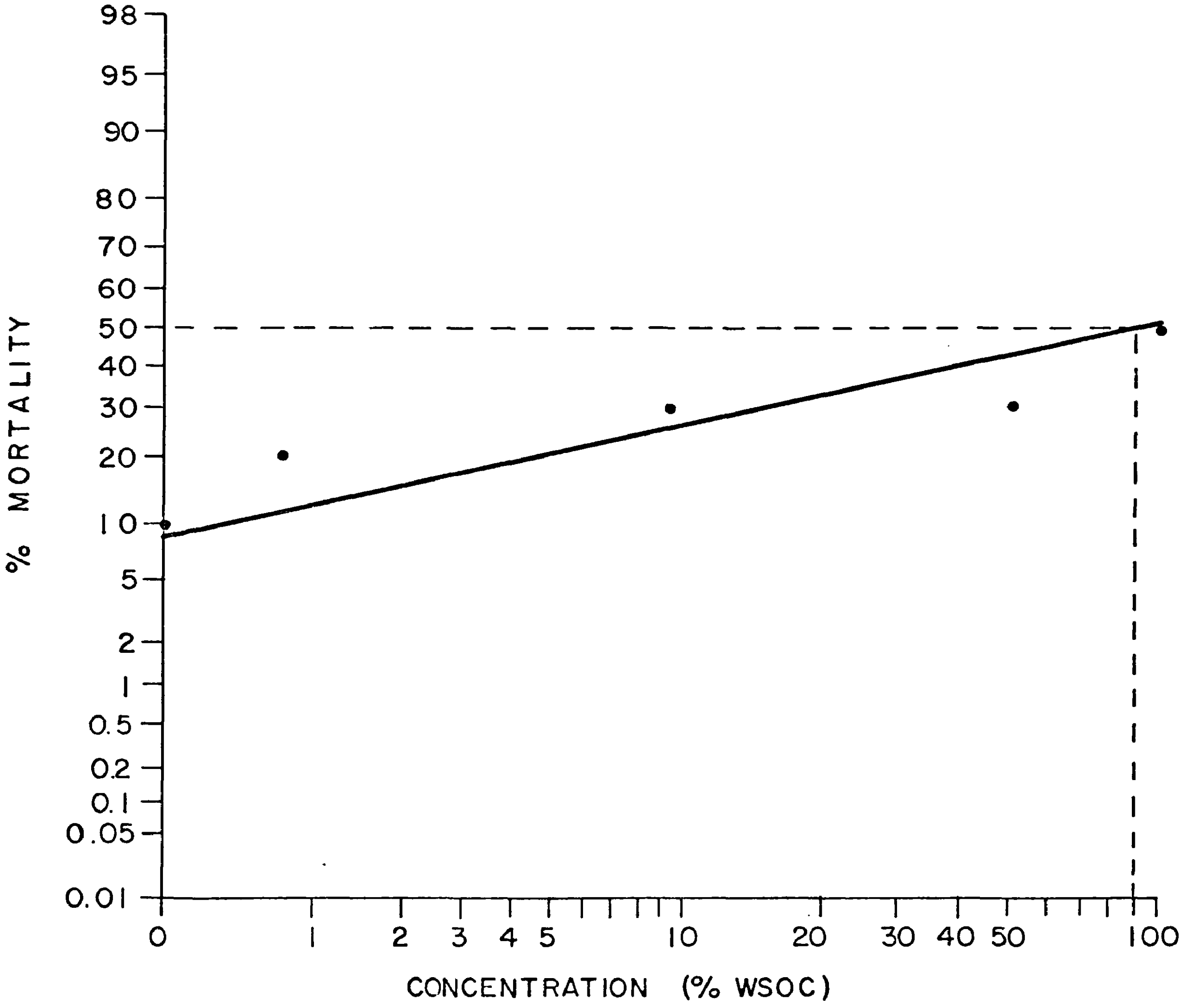

98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
% MORTALITY
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 9

Relation between percentage deaths in 48 hours and WSOC concentraton for *Spiochaetopterus costarum oculatus*, (replicate 1).



% MORTALITY
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 10

Relation between percentage deaths in 48 hours and WSOC concentration for *Spiochaetopterus costarum oculatus*, (replicate 2).

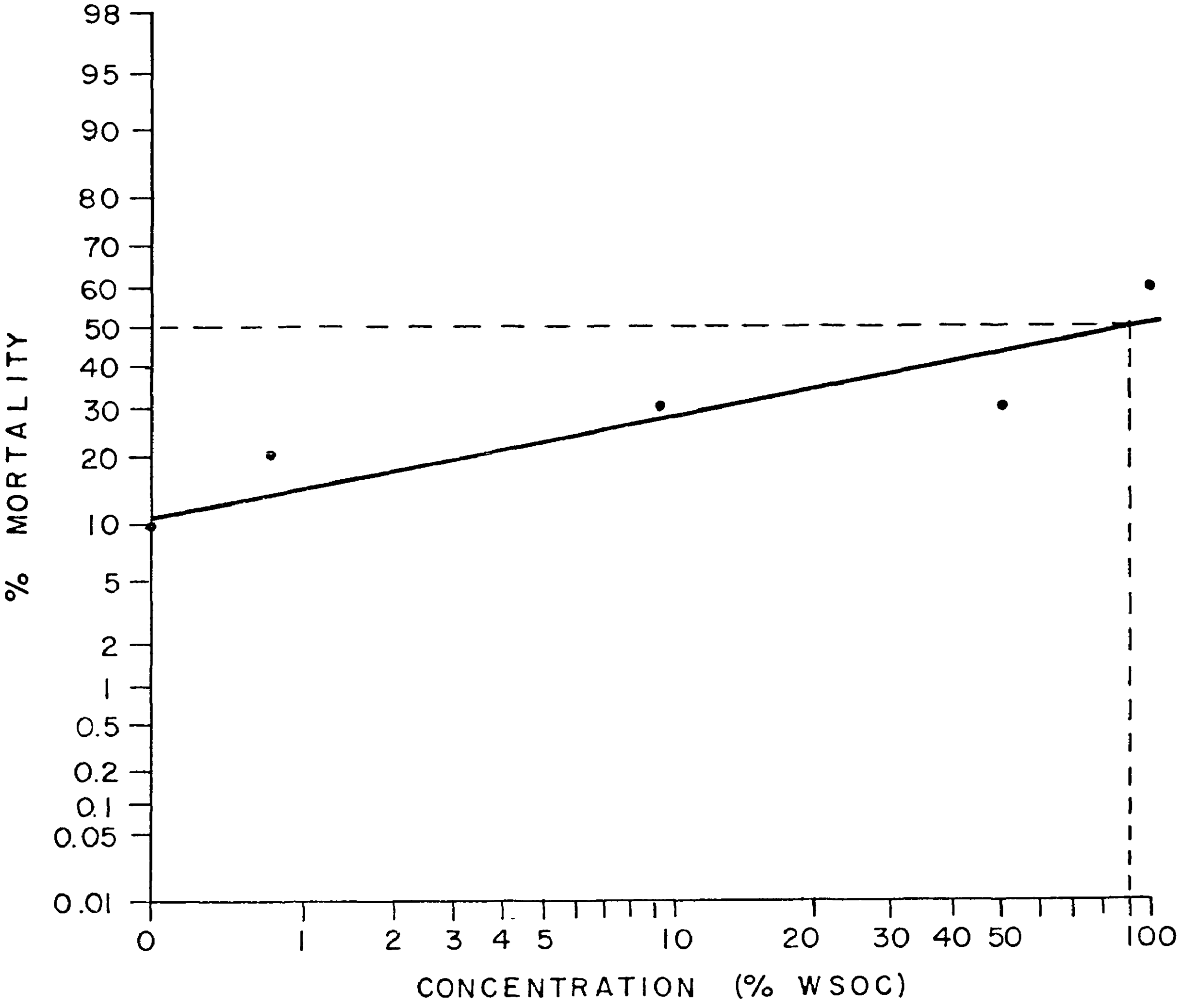

98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
0.05
0.01
% MORTALITY
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 11

Relation between percentage deaths in 48 hours and WSOC concentration for *Spiochaetopterus costarum oculatus*, (pooled observations).

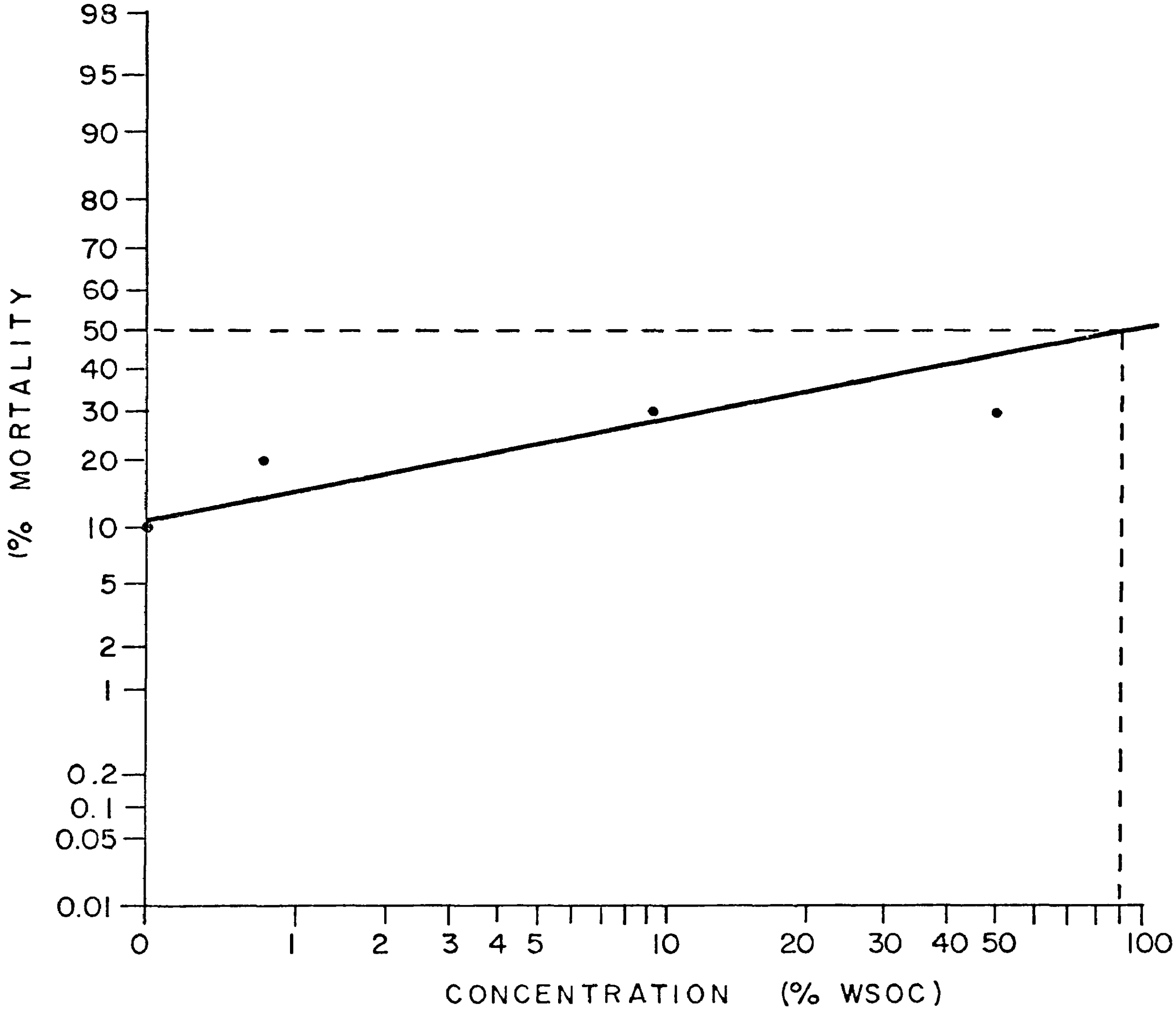

98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.2
0.1
0.05
0.01
(% MORTALITY
0
1
2
3
4
5
10
20
30
40
50
100
CONCENTRATION (% WSOC)

FIGURE 12

Relation between percentage deaths in 48 hours and WSOC concentration for Nassarius obsoletus, (replicates 1 and 2).

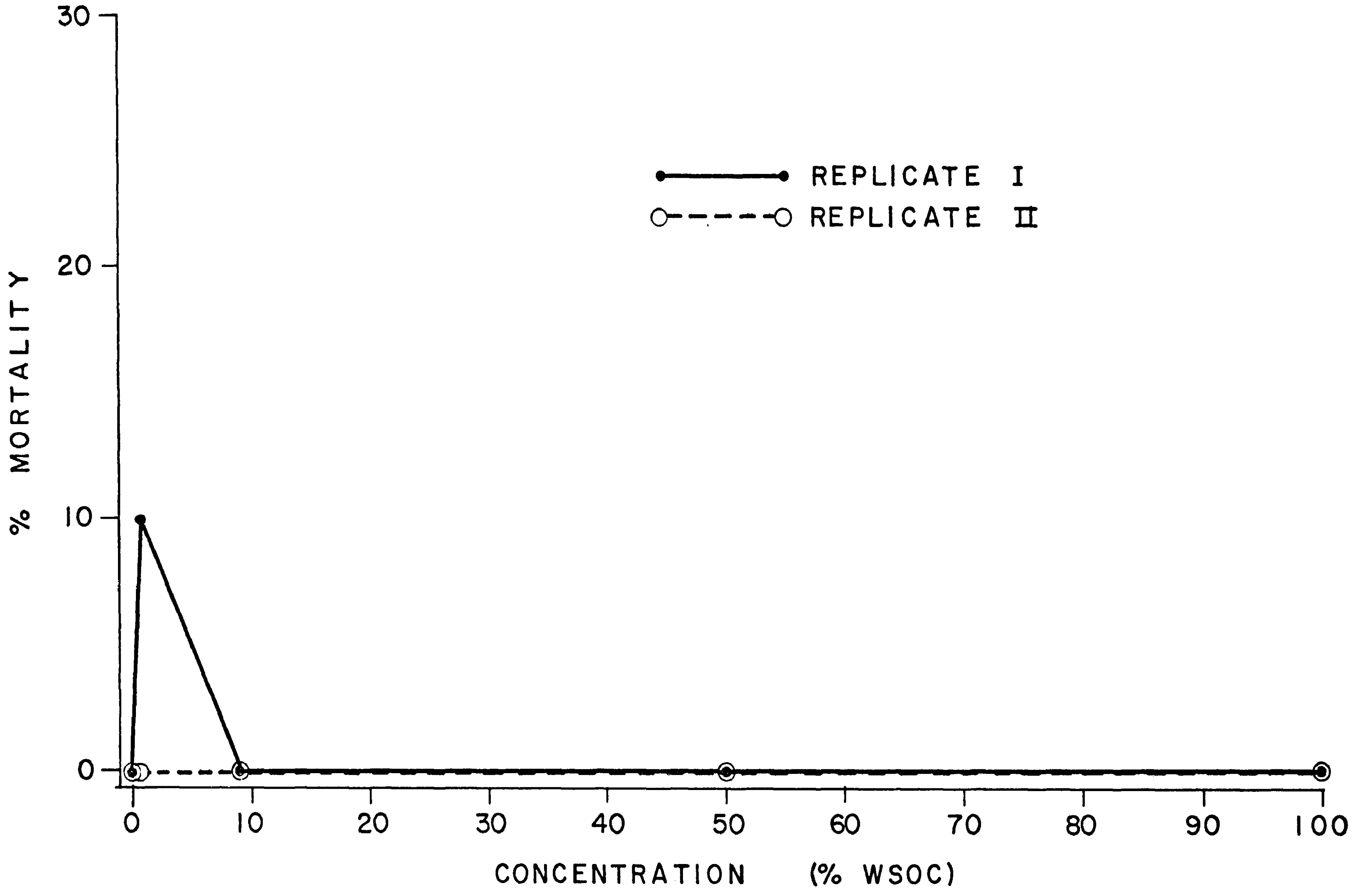

REPLICATE I
REPLICATE II
% MORTALITY
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
CONCENTRATION (% WSOC)

FIGURE 13

Relation between percentage deaths in 48 hours and WSOC concentration for *Edotea triloba*, (replicates 1 and 2).

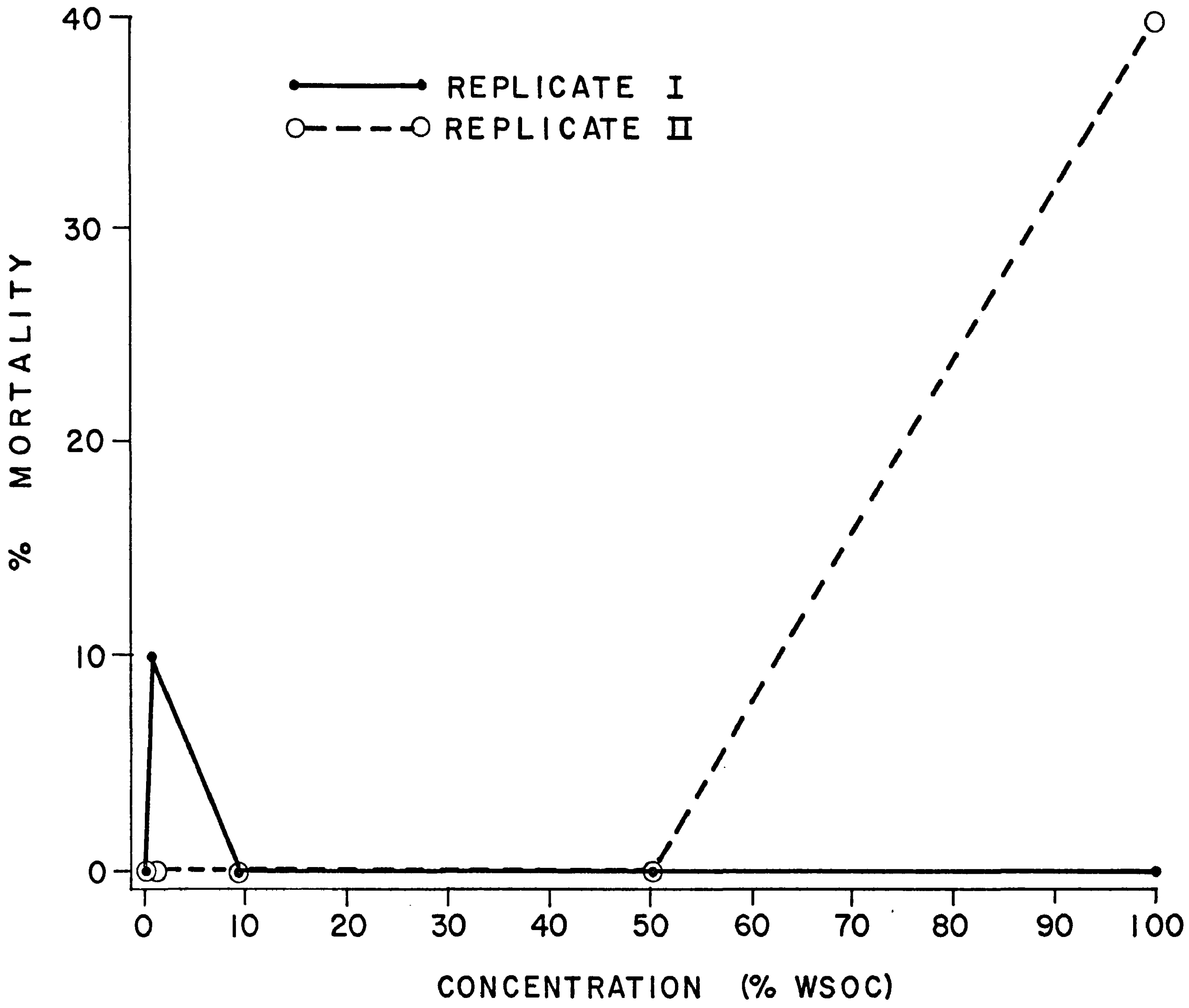

REPLICATE I
REPLICATE II
% MORTALITY
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
CONCENTRATION (% WSOC)

FIGURE 14

Relation between percentage deaths in 48 hours and WSOC concentration for *Nereis succinea*, (replicates 1 and 2).

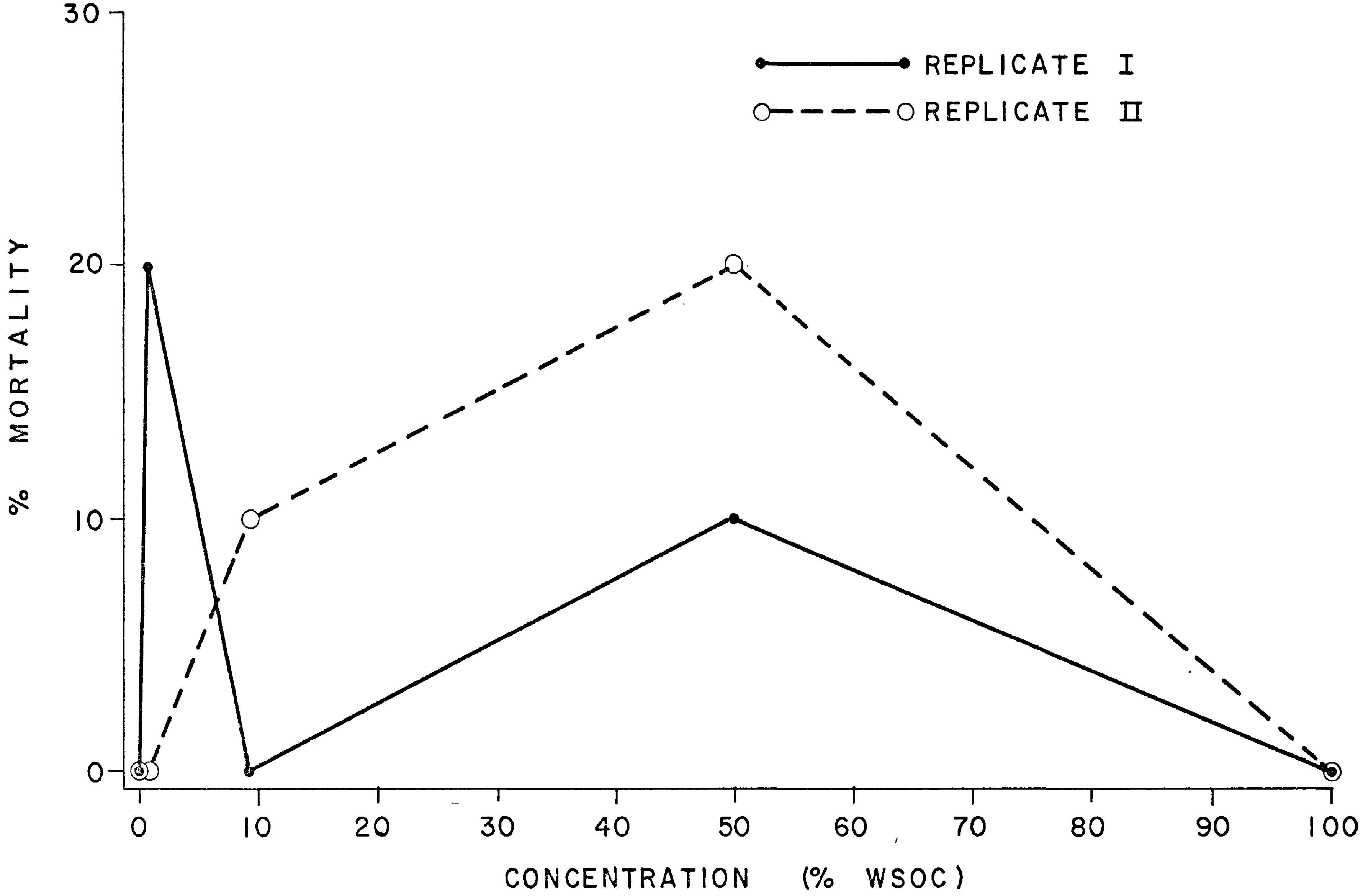
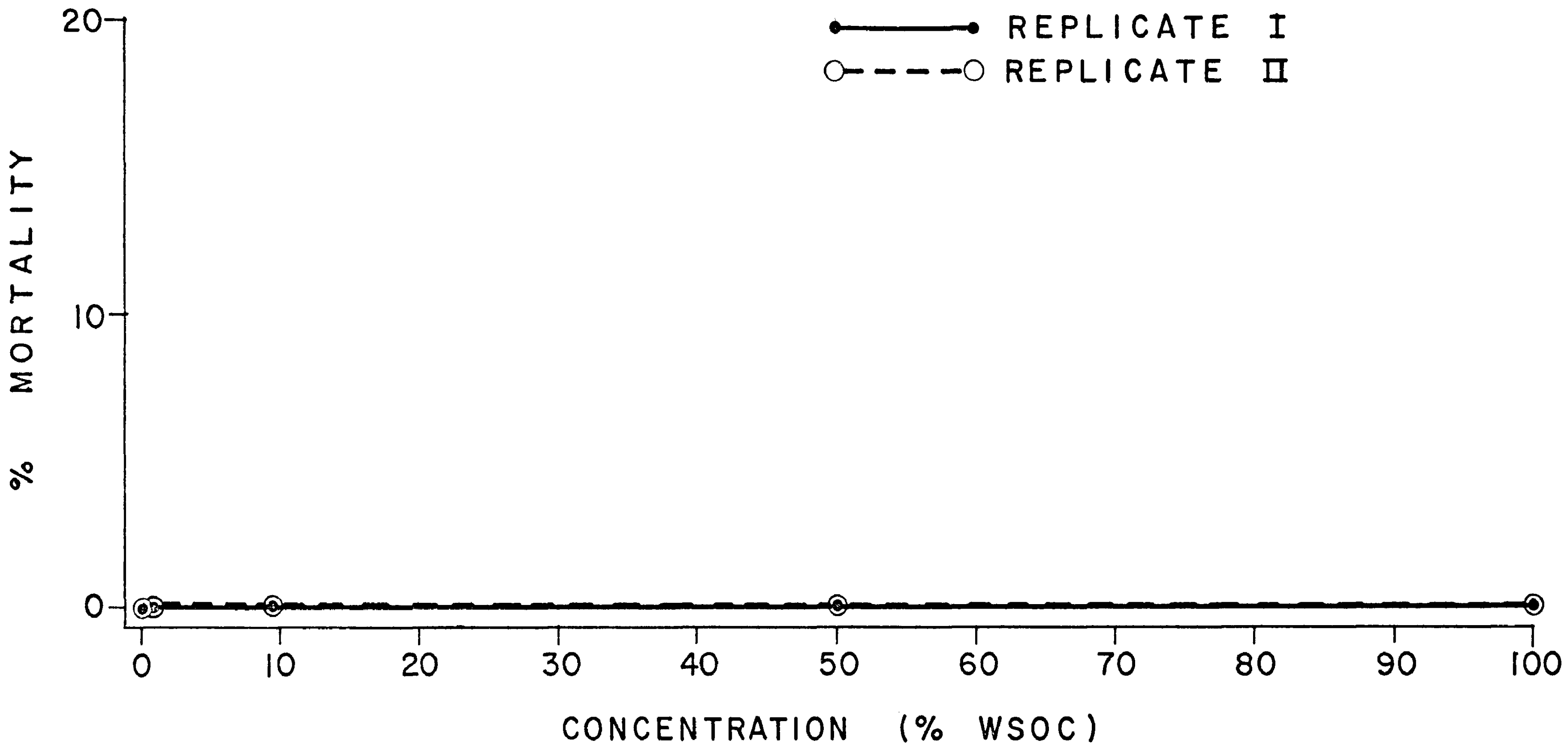

REPLICATE I
REPLICATE II
% MORTALITY
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
CONCENTRATION (% WSOC)

FIGURE 15

Relation between percentage deaths in 48 hours and WSOC concentration for *Modiolus demissus*, (replicates 1 and 2).


REPLICATE I
REPLICATE II
% MORTALITY
20
10
0
0
10
20
30
40
50
60
70
80
90
100
CONCENTRATION (% WSOC)

VITA

Jeffrey Lynn Hyland

Born in Alexandria, Virginia, April 13, 1949. Graduated from Thomas Jefferson High School, Alexandria, Virginia, June, 1967. Received B. S. in Biology from University of Miami, Coral Gables, Florida, June, 1971. Enrolled as a graduate student in the Department of Marine Science, College of William and Mary, September, 1971. Research assistant in the Ecology-Pollution Department, Virginia Institute of Marine Science.