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Acute Lethal Toxicity of Prudhoe Bay Crude Oil and Corexit 9527 to Arctic Marine Fish and Invertebrates

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ACUTE LETHAL TOXICITY OF PRUDHOE BAY CRUDE OIL AND COREXIT 9527 TO ARCTIC MARINE FISH AND INVERTEBRATES

by

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for the

Environmental Emergency Branch
Environmental Impact Control Directorate
Environmental Protection Service
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ABSTRACT

The toxicities of Prudhoe Bay crude oil, the dispersant Corexit 9527 and mixtures of these, to several arctic marine amphipods, one arctic marine copepod and one arctic marine fish were investigated. Toxicities were evaluated in semi-static 96 h bioassays in which exposure concentrations of hydrocarbons were measured by fluorescence spectroscopy. Mortality results were analyzed by probit analysis to determine the concentration which would be expected to cause 50 percent mortality in 96 hours (96 h LC_{50}). In addition, the toxicity of a reference toxicant, sodium lauryl sulphate, was determined.

Generally, sensitivities of all species were remarkably similar for any one toxicant. In instances where they could be determined, 96 h LC_{50} values ranged from 28 to 66 ppm (mg/L) sodium lauryl sulphate (SLS); from 104 to 175 ppm (µL/L) Corexit 9527 concentrate; from 32 to 73 ppm (µL/L) Prudhoe Bay crude oil dispersed mechanically; and from 45 to 196 ppm (µL/L) Prudhoe Bay crude oil dispersed with Corexit 9527. The LC_{50} values for "young-of-the-year" sculpins in SLS and Corexit, although not precisely determinable, were lower than the above values.

Mortality in oil-Corexit-water mixtures was much higher than in oil-water mixtures of the same nominal oil concentration. Measured oil concentrations in the water column, however, were much higher in mixtures dispersed with Corexit than in mechanically dispersed mixtures when the same amounts of oil and water were mixed initially. Therefore, the higher mortality observed in the oil-Corexit-water mixtures, relative to the oil-water mixtures, is thought to reflect the higher concentrations of oil to which the organisms were exposed.

Based on measured hydrocarbon concentrations in the water column (as determined by fluorescence spectroscopy), the toxicity of the oil-Corexit-water mixtures was less than that found for the oil-water mixtures. This is thought to be a result of the smaller ratio of dissolved hydrocarbons (including toxic water-soluble aromatics) to total measured hydrocarbons in the oil-Corexit-water mixtures, than that in the oil-water mixtures.

RÉSUMÉ

On a étudié la toxicité du pétrole brut de la baie Prudhoe, du dispersant Corexit 9527 et de mélanges de ces substances pour plusieurs amphipodes marins arctiques, un copépode marin arctique et un poisson marin arctique. La toxicité a été évaluée par des essais biologiques de 96 heures effectués dans des conditions semi-statistiques au cours desquelles la concentration d'exposition aux hydrocarbures a été mesurée par spectroscopie par fluorescence. Les résultats relatifs aux taux de mortalité ont été traités par analyse probit pour déterminer la concentration susceptible de causer 50 p. 100 de mortalité en 96 heures (CL_{50} de 96 heures). De plus, on a déterminé la toxicité d'un produit toxique de référence, soit le lauryl-sulfate de sodium.

En général, les sensibilités de toutes les espèces étaient remarquablement semblables pour n'importe laquelle des substances toxiques étudiées. Lorsqu'il a été possible de les déterminer, les valeurs de la CL_{50} de 96 heures ont varié de 28 p.p.m. à 66 p.p.m. (mg/l) pour le lauryl-sulfate de sodium (LSS); de 104 p.p.m. à 175 p.p.m. (µl/l) pour le concentré de Corexit 9527; de 32 p.p.m. à 73 p.p.m. (µl/l) pour le pétrole brut de la baie Prudhoe dispersé mécaniquement; et de 45 p.p.m. à 196 p.p.m (µl/l) pour le pétrole brut de la baie Prudhoe dispersé chimiquement avec du Corexit 9527. La CL_{50} du LSS et du Corexit pour des chabots âgés de moins d'un an, bien que non déterminables d'une façon précise, était inférieure aux valeurs précédentes.

Le taux de mortalité dans des mélanges pétrole-Corexit-eau était supérieur à celui obtenu dans des mélanges pétrole-eau pour la même concentration nominale de pétrole. Cependant, la concentration mesurée de pétrole dans la colonne d'eau était beaucoup plus élevée dans les mélanges dispersés avec du Corexit que dans les mélanges dispersés mécaniquement lorsqu'on mélangeait au départ des quantités égales de pétrole et d'eau. On estime donc que le taux de mortalité supérieur observé avec des mélanges pétrole-Corexit-eau par rapport à celui avec des mélanges pétrole-eau reflète la concentration plus élevée de pétrole à laquelle les organismes étaient exposés.

Si l'on se base sur la concentration mesurée d'hydrocarbures dans la colonne d'eau (déterminée par spectroscopie par fluorescence) on constate que la toxicité des mélanges pétrole-Corexit-eau était inférieure à celle des mélanges pétrole-eau. Cette différence de toxicité proviendrait du rapport plus faible entre les hydrocarbures dissous (comprenant les composés aromatiques toxiques hydrosolubles) et l'ensemble des hydrocarbures dosés dans les mélanges pétrole-Corexit-eau que dans des mélanges pétrole-eau.

FOREWORD

In 1977, as part of the Arctic Marine Oilspill Programme (AMOP), LGL Limited was contracted by the Environmental Protection Service (EPS) to determine the acute lethal toxicity of crude oil (Prudhoe Bay crude oil), dispersant (Corexit 9527) and crude oil-dispersant mixtures to a variety of arctic marine invertebrates and fish. Due to difficulties anticipated in transporting test organisms to, and maintaining them in a southern laboratory, it was felt that the quantity and quality of results could be maximized by conducting the study in close proximity to the collection sites. As a result, the study was performed in Resolute Bay, NWT, during the winter of 1977-78 and the results were reported to EPS in the spring of 1978.

A second study was planned with three objectives: (1) verifying and refining results of the first study; (2) increasing the number of test species; and (3) identifying (possibly) any seasonal or geographical variations in sensitivity of selected test organisms. This was carried out in Frobisher Bay, NWT, during the summer of 1978, and the results were reported to EPS in the spring of 1979.

The present report synthesizes the results of both studies. In doing so, much of the raw data have been omitted. These data are available for inspection at the Environmental Emergency Branch, EPS, Ottawa.

ACKNOWLEDGEMENTS

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PRINCIPAL FINDINGS

The toxicities of four toxicant mixtures (sodium lauryl sulphate, Corexit 9527 concentrate, Prudhoe Bay crude oil and Prudhoe Bay crude oil dispersed with Corexit 9527) were determined for seven species of arctic amphipods (Anonyx laticoxae, Anonyx nugax, Boeckosimus edwardsi, Boeckosimus sp., Gammarus oceanicus, Gammarus setosus and Onisimus litoralis); one species of copepod (Calanus hyperboreus); and one species of fish (Myoxocephalus quadricornis). Tests were run as 96 h LC_{50} semi-static bioassays in which the animals were placed in freshly prepared toxicant mixtures every 24 hours. Concentrations of Prudhoe Bay crude oil used to determine 96 h LC_{50} values, were concentrations in the toxicant mixtures measured using fluorescence spectroscopy. Concentrations of sodium lauryl sulphate and Corexit 9527 used to determine 96 h LC_{50} values, are reported as the nominal concentrations (expected concentration if all the added substance is in true solution).

The principal findings were as follows:

1. The large amphipod, Anonyx nugax, and "young-of-the-year" fourhorn sculpins (Myoxocephalus quadricornis) were the species most sensitive to all four toxicant mixtures. Anonyx nugax was more sensitive to oil and oil-Corexit mixtures than was Myoxocephalus quadricornis but less sensitive to the detergents, sodium lauryl sulphate and Corexit 9527.
2. With the exception of Myoxocephalus quadricornis in sodium lauryl sulphate (96 h LC_{50} = 3-9 ppm) and, possibly, in Corexit 9527 (96 h LC_{50} <40 ppm), sensitivities of all species were remarkably similar for any one toxicant. In instances where they could be determined, 96 h LC_{50} values ranged from 28 to 66 ppm (mg/L) sodium lauryl sulphate; from 104 to 175 ppm (µL/L) Corexit 9527; from 32 to 73 ppm (µL/L) Prudhoe Bay crude oil dispersed mechanically; and from 45 to 196 ppm (µL/L) Prudhoe Bay crude oil dispersed with Corexit 9527.
3. The addition of Corexit 9527 to Prudhoe Bay crude oil (1:10, vol:vol) increased the measured exposure concentrations (concentration determined by actual measurement) of hydrocarbons in test mixtures by as much as seven times over those of identical test mixtures dispersed solely by mechanical means.
4. For the same nominal oil concentration, mortality was much higher in the Corexit-dispersed oil mixtures, than in the mechanically dispersed oil

mixtures. This is thought to be the result of far greater hydrocarbon concentrations in Corexit-dispersed oil mixtures than in mechanically dispersed oil mixtures.

5. Using measured exposure concentrations of oil in calculating 96 h LC_{50} values, Corexit-dispersed oil mixtures appeared to be **less** toxic than mechanically dispersed mixtures.

6. On the basis of gas chromatography results it would appear that the large increase in hydrocarbons in Corexit-dispersed oil-water mixtures over mechanically dispersed oil-water mixtures is due primarily to a large increase in the particulate oil fraction. Concentrations of dissolved hydrocarbons do not increase to nearly the same degree when Corexit is added.

7. Of the chemical components of oil, the water soluble aromatics are generally considered to be the most toxic, partially because being dissolved in the water phase, they are easily incorporated by aquatic organisms. It is felt that the lower proportion of these compounds (water soluble aromatics: total hydrocarbons) in oil-Corexit-water mixtures as opposed to oil-water mixtures, resulted in lower toxicities for the oil-Corexit-water mixtures than for the oil-water mixtures, when based on total measured hydrocarbons.

1 INTRODUCTION

Development, production and transportation of petroleum resources in the Canadian Arctic will almost certainly result in accidental spillages of petroleum hydrocarbons into the marine environment. High priority must be given to the development of technology to minimize such accidents. However, we must also be prepared to deal with large-scale hydrocarbon contamination resulting from imperfections in technology or from human error.

The costs of oil spill cleanup in temperate latitudes are enormous. Because of remoteness and inhospitable climatic conditions, costs of cleanup in arctic regions can be expected to be even greater. In all likelihood, the question will not be one of how to remove the contaminant completely, but one of which habitats and geographical areas have priority in cleanup and protection. The identification of priority areas or habitats must be based on the sensitivity and vulnerability of those areas to hydrocarbon contamination.

To identify ecologically sensitive areas, one must have a sound knowledge of the individual components of the community (e.g. their life histories, physiology, distribution, etc.) and of the interaction among individual components of the community. In addition, it is important to know how and to what degree individuals and, ultimately, the community react to the presence of hydrocarbons.

While there is voluminous literature on the effects of crude oil and refined petroleum products on temperate marine organisms, little such information is available on arctic marine organisms. The only reports of such investigations in the Canadian Arctic are those of Percy (1977) and Percy and Mullin (1975, 1977) who based their work on invertebrates from the Beaufort Sea area. In determining concentrations of crude oil (alone and in combination with a chemical dispersant) that are lethally toxic to individual species, the work reported herein is a first step in filling this information gap.

It must be emphasized, however, that acute toxicity tests are useful only if their limitations are recognized. Their main values lie in establishing relative sensitivities of different species and life stages, and in determining the concentrations of contaminants to be used in subsequent studies of sublethal effects. It is not appropriate to use the results of these studies to predict the effects of contamination in a natural situation or to use 96 h LC_{50} values to derive meaningful water quality criteria.

1.1 Chemical Dispersion of Oil in Arctic Waters

Cleanup of oil in temperate latitudes has proven to be an expensive undertaking. Cleanup under arctic conditions, because of the logistic difficulties, can be expected to be even more expensive. Coupled with this is an inadequate technology for dealing with oil spilled in a cold-water and ice-dominated environment. It is anticipated that only a partial cleanup of oil or protection of some priority ecological or geographical areas would be attempted. The characteristics of such areas would determine, to some extent, the cleanup methods used in the event of a spill.

One method used in dealing with oil spills is the application of oil dispersants before the slick reaches nearshore regions. Ever since the 'Torrey Canyon' spill, the use of dispersants has been a controversial subject; much of the mortality observed after that spill was attributable to the highly toxic dispersants used in cleanup operations (Smith, 1968). Since that time, 'second generation' dispersants, which are much lower in inherent toxicity, have been developed. The acute toxicity of Corexit 9527, used in the present study, was comparatively low and direct mortality from concentrations used to disperse oil in a spill situation would be expected to be low. The toxicity of dispersants themselves may no longer be a strong argument against using them.

The use of dispersants on oil spilled in arctic waters might be indicated in some instances. Birds have been the most obvious and immediate victims of surface oil spills in waters of other latitudes. Holmes and Cronshaw (1977) state that between 1955 and 1972, one million bird deaths were recorded following oil spills. Nettleship (1977) states that 'pollution of the seas by oil poses the largest single threat to seabirds'. The large populations of seabirds that inhabit some areas of the Arctic, such as Lancaster Sound, and the leads in the Beaufort Sea in spring, could be severely affected by a large spill of oil. Rapid use of dispersants to break up and emulsify the surface slick might reduce bird mortality in such a case.

Dispersal of oil in offshore areas may also, in some cases, seem preferable to contamination of the shoreline and near-shore waters. In arctic regions, littoral areas and shallow water regions are sometimes important as feeding areas for vertebrates. Several of the amphipod species (i.e. Gammarus setosus, Gammarus oceanicus, Onisimus litoralis) tested in the present study are found in large numbers in the intertidal zone and have been identified as important food items for a number of fish, birds and, to a lesser degree, marine mammals. The greatest damage to these communities may not be caused by direct lethal toxicity of the petroleum hydrocarbons. In many cases, oil slicks that reach the shoreline will have been weathered and will have lost a large proportion of their most

toxic components as a result of several processes, including dissolution and evaporation (Clark and MacLeod, 1977). However, oil that reaches the shoreline in arctic areas may be persistent. Owens (1978) states that a heavy oil, stranded on a low-energy shoreline in arctic regions, may be expected to last for decades with little loss of volume. The alteration of habitat may result in desertion of the area by its residents or in a gradual decline in numbers of the inhabitant populations as a result of damage caused by chronic exposure to sublethal levels of hydrocarbons. The ultimate consequence is a disruption of the food chain.

The use of chemicals to disperse petroleum hydrocarbons in offshore waters does present some hazards, however. The results detailed in the present report, and the results of other toxicity studies in which exposure concentrations of hydrocarbons were measured, indicate that the use of a 'second generation' dispersant does not increase the toxicity of a crude oil. The use of a dispersant does, however, greatly increase the amount of oil in the water column, and thus its availability to aquatic organisms. If left as a slick on the water surface, a significant proportion of the volatile and most toxic components would normally be lost in a short time as a result of evaporation. Virtually all hydrocarbons smaller than C_{15} can evaporate from a sea surface within 10 days and the lightest compounds can completely volatilize within hours (Clark and MacLeod, 1977). The rate of evaporation varies with conditions such as the type of petroleum, wave state, wind speed and water temperature (Clark and MacLeod, 1977). If the oil is dispersed, a greater quantity of the more toxic compounds becomes dissolved in the water column. In the dissolved form, these compounds are easily incorporated by aquatic organisms.

After a large spill of petroleum hydrocarbons, one would expect some initial and localized mortality of planktonic organisms in the area of the spill. However, because of natural variability in planktonic populations and the dynamic nature of the aquatic environment, in which concentrations of pollutants and populations of planktonic organisms are constantly changing in time and space, it is extremely difficult to quantify the impact.

Some proportion of petroleum hydrocarbons dispersed in offshore waters will eventually reach the bottom sediments. As the lighter fractions of the petroleum dissolve or evaporate, the specific gravity of the oil increases. Dispersed particles may agglomerate, and absorption or adsorption of oil by particulate matter may occur. The result is a net downward movement of the oil (Clark and MacLeod, 1977; Karrick, 1977). The effect of such contamination on the sublittoral benthos is not well known. Possible

effects are direct mortality, sublethal damage and mechanical injury caused by coating. Sanborn (1977) has speculated that anoxia may result in some areas because of the high biochemical oxygen demand of oil. In any case, the ultimate consequences of such pollution, although not likely to be immediately obvious, may be significant, nonetheless.

Thus, despite the low toxicity of recently developed dispersants, the consequences of using them are still uncertain. In arctic regions, for which an understanding of basic biological relationships is often inadequate, the problem is compounded.

2 MATERIALS AND METHODS

2.1 Study Areas

Study 1 was performed in Resolute Bay, NWT, between the 28 November, 1977 and the 24 January, 1978. Animals were collected through the ice from two locations, Resolute Bay and near Cape Martyr in Resolute Passage (see Figure 1). Site 1 in Resolute Bay was located approximately 100 m east of Tide Gauge Jetty and Site 2 was located approximately 100 m from the shore in Resolute Passage in an ice-crack that forms annually and extends from Cape Martyr to the southeast end of Griffith Island.

The second study was executed in Frobisher Bay, NWT. Toxicity tests were carried out between the 2 August and the 25 September, 1978. Capture sites of the test organisms are illustrated in Figure 2.

2.2 Collection Methods and Test Species

Several collection methods were employed in both studies in order to capture a variety of test organisms. Because of the time of year, methods used in the first study were necessarily restricted to those that could be performed through holes in the ice. Standard cylindrical minnow traps covered with nylon stocking material and baited with seal meat or char were successful in capturing the amphipods Boeckosimus edwardsi (Krøyer), Onisimus litoralis (Krøyer) and Anonyx nugax (Phipps). Traps set on the bottom were more successful than those set at mid-depth or directly under the ice. The copepod Calanus hyperboreus (Krøyer) was captured by allowing a 1/4 m diameter plankton net (mesh size 239 µm), set 3 m under the ice at Site 2 near Cape Martyr, to stream for 24 h periods in the current.

In Study 2, amphipods were collected by a variety of methods. Gammarus oceanicus (Segerstråle) and G. setosus (Dementieva) were collected from under rocks and from shallow tide pools. Onisimus litoralis were collected in basically the same way, but small pieces of bait (sardines, seal meat, char, etc.) were sometimes thrown into the pools to attract and concentrate this species. Anonyx nugax, A. laticoxae (Gurjanova) and Boeckosimus sp. were collected in minnow traps which were covered with 1 mm mesh netting. These traps were baited with seal meat or char and left for at least 24 h in 10 m of water. Young-of-the-year (YOY) fourhorn sculpins, Myoxocephalus quadricornis quadricornis (Linnaeus), were collected with dip nets when observed in large numbers in surface waters.

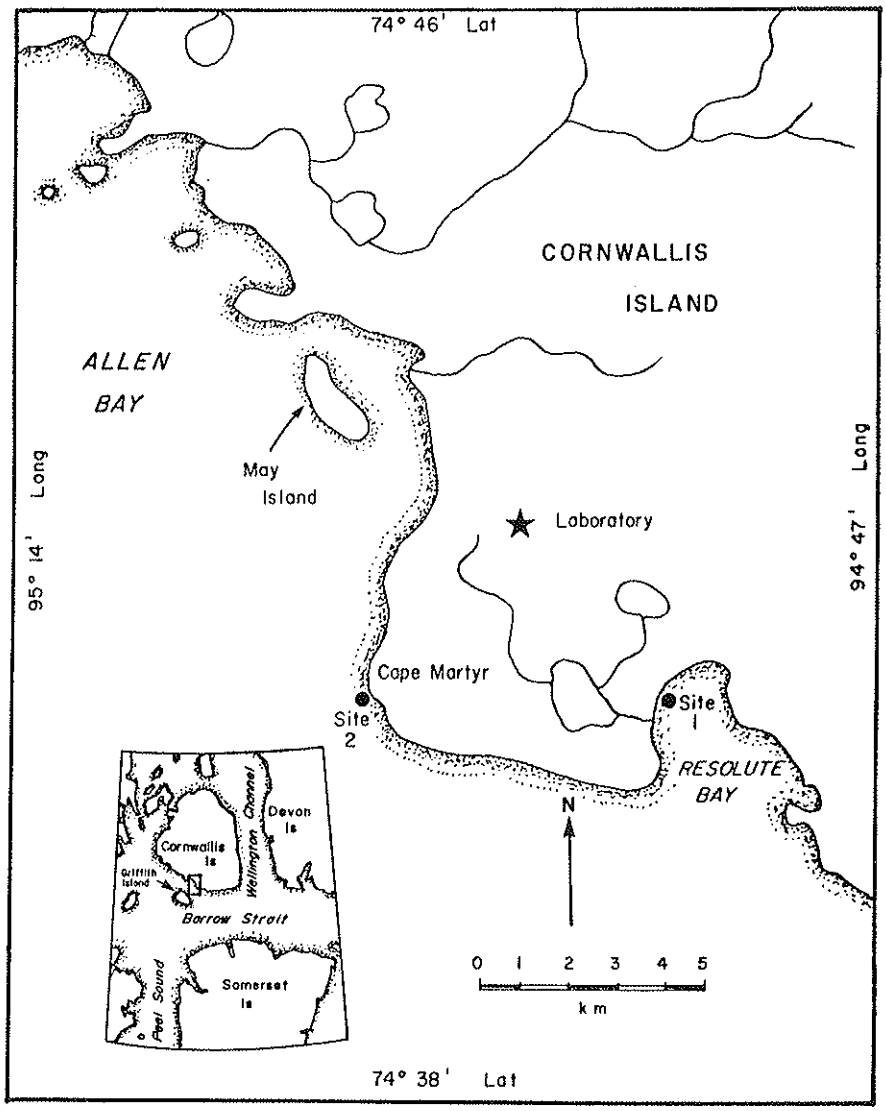


FIGURE 1 LOCATION OF STUDY 1.

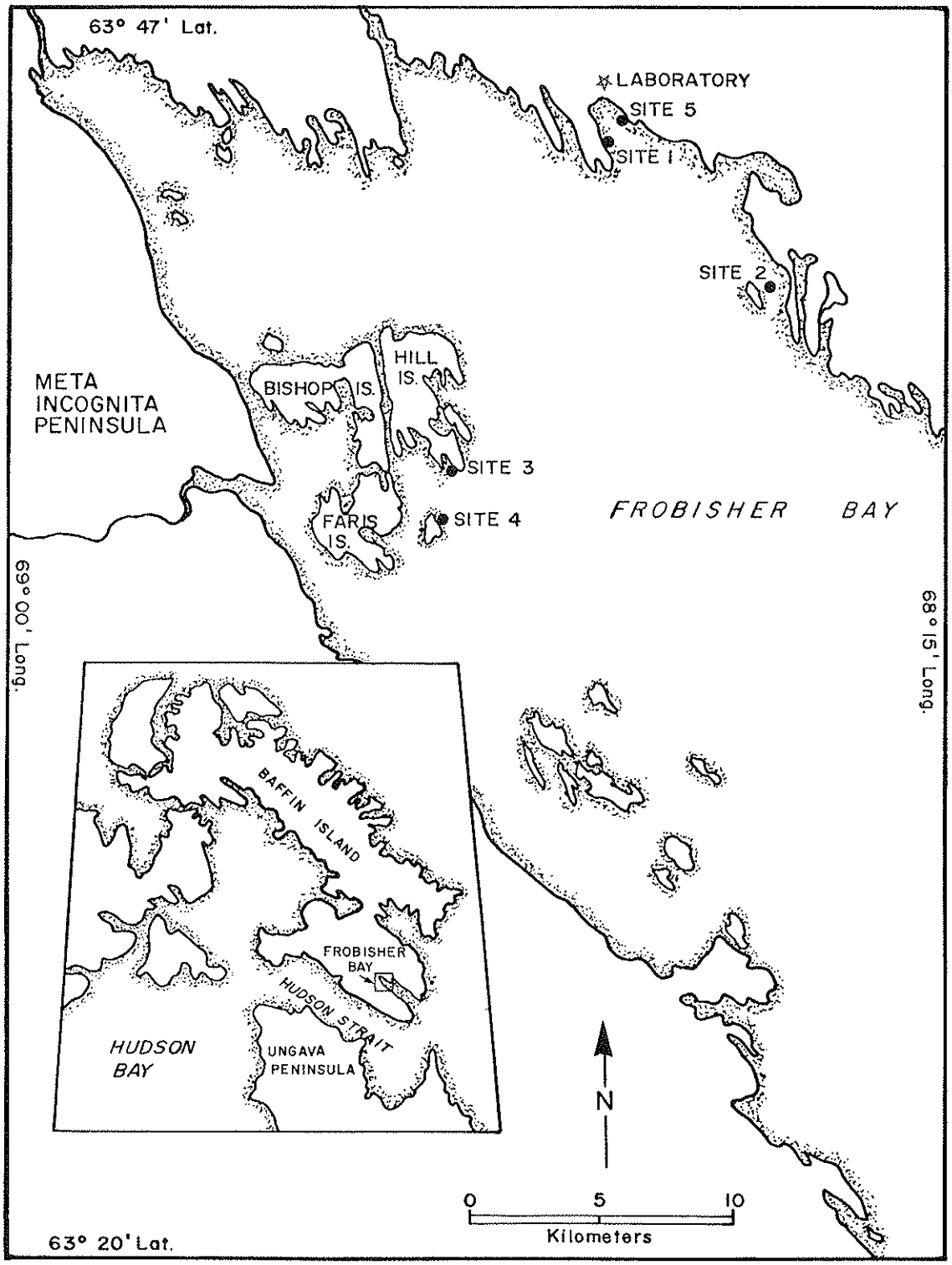


FIGURE 2 LOCATION OF STUDY 2.

Collection information for all test species is summarized in Table 1.

TABLE 1 TEST SPECIES COLLECTION INFORMATION

Site	Species	Depth	Time of Year
Study 1:	Resolute Bay, NWT		
1	Onisimus litoralis	bottom (10 m)	December
1	Anonyx nugax	bottom (10 m)	December-January
2	Boeckosimus edwardsi	bottom (15 m)	December-January
2	Anonyx nugax	bottom (15 m)	December-January
2	Calanus hyperboreus	3 m	December-January
Study 2:	Frobisher Bay, NWT		
1	Gammarus oceanicus	intertidal	August
1	Gammarus setosus	intertidal	August
1	Onisimus litoralis	intertidal	August-September
2	Anonyx laticoxae	bottom (10 m)	August-September
2	Anonyx nugax	bottom (10 m)	August-September
3	Anonyx laticoxae	bottom (10 m)	September
3	Anonyx nugax	bottom (10 m)	September
3	Boeckosimus sp.	bottom (10 m)	September
3	Myoxocephalus quadricornis	surface (0 m)	September
4	Anonyx laticoxae	bottom (10 m)	September
4	Anonyx nugax	bottom (10 m)	September
5	Onisimus litoralis	intertidal	September

2.3 Animal Transportation and Maintenance

Animals were transported to the laboratory, in water collected from the location of their capture, using insulated containers. In the laboratory, test organisms were separated by species and placed in freshly collected seawater in polystyrene animal cages (27 x 21 x 16 cm) which were kept at experimental temperature in refrigerators

(Study 1; mean temperature 2.9°C + s.d. 3.0°C, N = 79) or cold-water bioassay baths (see Study 2). Water in the cages was changed every one to two days and aerated continuously using an aquarium air pump (Silent Giant). Animals were not fed during this acclimation period.

During Study 2 it was sometimes necessary to hold animals for extended periods (up to seven days) before bioassays began. In these cases test animals (amphipods) were held in submerged minnow buckets at Site 1 and were fed periodically with sardines, seal meat and char. When needed for bioassays, they were brought into the laboratory and held for acclimation as described above. It was never necessary to maintain sculpins in this way; upon capture, they were always brought immediately into the laboratory for acclimation and subsequent bioassays.

2.4 Toxicants

Three test toxicants were used -- Prudhoe Bay crude oil (Atlantic Richfield Co., Ferndale, Washington), Corexit 9527 (ESSO Chemicals, Sarnia, Ontario) and a reference toxicant, sodium lauryl sulphate (BDH chemicals, specially pure). In addition, Prudhoe Bay crude oil-Corexit 9527 mixtures in a 10:1 concentration ratio (vol:vol) were tested for toxicity.

2.4.1 Prudhoe Bay Crude Oil. A typical Prudhoe Bay crude oil is a brownish-black, intermediate gravity, high-sulphur, high-nitrogen oil (Clark and Brown, 1977). It contains a relatively high percentage of aromatics and has a pour point of -10°C; a specific gravity of 0.888 at 15°C; and a viscosity of ($1.4 \times 10^{-5} m^2/s$ Kinematic, 38°C).

The oil was received in two tightly capped metal containers (approximately 19 L each). Immediately prior to each study, oil from one of the containers was mixed and distributed into 11 mL glass vials which were tightly capped and refrigerated until use. The oil used in both studies, therefore, was from the same batch but from different shipping containers. A new vial of oil was used each day for the bioassays. Test concentrations ranged from 30 to 800 ppm (µL/L).

2.4.2 Corexit 9527. Corexit 9527 Oil Spill Dispersant Concentrate is soluble in freshwater and hydrocarbon solvents (ESSO Chemicals, 1976). It is not, however, totally soluble in salt water (i.e. salinity of approximately 30 ppt). Corexit 9527 contains, as its main solvent, glycol ether (Lönning and Hagström, 1976).

Upon receipt of the Corexit, it was distributed into 11 mL glass vials, which were capped tightly and refrigerated until use. A new vial of Corexit was used in each

day's work. Its concentration in experimental solutions ranged from 5 to 640 ppm (µL/L) and, when used to disperse the oil, it was used in a volume equal to 1/10 that of the oil. All concentrations of Corexit referred to in this study are µL of Corexit concentrate per litre of water and not concentrations of a diluted formulation.

2.4.3 Sodium Lauryl Sulphate. Sodium lauryl sulphate (SLS), or dodecyl sodium sulphate, is an anionic detergent that has been recommended for use as a reference toxicant in oil/dispersant toxicity bioassays (Tarzwell, 1969; LaRoche et al., 1970; Wilson et al., 1973). In this study SLS was used in concentrations ranging from 0.5 ppm to 50.0 ppm (mg/L). This highest concentration was determined by the limited solubility of SLS in seawater at the low temperatures used in the bioassays.

2.5 Test Mixture Preparation

Surface seawater used in the preparation of all test mixtures was collected daily from one site (Site 1, Study 1; Site 1, Study 2) and filtered through 0.65 µm Millipore filters before use. Salinities were determined periodically using an Endeco hand-held salinometer/refractometer accurate to ± 0.5 ppt. Salinities varied from 29.1 to 34.6 ppt in Study 1 and from 24.1 to 32.2 ppt in Study 2.

Methods of preparation of oil-water mixtures were modified from those of Percy and Mullin (1975). Oil-water mixtures were prepared in 1500 mL quantities in 1900 mL wide mouth jars. The appropriate volume of oil was pipeted (Pipetman disposable tip automatic pipet, ±0.02 µL at 25 µL and ±5.0 µL at 1000 µL) into the filtered seawater; the pipet tip was rinsed 10 times with seawater in order to extract as much oil as possible from the tip. When oil-Corexit-water mixtures were prepared, Corexit was added after the oil.

After the addition of oil or oil and Corexit, the jar mouths were lined with tinfoil, capped tightly and shaken on a reciprocating shaker (280 oscillations/minute) for 0.5 h. The mixtures were immediately transferred to 2000 mL separatory funnels and allowed to stand undisturbed for 210 min (3.5 h). This period was selected as an acceptable settling time on the basis of the results illustrated in Figure 3. Percy and Mullin (1975) described the lower phase of such a mixture as a 'semi-stable dispersion'.

After 210 min had elapsed, the bottom 1350 mL of each mixture was drained into a large beaker, quickly hand-stirred, subsampled for a determination of 0 h oil concentration, and distributed evenly into three glass jars (Study 1) or polyethylene food bags (Rapid Packaging Systems Ltd., Scarborough, Ontario) supported in glass jars (Study 2). The final volume of test mixture in each jar was between 430 and 445 mL.

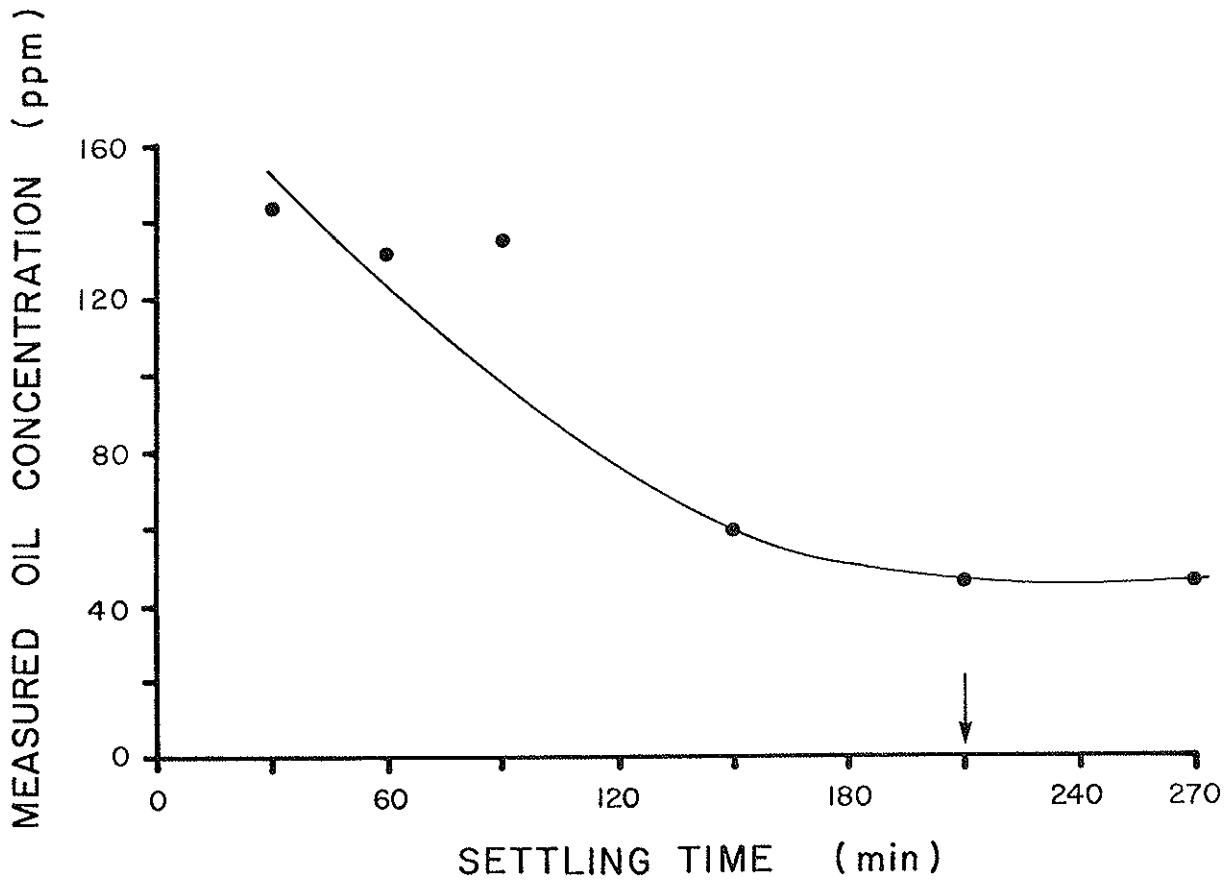


FIGURE 3 EFFECT OF SETTLING TIME ON PRUDHOE BAY CRUDE OIL CONCENTRATION IN OIL-WATER MIXTURES. The initial concentration of oil added was 600 ppm. The arrow indicates the settling time chosen for all oil-water and oil-Corexit-water mixtures.

Wide mouth jars (710 mL) were used as experimental vessels (Study 1) or support vessels (Study 2) for Boeckosimus spp., Onisimus litoralis, Calanus hyperboreus and Myoxocephalus quadricornis; bioassays using large amphipod species (Anonyx and Gammarus spp.) were run in 1900 mL wide mouth jars.

Test solutions of Corexit 9527 and sodium lauryl sulphate were prepared in the experimental vessels and were hand mixed.

Control solutions consisted of filtered seawater taken from the same lot that was used to prepare toxicant mixtures. Control solutions for the oil experiments were shaken on a reciprocating shaker for 30 min; they were not settled in separatory funnels. Control solutions for Corexit and SLS bioassays were agitated by hand.

All mixture preparations were carried out at laboratory temperatures (between 3 and 10°C, Study 1; and approximately 20°C, Study 2).

Between each use, all glassware was washed. Oiled glassware was rinsed three times with hot water and three times with filtered seawater. All other glassware was rinsed five times with filtered seawater. At no time was a detergent used for cleaning purposes. In addition, glassware used for one concentration of toxicant mixture, was subsequently used only for that concentration of that particular toxicant. In Study 2, polyethylene bags used in the bioassays were replaced when a fresh toxicant mixture was prepared (every 24 h).

2.6 Hydrocarbon Analysis

2.6.1 Fluorescence Spectroscopy. Hydrocarbon concentrations in the experimental solutions were monitored by fluorescence spectroscopy, using a Turner Model 430 Spectrofluorometer.

Optimum excitation and emission wavelengths for Prudhoe Bay crude oil were determined. A solution of 0.15 µL oil/mL hexane (J.T. Baker Chemical Co., spectrophotometric grade) was prepared. The emission wavelength was set at 395 nm and the excitation wavelength was scanned from 400 to 300 nm; readings of relative fluorescence were recorded at 10 nm or (in the range of peak values) 5 nm intervals. The results were graphed and the optimum excitation wavelength was chosen. The excitation wavelength was then set at optimum and the emission wavelength was scanned from 450 to 350 nm. Readings of the relative fluorescence were recorded at 10 or 5 nm intervals and the results graphed. Optimum excitation and emission wavelengths were found to be 350 and 410 nm, respectively, in Study 1 (see Figures 4 and 5). In Study 2, optimum excitation and

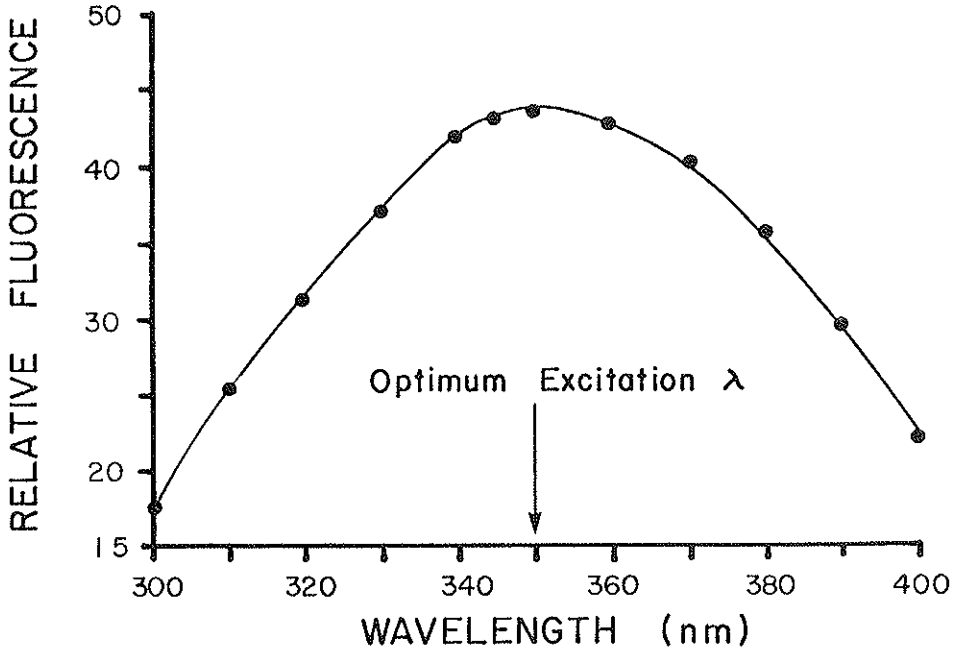


FIGURE 4 FLUORESCENCE EXCITATION SCAN OF PRUDHOE BAY CRUDE OIL IN STUDY 1. Emission wavelength set at 395 nm.

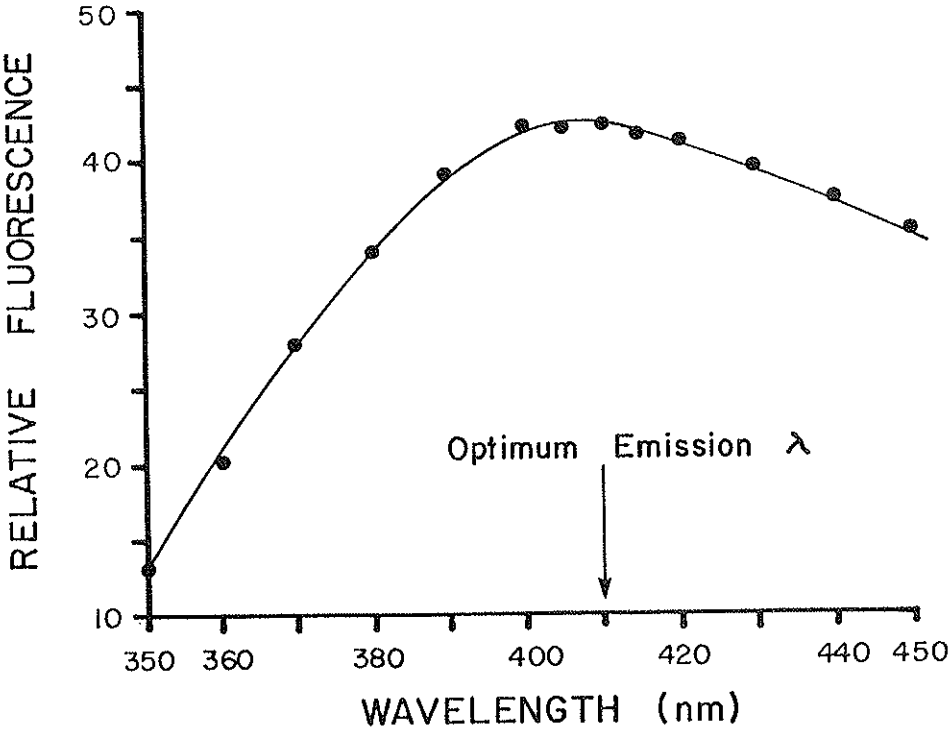


FIGURE 5 FLUORESCENCE EMISSION SCAN OF PRUDHOE BAY CRUDE OIL IN STUDY 1. Excitation wavelength set at 350 nm.

emission wavelengths were 355 and 410 nm, respectively. All subsequent determinations of oil concentrations were made using these optimum wavelengths.

During each study, calibration curves of known concentrations of oil in spectrophotometric grade hexane were used to determine concentrations of oil in the experimental mixtures. Relative fluorescence of known concentrations was determined, starting with the lowest concentration. The same cuvette was used for all readings and between readings was rinsed three times with hexane and twice with the solution to be read. Quenching of fluorescence was found to occur at and above 0.2 µL oil/mL hexane. In each study, several calibrations were performed and the results combined to produce one calibration curve in Study 1 (Figure 6) or one linear regression equation (eliminating concentrations that produced quenching of fluorescence) in Study 2 ($y = -0.00008 + 0.00711\ x$, where y = oil concentration [µL/mL hexane] and x = relative fluorescence).

The effect on the relative fluorescence readings of adding Corexit 9527 to the oil (1:10 ratio) was investigated. There was no significant difference between relative fluorescence of the oil alone and that of the oil-Corexit for the same oil concentration. Corexit alone, in concentrations to be used in the study, was checked for relative fluorescence; readings were minimal and not reliably distinguishable from zero.

At 0 h, after oil-water or oil-Corexit-water mixtures had been standing in 2000 mL separatory funnels for 210 min, the bottom 1350 mL of each mixture was withdrawn into a beaker, hand-stirred and a subsample taken for oil concentration determination. Subsamples of the oil-water and oil-Corexit-water mixtures were withdrawn using a volumetric pipet. After the 24 h exposure period, subsamples were taken from one or more of the replicates of each experimental concentration before animals were transferred to new solutions (in order to minimize disturbance to the oil-water mixtures). All subsamples were taken from approximately mid-depth. The volume of the subsample taken depended on the initial added oil concentration.

Twenty mL of spectro-grade hexane were added to each subsample and the mixture stirred for 5 min at maximum speed on a magnetic stirrer. The oil-hexane layer was then allowed to surface and a portion, depending on the original oil concentration, was transferred by pipet to a graduated cylinder. There it was diluted with hexane in order to ensure that concentrations were below those that caused quenching. The dilution factor depended on the initial oil concentration. A portion of the diluted mixture was then placed in the cuvette and analyzed fluorometrically. Concentration of oil was

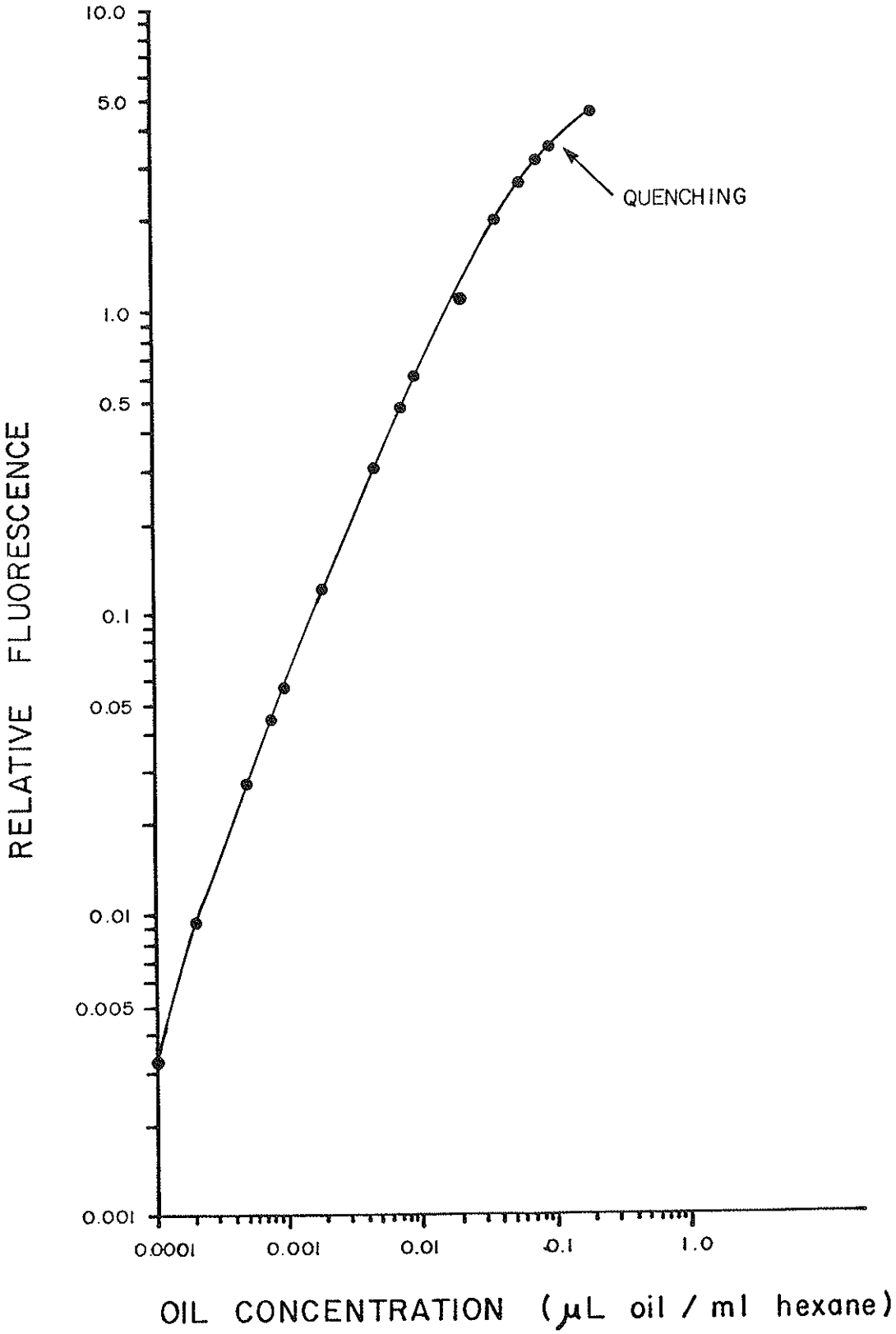


FIGURE 6 CALIBRATION CURVE USED FOR DETERMINING CONCENTRATION OF HYDROCARBONS IN OIL-WATER AND OIL-COREXIT-WATER MIXTURES IN STUDY 1.

quantified by applying the relative fluorescence reading to the calibration curve or regression equation and multiplying by the appropriate dilution factor.

Since hydrocarbon concentrations were determined from a single hexane extraction, the efficiency of a single extraction was determined. After the initial extraction, the seawater layer and remaining oil-hexane layer were poured into a 100 mL separatory funnel. The reagent jar was rinsed with 10 mL spectro-grade hexane and the rinse was added to the separatory funnel. After the water and hexane layers had separated, the water layer was returned to the reagent jar and extracted with 20 mL hexane in the same manner as the initial extraction. The sample was extracted repeatedly until the relative fluorescence was 0.01 or lower (Study 1), or until the hydrocarbon concentration in the final extract approximated 10% of that in the initial extract (Study 2). The resulting concentrations of hydrocarbons were summed and the concentration in the first extract expressed as a percentage of the total. In Study 1 relatively few extraction efficiency tests were performed and as a result the effects of initial oil or oil-Corexit concentration on extraction efficiency could not be determined. In Study 2 the tests were increased to determine the effects of initial oil concentration on extraction efficiency.

2.6.2 Gas Chromatography. In order to gain additional information about the composition of the toxicant mixtures, samples of oil-water and oil-Corexit-water mixtures with nominal (added) concentrations of 200 ppm Prudhoe Bay crude oil were analyzed by gas chromatography in the second study. These analyses were performed by the Oil and Gas Working Group, Institute for Environmental Studies, University of Toronto. Oil-water and oil-Corexit-water mixtures were prepared as described for bioassay mixtures, and sampled at 0 h and after 24 h under bioassay conditions but without test animals. Artificial seawater (35 ppt) was used to make up the mixtures. In addition, a sample of the oil was analyzed by gas chromatography to determine if it had been weathered to any extent prior to use in bioassays.

Hydrocarbons were extracted from the oil-water or oil-Corexit-water mixtures using the gas stripping technique described by Mackay and Shiu (1976). This method extracts efficiently only those hydrocarbons of volatility greater than naphthalene, which are generally low and medium molecular weight hydrocarbons (Mackay and Shiu, 1976).

Chromatography was performed using a Hewlett-Packard Model 5750 gas chromatograph as described by Mackay and Shiu (1976). The temperature of the column was increased from 50 to 280°C at 15°C/min and held at 280°C for 20 min. Concentra-

tions were determined by measuring peak areas using a Hewlett-Packard Model 3371 integrator. The error in these quantities is probably in the order of ±50% (D. Mackay, University of Toronto, pers. comm.).

In order to differentiate between dissolved and particulate hydrocarbons, oil-water or oil-Corexit-water mixtures were filtered through a 5 µm pore size filter. Hydrocarbon concentrations in the filtrate (determined by gas chromatography) were considered to be concentrations of dissolved hydrocarbons, and the concentration of the particulate (dispersed) hydrocarbon fraction was calculated by subtraction.

2.7 Bioassays

Bioassays were conducted as semi-static 96 h acute bioassays. Each experiment consisted of three replicates at each of three (Study 1) or four (Study 2) toxicant concentrations, in addition to five control replicates.

The test species were separately exposed to the toxicants (except for Gammarus oceanicus-G. setosus and Anonyx nugax-A. laticoxae mixtures). Only animals that were actively swimming were selected from the holding tanks. When possible, we tried to select animals of similar size for each experiment. To minimize placing all animals of one condition (e.g. the easiest or most difficult to catch) in the same experimental vessel, we first placed one animal in each vessel, then a second in each vessel, and so on until all vessels contained the alloted number of animals. Depending on the size of the test species, each replicate normally contained from 5 to 10 animals. Animals were not fed during the bioassays. At the end of every 24 h period during the tests, dead animals were counted, removed and preserved, and the living organisms transferred to freshly prepared toxicant mixtures. Animals were considered dead if they displayed no movement of appendages in response to touch. After the 96 h exposure period the living animals were counted and preserved (Study 1) or transferred to toxicant-free seawater for a 24 h post-exposure period (Study 2) before being preserved for subsequent positive identification.

During Study 2, three replicates of a single concentration of SLS were included in most tests of toxicity of oil, oil-Corexit and Corexit. The concentration was chosen to produce a partial mortality of the test species. It was hoped that by comparing the mortality in this control with that in other experiments or with that expected from the results of the SLS bioassays, gross differences in the condition or 'healthiness' of the test animals during different experiments would be detected if present.

In Study 1, standard refrigerators (which were vented, by leaving the doors slightly ajar, to prevent a build up of explosive gasses from oil mixtures) were utilized to maintain the temperature during bioassays. To prevent temperature stratification, air circulation was provided by a small desk fan located within each refrigerator. Air temperatures were monitored using maximum-minimum thermometers. Except when refrigerator doors were opened for transferring bioassay vessels, lights were off in the refrigerators. All vessels of each experiment were placed in mixed order in the same refrigerator for the duration of the bioassay.

In Study 2, we hoped to obtain more adequate temperature control than had been achieved in Study 1, by maintaining experimental temperatures using water baths rather than refrigerators. Water was cooled with a water chilling unit (Model D-100, Frigidunits Inc., Toledo, Ohio) and circulated through the water baths with a Model 3E-12NR Little Giant submersible water pump. Water temperatures were continuously monitored in the water baths using recording thermometers (United Electric Co.) with an accuracy of $\pm$0.7°C. Differences between water bath temperatures and bioassay mixture temperatures were checked with hand-held laboratory thermometers having an accuracy of $\pm$0.5°C.

Test mixtures were not aerated during the bioassays. However, dissolved oxygen concentrations were checked periodically, after 0 h and 24 h exposure, using a HACH oxygen kit (accuracy of $\pm$ 1 ppm).

2.8 Calculation of 96 h LC_{50} Values

In determining median lethal concentrations, mortality data from all replicates at each concentration were combined and averaged. Corrections for mortality in control vessels were made using Abbot's formula (APHA, 1976). In Study 1, exposure concentration for each nominal oil concentration in oil-water and oil-Corexit-water mixtures was calculated as the average of the mean 0 h and mean 24 h oil concentration determinations for all experiments and was not corrected for extraction efficiency. In Study 2, exposure concentration was calculated as the average of the mean 0 h and mean 24 h oil concentration determinations for each experiment and was corrected for extraction efficiency. Nominal concentrations of SLS and Corexit were regarded as exposure concentrations for these toxicants.

The graphical probit analysis of Litchfield and Wilcoxon (1949) was used in calculating most 96 h LC_{50} values in Study 1. In Study 2, for comparative purposes, estimates of 96 h LC_{50} values were calculated by three methods when possible: a

computerized probit analysis (Davies, 1971); a graphical probit analysis (Litchfield and Wilcoxon, 1949); and a 10% trimmed Spearman-Karber calculational method (Hamilton et al., 1977). In most experiments one or more of the three methods could not be used because of methodological limitations of the various analysis techniques. Only in one instance did the 96 h LC_{50} estimated by graphical probit analysis differ by more than 10% from the estimate obtained using computerized probit analysis. This occurred in Experiment 42, in which the toxicity of SLS to young-of-the-year sculpins was tested. In this instance, the 96 h LC_{50} values were low (8.5 and 4.8 ppm using computerized probit analysis and graphical probit analysis, respectively) and the highest mortality observed was 30.3%. This required that the 96 h LC_{50} be estimated by extrapolation. In thirteen other comparisons of 96 h LC_{50} estimates obtained by computerized and graphical probit analyses, percent differences ranged from 0.4 to 9.7%. Seven 96 h LC_{50} values were estimated using both the computerized probit analysis and the 10% trimmed Spearman-Karber method. In these comparisons, the percent differences between LC_{50} estimates ranged from 0.0 to 5.9%.

The LC_{50} estimates obtained by all three methods were similar and because more estimates could be calculated using the computerized probit analysis, LC_{50} values and confidence limits reported from Study 2 are from computerized probit analyses unless otherwise indicated. In some instances, 96 h LC_{50} values could not be calculated by that method, but could be estimated by one of the other two methods. In other cases, too few partial mortalities occurred to use any of the above methods. In these cases, whenever possible, median mortality was interpolated from a graph of percent mortality versus oil concentration on semi-log paper. However, this method did not allow the calculation of confidence limits.

Some bioassays were inadvertently conducted with mixtures of two species (i.e. Anonyx nugax and Anonyx laticoxae; Gammarus setosus and Gammarus oceanicus). In analyzing results from these tests, percent mortalities and median lethal concentrations were calculated separately for each species. As a result, numbers of test organisms for each species were sometimes small, and the statistical reliability of the results questionable. However, since consistent patterns of relative species sensitivity were observed in these results, it was considered worthwhile to include them.

2.9 Study 1 versus Study 2 - Methodological Differences

Several differences in methods of conducting the toxicity bioassays and in methods of analyzing the data occurred between Study 1 and Study 2. These are summarized in Table 2.

TABLE 2 MAJOR DIFFERENCES IN METHODS AND MATERIALS BETWEEN STUDY 1 AND STUDY 2

Study 1	Study 2
Conducted in winter.	Conducted in summer.
Located in Resolute Bay, NWT.	Located in Frobisher Bay, NWT.
Glass jars used as bioassay vessels.	Plastic bags used as bioassay vessels.
Temperature controlled by refrigerators.	Temperature controlled by cold water baths.
3 toxicant concentrations.	4 toxicant concentrations.
No post-exposure period.	24 h post-exposure period.
No SLS controls.	SLS controls of one concentration per experiment.
Measured oil exposure concentration calculated from averages of concentrations in all experiments.	Measured oil exposure concentration calculated from averages of concentrations in each experiment.
Measured oil exposure concentration not corrected for extraction efficiency.	Measured oil exposure concentration corrected for extraction efficiency.
Graphical probit analysis used to calculate 96 h LC_{50}.	Computerized probit analysis used to calculate 96 h LC_{50}.

3 RESULTS AND DISCUSSION

3.1 Test Species

The sensitivity of many animals to toxicants depends greatly on their life stages (Rice et al., 1975; Linden, 1976; Wilson, 1977). Moreover, details of their biology, including reproduction, feeding habits and preferred habitats, all affect their vulnerability and/or their ability to recover from adverse impacts.

Test organisms used in the toxicity bioassays included seven species of benthic or intertidal amphipods, one copepod species and one species of fish. The following discussion assesses the life stages of the organisms used in the bioassays in terms of what is known of their life histories, and describes their importances in the food web.

3.1.1 Anonyx nugax. Anonyx nugax, the largest of the amphipods used in these studies, is usually classified as a benthic scavenger. It has, however, been found on occasions in the water column (Dunbar, 1954; Green and Steele, 1975) and on the undersurface of ice where it consumed diatoms (LGL Ltd. unpubl. data).

Large numbers of Anonyx nugax were caught in both studies. Test animals appeared to include individuals of two size classes in each study: 23 to 28 mm and 30 to 42 mm in Study 1, and 18 to 34 mm and 36 to 45 mm in Study 2 (see Figure 7). The wide size ranges of animals collected over relatively short periods are thought to be indicative of a lengthy breeding period (Steele, 1961). This is corroborated by the presence of ovigerous females (40 to 41 mm long) in collections from Study 1, mature males in Barrow Strait in May (LGL Ltd. unpubl. data), and brooding females in Brentford Bay, Boothia Peninsula, in August (LGL Ltd. unpubl. data).

Anonyx nugax has been recorded, generally in small numbers, from stomachs of eider ducks, cod, bearded seals (Dunbar, 1954), ringed seals (McLaren, 1958) and arctic char (Grainger, 1953). However, these reports preceded the genus revision of Steele and Brunel (1968) and it is possible that some amphipods identified as A. nugax were actually other, less common, species known to occur in arctic waters. More recently, A. nugax has been found among the stomach contents of arctic cod, fourhorn sculpins and arctic sculpins (LGL Ltd. unpubl. data).

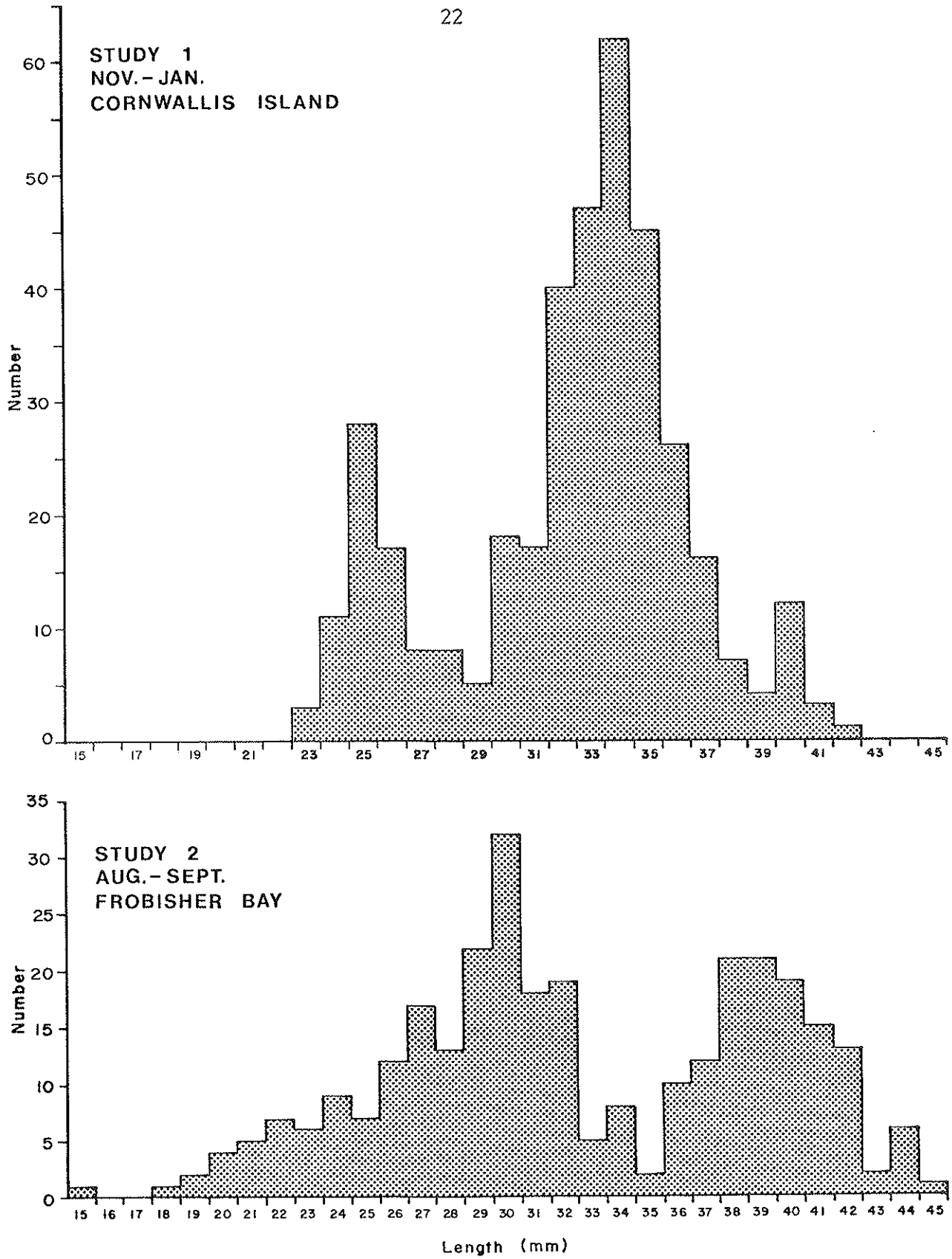


FIGURE 7 LENGTH FREQUENCY OF Anonyx nugax USED IN 96 h BIOASSAYS.

3.1.2 **Anonyx laticoxae.** Anonyx laticoxae was caught with Anonyx nugax from depths of approximately 10 m in Frobisher Bay during Study 2. It had not been collected during Study 1. During the bioassays, it was not recognized as being a separate species and, therefore, occurred as a 'contaminant' in toxicity tests using A. nugax. Separate data analyses were conducted using A. laticoxae, but the numbers of animals were too small to yield reliable results from individual bioassays.

Body lengths of Anonyx laticoxae used in these experiments ranged from 21 to 35 mm, with 89% falling between 26 and 35 mm (see Figure 8). Very little is known about the life history of this amphipod. Steele and Brunel (1968) state that this species apparently matures when it is greater than 30 mm in length and D. Thomson (LGL Ltd., pers. comm.) found a brooding female, 32 mm in length, near Cape Warrender, Devon Island. No ovigerous or brooding females were found in the present collection, although several exceeded 32 mm in length.

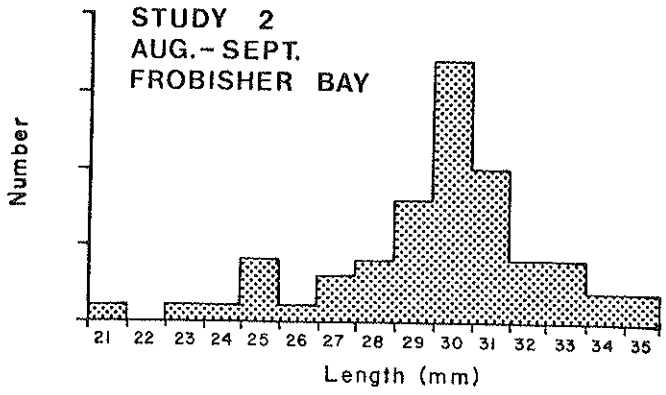


FIGURE 8 LENGTH FREQUENCY OF Anonyx laticoxae USED IN 96 h BIOASSAYS.

There do not appear to be any published reports identifying Anonyx laticoxae as a food item for other marine organisms in eastern arctic waters. However, some food organisms identified as A. nugax before the genus revision of Steele and Brunel (1968) could have been A. laticoxae.

3.1.3 Boeckosimus edwardsi. Stephensen (1923) described Boeckosimus edwardsi as a boreo-arctic amphipod. Within arctic North America, B. edwardsi appears to be restricted to eastern arctic marine waters, since this species has not been recorded in either Alaskan waters (Shoemaker, 1955) or near the Mackenzie Delta (Wacasey, 1974, 1975). Although it is considered a predominantly shallow water species, B. edwardsi has been collected at a depth of 250 m (Stephensen, 1923, 1933, 1935; Dunbar, 1954).

Boeckosimus edwardsi was collected only during Study 1 from Resolute Passage. Virtually nothing is known about the life history of this species and the few specimens that were examined for this study were not collected over a sufficiently long time period (i.e. a minimum of one year) to enable a proper elucidation of the life history. However, unpublished information (LGL Limited) tentatively suggests that the life cycle of B. edwardsi is two years in duration and that during winter mature adults (14 to 17 mm) coexist with young-of-the-year (5 to 7 mm) and one-year-olds (9 to 12 mm). No ovigerous or brooding females were found during Study 1. Ninety-seven percent of the B. edwardsi specimens used in the toxicity experiments of Study 1 were from the intermediate size class (8 to 11 mm long), and were probably one-year-olds (see Figure 9a).

Stephensen (1923) and Dunbar (1954) described Boeckosimus edwardsi as a scavenger, and during Study 1 this species was attracted to traps baited with arctic char and seal meat. Unfortunately, as far as we know, no information is available regarding the role of B. edwardsi in arctic food chains.

3.1.4 Boeckosimus sp. The Boeckosimus amphipod species was collected from Frobisher Bay during Study 2, but was not found in Resolute Bay or Resolute Passage during Study 1. On the basis of the shape of the telson, lateral head lobes and epimeron 3, and on the basis of current distribution records, this species most clearly resembled Boeckosimus affinis (Hansen). However, it also resembled Boeckosimus derjugini (Gurjanova) and Boeckosimus dubius (Schellenberg) in some characteristics. Until the taxonomic problems of this group of lysianassids are solved, it is impossible to assign a specific name to this organism (D. Laubitz, National Museum of Natural Sciences, pers. comm.).

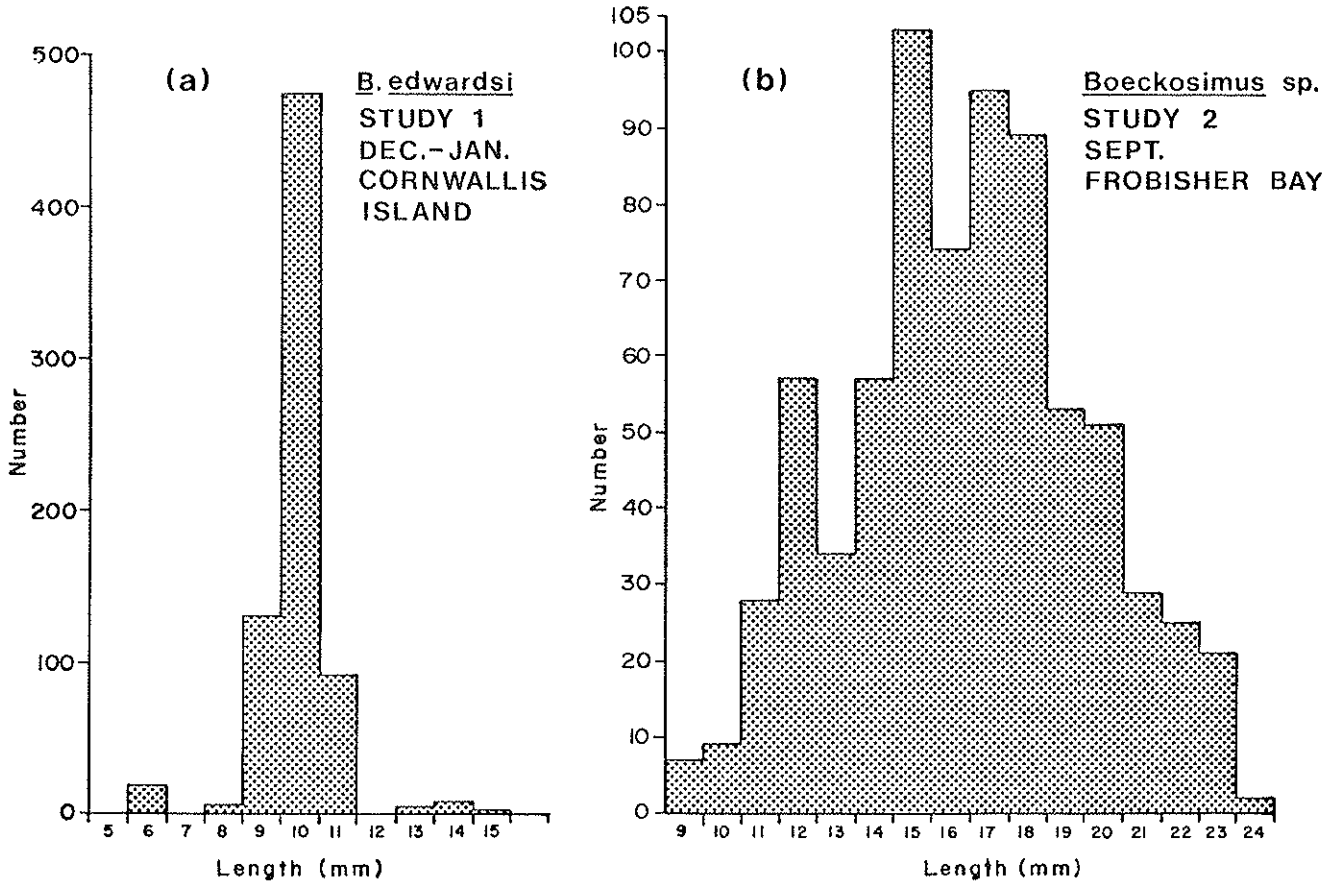


FIGURE 9 LENGTH FREQUENCY OF (a) Boeckosimus edwardsi AND (b) Boeckosimus sp. USED IN 96 h BIOASSAYS.

Individuals of this species ranged in length from 9 to 24 mm (see Figure 9b). A few mature brooding females were found in the 22 to 24 mm size range. This species appeared to favour the same habitat as Anonyx spp. and was collected only in traps set at about a 10 m depth.

3.1.5 Gammarus oceanicus. Gammarus oceanicus is a euryhaline intertidal amphipod which is confined to the Atlantic Ocean and adjacent seas. It is the most numerous Gammarus species south of Newfoundland. However, it is less abundant than G. setosus in the northern region of its range (Steele and Steele, 1972). In Study 2, G. oceanicus was collected, along with G. setosus, in the intertidal region from under small rocks and algae.

Like Anonyx laticoxae, this species appeared as a 'contaminant' species; it was found upon positive identification of the test organisms used in toxicity tests on

Gammarus setosus. Interestingly, it only appeared in the early stages of Study 2. Although separate analyses of the data on G. oceanicus have been performed, it is emphasized that the number of organisms was small and that the reliability of the results is, therefore, questionable.

Individuals of G. oceanicus varied from 21 to 37 mm in length (see Figure 10). Steele and Steele (1972) speculate that in the northern part of its range, G. oceanicus may take 4 years to reach lengths of 30 mm for females and 35 mm for males.

Because the habitat of Gammarus oceanicus is similar to that of G. setosus, it is probably utilized as food by inshore feeding birds, fish and mammals, as is G. setosus. McLaren (1958) found G. oceanicus in the stomach of one ringed seal taken off southwest Baffin Island and recorded unidentified gammarids in stomachs of ringed seals from Ungava Bay and Northern Labrador. Grainger (1953) reported relatively large numbers of Gammarus sp. in the stomach contents of arctic char taken in Frobisher Bay. It is possible that some of these unidentified gammarids were G. oceanicus. However, it is expected that G. setosus, because of its greater numbers, is a more important food item in these regions.

3.1.6 Gammarus setosus. The amphipod Gammarus setosus has a circumpolar distribution and is the most common species of Gammarus from Labrador north (Steele and Steele, 1974). It is found primarily in intertidal and shallow water regions (Ellis and Wilce, 1961; Steele and Steele, 1970). Gammarus setosus, like G. oceanicus, was found only in Study 2.

Body lengths of G. setosus used in the toxicity bioassays varied from 16 to 40 mm (see Figure 11). Steele and Steele (1970) reported that at 3°C, in the laboratory, G. setosus reached maturity at mean body lengths of 14 mm for males and 12 mm for females. It is likely that the individuals used in this study were adults, some of which would breed in the fall and winter.

Gammarus setosus is an important food item for many species of vertebrates. Fish which have been found to consume this amphipod include arctic cod, arctic char, arctic cisco and several species of sculpins (LGL Ltd. unpubl. data). Gammarus setosus is also consumed by several bird species, including the oldsquaw duck, red phalarope, northern fulmar, thick-billed murre and black guillemot (LGL Ltd. unpubl. data). Ringed seals have also been known to consume this species (McLaren, 1958).

3.1.7 Onisimus litoralis. Onisimus litoralis is a common arctic littoral and intertidal amphipod, generally restricted to near-shore shallow waters 30 m or less in depth

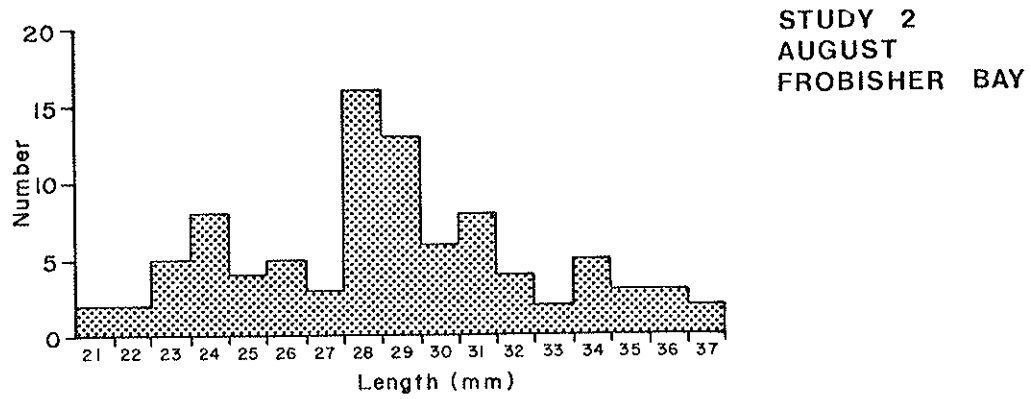


FIGURE 10 LENGTH FREQUENCY OF Gammarus oceanicus USED IN 96 h BIOASSAYS.

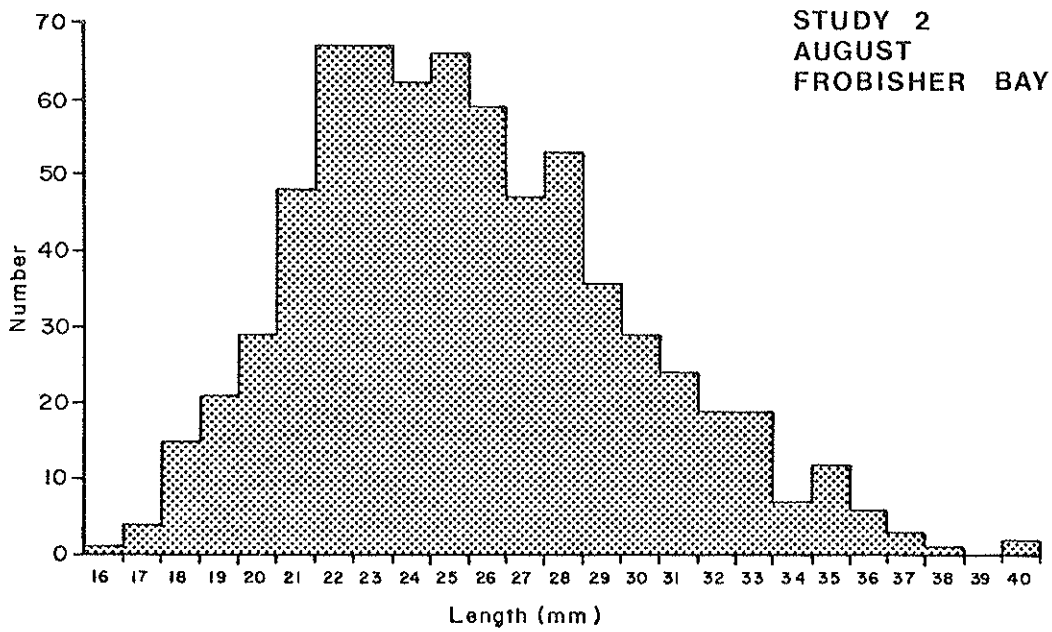


FIGURE 11 LENGTH FREQUENCY OF Gammarus setosus USED IN 96 h BIOASSAYS.

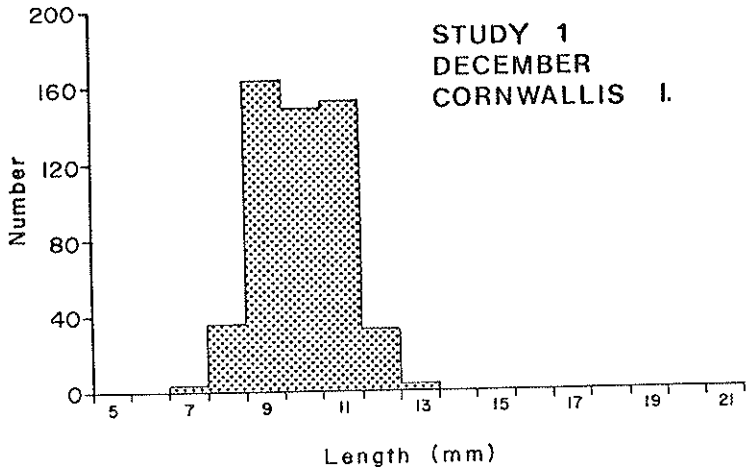
(Stephensen, 1935; Dunbar, 1954; MacGinitie, 1955). It has generally been regarded as a scavenger (Steele, 1961), but has been known to feed on ice algae (LGL Ltd. unpubl. data). This species was caught using baited minnow traps set on the bottom of Resolute Bay during Study 1. In Frobisher Bay, during Study 2, this organism did not appear to be attracted to such traps but was collected from tidal pools. Individuals were frequently buried in sand, as has been noted by Steele (1961), and would sometimes move from the sand into the open after small pieces of raw fish or seal were placed in the pools.

The size distribution of Onisimus litoralis used in the two studies is illustrated in Figure 12. Individuals used in the first study were all between 7 and 13 mm in length, with 87% having lengths of 9 to 11 mm. In the second study two distinct size classes (6 to 9 mm and 13 to 19 mm) were tested separately in the toxicity bioassays. Based on the life history information compiled by Steele (1961) and on unpublished data of LGL Ltd., O. litoralis appears to have a two year breeding cycle with spawning occurring in winter-spring. Animals from Study 1 and those of the smaller size class in Study 2 were immature young (juveniles) that had been spawned in the winter or spring. The larger class used in Study 2, referred to as adults in this report, would likely mate in the coming autumn or winter.

Like Gammarus setosus, Onisimus litoralis appears to be an important food item for several species of vertebrates. It has been found to be a major food source for arctic char (Grainger, 1953; LGL Ltd. unpubl. data), arctic cod, arctic cisco and several species of sculpins (LGL Ltd. unpubl. data). Onisimus litoralis is also an important food item for several species of birds including thick-billed murre, black guillemot, nothern fulmar, black-legged kittiwake, oldsquaw duck and several species of shorebirds (LGL Ltd. unpubl. data). The ringed seal has also been found to consume this amphipod species (McLaren, 1958).

3.1.8 Calanus hyperboreus. The only copepod used in the toxicity bioassays was Calanus hyperboreus, which was caught in Study 1. This species is one of the most abundant of the arctic copepods and is found throughout the Canadian arctic archipelago (Shih et al., 1971; Mohammed and Grainger, 1974; LGL Ltd. unpubl. data).

The life cycle of Calanus hyperboreus consists of six naupliar and then six copepodite stages; the last of these stages is the adult. During Study 1, adult C. hyperboreus (both male and female) and copepodite stages III through V were used in the bioassays (Table 3).



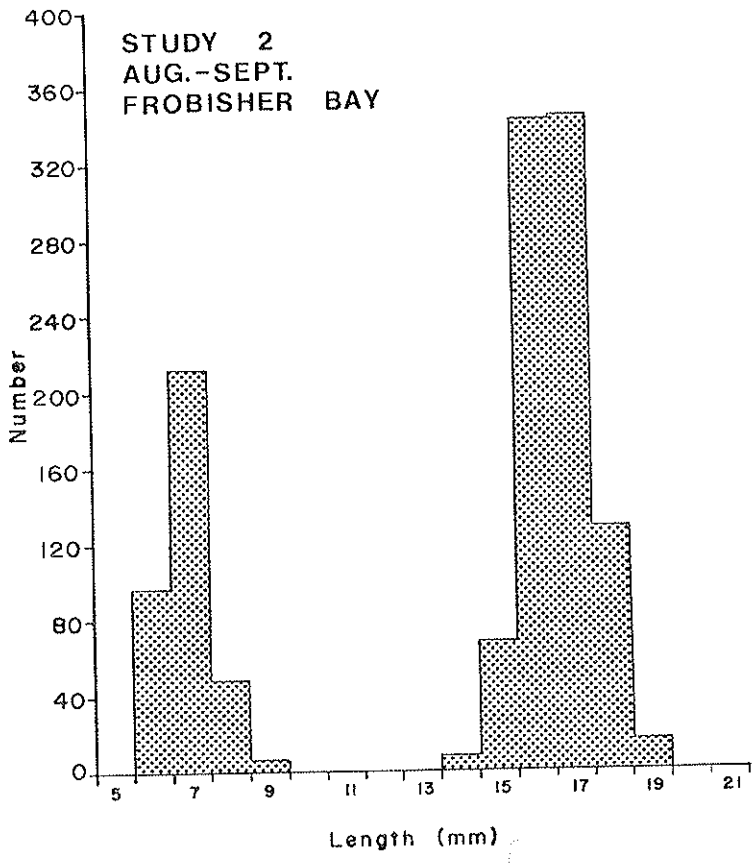


FIGURE 12 LENGTH FREQUENCY OF Onisimus litoralis USED IN 96 h BIOASSAYS.

TABLE 3 STAGES OF Calanus hyperboreus USED IN 96 h BIOASSAYS

Stage	Number	% of Total
Adults	71	36.8
male	23	11.9
female	48	24.9
Copepodites	122	63.2
V	83	43.0
IV	37	19.2
III	2	1.0

3.1.9 Myoxocephalus quadricornis. The fourhorn sculpin, Myoxocephalus quadricornis quadricornis, was used as a test organism in Study 2. This fish has a circumpolar distribution, commonly inhabiting cold brackish coastal waters (Kendel et al., 1975; Percy, 1975). Adults are generally bottom feeders, while fry may be pelagic as well as bottom feeders (Kendel et al., 1975).

Body lengths of the M. quadricornis used in the toxicity bioassays varied from 14 to 23 mm (Figure 13). Fish of this size class would be young-of-the-year that had probably been spawned during late winter or early spring (Khan and Faber, 1974; Kendel et al., 1975).

Kendel et al. (1975) found that fry of 12 to 20 mm in length, taken off the Yukon coast in late July, had not commenced feeding. Since individuals used in the toxicity tests reported here were only slightly longer, it is reasonable to assume that they had not, or had only recently, started to feed. Fish used in the present study may have been at the stage of greatest sensitivity to pollutants. Rice et al. (1975) found that alevins of pink salmon were most sensitive to crude oil at the completion of yolk absorption. In studies on the effects of dispersants, larvae of several marine fish were most sensitive at, and immediately subsequent to, their first feeding (Wilson, 1977). Sensitivity declined once successful feeding had been established.

At some times, fourhorn sculpins may be important as food for other vertebrate species. Kendel et al. (1975) describe them as being an important forage food for other species of fish along the Yukon coast and they have been found, sometimes in large numbers, in the stomachs of arctic char (Grainger, 1953; LGL Ltd. unpubl. data) and

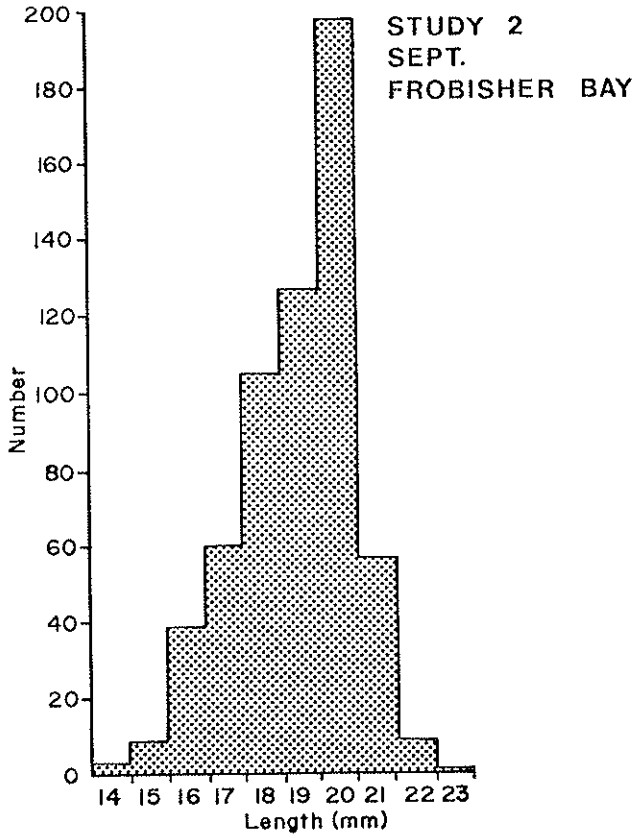


FIGURE 13 LENGTH FREQUENCY OF Myoxocephalus quadricornis USED IN 96 h BIOASSAYS.

arctic cod (LGL Ltd. unpubl. data). Black-legged kittiwakes, thick-billed murres, black guillemots (LGL Ltd. unpubl. data) and ringed seals (McLaren, 1958) have also been known to consume this organism.

3.2 Bioassays

The total number of some test species (i.e. Anonyx laticoxae and Gammarus oceanicus) used in the bioassays was extremely small and the 96 h LC_{50} values given for these species can only be considered gross estimates.

3.2.1 Bioassay Conditions. During Study 1, bioassay temperatures were variable. Air temperatures in the refrigerators fluctuated by as much as 12°C in some experiments (5.0°C to -7.0°C); it is expected, however, that temperatures in bioassay vessels varied by a lesser amount (e.g. when air temperatures were -7.0°C only slight freezing had occurred on the surface of the experimental mixtures).

In an attempt to improve temperature control, cold water baths were used in Study 2 to maintain experimental temperature. In addition, temperature of the circulating water was continuously monitored with recording thermometers. The temperature at which bioassays were conducted was intended to be 4.5°C. Temperature fluctuation, attributable to on-off cycling of the cooling unit, was approximately $\pm$0.5°C. Further temperature variation occurred when bioassay vessels were added to or removed from the cooling tanks. The range of temperature fluctuation in single experiments was 1.0 to 2.9°C, except in two experiments. In the two exceptions (Experiments 23 and 24), conducted at the beginning of the study when the temperature control system was still being stabilized, temperatures rose to approximately 13°C on two occasions for periods of 2 to 3 h.

Experimental temperatures of individual toxicity bioassays are reported in the tables summarizing 96 h LC_{50} results in following sections of this report.

Salinities of filtered seawater used in preparing the toxicant mixtures were spot-checked throughout the study. Salinities varied from 29.1 to 34.6 ppt in Study 1 and from 24.1 to 32.2 ppt in Study 2.

Dissolved oxygen levels were always greater than 4.0 ppm, the minimum level recommended by LaRoche et al. (1970) for oil-dispersant bioassays. In most cases, oxygen concentrations increased during the 24 h exposure periods, apparently regaining oxygen lost during the filtration process. Only in bioassays using the large amphipod species (Anonyx laticoxae and Anonyx nugax) was the animal:mixture volume ratio large enough

to affect dissolved oxygen concentration noticeably. In these cases, dissolved oxygen concentrations remained constant or decreased slightly from the post-filtration levels, but never fell below 4.0 ppm.

3.2.2 Sodium Lauryl Sulphate. The use of a reference toxicant has been recommended as a basis for comparing results of different investigations and for checking the relative 'healthiness' of test organisms (Tarzwell, 1969; LaRoche et al., 1970; Wilson et al., 1973). Sodium lauryl sulphate (dodecyl sodium sulphate), a synthetic anionic detergent of the linear alkylate sulphonate type (Tatem et al., 1976), has been suggested for use as a reference toxicant in oil and oil-dispersant toxicity bioassays (Tarzwell, 1969; LaRoche et al., 1970).

Results of the 96 h bioassays with sodium lauryl sulphate (SLS) are shown in Table 4. The fourhorn sculpin, Myoxocephalus quadricornis, was more sensitive to SLS than were the amphipod species tested. From the results of two bioassays, it appeared that the 96 h LC_{50} of SLS for young M. quadricornis was between 3 and 9 ppm. However, SLS concentrations of 4 and 5 ppm used as controls in other experiments caused 100% mortality (see Table 5).

For the most sensitive amphipod tested, Anonyx nugax, the 96 h median lethal concentration of SLS was between 15 and 25 ppm. The 96 h acute toxicity of SLS to juvenile Onisimus litoralis was found to be between 4 and 40 ppm in Study 1, and in one experiment in Study 2, was found to be 22 ppm. Adult O. litoralis appeared to be slightly less sensitive to SLS than were the juveniles; in two separate tests during Study 2, the 96 h LC_{50} value was found to be 28 ppm. Gammarus setosus exhibited different sensitivities to SLS in two separate bioassays (96 h LC_{50} estimates of 89 and 42 ppm). It is possible that in Experiment 34 the relatively long acclimation period of 120 h contributed to a greater sensitivity and thus a lower 96 h LC_{50}. Based on one bioassay with relatively few animals, Gammarus oceanicus appeared to be more sensitive to SLS than was G. setosus (96 h LC_{50} estimate of 30 ppm). Of all the species tested, Boeckosimus edwardsi and Boeckosimus sp. were the most resistant to SLS; neither exhibited 50% mortality within 96 h in the highest concentrations tested (40 and 50 ppm, respectively).

The acute response of Myoxocephalus quadricornis to SLS was similar to that which has been reported for fish species from other latitudes. Doe and Harris (1976) reported 96 h LC_{50} values of SLS for Salmo gairdneri fingerlings of 3.2 to 5.0 mg/L. For this same species and toxicant, Fogels and Sprague (1977) calculated a 96 h LC_{50} of

TABLE 4 MEDIAN LETHAL CONCENTRATIONS (96 h LC_{50}) OF SODIUM LAURYL SULPHATE, FOR TEST SPECIES, AND CONDITIONS UNDER WHICH THEY WERE DETERMINED. Unless otherwise indicated, 96 h LC_{50} values were calculated using a computerized probit analysis (Davies, 1971). Values within parentheses after the 96 h LC_{50} are 95% confidence limits.

Species	Study	Exp. No.	N	96 h LC_{50} (ppm)	Animal Length (mm)			Temperature Range (°C)[1]	A.P. (h)
					Mean	s.d.	Range		
Amphipods									
Anonyx laticoxae	2	26	19	41 (35-45)[2]	28.0	3.1	21-34	4.1-7.0	70
Anonyx nugax	1	12	45	24 (19-31)[3]	32.2	4.7	23-42	-2.5-3.0	24-96
	2	26	38	16 (15-25)[2]	32.6	5.7	22-44	4.1-7.0	70
Boeckosimus edwardsi	1	15	90	>40	10.1	0.6	9-11	-7.0-5.0	24-96
Boeckosimus sp.	2	50	30	>50	17.4	2.7	12-23	4.5-5.5	196
Gammarus oceanicus	2	21	19	30 (25-35)[2]	29.4	3.8	23-36	3.0-5.0	16
Gammarus setosus	2	21	40	89 (42-186)[3]	26.2	2.9	20-33	3.0-5.0	16
	2	34	83	42 (39-46)	22.7	3.5	16-33	4.5-6.5	120
Onisimus litoralis	2	25	120	28 (26-30)	16.8	1.0	13-19	3.9-6.0	52
(adult)	2	29	120	28 (26-29)	16.6	0.9	15-19	3.8-5.3	44
Onisimus litoralis	1	1	81	>4	10.1	1.0	8-12	-2.0-4.0	24-96
(juvenile)	1	5	54	4-40	9.4	1.1	7-12	0.5-8.0	24-96
	2	33	134	22 (19-26)	6.9	0.7	6-9	4.0-5.5	32
Fish									
Myoxocephalus	2	39	72	<5	17.1	1.3	14-20	4.5-5.5	36
quadricornis	2	42	60	5 (3-9)[3]	19.6	1.1	17-22	3.5-5.7	24

Abbreviations: N = number of animals; s.d. = standard deviation; A.P. = acclimation period.

[1] Refrigerator air temperatures, Study 1; experimental mixture temperatures, Study 2.

[2] Interpolated from data plotted on semi-log paper; figures in brackets give the range of concentrations within which 50% mortality would occur.

[3] Estimate from graphical probit analysis (Litchfield and Wilcoxon, 1949).

TABLE 5 DIFFERENCES BETWEEN EXPECTED AND OBSERVED MORTALITY IN SODIUM LAURYL SULPHATE CONTROLS

SLS Concentration for each Species	Exp. No.	N	Expected Mortality[1] (%)	Observed Mortality[2] (%)	Difference (%)
Amphipods					
Anonyx laticoxae					
25 ppm	51	3	0	0	0
25 ppm	40	3	0	0	0
Anonyx nugax					
25 ppm	51	12	100	67	-33
25 ppm	40	12	100	100	0
25 ppm	48	15	100	49	-51
Boeckosimus sp.					
50ppm	46	30	3	3	0
50 ppm	45	30	3	3	0
Gammarus oceanicus					
35 ppm	23	5	100	60	-40
35 ppm	22	4	100	100	0
35 ppm	24	3	100	22	-78
Gammarus setosus					
35 ppm	23	16	22	42	+20
35 ppm	31	15	22	40	+18
35 ppm	22	11	22	27	+ 5
35 ppm	24	12	22	48	+26
35 ppm	35	21	22	14	- 8
Onisimus litoralis (adult)					
35 ppm	27	30	88	90	+ 2
25 ppm	32	30	29	63	+34
25 ppm	30	30	29	40	+11
Onisimus litoralis (juvenile)					
25 ppm	36	21	59	80	+21
Fish					
Myoxocephalus quadricornis					
5 ppm	38	18	100	100	0
1 ppm	43	15	0	0	0
1 ppm	44	15	0	0	0
4 ppm	55	18	100	100	0

Abbreviations: N = number of animals.

[1]Expected mortality calculated from equation of probit line, when possible. Otherwise, expected mortality was that observed for the same concentration in the SLS bioassay.

[2]Observed mortality calculated from combined replicate data, including adjustment for mortality in seawater controls.

4.6 mg/L. LaRoche et al. (1970) found the 96 h LC_{50} of SLS for the estuarine fish Fundulus heteroclitus to be between 4.5 and 5.6 mg/L. Wells and Doe (1976) reported a 96 h LC_{50} of 6.2 mg/L for the same species. Menidia beryllina, Fundulus similus and Cyprinodon variegatus exhibit similar sensitivities to this chemical; reported 96 h LC_{50} determinations are 3, 5 and 10 ppm, respectively (Anderson, 1975). Fogels and Sprague (1977) reported the 96 h LC_{50} levels of SLS to be 8.0 and 8.1 mg/L for zebrafish (Brachydanio rerio) and flagfish (Jordanella floridae), respectively. In the list given by Abel (1974), 96 h median lethal concentrations of linear alkylate sulphonate detergents for seven species of fish vary from 0.6 to 6.4 mg/L.

There is little information on the effects of SLS on marine amphipods. Sensitivities of other aquatic invertebrates to SLS vary considerably. Tatem et al. (1976) reported 96 h TLm values (median tolerance limit; has the same numerical value as LC_{50}), for the grass shrimp, Palaemonetes pugio, of 69 to 162 ppm. For the sandworm, Nereis virens, LaRoche et al. (1970) reported a 96 h median lethal concentration of 13.5 ppm. Wells and Sprague (1976) found that first stage larvae of the American lobster (Homarus americanus) were comparatively sensitive to SLS (96 h LC_{50} of 0.72 ppm), whereas Wells and Doe (1976) found that fourth stage larvae were much less sensitive (96 h LC_{50} of 18.7 ppm). Tatem et al. (1976) cited Zillioux et al. (1973) as finding a 48 h TLm of 3.4 ppm SLS for the brine shrimp, Artemia sp., and Anderson (1975) reported a 24 h TLm of 2 ppm SLS for the opossum shrimp, Mysidopsis almyra.

During Study 2, in addition to SLS bioassays, three replicates of a single concentration of SLS were used concurrently with most bioassays of other toxicants. Expected mortality for the single concentration was estimated from the SLS bioassay results for each species and compared to the mortality observed in each test with a single concentration. Results are shown in Table 5.

Because Anonyx laticoxae and Gammarus oceanicus were found as 'contaminant' species, the concentrations of SLS used as controls in oil, Corexit and oil-Corexit bioassays were those chosen for A. nugax and G. setosus, respectively. Therefore, A. laticoxae, on the basis of SLS bioassay results, was not expected to exhibit any mortality in 25 ppm SLS and, indeed, did not. Gammarus oceanicus, on the basis of SLS bioassay results, was expected to exhibit 100% mortality in control SLS concentrations of 35 ppm. Observed mortality, however, was up to 78% less than expected. It is not possible to determine if these differences between observed and expected mortality reflected differences in the condition of test animals, because the number of animals used in the

control SLS mixtures was small, and the death of a single animal, therefore, made a large difference in percent mortality.

Gammarus setosus and Onisimus litoralis appeared to be of relatively uniform condition in different experiments. In the SLS controls of all toxicity tests with these organisms, the maximum difference in mortality was 34% of that which was expected on the basis of the SLS bioassays. The condition of Boeckosimus sp. appeared to be even less variable; this species demonstrated 3% mortality in 50 ppm SLS in three experiments, including the SLS bioassay.

The SLS concentrations used as controls in bioassays using Myoxocephalus quadricornis were all either above or below the range of concentrations which produced partial effects. Therefore, the difference of 0% between observed and expected effects does not indicate a uniform sensitivity of the test animals in these cases.

Recently, there has been some question as to the suitability of sodium lauryl sulphate as a reference toxicant. Fogels and Sprague (1977) cite Pessah et al. (1975) as reporting that SLS in solution decreased in toxicity with time and that SLS bioassays failed to detect differences in sensitivity between healthy and diseased fish. Five-year-old SLS, stored as a powder, has been reported to be approximately one-third as toxic as one-year-old SLS to zebrafish, flagfish and rainbow trout (Fogels and Sprague 1977). In solution, SLS may not have a homogeneous distribution; detergents tend to concentrate at the sides of the vessels, at the air-water interface, and perhaps on the surfaces of test organisms (Abel, 1974). In the present study, the formation of a precipitate was noted in SLS concentrations of 50 ppm and higher. Such precipitate formation has also been reported by Rice et al. (1977). When precipitate formation occurs, the concentration of SLS is not known with certainty. It is evident from the foregoing that the actual concentrations of SLS to which test animals are exposed in a bioassay, inevitably are somewhat uncertain and variable.

3.2.3 Corexit 9527. Results of bioassays conducted to determine the acute effects of Corexit 9527 on the test species are summarized in Table 6. The one fish species tested, Myoxocephalus quadricornis, was far more sensitive to this dispersant than were the amphipod species tested; the calculated 96 h LC_{50} was less than 40 ppm (µL/L), or less than 39.8 mg/L based on a density of 0.996 g/cm^3 (ESSO Chemicals, 1976). This is a greater sensitivity than that found by Doe and Harris (1976) for Salmo gairdneri

TABLE 6 MEDIAN LETHAL CONCENTRATIONS (96 h LC_{50}) OF COREXIT 9527 FOR TEST SPECIES, AND CONDITIONS UNDER WHICH THEY WERE DETERMINED

Unless otherwise indicated, 96 h LC_{50} values were calculated using a computerized probit analysis (Davies, 1971). Values within parentheses after the 96 h LC_{50} are 95% confidence limits.

Species	Study	Exp. No.	N	96 h LC_{50} (ppm)	SLS Control		Animal Length (mm)			Temperature Range (°C)[1]	A.P. (h)
					C (ppm)	%D	Mean	s.d.	Range		
Amphipods											
Anonyx laticoxae	2	40	13	>140	25	0.0	30.1	2.0	25-34	4.0-5.5	18
Anonyx nugax	2	40	47	104 (97-111)	25	100.0	35.1	5.2	25-44	4.0-5.5	18
Boeckosimus edwardsi	1	7	90	>80	-	-	9.9	0.8	9-14	-0.5-5.5	24-96
Boeckosimus sp.	2	53	96	>175	-	-	13.9	3.2	9-23	4.4-5.5	120
Gammarus oceanicus	2	22	15	>80	35	100.0	29.8	3.2	22-36	3.0-5.2	16
Gammarus setosus	2	22	44	175 (38-803)	35	27.3	26.9	3.8	20-35	3.0-5.2	16
Onisimus litoralis	2	32	119	115 (80-160)[2]	25	63.3	16.6	0.9	14-19	3.8-6.3	36
Onisimus litoralis (juvenile)	1	2	80	>70	-	-	10.2	1.1	8-12	-2.0-6.0	24-96
Fish											
Myoxocephalus quadricornis	2	44	60	<40	1	0.0	19.8	1.0	17-22	3.5-5.7	16

Abbreviations: N = number of animals; C = concentration; %D = percent mortality; s.d. = standard deviation; A.P. = acclimation period.

[1]Refrigerator air temperatures, Study 1; experimental mixture temperatures, Study 2.

[2]Interpolated from data plotted on semi-log paper; figures in brackets give the range of concentrations within which 50% mortality would occur.

fingerlings (96 h LC_{50} of 140 to 233 mg/L). It is probably also greater than that found for the freshwater zebrafish (Brachydanio rerio), for which a 48 h LC_{50} was calculated as 550 ppm (ESSO Chemicals, 1976).

Amphipods were more resistant to Corexit 9527. The 96 h LC_{50} determinations for the species tested were all greater than 70 ppm. Median lethal concentrations for three amphipod species were calculated in Study 2. Ninety-six h LC_{50} values of Corexit 9527 for Anonyx nugax, Onisimus litoralis and Gammarus setosus were 104, 115 and 175 ppm, respectively. Boeckosimus edwardsi displayed no mortality in the highest concentration (80 ppm) used in Study 1; Boeckosimus sp., in Study 2, displayed no mortality at 175 ppm Corexit 9527.

Little information on the acute toxicity of Corexit 9527 to marine invertebrates can be found in the literature. A 48 h LC_{50} of 6600 ppm of Corexit 9527 was reported by ESSO Chemicals (1976) for the brown shrimp, Crangon crangon, but it is not clear whether this was calculated as ppm of Corexit 9527 concentrate or as ppm of a diluted formulation.

3.2.4 Prudhoe Bay Crude Oil and Oil-Corexit Mixtures. Analysis by gas chromotography, in Study 2, confirmed the presence of volatile aromatics in the Prudhoe Bay crude oil used in the toxicity bioassays and indicated that the oil had not been 'weathered' significantly prior to use (see Figure 14).

During Study 1, relatively few tests were performed to check efficiencies of oil extraction at different added oil concentrations. As a result, efficiencies were lumped into four categories:

1) oil-water mixtures, 0 h after preparation;
2) oil-water mixtures after 24 h animal exposure;
3) oil-Corexit-water mixtures, 0 h after preparation; and
4) oil-Corexit-water mixtures after 24 h animal exposure.

Extraction efficiencies of hydrocarbons depended on the type of mixture (oil-water or oil-Corexit-water) and the sampling time (0 h after preparation or after 24 h animal exposure). In the oil-water mixtures there was a significant difference between the extraction efficiencies at 0 h and at 24 h; mean efficiencies at the two times were 96.2% and 86.7%, respectively (see Table 7). This temporal difference was not apparent in the oil-Corexit-water mixtures (73.9 vs 71.5%). Efficiencies also differed significantly

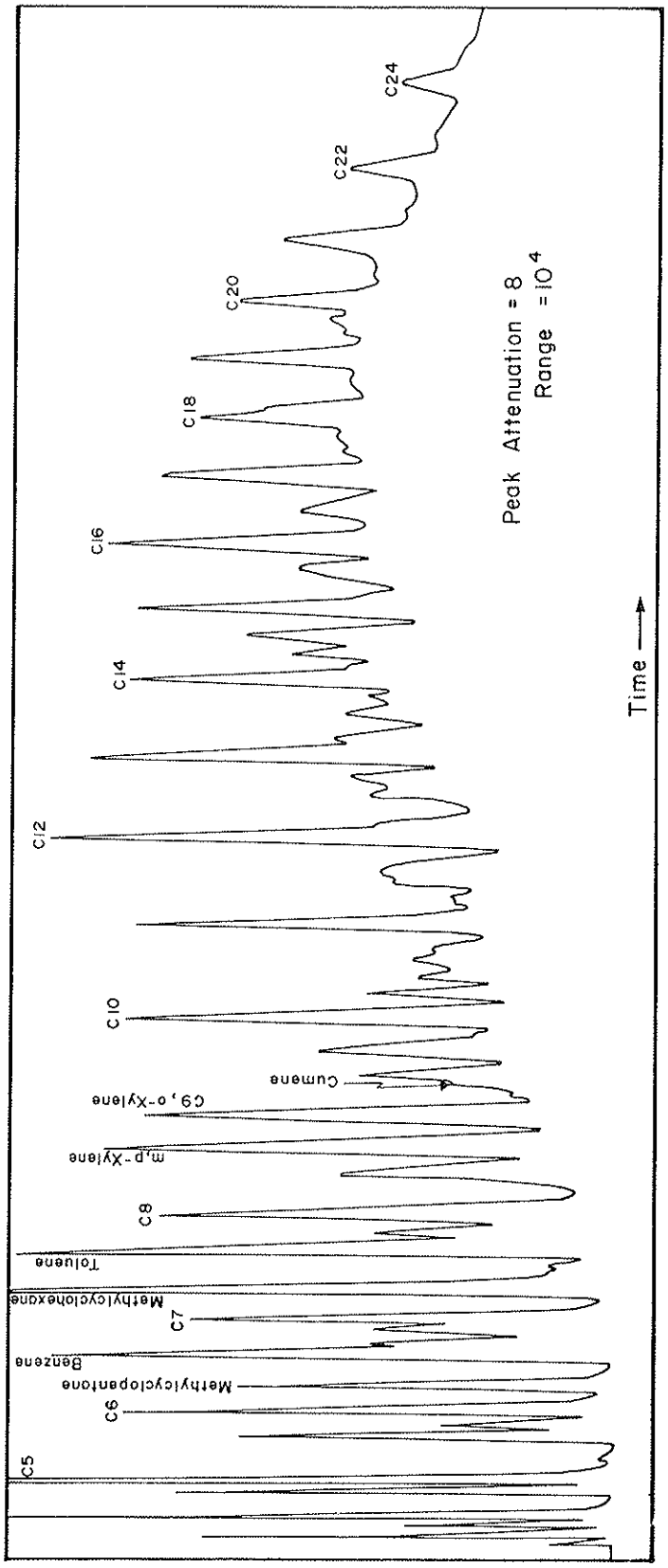


FIGURE 14 GAS CHROMATOGRAM OF PRUDHOE BAY CRUDE OIL USED IN TOXICITY TESTS.

TABLE 7 COMPARISON OF HYDROCARBON EXTRACTION EFFICIENCIES IN OIL-WATER AND OIL-COREXIT-WATER MIXTURES AT 0 h AND 24 h USING TWO-SIDED MANN-WHITNEY U TESTS

	Individual Statistics			Test Statistics	
Comparison	x(%)	s.d.	n	U	P
Oil-Water, 0 h and Oil-Water, 24 h	96.2 86.7	2.2 12.9	9 17	9.5	P<0.002
Oil-Corexit-Water, 0 h and Oil-Corexit-Water, 24 h	73.9 71.5	16.3 18.6	19 9	82.0	P>0.05
Oil-Water, 0h and Oil-Corexit-Water, 0 h	96.2 73.9	2.2 16.3	9 19	5.5	P<0.002
Oil-Water, 24 h and Oil-Corexit-Water, 24 h	86.7 71.5	12.9 18.6	17 9	20.5	P<0.002

Abbreviations: x = mean; s.d. = standard deviation; n = number of tests; U = test statistic; P = probability.

between samples taken from oil-water mixtures and oil-Corexit-water mixtures at 0 h (96.2% vs 73.9%) and at 24 h (86.7% vs 71.5%). Oil extraction efficiencies were greatest in 0 h oil-water mixtures (mean of 96.2%), less in oil-water mixtures at 24 h (86.7%), and lowest in the oil-Corexit-water mixtures (73.9% and 71.5%). Due to lack of information about the effects of oil concentration on extraction efficiency and due to the relatively few extraction efficiency tests performed, it was not felt advisable to correct oil exposure concentrations for extraction efficiency in Study 1.

In Study 2, more tests were done in order to evaluate the effects of nominal oil concentration on extraction efficiency. The efficiency of the first hexane extraction of oil from the oil-water mixtures varied from 85.4% to 97.6% in the samples taken at 0 h (n = 27) and from 82.1% to 100% in the samples taken at 24 h (n = 24). Efficiencies for samples taken at either 0 h or 24 h showed no significant relationship to added oil concentrations when the data were analyzed by either linear or exponential regression (see Table 8).

TABLE 8 SIGNIFICANCE (two-tailed t-Test) OF REGRESSION OF EFFICIENCY OF EXTRACTION ON NOMINAL OIL CONCENTRATION IN OIL-WATER MIXTURES AT 0 h AND 24 h.

Exposure Time	d.f.	Linear Regression		Exponential Regression	
		t	P	t	P
0 h	2	0.874	>0.4	0.893	>0.4
24 h	2	1.809	>0.2	1.802	>0.2

Abbreviations: d.f. = degrees of freedom; t = t-statistic; P = probability.

In contrast to Study 1, a two-sided Mann-Whitney U-test detected no significant difference between the values from 0 h and 24 h samples (P>0.2). Therefore, extraction efficiencies from all added oil concentrations and from both 0 h and 24 h determinations were combined and averaged (mean = 91.2%, s.d. = 4.8%) and a single correction factor (1.096) was determined. This factor was applied to all oil concentrations measured in the extracts of oil-water mixtures during Study 2, to determine more realistic exposure concentrations.

Extractions of hydrocarbons from oil-Corexit-water mixtures, as in Study 1, were less efficient and the efficiencies more variable than those of the oil-water mixtures. Efficiency depended upon nominal oil concentration as well as exposure time. In order to determine factors to correct for extraction efficiencies, exponential curves were fitted by regression analysis to the data from samples taken at 0 h (Figure 15) and 24 h (Figure 16). Correction factors for each nominal oil concentration were determined from these curves (see Table 9).

It is emphasized that results from Study 1 have not been corrected for extraction efficiency, whereas those of Study 2 have been corrected. The effect of this methodological difference is most pronounced in the results of the oil-Corexit-water bioassays in which efficiency of oil extraction was lowest.

The relationships between nominal (added) and measured (exposure) oil concentration in the oil-water and oil-Corexit-water mixtures are illustrated in Figure 17. The measured value for an experiment is the average of mean 0 and mean 24 h concentration determinations. Measured hydrocarbons in oil-Corexit-water mixtures increased in an almost linear fashion, as the nominal oil concentration increased, throughout the range of

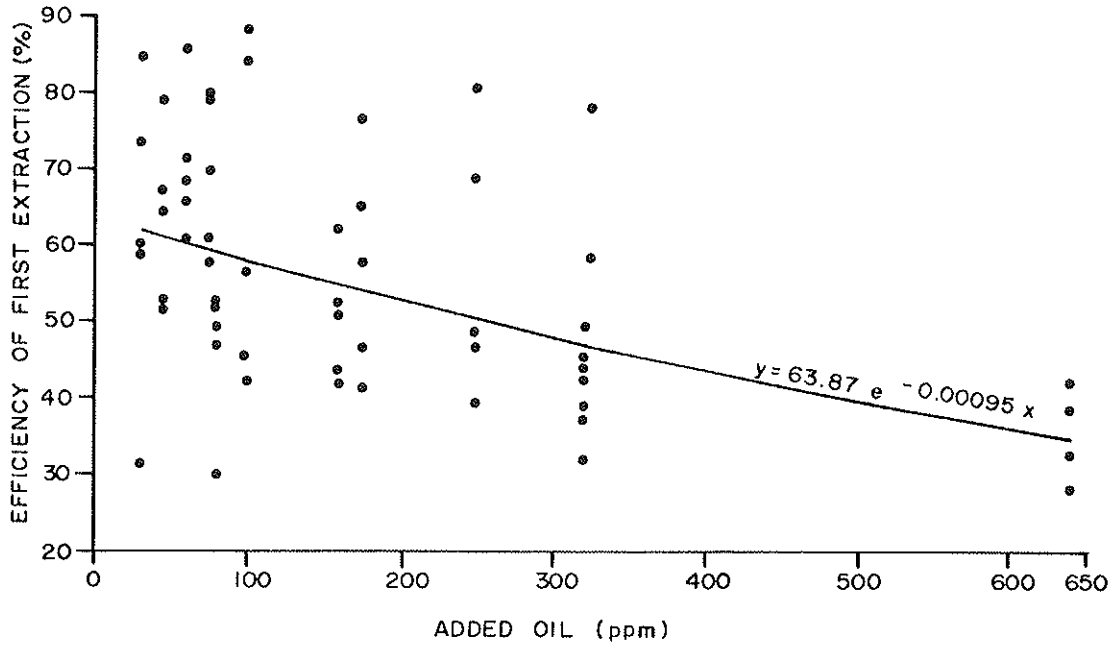


FIGURE 15 EFFICIENCY OF INITIAL OIL EXTRACTION FROM OIL-COREXIT-WATER MIXTURES AT 0 h. Regression coefficient = - 9.52 x 10^{-4}, P <0.001 (two-tailed t-test)

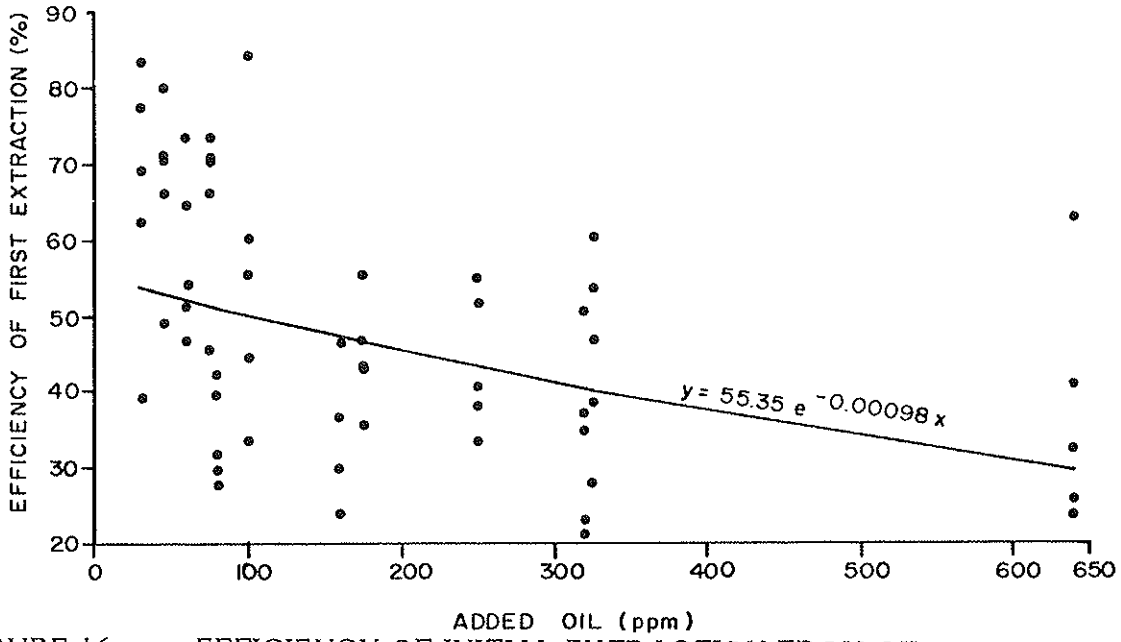


FIGURE 16 EFFICIENCY OF INITIAL EXTRACTION FROM OIL-COREXIT-WATER MIXTURES AFTER 24 h. Regression coefficient = - 9.76 x 10^{-4}, P <0.01 (two-tailed t-test)

TABLE 9 EFFICIENCIES OF INITIAL OIL EXTRACTION AND RESULTANT CORRECTION FACTORS FOR OIL-COREXIT-WATER MIXTURES AT EXPOSURE TIMES OF 0 h AND 24 h.

Added Oil (ppm)	Exposure Time 0 h		24 h	
	Efficiency(%)[1]	Correction Factor	Efficiency(%)[1]	Correction Factor
30	62.1	1.6103	53.8	1.8587
45	61.2	1.6340	53.0	1.8868
60	60.3	1.6584	52.2	1.9157
75	59.5	1.6807	51.4	1.9455
80	59.2	1.6892	51.2	1.9531
100	58.1	1.7212	50.2	1.9920
160	54.9	1.8215	47.3	2.1142
175	54.1	1.8484	46.7	2.1413
250	50.4	1.9841	43.4	2.3041
320	47.1	2.1231	40.5	2.4691
325	46.9	2.1322	40.3	2.4814
640	34.7	2.8818	29.6	3.3784

[1]Based on curves in Figures 15 and 16.

nominal oil concentrations used in the toxicity bioassays. In contrast, measured hydrocarbons in the oil-water mixtures increased only slightly with increases in nominal concentrations. It is evident that the addition of Corexit to oil-water mixtures substantially increased the concentrations of hydrocarbons in the water column over those in oil-water mixtures that had been dispersed solely by mechanical means.

One oil-water mixture and one oil-Corexit-water mixture (both with a nominal oil concentration of 200 µL/L) were analyzed by gas chromatography. The results indicate that the concentration of particulate (dispersed) hydrocarbons was much higher in the oil-Corexit-water mixture than in the oil-water mixture (see Table 10). Concentrations of 'dissolved' hydrocarbons were also higher in the oil-Corexit-water mixture. However, 'dissolved' hydrocarbons were defined as those hydrocarbons that passed through a filter which had a 5 µm diameter pore size, and dispersed oil droplets can be smaller than 5 µm. Stokes and Harvey (1973) found that the addition of detergents to oil-water

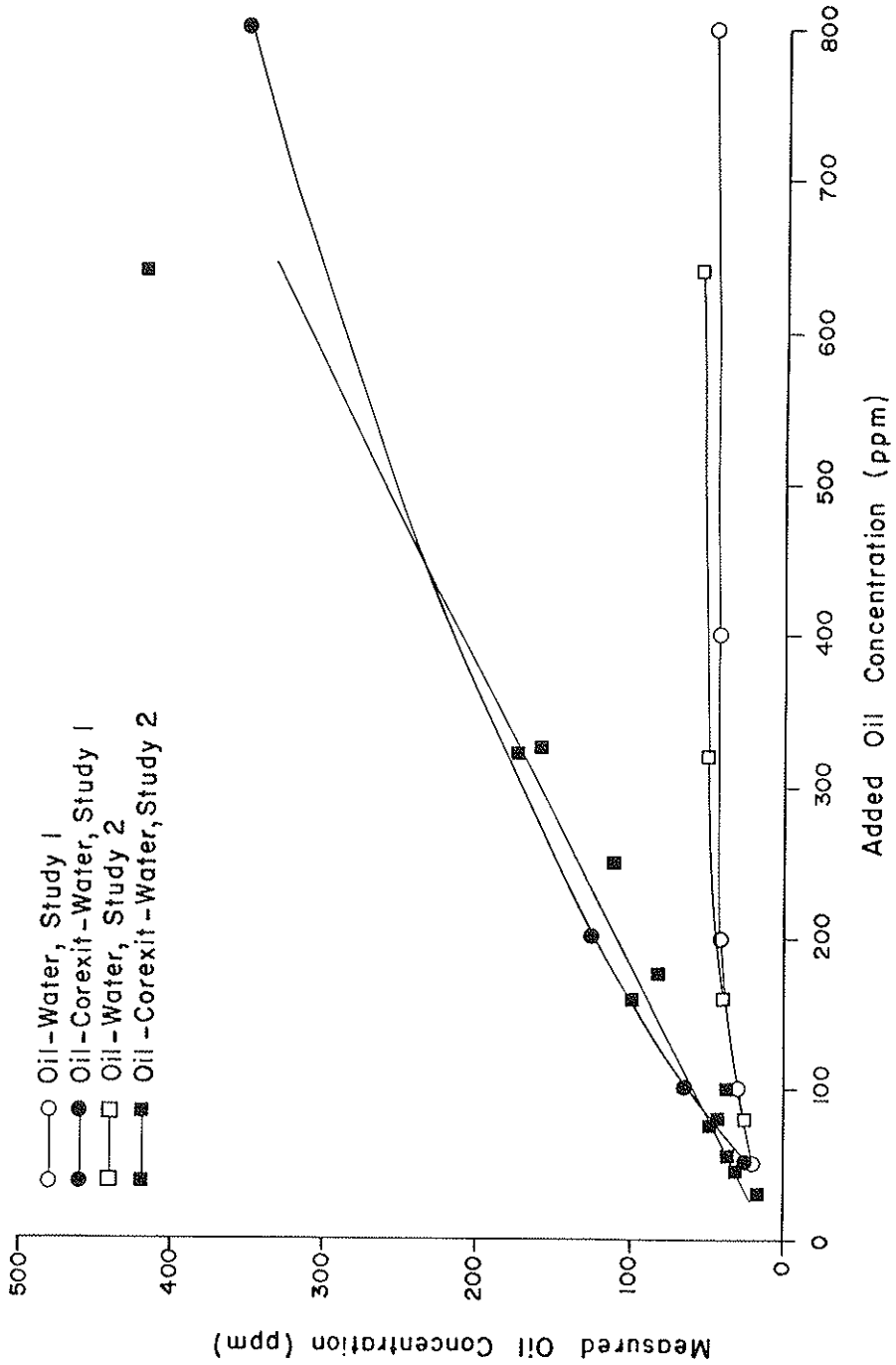


FIGURE 17 ADDED OIL CONCENTRATION vs MEASURED OIL CONCENTRATION IN (average of means 0 h and 24 h concentrations) IN STUDIES 1 AND 2.

TABLE 10 CONCENTRATION OF GAS-STRIPPABLE HYDROCARBONS (mg/L), AS DETERMINED BY GAS CHROMATOGRAPHY, IN OIL-WATER AND OIL-COREXIT- WATER MIXTURES AT 0 h AND AFTER 24 h. Nominal oil concentration was 200 µL/L.

Sample	Concentration of 'Dissolved' Hydrocarbons	Concentration of Particulate Hydrocarbons	Total
Oil-Water, 0 h	1.20	4.20	5.40
Oil-Water, 24 h	0.32	2.80	3.12
Oil-Corexit-Water, 0 h	3.50	17.90	21.40
Oil-Corexit-Water, 24 h	0.81	12.30	13.11

mixtures increased the number of oil droplets in the small-diameter size range (as small as 2 to 3 µm). It is thought, therefore, that the apparently higher 'dissolved' hydrocarbon concentration in the oil-Corexit-water mixture was due, at least in part, to particulate oil less than 5 µm in diameter that passed through the filter.

The much higher concentration of total hydrocarbons, as measured by gas chromatography, in the oil-Corexit-water mixture than in the oil-water mixture, was due primarily to the higher concentration of particulate hydrocarbons in the oil-Corexit-water mixture. Total hydrocarbon concentration was approximately four times greater in the oil-Corexit-water mixture than in the oil-water mixture, and approximately 90% of the total hydrocarbons in the oil-Corexit-water mixture were of a particulate nature (see Table 10). The ratio of dissolved to particulate hydrocarbons was greater in the oil-water mixture (1:3.5 at 0 h and 1:8.8 at 24 h) than in the oil-Corexit-water mixture (1:5.1 at 0 h and 1:15.2 at 24 h).

Aromatic compounds were the predominant gas-strippable hydrocarbons in both the oil-water and oil-Corexit-water mixtures (see Figures 18 and 19). They were found in the following approximate proportions:

benzene	4
toluene	4
m- and p-xylenes	2
o-xylene	1
naphthalene	1

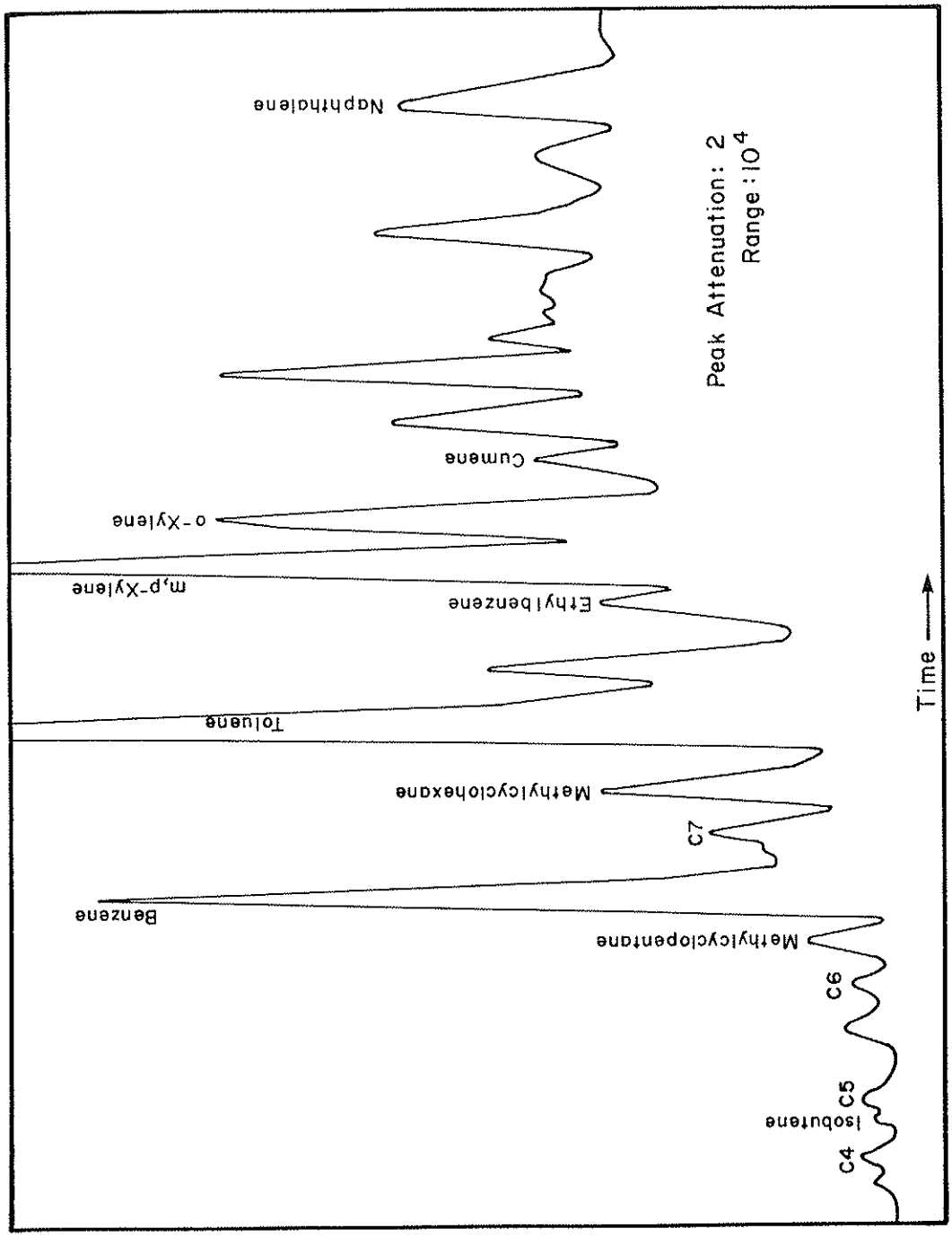


FIGURE 18 GAS CHROMATOGRAM OF AN UNFILTERED SAMPLE OF A MIXTURE OF PRUDHOE BAY CRUDE OIL AND WATER. The sample was taken at 0 h.

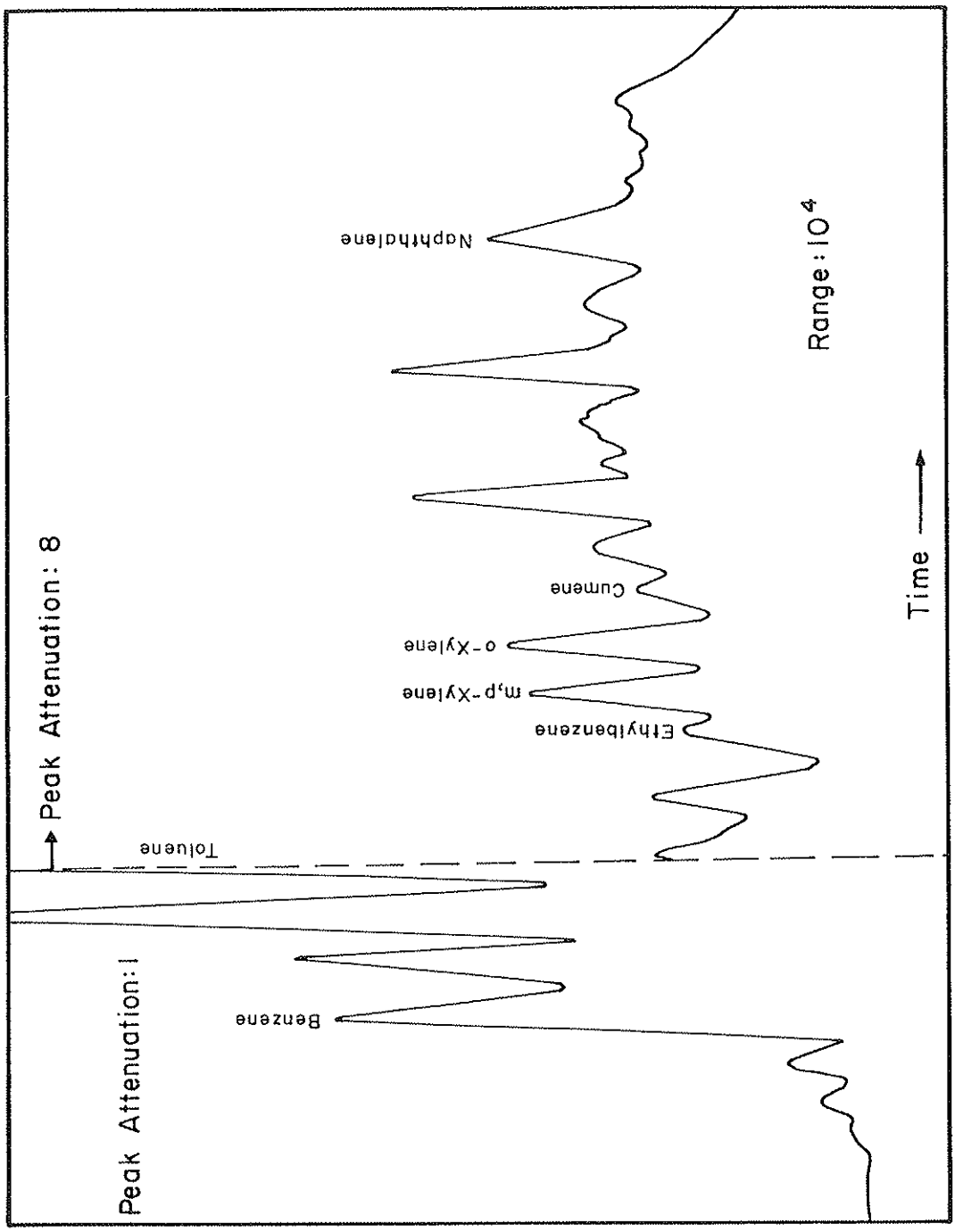


FIGURE 19 GAS CHROMATOGRAM OF AN UNFILTERED SAMPLE OF A MIXTURE OF PRUDHOE BAY CRUDE OIL, COREXIT 9527 AND WATER. The sample was taken at 0 h.

The oil-Corexit-water mixture contained a higher concentration of alkanes than did the oil-water mixture. These alkanes, because of their low aqueous solubility, would presumably be present in the particulate fraction.

Water soluble aromatics are generally considered to be the compounds that cause toxicity in oil-water mixtures. Their toxicity, in the short-term at least, is greatest when they are dissolved in the aqueous phase, from which they can be readily incorporated by aquatic organisms. Therefore, oil-water emulsions with a high dissolved to particulate hydrocarbon ratio would be expected to be more toxic than emulsions with the same actual measured hydrocarbon concentration but with a lower dissolved to particulate hydrocarbon ratio.

Results of the bioassays using Prudhoe Bay crude oil in oil-water mixtures are summarized in Table 11. In several cases, the highest hydrocarbon concentrations attained in the water column, in oil-water mixtures, were not high enough to produce mortality. In these instances 96 h LC_{50} values could not be determined.

Of all the species tested, Anonyx nugax appeared to be most sensitive to hydrocarbons in oil-water mixtures. It displayed a similar sensitivity in both studies (96 h LC_{50} values of 32-43 ppm in Study 1 and 32 ppm in Study 2). The young fourhorn sculpins and the amphipod Boeckosimus edwardsi appeared to be similar in sensitivity to Prudhoe Bay crude oil (96 h LC_{50} values of 44 ppm and 42-43 ppm, respectively) and were the next most sensitive species. Onisimus litoralis juveniles were slightly more resistant (96 h LC_{50} values of 49 and 68 ppm in Study 1 and Study 2, respectively) and were similar in sensitivity to the amphipod Gammarus setosus (96 h LC_{50} value of 56 ppm). For the copepod, Calanus hyperboreus, the 96 h median lethal concentration of Prudhoe Bay crude oil was 73 ppm within broad 95% confidence limits of 51 to 103 ppm.

It was not possible to calculate reliable 96 h LC_{50} estimates for the remaining test organisms since, generally, mortality was observed in only the highest oil concentrations used.

Comparison of these results with those of other studies is difficult because of varying methods and analytical procedures. Percy and Mullin (1975), using methods and analytical techniques similar to mine, investigated the effects of crude oils on arctic marine invertebrates. Calculated from their data, the 96 h LC_{50} of Norman Wells crude oil for the amphipod Boeckosimus affinis was 32 ppm. Linden (1976) found that the 96 h median lethal concentration of a Venezuelan crude oil, in the form of an oil-water mixture, for adult Gammarus oceanicus, was 550 µL/L (calculated on the basis of nominal

TABLE 11 MEDIAN LETHAL CONCENTRATIONS (96 h LC_{50}) OF PRUDHOE BAY CRUDE OIL IN OIL-WATER MIXTURES FOR TEST SPECIES, AND CONDITIONS UNDER WHICH THEY WERE DETERMINED.

Unless otherwise indicated, 96 h LC_{50} values were calculated using a computerized probit analysis (Davies, 1971). Values within parentheses after the 96 h LC_{50} are 95% confidence limits.

Species	Study	Exp. No.	N	96 h LC_{50} (ppm)	SLS Control C (ppm)	SLS Control %D	Animal Length (mm) Mean	s.d.	Range	Temperature Range (°C)[1]	A.P. (h)
Amphipods											
Anonyx laticoxae	2	51	6	>51	25	0.0	31.3	2.1	28-35	4.5-6.5	72
Anonyx nugax	1	13	44	32-43	-	-	32.5	5.0	24-41	-1.0-3.0	24-96
	1	18	42	32-43	-	-	33.2	3.9	25-43	-1.5-5.0	24-96
	2	51	54	32 (27-37)	25	66.7	26.8	4.6	15-40	4.5-6.5	72
Boeckosimus edwardsi	1	6, 16[2]	177	44 (43-45)[3]	-	-	9.7	1.1	6-15	-0.5-7.0	24-96
Boeckosimus sp.	2	46	119	>59	50	3.3	16.4	2.9	11-24	4.2-5.5	100
Gammarus oceanicus	2	23	10	>55	35	60.0	28.3	3.0	23-36	5.0-13.0	37
Gammarus setosus	2	23	47	56 (51-62)	35	41.6	29.1	3.6	22-38	5.0-13.0	37
	2	31	84	>53	35	26.5	23.9	3.3	18-36	3.8-5.2	24
Onisimus litoralis (adult)	2	27	108	>47	35	90.0	16.6	0.8	14-19	3.9-5.5	85
Onisimus litoralis	1	3	82	49 (44-55)[3]	-	-	10.1	0.9	9-12	-1.5-8.0	24-96
(juvenile)	2	36	124	68 (62-75)[3]	25	80.0	6.9	0.7	6-9	4.2-5.5	70
Copepods											
Calanus hyperboreus	1	11	75	73 (51-103)	-	-	No Length Data			-0.5-5.0	24-96
Fish											
Myoxocephalus	2	38	72	43 (37-49)	5	100.0	18.0	1.3	14-21	4.5-6.0	72
quadricornis	2	43	60	42 (39-46)	1	0.0	19.8	1.1	16-23	3.5-5.7	64

Abbreviations: N = number of animals; C = concentration; %D = percent mortality; s.d. = standard deviation; A.P. = acclimation period.

[1] Refrigerator air temperatures, Study 1; experimental mixtures, Study 2.

[2] Results of two experiments combined to calculate one 96 h LC_{50} value.

[3] Estimate from graphical probit analysis (Litchfield and Wilcoxon, 1949).

oil concentration). This appears to be similar to the 96 h LC_{50} of Prudhoe Bay crude oil determined in the present study for Gammarus setosus; a measured concentration of 56 ppm (Table 11) represents a nominal concentration of approximately 575 ppm (see Figure 17).

Results of bioassays determining the toxic effects of Prudhoe Bay crude oil dispersed in oil-Corexit-water mixtures are summarized in Table 12. The 96 h LC_{50} of Prudhoe Bay crude oil dispersed by Corexit on Anonyx nugax appeared to vary somewhat between the two studies. In Study 1 it occurred between 64 and 213 ppm, whereas in Study 2 it was calculated as 45 ppm. This difference may be larger in reality, since concentrations of oil were not corrected for extraction efficiency in Study 1. The fourhorn sculpin was relatively sensitive to the oil-Corexit-water mixtures (96 h LC_{50} = 59 ppm). Gammarus setosus displayed differing sensitivities in two separate experiments (96 h LC_{50} values of 83 and 138 ppm). The 96 h LC_{50} values for other amphipod species (Anonyx laticoxae, Boeckosimus sp., Gammarus oceanicus and adult Onisimus litoralis) varied from 105 to 162 ppm. The one copepod tested, Calanus hyperboreus, appeared to be among the most resistant of the species tested (96 h LC_{50} = 196 ppm).

The 96 h LC_{50} determinations, when based on hydrocarbon concentrations measured by fluorescence spectroscopy, were lower, for at least five species (Anonyx nugax, Boeckosimus edwardsi, Gammarus setosus, Calanus hyperboreus and Myoxocephalus quadricornis), in oil-water mixtures than in oil-Corexit-water mixtures (Tables 11 and 12). Results for the other tested species are less well defined; however, mortality data are generally consistent with the conclusion that the toxicity of Prudhoe Bay crude oil hydrocarbons is somewhat greater in oil-water mixtures than in oil-Corexit-water mixtures, based on measured as opposed to nominal oil concentrations.

The higher toxicity of hydrocarbons (based on measured exposure concentration) in oil-water mixtures than in oil-Corexit-water mixtures may have resulted from a higher proportion of water-soluble aromatic compounds in the oil-water mixtures. It is generally thought that the water soluble aromatics in crude oils are, in oil-water mixtures, the agents that cause mortality.

In the present study, mortalities observed in oil-Corexit-water mixtures were higher than those in oil-water mixtures of the same nominal oil concentration. Similar results have been obtained in other studies (Swedmark et al., 1973; Linden 1975; Percy and Mullin, 1975). This higher mortality observed in oil-dispersant mixtures is thought to be

TABLE 12 MEDIAN LETHAL CONCENTRATIONS (96 h LC_{50}) OF PRUDHOE BAY CRUDE OIL IN OIL-COREXIT-WATER MIXTURES FOR TEST SPECIES, AND CONDITIONS UNDER WHICH THEY WERE DETERMINED.

Unless otherwise indicated, 96 h LC_{50} values were calculated using a computerized probit analysis (Davies, 1971). Values within parentheses after the 96 h LC_{50} are 95% confidence limits.

Species	Study	Exp. No.	N	96 h LC_{50} (ppm)	SLS Control C (ppm)	SLS Control %D	Animal Length (mm) Mean	s.d.	Range	Temperature Range (°C)[1]	A.P. (h)
Amphipods											
Anonyx laticoxae	2	48	5	112 (73-169)[2]	-	-	30.7	1.8	28-33	4.4-6.5	20
Anonyx nugax	1	17	45	64-213	-	-	32.9	3.1	25-38	-1.0-7.0	24-96
	1	18	45	64-213	-	-	31.5	4.1	24-40	-1.5-5.0	24-96
	2	48	55	45 (34-61)	25	48.9	35.9	5.3	25-45	4.4-6.5	20
Boeckosimus edwardsi	1	14	82	64-213	-	-	10.0	0.8	6-11	-7.0-5.0	24-96
Boeckosimus sp.	2	45	120	162 (135-195)	50	3.3	17.8	2.4	13-23	4.0-5.5	52
Gammarus oceanicus	2	24	20	105 (73-153)[3]	35	33.3	27.9	4.7	21-37	5.0-13.0	41
Gammarus setosus	2	24	55	138 (114-168)	35	48.2	28.3	4.0	21-40	5.0-13.0	41
	2	35	84	83 (70-98)	35	14.3	24.5	4.1	17-37	4.8-6.5	120
Onisimus litoralis (juvenile)	1	3	81	24-213	-	-	10.1	1.1	8-13	-1.5-8.0	24-96
Onisimus litoralis (adult)	2	30	118	138 (118-161)	25	40.0	16.5	0.9	14-18	3.7-5.2	72
Copepods											
Calanus hyperboreus	1	11	74	196 (161-238)	-	-	No Length Data			-0.5-5.0	24-96
Fish											
Myoxocephalus quadricornis	2	55	71	59 (40-87)	4	100.0	19.6	0.9	16-21	4.4-5.5	96

Abbreviations: N = number of animals; C = concentration; %D = percent mortality; s.d. = standard deviation; A.P. = acclimation period.

[1] Refrigerator air temperatures, Study 1; experimental mixture temperatures, Study 2.

[2] Interpolated from data plotted on semi-log paper; figures in brackets give the range of concentrations within which 50% mortality would occur.

[3] Estimate from 10% trimmed Spearman-Karber analysis (Hamilton et al. 1977).

merely a result of much higher oil concentrations in the water column of chemically dispersed mixtures. The results of some other studies support this interpretation. Trudel (1978) found that Lago Medio crude oil was equally toxic, based on measured concentrations, to natural phytoplankton populations (based on inhibition of carbon-14 uptake) whether chemically dispersed using Corexit 9527, or mechanically dispersed. Vaughan (1973) found that, based on nominal concentration, South Louisiana crude oil had a 96 h TLm for shiner perch (Cymatogaster aggregata) juveniles of 100 ppm when chemically dispersed and 840 ppm when mechanically dispersed. However, based on estimated exposure concentrations of oil, chemically and mechanically dispersed oil had 96 h TLm levels of 14 ppm and 15 ppm, respectively. Venezia and Fossato (1977) concluded from a study of the effects of Kuwait crude oil and Corexit 7664 on the harpacticoid copepod, Tisbe bulbisetosa, that 'the reinforcement of the toxic effect of oil plus Corexit is due...to the higher actual hydrocarbon concentration in a suspension resulting from such a mixture'.

3.3 Post-Exposure Period

In many of the bioassays conducted during Study 2, animals remaining alive after the 96 h exposure period were transferred to fresh seawater for a 24 h observation period. Mortality data recorded during this period are summarized in Table 13.

The sole purpose of such a post-exposure period was to determine if mortality of animals continued after they were returned to clean water, following the 96 h exposure period. In many cases, mortality exceeding that of the seawater controls, was observed during the post-exposure period, indicating that irreversible damage had been caused by exposure to the toxicants for 96 h. The absence of mortality during the 24 h post-exposure period does not necessarily indicate that the effects of the toxicant had been nil or temporary; mortality might have occurred if the animals had been kept for longer than 24 h after exposure to the toxicants, and sublethal effects were not studied.

3.4 Relative Species Sensitivity

It must be emphasized that the sensitivity of an organism to a given toxicant is not constant. Fogels and Sprague (1977) cite Brown (1968) as finding that 48 h LC_{50} values of numerous toxicants varied by a factor of 2.5 over a period of 9 months. Tatem et al. (1976) found that grass shrimp (Palaemonetes pugio), collected at different times of year, varied in their sensitivity to SLS by as much as a factor of 2.3. Sensitivity to a given toxicant may even vary at different times of day (Spieler et al., 1977). Fogels and

TABLE 13 SUMMARY OF PERCENT MORTALITY, DURING POST-EXPOSURE PERIOD, OF ANIMALS REMAINING ALIVE AFTER 96 h EXPOSURE PERIOD.

Toxicant and Species	Exp. No.	Increasing (→) Toxicant Concentration				
		Control	1	2	3	4
Sodium Lauryl Sulphate						
Anonyx laticoxae	26	0	0	0	0	0
Anonyx nugax	26	0	0	-	-	-
Gammarus oceanicus	21	0	25	29	-	100
Gammarus setosus	21	0	0	29	0	50
" "	34	0	0	0	0	25
Onisimus litoralis (adult)	25	0	0	4	0	-
" " "	29	2	4	4	13	20
Onisimus litoralis (juvenile)	33	0	10	7	10	40
Myoxocephalus quadricornis	42	5	8	0	9	10
Corexit 9527						
Anonyx laticoxae	40	0	0	0	0	80
Anonyx nugax	40	0	9	0	33	-
Gammarus oceanicus	22	0	0	0	0	0
Gammarus setosus	22	0	0	0	8	0
Onisimus litoralis (adult)	32	0	7	0	-	-
Prudhoe Bay Crude Oil						
Gammarus oceanicus	23	0	0	0	0	0
Gammarus setosus	23	0	0	0	0	0
" "	31	0	0	0	0	10
Onisimus litoralis (adult)	27	0	0	4	0	0
Onisimus litoralis (juvenile)	36	0	0	0	13	9
Myoxocephalus quadricornis	38	0	0	0	11	-
Prudhoe Bay Crude Oil - Corexit 9527						
Gammarus oceanicus	24	14	0	0	-	-
Gammarus setosus	24	0	0	25	0	0
" "	35	0	5	18	14	0
Onisimus litoralis (adult)	30	0	0	0	25	100

Sprague (1977) state that 'results of short-term bioassays should...be regarded only as estimates, within approximately one order of magnitude, of the acute toxicity of a substance'.

With this variability in mind, the sensitivities, to a particular toxicant, of all species tested in this study were remarkably similar (see Table 14). Only the sensitivity of the fourhorn sculpin to the detergent SLS and possibly to Corexit 9527 was markedly different from the reactions of the amphipod species.

Some consistent patterns in relative sensitivity can be discerned, however. Of the organisms tested, fourhorn sculpin (Myoxocephalus quadricornis) "young-of-the-year" and the large amphipod Anonyx nugax appeared to be the most sensitive to all four toxicant mixtures (Table 14). Myoxocephalus quadricornis was the species most sensitive to sodium lauryl sulphate and Corexit 9527, while Anonyx nugax appeared to be the species most sensitive to the Prudhoe Bay crude oil in both oil-water and oil-Corexit-water mixtures. This reversal in order of sensitivity may be a result of different modes of toxic action for the detergents and oil mixtures. There is no reason to expect that invertebrates and fish will react in the same way to a given toxicant (Sprague, 1970).

Other amphipods tested were slightly more resistant to the toxicant mixtures. Boeckosimus sp. appeared to be one of the most resistant species tested and Anonyx laticoxae also appeared to be relatively resistant, especially when compared to A. nugax. It is interesting to note that, while these three species were all collected from approximately the same depth in the sublittoral zone, their sensitivities to the toxicant mixtures were different. This seems to contradict the theory that sublittoral species are generally more sensitive to pollution than littoral species, because of their adaptation to a less variable environment (Swedmark et al., 1973). However, the biology of Boeckosimus sp. and Anonyx laticoxae is not well known and it may be that these species do inhabit some areas of more variable nature. The three intertidal amphipods tested, Onisimus litoralis, Gammarus oceanicus and G. setosus, appeared to be relatively similar in sensitivity to the toxicant mixtures. None of these species appeared to be the most or least sensitive to any of the toxicants. The copepod Calanus hyperboreus, despite its delicate appearance, was remarkably resistant to Prudhoe Bay crude oil. However, it is felt that, due to fouling, if nothing else, these copepods would not have survived, had they been placed in a clean environment.

TABLE 14 BEST ESTIMATES OF 96 h LC_{50} VALUES, RANGES WITHIN WHICH 96 h LC_{50} VALUES ARE EXPECTED TO OCCUR AND RANKING IN SENSITIVITY OF TESTED SPECIES. The most sensitive species is given the rank of 1.

Species	Sodium Lauryl Sulphate			Corexit 9527			Prudhoe Bay Crude Oil			Prudhoe Bay Crude Oil Dispersed with Corexit 9527		
	LC_{50}[1]	Range[2]	Rank	LC_{50}[1]	Range[2]	Rank	LC_{50}[1]	Range[2]	Rank	LC_{50}[1]	Range[2]	Rank
Amphipods												
Anonyx laticoxae	41	35-45	6	>140	-	4-9	>51	-	4-10	112	73-169	5
Anonyx nugax	20	15-31	2	104	97-111	2	32	27-43	1	45	34-213	1
Boeckosimus edwardsi	>40	-	5-9	> 80	-	2-9	44	43-45	3	-	64-213	3-10
Boeckosimus sp.	>50	-	6-9	>175	-	4-9	>59	-	6-10	162	135-195	7
Gammarus oceanicus	30	25-35	5	> 80	-	2-9	>55	-	4-10	105	73-153	3
Gammarus setosus	66	39-186	7	175	38-803	4	56	51-62	4	111	70-168	4
Onisimus litoralis (juvenile)	22	19-26	3	> 70	-	2-9	59	44-75	5	-	24-213	1-10
Onisimus litoralis (adult)	28	26-30	4	115	80-160	3	>47	-	4-10	138	118-161	6
Copepods												
Calanus hyperboreus	-	-	-	-	-	-	73	51-103	6	196	161-238	8
Fish												
Myoxocephalus quadricornis	-	3-9	1	< 40	-	1	43	37-49	2	59	40-87	2

[1]Average, if more than one determination.

[2]Based on 95% confidence limits or, lacking these, on ranges of concentrations within which 50% mortality occurred.

3.5 Relative Life Stage Sensitivity

Many researchers have indicated that juvenile or larval stages of many marine organisms are more sensitive to pollutants than the adults (Moore and Dwyer, 1974; Rice et al., 1975; Linden, 1976; Wilson, 1977). In Study 2, the 96 h LC_{50} of sodium lauryl sulphate for juvenile Onisimus litoralis was only slightly, but significantly (test of significant difference, APHA, 1976, p. 737) lower than that for adult O. litoralis (see Table 4). Juvenile O. litoralis also appeared to be more sensitive to Prudhoe Bay crude oil than adults (see Table 11). Linden (1976) found that four to six day old Gammarus oceanicus (approximately 1 mm in length) were about 700 times more sensitive to crude oil, based on added oil concentrations, than adult G. oceanicus. It is probable that the juvenile O. litoralis used in this study were well past the stage of greatest sensitivity.

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