

## PART III

BEHAVIOURAL RESPONSES OF BENTHIC INVERTEBRATES  
EXPOSED TO DISPERSED CRUDE OIL

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A report prepared for the Baffin Island Oil Spill  
Project

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### ABSTRACT

A series of experiments were carried out with the purpose of defining behavioral responses in three species of benthic invertebrates, *Strongylocentrotus droebachiensis* (STR), *Serripes groenlandicus* (SER) and *Mya truncata* (MYA), when exposed to Lago medio crude oil. The dosing protocol was designed to correlate with the 1981 BIOS experimental spill program but relied on tank exposures using a flow-through seawater system on site at Cape Hatt, N.W.T. Oil was added to test systems as an oil/dispersant 10/1 mix at 0.5 to 500 ppm, as well as regimes similar to the field spills. Exposures were static for 6-hour periods. Samples were taken for hydrocarbon analysis of seawater and tissues. Each experiment was followed to a 7-day clearance period.

MYA responded to the addition of dispersed oil by ostial closure and siphon retraction. At high doses, there was a decrease in the effectiveness of mantle and siphon retraction following mechanical stimulation.

SER also exhibited sensitivity to oil by ostial closure and siphon retraction. Emergence out of the sediment occurred at concentrations greater than 100 ppm. Increased locomotion was observed. High doses resulted in latent responses of shell gaping and foot extrusion. An earlier response was extreme closure of the valves, resulting in breakage at the margins. A loss of stimulus responsiveness occurred at the 500 ppm dose with a decrease in the effectiveness of siphon and foot retraction, as well as shell closure when stimulated by touch.

STR showed a rapid onset of behavioral changes at 5 ppm and greater. These included a loss of adherence to substrate and a loss of decoration with shells and seaweed. Spines became rigid at low doses and drooped at higher doses even after the removal of the oil. Tube feet retraction was common. Narcosis was evident with a loss in spine and tube feet responsiveness to mechanical stimuli. A dose-response relationship was demonstrated. Recovery was good except at the 500 ppm dose.

Limited observations were carried out to assess the effects of the dispersant Corexit, and to differentiate sensitivities of small and large individuals. All three species took up petroleum hydrocarbons at a level which was dose-related. STR continued to load soft tissues with hydrocarbon transferred from the external body surface even during the clearing periods.

The behavioral data are interpreted in the context of individual survival, of potential for hydrocarbon loading, and generally in relation to the BIOS experimental spill data.

### INTRODUCTION

The invertebrate biota inhabiting the Arctic seafloor has been shown to be affected by petroleum. Polychaetes, isopods, and amphipods showed a number of negative responses when exposed (Atlas et al., 1978; Percy, 1977). These included an inability to recolonize contaminated sediments, increased respiration, and death. Observations made during the experimental spills of the Baffin Island Oil Spill (BIOS) project demonstrated that bivalves and urchins were affected, showing emergence out of the sediment and apparent narcosis (Cross and Thomson, 1982).

There is little doubt that acute exposures can have a significant effect on benthic biota. Depending on the location, this may have direct effects on other components of the ecosystem. If the benthic population becomes reduced in feeding areas for marine mammals such as walrus (*Odobenus marinus*) or for seabirds such as eiders (*Somateria* spp.), the results may be locally significant.

The 1981 experimental spill study was successful in differentiating the short-term effects of a dispersed oil spill from that of an untreated oil discharge. A large amount of important information was generated by the study in this regard. A consequence of the limitations of an experimental study carried out in the field, however, is that it was not possible to account for and control all impinging variables. A full assessment of fate and effects has to use extrapolation and interpretation of the data generated. The study described here will attempt to define, in a controlled manner, a number of behavioral effect indices which were suggested in the 1981 BIOS field spill, as well as describe more broadly the responses of Arctic marine benthos to oil.

## METHODS AND MATERIALS

### Location

The field portion of this study was carried out at Cape Hatt in Eclipse Sound, N. W.T. (72° 28'N, 79° 50'W), at the location of the 1981 Baff in Island Oil Spill study. on-site laboratory experiments were designed to investigate the behavioural responses of three selected species of benthic invertebrates to chemically dispersed crude oil. These tank studies were conducted on the shores of Cape Hatt during the period of August through early September 1982.

### Experimental Design

**Holding and Species Parameters.** A flow-through system was constructed on-site to provide a number of 50 L aquaria simultaneously with a continuous flow of seawater at a rate of approximately 100 mL/min per tank. Water originated offshore from an 8 m water depth (high tide), taken 1 m above the bottom sediment. During dosing periods, the flow-through system was temporarily interrupted and individual circulators were used on each test and control tank to recirculate the total water volume at a rate of 8 L/rein.

**Temperature and salinity were verified regularly and were found to be  $6 \pm 1^\circ\text{C}$  and  $26 \pm 3 \text{ ‰}$ , respectively, throughout the experimental period. This was similar to conditions recorded in offshore waters where the test species were collected. Further normalization of holding conditions was approached by the use of subdued natural lighting in the onshore laboratory.**

The three test species selected were two filter-feeding bivalves, *Mya truncata* and *Serripes groenlandicus*, and a surface deposit-feeder, the green sea urchin *Strongylocentrotus droebachiensis*. All three species were collected by divers in 7 m water depths off the western shore of Ragged Island, a site near Cape Hatt but removed from potential oil contamination during the 1981 experimental spill. The test animals were transferred to the field laboratory, where they were acclimated in the flow-through seawater system for at least 7 days prior to the exposure studies. Mortalities during this acclimation period were less than 3%. Fresh seaweed (*Laminaria* sp.) and broken shell material were supplied regularly to the urchins during the pre-exposure period. *Serripes groenlandicus* were kept in a 10 cm layer of washed and sifted coarse beach sand, while both *M. truncata* and *S. droebachiensis* were held without sediment in the test tanks.

Except as noted below, the three species were of a medium size range, as identified in previous BIOS studies (Cross and Thomson, 1981). *Mya truncata* were  $4.6 \pm 0.4$  cm, *S. groenlandicus* were  $4.3 \pm 0.6$  cm, and urchins were  $4.6 \pm 0.5$  cm. One test series also used small and large individuals (*M. truncata*,  $2.7\text{-}4.4 \pm 0.3$  cm; *S. groenlandicus*,  $2.7\text{-}5.1 \pm 0.4$  cm; *S. droebachiensis*,  $3.0\text{-}5.4 \pm 0.3$  cm). Holding densities were calculated to not exceed field densities and were limited to 20 individuals of medium size for each species per 50 L aquarium.

**Dosing Protocols.** Dispersed oil concentrations were prepared by dilution of a stock oil/dispersant mixture. This was prepared by vortex stirring of the BIOS-stock aged Lago medio crude oil with Corexit 9527, 1:10, in seawater, as a 25,000 ppm solution. All concentrations are expressed on a basis of weight of crude oil.

Eight experimental conditions were tested, as outlined in Table 1. In all instances, the dosing procedure was similar, with concentrations, exposure times and number or size of animals being varied in accordance with the intent of the test. Single 6-hour exposures were carried out to assess dose-responses or experimental variables. Sequential doses (three at 6 h each) were designed to correlate with conditions recorded in Bays 9 and 10 after the field spill experiment. Corexit was tested alone at one concentration. Other experiments were carried out to differentiate the effect of a probable partial loss of volatiles from the dispersed oil during the exposure period. A post-exposure period of 7 days was used to assess recovery. Controls for each species were monitored concurrently with the exposure experiments.

**Sampling Scheme.** Water samples were taken from each exposure system during the first 5-10 rein, and approximately 2, 4 and 6 h after the addition of each oil dispersion. A further sample was taken 1 h and 12 h after the exposure periods, when the tanks had undergone a rapid flushing with clean seawater. These 50 mL samples were analyzed for hydrocarbons by real-time fluorometry. A few additional 10 mL water samples were taken during the exposures for gas chromatographic analysis.

Samples for tissue hydrocarbons were taken at the end of the exposure periods (6 or 18 h) and at 7 d. The soft tissues of five individuals of each species were removed and pooled as a single sample for each time period. The tissues were wrapped in aluminum foil, sealed in Whirl-paks, and frozen.

All glassware and instruments used for hydrocarbon residue samples were prerinse and dried with high purity hexane-methylene chloride solvent.

TABLE I DOSING PROTOCOL

Experimental Condition	Concentrations (ppm)	Exposure Time (h)	Test Organisms <sup>a</sup>
Single exposure	0.5, 5, 50, 100, 500	6	MYA SER STR
Sequential exposure Bay 10 simulation	0.5/5.0/0.2	6 each	MYA SER STR
Sequential exposure Bay 9 simulation	10/100/5.0	6 each	MYA SER STR
Single exposure (covered and uncovered comparisons)	250	6 6	STR STR
Sequential exposure (covered and uncovered comparisons)	10/100/5.0	6 each	MYA SER STR
Single exposure (size comparisons)	250	6	MYA SER STR
Single exposure (predation test)	250	6	SER
Corexit exposure	50	6	MYA SER STR

<sup>a</sup>SER = *Serripes groenlandicus* MYA = *Mya truncata* STR = *Strongylocentrotus droebachiensis*

n = 20 per test, except 15 for size comparison tests.

Analyses. Water samples were analyzed by real-time fluorometry and by gas chromatography on stored samples. The fluorometric method used a Turner model 240 flow-through fluorometer, calibrated and blank adjusted before and during use. Dispersed Lago medio crude was used for calibration. All readings were carried out in the linear range of the fluorescence calibration, at times necessitating dilution of test samples with seawater. Reproducibility of duplicate samples was found to be 5% or less.

Water analysis by gas chromatography was used in a limited manner to obtain preliminary data on hydrocarbon fraction composition in the test systems and to serve as a check on total oil concentrations determined by fluorometry.

The analyses were carried out under contract by Seakem Oceanography Ltd., using a Hewlett-Packard Model 5830A gas chromatography, equipped with a J & W fused silica column with a SE-30 stationary phase. Standard methods were employed for the characterization of hydrocarbons in hexane extracts of the 10 mL water samples. Hydrocarbon concentrations were expressed as ppm of crude oil, calculated on the basis of the C-21 to C-24 alkane peaks relative to an internal standard (cetylbenzene).

Tissue hydrocarbon residues were analyzed as follows. Extraction of the samples was carried out according to the method of Engelhardt et al. (1982). Fused silica capillary GC (GC<sup>2</sup>) of an aromatic hydrocarbon fraction (f2) obtained through alumina/silicic acid fractionation of the total extract was used (Boehm et al., 1982). Naphthalene (N), methyl (C1) naphthalenes, ethyl/dimethyl (C2) naphthalenes and trimethyl (C3) naphthalenes were quantified. The ratio of C3N to total aromatics, and in turn the ratio of total aromatics to total oil (as determined by a combination of electrobalance gravimetric measurement on total oil and aromatic (f2) fraction and total C3N peak area to total f2 peak area) were used to convert C3N to "total oil" values for neat oil. As each sample was analyzed by GC<sup>2</sup>, the total amounts of N, C1N, C2N, C3N, and alkylated benzenes were obtained directly from the GC trace. The C3N:total oil conversion was used to arrive at the "total oil" values reported for the animals.

A variety of behavioral responses were recorded and quantified. The choice of particular behavioral indices of petroleum effect was based on extensive observations of the three species during the acclimation periods, and was predicated on being both quantifiable and significant to the survival of the individual. Characteristics of the index behavioral responses are detailed in Table 2.

TABLE 2 BEHAVIORAL RESPONSES OF *MYATRUNCATA*,  
*SERRIPES GROENLANDICUS* AND *STRONGYLOCENTROTUS*  
*DROEBACHIENSIS* EXPOSED TO CRUDE OIL DISPERSIONS

Target species	Behavioral Responses	Description
<i>Strongylocentrotus droebachiensis</i>	direct observations	
	cover	shell fragments and pieces of algae cover test on aboral surface
	attachment	attachment to glass of aquarium floor and sides by tube feet
	spine droop	depression of spine downward along test surface
	spine rigidity	"pincushion" orientation and loss of flexibility
	curling of tube feet	aboral tube feet showing curling of distal ends
	retraction of tube feet	aboral tube feet not existent
	<u>stimulus response</u>	
	tube feet	tube foot retraction following mechanical stimulus laterally on test
	spine	re-orientation towards source of mechanical stimulus laterally on test
<i>Mya truncata</i>	direct observations	
	ostial closure	closure of both inhalent and exhalent siphons
	siphon retraction	retraction of siphon to 1/2 or less of normal extended length
	<u>stimulus response</u>	
	siphon	siphon retraction following mechanical stimulus to siphon
	mantle	retraction of mantle and closure of shell following mechanical stimulus of mantle edge
<i>Serripes groenlandicus</i>	<u>direct observation</u>	
	buried	burial to depth where ostia are exposed
	ostial closure	closure of one or both ostia
	mantle gape	mantle edges not closed and shell gaping
	<u>stimulus response</u>	
	siphon	siphon retraction following mechanical stimulus to siphon

TABLE 3 PETROLEUM HYDROCARBON CONCENTRATIONS IN WATER OF SINGLE TEST SYSTEMS. COMPARING NOMINAL CONCENTRATIONS TO VALUES DETERMINED BY REAL-TIME FLUOROMETRY AND BY GAS CHROMATOGRAPHIC ANALYSIS OF SELECTED SAMPLES (cont'd)

Nominal Concentration (ppm)	Time <sup>b</sup> (hours)	Test Species <sup>c</sup>	Measured Concentration (ppm)	
			Fluorometry	Gas Chromatography
250	6	MYA	225	(96)
		SER	225	(86)
		STR	200 (225)	269 (153)
500	6	MYA	310	307
		SER	290	
		STR	290	
	12	MYA	nd	
		SER	nd	
		STR	nd	

<sup>a</sup> Calculated as dilution of Lago medio crude-Corexit stock dispersion.

<sup>b</sup> Elapsed time from start of 6-h continuous exposure period.

<sup>c</sup> MYA - *Mya truncata*; SER - *Serripes groenlandicus*; STR - *Strongylocentrotus droebachiensis*.

<sup>d</sup> Numbers in parentheses are concentration values from duplicate tests in which exposure systems were covered.

nd = not detectable

= no data collected

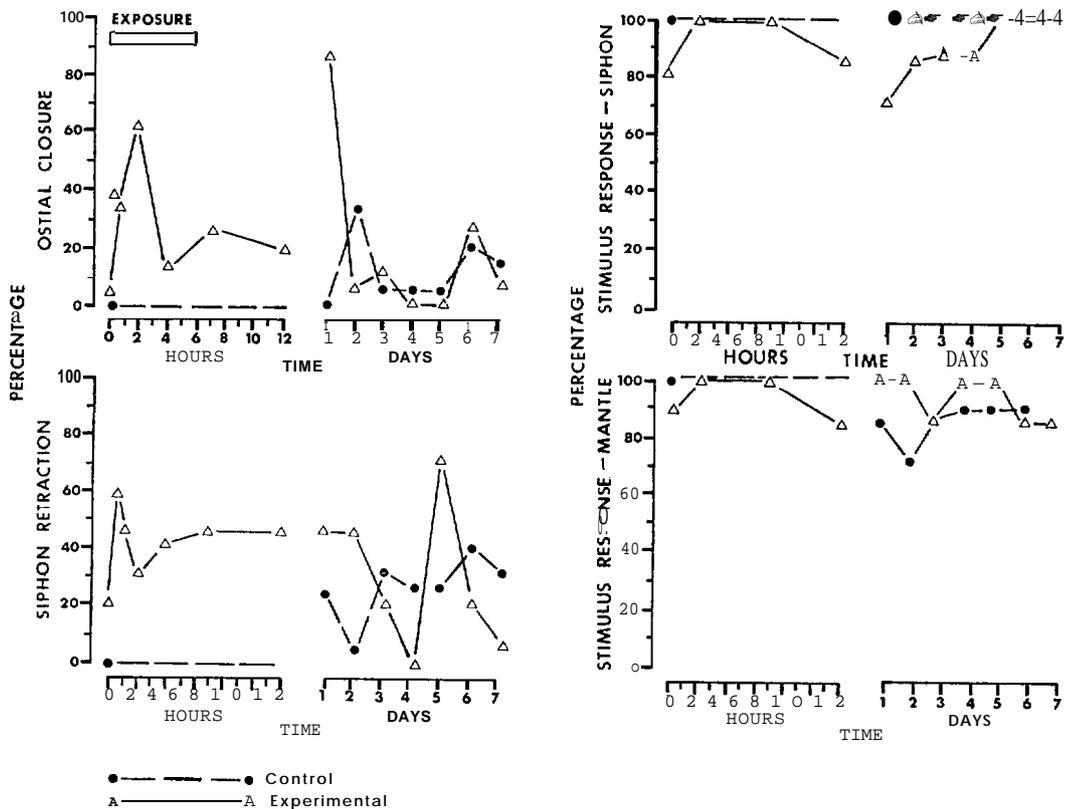


FIGURE 1 SELECTED BEHAVIORAL RESPONSES OF MYA TRUNCATA TO 50 PPM DISPERSED OIL

**Quantitation** of these responses was on the basis of proportion of the test population (n = 20) showing a change as related to exposure concentration and elapsed time. A standard point of comparison was the time at which 50% of the starting population showed a response (**ET<sub>50</sub>**). Recovery was assessed as that point in elapsed time at which the proportion of animals showing the response had returned to pre-exposure levels. The reported **ET<sub>50</sub>** and recovery time values were obtained from data plots made for each species and **exposure test**, comparing percentage of test population showing the response to elapsed time.

Behavioral changes in addition to quantified responses were also described. Such changes tended to be less consistent, or could not be observed to involve at least 50% of the test population at any concentration.

## RESULTS

### Exposure Concentrations

A comparison of nominal and **fluorometry** measured concentrations of crude oil hydrocarbons in the various test systems showed deviations of as much as 50% from the expected starting concentrations. The deviation was usually an increase in both single exposure (**Table 3**) and **sequential exposure tests** (**Table 4**). **In all instances, the measured concentrations declined over the 6-h periods of static recirculated exposure to an average of two-thirds of starting concentrations. Subsequent extrapolations of concentrations to 6 h averages indicated that total exposures were very similar to nominal concentrations.**

**A small number of water samples were assessed by gas chromatography (GC) for both total oil concentration and fraction characteristics. Quantitation using C-21 to c-24 alkanes and an internal standard showed similar concentrations of oil in water as assessed by both methods. Both fluorometry and GC demonstrated similar declines in total oil concentration whether the systems were run covered or uncovered, as was the case for the [majority of the tests.**

### Behavioural Responses

*Mya truncata*. This species responded to the presence of oil somewhat inconsistently, especially at the lower concentrations. **Ostial** closure and siphon retraction were common at concentrations above 10 ppm (**Table 5; Figure 1**). Loss of response capability to mechanical stimulation by retraction of the siphon or mantle was noted at concentrations of 50 ppm and above. This response tended to be **latent**. Recovery was rapid at low-level exposures even during the dosing period. Exposure to higher concentrations required longer recovery times. **Corexit** at 50 ppm elicited **all** four quantitated behavioral changes soon after exposure. Recovery times, however, were much shorter, with a return to normal behaviour within 1 d of the exposure.

The sequential dosing experiments suggested that responses were delayed relative to single exposures. These responses often did not occur during the period of highest concentration (for example, the 100 ppm phase (**Table 6**)); recovery required 1 to several days, as in the case of the **single** 100 ppm exposures. Neither size differences nor the effect of covering the exposure tanks could be differentiated in the *Mya truncata* tests.

Other behavioral changes were observed to occur as a consequence of oil exposure. At low concentrations, or early in the exposure periods, the diameters of siphon **ostia** tended to be increased. Occasional extensions of the foot were common. Elongation of the siphon was observed at high concentrations, especially late in the post-exposure period. No lethality was observed.

TABLE 3 PETROLEUM HYDROCARBON CONCENTRATIONS IN WATER OF SINGLE TEST SYSTEMS, COMPARING NOMINAL CONCENTRATIONS To VALUES DETERMINED BY REAL-TIME FLUOROMETRY AND BY GAS CHROMATOGRAPHIC ANALYSIS OF SELECTED SAMPLES

Nominal Concentration (ppm)	Time <sup>b</sup> (hours)	Test Species <sup>c</sup>	Measured Concentration (ppm)	
			Fluorometry	Gas Chromatography
0	0.1	MYA	nd	
		SER	nd	
		STR	nd	
	12	MYA	nd	
		SER	nd	
		STR	nd	
0.5	0.1	MYA	0.70	
		SER	0.55	
		STR	0.80	
	6	MYA	nd	
		SER	0.40	
		STR	0.40	
	12	MYA	nd	
		SER	nd	
		STR	nd	
5.0	0.1	MYA	8.0	
		SER	8.7	
		STR	7.8	
	6	MYA	6.0	
		SER	6.4	
		STR	6.8	
	12	MYA	nd	
		SER	nd	
		STR	nd	
50	0.1	MYA	60	
		SER	60	
		STR	64	
	6	MYA	26	26
		SER	22	
		STR	23	
	12	MYA	nd	
		SER	nd	
		STR	nd	
100	0.1	MYA	140	
		SER	128	
		STR	136	
	6	MYA	86	75
		SER	80	86
		STR	78	44
	12	MYA	nd	
		SER	nd	
		STR	nd	
250	0.1	MYA	380	(295) <sup>d</sup>
		SER	350	(133)
		STR	310 (350)	320 (183)

**TABLE 4**      **PETROLEUM HYDROCARBON CONCENTRATIONS IN WATER OF SEQUENTIAL TEST SYSTEMS, COMPARING NOMINAL CONCENTRATIONS TO VALUES DETERMINED BY REAL-TIME FLUOROMETRY AND BY GAS CHROMATOGRAPHIC ANALYSIS OF SELECTED SAMPLES**

Nominal Concentrations (ppm)	Time <sup>b</sup> (hours)	Test Species <sup>c</sup>	Measured Concentration (ppm)			
			Fluorometry		Gas Chromatography	
			ncv <sup>d</sup>	cv	ncv	cv
0.5/5/0.2	0.1	MYA	0.40		nd	
		SER	0.35			
		STR	0.40			
	6	MYA	0.20		nd	
		SER	nd			
		STR	nd			
6.2	MYA	10		nd		
	SER	5.0				
	STR	6.5				
12	MYA			nd		
	SER	5.0				
	STR	4.0				
12.2	MYA	1.2		nd		
	SER	1.1				
	STR	0.65				
18	MYA	1.0		nd		
	SER	1.0				
	STR	0.80				
10/100/5	0.1	MYA	12, 6.5	7.5	nd	nd
		SER	10, 8.0	8.0		
		STR	10, 7.5	8.0		
	6	MYA	8.5, 6.5	6.5	nd	nd
		SER	10, 5.0	6.2		
		STR	6.5, 5.0	5.0		
	6.2	MYA	150, 80	65	73, 159	130
		SER	150, 80	75		
		STR	100, 90	65		
	12	MYA	95, 65	50	110, 171	105
		SER	70, 50	50		
		STR	65, 50	50		
	12.2	MYA	10, 4.0	6.5	nd	nd
		SER	8.5, 5.0	6.5		
		STR	8.5, 5.0	6.5		
	18	MYA	7.0, 4.0	4.0	nd	nd
		SER	7.0, 3.7	4.0		
		STR	7.5, 3.7	4.0		

<sup>a</sup> Calculated as three dilutions of Lago medio crude-Corexit stock dispersions, presented sequentially.

<sup>b</sup> Elapsed time from start of three sequential exposure periods, each of 6-hour duration.

<sup>c</sup> MYA - *Mya truncata*; SER - *Serripes groenlandicus*; STR - *Strongylocentrotus droebachiensis*.

<sup>d</sup> ncv - tanks not covered during exposure period; cv - tanks covered during exposure period.

nd = not detectable; - = no data collected

TABLE 5 BEHAVIORAL RESPONSES OF *MYA TRUNCATA* EXPOSED TO CRUDE OIL DISPERSIONS IN SINGLE TESTS

Exposure <sup>a</sup> Condition	Ostial Closure	Siphon Retraction	Stimulus Response - Siphon	Stimulus Response - Mantle
<u>ET50</u>				
<b>Oil</b>				
0.5	NC	3 h	NC	NC
5.0	<0.5 h	NC	NC	5 d
50	1 h	1 h	NC	NC
100	0.5 h	0.5 h	NC	1 d
250 (cv, sml)			2 d	
250 (cv, lg)	<2 h			
500			12h	6 h
<b>Corexit</b>				
50	<0.5 h	6h	<0.5 h	<0.5 h
<u>RECOVERY</u>				
011				
0.5	NC	6h	NC	NC
5.0	1 h	NC	NC	6 d
50	2 d	3 d	NC	NC
100	12 h	3 d	NC	2 d
250 (cv, sml)	1 d	1 d	>2 d	1 d
250 (cv, lg)	1 d	1 d	5 d	1 d
500	2 d	5 d	1 d	1 d
<b>Corexit</b>				
50	1 d	1 d	4 h-1 d	4 h-1 d

<sup>a</sup> 6 Hours of exposure to Lago medio crude oil or Corexit 9527 dispersions, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as cv; animal size medium except where noted as sml or lg.

NC . no change

- = no data, due to inaccessibility of animals because of covered system or to inability to observe behavioral change in dispersion-clouded water

TABLE 6 BEHAVIORAL RESPONSES OF *MYA TRUNCATA* EXPOSED TO CRUDE OIL DISPERSIONS IN SEQUENTIAL TESTS

Exposures Condition	Ostial Closure	Siphon Retraction	Stimulus Response - Siphon	Stimulus Response - Mantle
<u>ET<sub>50</sub></u>				
0.5/5 .0/0.2	NC	NC	NC	NC
10/100/5.0	3 h	6 h	1 d	13 h
10/100/5.0	7 h	NC		
10/100/5.0 (CV)	7 h	NC		
<u>RECOVERY</u>				
0.5/5/0.2	NC	NC	NC	NC
10/100/5	1 d	1 d	5 d	1 d
10/100/5	15 h	NC		
10/100/5 (Cv)	18 h	NC		

\*18 Hours of exposure to Lago medio crude oil dispersions, given in three sequential concentrations of 6 hours each, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as cv.

NC . no change

- . no data

**Serripes groenlandicus.** At concentrations below 50 ppm, only ostial closure occurred as a response to oil (Table 7; Figure 2). Ostial closure showed a concentration-response relationship from 0 to 100 ppm, the time to affect closure decreasing with increased concentration. A discontinuity in this response occurred above 100 ppm, where ostia were closed in 50% of the test population only after several hours. At 250 ppm, both emergence from the sediment and an increased gaping of the mantle opening (and shell) became major responses. At 500 ppm, all behavioral indices were affected, with biphasic responses noted in mantle/shell gape, foot protrusion, and reaction to mechanical stimuli. The second phase of these responses was latent, occurring after the exposure period. Lethality as well was latent at 500 ppm, with an ET<sub>50</sub> of 6 d. Recovery took 1 to several days, suggesting a dose response of increased recovery time with increasing concentrations. The data on the effect of differential sizes of test organisms to 250 ppm exposure were inconclusive.

Exposure to 50 ppm of Corexit resulted in emergence from the sediment, ostial closure, and mantle gape responses similar to the 500 ppm dispersed oil dose. Time to recovery from this exposure was shorter, however, and there was no lethality.

The 18-h three-phase exposure regime resulted in unique response patterns (Table 8). An initial 6-h exposure to 0.5 or 10 ppm resulted in an enhanced response to the mid-sequence high concentration, as compared to single doses of 5 and 100 ppm. Recovery as well was markedly delayed in the sequential exposure tests. Only the 500 ppm exposure resulted in lethality. The survivors recovered normal behaviour patterns. The use of tank covers to minimize evaporative hydrocarbon loss did not consistently alter the responses to sequential exposures. Most indices remained similar.

An additional response had to be inferred from the observations of ostial closure. Siphon retraction was a probable accompanying response which could not be quantified given the normal attitude of the bivalve. This attitude is characterized by burial to various depths in the sediment with only the tip of the siphon and ostia showing. Siphon retraction and forceful closure were probable occurrences at concentrations of 50 ppm and greater, as evidenced by a high incidence of shell damage. Individuals which emerged from the sediment tended to be cracked along the margins of their valves. At times this involved as much as one-third of the shell surface, exposing soft tissues. Forceful closure of the two valves was an additional latent response during both the 500 ppm single dose and the 10/100/5 ppm sequential exposure. Shell damage resulted from tight opposition of the valves, pinching an extruded foot. Increased locomotion occurred as a result of the extruded foot contacting the sediment and straightening rapidly to propel the shell. Locomotion was erratic and was observed primarily in emerged individuals early on during exposure to single concentrations of 250 and 500 ppm, and to the serial 10/100/5 ppm exposure. The style of locomotion was similar but was observed only rarely during the pre-exposure period.

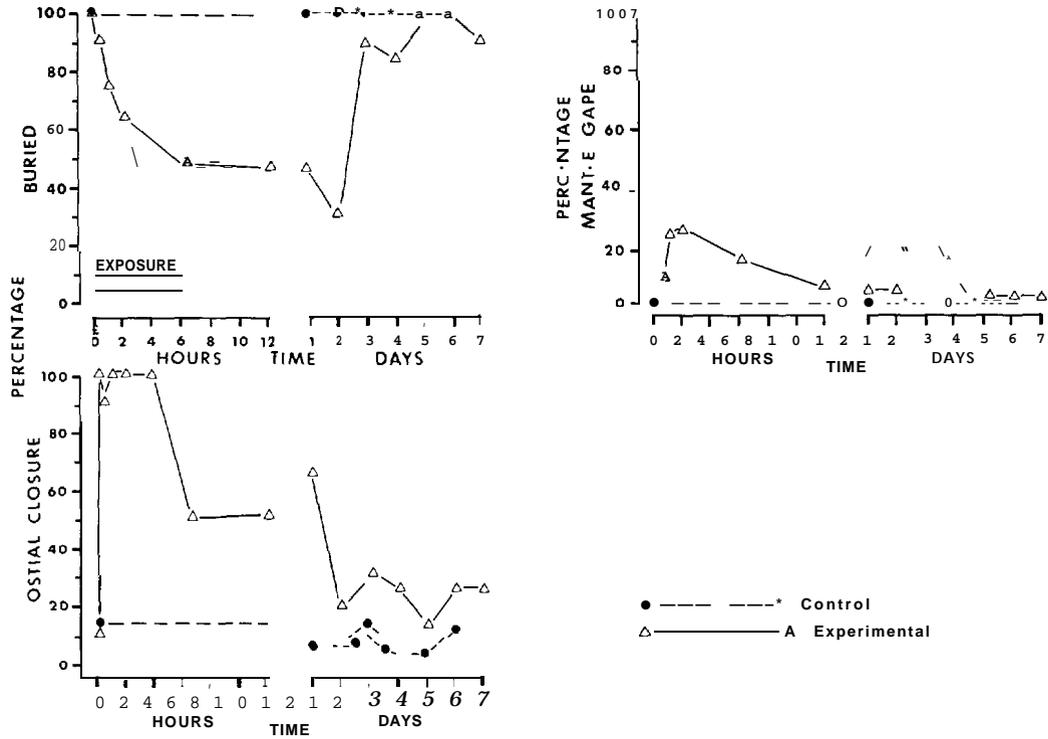


FIGURE 2 SELECTED BEHAVIORAL RESPONSES OF *SERRIPES GROENLANDICUS* TO 50 PPM DISPERSED OIL

TABLE 8 BEHAVIOURAL RESPONSES OF *SERRIPES GROENLANDICUS* EXPOSED TO CRUDE OIL DISPERSIONS IN SEQUENTIAL TESTS

Exposures Condition	Emergence	Ostial Closure	Mantle Gape	Foot Protrusion	Stimulus Response - Siphon	Lethality
<u>ET<sub>50</sub></u>						
0.5{5/0.2	18 h	6 h	NC	NC	NC	NC
10/100/5	13 h	<0.5 h	7 h, 2 d	7 h, 1 d	NC	
10/100/5	18 h	<0.5 h	7 h, 2 d	10 h	7 h	NC
10/100/5 (cv)	14 h	1 h	7 h, 2 d	0.5 h		NC
<u>RECOVERY</u>						
0.5/5/0.2	2 d	6d	NC	NC	NC	NC
10/100/5	>7 d	5d	13 h	12 h, 2 d	NC	NC
10/100/5	6 d	3 d	8 h, >7 d	13 h	3 d	NC
10/100/5 (cv)	4 d	1 d	8 h, >7 d	2h	2 d	NC

<sup>a</sup> 18 Hours of exposure to Lago medio crude oil dispersions, given in three sequential concentrations of 6 hours each, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as CV.

NC = no change

. no data

TABLE 7 BEHAVIORAL RESPONSES OF *SERRIPES GROENLANDICUS* EXPOSED TO CRUDE OIL DISPERSIONS IN SINGLE TESTS

Exposures Condition	Emergence	Ostial Closure	Mantle Gape	Foot Protrusion	Stimulus Response - Siphon	Lethality
<u>ET<sub>50</sub></u>						
<b>Oil</b>						
0.5	NC	6 h	NC	NC	NC	NC
5.0	NC	1 h	NC	NC	NC	NC
50	6 h	<0.5 h	NC	NC	NC	NC
100	12 h	<0.5 h	NC	NC	NC	NC
250 (cv, sml)	6 h	<5 h	1 d <sup>b</sup>	NC		NC
250 (cv, lg)	6 h	<6 h	3d <sup>b</sup>	NC		NC
500	4 h	3 h	<0.5 h, 3 d	<0.5 h, 1 d	<0.5 h, 7 d	6 d
<b>Corexit</b>						
50	4 h	3 h	4 h	NC		NC
<u>RECOVERY</u>						
<b>Oil</b>						
0.5	NC	1 d	NC	NC	NC	NC
5.0	NC	2 d	NC	NC	NC	NC
50	3 d	2 d	NC	NC	NC	NC
100	3 d	2 d	NC	NC	NC	NC
250 (cv, sml)	5 d	5 d	5 d	NC		NC
250 (cv, lg)	3 d	6 d	5 d	NC		NC
500	>7 d	6 d	1 d <sup>b</sup> , 7 d	2 h, 7 d	3d	-
<b>Corexit</b>						
50	2 d	2 d	2 d	NC		NC

<sup>a</sup>6 Hours of exposure to Lago medio crude oil or Corexit 9527 dispersions, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as cv; animal size medium except where noted as sml or lg.

<sup>b</sup> Indicates the maximum response shown by less than 50% of starting test population, generally 20-40%.

NC = no change.

- = no data, usually because of use of a covered test system.

**Strongylocentrotus droebachiensis.** The urchin was the most responsive benthic species examined in terms of the number of behavioral indices affected by oil. The most sensitive response was the loss of covering material as the aboral tube feet released pieces of macro algae and shell fragments (Table 9; Figure 3).

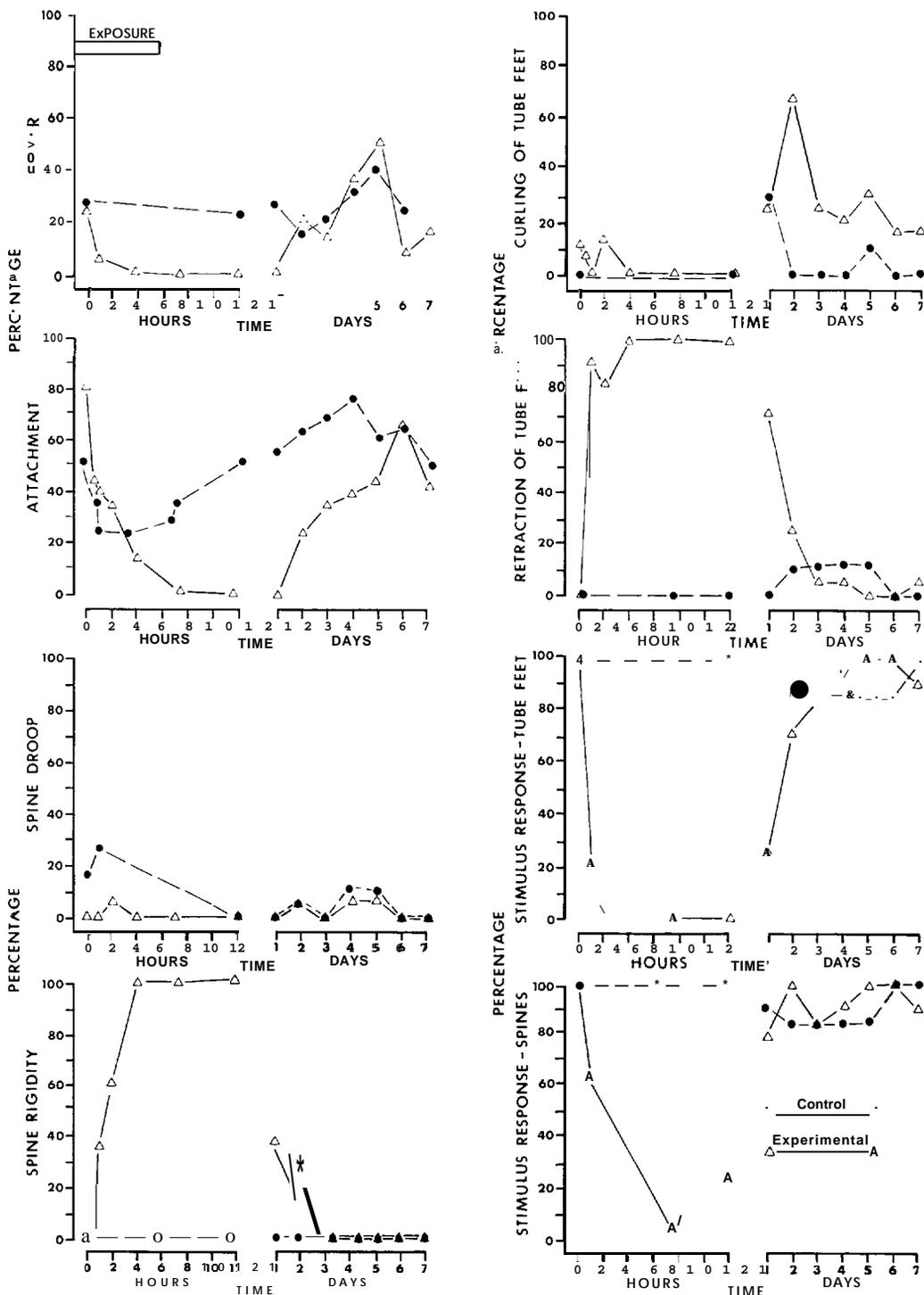


FIGURE 3 SELECTED BEHAVIORAL RESPONSE OF *STRONGYLOCENTROTUS DROEBACHIENSIS* TO 50 PPM DISPERSED OIL

**TABLE 9 BEHAVIORAL RESPONSES OF *STRONGYLOCENTROTUS DROEBACHIENSIS* EXPOSED TO CRUDE OIL DISPERSIONS IN SINGLE TESTS**

Exposure Condition	Loss of Cover	Loss of Attachment Ability	Spine Droop	Spine Rigidity	Curling of Tube Feet	Retraction of Tube Feet	Stimulus Response Tube Feet	Stimulus Response Spines	Lethality
<b><u>ET<sub>50</sub></u></b>									
Oil									
0.5	NC	NC	8 hb	NC	1 h	1 hb	NC	NC	NC
5.0		NC	4 h	3 h	4 h	4 h	5 h	5 h	NC
50	<1 h	3 h	NC	2 h	2 d	<1 h	<1 h	2 h	NC
100	2 h	3 h		3 h	2 d	<1 h	(2 h)	(2 h)	NC
250	<0.5 h	<0.5 h	0.5 h, 1 d	<0.5 h	0.5 h, 2 d	1 h			NC
250 (cv)	<0.5 h	1 h	0.5 h, 1 d	<0.5 h	2 d	<0.5 h			NC
250 (cv,sml)	1 h	1 h	<0.5 h	<0.5 h	<0.5 h, 3 d	<0.5 h			NC
250 (cv,lg)	1 hb	1 h	<0.5 h, 1 d	0.5 h	<0.5 h, 3 d	<0.5 h			NC
500	<0.5 h	1 h	1 d	<0.5 h	2 d		<1 h	<1 h	3 d
Corexit									
50	NC	<0.5 h	<0.5 hb	<0.5 hb	NC		NC	NC	NC
<b><u>RECOVERY</u></b>									
Oil									
0.5	NC	NC	2 d	NC	12 h	NC	NC	NC	NC
5	3 d	NC	NC	1 d	1 d	2 d	1 d	2 d	NC
50	4 d	3 d	NC	2 d	3 d	3 d	4 d	2 d	NC
100	4 d	3 d	NC	2 d	4 d	3 d	2 d	2 d	NC
250	3 d	>4 d	4 d	1 d	4 d	2 d	2 d	1 d	NC
250 (cv)	3 d	4 d	4 d	2 d	7 d	3 d	3 d	1 d	NC
250 (cv,sml)	3 d	5 d	1 h	3 d	1 h, 5 d	3 d	3 d	1 d	NC
250 (cv,lg)	3 d	4 d	1 h, 3 d	2 d	1 h, 5 d	3 d	3 d	1 d	NC
500	3 d	Nil	Nil	2 d	7 d	5 d	>5 d	>4 d	Nil
Corexit									
50	NC	12 h	2 h	2 h	NC		NC	NC	NC

<sup>a</sup> 6 Hours of exposure to Lago medium crude oil or Corexit 9527 dispersions, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as cv; animal size medium except where noted as sml or lg.

b Indicates that maximum response shown by less than 50% of starting test population, generally 20-40%.

NC = no change; -. no data.

Loss of covering showed a concentration response beginning within 4 h at 10 ppm (Table 10) and occurring much earlier at higher concentrations. Loss of ability by the urchins to remain attached to the walls of the aquaria with their tube feet was also a sensitive response to oil and was also dose-related. Recovery of these tube feet functions required 3 to 5 d, irrespective of concentrations to 500 ppm. At 500 ppm, however, there was no recovery of attachment ability within the 7-d experimental period.

TABLE 10 BEHAVIORAL RESPONSES OF *STRONG YLOCENTROTUS DROEBACHIENSIS* EXPOSED TO CRUDE OIL DISPERSIONS IN SEQUENTIAL TESTS

Exposure Conditiona	Loss of Cover	Loss of Attachment Ability	Spine Droop	Spine Rigidity	Curling of Tube Feet	Retraction of Tube Feet	Stimulus Response Tube Feet	Stimulus Response Spines	Lethality
<u>ET<sub>50</sub></u>									
0.5/5/0.2	NC	7 h	3 h	1 h	12 h	7 h	7 h	7 h	NC
10/100/5	4 h	5 h	0.5 h	1 h	1 h	0.5 h	2 h	2 h	NC
10/100/5	-	6 h	5 h	6 h	5 h	6 h			7 d
10/100/5 (Cv)	-	6 h	4 h	7 h	3 h	6 h			NC
<u>RECOVERY</u>									
0.5/5/0.2	NC	18 h	13 h	15 h	15 h	18 h	16 h	13 h	NC
10/100/5	4 d	3 d	2 h	1 d	6 d	6 d	6 d	6 d	NC
10/100/5	-	5 d	>7 d	1 d	9 h	1 d	1 d	1 d	Nil
1 0/100/5 (cv)	-	5 d	>7 d	2 d	13 h	1 d	1 d	2 d	NC

<sup>a</sup>18 Hours of exposure to Lago medio crude oil dispersions, given in three sequential concentrations of 6 hours each, followed by 7 days of post-exposure observation; numerical entries identify concentration; all tanks not covered except where noted as cv.

NC . no change

. no data

Curling and retraction of aboral tube feet was a further response to oil exposure (Table 9). At 250 ppm, the curling of the ostial ends of the tube feet was bi-phasic. Retraction of tube feet occurred rapidly at high doses, although not all tube feet were retracted simultaneously. A small number of oral and aboral tube feet often continued to attach the urchin to the glass surface. The higher the single exposure dose, the longer was the time required for recovery of these two indices, especially that of curling.

Changes in spine behaviour were observed at exposures to 5 ppm and greater; they occurred more rapidly and recovered more slowly with increasing exposure concentration. Drooping of aboral spines at times occurred in two phases, during both exposure and recovery periods. The "pincushion" effect characterizing spine rigidity tended to be a rapid response. Spine attitude recovery was generally more rapid than tube foot recovery.

Exposures to concentrations of 5 ppm and greater resulted in a loss of response capacity to mechanical stimuli, with ET<sub>50</sub> times decreased from 5 h to less than 1 h (Table 9). Recovery times were not well correlated with exposure concentration, 1 to 5 d or more being required. Spine responsiveness recovered more rapidly than did tube foot responses. A lethal effect was recorded only when spine and tube foot responses to tactile stimulation were unobtainable. A 3-d ET<sub>50</sub> for lethality was recorded for the 500 ppm single exposure.

Additional observations of *S. droebachiensis* responses to oil were made. Rapid linear movement along the substrate was noted in some instances at 50 to 250 ppm doses during the first few minutes following the addition of dispersed oil to the test systems. Also, a darkening in surface color was a common response to high-level exposures. Shedding of gametes occurred frequently within 2 d of exposure. Spines tended to drop off several days prior to a determination of death in the individual urchin.

No size specific effects were found in urchins. Exposure to 50 ppm of Corexit resulted in loss of adhesion and spine rigidity, but recovery of both functions was much more rapid than for any dispersed oil exposure.

The data shown in Table 10 suggest that 6 h of exposure to 0.5 ppm enhanced the effect of 5 ppm given in a subsequent dose, as compared to a 5 ppm single exposure. Since 10 ppm exposures were effective in eliciting all behavioral changes within a 6-h time period, it was not possible to differentiate the behavioral responses of 100 ppm single and sequential doses. A comparison test between covered and noncovered systems showed that the urchin response and recovery were similar.

#### Tissue Hydrocarbons

The results of tissue hydrocarbon determinations are presented in Table 11. Total aromatic concentrations averaged one-third of the total hydrocarbon levels. Total hydrocarbon values were mathematically derived from the sum of alkylbenzenes and naphthalenes, measured from the gas chromatograms, connected by an aromatic ratio of 0.0056. Total oil values were derived from a total aromatic to total oil ratio of 0.37.

*M. truncata* and *S. groenlandicus* tissues had a greater hydrocarbon load than did *S. droebachiensis* tissues when the samples were taken at 6 h of a single exposure, or 18 h of the sequential test. This relationship was reversed for the 7 d samples in which urchin tissue loads were not only greater than those of the bivalves, but also much higher than they were at 6 or 18 h. Urchin tissues additionally showed a greater capacity to take up alkyl benzenes during the dosing period, although the emphasis on these hydrocarbons tended to be gone in 7-d samples. An overview of tissue hydrocarbons demonstrated fractional compositions qualitatively similar to that of Lago medio crude oil (Figure 4).

## DISCUSSION

### Exposure Concentrations

Fluorescence analysis has shown itself to be a useful method for detecting hydrocarbons in water, especially when used as a real-time measure of experimental concentrations. The 0.5 ppm sensitivity limit is useful, since samples do not require any concentrating or other special handling, thereby minimizing procedural losses of volatile hydrocarbons in particular. The GC method, as employed in this phase of the study, generally verified the fluorometry concentrations. Both the covered and uncovered set-ups are likely to have undergone volatile losses. It is probable that the average one-third decline in starting concentration was mainly due to volatile losses. Nonetheless, averaging of measured concentrations over a 6-h exposure period showed values to be very similar to the nominal concentrations.

The choice of the range of exposure concentrations was predicated by the desire to encompass water concentrations of dispersed Lago medio crude as observed in the 1981 field spill. This condition was met by the series of single exposure tests. Closer similarity with the field concentration scenario was accomplished with the three-phase sequential exposure schemes. The 10/100/5 ppm concentration series was similar in level and sequence to what had been observed in Bay 9, the site of the dispersed oil spill, for the 18-h period subsequent to oil release (Green et al., 1982). The 0.5/5/0.2 ppm concentration sequence approximated the levels during the first 18 h post-spill in Bay 10, an area adjacent and down-channel from the dispersed oil spill site. In both the Bay 9 and 10 simulations, flushing with clean seawater after 18 h was appropriate to match the trace and undetectable concentration levels of hydrocarbons recorded in field observations after this time.

### Behavioral Responses

The behavioral responses shown by the three species can be interpreted from the point of view of: direct toxicity effects of oil, the significance of these effects on survival to oil exposure, and finally interpreting the observations and conclusions originating from the field oil spills. In the latter case, meaningful correlations between this on-site controlled exposure study and the field spill cannot be made effectively until the benthic field data have been finally assembled and interpreted.

The description of behavioral changes in this study emphasizes ET50 values and time to recovery to pre-exposure behaviour. It should be recognized that other behavioral changes occurred which may either not have affected 50% of the test population or else were sporadic in occurrence. These will be described only to round out the observations of behavioral effects.

*Mya truncata* was found to be a difficult species for an assessment of oil effects. Behavioral changes tended to be "noisy" insofar as they were quite variable and easily set off by disturbances other than oil. A clear dose-response could not be discerned using the above indices, but the higher concentrations were certainly more effective in inducing behavioral changes and in delaying recovery times. The trend of this bivalve to close ostia rapidly, and thereby arrest water flow, filtration and oil input, may be considered an advantage in surviving exposure to oil in seawater. The loss of responsiveness to mechanical stimuli may be considered as a narcotization response, although other physiological causes such as neural control override or muscle fatigue may have been contributory. Altogether, *M. truncata* was more resistant to oil than either *S. groenlandicus* or *S. droebachiensis*.

TABLE 11 HYDROCARBON CONCENTRATIONS IN TISSUES OF MYA TRUNCATA (MYA), SERRIPES GROENLANDICUS (SER), AND STRONGYLOCENTROTUS DROEBACHIENSIS (STR)

Exposure Conditions	Time	Species	$\mu$ g/g wet weight					Total Aromatic Oil	Total
			$\Sigma$ AB	N	$\Sigma$ C <sub>1</sub> N	$\Sigma$ C <sub>2</sub> N	$\Sigma$ C <sub>3</sub> N		
0	6 h	MYA	0.08	0.001	0.001	0.004	0.050	8.9	24
		SER	nd	nd	nd	nd	nd	nd	nd
		STR	nd	nd	nd	nd	0.093	17	44
0.5	6 h	MYA	nd	nd	nd	nd	0.028	5.0	13
		SER	nd	nd	nd	0.011	0.032	5.7	15
		STR	nd	nd	nd	nd	0.116	21	55
5	6 h	MYA	0.36	0.017	0.120	0.267	0.269	48	130
		SER	0.64	0.024	0.194	0.457	0.455	81	217
		STR	nd	0.005	0.063	0.059	0.137	25	65
50	6 h	MYA	0.04	0.009	0.271	0.701	0.755	135	360
		SER	0.12	0.01	0.33	0.91	0.76	136	360
		STR	3.5	0.03	0.34	0.56	0.33	59	160
100	6 h	MYA	nd	nd	0.10	0.61	0.50	89	240
		SER	nd	0.1	0.21	0.60	0.37	66	180
		STR	nd	0.01	0.22	0.12	0.06	11	29
250	6 h	STR	nd	nd	0.03	0.03	0.04	7.7	21
250 (cv,lg)	6 h	MYA	nd	nd	0.23	0.53	0.38	67	180
		SER	nd	nd	0.26	0.67	0.44	79	210
		STR	nd	nd	0.01	0.09	0.04	7.0	19
250 (cv,sml)	6 h	SER	nd	0.01	0.12	0.22	0.12	22	59
		STR	nd	nd	0.70	0.10	0.08	14	37
500	6 h	MYA	0.05	0.01	0.54	1.9	1.5	270	710
		SER	1.7	0.16	1.2	2.1	1.1	200	540
		STR	0.5	0.05	0.30	0.20	0.06	11	31
0.5/5/0.2	18 h	MYA	nd	nd	nd	nd	0.03	5.9	16
		SER	nd	nd	nd	0.04	0.27	48	130
		STR	nd	nd	0.04	0.06	0.04	7.9	21
	7 d	MYA	0.01	nd	0.01	0.02	0.10	17	45
		SER	nd	nd	nd	0.01	0.05	8.4	22
		STR	nd	nd	nd	0.18	0.45	81	220
10/100/5	18 h	MYA	0.03	0.36	0.42	1.6	1.3	240	630
		SER	nd	nd	0.26	1.2	1.1	200	530
		STR	nd	nd	0.11	0.48	0.32	56	150
	7 d	MYA							
		SER	nd	nd	nd	0.10	0.31	55	150
		STR	0.08	0.01	0.24	0.88	1.1	190	510
10/100/5 (cv)	18 h	MYA	1.0	0.05	0.58	1.7	1.1	200	540
		SER	0.39	0.05	0.05	0.18	0.20	37	99
		STR	2.7	0.12	0.58	0.52	0.13	23	61
	7 d	MYA	0.07	nd	0.32	0.31	0.72	130	340
		SER	nd	0.10	0.20	0.10	0.36	65	170
		STR	nd	nd	0.08	0.48	0.80	140	380

TABLE 11 HYDROCARBON CONCENTRATIONS IN TISSUES OF *MYA TRUNCATA* (MYA), *SERRIPES GROENLANDICUS* (SER), AND *STRONGYLOCENTROTUS DROEBACHIENSIS* (STR) (cent'd)

Exposure Conditiona	Time	Species	µg/g wet weight					Total Aromatic	Total Oil
			ΣAB	N	ΣC <sub>1</sub> N	ΣC <sub>2</sub> N	ΣC <sub>3</sub> N		
10/100/5	18 h	MYA	2.7	0.11	0.73	2.0	1.2	210	570
		SER	0.03	0.01	0.43	1.9	1.8	320	860
		STR	2.2	1.0	0.50	0.56	0.24	43	120
	7rf	MYA	ND	ND	ND	0.01	0.10	18	47
		SER	ND	ND	ND	0.01	0.07	12	33
		STR	0.80	0.08	0.15	0.59	0.74	130	350

<sup>a</sup>In order: Exposure concentration (ppm); where indicated, cv specifies tanks were covered during exposure; animal size, large or small where indicated, otherwise medium size range.

nd . not detectable

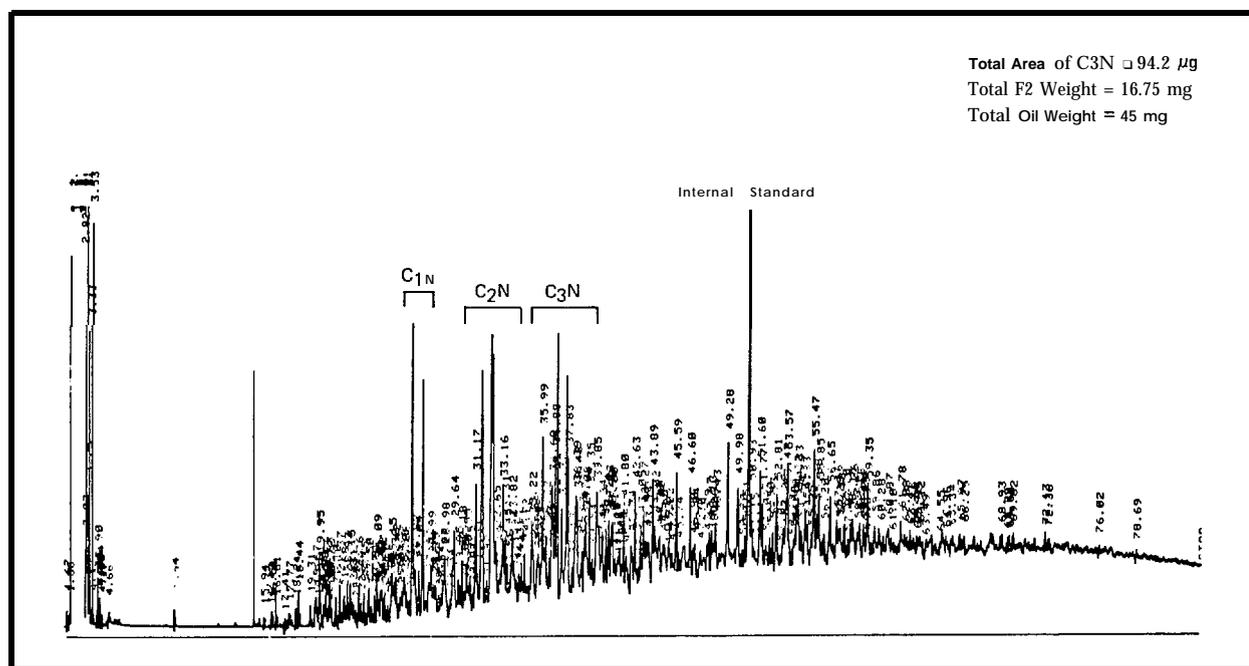


FIGURE 4 GC<sup>2</sup> TRACE OF AROMATIC FRACTION OF LAGO MEDIO CRUDE OIL

*Serripes groenlandicus* exhibited a more consistent and a wider behavioral response repertoire to oil exposure than did *M. truncata*. Both ostial closure and mantle/shell gape have relevance to the exposure of soft tissues to hydrocarbons. Closure of the ostia at even low concentration; of oil shut off filtration and "limited the input of oil, explaining why the species showed few other responses at concentrations below 50 ppm. Conversely, rather than being an avoidance response, the mantle and shell gape which occurred both at higher concentrations and later in the experimental period are more likely to have been direct toxic effects. Both hydrocarbon narcosis and muscle fatigue are possible explanations.

The sporadic bursts of locomotion recorded at higher concentrations may be expected to expose sensitive tissues to oil. Exposure of soft tissues would also result from shell damage. The possibility of increased predation on the bivalve because of its emergence from the sediment, shell gapping, and shell damage has to be considered as a potential oil exposure effect on the benthic population. The decreased capacity to respond normally to tactile stimuli, as shown during exposure, suggests an early narcotizing effect. The latent recurrence of this negative response is more likely to have been of pathological origin.

Sequential dosing in *S. groenlandicus* pointed out that while the first low concentration phase was innocuous, similar to single exposures at the same concentration, a second exposure at a higher concentration elicited a markedly greater response than what would have been predicted from single exposures. This type of sequential exposure and corresponding response is similar to the situation reported for Bays 9 and 10 following the 1981 spill at Cape Hatt. Emergence and shell/mantle gaping were outstanding behaviour indices. The simplest explanation for the sensitivity of the species to sequential exposures may be that the animal has only a limited capacity to remain with shells closed. Once this time is exceeded (somewhat longer than 6 hours), the valves open and soft tissues become exposed to oil.

*Strongylocentrotus droebachiensis* served as a useful benthic species in which to observe the behavioral effects of dispersed crude oil. It was sensitive, showing responses even to 0.5 ppm of oil and demonstrating a fairly consistent dose response. A primary effect of oil exposure was debilitation of tube feet functions, causing loss of covering material and ability to adhere to substrate. The natural consequences of this may include an increased susceptibility to predation, displacement by currents, resulting in physical damage or removal from food source and habitat. This may be especially significant since this debilitating condition may last for several days even though the initial exposure may have been small and of short duration. The early responses of the tube feet, curling of aboral tube feet and loss of ability to respond to tactile stimulation, may result from the narcotizing effect of hydrocarbons in water. Distribution of hydrocarbons by the water vascular system of the urchin would generalize the response. Although interruption of water exchange was not verified, observations of the tube feet changes imply it was likely. Also, throughout the affected period, the peristomal membrane and the periproct regions displayed a sunken and flaccid appearance suggesting an impaired hydrostatic system.

A neuromuscular effect is implicated in the spine responses, again at least initially narcotic in nature. Spine drooping and spine rigidity alternated with each other and with a more normal radial but non-rigid arrangement. While spine drooping would increase the potential for damage and perhaps predation, spine rigidity would counter that effect. The response of assuming a more uniformly round, almost ball-like, appearance with rigid spines would, however, enhance the potential for physical displacement by currents.

Recovery of the spine system to respond to mechanical stimulation occurred sooner than did tube foot response, lending support to the suggestion that two different physiological systems were affected. Lethality occurred only during the 500 ppm single exposure test and during one of the Bay 9 simulating sequential tests. Recovery from the 500 ppm exposure was very limited, however; only two of the original 20 animals survived.

The responses to sequential dosing correlate well with divers' descriptions of the state of urchins in Bays 9 and 10 within a few days of the BIOS spill. Loss of adherence, narcotization, free movement in currents, and a decrease in the number of urchins in the spill area were all reported for that time period.

Tests of the effects of Corexit at 50 ppm, the amount used in 500 ppm oil dispersions, showed that all three species were responsive to the compound. The dispersant had a narcotizing effect on all three species, usually shortly after exposure. Recovery from all the noted responses for Corexit was rapid, however, and the overall effect of Corexit was much less than that of oil dispersions.

In all, the behavioral observations have shown that in these three major Arctic benthic species, the direct effects of short-term (6-h) exposure to dispersed oil are not likely to be fatal at 250 ppm or less. Behavioral changes may predispose these species, particularly *S. groenlandicus* and *S. droebachiensis*, to increased predation. Removal from habitat is another indirect effect, potentially most important to urchins.

#### **Tissue f-hydrocarbons**

The tissue hydrocarbon part of this study was little emphasized in this phase of the program, and was limited in terms of both sample size and analytical detail. It was evident, however, that all three species accumulated crude oil hydrocarbons.

The two bivalve species showed maximum levels by 6 h or 18 h, corresponding to the end points of exposure regimes. By 7 d, these levels generally had decreased, even to background concentrations in some instances. In urchins, however, a very different response was noted. Samples of tissues taken at 7 d showed an increased accumulation of hydrocarbons. The source of these is likely to have been from residues accumulated on the external body surface which were then absorbed even during the recovery period. This pattern may serve to explain the latent mortalities in the test populations. Further, an emphasis on volatile hydrocarbons such as the water-soluble alkyl benzenes in the 6 h samples may have been a major factor in the rapid early narcosis which was recorded for urchins.

The pattern of accumulation and release observed in these three species is the same as that observed in the field following the experimental spill (Boehm et al., 1982). The actual levels do not and cannot be expected to correlate since sampling times were not the same. The higher values recorded in this study at 6 h as compared to the first post-spill samples taken in the field may be attributed to differences in sampling times. During the tank study, sampling was carried out immediately after the oil exposure, whereas field study samples were taken 1 to 4 d after exposure.

A further phase of the study program is planned to define hydrocarbon uptake and clearance characteristics, and correlate them with the behavioral effects of dispersed oil exposure.

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