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Histopathologic and Biochemical Responses in Arctic Marine Bivalve Molluscs Exposed to Experimentally Spilled Oil

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ABSTRACT. Following two experimental spills of chemically dispersed and undispersed crude oil in shallow bays on the northwest coast of Baffin Island, Canadian Arctic, the bivalve molluscs *Mya truncata* and *Macoma calcarea* accumulated significant amounts of petroleum hydrocarbons in bays receiving dispersed oil and in those receiving crude oil alone (Boehm *et al.*, 1987). Following the spills, *Mya* released accumulated hydrocarbons more rapidly than *Macoma*.

Specimens of *Mya truncata* and *Macoma calcarea* for histopathologic examination were collected immediately before, immediately after and one year after the experimental oil spills. Immediately after the spill there was an increased incidence of gill and digestive tract necrosis in *Mya* from the bays receiving chemically dispersed oil (Bays 7, 9 and 10). This was accompanied by an increase in the number of mucus cells in the digestive tract epithelium. After one year a few clams had granulocytomas throughout the tissues. Three clams from Bay 11 (receiving oil alone) collected one year after the spill had invasive neoplasias (probably cancer). One clam collected from Bay 7 immediately after the spill had a similar lesion.

There were few lesions in *Macoma* from Bays 7 and 9 immediately after or one year after the spill. One year after the spill, animals from Bay 11 had a high incidence of vacuolization of the digestive tubule epithelium. The incidence of parasitism and hemocytic infiltration also was higher in *Macoma* from Bay 11 than from the other bays. One specimen had a blood neoplasm.

Clams *Mya truncata* were collected for biochemical analysis immediately before, immediately after and about two weeks after the simulated oil spills. Concentrations in the clam tissues of glucose, glycogen, trehalose, total lipid and free amino acids were measured. Concentrations and ratios of free amino acids in adductor muscles were the most useful indices of pollutant stress.

The results of the biochemical analyses indicate that *Mya* from the four bays were not severely stressed by either dispersed oil or oil alone. Immediately after the spill, clams from the two major dispersed oil bays, and particularly Bay 10, appeared to be more severely stressed than clams from Bay 11. After two weeks, clams from the dispersed oil bays were nearly normal, while those from the bay receiving oil alone appeared stressed. These results seem to corroborate results from analytical chemistry and histopathology: that the acute effects of dispersed oil are greater than those of undispersed oil, but effects of undispersed oil on infaunal molluscs develop more slowly and persist longer than those from dispersed oil.

Key words: oil spill, dispersant, *Mya truncata*, *Macoma calcarea*, histopathology, biochemistry, neoplasia, free amino acids, glycogen, parasites

RÉSUMÉ. A la suite de deux déversements expérimentaux de pétrole chimiquement dispersé et de pétrole brut non dispersé dans des baies peu profondes de la côte nord-ouest de l'île Baffin, dans l'Arctique canadien, les mollusques bivalves *Mya truncata* et *Macoma calcarea* ont accumulé des quantités significatives d'hydrocarbures de pétrole dans les baies traitées au pétrole dispersé et dans celles recevant seulement du pétrole brut (Boehm *et al.* 1987). A la suite de ces déversements, les *Mya* ont libéré les hydrocarbures accumulés plus rapidement que les *Macoma*.

On a recueilli des spécimens de *Mya Truncata* et de *Macoma calcarea* pour un examen histopathologique tout de suite avant les déversements expérimentaux, tout de suite après, et au bout d'un an. Immédiatement après le déversement, il y a eu une incidence accrue de nécrose des branchies et du tube digestif chez les *Mya* des baies ayant reçu du pétrole chimiquement dispersé (baies 7, 9 et 10). Cela s'est accompagné d'une augmentation du nombre de cellules muqueuses dans l'épithélium du tube digestif. Au bout d'un an, quelques myes présentaient des granulocytes dans tous leurs tissus. Trois myes de la baie 11 (ayant reçu le pétrole brut) recueillies un an après le déversement, présentaient des néoplasmes envahissants (probablement cancéreux). Une mye recueillie dans la baie 7 immédiatement après le déversement présentait une lésion semblable.

Les *Macoma* des baies 7 et 9 ne présentaient que peu de lésions tout de suite après le déversement ou un an plus tard. Un an après, les animaux de la baie 11 présentaient une incidence élevée de vacuolisation de l'épithélium du tube digestif. L'incidence de parasitisme et d'infiltration hémocytique était aussi plus élevée chez les *Macoma* de la baie 11 que chez ceux des autres baies. Un spécimen avait un néoplasme sanguin.

Les myes *Mya truncata* ont été ramassées en vue d'analyses biochimiques tout de suite avant qu'on ne procède à la simulation de marées noires, tout de suite après, et au bout de deux semaines. On a mesuré dans les tissus des myes les concentrations en glucose, en glycogène, en tréhalose, en lipides totaux et en acides aminés libres. Les concentrations et les taux d'acides aminés libres dans les muscles adducteurs ont été les indices les plus utiles pour évaluer le stress dû à la pollution.

Les résultats des analyses biochimiques indiquent que les *Mya* des quatre baies n'ont pas été gravement traumatisées ni par le pétrole dispersé, ni par le pétrole brut. Tout de suite après le déversement, des myes des deux plus grandes baies ayant reçu du pétrole dispersé, en particulier de la baie 10, semblaient plus gravement atteintes que celles de la baie 11. Après deux semaines, des myes provenant des baies ayant reçu du pétrole dispersé, étaient presque normales, alors que celles de la baie ayant reçu du pétrole brut semblaient traumatisées. Ces résultats paraissent corroborer ceux de l'analyse chimique et de l'histopathologie, à savoir que les effets immédiats et intenses du pétrole dispersé sont plus importants que ceux du pétrole non dispersé, mais que les effets du pétrole non dispersé sur les mollusques de l'endofaune se manifestent plus lentement et persistent plus longuement que ceux du pétrole dispersé.

Mots clés: marée noire, agent de dispersion, *Mya truncata*, *Macoma calcarea*, histopathologie, biochimie, néoplasme, acides aminés libres, glycogène, parasites

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INTRODUCTION

More than 10 000 tons of chemical dispersant were used to clean the coast of Cornwall, England, of Kuwait crude oil following the *Torrey Canyon* oil spill in 1967. It is now generally agreed that the dispersant caused more damage to the intertidal fauna and flora than did the oil itself (Southward and Southward, 1978). The most frequently used dispersant during the *Torrey Canyon* cleanup contained 12% nonionic surfactant and 3% stabilizer in a high aromatic solvent (kerosene extract). This mixture was highly toxic to nearly all forms of marine life. Because of the disastrous consequences of dispersant use in this and a few other spills, use of chemical dispersants for oil spill cleanup fell into disfavor.

Since the *Torrey Canyon* incident, considerable progress has been made in developing dispersants that have a very low

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toxicity to marine organisms. Since dispersal may be the method of choice for treating spilled oil in many cases, there is an urgent need for information about the toxicity and environmental impact of oil that has been dispersed with the new generation of "low-toxicity" dispersants (Sprague *et al.*, 1982).

Objectives

In this paper, we report the results of a research project performed in concert with the Baffin Island Oil Spill (BIOS) Project (see Sergy and Blackall, 1987). This issue of *Arctic* contains the results of several study components of the BIOS Project.

The primary objective of the investigation described here was to assess and compare sublethal biological effects of chemically dispersed and non-dispersed spilled oil on benthic infaunal bivalve molluscs from the Arctic. This research project had three components: accumulation by three species of molluscs (*Mya truncata, Serripes groenlandicus* and *Astarte borealis*) of hydrocarbons from dispersed and non-dispersed spilled crude petroleum; sublethal biochemical responses of *Mya truncata* to dispersed and non-dispersed spilled crude petroleum; and histopathology of *Mya truncata* and *Macoma calcarea* up to one year after the simulated oil spills. The program was designed to determine if chemically dispersed oil is more or less bioavailable than undispersed oil to benthic infaunal bivalve molluscs and whether dispersed oil is more harmful than undispersed oil to these animals. The results of the bioaccumulation studies are reported elsewhere in this volume (Boehm *et al.*, 1987).

Background

Oil Spills: In 1981, as part of the Baffin Island Oil Spill (BIOS) Project, oil was released for experimental purposes into the nearshore arctic environment (see Sergy and Blackall, 1987, for experimental design and summary). A Lagomedio crude oil and the dispersant Corexit 9527 were used in the study. A crude oil-dispersant mixture was discharged to Bay 9 with a resulting exposure of 300 ppm·h^{-1} and sustained concentrations of 55 ppm oil in the water column. An adjacent Bay 10 received 30 ppm·h^{-1} and 5 ppm exposure, while the rest of Ragged Channel, including the reference Bay 7, received an average of only 0.05 ppm. An untreated oil slick released in Bay 11 was left to natural processes after stranding on the beach. While contamination of the water column was minimal, oil from the beach was deposited in nearshore subtidal sediments over time.

Hydrocarbon Accumulation: Marine animals readily accumulate petroleum hydrocarbons in their tissues from dispersion or solution in seawater and to a lesser extent from petroleum-contaminated sediments and food (Neff *et al.*, 1976; Boehm and Quinn, 1977; Malins and Hodgins, 1981; Neff and Anderson, 1981; Augenfeld *et al.*, 1982). Bivalve molluscs, apparently because they have a limited ability to metabolize aromatic hydrocarbons to water-soluble and easily excreted metabolites (Stegeman, 1981, 1985; Lee, 1981), tend to accumulate petroleum hydrocarbons to higher concentrations and retain them longer than do other phyla of marine organisms (Neff *et al.*, 1976; Boehm and Quinn, 1977; Neff and Anderson, 1981; Elmgren *et al.*, 1983). Dispersants favor the formation of very small oil droplets in the water column. The oil droplets are of a size that might be readily filtered from the water and ingested by bivalve molluscs during normal filter feeding. Thus, the use of dispersant could increase the bioavailability of petroleum hydrocarbons and, of particular importance, the poorly soluble medium molecular weight polycyclic aromatic hydrocarbons and heterocyclics (azaarenes, dibenzothiophenes, etc.) to bivalve molluscs.

In the BIOS studies, *Mya truncata* and *Serripes groenlandicus*, which are filter-feeders, rapidly accumulated dispersed oil in Bays 9 and 10 immediately after the spill but released much of the hydrocarbons by the time of the second post-spill sampling about two weeks after the spill (Boehm *et al.*, 1987). In Bays 9 and 10, the deposit-feeders, *Macoma calcarea*, *Astarte borealis* and *Nuculana minuta*, accumulated more oil than did the filter-feeders (presumably from the sediments) and retained it longer. In Bay 11, receiving undispersed oil, all five species accumulated very little oil immediately after the spill but became heavily contaminated within about two weeks. Bay 7, considered a reference bay, actually received about 50-100 ppb dispersed oil in the first few days after the dispersed oil spill. This was about 1000-fold less than the amount in the water of Bay 9. Nevertheless, the molluscs, especially *Serripes*, from Bay 7 accumulated some oil at the one-day post-spill sampling time.

Both *Mya* and *Serripes* depurated oil during the two-week post-spill period, in part through an *in vivo* biodegradation presumably by microbial activity in the guts of the animals. However, *Serripes* preferentially retained the high molecular weight saturated hydrocarbon assemblage as well as the higher alkylated naphthalene, phenanthrene and dibenzothiophene compounds, whereas *Mya* depurated all hydrocarbon components, although the water-soluble alkyl benzenes and naphthalenes were depurated somewhat faster than other components. The filter-feeders depurated oil even though the sediments in which they resided still contained oil. However, the deposit-feeders continued to accumulate oil from the sediments, at least for the two weeks after the spills.

Histopathology: Petroleum hydrocarbons, and particularly the more toxic aromatics and heterocyclics, accumulated by marine animals interact with cells and tissues to produce a variety of lesions. Aromatic hydrocarbons bind to the surface of cell membranes and interfere with cell membrane-mediated biological processes (Roubal, 1974; Roubal and Collier, 1975). Many hydrocarbons are irritants and cause localized inflammatory responses. In oysters *Crassostrea gigas* from the *Amoco Cadiz* oil spill site, the most common histopathology was leucocytosis (an inflammatory response) in mantle and gill tissues (Neff and Haensly, 1982). Cockles *Cerastoderma edule* and mussels *Mytilus edulis*, transplanted to a bay that was heavily contaminated with oil from the *Amoco Cadiz* spill, developed accumulations of lipid droplets and lysosomal granules in the digestive diverticula (Wolfe *et al.*, 1981). Stainken (1976) reported generalized leucocytosis in the mantle of soft-shell clams *Mya arenaria* exposed in the laboratory to oil. He also observed glycogen depletion and cellular vacuolization in several tissues of exposed clams. A wide variety of other histopathological lesions have been reported in invertebrates and fish exposed to petroleum in the laboratory or field (Malins, 1982).

Crude petroleum and heavy refined oils (e.g., bunker C residual oil) contain known carcinogens including benzo(a)pyrene, dimethylbenz(a)anthracene and methyl chrysene (Neff, 1979). There are several reports in the literature of increased incidence of apparently cancerous tumors in populations of bivalve molluscs from oil-contaminated environments (Bayne *et al.*, 1985). However, in no case has it been unequivocally

demonstrated that oil was the immediate cause of the cancerous lesions.

Immunosuppression and the resulting increased susceptibility to disease, including parasitism, have been observed in molluscs and other marine animals exposed to oil spills (Hodgins *et al.*, 1977; Sindermann, 1982). Since some hyperplastic or neoplastic (cancer-like) lesions in molluscs are known or suspected of being caused by viruses, bacteria, or fungi (Couch and Winstead, 1979), similar cancer-like lesions in bivalves from oil spill sites may result from petroleum-mediated decrease in resistance to infection with pathogenic organisms.

Biochemistry/Physiology: Several physiological or biochemical measures of metabolic energy partitioning and nutritional status may be sensitive indices of sublethal pollutant stress in marine invertebrates. Typical responses of bivalve molluscs to chronic exposure to sublethal concentrations of petroleum include alterations in respiration rate or ratio of oxygen consumed to nitrogen excreted (Widdows *et al.*, 1982; Carr and Linden, 1984), reduction in nutrient assimilation and scope for growth (Gilfillan and Vandermeulen, 1978; Stekoll *et al.*, 1980; Bayne *et al.*, 1985), depletion of glycogen reserves (Stainken, 1976), changes in tissue free amino acid concentrations and ratios (Jeffries, 1972; Roesijadi and Anderson, 1979) and decrease in condition index (Roesijadi and Anderson, 1979; Augenfeld *et al.*, 1980). All of these responses are indicative of a pollutant-mediated increase in metabolic load (loading stress) on the animals. In oysters from the *Amoco Cadiz* oil spill site, we have observed statistically significant long-term (more than two years) changes in tissue free amino acid ratios, blood glucose concentration and reserves of glycogen and ascorbic acid (Neff and Haensly, 1982; Neff *et al.*, 1985).

MATERIALS AND METHODS

Four experimental bays were sampled in these experiments (Sergy and Blackall, 1987). Bay 7 was considered a reference bay (though it received a small amount of dispersed oil), Bays 9 and 10 received dispersed oil and Bay 11 received oil alone.

Mollusc specimens were collected by BIOS Project divers, using an air-lift system (Snow *et al.*, 1987), at ten stations located along two transects paralleling the shore at the 3 m and 7 m isobaths in each bay. Specimens were collected several days before the spill, one to four days after the spill, approximately two weeks after the spill and one year after the spill. Some samples were kept over night in the cold before freezing or preserving.

For histopathology investigations, *Mya truncata* and *Macoma calcarea* were collected immediately before, immediately after and one year after the experimental spills. *M. truncata* for biochemical investigations were collected immediately before, immediately after and two weeks after the experimental spills. Only small numbers of *M. calcarea*, *Astarte borealis* and *Serripes groenlandicus* were available from a few bays on a few sampling occasions, so they could not be used for the biochemical investigations.

Specimens for histopathology were fixed at the Baffin Island study site by the collectors. Fixation for the 1981 collections was in Carson's modified Millonig's phosphate-buffered formalin. Neutral buffered 10% formalin was used for the 1982 collections.

For fixation of larger specimens such as *Mya truncata*, one valve was removed before the animal was placed in the fixative. Smaller specimens, such as *Macoma calcarea*, were treated similarly if possible, or at least one shell was cracked slightly to permit entry of the fixative. The specimens were placed in fixative in small plastic tissue bags, in which were also placed coded identification tags. The bags were then sealed, packed in shipping containers and shipped to the Battelle laboratory in Duxbury, Massachusetts, for histopathological analysis.

Upon receipt at Battelle, the samples were removed from the shipping containers and logged in according to the coded label by station number, species and date collected. The specimens were then washed in running tap water for several hours and transferred to 70% ethyl alcohol until histological processing.

For processing, the specimens were trimmed to provide cross-sectional pieces of tissue, which were dehydrated and embedded in Paraplast Plus.

The embedded tissues were sectioned at 5-6 μm and stained with hematoxylin and eosin using standard procedures. The stained sections were examined for any pathological conditions.

Mollusc samples for biochemical analysis were frozen in the field and shipped to the laboratory on dry ice. In the laboratory, individual clams were thawed, shucked and weighed. Tissue glucose, glycogen and other glucose-containing carbohydrates (mainly trehalose, a glucose disaccharide) were analyzed with the Beckman automatic glucose analyzer after selective hydrolysis according to the method of Carr and Neff (1984). Total lipids were determined in mollusc soft tissues by the methods of Holland and Gabbott (1971).

Tissue free amino acids from adductor muscles of clams were extracted and analyzed by methods similar to those described by Roesijadi and Anderson (1979). The tissues were homogenized in 7% trichloroacetic acid. The homogenate was centrifuged and the supernatant was washed three times with diethyl ether to remove trichloroacetic acid. The supernatant was then lyophylized and dissolved in 0.1N HCl. Samples were analyzed with a Waters Associates gradient high-performance liquid chromatograph equipped for post-column derivatization with O-phthalaldehyde and fluorescence detection.

Data were analyzed for statistically significant differences between reference and treatment means by the Mann-Whitney one-tailed U-test, Student's t-test and Kruskal-Wallis one-way analysis of variance. The Spearman rank correlation test was used to detect association between pairs of biochemical parameters among animals from different sampling times and treatment groups.

RESULTS

Molluscs from all four bays, including Bay 7, the supposed reference bay, were exposed to some oil, and molluscs from all four bays accumulated some oil (Boehm *et al.*, 1987). Highest concentrations of oil in tissues of *Mya truncata* were observed in animals from Bay 10, followed in order of decreasing maximum hydrocarbon concentration by animals from Bays 9, 7 and 11 (see Table 3). Highest concentrations of oil in tissues of *Macoma calcarea* were observed in animals from Bay 9, followed by Bays 10, 11 and 7. Because all experimental animals were exposed to oil and accumulated some hydrocarbons in their tissues, there were no true reference animals in this investigation. This does not pose a serious problem with respect to interpretation of the results of the histopathological analysis, because it is still possible to compare incidences of different types of lesions among populations exposed for different lengths

of time to different concentrations and forms (dispersed and undispersed) of oil. In the case of the biochemical parameters, it is always advisable to compare values for exposed populations to those from nearby unexposed (normal) populations sampled at the same time in order to compensate for natural seasonal variations in the values for these biochemical parameters. Because no true reference populations of *Mya* were available in this investigation, it is more difficult to attribute changes observed in biochemical parameters among populations of *Mya truncata* to natural variation or effects of oil. To a limited extent, pre-spill samples from each bay can be used as unexposed reference samples.

Histopathology

Results of histopathologic observations on tissues of 856 specimens of *Mya truncata* and 519 specimens of *Macoma calcarea* from the four bays involved in the BIOS Project are summarized in Tables 1 and 2 respectively.

Many of the tissues showed indications of poor fixation, probably due to handling in the field, rather than the fixative itself. These indications were taken into account in assessing pathology due to factors other than fixation. It was difficult, however, to determine whether conditions such as sloughing of gill epithelium or general gill necrosis in the immediate post-spill specimens resulted from oil, dispersed oil or poor fixation, since the condition was common in many of the specimens from every bay at every sampling.

Mya truncata: The most common histopathologic conditions observed in *M. truncata* were hemocytic infiltration, or inflammation, and the occurrence of an unidentified trematode parasite (Table 1). The incidence of hemocytosis and parasitism in clams from Bays 7 and 11, but not from Bays 9 and 10, decreased markedly between the pre-spill and first post-spill sampling. Immediately following the spill, the incidence of necrotic tissue, particularly in the gills and digestive tract, increased in Bays 7, 9 and 10. A year later this incidence had decreased considerably. Necrotic lesions in the digestive tract were accompanied by an increase in the number of mucus-producing cells in the gastrointestinal tissues, and in Bay 10 by unidentified basophilic inclusions in the digestive gland tubules. Bays 9 and 10 produced a few one-year post-spill clams with granulocytomas throughout the tissues (Fig. 1).

One specimen with a hematopoietic neoplasm was collected from Bay 7 immediately after the spill, and three specimens with neoplasias were taken from Bay 11 one year after the spill. Figure 2 shows one of the clams with a neoplastic lesion. Invasion of the digestive tubules is evident.

Hyperplastic gill epithelium was observed in two specimens from Bay 7 and one specimen from Bay 9 prior to the spill but was not observed at any other time.

Macoma calcarea: The only pre-spill collections of *M. calcarea* were from Bay 9. Specimens of *M. calcarea* were collected from Bay 7 shortly after the applications of dispersed oil. No major pathological conditions were noted in these collections of specimens from either Bay 9 or 7. The Bay 7 group showed more necrotic foci and parasites (primarily due to a trematode) than the Bay 9 group, but in other respects appeared to be quite normal (Table 2).

There was little change in the condition of *M. calcarea* from Bay 9 one year after the spill. One specimen showed some hyperplastic growth on the gill, but it did not appear to have any effect on function. A decrease in the number of necrotic lesions was observed in specimens from Bay 7.

TABLE 1. Summary of histopathologic observations of tissues of the clam *Mya truncata* from the BIOS Project site

			Histopathologic condition								
Bay[a]	Collection[b]	N[c]	Hemocytosis	Necrosis	Abcesses	Digestive tissue vacuolization	Metaplasia	Hyperplasia	Neoplasia	Parasites	Other
	1	40	10	0	0	0	0	5	0	38	
7(Ref.)	2	47	0	2	0	0	0	0	2	6	
	3	75	21	3	1	0	0	0	0	29	
	1	94	1	0	1	0	0	1	0	29	
9(D.O.)	2	80	5	5	2	0	0	0	0	36	1 fibrous connective tissue
	3	75	12	3	0	0	1	0	0	40	2 granulocytoma, 1 fibrous connective tissue
	1	84	5	0	0	0	1	0	0	29	1 hypertrophic hemocyte mass in stomach
10(D.O.)	2	102	6	13	1	0	1	0	0	35	1 mucus cell in GI epithelium, 4 basophilic inclusions in digestive tubules
	3	75	7	1	0	0	0	0	0	31	2 granulocytomas, 1 inclusion in digestive tubule epithelium
	1	48	12	0	2	2	0	0	0	17	
11(O.A.)	2	59	3	0	0	0	0	0	0	37	
	3	77	3	4	0	0	0	0	4	53	1 gregarine-like cyst on gill

Incidence of lesions is given as percent of all animals examined (N) in that collection.
[a]Ref., reference bay; D.O., dispersed oil bay; O.A., oil alone bay.
[b]Collection 1, immediate pre-spill; Collection 2, immediate post-spill; Collection 3, one year post-spill.
[c]N = number of clams examined.

TABLE 2. Summary of histopathologic observations of tissues of the clam *Macoma calcarea* from the BIOS Project site

			Histopathologic condition								
Bay[a]	Collection[b]	N[c]	Hemocytosis	Necrosis	Abcesses	Digestive tissue vacuolization	Metaplasia	Hyperplasia	Neoplasia	Parasites	Other
7(Ref.)	2	72	0	12	1	1	0	0	0	6	1 granulocytoma, 1 small cyst in digestive tubule
	3	86	0	1	1	0	0	0	0	7	1 unidentified inclusion in digestive tract, 1 encysted inclusion
9(D.O.)	1	83	1	1	2	0	0	0	0	1	1 unidentified inclusion in testes
	3	75	0	9	0	0	0	1	0	3	
10(D.O.)	3	83	4	5	2	10	0	0	0	5	
11(O.A.)	3	120	5	3	3	52	1	0	1	20	2 inclusions in gonads

Incidence of lesions is given as percent of all animals examined (N) in that collection.
[a]Ref., reference bay; D.O., dispersed oil bay; O.A., oil alone bay.
[b]Collection 1, immediate pre-spill; Collection 2, immediate post-spill; Collection 3, one year post-spill.
[c]N = number of clams examined.

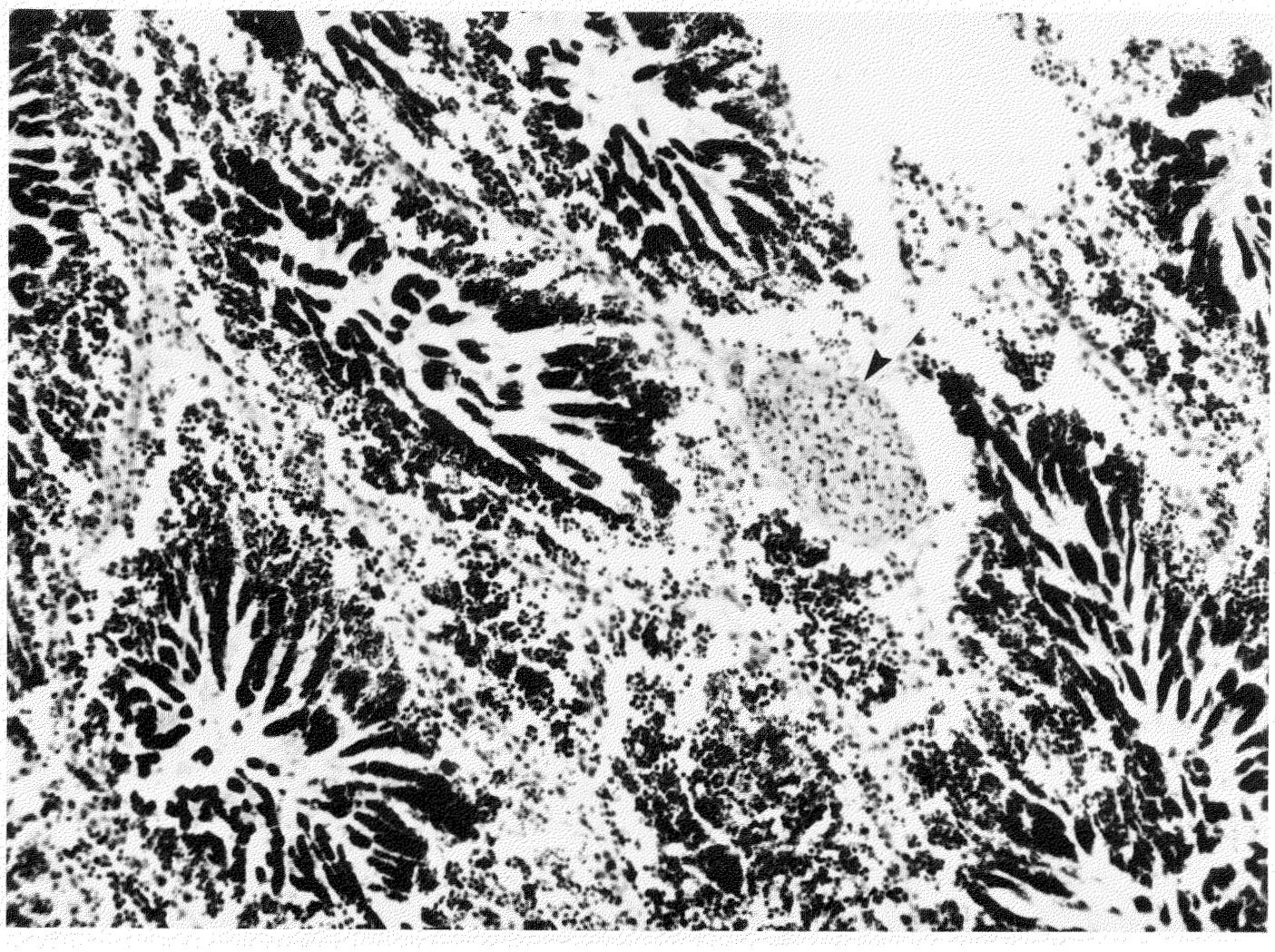

FIG. 1. Granulocytoma (arrow) in testis of truncate soft-shelled clam, *Mya truncata*, from Bay 9 one year following oil spill.

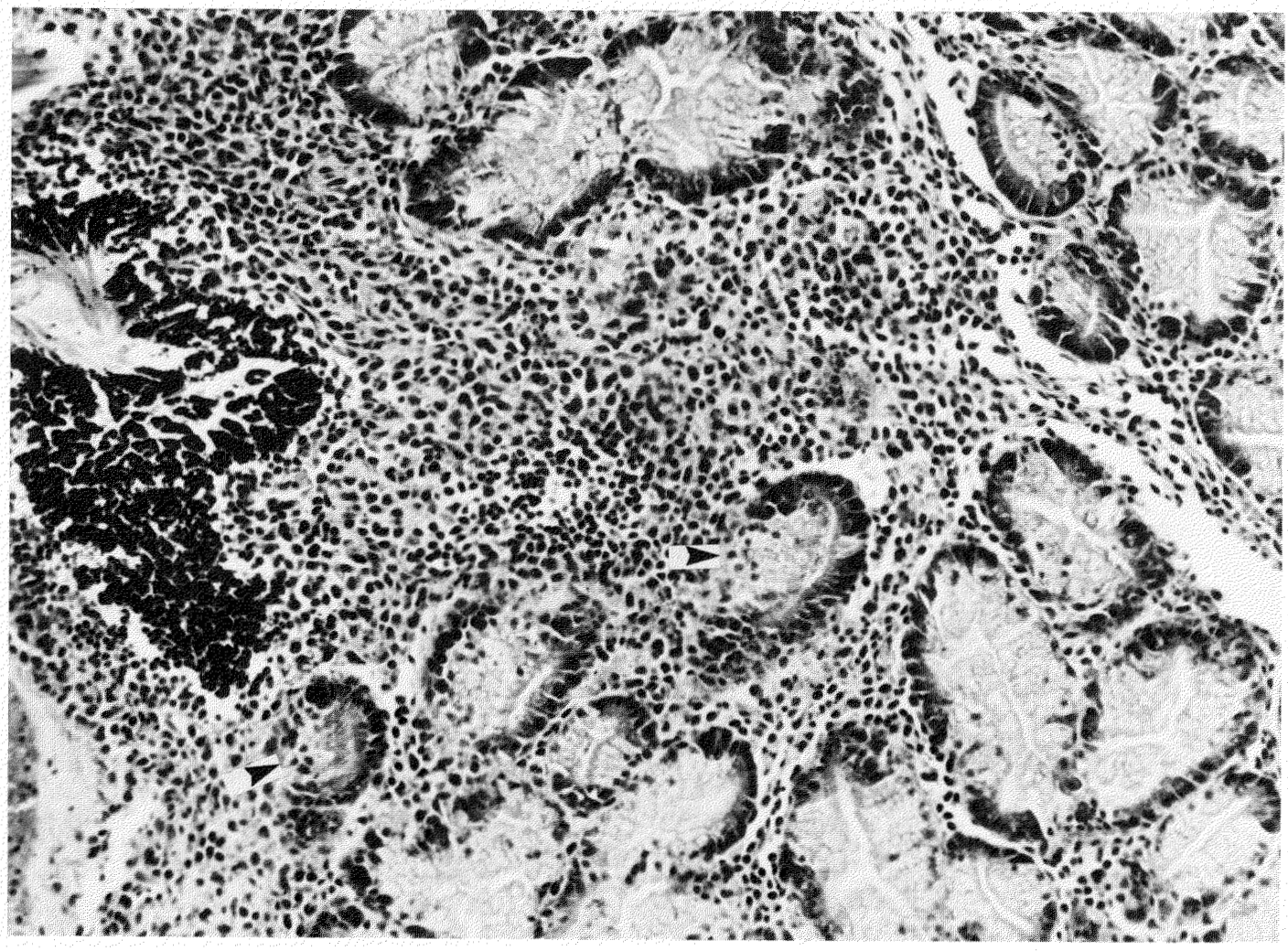

FIG. 2. Hematopoietic neoplasm in digestive tubule area of truncate soft-shelled clam, *Mya truncata*, collected from Bay 11 one year after oil spill. Note invasion of digestive tubules by neoplastic cells (arrows).

Specimens collected from Bay 11 one year after the spill showed the largest number of pathological conditions, especially in the degree of vacuolization of the digestive tubule epithelium. In addition, the parasite burden in *M. calcarea* from Bay 11 was higher than in specimens from the other bays, as was the incidence of hemocytic infiltration and abscesses. One specimen from Bay 11 also had a blood neoplasm. Unfortunately, there are no specimens for comparison from Bay 11 prior to or immediately after the oil application.

Biochemistry

There was a great deal of variation among replicates, experimental bays and sampling times in the concentration of carbohydrates and lipids in the tissues of *Mya truncata* (Table 3). In the pre-spill samples, mean concentrations of free glucose in clams from the three bays receiving dispersed oil were significantly lower than in clams from the bay receiving oil alone. Concentrations of free glucose in clams from Bays 9 and 10 (dispersed oil) and Bay 11 (oil alone) were lower than in clams from Bay 7 at the time of the first post-spill sampling. There was no clear relationship between the amount of oil accumulated by the clams in the day following the spills and the direction or magnitude of change in tissue free glucose concentrations. By the second post-spill sampling, mean tissue glucose concentrations were similar in all four clam populations.

Concentrations of tissue glycogen were quite uniform within populations over time and among populations from the four bays. There was no relationship between body burdens of oil in different clam samples and concentrations in the samples of tissue glycogen. Concentrations of total other carbohydrates, which consist of trehalose and other non-glycogen oligosaccharides, were highly variable and no obvious trends were apparent among samples from different bays or sampling times.

Concentrations of total lipids in clams from Bays 7, 10 and 11 dropped between the pre-spill and first post-spill samples and then returned to pre-spill or higher values by the time of the second post-spill sampling. In clams from Bay 9, total lipid concentration increased between pre-spill and first post-spill and then dropped to the pre-spill range by the time of the second post-spill sampling.

Fourteen different free amino acids were identified and quantified in the adductor muscles of *Mya truncata* from the four bays. The mean concentration of total free amino acids ranged from 12.45 to 23.25 $\mu m \cdot mg^{-1}$ wet weight in clams from different bays at different sampling times (Table 4). In clams from two of the bays receiving dispersed oil (Bays 9 and 10), the mean concentration of tissue total free amino acids dropped

TABLE 3. Carbohydrates and lipids in tissues of the truncated soft-shell clam, *Mya truncata*, collected from the BIOS site before and after the simulated oil spill

Bay/Collection	Petroleum[d] (ppm)	($mg \cdot g^{-1}$ wet wt. + SE) Glucose	Glycogen	Other carbohydrates	Total lipids
7 (reference)					
Pre-spill	0.34	0.642±0.037	11.68±0.77	3.11±1.20	158.08±17.76
1st post-spill	114	1.272±0.127	13.85±0.96	0.26±0.19	141.07±15.29
2nd post-spill	47	1.608±0.125	16.84±1.30	0.74±0.41	163.14±22.02
9 (dispersed oil)					
Pre-spill	0.38	0.742±0.044[c]	10.32±0.64[b]	1.12±0.58[c]	148.39±17.18
1st post-spill	168	0.722±0.032[abc]	11.31±1.02[c]	2.11±0.60	183.52±10.21[bc]
2nd post spill	124	1.517±0.098	12.88±1.40[a]	1.66±0.52	166.01±13.16
10 (dispersed oil)					
Pre-spill	0.68	0.744±0.058[c]	14.58±1.02	0.13±0.07[c]	170.31±11.98
1st post-spill	322	0.428±0.048[a]	17.39±1.75	0.63±0.25	133.40± 8.59
2nd post-spill	144	1.515±0.140	14.50±1.01	0.95±0.29	215.50±12.87
11 (oil alone)					
Pre-spill	0.43	1.482±0.155[a]	12.29±0.99	1.67±0.53	169.65±12.47
1st post-spill	2.0	0.514±0.075[a]	15.03±0.60	0.27±0.26	118.52±12.10
2nd post-spill	93	1.459±0.071	13.86±1.02	1.34±0.39	166.34±18.42

All values are in $mg \cdot g^{-1}$ wet tissue. Mean concentrations of petroleum hydrocarbons in tissues of the clams also are given.
[a]Significantly different from Reference (Sta. 7), Student's T-test, or Kruskal-Wallis one-way ANOVA.
[b]Significantly different from Disp. Oil (Sta. 10), Student's T-test, or Kruskal-Wallis one-way ANOVA.
[c]Significantly different from Oil Alone (Sta. 11), Student's T-test, or Kruskal-Wallis one-way ANOVA.
[d]Data from Boehm *et al.*, 1987.

between the pre-spill and first post-spill samples and then rose again in the second post-spill samples. The opposite trend was observed in clams from the bay receiving oil alone (Bay 11), while tissue total free amino acid concentrations in clams from Bay 7 remained relatively constant (range 15.37-17.65 $\mu M \cdot mg^{-1}$).

In clams collected immediately before the BIOS oil spills, concentrations of several tissue free amino acids were significantly different in clams from the four bays. Immediately after the spills, concentrations of one to three amino acids were significantly different in clams from the four bays receiving dispersed or undispersed crude oil. Concentrations of total and most individual tissue free amino acids were lower in clams from Bay 10 than in clams from the other three bays. In clams collected during the second post-spill sampling approximately two weeks after the spills, there were many statistically significant differences in concentrations of individual tissue free amino acids among clams from the four bays. Values for clams from Bay 10 varied most from the corresponding values for clams from the other three bays.

Two parameters that have been recommended as indices of sublethal stress in marine invertebrates are the molar ratio of taurine to glycine and the sum of the concentrations of threonine plus serine (Bayne *et al.*, 1985). Stressed animals should have a higher taurine/glycine ratio and lower threonine plus serine concentration than unstressed animals. The taurine/glycine molar ratio in adductor muscles of clams from Bay 9 increased between the pre-spill and first post-spill samples, and then decreased to the pre-spill value at the time of the second post-spill sample. Values for clams from Bay 9 generally were lower than the corresponding values for clams from the other bays. Threonine plus serine concentrations increased in clams from Bay 9 between the pre-spill and first post-spill samples, and then returned to the pre-spill value by the time of the second post-spill sample. These results suggest that clams from Bay 9 were slightly stressed at the time of the first post-spill sample when they contained a mean of 168 ppm oil residues in their tissues.

In clams from Bay 10, there was an increase in adductor

TABLE 4. Molar ratio of taurine to glycine and the sum of the concentrations of threonine plus serine in the free amino acid pool of adductor muscles of truncate soft-shell clams, *Mya truncata*, from the four BIOS experimental bays

Parameter	Pre-spill	1st post-spill	2nd post-spill	Pre-spill	1st post-spill	2nd post-spill
	Bay 9			Bay 10		
Total free amino acids	18.89	14.74	16.32	14.45	12.45	23.25
Taurine/glycine	0.108±0.007 (9)	0.134±0.006 (19)	0.109±0.006 (20)	0.137±0.007 (13)	0.141±0.007 (18)	0.297±0.036 (16)
Threonine + serine	0.577±0.065 (9)	0.365±0.040 (20)	0.533±0.067 (20)	0.389±0.033 (13)	0.359±0.024 (18)	0.191±0.047 (13)
	Bay 11			Bay 7		
Total free amino acids	13.51	16.12	13.00	15.37	17.65	16.14
Taurine/glycine	0.109±0.007 (10)	0.263±0.109 (10)	0.112±0.006 (10)	0.141±0.012 (10)	0.141±0.012 (7)	0.109±0.006 (10)
Threonine + serine	0.343±0.045 (10)	0.424±0.035 (10)	0.384±0.038 (10)	0.525±0.070 (10)	0.367±0.056 (7)	0.479±0.104 (10)

Concentrations of threonine plus serine are in $\mu M \cdot mg^{-1}$ dry weight and are the mean and standard error of 7-20 replicate animals per treatment. The number of clams analyzed is given in parentheses.

muscle taurine/glycine ratio and a decrease in the concentration of threonine plus serine at the time of the second post-spill sampling. These results suggest that clams from the second post-spill sample in Bay 10 were slightly stressed. The pattern is less clear for clams from Bays 11 and 7. In clams from Bay 11, the taurine/glycine ratio increased at the time of the first post-spill sample but there was no real change in the threonine plus serine concentration. In clams from Bay 7, the taurine/glycine ratio dropped at the time of the second post-spill sample, and the threonine plus serine ratio dropped at the time of the first post-spill sample.

Spearman rank correlation tests were performed on all biochemical parameters measured for clams from the three sampling times and four experimental bays. Parameters that showed a high ($\alpha<0.05$) degree of interassociation, positive or negative, are tabulated according to sampling time and inter-bay association in Table 5. Clams from the second post-spill sampling had the largest number of associated pairs of biochemical parameters (Table 6). One hundred and five associated pairs were shared by all four bays, indicating that in these samples clams from the four bays were very uniform in relative (though not necessarily absolute) values for the biochemical parameters measured. Clams from the first post-spill sampling had the lowest number of associated pairs and the greatest inter-bay diversity. Clams from the pre-spill sample were intermediate. At all three sampling times there was little association among values for carbohydrate, lipid and free amino acid parameters. In clams from the first post-spill sampling there were no associated pairs shared by Bay 11 (receiving oil alone) and the other three bays.

TABLE 6. The number of associated pairs of biochemical parameters in the truncate clam *Mya truncata* collected from the BIOS experimental bays at three sampling times

Bay combinations[1]	Pre-spill	First post-spill	Second post-spill
7,9,10,11	9		105
7,9,10	5	5	
7,9,11	7		
7,10,11			1
9,10,11	10		
7,9	9	9	
7,10		3	
7,11			
9,10	16	6	
9,11	11		
10,11	1		
7	1	5	
9	26	36	
10	1	4	
11	1		
Total associated pairs	97	68	106

[1]Bay combinations denote paired associations that are shared.

DISCUSSION

Histopathology

Although no clear-cut effects of either the oil or dispersed oil on the pathology of *Mya truncata* or *Macoma calcarea* from the Baffin Island region have been demonstrated up to a year following the application of the oil and dispersant, there are strong indications of more histopathological problems in molluscs from Bay 11 than from the other bays. For example, of the five observed incidences of neoplasms, four occurred in Bay 11 a year after the spill. The parasite burden in the bivalves also appears to be higher there than in the other bays.

A small amount of vacuolization of digestive tubule epithelium is not uncommon and may be normal. The degree to which the digestive tubules of *M. calcarea* from Bay 11 were vacuolated a year after the oil spills seems excessive when compared to the condition of the tubules from *M. calcarea* specimens at other times and at other sites. Increased vacuolization is undoubtedly related to diet or feeding, but whether there is an effect of the oil is not fully understood at this time. Similar conditions of the digestive tubule epithelium were reported in bivalve molluscs contaminated by the *Amoco Cadiz* oil spill (Wolfe *et al.*, 1981; Neff and Haensly, 1982).

The presence of granulocytomas in several specimens of *Mya truncata* from Bays 9 and 10 after a year also is of interest. Lowe and Moore (1979) suggest a relationship between this non-

TABLE 5. A summary of associated pairs of biochemical parameters in *Mya truncata* determined by the Spearman Rank Correlation Test

	Taurine	Aspartate	Threonine	Serine	Glutamate	Glycine	Alanine	Valine	Methionine	Isoleucine	Phenylalanine	Histidine	Lysine	Arginine	Total AA	Glycogen
Taurine																
Aspartate	3A															
Threonine	2B,3A	3A														
Serine	1E,3A	3A	1B,3A													
Glutamate	3A	3A	3A	3A												
Glycine	1E,3A	1A,3A	1A,3A	1A,3A												
Alanine	1E,3A	3A	1A,3A	1A,2B,3A	1C,3A	1E,3A										
Valine	3A	3A	3A	3A	3A	3A	3A									
Methionine	3A	3A	3A	3A	3A	3A	3A	3A								
Isoleucine	3A	3A	3A	3A	3A	3A	3A	3A								
Phenylalanine	3A	3A	3A	1C,3A	3A	3A	3A	3A	3A	3A						
Histidine	3A	3A	3A	3A	3A	3A	3A	3A	3A	3A	3A					
Lysine	3A	3A	1E,3A	2B,3A	3A	3A	1E,3A	3A	3A	3A	3A	1B,3A				
Arginine	3A	3A		3A	3A	3A	3A	3A	3A	3A	3A	3A	3A			
NH_3	3A	3A		1A,3A	1C,3A	1E,3A	3A	3A	3A	3A	3A	3A	3A	3A		
Total AA			1C	1A			1C				1C	1B	1E			
Taurine/glycine																
Threonine + serine	1E		1A,2B	1A			1E					1B	1B		1C	
Glycogen																
Glucose																
Other carbons																
Lipids																3D

Data are tabulated by pairs shared among bays and by sampling times.
Sampling periods: 1, pre-spill; 2, first post-spill; 3, second post-spill.
Bay associations: A, Bays 7,9,10,11; B, Bays 7,9,10; C, Bays 7,9,11; D, Bays 7,10,11; E, Bays 9,10,11.

neoplastic inflammatory cellular condition, which they describe in the marine mussel *Mytilus edulis*, and water quality. They point out that mussels from areas of chronic domestic and industrial pollution have a high incidence of granulocytomas, whereas mussels exposed to low-level pollution exhibit a low to zero incidence of the condition.

Both species, but especially *Mya truncata*, were quite heavily parasitized before and after the oil spill. This degree of parasitism might have an effect on the ability of the clams to withstand toxic effects of the oil and, conversely, the effects of the oil could lead to an increased parasite burden. More needs to be known of how toxicants affect a mollusc's ability to mobilize its own natural defense mechanisms.

Biochemistry

There was a high degree of variability in the values for different biochemical parameters in replicate clams from the same sample, among samples from different bays and in samples collected at different times. This variability makes it difficult to identify biochemical responses of clams to the oil spills. There are several possible explanations for the observed variability.

Bivalve molluscs, like many other marine invertebrates, typically show a wider range of normal (unstressed) values for many biochemical parameters than do fish and other "higher" animals (Carr and Linden, 1984; Bayne *et al.*, 1985; Gabbott, 1976). In species such as the blue mussel, *Mytilus edulis*, for which an extensive body of basic biochemical and physiological information is available (Bayne, 1976), some of this variability can be accounted for or controlled (Widdows *et al.*, 1984). Practically no data are available on the normal biochemistry, physiology and seasonal cycles of *Mya truncata*.

Perhaps more important, and a major problem in a remote field experiment of this sort, are the methods used to sample and handle animals in the field. A substantial time delay between collecting the clams and freezing them can result in large and unpredictable changes in several of the biochemical parameters studied, particularly concentrations of tissue glucose and free amino acids. Ideally, samples should be frozen in liquid nitrogen or dry ice immediately upon collection. This was not feasible in the BIOS study.

Despite these problems, some conclusions can be drawn from the results of these histopathologic and biochemical studies on *Mya truncata*. Based on results of the biochemical analyses, truncate soft-shell clams were not severely stressed by either dispersed or undispersed oil at the contaminant levels attained in the BIOS experiment. Because all treatment groups were exposed to and subsequently accumulated some petroleum, there was no true control or reference group of animals. However, it is possible to compare clam populations exposed to different concentrations of dispersed or undispersed oil and showing different patterns and degrees of hydrocarbon accumulation and release. Clams from Bay 11 (undispersed crude oil) differed the most from clams from other bays, particularly in the second post-spill sample. Clams from Bay 10 (dispersed crude oil) became more heavily contaminated with petroleum hydrocarbons than clams from the other dispersed oil bays (Bays 9 and 7) and showed greater differences than the latter bays in several biochemical parameters, when comparing pre- and post-spill samples from the respective bays. These differences were most marked in the first post-spill survey.

These results are somewhat surprising in that the dispersed oil was discharged into Bay 9 and higher concentrations of dispersed oil were observed over a longer period of time in the water column of this bay than in Bay 10 (Humphrey *et al.*, 1987). Divers observed narcosis in the benthos, including bivalve molluscs, upon contact with the dispersed oil immediately after the release of dispersed oil in Bay 9 (Cross and Thomson, 1987; Mageau *et al.*, 1987). This narcosis was not observed in the benthos of Bay 10. In laboratory simulations of the dispersed oil spill, Mageau *et al.* (1987) observed far greater narcotic effects in benthic animals under simulated Bay 9 conditions than under simulated Bay 10 conditions. Apparently, the narcotized bivalves accumulated less oil in their tissues and thereby experienced fewer physiological and biochemical alterations than the non-nasiotized molluscs in Bay 10.

We can conclude from the histopathologic and biochemical results that chemically dispersed oil may cause more severe acute effects than undispersed oil in benthic infaunal molluscs, but longer term impacts of undispersed crude oil may be more severe than those of chemically dispersed oil. This is undoubtedly related to the observations documented by Boehm *et al.*, (1987) that petroleum contamination of filter-feeding molluscs was greatest in the bays receiving dispersed oil and reached a peak in the first post-spill samples, decreasing in the second post-spill samples. On the other hand, contamination of clams in the bay receiving oil alone was more gradual and reached a peak in the second post-spill sample. In bottom sediments, undispersed crude oil may be more persistent than chemically dispersed oil and so lead to more serious long-term effects in benthic animals. We have obtained similar results in recent mesocosm experiments with chemically dispersed oil (Carr *et al.*, 1985). Benthic animals in tanks receiving chemically dispersed crude oil experienced higher short-term mortality and sublethal effects than animals receiving oil alone. However, after a month, sublethal physiological and biochemical responses were more marked in animals from the undispersed oil treatment groups than the dispersed oil treatment groups.

In this investigation, several biochemical parameters were evaluated as indices of pollutant stress in truncate soft-shell clams exposed to dispersed and non-dispersed crude oil in the BIOS experiment. The parameters used were chosen based on their proven utility for this purpose and because they could be measured in frozen samples, an important consideration in view of the remoteness of the sampling site and lack of facilities to make measurements on-site on fresh tissue. Values for some of the biochemical parameters were significantly different in the four populations of *Mya* samples. Tissue free amino acid concentrations and ratios showed the most changes. Tissue free amino acids also were the most useful index of pollutant stress in oysters *Crassostrea gigas* from bays contaminated with crude oil from the *Amoco Cadiz* crude oil spill (Neff and Haensly, 1982; Neff *et al.*, 1985).

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