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Influence of a Crude Oil Dispersant, Corexit 9527, and Dispersed Oil on Capelin (*Mallotus villosus*), Atlantic Cod (*Gadus morhua*), Longhorn Sculpin (*Myoxocephalus octodecemspinosus*), and Cunner (*Tautogolabrus adspersus*)

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Chemical dispersants are used to emulsify and disperse crude oils after spills in order to prevent coating of inshore areas inhabited by a variety of marine organisms. Water-accommodated fractions (WAF), especially polycyclic aromatic hydrocarbons (PAH), of crude oils are toxic to all life stages of fish and several species of invertebrates (Anderson 1979; Black et al. 1983; Pelletier et al. 1997). Dispersants have also been reported to cause adverse effects as well as interact with crude oil releasing a greater concentration of PAHs in the water column and increasing the concentration of toxic components (Pollino and Holdway 2002; Barron et al. 2003; Koyama and Kakuno 2004). Wolfe et al. (2001) observed that the breakdown products, following the application of dispersants to crude oil, resulted in increased toxicity to eggs and early life stages of a fish, top smelt (*Atherinops affinis*). Moreover, Ramachandran et al. (2004) observed that an oil dispersant increased PAH uptake in fish exposed to crude oil as the induction of the detoxification enzyme CYP1A increased by 6 to 1100 fold than WAF alone.

Several species of finfish inhabit inshore localities of Newfoundland where spills and discharges of crude oil and its derivatives have been reported over several decades, sometimes causing high mortality in seabirds (Khan and Ryan 1991). Dispersants have been applied, occasionally, but there are no reports on their effects after application, either alone or in conjunction with crude oil, on fish. However, studies have observed physiological, biochemical and pathological changes after exposure of indigenous species to WAF (Payne et al. 1988; Khan and Kiceniuk 1984; Kiceniuk and Khan 1987). In view of the potential for Corexit 9527 to increase the toxicity of crude oil, the purpose of the present study was to assess histopathology and/or mortality on the influence of WAF and a mixture of the dispersant and WAF simultaneously in groups of mature fish, viz., capelin (*Mallotus villosus*), Atlantic cod (*Gadus morhua*), longhorn sculpin (*Myoxocephalus octodecemspinosus*) and cunner (*Tautogolabrus adspersus*) following 4-day exposures. Mortality was assessed for capelin after 4 days but the other species were depurated for an additional 4 weeks when the experiments were terminated. These results are reported herein.

MATERIALS AND METHODS

Capelin were caught with a dipnet in mid-June onwards during migration inshore to spawn on the northeastern coast of Newfoundland. The fish (length, 18.0+2.1 cm; mass, 39.4+4.1 g) were transported in aerated, ambient (water temperature, WT 6-

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8°C, pH 7.7, salinity 3.2%) sea water to the Ocean Sciences Centre (OSC) where they were held indoor under fluorescent light (12-h photoperiod) in 5,000L flow-through aquaria supplied with ambient sea water (temperature, ~6°C). The fish were not fed as feeding does not occur during the spawning migration (Scott and Scott 1988). Prior to experimentation, groups of fish, males only, were transferred to 300L (surface area $1m^2$) flow-through aquaria (filled to 250L capacity) supplied with ambient sea water and acclimated for 1 wk.

Atlantic cod (length, 39.8+4.3 cm; mass, 507.4+5.7g) were captured in cod traps set 5-10 m deep in Conception Bay (47°31'N, 53°05'W). The fish were transported to the OSC where they were held initially in a flow-through raceway (11.0 x 2.5 x 1.2 m) receiving ambient seawater at 10L/min. All fish were acclimated about 6 wk prior to experimentation and fed to satiation, freshly-thawed capelin thrice weekly. Groups of cod were subsequently transferred to 300L flow-through aquaria supplied with ambient sea water at a rate of 5L/min.

Longhorn sculpin (length, 33.9+ 3.7 cm; mass, 489.0+5.4g) and cunner (length, 33.4+3.7 cm; mass, 43.4+4.5g) were captured by SCUBA divers using a dipnet in Conception Bay. After transportation to the OSC, they were held in 300L flow-through aquaria into which ambient seawater flowed. The fish were fed to satiation with capelin and acclimated for 6 wk before exposure.

A light crude oil, Hibernia, originating from the Grand Banks of Newfoundland, was used in this study. The predominant components in the unsaturated fraction of the crude oil included the low molecular weight hydrocarbons, naphthalene, phenanthrene, fluorene, chrysene and dibenzothiophene (Hellou et al. 1994). The water-accomodated fraction (WAF) was prepared by introducing the crude oil into a head tank (80L) daily, mixing it with a constant spay of seawater and drawing off the bottom contents into the fish tank (Kiceniuk and Khan 1987). The flow rate was adjusted to 2.5L/min and was identical to an additional source of uncontaminated water that flowed into the fish aquarium. Hydrocarbon analysis was determined by HPLC and expressed as µg/L (ppb) as total hydrocarbon concentration (Kiceniuk and Khan 1987). Initial concentration varied from 50-100 ppb and declined subsequently.

A dispersant, Corexit 9527, was placed in an uncontaminated head tank (80L) supplied with a constant spray of seawater (2.5L/min), agitated and the bottom contents drawn off into the fish aquarium. An additional 2.5L/min seawater also flowed into the fish aquarium. The dispersant was discontinued after 4 days and the flow rate of seawater increased to 5L/min when the fish were held for an additional 4 wk. When the dispersant and crude oil were used simultaneously, equal volumes of both were placed in the head-tank over which a constant spray of sea water flowed (2.5L/min) and the contents (flow rate, 2.5L/min) allowed to flow into the fish aquarium. Exposure period was 4 days again and then additional seawater (2.5L/min) was supplied to the aquarium.

Each fish group was held in a separate aquarium and acclimated for one week. The dispersant, WAF and dispersed oil (DO) were administered daily for 4 days. An equal number of controls were held in an untreated flow-through aquarium supplied with ambient sea water. Capelin that survived were autopsied at the end of the fourth day as well as fish that succumbed. Changes in gill architecture were the only criterion that was used to assess pathological anomalies following exposure (Mallat

1985). Consequently, the gills were fixed in 10% buffered formalin, processed by conventional histological methods, cross sections, 8µm in thickness and stained with hematoxylin and eosin (see Khan and Kiceniuk 1984). Atlantic cod, longhorn sculpin and cunner that were still alive after 4 days, were held until the end of 4 wk following exposure before necropsy. Gills for histological assessment were taken from all fish groups and processed as mentioned previously. The results were expressed as a percentage (prevalence) of occurrence of commonly-occurring lesions and their intensity. A scoring method, varying from 0 to ++++, was used to compare intensity among fish groups and the data analysed by a 1-way ANOVA for significant differences set at $p < 0.05$. Chi square and Fisher's exact probability tests were used to compare prevalence (%) of lesions between groups of fish.

RESULTS AND DISCUSSION

Three preliminary trials were conducted on capelin using the dispersant while a second untreated group served as controls. In the first trial, 50ml of dispersant were introduced into the head tank. Shortly after entry into the fish aquarium, the fish responded by swimming erratically in all directions rather than remaining in a group formation. This continued until all 10 succumbed on the second day. Hemorrhaging from the operculum was observed in all capelin. A second trial, using 25ml of dispersant resulted in the death of eight of 10 fish exposed. Hemorrhaging through the operculum was observed again. In a third trial using 20ml of dispersant, six of the 10 fish exposed died. Hemorrhaging was observed in the fish that succumbed. No controls died during the three experiments. Examination of histological sections of the gills revealed separation and rupture of the secondary branchial lamellae in fish that died. Lifting of the respiratory epithelial cells and lamellar telangiectasis were the most common anomalies noted in surviving fish. None of the above abnormalities was seen in the controls.

Two trials to assess toxicity of the dispersant were conducted on groups of cunner. In the first trial, all 10 experimental fish were alive at the end of 4 days but 5 died during the 4 weeks of depuration. All controls survived. In a second trial, all were alive after 4 days but 4 died during depuration. Epithelial separation and rupture of the secondary branchial lamellae, telangiectasis and hyperplasia in the lamellae were observed in histological sections of fish that succumbed. In surviving fish, epithelial hyperplasia at the base and distal extremities of the lamellae resulted in an increase in thickness and fusion. None of these abnormalities was noted in the controls.

Two trials were conducted to assess the effects of WAF on capelin. In first trial using 50ml of crude oil, the capelin showed no response to the WAF following its entry as it was visible as a sheen at the surface of the aquarium. Nine of 10 fish died at the conclusion at 4 days while all controls remained alive. In a second trial, using 25ml of crude oil, no change in behavior was observed again. All 10 exposed fish died by day 4 while all controls remained alive. Examination of histological sections revealed epithelial rupture and separation from the underlying tissue of the gill lamellae in fish that died while epithelial lifting and telangiectasis were noted in the specimen that survived. None of these anomalies was noticed in the controls.

Three trials were conducted on capelin with the dispersant, WAF and dispersant-WAF (1:1), using 20ml of each. A fourth group was held without any additives as controls. Initial exposure to the dispersant elicited a change in behavior characterized

Table 1. Mortality (%) in capelin, Atlantic cod, longhorn sculpin and cunner following exposure to an oil dispersant (DISP), water-accommodated fractions (WAF) of a crude oil and dispersed oil (a mixture of DISP and WAF) for 4 days and depuration of cod, sculpin and cunner for 4 weeks until necropsy.

Fish species	No. of trials	Fish Groups No. died/No. exposed			
		Control	DISP	WAF	DISP/WAF
Capelin	3	1/42	15/42	28/42	25/42
% died		2	36	67	60
Atlantic cod	2	0/30	16/30	12/30	21/30
% died		0	53	40	70
Longhorn sculpin	2	0/25	2/25	125	5/25
% died		0	8	4	20
Cunner	2	0/20	0/20	6/20	0/20
% died		0	0	30	0

by swimming erratically in the aquarium. This occurred continually until the fourth day in surviving fish. Mortality, however, was greatest in the two groups exposed to WAF (67%) and dispersant-WAF (60%), considerably less in the dispersant group (36%) and least in the controls (Table 1). Hemorrhaging from the operculum and increased pigmentation in the skin were observed only in the dispersant group. Epithelial rupture and lifting were observed in all capelin that succumbed while lifting of the secondary lamellae and telangiectasis occurred more often in surviving fish (Table 2).

Two trials each, involving the exposure to the dispersant, WAF and a mixture of dispersant-WAF, were conducted on Atlantic cod, longhorn sculpin and cunner. A fourth untreated group served as controls. No changes in behavior occurred in any species following exposure. Increased pigmentation of the skin was observed in only cod surviving the period of depuration. Mortality was greater in both cod and sculpin (70 and 20% respectively) exposed to the dispersant-WAF mixture than in any of the other groups (Table 1). However, both the dispersant and the WAF also caused mortality in cod while it was considerably less in the sculpin. Mortality(30%) was noted only in the group of cunner that was exposed to WAF.

Examination of histological sections of the gills showed a consistent pattern observed previously. Epithelial rupture and lifting were observed in all fish that died or survived (Table 2). Epithelial hyperplasia and telangiectasis occurred less often in both groups of fish that either succumbed or survived. Lamellar thickening resulting in fusion of the secondary lamelae and hyperplasia at the basal portion of the lamellae was noted only in fish that survived after depuration. Moreover intensity of the four major gill lesions was 5 to 11^{+} times more severe in cod, sculpin and cunner exposed to the dispersed oil than to either the WAF or dispersant alone (Table 3).

Results from the present study have revealed that the two mature pelagic species of fish, viz., capelin and cod, were more susceptible to the dispersant, WAF and DO than the two subtidal species. In ascending order of susceptibility, cunner appeared to be more resistant than sculpin while cod and capelin were the most prone species.

Table 2. Prevalence (%) of gill lesions in Atlantic cod (COD), longhorn sculpin (LHS) and Cunner (Cu) that died or survived after exposure to a dispersant (DISP), water-accommodated fractions (WAF) of crude oil and dispersed oil (DISP-WAF) for 4 days and depurated subsequently for 4 weeks.

Died/ Survived	Gill Lesions	Control			DISP			WAF			DISP -WAF		
		COD	LHS	Cu	COD	LHS	Cu	COD	LHS	Cu	COD	LHS	Cu
Died													
	Epithelial rupture/lifting	–	–	–	100	100	–	100	100	100	100	100	–
	Epithelial hyperplasia	–	–	–	44	100	–	100	100	100	47	100	–
	Telangiectasis	–	–	–	38	50	–	42	100	83	86	95	–
Survived													
	Epithelial hyperplasia	0	4	5	100	100	100	100	100	100	100	100	100
	Fusion of secondary lamellae	0	0	0	64	52	90	89	80	43	100	100	35
	Basal hyperplasia	0	0	0	43	43	80	100	80	86	100	100	100
	Telangiectasis	0	0	0	21	17	30	17	25	29	22	30	16

Fish that inhabit the subtidal zone tend to be exposed to unstable environmental conditions and are capable of surviving stress changes in contrast to pelagic species. In the event of an oil spill off the northeastern coast of Newfoundland where the Labrador current flows into all embayments, it can be anticipated that the WAF will have a severe impact on capelin that is a major food source of Atlantic cod, the most important commercial species. Should a dispersant such as Corexit 9527 be applied to emulsify the crude oil, mortality can be anticipated not only in capelin but also in cod. Dilution of the DO in the ocean will most likely occur before it reaches the subtidal areas reducing its toxicity and having little or no impact on fish such as sculpin and cunner. The long term effect of a spill will most likely hinge on the quantity of oil spilled and also the dispersant that is applied to prevent fouling of the inshore zone.

Capelin, in the present study, responded adversely to the dispersant, WAF and the DO by swimming in an erratic manner following exposure. Previous studies have also reported changes in swimming activity in both larval and adult stages of fish after exposure to dispersants (Pollino and Holdway 2002). Gulec and Holdway (2002) noted that DO was more toxic than WAF to ghost shrimp (*Palaemon serenus*) and larval Australian bass (*Macquaria novemaculeata*). Wolfe et al. (2001) subsequently observed that chemical dispersants alter the behavior of fish by increasing the solubility of the oil fractions in water resulting ultimately in enhanced bioavailability and eventually affecting biological membranes. The same authors also noted that uptake of naphthalene, a major component in WAF, was enhanced significantly in the presence of the dispersant within hours of exposure. Moreover, photoenhansed toxicity was observed in eggs and larvae of Pacific herring (*Clupea pallasi*) following exposure to chemically dispersed oil in sunlight (Barron et al. 2003).

Epithelial separation and rupture of the secondary lamellae of the gills were observed in fish that succumbed following exposure to the dispersant, WAF and chemically dispersed oil. These changes culminated in hemorrhage, blood and most likely electrolyte loss and death in capelin. The three species that survived and were

Table 3. Intensity of gill lesions in Atlantic cod that survived 4 weeks after 4 days of exposure to a dispersant (DISP), water-accommodated oil fractions (WAF) and dispersed oil (DO).

	Fish Group-Intensity of Lesion*		
	DISP	WAF	DO
Epithelial hyperplasia	+**	+	+++***
Fusion of secondary lamellae	+	+	++
Basal hyperplasia	+	+	++
Telangiectasis	+	+	++

*intensity based on examination of 20 secondary lamellae of a fish from each group
**+(1-4), ++(5-10), +++(11^+)
***significantly different ($p < 0.05$) from other groups and between ++ and +

depurated exhibited evidence of lamellar hyperplasia and fusion. These lesions have been observed primarily in fish after chronic exposure to toxic pollutants (Khan and Kiceniuk 1984; Mallat 1985). Similar observations were made when cod and sculpin were exposed to WAF or oil contaminated sediment (Khan and Kiceniuk 1984). There is evidence that chemical dispersants increase the concentration of chemically dispersed oil fractions with enhanced toxicity (Barron et al. 2003). Consequently, an increase in gill lesions in fish, in the present study, that were exposed to the dispersed oil was probably the result of enhanced toxicity noted in other studies.

Our studies on the dispersant and chemically dispersed oil were conducted only on mature fish species. In contrast, most recent studies have focused on short term exposure of eggs, early life stages or juvenile fish because of their sensitivity to toxic compounds, especially PAHs (Moles et al. 2002; Singer et al. 1998; Barron et al. 2003; Koyama and Kakuno 2004; Ramachandran et al. 2004). Since the Exxon Valdez oil spill in 1989 in the Gulf of Alaska and the extensive use of dispersants, toxic subsurface oil still persists at sublethal levels, exerting chronic, delayed and indirect effects on wildlife (Peterson et al. 2003). This has resulted in a decline of fish, seabird and mammal populations. Future studies on dispersants and crude oil should focus on chronic rather than short term exposure to assess the impact on pelagic and subtidal fish species.

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