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Petroleum Hydrocarbons and Their Effects in Subtidal Regions after Major Oil Spills

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The question often arises after large oil spills as to the extent and effect of oil entering the subtidal zones adjacent to heavily oiled shorelines. Estimates for a number of large spills suggest that 1 to 13% of the spilled oil can enter subtidal regions. Hydrocarbon concentrations in these subtidal zones are generally orders of magnitude lower than shoreline sediments. For example, in the *Exxon Valdez* oil spill, subtidal sediment hydrocarbon concentrations attributable to the spill were very low in the first year after the spill and barely detectable in the second year. The conditions necessary to produce high concentrations of hydrocarbons in the subtidal region include large amounts of oil in a semi-enclosed estuary or bay and high concentrations of fine particulate matter to associate with hydrocarbons to allow them to disperse and sink. Such conditions do not often occur after spills, with some exceptions, such as low energy tidal estuaries in the *Amoco Cadiz* spill in Brittany, France. More commonly sea floor sediment hydrocarbon concentrations, where sediment-associated hydrocarbons have settled, are generally near to background levels, due to dilution and weathering.

A number of methods have been used to evaluate the biological effects of oil spills on subtidal fauna. These include toxicity to amphipods, increases in the concentrations of fluorescent aromatic metabolites in the bile of fish, histopathology of fish, increases in opportunistic species and infaunal succession. Sediments collected from the subtidal zone below heavily oiled shorelines of the *Exxon Valdez* spill showed low toxicity using standard amphipod bioassays. Well documented effects on the subtidal biota adjacent to heavily oiled shorelines are the increases in the number of hydrocarbon degrading microbes which are fed on by opportunistic species of meiofauna which in turn are food for macrofauna. The documented biological effects of oil in the subtidal region are generally of short duration and recovery back to an equilibrium or 'normal' condition is typically quite rapid. © 1997 Elsevier Science Ltd

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A common feature of major spills in coastal areas are sites where, because of the topography and the action of various physical processes, the shorelines are heavily oiled. Hydrocarbon concentrations in the intertidal regions of these sites can be as high as 10 000 to 30 000 μg g^{-1} (Gundlach *et al.*, 1983; Page *et al.*, 1989; Sauer *et al.*, 1993). The question often arises after such spills as to the extent and effect of oil entering the subtidal zones. Estimates made on the relative amount of the oil entering the subtidal zones after spills include the following: *1.* 8% for the *Amoco Cadiz* on the Brittany Coast (English Channel), France (Gundlach *et al.*, 1983); *2.* 3–6% for the *Tsesis* spill in the Baltic Sea, Sweden (Linden *et al.*, 1979; Johansson *et al.*, 1980); *3.* 13% for the *Exxon Valdez* spill in Prince William Sound, Alaska (Wolfe *et al.*, 1994); *4.* 0.5–3% of the oil from the *Ixtoc* blowout in the Gulf of Mexico (Boehm and Fiest, 1982). These estimates suggest that the total volume of oil entering the subtidal regions after spills can be quite high. However, as discussed in this paper, it is not the total amount of oil which is important when evaluating the possible biological effects, but the types and concentrations of hydrocarbons in the subtidal sediments. It appears that in most large oil spills, the hydrocarbon concentrations in the subtidal regions are many orders of magnitude lower than heavily oiled intertidal regions.

In this paper we review the literature on major oil spills to determine the hydrocarbon concentrations found in subtidal sediments after spills and the effects of this oil on the subtidal biota. Total hydrocarbon concentrations in sediments and tissues after oil spills are often reported. However, the aromatic hydrocarbons are considered to be the components causing most observed biological effects after spills. Thus, for many recent spills, the concentrations of polycyclic aromatic hydrocarbon (PAH) concentrations in sediments and tissues are reported and are presented in this review. The relative amount of PAHs is highly variable depending on the particular crude oil. For example, Alaska North Slope (ANS) crude, the oil from the *Exxon Valdez* spill, has between 1.0 and 1.25% resolved

PAH depending on its degree of weathering. As a general rule, there is more than one hydrocarbon source in an oil spill zone (e.g. Page *et al.*, 1995). One difficulty with the analysis of subtidal hydrocarbon data is distinguishing oil spill hydrocarbons from other sources, both natural and anthropogenic. Background hydrocarbon concentrations can be quite high, particularly in petroleum producing regions. Discussed in this paper are methods that have successfully been used to distinguish oil spill hydrocarbons in subtidal sediments from background hydrocarbons. One important question which we address is the extent and effect of oiling in the deeper subtidal (more than 10 m depth). Most subtidal oil studies have focused on shallow water depth (less than 10 m depth), but in the case of the *Exxon Valdez* spill, there were several studies of the input and effects of oil in subtidal regions deeper than 10 m (Armstrong *et al.*, 1995; Page *et al.*, 1995, 1996a; Jewett *et al.*, 1996; O'Clair *et al.*, 1996). For biological studies on oil spill effects in the subtidal, there are 'background' community changes which are difficult to document, since non-oiled reference sites can often have very different community structure from oiled sites.

Hydrocarbon Concentrations in the Subtidal Regions after Major Oil Spills

Hydrocarbon concentrations in subtidal zones of heavily oiled shorelines are presented in Tables 1 and 2 and Fig. 1. For most spills the hydrocarbon concentrations found in the subtidal sediments were orders of magnitude lower than sediments from oiled shores. An interesting exception is the *Amoco Cadiz* spill where certain estuaries, called abers, near the wreck site, had heavily oiled subtidal sediments. The abers with high subtidal hydrocarbons were shallow semi-enclosed with clay–silt bottoms, very high (~10 m) tidal ranges and high concentrations of suspended sediments (Page *et al.*, 1989). In the case of the *Exxon Valdez* spill in Alaska, even the subtidal sediments associated with heavily oiled low energy environments, e.g. Bay of Isles (Table 1), showed subtidal hydrocarbon concentrations very much lower than the intertidal hydrocarbon concentrations (Boehm *et al.*, 1995). The decreases in the hydrocarbon concentrations in going from the upper intertidal zone, to middle intertidal zone, to lower intertidal zone and to the sub-tidal zone after the *Exxon Valdez* spill, are shown in Fig. 1 for three heavily oiled sites. Although not reported in Tables 1 and 2, some of the studies (Page *et al.*, 1995, 1996a) analysed hydrocarbons in deep subtidal (more than 10 m depths) of the spill zone and found little or no hydrocarbons attributable to the spill. Where present, spill hydrocarbon residues were generally a small increment to a large seep-derived natural petroleum hydrocarbon background (Page *et al.*, 1996a). Figure 2 shows the hydrocarbon concentrations in the shoreline and subtidal regions of a site on Baffin Island, Northwest Territories (Canada) where a controlled crude oil spill was carried out (Owens *et al.*, 1987). While the hydrocarbon concentrations in the subtidal zones were always several orders of magnitude lower than the intertidal area of the Baffin Island spill, it

TABLE 1

Concentrations of polycyclic aromatic hydrocarbons (PAH) in subtidal sediments adjacent to several heavily oiled intertidal sediments of Prince William Sound, Alaska listed by shoreline type. The spill took place in March 1989. Taken from Boehm *et al.* (1995).

		PAH concentrations (ng g^{-1})*		
			Subtidal sediments	
Substrate/site	Sampling date	Intertidal sediments	3 m depth	3–10 m depth
Boulder/Cobble				
Point Helen	5/89	10 000–644 000	510	130
Knight Island	8/89	720–7700	330	20
	8/90	100–510	20	10
	8/91	60–630	10	< 10
Latouche Island	9/89	ND–3100	20	< 10
	9/90	20–420	< 10	10
	9/91	10–1300	< 10	< 10
Pebble/Gravel				
Herring Bay	5/89	680–333 000	240	110
Knight Island	9/89	40–17 000	10	–
	9/90	10–43 000	< 10	< 10
	9/91	10–5400	< 10	< 10
Snug Harbor	9/89	190–20 000	200	60
Knight Island	8/90	140–20 000	80	90
	9/91	660–2000	80	160
Clay/Sand				
Bay of Isles	8/90	14 000–142 000	260	190
Knight Island	8/91	3000–370 000	2000	600

*Resolved PAH in Alaska North Slope crude comprises 1.0–1.25% of the total petroleum hydrocarbon fraction. To compare these data with total petroleum hydrocarbon data presented elsewhere in this paper, multiply the numbers in Table 1 by 100.

TABLE 2

Concentrations of total petroleum hydrocarbons (PHC) in subtidal sediments adjacent to heavily oiled intertidal sediments from various large oil spills.

Spill description (location, oil type spill volume (liters), date of spill)	Description of heavily oiled station sediments	PHC conc. of intertidal sediments ($\mu g\ g^{-1}$)	PHC conc. of subtidal sediments ($\mu g\ g^{-1}$)	Sample time (months after spill)	Subtidal water depth (m)	Reference
Arco Anchorage (Alaska) North Slope crude, 960 000 l	Sandy Beach	2200–9000	460	9	4	Lindstedt-Siva *et al.* (1987) Miller (1989)
12/85			260	18	4	
1991 Gulf War, Kuwait crude, 640 to 1360×10^6 l 2/91	Abu Ali Is. (sand beach)	23 000–30 000	0.31	12	<7	Sauer *et al.* (1993)
	Station 12N (tidal flat)	3000–35 000	43	12	<7	
	Station T16 (sand)	7900	0.76	12	<7	
	Station 19SA	15 000	1.4–65	12	<7	
Nestucca (Washington Bunker C fuel oil 920×10^3 L, 12/88	Sand Beach	570–1800	1.0	2	1	Strand *et al.* (1992)
Amoco Cadiz (France) Arabian and Iranian Crude, 247×10^6 L 3/78	Estuary with clay-silt bottom (Aber Benoit, Aber Wrach'h)	22 000	746–28 000	20	<10	Page *et al.* (1989) Gundlach *et al.* (1983)
Florida, Massachusetts Fuel oil No. 2	Wild Harbor Station	1100–5000	140–240	2	10	Sanders *et al.* (1980)
700,000 L, 9/69			20–61	10	10	
BIOS, Baffin Island NWT, (Canada)	Sand-gravel Beach	6000–17 000	0.6	10	3	Owens *et al.* (1987)
Lagomedio		6000–17 000	27.0	36	3	
crude, 15×10^3L,		6000–17 000	2.1	1	7	
8/81		6000–17 000	15.0	36	7	

is of interest that one and two years after the spill there was an increase in the subtidal hydrocarbon concentrations reflecting offshore transport and sedimentation of hydrocarbons from the heavily oiled shorelines into the subtidal regions.

The question of the background hydrocarbons is not always addressed in subtidal hydrocarbon studies. Sediments from the *Exxon Valdez* spill area had a variety of petrogenic (petroleum-derived) and pyrogenic (combustion-derived) hydrocarbons as background. The dominant petrogenic hydrocarbon source in Prince William Sound are natural seep-derived petrogenic hydrocarbons carried by the Alaskan Coast current into Prince William Sound from eastern Gulf of Alaska oil seep sources in association with suspended sediment (Page *et al.*, 1995, 1996b). Suspended sediment and associated seep hydrocarbons are swept into Prince William Sound and are sedimented in depositional parts of the sound. The passage of suspended particulates into Prince William Sound from outside the sound has been well demonstrated (Sharma, 1979). A variety of marker hydrocarbons were used in Prince William Sound to separate background hydrocarbons from *Exxon Valdez* spill hydrocarbons. When background hydrocarbons were subtracted from total hydrocarbons found in subtidal zones, *Exxon Valdez* hydrocarbon concentrations were very low in the first year after the spill and barely detectable in the second year (Armstrong *et al.*, 1995; Page *et al.*, 1995, 1996a,b). Similar approaches should be used in the future to separate background hydrocarbons in subtidal sediments after a large spill has occurred.

Sedimentation of Oil in the Subtidal Zone

Adsorption of oil to particles in the water

Surface oil slicks or dispersed oil droplets can adsorb to suspended particulates (Poirier and Thiel, 1941; Bassin and Ichiye, 1977; Gearing *et al.*, 1979; Lee, 1980). A mathematical model for the reaction of oil droplets with suspended particulate matter was developed by Payne *et al.* (1989) which required determination of oil-droplet-suspended particle interaction rate constants. Some of the important factors affecting interaction rates were oil droplet concentrations, particulate concentrations, turbulent energy, particle sticking coefficients, and geometric factors. Since the reaction rates are proportional to both oil droplet and

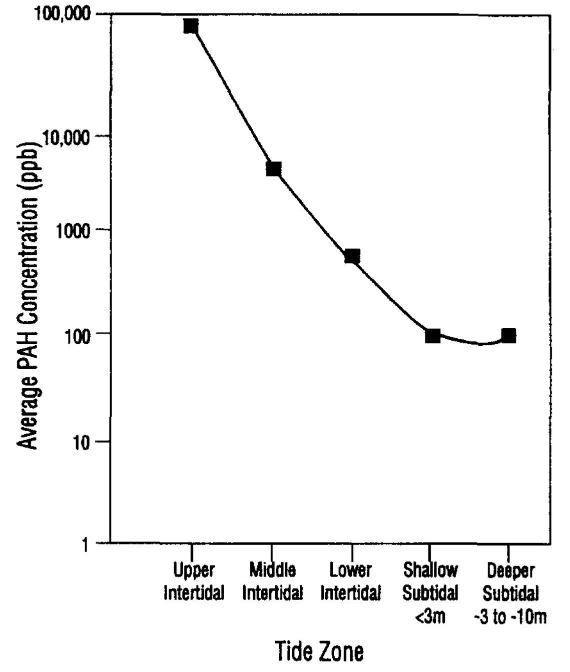

Fig. 1 Comparison of polycyclic aromatic hydrocarbon concentrations after *Exxon Valdez* spill in intertidal and subtidal sediments. The data are geometric means for sediment hydrocarbon data from three heavily oiled sites in Prince William Sound collected in 1990 (one year after spill). Modified from Boehm *et al.* (1995).

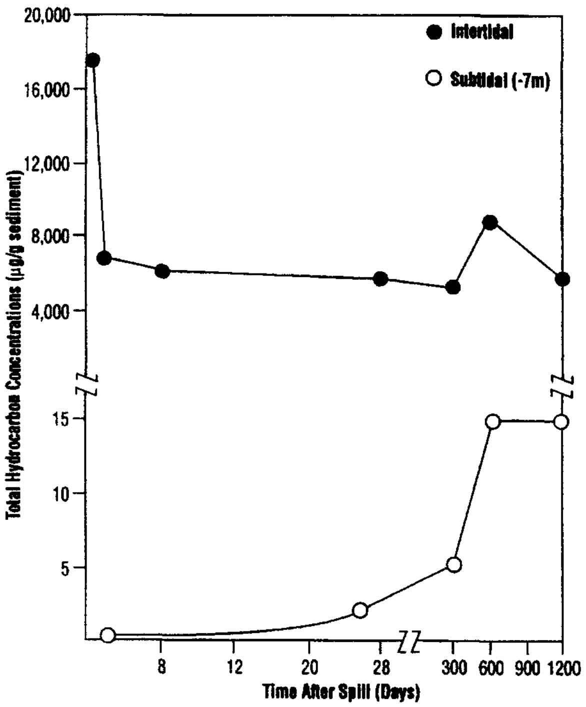

Fig. 2 Changes in the hydrocarbon concentrations of oiled shorelines and subtidal regions at different times after a controlled oil spill on an Arctic Island. A controlled spill of 15 000 l of crude Lagomedio oil took place on Baffin Island, Northwest Territories (Canada) on August 1981. The hydrocarbon concentrations in the subtidal and oiled shorelines were determined at different times after the spill. Data taken from Owens *et al.* (1987).

particulate concentrations, high particulate concentrations, 10–100 mg l^{-1}, are required to produce significant amounts of oiled particulates. The particulate concentrations in most open ocean areas are 2 mg l^{-1} or less (Schubel, 1974) and thus, the adsorption to particles in the water followed by sedimenting of these oiled particulates is assumed to be relatively unimportant in most coastal regions (Payne *et al.*, 1989). At high particulate concentrations, such as where rivers empty into coastal regions, particles can flocculate and rapidly precipitate (Duinker, 1980; Postma, 1980). In the sedimentation of micron sized particulates, which have been shown to help stabilize oil-in-water emulsions (Bragg and Yang, 1995), the settling velocity varies with the square of the diameter, *d*, following Stokes Law

$$W_S = Cd^2$$

where W_S is the settling velocity and C includes various constants including particle density, fluid density, acceleration of gravity and fluid viscosity (Gibbs, 1985; Nichols and Biggs, 1985). The oiled particles have a lower density than non-oiled particles and thus a lower settling velocity. After weathering, i.e. oxidation of the oil, the oil becomes denser due to addition of polar groups and the weathered oiled particles would have an increased settling velocity. Soon after the oil spill off Santa Barbara, California there was a period of heavy rain followed by heavy runoff which produced high suspended sediment concentrations along the coast (up to 10 mg l^{-1}, Drake *et al.*, 1971). Hydrocarbon analysis of the sediment, although difficult to quantify because of the non-specific infrared analytical method used, suggested that a significant amount of the oil entered the deep water sediments (Kolpack *et al.*, 1971). For the *Exxon Valdez* spill in Prince William Sound, the suspended particulate concentrations were 2 mg l^{-1} or less in most areas, so formation of oiled particulates in the water and subsequent sedimentation would not be expected to be an important process at most sampling stations (Bragg and Yang, 1995).

Sedimentation trap data

Elevated hydrocarbon concentrations have been found on particles from sediment traps placed in subtidal waters at several oil spill sites (Table 3). Sediment traps deployed for 1–2 weeks after the *Tsesis* oil spill (Table 3) collected particles with high hydrocarbon concentrations (4800–7300 µg g^{-1}) but for traps deployed four weeks post-spill, the hydrocarbon concentrations of particles returned to background (Boehm *et al.*, 1982). Similarly, after the *Exxon Valdez* spill, particles collected in sediment traps deployed near heavily oiled shorelines for a seven month period after the spill had elevated concentrations (30–75 µg g^{-1}) of resolved PAHs in sediment traps while sediment traps placed in the water after this seven month period had near background concentrations (2 µg g^{-1}) of PAHs (Short *et al.*, 1996). In the Persian

TABLE 3

Hydrocarbons concentrations in particles collected in sediment traps after oil spills.

Spill, location, spill volume (l)	Station	Time after spill (weeks)	Collecting time (weeks)	Sedimentation rate (g m^{-2} day^{-1})	Hydrocarbon concentration on particles (μg/g)	Reference
Tsesis (Sweden) 12×10^6	Site 5	1	1	6 to 9	7300	Boehm *et al.* (1982)
		2	1		2000	
		4	1		80	
	Site 2	1	1		4800	Johansson *et al.* (1980)
		2	1		2800	
		4	1		190	
Exxon Valdez (Alaska, USA) 4.1×10^7	NW Bay	32	28	–	75 (TPAH)	Short *et al.* (1996)
		60	8		2 (TPAH)	
	Snug Harbor	32	28		30 (TPAH)	
		60	8		2 (TPAH)	
Gulf War (Middle East) 640 to 1360×10^6		52			170 to 250	Michel *et al.* (1993)

*Resolved TPAH in Alaska North Slope crude comprises 1.0–1.25% of the total petroleum hydrocarbon fraction. To compare these TPAH data with total petroleum hydrocarbon data presented elsewhere in this table and this paper, multiply the TPAH numbers in Table 3 by 100.

Gulf War, sediment traps were not placed in the water until one year after the spill and hydrocarbon concentrations of particles collected at this time were near background, even though the adjacent shorelines were still heavily oiled (Sauer *et al.*, 1993). Thus, after a large oil spill, it appears that even with low particulate concentrations there can be production, and sedimentation of oiled particulate matter in the nearshore subtidal environment for a few weeks to a few months after the spill.

While the *Tsesis* and *Exxon Valdez* spills had hydrocarbons in suspended particulates attributable to the spills, the bottom sediments below the sediment traps showed background hydrocarbon concentrations (Boehm *et al.*, 1982; Sale and Short, 1995; Page *et al.*, 1996a,b; Short *et al.*, 1996). Two explanations can explain the low hydrocarbon concentrations in these bottom sediments:

- degradation of the particulate hydrocarbons before or shortly after entering the bottom sediments;
- dilution of the oiled particulates by particles with lower hydrocarbon concentrations.

Subtidal sediment samples are normally taken from the 1–2 cm thick surface layer. This can correspond to a period of 15–20 years depending on the sedimentation rate. Sediment trap data are from a much shorter and more recent time period. If oiled particles are only found for a few weeks after an oil spill, then the oiled particles entering the bottom sediments will be quickly diluted by particles in the bulk of the sediments with background hydrocarbon concentrations. Gearing *et al.* (1979) found that approximately 15% of the fuel oil added to a sediment/water mesocosm entered the sediment and these sediment hydrocarbons were associated with a very unstable flocculent layer on the surface of the bottom sediments. This flocculent layer probably mixes with the bulk of the sediments at very low rates, thus allowing hydrocarbon degradation by sediment microbes. The floc can also be broken down by physical/chemical processes in the water column during the process of transport and dispersal by currents so that further dilution of the hydrocarbons would take place before incorporation into the bottom sediments.

The flocculent hydrocarbons may be taken up by invertebrates and fish on or near the bottom and might explain the hydrocarbons or hydrocarbon metabolites found in invertebrates and fish (bile metabolites) from deeper water sample stations at oil spill sites, in spite of very low sediment hydrocarbon concentrations (Krahn *et al.*, 1992; Armstrong *et al.*, 1995; Collier *et al.*, 1996; Page *et al.*, 1996a).

Resuspension of oiled particles formed in the intertidal

The adsorption of oil to suspended particulates in the water, as noted above, is important only when suspended particulate concentrations are quite high. Another process is the interaction of stranded oil with sediment particles in the intertidal zone. Bragg and Yang (1995) found that in the *Exxon Valdez* spill the oil reacted with one micron or smaller particles in the intertidal sediments to form stable oil-in-water emulsions. These clay-oil flocs were formed and, because of their buoyancy, were dispersed from the intertidal zone and carried away by currents. Further weathering of the clay-oil flocs washes away lower molecular weight components and degrades the less refractory components of the oil, thereby increasing the density and allowing the floc to sediment to the bottom. Thus, clay-oil flocs can be transported and dispersed over long distances before entering the subtidal bottom sediments. Chemical and biological degradation can occur during the transport of the clay-oil flocs. Such physical, chemical and biological processes acting on flocs explains why subtidal regions, adjacent to heavily oiled shorelines, generally do not have high hydrocarbon concentrations. Experimental verification of this subtidal input model was presented by Page *et al.* (1996a) in a stratified random sampling study of two embayments in the *Exxon Valdez* spill zone. In the heavily oiled Bay of Isles, they found a uniform average concentration of highly weathered petroleum residues attributable to the spill of ~60 ng g^{-1} total resolved PAH for all depth zones from 10–150 m water depth. This even distribution of petroleum residues over all depth zones argues for an even dispersal and sedimentation of suspended particulate-associated petroleum as described above.

To form stable clay-oil flocs requires micron-sized mineral particles, oil with low viscosity and sufficient polar groups and turbulence for mixing energy. A study by Bragg and Owens (1994) found that such conditions are not uncommon in oil spill areas and showed that clay-oil flocs could be formed from sediments collected from a number of spill sites including the *Arrow* (1970, Nova Scotia), *Metula* (1974, Straits of Magellan, Chile), *Nosac Forest* (1993, Tacoma, WA) and *Fred Bouchard* (1993, Tampa Bay, FL). The importance of sediment characteristics was noted in studies on the transport of oil from oiled intertidal areas into the subtidal in Milford Haven, UK (Little and McLaren, 1989). On beaches with particles too large to form stable flocculated emulsions, particles can still mix with oil droplets but without emulsification. This could promote the formation of tarballs rather than clay-oil flocs.

A sample calculation will illustrate the effect of dispersing clay-oil flocs over a large area. Assume a shoreline of 1 km is heavily oiled (10 000 μg g^{-1}) to a depth of 2 cm in a 10 m wide intertidal zone. Thus, the amount of oil in the oiled shore is 20×10^{13} μg of oil. Next, assume 15% of this oil on the shoreline forms clay-oil flocs which are dispersed over a subtidal area of 100 km^2 to a depth of 1 cm. Assuming no loss of the oil due to weathering, hydrocarbon concentrations in this subtidal area would be 200 μg g^{-1}. When weathering is taken into account, the hydrocarbon concentrations in the subtidal sediments after a spill are likely to remain near background levels.

Bottom sediment hydrocarbons after deep water discharge

The discharge of large quantities of oil at deeper depths can occur when oil tankers sink in deep water, well blows out from deep water drilling accidents, and from natural deep water oil seeps. In each of these examples, high concentrations of hydrocarbons are found in the bottom sediments, but generally only adjacent to the point of discharge. Tankers which have sunk in deeper waters and discharged much of their contents near the bottom include the *Argo Merchant* in Rhode Island (USA), *Bahia Paraiso* (Antarctica), *Braer* North Sea, (UK). The hydrocarbon concentrations were high only near the wrecks and generally a kilometre or more away from the wrecks, the hydrocarbon concentrations were at or near background (Hoffman and Quinn, 1980; Kennicutt *et al.*, 1991; Richie and O'Sullivan, 1994). Similarly, the sediments around oil seeps or blowouts from drilling accidents are characterized by high hydrocarbon concentrations near the discharge area (Boehm and Fiest, 1982; Spies *et al.*, 1980; Kennicutt *et al.*, 1988). Most of the oil enters the water column as a result of its buoyancy and will be degraded or transported away.

Effects of clean-up on subtidal oil transport

Resuspension and movement of sediment-associated petroleum from cleaned oiled intertidal areas to subtidal areas was observed for the *Amoco Cadiz* oil spill (Morel and Courtot, 1981; Page *et al.*, 1989) and for the *Exxon Valdez* oil spill (Jahns *et al.*, 1991; Payne *et al.*, 1991; Bragg and Yang, 1995; Sale and Short, 1995; Page *et al.*, 1996a). The linkage of subtidal transport with clean-up activities was generally circumstantial. In the case of the *Exxon Valdez* oil spill the shorelines that were most heavily cleaned were those most heavily oiled (Teal, 1990; Jahns *et al.*, 1991; Owens, 1991). This means that natural petroleum transport processes were difficult to separate from clean-up-related processes. Transport of petroleum residues from cleaned heavily oiled shorelines to nearshore subtidal areas in the *Exxon Valdez* spill was a localized phenomenon (Sale and Short, 1995; Page *et al.*, 1996a). Sediment traps were successful in detecting subtidal transport of sediment-associated petroleum residues in the *Tsesis* spill (Boehm *et al.*, 1982) and in the *Exxon Valdez* oil spill (Sale and Short, 1995). Subtidal sediment trap studies carried out after the *Exxon Valdez* spill showed less weathered PAH in traps deployed in 10 m of water off heavily oiled shorelines that were agressively cleaned during the sampling period than traps used during the preceding winter period, even though there was an overall decreasing trend in PAH concentration (Sale and Short, 1995). This suggests that some clean-up-related transport of petroleum residues to nearshore benthic sediments did occur as a part of an overall declining concentration trend over time but were not large scale contributors of petroleum residues to nearshore subtidal sediments.

Effects of Oil Spills on Subtidal Biota

Input of petroleum from oiled shorelines to benthic biota

Hydrocarbons enter subtidal sediments from heavily oiled intertidal sediments as a result of transport and sedimentation. As discussed earlier, hydrocarbon concentrations in the subtidal sediments are generally much lower than the adjacent intertidal regions. Filter feeding benthic invertebrates are likely to take up hydrocarbons from flocculents and thus would have higher concentrations of spill hydrocarbons than the surrounding bulk sediment since much of the hydrocarbons in flocs are not incorporated into the sediment but remain on the surface. The presence of petroleum hydrocarbons or their metabolites in invertebrates and fish from subtidal regions is a measure of the extent to which petroleum has entered the biota of these regions. Because oil spills are transitory events and recovery of oiled intertidal areas takes place (National Research Council, 1985; Gould, 1988), the input of petroleum residues from oiled shorelines to nearshore subtidal areas is also a transitory process. One difficulty, as noted earlier for sediment hydrocarbons, is distinguishing between background and spill hydrocarbons or their metabolites in the biota. One method used after the *Exxon Valdez* spill was to compare the concentrations of alkylated PAHs, which were assumed to be derived from crude oil, in deep water bivalves from oiled and non-oiled bays (Armstrong *et al.*, 1995). The presence of low concentrations of alkylated aromatics in bivalves from oiled bays as contrasted with very low or nondetectable concentrations in bivalves from non-oiled bays, led to the conclusion that most of the alkylated aromatics in the bivalves from the oiled bays were derived from the *Exxon Valdez* oil. Care must be taken in interpreting such data since the uptake by biota of refined petroleum products, such as diesel fuel, from the same feed stock as spill oil can lead to erroneous conclusions as was the case for the *Exxon Valdez* oil spill (Bence and Burns, 1995; Bence *et al.*, 1996). The entrance of hydrocarbons into the subtidal microbial community can be inferred if there is an increase in the hydrocarbon degrading microbial activity of subtidal sediments after a spill. This method was used in subtidal sediments of Prince William Sound after the *Exxon Valdez* spill (Braddock *et al.*, 1995Braddock *et al.*, 1996). Once hydrocarbons have been shown to enter subtidal biota after a spill, a variety of methods (summarized in Table 4) can be used to determine biological effects and are discussed below.

Much of our quantitative understanding of subtidal oil spill fate and effects has been gained from quantitative chemical and biological studies of oil spills in temperate and subarctic environments. Similar studies in tropical environments are rare. One study is the 1986 crude oil spill from a ruptured storage tank in Bahia las Minas, Panama, where oil entered a sheltered tropical coastal mangrove/seagrass/coral coastal habitat. Extensive pre-spill baseline data existed for this area and follow-up studies found a significant decrease in live coral cover for the 0–3 m depth zone of a heavily oiled reef and decreases in coral cover for depths up to 12 m (Jackson *et al.*, 1989). This spill was noteworthy because, as a land-based spill, the oil did not undergo the physical weathering processes that generally lowers the toxicity of oil spilled at sea (Koons and Jahns, 1992) and extreme low tides after the spill exposed subtidal biota to increased concentrations of water soluble fractions of the spilled oil (Jackson *et al.*, 1989). A summary of the effects of oil on corals were discussed in a review by Knap (1992).

Sediment toxicity

Sediment toxicity is one of the first tests that can be carried out after a spill. The most toxic components of oil, i.e. one and two ringed aromatics, are generally removed before the oil enters the subtidal zones as a

TABLE 4

Methods used to evaluate biological effects in subtidal regions of oil spills.

Method	Oil spill and location	Reference
Biochemical/Physiological Methods		
Sediment toxicity	*Exxon Valdez*, Alaska (USA)	Boehm *et al.* (1995)
Amphipod bioassay		Wolfe *et al.* (1996)
Fish histopathology	*Amoco Cadiz*, France	Haensley *et al.* (1982) Stott *et al.* (1983)
	Exxon Valdez, Alaska (USA)	Armstrong *et al.* (1995)
Bile metabolites (Fluorescent aromatic compounds-FACs)	Gulf War, Saudi Arabia	Krahn *et al.* (1993)
	Exxon Valdez, Alaska (USA)	Krahn *et al.* (1992) Armstrong *et al.* (1995)
	Mobiloce, Oregon (USA)	Krahn *et al.* (1986)
	Bahia Paraise, Antarctic	McDonald *et al.* (1992)
Fecundity of crustaceans	*Exxon Valdez*, Alaska (USA)	Armstrong *et al.* (1995)
Population Level Methods		
Crustacean and echinoderm population structure	*Exxon Valdez*, Alaska (USA)	Armstrong *et al.* (1995) Dean *et al.* (1996a)
Increases in opportunistic species	*Amoco Cadiz*, France	Glemarec and Hussenot (1982)
	West Falmouth, MA (USA)	Sanders *et al.* (1980)
	API Test Spills, MA (USA)	Gilfillan *et al.* (1986)
Infauna succession	*Amoco Cadiz*, France	Glemarec and Hussenot (1982)
	West Falmouth, MA (USA)	Sanders *et al.* (1980)
Meiofaunal population structure	Agip Abruzzo, Italy	Donovaro *et al.* (1995)
	Exxon Valdez, Alaska (USA)	Gilfillan *et al.* (1995)
Fish populations	*Exxon Valdez*, Alaska (USA)	Laur and Haldorson (1996)
Macroalgae populations	*Exxon Valdez*, Alaska (USA)	Dean *et al.* (1996b)

result of weathering, such as evaporation and degradation. Thus, the amphipod assay for sediment toxicity showed much reduced toxicity of oiled subtidal sediments compared with the toxicity of oiled intertidal sediments after the *Exxon Valdez* spill (Fig. 3). One year after the spill, intertidal sediments from a heavily oiled soft sediment location in the Bay of Isles, were toxic to amphipods, while sediments from the nearby subtidal zone were only slightly toxic. A second study using subtidal (6–40 m depth) sediments from reference and oiled sites in Prince William Sound found no significant toxicity to amphipods or oyster larvae (Wolfe *et al.*, 1996).

A useful measure of potential for toxic effect is the Effects Range-Low (ER-L) sediment toxicity threshold of 4000 ng g^{-1} for total resolved PAH proposed by Long and Morgan (1990). The concentrations of spill-related PAH in subtidal sediments observed after the *Exxon Valdez* oil spill rarely exceeded 4000 ng g^{-1} and were generally 1–2 orders of magnitude lower (Boehm *et al.*, 1995; Page *et al.*, 1995, 1996a). Thus, the lack of sediment toxicity in the subtidal sediments of Prince William Sound was not unexpected. In discussing threshold values for PAH effects after an oil spill, it should be noted that in contrast to chronically polluted sites, the oil spill hydrocarbon concentrations in the sediment are decreasing due to biodegradation, other weathering processes and sediment associations that decreases bioavailability (i.e. Page *et al.*, 1996a). Similar results were found at other heavily oiled sites in Prince William Sound when comparing the toxicity of subtidal and intertidal sediments with those of unoiled reference sites (Boehm *et al.*, 1995).

Histopathology of fish

Following both the *Amoco Cadiz* and *Exxon Valdez* spills, there were histological examinations of fish collected from subtidal regions of heavily oiled sites. In the *Amoco Cadiz* spill, histopathogical lesions were observed in the ovaries, kidneys and gills of plaice, *Pleuronectes platessa*, from Aber Wrac'h and Aber Benoit, which as earlier noted, were sites with very high hydrocarbon concentrations in the shallow subtidal regions (Haensley *et al.*, 1982; Stott *et al.*, 1983). In addition to histopathology, some of these fish showed biochemical changes, including depressed liver glycogen and ascorbic acid, hypoglycemia and altered muscle amino acid ratios indicating alteration in energy metabolism and balance (Neff, 1985).

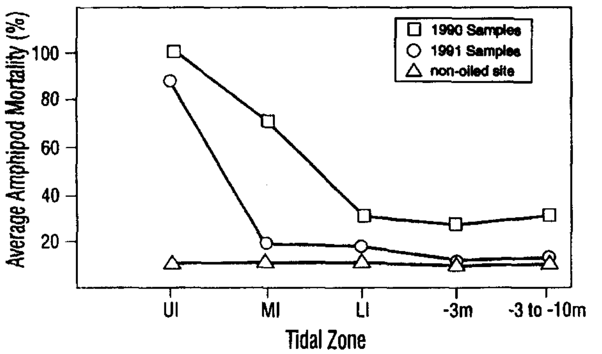

Fig. 3 Sediment toxicity of sediments from *Exxon Valdez* spill. For a heavily oiled soft sediment shoreline location in the Bay of Isles, Knight Island, Prince William Sound. The percent mortality averaged over triplicate samples from each tide zone. (UI-upper intertidal; MI-middle subtidal; LI-lower intertidal; subtidal −3 m water depth and randomly selected stations in the −3 to −10 m water depth zone (modified from Boehm *et al.*, 1995)). Data from the same study for non-oiled reference site sediment toxicity averages are included for comparison.

After the *Exxon Valdez* spill, flathead sole (*Hippoglossoides elassodon*) were collected from heavily oiled bays and from unoiled reference sites. These fish were examined for idiopathic hepatic lesions since these are correlated with chronic exposure to contaminants (Meyers *et al.*, 1987). No hepatocellular neoplasms were detected. Foci of cellular alteration (putative preneoplasms) were observed in low frequency ($\leq 1\%$) and were present in fish from both oiled and nonoiled bays (Armstrong *et al.*, 1995). Among the specific degenerative conditions, nuclear pleomorphisms were significantly ($p < 0.05$) higher among fish from oiled compared to non-oiled bays in the first year after the spill. In the second year, there were no statistically significant differences in the incidence of histopathological lesions between fish from oiled and non-oiled bays. These differences in the incidence of histopathological lesions between fish from the two spills were not unexpected since subtidal hydrocarbon concentrations in the heavily oiled abers of the *Amoco Cadiz* were as high as 28 000 μg g^{-1} (Table 2) at least an order of magnitude greater than heavily oiled locations in the *Exxon Valdez* oil spill. In the heavily oiled bays of the *Exxon Valdez* spill, the subtidal PAH concentrations were as high as 2000 ng g^{-1} for one bay (Bay of Isle) but generally subtidal PAH concentrations of the bays were below 600 ng g^{-1} (Table 1). Also, production of hepatic lesions in fish requires exposure to high hydrocarbon concentrations for an extended period. Hydrocarbon concentrations in the subtidal zone after the *Exxon Valdez* spill rapidly decreased (Boehm *et al.*, 1995; O'Clair *et al.*, 1996), while hydrocarbon concentrations remained high in a few of the subtidal regions of the abers after the *Amoco Cadiz* spill due to tidal-driven resuspension and transport of oiled sediment in the abers (Gundlach *et al.*, 1983; Page *et al.*, 1989).

Cytochrome P450/mixed function oxygenase/bile metabolites

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A response of fish from oil spill sites are increases in activity and level of the mixed function oxygenase (MFO) system (Kurelec *et al.*, 1977; Payne *et al.*, 1983). This cytochrome P450 enzyme system with highest activity in the liver, is responsible for the metabolism of petroleum hydrocarbons by fish. After metabolism by phase I (MFO) and phase II (conjugating enzymes) enzymes in the liver, hydrocarbon metabolites accumulate in the bile before elimination via urine or faeces. Thus, increased concentrations of hydrocarbon metabolites in fish bile, so called fluorescent aromatic compounds (FACs), from fish at oiled sites is taken as an indication of hydrocarbon uptake from the food or water (Krahn *et al.*, 1986, 1992, 1993; McDonald *et al.*, 1992; Armstrong *et al.*, 1995; Collier *et al.*, 1996). As discussed earlier for hydrocarbons in sediments, it is necessary to determine the background FACs for fish from oiled sites. Concentrations of bile FACs were significantly higher (approximately two-fold) in flathead sole from oiled bays compared with non-oiled bays collected 1 and 2 years after the *Exxon Valdez* spill (Armstrong *et al.*, 1995). Bile FACs were found in all fish from both oiled and non-oiled bays. Induction of cytochrome P-450 and increased levels of fluorescent aromatic compounds in fish bile were found in Dolly Varden (*Salvelinus malma*) from oiled subtidal region of Prince William Sound compared with non-oiled sites (Collier *et al.*, 1996).

Ariese *et al.* (1993) have recommended using 1-hydroxypyrene in bile as an indicator of exposure by fish to pyrolytic PAHs. Given the variability in PAH analytes from pyrogenic sources (e.g. Burns *et al.*, 1997) metabolites from other pyrogenic PAHs such as fluoranthene and benz(a)anthracene should also be measured. Krahn *et al.* (1992) has suggested that the presence of dibenzothiophenols in bile is an indication of crude oil exposure. However, some crude oils are very low in dibenzothiophenes while some refined petroleum products contain dibenzothiophenes. In the case of the *Exxon Valdez* oil spill, the widespread use and presence of diesel fuel from an Alaskan North Slope feedstock in Prince William Sound meant dibenzothiophenols in bile were not necessarily an indication of crude oil exposure (Bence and Burns, 1995; Bence *et al.*, 1996). While FACs are a good indicator of PAH exposure, they are at best, semi-quantative and more studies are necessary to find good indicator metabolites and approaches to data interpretation.

Benthic macrofauna population studies

After the *Exxon Valdez* spill, Armstrong *et al.* (1995) investigated the benthic crustacean populations in deeper subtidal regions of oiled and unoiled bays. Increases in both fish bile FACs and petroleum hydrocarbons in bivalves from these regions showed that *Exxon Valdez* oil had entered the biota of the deep subtidal. Concentrations of PAHs associated with *Exxon Valdez* oil in both sediment and biota were very low and generally less than a few nanograms per gram. Based on larval distribution, fecundity, settlement during and after the spill, growth of juveniles and subadults and recruitment there was no indication of impacts due to the spill on the deep subtidal crustacean populations. Crustaceans studied included tanner crabs (*Chionoecetes hairdi*) and pandalid shrimp (*Pandalus plyceros, P. hysinotus* and *P. borealis*). Dean *et al.* (1996a) studied epibenthic invertebrates from oiled and non-oiled shallow subtidal sites after the *Exxon Valdez* spill. They found that the subtidal taxa included five species of sea stars (*Dermasterias imbricata, Evasterias troshelii, Pycnopodia helianthoides, Orthasterias koehleri* and *Henricia leviuscula*) and one species of crab (*Telmessus cheiragonus*). Oiled subtidal regions had decreased population densities of *D. imbricata* and *T. cheiragonus* which the authors concluded was due to the oil toxicity. Within three years, the population of these species had recovered in the shallow portions of the bays. The possible decrease of these species as a result of organic enrichment in the bays was not considered. Population density, biomass and cover of macroalgae were compared between oiled and controls sites from both shallow and deep subtidal regions of Prince William Sound after the *Exxon Valdez* spill (Dean *et al.*, 1996b). There were no differences in the total density, biomass or percentage cover of macroalgae between oiled and reference sites. Dean *et al.* (1996b) concluded that there were no long-term impacts from the spill on subtidal populations of macroalgae. A study of the subtidal (<20 m depth) fishes in Prince William Sound after the spill found that at some oiled sites there were higher densities of fish than at reference sites (Laur and Haldorson, 1996). It was not clear if the increased number of fish at oiled sites was due to some subtle effect of oil, such as decreasing predation or more available food.

An interesting series of observations were made in heavily oiled embayments after the *Exxon Valdez* oil spill by Jewett *et al.* (1996). For the first year after the spill, there was a large reduction in the number of taxa of subtidal benthic invertebrates in several of the embayments. By the second year there was recovery of biota along with a large reduction in hydrocarbon concentrations. However, in year three, there was again, a large decrease in numbers and taxa even though hydrocarbon concentrations were very low. Apparently, these embayments have periods of natural hypoxia-anoxia which can result in large decreases in the invertebrate populations. These bays are characterized by heavy deposition and restricted water flows. Thus, they concluded that their first hypothesis that oil caused the observed population decrease in year one was in error and more likely due to the natural occurrence of very low oxygen concentrations. These observations point out the need for an understanding of natural population changes in an oil spill area before attributing all changes after an oil spill to oil effects.

Benthic infaunal community structure

Two studies documenting oil effects on the shallow subtidal infauna were the oil spills off West Falmouth, Massachusetts and the *Amoco Cadiz* off the Brittany coast of France. Faunal succession in the subtidal took place after the spill off West Falmouth (Sanders *et al.*, 1980). *Mediomastus ambiseta*, a semi-opportunistic capitellid polychaete, became common a year after the spill at offshore stations and remained so at intermediately oiled stations, but rapidly declined at lightly oiled stations.

In the case of the *Amoco Cadiz*, there were several sheltered tidal rivers and bays, such as Aber W'rach and Aber Benoit, where large amounts of oil entered the subtidal regions resulting in high concentrations of hydrocarbons in subtidal sediments and associated mortality of biota (Table 2). In subtidal sediments where hydrocarbon concentrations were less than 50 $\mu g\ g^{-1}$ there was no evidence of changes in the community structure. When hydrocarbon concentrations were 100 to 1000 $\mu g\ g^{-1}$ opportunistic species of polychaetes became dominant while at concentrations greater than 10 000 $\mu g\ g^{-1}$, there was very little species diversity and only the opportunistic polychaete species were present (Glemarec and Hussenot, 1981). In heavily oiled parts of these two abers, there was a pattern of succession from initial mortality to opportunistic species to an equilibrium or 'normal' condition (Glemarec and Hussenot, 1981, 1982; Cabioch *et al.*, 1982; Gilfillan *et al.*, 1991). For the very heavily oiled parts of these abers, this succession was not complete for several years. For example, the repopulation of the amphipod communities in the outer channel of the Aber W'rach was not complete until 10 years after the spill (Dauvin and Gentil, 1990). Gilfillan *et al.* (1986) found an increase in semi-opportunistic polychaete species in subtidal sediments a year after a series of test oil spills in intertidal areas, presumably due to an increase in sedimented biomass available for food. The succession documented for these two spills are likely due to the increase in hydrocarbon degrading microbes in the oiled subtidal sediments. These microbes would in turn provide food for opportunistic fauna, such as certain species of nematodes and polychaetes.

Prince William Sound, where the *Exxon Valdez* spill took place, has relatively few soft-sediment environments, that are described for the *Amoco Cadiz* and West Falmouth spill where, spill-induced anoxia can result in large mortalities and alterations in community structure. Thus, the subtidal regions of the *Exxon Valdez* spill sites did not show the succession of opportunistic invaders observed in other spills (Gilfillan *et al.*, 1995).

Studies on communities around natural oil seeps, which are found in many parts of the world's oceans, have shown high abundances of nematodes and bacteria near oil seeps which results in higher abundances of both macrofauna and meiofauna (Montagna *et al.*, 1987). A more detailed study of oil seep communities has shown that where hydrocarbons were at the highest concentrations there were reduced infaunal densities (toxic effects) while at intermediate hydrocarbon concentrations infaunal densities were higher than reference sites due to organic enrichment (Steichen *et al.*, 1996). A series of field experiments, where oil and kelp were added to sediment, showed that benthic infauna responded similarly to oil and organic enrichment (Spies *et al.*, 1988). Thus, the results from both spill and seep studies suggest that at very high hydrocarbon concentrations, there can be toxicity. In most spills, this toxicity phase, if it occurs in the subtidal regions, would likely take place during the first few months after the spill. When there are lower, but significant concentrations of hydrocarbon, the effect of the oil is to produce an environment suitable for opportunistic species which can take advantage of increases in the microflora feeding on hydrocarbons. The final phase is the equilibrium or 'normal' condition where most of the subtidal hydrocarbons are degraded or unavailable for microbial degradation and opportunistic species are reduced in numbers and so-called 'sensitive' species return.

Summary and Conclusions

Oil from large oil spills can enter subtidal zones. Generally, hydrocarbon concentrations in the subtidal zones are many orders of magnitude lower than adjacent heavily oiled shorelines. The conditions necessary to produce high concentrations of hydrocarbons in the subtidal region include large amounts of oil in a semi-enclosed estuary or bay, high concentrations of micron sized particulates, and sufficient turbulence to mix particles with oil. Such conditions do not often occur after spills with the notable exception of the abers of the *Amoco Cadiz* spill. Particulates, either in the water or on oiled shorelines, can adsorb to oil to form oil-in-water emulsions with high hydrocarbon concentrations. However, these oiled particulates or clay-oil flocs, are generally highly dispersed in the subtidal zone, as well as being degraded by various weathering processes, so that the sedimentation of such oiled particulates often does not produce hydrocarbon concentrations in the bottom sediments above background levels. Studies with sediment traps suggest that oiled particulates are found for brief periods after spills, possibly only a few weeks, and these oiled particulates enter the flocculent layers on the bottom. Because of dispersion and degradation, only a very small fraction of flocculent hydrocarbons are incorporated into the bottom sediments. In contrast, continuous input into the subtidal, such as from oil seeps or sewage outfalls, can produce elevated hydrocarbon concentrations due to the continuous input of oiled particulates.

A number of methods have been used to evaluate the biological effects of an oil spill on subtidal fauna. These include toxicity to amphipods, increases in concentrations of fluorescent aromatic metabolites in the bile, histopathology of fish, increases in opportunistic species and infaunal succession.

The more toxic components of the oil, e.g. one and two-ringed aromatics, are generally lost by weathering before entering the subtidal. Sediments collected from the subtidal zone below heavily oiled shorelines of the *Exxon Valdez* spill showed low toxicity in amphipod toxicity tests. One of the major effects on the subtidal biota are organic enrichment effects which are likely due to increases in the number of hydrocarbon degrading microbes which can be fed on by opportunistic species of meiofauna, which, in turn, can be fed on by the macrofauna.

Thus, while each oil spill is different, as a general rule, oil entering the subtidal zone after an oil spill produces sediment concentrations near or slightly above background concentrations. However, there can be special situations where because of the topography, subtidal regions can contain high hydrocarbon concentrations, e.g. abers of the *Amoco Cadiz*. The biological effects of oil in the subtidal region are generally of short duration and recovery back to an equilibrium or 'normal' condition is typically quite rapid.

Ariese, F., Krok, S. J., Verkaik, M., Gooijer, C., Velthorst, M. H. and Hofstraat, J. W. (1993) Synchronous fluorescence spectrometry of fish bile: a rapid screening method for the biomonitoring of PAH exposure. *Aquatic Toxicology* **26**, 273–286.

Armstrong, D. A., Dinnel, P. A., Orensanz, J. M., Armstrong, J. L., McDonald, T. L., Cusimano, R. F., Nemeth, R. S., Landolt, M. L., Skalski, J. R., Lee, R. F. and Huggett, R. J. (1995) Status of selected bottomfish and crustacean species in Prince William Sound following the *Exxon Valdez* oil spill. In Exxon Valdez *Oil Spill: Fate and Effects in Alaskan Waters*, eds P. G. Wells, J. N. Butler and J. S. Hughes, pp. 485–547. American Society for Testing and Materials (1219), Philadelphia, PA.

Bassin, N. J. and Ichiye, T. (1977) Flocculation behavior of suspended sediments and oil emulsions. *Journal of Sedimentary Petrology* **47**, 671–677.

Bence, A. E. and Burns, W. A. (1995) Fingerprinting Hydrocarbons in the Biological Resources of the *Exxon Valdez* Spill Area. In Exxon Valdez *Oil Spill: Fate and Effects in Alaskan Waters*, eds P. G. Wells, J. N. Butler and J. S. Hughes, pp. 84–140. American Society for Testing and Materials (1219), Phildelphia, PA.

Bence, A. E., Kvenvolden, K. A. and Kennicutt, M. C. (1996) Organic geochemistry applied to environmental assessments of Prince William Sound, Alaska, after the *Exxon Valdez* oil spill—a review. *Organic Geochemistry* **24**, 7–42.

Boehm, P. D. and Fiest, D. L. (1982) Subsurface distributions of petroleum from an offshore well blowout. The Ixtoc I blowout, Bay of Campeche. *Environmental Science and Technology* **16**, 67–74.

Boehm, P. D., Barak, J. E., Fiest, D. L. and Elskus, A. A. (1982) A chemical investigation of the transport and fate of petroleum hydrocarbons in littoral and benthic environments: the *Tsesis* oil spill. *Marine Environmental Research* **6**, 157–188.

Boehm, P. D., Page, D. S., Gilfillan, E. S., Stubblefield, W. A. and Harner, E. J. (1995) Shoreline ecology program for Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Part 2: Chemistry and toxicology. In Exxon Valdez *Oil Spill: Fate and Effects in Alaskan Waters*, eds P. G. Wells, J. N. Butler and J. S. Hughes, pp. 347–397. American Society for Testing and Materials (1219), Philadelphia, PA.

Braddock, J. F., Lindstrom, J. E. and Brown, E. J. (1995) Distribution of hydrocarbon-degrading microorganisms in sediments from Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. *Marine Pollution Bulletin* **30**, 125–132.

Braddock, J. F., Lindstrom, J. E., Yeager, T. R. and Rasley, B. T. (1996) Patterns of microbial acitivty in oiled and unoiled sediments in Prince William Sound. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Speis, D. A. Wolfe and B. W. Wright, pp. 94–108. American Fisheries Society, Bethesda, MA.

Bragg, J. R. and Owens, E. H. (1994) Clay-oil flocculation as a natural cleansing process following oil spills. Part 1: Studies of shoreline sediments and residues from past spills. In *Proceedings of the Seventeenth Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*, Vol. 2, pp. 1–23. Environment Canada, Ottawa, ON.

Bragg, J. R. and Yang, S. H. (1995) Clay-oil flocculation and its role in natural cleansing in Prince William Sound following the *Exxon Valdez* oil spill. In Exxon Valdez *Oil Spill: Fate and Effects in Alaskan Waters*, eds P. G. Wells, J. N. Butler and J. S. Hughes, pp. 178–214. American Society for Testing and Materials (1219), Philadelphia, PA.

Burns, W. A., Mankiewicz, P. J. Bence, A. E., Page, D. S. and Parker, K. (1997) A least squares method for allocating sediment hydrocarbons to multiple sources. *Environmental Toxicology and Chemistry* (in press).

Cabioch, L., Dauvin, J. C., Retiere, C., Rivain, V. and Archambault, D. (1982) Evolution de peuplements benthiques des fonds sedimentaires de la region de Roscoff, perturbes par les hydrocarbures de *L'Amoco Cadiz*. *Netherlands Journal of Sea Research* **16**, 491–501.

Collier, T. K., Krone, C. A., Krahn, M. M., Stein, J. E., Cahn, S. L. and Varansi, U. (1996) Petroleum exposure and associated biochemical effects in subtidal fish after the Exxon Valdez oil spill. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Spies, D. A. Wolfe, B. W. Wright, pp. 671–683. American Fisheries Society, Bethesda, MA.

Dauvin, J. C. and Gentil, F. (1990) Conditions of the peracarid populations of subtidal communities in Northern Brittany ten years after the *Amoco Cadiz* oil spill. *Marine Pollution Bulletin* **21**, 123–130.

Dean, T. A., Jewett, S. C., Laur, D. R. and Smith, R. O. (1996a) Injury to epibenthic invertgrates resulting from the Exxon Valdez oil spill. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Spies, D. A. Wolfe and B. W. Wright, pp. 424–439. American Fisheries Society, Bethesda, MA.

Dean, T. A., Stekoll, M. S. and Smith, R. O. (1996b) Kelps and oil: the effects of the *Exxon Valdez* oil spill on subtidal algae. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Spies, D. A. Wolfe and B. W. Wright, pp. 412–423. American Fisheries Society, Bethesda, MA.

Donovaro, R., Fabiano, M. and Vincx, M. (1995) Meiofauna response to the Agip Abruzzo oil spill in subtidal sediments of the Ligurian Sea. *Marine Pollution Bulletin* **30**, 133–143.

Drake, D. E., Fleischer, P. and Kolpack, R. L. (1971) Transport and deposition of flood sediment, Santa Barbara Channel, California. In *Biological and Oceanographic Survey of the Santa Barbara Channel Oil Spill, 1969–1970*, ed. R. L. Kolpack, pp. 181–217. Allen Hancock Foundation, University of Southern California, Los Angeles, CA.

Duinker, J. C. (1980) Suspended matter in estuaries: adsorption and desorption processes. In *Chemistry and Biogeochemistry of Estuaries*, eds E. Olausson and I. Cato, pp. 121–151. John Wiley, New York.

Gearing, J. N., Gearing, P. J., Wade, T., Quinn, J. G., McCarty, H. N. B., Farrington, J. and Lee, R. F. (1979) The rates of transport and fates of petroleum hydrocarbons in a controlled marine ecosystem and a note on analytical variability. In *Proceedings of the 1979 Oil Spill Conference*, pp. 555–564. American Petroleum Institute, Washington, DC.

Gibbs, R. J. (1985) Estuarine flocs: their size, settling velocity and density. *Journal of Geophysics Research* **90**, 3249–3251.

Gilfillan, E. S., Page, D. S. and Foster, J. C. (1986) *Tidal Area Dispersant Project: Fate and Effects of Chemically Dispersed Oil in the Nearshore Benthic Environment. American Petroleum Institute Publication No. 4440.* American Petroleum Institute, Washington, DC.

Gilfillan, E. S., Page, D. S., Krejsa, C. M., Hanson, S. A., Foster, J. C., Trussell, G. C. and Whalon, B. S. (1991) Use of ordination techniques to follow community succession from oil impact to recovery in the field. *Chemistry and Ecology* **5**, 85–97.

Gilfillan, E. S., Page, D. S., Harner, E. J. and Boehm, P. D. (1995) Shoreline ecology program for Prince William Sound, Alaska, following the *Exxon Valdez* oil spill. Part 3: Biology. In Exxon

Valdez *Oil Spill: Fate and Effects in Alaskan Waters*, eds P. G. Wells, J. N. Butler and J. S. Hughes, pp. 398–443. American Society for Testing and Materials (1219), Philadelphia, PA.

Glemarec, M. and Hussenot, E. (1981) Definition d'une Secession Ecologique en Milieu Meuble Abnormallement Enrichi en Matieres organique a la Suitre de la Catastrophe de l'Amoco Cadiz. In Amoco Cadiz: *Conseuquences d'une Pollution Accidentelle par les Hydrocarbures; Fates and Effects of the Oil Spill*, pp. 499–525. Centre National Pour L'Exploitation des Oceans, Paris.

Glemarec, M. and Hussenot, E. (1982) A three-year ecological survey in Benoit and Wrac'h Abers following the *Amoco Cadiz* oil spill. *Netherlands Journal of Sea Research* **16**, 483–490.

Gould, J. R. (1988) *Oil Spill Studies: Measurement of Environmental Effect and Recovery*. Report Number DR199, Environmental Sciences Department, American Petroleum Institute, Washington, DC.

Gundlach, E. R., Boehm, P. D., Marchand, M., Atlas, R. M., Ward, D. M. and Wolfe, D. A. (1983) The fate of *Amoco Cadiz* oil. *Science* **221**, 122–129.

Haensley, W. E., Neff, J. M., Sharp, J. R., Morris, A. C., Bedgood, M. F. and Boehm, P. D. (1982) Histopathology of *Pleuronectes platessa* L. from Aber Wrac'h and Aber Benoit, Brittany, France: long-term effects of the *Amoco Cadiz* oil spill. *Journal of Fish Diseases* **5**, 365–391.

Hoffman, E. and Quinn, J. G. (1980) The *Argo Merchant* oil spill and the sediments of Nantucket shoals. In *Contaminants and Sediments*, ed. R. A. Baker, Vol. I, pp. 185–218. Ann Arbor Science, Ann Arbor, MI.

Jackson, J. B. C., Cubit, J. D., Keller, B. D., Batista, V., Burns, K., Caffey, H. M., Caldwell, R. L., Garrity, S. D., Getter, C. D., Gonzalea, C., Guzman, H. M., Kaufman, K. W., Knap, A. H., Levings, S. C., Marshall, M. J., Steger, R., Thompson, R. C. and Weil, E. (1989) Ecological effects of a major oil spill on panamanian coastal marine communities. *Science* **243**, 37–44.

Jahns, H. O., Bragg, J. R., Dash, L. C. and Owens, E. H. (1991) Natural cleansing of shorelines following the *Exxon Valdez* spill. In *Proceedings of the 1991 Oil Spill Conference*, pp. 167–176. American Petroleum Institute, Washington, DC.

Jewett, S. C., Dean, T. A. and Laur, D. R. (1996) Effects of the *Exxon Valdez* oil spill on benthic invertebrates in an oxygen-deficient embayment in Prince William Sound, Alaska. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Spies, D. A. Wolfe and B. W. Wright, pp. 440–447. American Fisheries Society, Bethesda, MA.

Johansson, S., Larsson, U. and Boehm, P. (1980) The *Tsesis* oil spill. *Marine Pollution Bulletin* **11**, 284–293.

Kennicutt, M. C., Brooks, J. M., Bidigare, R. R. and Denoux, G. J. (1988) Gulf of Mexico hydrocarbon seep communities. I: Regional distribution of hydrocarbon seepage and associated fauna. *Deep-Sea Research* **35**, 1639–1651.

Kennicutt, M. D., Sweet, S. T., Fraser, W. R., Culver, M. and Stockton, W. L. (1991) The fate of diesel fuel spilled by the Bahia Paraiso in Arthur Harbor, Antarctica. In *Proceedings of 1991 Oil Spill Conference*, pp. 493–500. American Petroleum Institute, Washington, DC.

Knap, A. H. (1992) *Biological Impacts of Oil Pollution: Coral Reefs, Vol. 3*. International Petroleum Industry Environmental Conservation Association, London.

Kolpack, R. L., Mattson, J. S., Mark, H. B. and Yu, T.-C. (1971) Hydrocarbon content of Santa Barbara Channel Oil Sediments. In *Biological and Oceanographic Survey of the Santa Barbara Oil Spill, 1969–1970*, ed. R. L. Kolpack, pp. 276–295. Allen Hancock Foundation, University of Southern California, Los Angeles, CA.

Koons, C. B. and Jahns, H. O. (1992) The fate of oil from the *Exxon Valdez*—a perspective. *Marine Technology Society* **26**, 61–69.

Krahn, M. M., Kittle, L. J. and MacLeod, W. D. (1986) Evidence for exposure of fish to oil spilled into the Columbia River. *Marine Environmental Research* **20**, 291–298.

Krahn, N. M., Burrows, D. G., Hlitalo, G. M., Brown, D. W., Wigren, C. A., Collier, T. K. K., Chan, S. L. and Varanasi, U. (1992) Mass spectrometric analysis for aromatic compounds in bile of file sampled after the *Exxon Valdez* oil spill. *Environmental Science and Technology* **26**, 116–126.

Krahn, N. M., Ylitalo, G. M., Buzitis, J., Bolton, J. L., Wigren, C. A., Chan, S. L. and Varanasi, U. (1993) Analyses for petroleum-related contaminants in marine fish and sediments following the Gulf Oil spill. *Marine Pollution Bulletin* **27**, 285–292.

Kurelec, B., Britvic, S., Rijavec, M., Muller, W. E. G. and Zahn, R. K. (1977) Benzo(a)pyrene monooxygenase induction in marine fish-molecular response to oil pollution. *Marine Biology* **44**, 211–216.

Laur, D. and Haldorson, L. (1996) Coastal habitat studies: the effects of the *Exxon Valdez* oil spill on shallow subtidal fishes in Prince William Sound. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Spies, D. A. Wolfe and B. W. Wright, pp. 659–670. American Fisheries Society, Bethesda, MA.

Lee, R. F. (1980) Processes affecting the fate of oil in the sea. In *Marine Environmental Pollution. 1: Hydrocarbons*, ed. R. A. Geyer, pp. 337–351. Elsevier, Amsterdam.

Linden, O., Elmgren, R. and Boehm, P. (1979) The *Tsesis* oil spill. Its impact on the coastal ecosystem of the Baltic Sea. *Ambio* **8**, 244–253.

Lindstedt-Siva, J., Chamberlain, D. W. and Mancini, E. R. (1987) Environmental aspects of the *Arco Anchorage* oil spill, Port Angeles, Washington. In *Proceedings of the 1987 Oil Spill Conference*, pp. 407–410. American Petroleum Institute, Washington, DC.

Little, D. I. and McLaren, P. (1989) Sediment and contaminant transport in Milford Haven. In *Ecological Impacts of the Oil Industry*, ed. B. Dicks, pp. 203–234. John Wiley, New York.

Long, E. R. and Morgan, L. G. (1990) *The Potential for Biological Effects of Sediment-sorbed Contaminants Tested in the National Status and Trends Program. NOAA Technical Memorandum NOS OMA 52*. National Atmospheric and Oceanic Administration, US Department of Commerce, Rockfille, MA.

McDonald, S. J., Kennicutt, M. C. and Brooks, J. M. (1992) Evidence of polycyclic aromatic hydrocarbon (PAH) exposure in fish from the Antarctic Peninsula. *Marine Pollution Bulletin* **25**, 313–317.

Meyers, M. S., Rhodes, L. D. and McCain, B. B. (1987) Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions, and other idiopathic hepatic conditions in English sole (*Parophyrs vetulus*) from Puget Sound, Washington. *Journal of the National Cancer Institute* **78**, 333–363.

Michel, J., Hayes, M. O., Keenan, R. S., Sauer, T. C., Jensen, J. R. and Narumalani, S. (1993) Contamination of nearshore subtidal sediments of Saudi Arabia from the Gulf War spill. *Marine Pollution Bulletin* **27**, 109–116.

Miller, J. A. (1989) Physical and chemical recovery of intertidal and shallow subtidal sediments impacted by the Arco Anchorage oil spill, Ediz Hook, Washington. In *Proceedings of 1989 Oil Spill Conference*, pp. 487–491. American Petroelum Institute, Washington, DC.

Montagna, P. A., Bauer, J. E., Toal, J., Hardin, D. and Spies, R. B. (1987) Temporal variability and the relationship between benthic meiofaunal and microbial populations of a nautral coastal petroleum seep. *Journal of Marine Research* **45**, 761–789.

Morel, G. and Courtot, P. (1981) Evolution des hydrocarbures dan l'eau et le sediments de l'Aber Benoit. In Amoco Cadiz: *Consequences d'une Pollution Accidentelle par les Hydrocarbures; Fate and Effects of the Oil Spill*, pp. 107–127. Centre National Pour L'Exploitation des Oceans, Paris.

National Research Council (1985) *Oil in the Sea: Inputs, Fates, and Effects*. National Research Council, National Academy Press, Washington, DC.

Neff, J. M. (1985) The use of biochemical measurement to detect pollutant-mediated damage to fish. In *Aquatic Toxicology and Hazard Assessment*, eds R. D. Cardwell, R. Purdy and R. C. Bahner, pp. 115–183. American Society for Testing and Materials, Philadelphia, PA.

Nichols, M. M. and Biggs, R. B. (1985) Estuaries. In *Coastal Sedimentary Environments*, ed. R. A. Davis, pp. 77–186. Springer, New York.

O'Clair, C. E., Short, J. W. and Rice, S. D. (1996) Contamination of intertidal and subtidal sediments by oil from the Exxon Valdez in Prince William Sound. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Spies, D. A. Wolfe, B. A. Wright, pp. 61–93. American Fisheries Society, Bethesday, MA.

Owens, E. H. (1991) Shoreline conditions following the *Exxon Valdez* spill as of fall 1990. In *Proceedings of 14th Annual Arctic and Marine Oilspill Program Technical Seminar*, pp. 579–606. Environment Canada, Ottawa, ON.

Owens, E. H., Humphrey, B. and Gergy, G. A. (1987) The migration of stranded oil from a sheltered beach into adjacent nearshore subtidal sediments. In *Fate and Effects of Oil in Marine Ecosystems*, eds J. Kuiper and W. J. Vanden Brink, pp. 231–241. Marinus Nijhoff, Dordrecht.

Page, D. S., Foster, J. C., Pickett, P. M. and Gilfillan, E. W. (1989) Long-term weathering of *Amoco Cadiz* oil in soft intertidal sediments. In *Proceedings of 1989 Oil Spill Conference*, pp. 401–405. American Petroleum Institute, Washington, DC.

Page, D. S., Boehm, P. D., Douglas, G. S. and Bence, A. E. (1995) Identification of hydrocarbon sources in the benthic sediments of Prince William Sound and the Gulf of Alaska following the *Exxon Valdez* oil spill. In Exxon Valdez *Oil Spill: Fate and Effects in Alaskan Waters*, eds P. G. Wells, J. N. Butler and J. S. Hughes, pp. 41–83. American Society for Testing and Materials (1219), Philadelphia, PA.

Page, D. S., Boehm, P. D., Gilfillan, E. S., Bence, A. E., Burns, W. A. and Mankiewicz, P. J. (1996a) Effects of the *Exxon Valdez* oil spill on the subtidal organic geochemistry of two bays in Prince William Sound, Alaska. In *Proceedings of the 19th Arctic and Marine Oil Spill Program (AMOP), Technical Seminar*, pp. 1195–1209. Environment Canada, Ottawa, ON.

Page, D., Boehm, P. D., Douglas, G. S., Bence, A. E., Burns, W. A. and Mankiewicz, P. J. (1996b) The natural petroleum hydrocarbon background in subtidal sediments of Prince William Sound, Alaska, USA. *Environmental Toxicology and Chemistry* **15**, 1266–1281.

Payne, J. F., Kiceniuk, J., Misra, R., Fletcher, G. and Thompson, R. (1983) Review and perspective on the use of mixed-function oxygenase enzymes in biological monitoring. *Comparative Biochemistry and Physiology* **86C**, 233–245.

Payne, J. R., Clayton, J. R., McNabb, G. D., Kirstein, B. E., Clary, C. L., Redding, R. T., Evans, J. S., Reimnitz, E. and Kempema, E. W. (1989) *Oil–ice–sediment interactions during freeze-up and break-up. US OCSEAP Final Report 64*. Department of Commerce, National Oceanic and Atmospheric Administration (NOAA).

Payne, J. R., Clayton, J. R., McNabb, G. D. and Kirstein, B. E. (1991) *Exxon Valdez* oil weathering fate and behavior: model predictions and field observations. In *Proceedings of the 1991 Oil Spill Conference*, pp. 641–654. American Petroleum Institute, Washington, DC.

Poirier, O. A. and Thiel, G. A. (1941) Deposition of free oil by sediments settling in seawater. *Bulletin of the American Association of Petroleum Geologists* **25**, 2170–2180.

Postma, H. (1980) Sediment transport and sedimentation. In *Chemistry and Biogeochemistry of Estuaries*, eds E. Olausson and I. Cato, pp. 153–186. John Wiley, New York.

Richie, W. and O'Sullivan, M. (1994) *The Environmental Impact of the Wreck of the* Braer. Environment Department, The Scottish Office, Edinburgh.

Sale, D. M. and Short, J. W. (1995) *Nearshore subtidal transport of hydrocarbons and sediments following the* Exxon Valdez *oil spill. Final Report*. Exxon Valdez Oil Spill State/Federal Natural Resources Damage Assessment (Subtidal Study Number 3B), Alaska Department of Environmental Conservation, Juneau.

Sanders, H. L., Grassle, J. F., Hampson, G. R., Morse, L. S., Garner-Price, S. and Jones, C. C. (1980) Anatomy of an oil spill: long-term effects from the grounding of the barge *Florida* off West Falmouth, Massachusetts. *Journal of Marine Research* **38**, 265–380.

Sauer, T. C., Brown, J. S., Boehm, P. D., Aurand, D. V., Michel, J. and Hayes, M. O. (1993) Hydrocarbon source identification and weathering characterization of intertidal and subtidal sediments along the Saudi Arabian coast after the Gulf War oil spill. *Marine Pollution Bulletin* **27**, 117–134.

Schubel, J. R. (1974) Effects of tropical storm Agnes on the suspended solids of the northern Chesapeake Bay. In *Suspended Solids in Water*, ed. R. Gibbs, pp. 113–130. Plenum, New York.

Sharma, G. D. (1979) *The Alaskan Shelf. Hydrographic, Sedimentary, and Geochemical Environment*, Springer, New York.

Short, J. W., Sale, D. M. and Gibeaut, J. C. (1996) Nearshore transport of hydrocarbons and sediments after the *Exxon Valdez* oil spill. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Spies, D. A. Wolfe and B. W. Wright, pp. 40–60. American Fisheries Society, Bethesda, MA.

Spies, R. B., Davis, P. H. and Stuermer, D. H. (1980) Ecology of a submarine petroleum seep off the California coast. In *Marine Environmental Pollution*, ed. R. A. Geyer, Vol. 1, pp. 229–263. Elsevier, Amsterdam.

Spies, R. B., Hardin, D. D. and Toal, J. P. (1988) Organic enrichment or toxicity? A comparison of the effects of kelp and crude oil in sediments on the colonization and growth of benthic infauna. *Journal of Experimental Marine Biology and Ecology* **124**, 261–282.

Steichen, D. J., Holbrook, S. J. and Osenberg, C. W. (1996) Distribution and abundance of benthic and demersal macrofauna within a natural hydrocarbon seep. *Marine Ecology Progress Series* **138**, 71–82.

Stott, G. G., Haensly, W. E., Neff, J. M. and Shar, J. R. (1983) Histopathologic survey of ovaries of plaice *Pleuronectes platessal* from Aber Wrac's and Aber Benoit, Brittany, France: long-term effects of the *Amoco Cadiz* crude oil spill. *Journal of Fish Diseases* **6**, 429–437.

Strand, J. A., Cullinan, V. I., Crecelius, E. A., Fortman, T. J., Citterman, R. J. and Fleischmann, M. L. (1992) Fate of Bunker C fuel oil in Washington coastal habitats following the December 1988 *Nestucca* oil spill. *Northwest Science* **66**, 1–14.

Teal, A. R. (1990) Shoreline clean-up follwing the *Exxon Valdez* spill-the decision process for clean-up operations. In *Proceedings of the 13th Arctic and Marine Oilspill Program Technical Seminar*, pp. 422–429. Environment Canada, Ottawa, ON.

Wolfe, D. A., Hameedi, M. J., Galt, J. A., Watabayashi, G., Short, J., O'Claire, C., Rice, S., Michel, J., Payne, J. R., Braddock, J., Hanna, S. and Sale, D. (1994) The fate of the oil spilled from the *Exxon Valdez. Environmental Science and Technology* **28**, 561A–568A.

Wolfe, D. A., Krahn, M. M., Casillas, E., Sol, S., Thompson, T. A., Lunz, J. and Scott, K. J. (1996) Toxicity of intertidal and subtidal sediments contaminated by the *Exxon Valdez* spill. In *Proceedings of the* Exxon Valdez *Oil Spill Symposium*, eds S. D. Rice, R. B. Spies, D. A. Wolfe and B. W. Wright, pp. 121–139. American Fisheries Society, Bethesda, MA.