Implications of Oil Pollution in Production of Disease in Marine Organisms [and Discussion]

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Implications of oil pollution in production of disease in marine organisms

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Contamination of marine waters by petroleum, whether as a consequence of acute or chronic events, constitutes an additional source of stress for marine organisms – one often reflected in pathological changes. Morphological, behavioural, physiological or biochemical abnormalities may result from exposure to petroleum or its component chemicals. Among the morphological changes that have been associated, at least circumstantially, with petroleum contamination of marine habitats are (for fish) fin erosion, fin ray deformation, ovarian histopathology, olfactory lesions, degeneration of ventricular myocardium and cytogenetic anomalies; and (for invertebrates) tissue hyperplasia, gill and gut epithelial necrosis, gonadal tumours and kidney tubule occlusion.

Literature on petroleum-associated diseases and abnormalities of fish and shellfish can best be summarized in four major categories: that dealing with integumental lesions of fish; that concerned with various other kinds of histopathologies, usually experimentally induced; that emphasizing neoplasms of molluscs; and that related to genetic and morphological abnormalities in eggs and larvae as a consequence of exposure to petroleum or its components. A general conclusion is that petroleum, in sufficient concentration, can be toxic to marine animals, and toxicity may be expressed as morphological changes.

Some of the effects of petroleum, such as the enhancement of latent viral infections in clams and immunosuppression in fish, are quite probably associated with increased stress. The development of neoplasia and hyperplasia in petroleum-exposed marine animals deserves further examination, as does the entire matter of pollution-related integumental lesions.

1. Introduction

There have been many attempts to elucidate the relations between pollution and disease. Some statistical association of certain abnormalities with pollutants has been made, but other data from coastal or estuarine waters must still be considered circumstantial. Before considering the effects of petroleum specifically, it might be worthwhile to summarize the more general information about pollution and disease. Integumental lesions in fish have been found to have a good statistical association with environmental contamination in several parts of the world. Pollutants have been shown to provoke latent viral infections into patency in several species. The degree of environmental degradation has been found in several studies to have a rough statistical association with prevalences of skeletal abnormalities. Prevalence of chromosomal anomalies has also been shown in one extensive study to have a general correlation with pollution of coastal waters.

So there is evidence, both from field observations and experimental studies, of an emerging

relation between particular categories of diseases and disease signs and pollution. The temptation to overextend the relation should be resisted, however, until many more data are available. The possible association of neoplasms in fish and shellfish with pollution needs much more critical and extensive examination, as does the entire matter of chromosomal damage in eggs and larvae.

In any attempt to frame the effects of petroleum on marine organisms within the larger context of coastal or estuarine pollution, it is important to consider the matter of stress. A basic concept, which is being reinforced repeatedly, is an obvious one: that pollutants constitute forms

TABLE 1. PHASES OF THE STRESS SYNDROME IN HUMANS (SELYE'S ORIGINAL CONCEPT)

1. *alarm*	→	2. *resistance*	→	3. *exhaustion*
adaptation not yet acquired		adaptation is optimum		adaptation lost; collapse and death

TABLE 2. GENERALIZATION OF THE STRESS PHENOMENON TO INDIVIDUALS AND POPULATIONS

1. *alarm*	→	2. *resistance* (*adaptation*)	→	3. *exhaustion*
short-term responses: behavioural, physiological and other responses to non-optimum environment		longer-term responses: adaptive enzymes protein binding of metals induction of mixed-function oxidases population selective action favouring survival of resistent individuals changes in reproductive strategy		failure of critical biochemical functions leading to functional disorders and death gradual diminution and disappearances of some populations due to reproductive failure

of environmental chemical stress, and much of what we call 'pathology' or 'disease' is a consequence of actions of environmental stressors. Since stress is a fundamental but easily misunderstood concept, a little attention to it at this point is relevant.

It would be reasonable at the outset to attempt to define stress – a significant if somewhat elusive concept in biology, which, like any concept, can be misunderstood, misinterpreted or overextended. Stress as a major factor in human disorders was first enunciated by Hans Selye, a Canadian, in 1936, and the concept was quickly extended to the population level. Selye's original concept of stress, as it applies to humans, included three phases, – alarm, resistance and exhaustion – as seen in table 1. A redescription of the concept, as it may apply to any organism or population, is presented in table 2. Selye (1950, 1952) defined stress as 'the sum of all the physiological responses by which an animal tries to main or reestablish a normal metabolism in the face of a physical or chemical force'. Others have proposed many modifications of this definition.

Brett (1958) defined stress as 'a state produced by any environmental or other factor which extends the adaptive responses of an animal beyond the normal range, or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are significantly reduced'. Another definition that clearly identifies stress as the product and not the cause of homoeostatic change is that of Esch *et al.* (1975): 'stress is the effect of any force which tends to extend any homeostatic or stabilizing process beyond its normal limit, at any level of

biological organization'. Current usage of the term seems to be tending toward its most general definition, as proposed by Gronow (1974): 'Stress is a strain or burden placed on an animal under the influence of extreme external or internal stimulation'.

All of these definitions are vaguely unsatisfying, but in its broadest sense stress represents *the sum of morphological, physiological, biochemical and behavioural changes in individuals that result from actions of stressors.*

Petroleum contamination of estuarine or coastal waters, either as an acute episode (a spill) or as a chronic stressor, clearly affects marine organisms. Toxicity exists, particularly for early life stages which are most likely to contact oil at or near the ocean surface. Sensitivity can vary with species, and possibly with the extent of previous contact of the parent population with oil contamination (in areas of chronic spills or seeps). Adult animals exhibit many physiological, biochemical and behavioural responses to oil contamination. Some histopathological signs result, enzyme activity may be modified, reproductive behaviour can be affected, and in cases of extreme toxicity, mortalities can occur.

This report attempts to summarize that part of the literature on oil pollution that is concerned with production of disease and mortality in marine organisms. It deals to a great extent with subacute chronic effects, and to a lesser extent with acute mortality-inducing events. Chronic effects may be expressed as physiological, behavioural, biochemical or morphological changes in exposed animals. Although all of these changes may be encompassed in the broadest definition of disease (defined as 'any departure from normal structure or function of an organism'), much of the content of this paper will be confined to morphological changes associated with oil pollution. This is a much narrower, but more tractable body of information.

To achieve some semblance of order, this paper will summarize information on disease conditions associated with petroleum contamination of the marine environment in four categories: (1) integumental lesions in fish, (2) other histopathology associated with oil exposure, (3) abnormalities in molluscs, and (4) chromosomal anomalies and mutagenesis.

2. Integumental lesions in fish

Probably the best information that exists (in a relatively poor field) for the induction of gross lesions in fish by exposure to petroleum concerns integumental abnormalities: fin erosion and skin ulceration. The recent (March 1978) wreck of the tanker *Amoco Cadiz* on the Brittany coast of France provided some interesting, if somewhat circumstantial, information about the development of such lesions. Six months after the spill, 50–80% of a catch of mullet (*Mugil cephalus*) taken near the spill area had lesions in the form of penetrating ulcers with necrotic margins and bases. A pronounced inflammatory response, with occasional muscle lysis, was seen histologically. Neither thrombosis nor haemorrhage was seen (Balouet & Blaudin-Laurencin 1980). A relation of ulcerations to the previous oil spill was suggested, but not demonstrated.

Other reports of integumental lesions circumstantially associated with petroleum from the wreck of the *Amoco Cadiz* were published by Desaunay (1979). Three species of fish, plaice (*Pleuronectes platessa*), sole (*Solea vulgaris*) and dab (*Limanda limanda*) from the bays of Morlaix and Lannion were examined in detail over an 18 month period (April 1978 to October 1979) and other species received some attention. Fin erosion was the most common anomaly found. In plaice, erosion was most pronounced on the posterior fins, especially in those areas in intimate contact with the bottom (figure 1). In gadoids (*Gadus morhua*, *Pollachius pollachius*) the caudal

fin was most affected. An associated phenomenon was called 'bent fin rays', which either accompanied or followed fin erosion (figure 1).

Proportions of these anomalies in samples of plaice from the two affected bays disclosed a remarkable progression, from nil in April 1978 (immediately after the spill) to 90% in December 1978, to 73% in May 1979, to 2.5% in October 1979. Desaunay pointed out possible

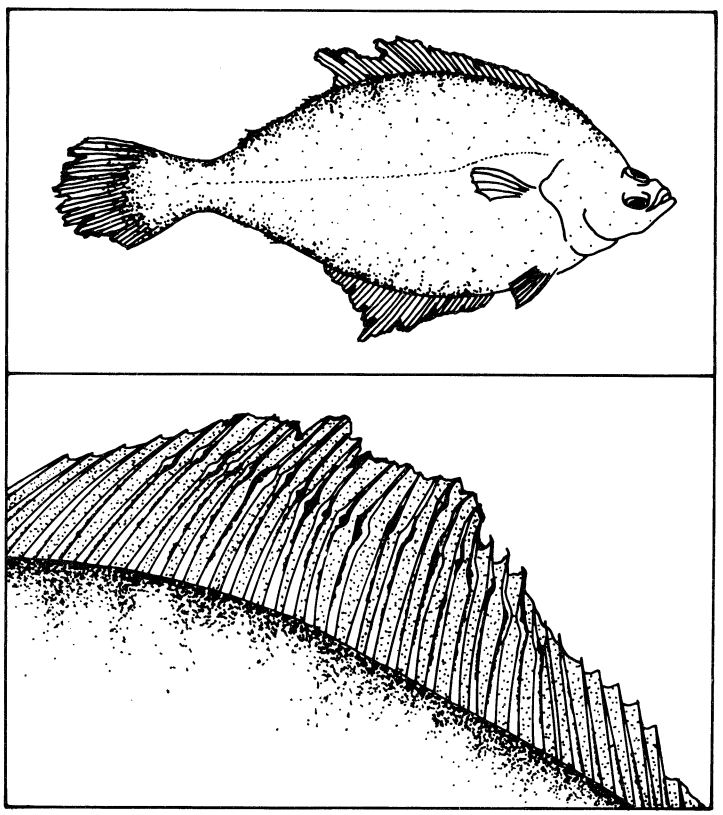

FIGURE 1. Abnormalities associated with the *Amoco Cadiz* spill: above, fin erosion in plaice; below, bent fin rays in plaice. (Redrawn from Desaunay (1979).)

weaknesses in the data – dilution by recruitment of a new year class, migration of adults, and possible differential mortality of affected fish – but the findings are certainly suggestive of a possible causal relation of fin erosion and bent fin rays with effects of petroleum contamination.

It is interesting and relevant that similar disease signs, fin erosion and the bent fin ray condition, have also been seen in fish from the highly contaminated New York Bight (Murchelano 1975; Ziskowski & Murchelano 1975; Ziskowski *et al.* 1980). Fin erosion is probably the best known but least understood disease of fish from polluted waters. It has been found in the New York Bight, the California coast, Puget Sound, Biscayne Bay, Escambia Bay, Irish Sea and Tokyo Bay, as well as the French coast (Sindermann 1979). It occurs in two types: (*a*) site-specific found especially in demersal fish, probably associated with contact with contaminated sediments, and (*b*) generalized, found especially in pelagic fish, with involvement of all fins but especially the caudal, and with occasional bacterial infections.

In the New York Bight, fin erosion has been found in 22 species, and has been demonstrated in flounders to be statistically more abundant in that Bight than in other comparable coastal

areas. It has a statistical association with high coliform bacteria and high heavy metal levels in sediments. In southern California, fin erosion has been found in many species, and is clearly associated with proximity to ocean outfalls of sewage systems.

Wherever studied, fin erosion disease signs include epidermal hyperplasia, dermal fibrosis, hyperaemia, haemorrhage (occasional), no consistent bacterial infection, and no pronounced inflammatory response.

The possible role of environmental chemical contamination in the aetiology of fin erosion emerges more clearly as additional studies are reported. Fish from the New York Bight, reported in studies by Mahoney *et al.* (1973), Murchelano (1975) and Ziskowski & Murchelano (1975), exist in a highly contaminated area, with chemicals such as heavy metals and petroleum residues in sediments far above background levels. McDermott & Sherwood (1975) in California found DDT levels to be significantly higher in fish with fin erosion, and polychlorinated biphenyl (PCB) levels slightly higher in such fish than in normal individuals. Wellings *et al.* (1976) found abnormally high concentrations of PCB in English sole and starry flounders with fin erosion from the Duwamish River in Washington.

Several authors have postulated that fin erosion in flatfish may be initiated by direct contact of tissues with contaminated sediments. Mearns & Sherwood (1974), for example, suggested that toxic substances such as sulphides, heavy metals and chlorinated hydrocarbons could remove or modify the protective mucous coat and expose epithelial tissues to the chemicals. Sherwood & Bendele (1975) reported that Dover sole from California with fin erosion produced much less mucus than normal fish.

It seems quite likely that the 'fin erosion' syndrome in fish includes chemical stress, probably acting on mucus and epithelium; stress resulting from marginal dissolved oxygen concentrations, possibly enhanced by a sulphide-rich environment; and secondary bacterial invasion in at least some instances.

Experimentally induced integumental lesions have been reported as a consequence of petroleum contamination. Fin erosion was produced in mullets (*Mugil cephalus*) held in brackish ponds (12 ng g^{-1}) in the southern United States and exposed to an experimental 'spill' of Empire Mix crude oil to provide a calculated slick of 0.0013–0.0023 cm or a calcuated 4.0–5.0 μg g^{-1} concentration (Minchew & Yarbrough 1977). Fin erosion was first noted within 12 days of the spill; by 13 days all samples of exposed fish had fin erosion. Fins were haemorrhagic; most fish had numerous small haemorrhages on the ventral body surfaces, and some fish were emaciated. The caudal fin was often the most extensively eroded. By 34 days after the spill there was some evidence of fin regeneration, a process that was further advanced at 56 days post spill (figure 2).

Bacteriological studies during the experiment (Giles *et al.* 1978) disclosed the presence of a *Vibrio* species (not further identified) as the suspected pathogen. Repeated isolates from eroded fins and kidneys supported a postulation that chemical stress from petroleum, combined with an altered microbial flora favouring a *Vibrio* population increase, probably produced the disease condition in experimental fish. Parallel aquarium experiments yielded similar results in terms of fin erosion and predominance of *Vibrio* infections. Of the microbial isolates screened, 40% were capable of utilizing crude oil.

3. Histopathology in various organs of fish and shellfish

Recently, a number of experiments have been reported in which marine animals were exposed to experimentally oiled sediments (see, for example, Anderson *et al.* 1974*a*). Among these reports, one from the northeast Pacific by McCain *et al.* (1978) described pathological consequences of exposure of English sole, *Parophrys vetulus*, to oiled sediments. In a 4 month exposure (initial concentration was a 0.2% mixture by volume of Alaskan crude oil and clean sediments) severe liver pathology was observed.

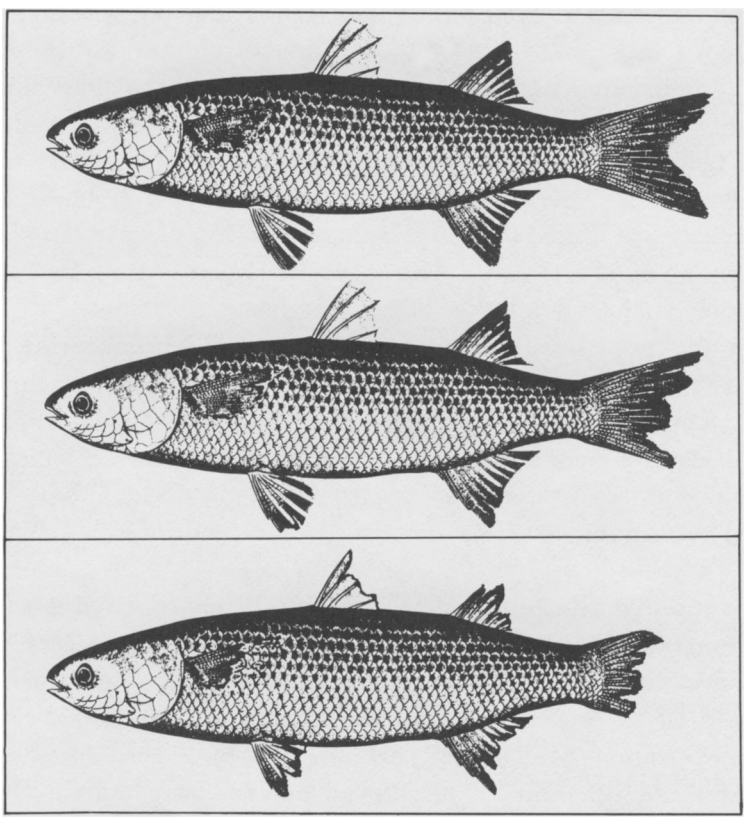

Figure 2. Fin erosion produced experimentally in mullet by exposure to oil. From top to bottom: initial fraying of caudal fin; moderate erosion; extensive erosion. (Redrawn from Minchew & Yarbrough (1977).)

During the first month the oil-exposed fish had severe hepatocellular lipid vacuolization, in which as much as 95% of the liver cell volume consisted of lipid droplets and vacuoles. This was accompanied by extensive proliferation of rough endoplasmic reticulum, leaving very little normal liver tissue. Bioavailability was seen to be dependent on aromatic structure, bioaccumulation, and degradation mechanisms in the fish tissues (suggested by the disappearance of tissue-bound crude oil hydrocarbons from all but liver, despite continued exposure to oiled sediments). The continuously oiled group experienced greater weight loss, and had significantly higher haematocrits and haemoglobin levels. All these effects declined markedly after the first month of the experiment. The most obvious liver pathology, hepatocellular lipid vacuolization, was suggested by McCain *et al.* to be a non-specific liver lesion induced by exposure to a variety of hydrocarbons at toxic levels. It may serve as an indicator of long-term environmental stress

from petroleum and other hydrocarbon contamination, and it has been observed in other experimental studies of pathological effects of petroleum (Eurell & Haensley 1981).

The experimental literature is replete with reports of this kind describing histopathology associated with exposure to petroleum and its water-soluble fractions. The polycyclic aromatic hydrocarbons (PAHs) are known toxic components of crude and refined oil–water mixtures, being highly soluble in water and taken up and accumulated rapidly by marine animals. Of the PAHs, naphthalene is among the most toxic (Anderson *et al.* 1974*b*; Neff *et al.* 1976), and is the component of choice in numerous exposure studies.

In a recently reported continuous low-level exposure of an euryhaline fish, *Fundulus heteroclitus*, to naphthalene at concentrations of 0.002–30 mg l^{-1} for up to 15 days (DiMichele & Taylor 1978) a number of interesting observations were made.

1. Necrosis of neurosensory cells, particularly those of the olfactory and lateral line systems, occurred at concentrations as low as 0.02 mg l^{-1}.

2. Generalized blood stasis was evident at higher exposure concentrations; small blood vessels were engorged and blocked.

3. Pathological changes – lesions and necrosis – were noted in brain, liver and pancreas at concentrations as low as 0.2 mg l^{-2}.

4. At highest concentrations, gut mucosal necrosis and skeletal muscle degeneration occurred.

5. Naphthalene was selectively accumulated in organs most susceptible to pathology.

6. Generalized signs of chemical stress were present at all concentrations: gill hyperplasia and haemorrhage were common, as were signs of metabolic stress.

Olfactory lesions were predominant pathological responses in another study (Gardner 1975), in which Atlantic silversides (*Menidia menidia*) were exposed to water-soluble and insoluble components of crude oil for 7 days. Test concentrations were stated to approximate 0.14–0.58 mg l^{-1}. Hyperplasia of the olfactory supporting epithelium and degeneration of the olfactory mucosa were common after exposure to crude oil. These exposures also produced degeneration of the ventricular myocardium of the heart and of the pseudobranch secretory cells. Additional histopathology was seen in *Menidia* exposed only to salt-water soluble components of crude oil, in the appearance of metaplasia in olfactory tissues, with replacement of neurosensory and other cells by less differentiated neoplastic cells.

In still another study (Hawkes 1980), degenerative changes in olfactory epithelium and chemosensory cilia of larval sand sole (*Psettichthys melanostictus*) were seen after exposure for 8 days to the water-soluble fraction of crude oil.

The work with *Menidia* was expanded by Gardner *et al.* (1975) to include an examination of morphological anomalies in silversides, oysters and scallops exposed to waste motor oil. Silversides exhibited vascular and pseudobranch lesions when exposed to 20 μg g^{-1} or greater concentrations for 36 days. The predominant pathology in oysters was the occlusion of blood vessels by amoebocyte accumulations, and the detachment and vacuolation of the intestinal mucosa. Branchial and renal lesions were seen in scallops, but only after exposure to 500 μg g^{-1} or greater concentrations of motor oil. Abnormal concretions – accumulations of amoebocytes – were observed in scallop kidneys: these were reminiscent of the concretions seen in clams from polluted areas, discussed in a later section of this paper.

Because of the extensive offshore production of petroleum in the Gulf of Mexico during the past half century, much attention has been directed to possible effects of chronic spills or

leakages on fish and shellfish. An extensive literature has developed, beginning with the exhaustively thorough work of Galtsoff *et al.* (1935). One general conclusion of that early study was that oysters growing on pilings of producing oil wells showed no gross or histological evidence of interference with gonad development and spawning (or with setting of larvae).

Studies conducted since then have demonstrated extremely localized effects on survival and growth of oyster and other benthic populations, but no negative effects on fishes. The most recent of these studies (Stott *et al.* 1980), examined 11 species of demersal and 'platform associated' fish species for petroleum-induced lesions in the ovaries. The conclusion reached was that there was 'minimal morphologically-apparent toxic effects on the ovaries resulting from the environment near the oil and gas production platforms...'. The only suggestions of differences (when compared with control sites) were in increased occurrence of atretic follicles and possible retardation of maturation in some species.

Other studies of pathology associated with exposure to petroleum have extended the range of effects. Gill epithelial sloughing has been observed (Blanton & Robinson 1973). Liver lesions in the form of increases in rough endoplasmic reticulum were seen in rainbow trout fed crude oil for 8 months (Hawkes 1977). Similar liver lesions were reported from the killifish, *Fundulus heteroclitus*, sampled in an area affected by an oil spill 8 years previously (Sabo & Stegeman 1977). Splenic discoloration was reported in salmon exposed to no. 2 fuel oil (Cardwell 1973), and lesions were observed in the intestines of salmon fed mixtures containing petroleum hydrocarbons at 5 $\mu g\ g^{-1}$ for 28 days.

While the preceding studies indicate various pathologies associated with exposure to petroleum or its components, there are numerous other studies that have led to inconclusive results, or to findings of no effects. As an example, Payne *et al.* (1978) reported on a 6 month exposure of cunner (*Tautogolabrus adspersus*) to crude oil in a flow-through system (the concentration of water-soluble components was not determined, but was low). No morphological or histochemical changes were observed, except for a slight increase in eye lens diameter and a slight reduction in testis mass.

4. Abnormalities in molluscs

(*a*) *Pollution stress and mortalities*

Clams and mussels in petroleum-contaminated bays and estuaries have received much attention, principally because of their commercial importance and the economic losses from the closure of harvesting areas, tainting, reduced growth, and mortality.

Among the more informative of the longer-term studies with bivalve molluscs is a 2 year examination of growth and survival of clams (*Mya arenaria*) in an oil spill site on the Maine coast of the United States (Dow 1975). Clams transplanted to the site suffered a 65% reduction in growth rate, with a survival of only 12.8% of the transplanted population. Production of clams from the residual native population at the spill site declined by 20% during the 2-year period, while production from neighbouring uncontaminated bed increased by 250%.

Another study of long-term effects of a localized oil spill on molluscan shellfish has been carried on by staff members of the Woods Hole Oceanographic Institution, after a spill of 720 m^3 of no. 2 heating oil on the Massachusetts coast in 1969 (Blumer *et al.* 1970; Sanders *et al.* 1972). Oysters, soft shell clams, quahogs and scallops took up oil, and effects reported included immediate mortality, persistence of oil in sediments, and continued closure of shellfish beds to harvesting because of persistent contamination.

In still another study – this one of hard clams, *Mercenaria mercenaria*, in Narragansett Bay, Rhode Island, an estuary long polluted with petroleum hydrocarbons – Jeffries (1972) found an entire syndrome that he associated with pollution stress. He found a black, tarlike discoloration, resistant to lipid solvents, in many clams from the most polluted upper estuary. Masses of amoebocytes containing the material were seen in histological sections of mantle and kidney, and the black material collected in renal tubules, where it appeared to plug the tubules and interfere with kidney function. The black discoloration was often (5–10%) accompanied by presence of mud blisters on the inner shell, resulting from infestation by the polychaete *Polydora* – an unusual event in sediment-burrowing molluscs. Jeffries suggested that the abnormal infestation may have been a consequence of the emergence of clams from the sediments, in response to conditions associated with pollution, thereby exposing themselves to attack by *Polydora* larvae. Shell abnormalities, apparently resulting from mantle recession, were also seen. These disease signs, constituting a stress syndrome, were often accompanied by mortalities, especially of smaller individuals. A mortality rate of 1% per day was estimated, and few clams were found during a resurvey 1 year later.

Further study of the discoloration and associated concretions in the kidneys of *M. mercenaria* by Rheinberger *et al.* (1979) produced evidence that a spectrum of heavy metals was present, probably as sulphides, in addition to hydrocarbons. The hypothesis was presented that the concretions resulted from an inability to excrete effectively in highly polluted zones.

(*b*) *Hyperplasia and neoplasia*

Neoplasia and hyperplasia have been described in many marine animals, both vertebrate and invertebrate. Exposure to carcinogens from petroleum may increase the incidence in susceptible marine species, but evidence that this occurs is minimal.

One of the most interesting attempts to understand the possible relationship of neoplasia or hyperplasia and pollutants concerns studies during the past decade of clams, *Mya arenaria*. A report by Barry *et al.* (1971) disclosed 'atypical hyperplasia' of gill and kidney epithelium in 37% of clams sampled on United States coasts (Maine, Rhode Island, Maryland and California). The lesions were regarded by the authors as 'pre-cancerous', but causative factors were not identified. This report seems to have been the precursor of a remarkable series of papers, spanning the period 1975 to the present, which has documented the occurrence of gonadal and haematopoietic neoplasms in clams (*Mya arenaria*) from the New England coast (Barry & Yevich 1975; Brown *et al.* 1977; Brown 1980; Appledoorn & Oprandy 1980; Oprandy *et al.* 1981).

Gonadal tumours were first noted in clams that had survived a coastal oil spill in Maine in 1971. Described as malignant, the neoplastic development frequently completely replaced normal gonadal tissues and occasionally invaded other tissues of the clam (Barry & Yevich 1975). Of over 2000 clams from the spill site examined during the period 1971–3, 124 (6%) had gonadal tumours. No comparable tumours were found in 781 control clams from three other sites on the Maine coast. The sampling was extended to an area of chronic jet fuel contamination (Freeport, Maine), in a subsequent study (Yevich & Barszcz 1976), and haematopoietic as well as gonadal tumours were reported.

Then, beginning in 1976, a greatly expanded study of tumour prevalence in *Mya arenaria* was instituted, to determine if neoplasia was associated with varying types and degrees of environmental hydrocarbon pollution (Brown *et al.* 1977). Ten coastal sites from Maine to Rhode Island were selected, based on existence of varying kinds and intensities of pollution. Examination of 1829 clams disclosed neoplasia in 159 (8%), but in only five of the ten sites.

Prevalences varied remarkably among sites: from 0 to 64%. Gonadal and haematopoietic neoplasias were found; the haematopoietic neoplasms were observed to cause poor condition and mortality. An interesting geographic variation was seen, with only haematopoietic neoplasms found in southern New England and predominantly gonadal neoplasms in northern New England. The relation of clam neoplasms to oil pollution was not clarified by the study. In the words of the authors (Brown *et al.* 1977), 'There is a surprising dichotomy of results from petroleum-derived hydrocarbon (PDH)-polluted sites. Clams from some polluted sites had no neoplasms, whereas other PDH-polluted sites had a high prevalence of neoplasms. These results suggested that the type and degree of hydrocarbon pollution are possibly related to the frequency of neoplasms and other lesions in *Mya*, but they are by no means the only causative factors.' The authors went on to state that 'the possibility that neoplastic disease in *Mya* has a genetic base or *is a result of an infectious agent* cannot be excluded by our results' [my italics].

Then in 1979 and 1980 the experimental induction of haematopoietic neoplasms in clams was reported (Brown *et al.* 1979; Brown 1980; Appledoorn & Oprandy 1980). Exposures of normal clams to water passed over populations of neoplastic clams, with and without the presence of petroleum hydrocarbons, resulted in significant levels of neoplasia in experimental animals. Prevalence exceeded 70% in one instance. This was the first transmission of neoplasia in marine invertebrates, and indicated the presence of an infectious agent. Virus-like particles were found in neoplastic clams, and they produced neoplasia when injected into normal clams. Reisolation of virus was achieved. Results of the experiment also indicated that stress enhanced susceptibility of clams to the disease.

The final step in the clam neoplasia story was published recently (Oprandy *et al.* 1981). A viral aetiological agent was described, and was reported to be similar to B-type retroviruses. Purified virus, when injected into clams, caused tumours within 2 months, and the virus was reisolated. No direct correlation between disease prevalence and type or extent of environmental pollution was demonstrated in any of the experiments, but the role of petroleum hydrocarbons as stressors was not discounted.

There are of course many other reports of neoplasms in molluscs (reviewed by Pauley (1969) and Farley (1976)). Haematopoietic neoplasms have been observed in a number of species in addition to *Mya arenaria* (Mix *et al.* 1979*a*; Farley & Sparks 1970). In one study by Mix *et al.* (1979*b*), mussels, *Mytilus edulis*, from polluted Oregon estuaries were found to have significant body benzo[*a*]pyrene (B[*a*]P) burdens and proliferative cell disorders, while those with low or undetectable levels of B[*a*]P has no proliferative disease. Other than this report, there are many others that fail to disclose any association or relation of molluscan neoplasia and pollution.

5. Chromosomal anomalies and mutagenesis

The mutagenic and carcinogenic properties of a number of chemical contaminants, including pesticides, heavy metals and certain petroleum hydrocarbons, have been demonstrated in experimental studies with terrestrial animals, and some information is available from studies of marine animals. Recent work suggests high percentages of chromosomal anomalies and high prevalence of dead fish eggs in polluted areas of the New York Bight (Longwell 1976). All degrees of chromosomal damage have been found, and higher percentages of anomalies seem generally associated with degree of environmental degradation. It may well be that a new and

significant mortality factor – increased genetic damage – may have been introduced with increasing chemical pollution. These genetic disturbances fall clearly within the definition of disease.

Longwell's initial studies concerned cytogenetic abnormalities in developing mackerel eggs from the Middle Atlantic Bight. Of approximately 19000 chromosome and mitotic figures scored in eggs from 14 coastal stations, over one-third showed some abnormality. Observed aberrations extended through the entire range of radiomimetic effects, and abnormal cells ranged from 13 to 79%. An important finding was the presence of several statistically significant variations among stations. Those with fewest abnormalities (background levels of 13–16%) were on the periphery of the Bight and along the relatively lightly polluted Long Island coast, with the exception of stations near a toxic chemical dump site 160 km offshore. Stations near the toxic dump site had the highest incidence of chromosomal and mitotic abnormalities, and at one numerous dead eggs were found. Eleven aromatic hydrocarbons were present at measurable or detectable levels in plankton from the heavily contaminated New York Bight Apex (Longwell & Hughes 1980).

The wreck of the *Argo Merchant* off Nantucket Island, U.S.A., in 1976 offered an opportunity for Longwell to examine specific effects of petroleum on developing eggs and larvae. Her findings were reported in a 1977 symposium (Longwell 1977, 1978). Eggs of cod and pollock taken in the vicinity of the spill were examined, with the following findings.

1. About 20% of cod eggs and 46% of pollock eggs from the spill zone were dead or moribund.

2. At a station within the slick, pollock embryos were grossly malformed in 18% of the eggs, and at the edge of the slick, 9% were grossly abnormal. No abnormal pollock embryos were found at stations more distant from the slick.

3. No cod embryos were deformed.

4. Eggs at all stations showed some oil contamination of membranes; fewer cod than pollock eggs were fouled.

5. Eggs in the dead and moribund categories displayed 'a combination of cytological abnormality of the embryo's cells or of the nuclear configurations, coupled with division arrest' (Longwell 1978).

Obviously, much more work is necessary to develop these initial insights into meaningful demonstrations of relationships of chromosomal abnormalities and pollutants, but the stage has been set by Longwell, even though she is careful to state (Longwell & Hughes 1980) that 'despite statistical associations with site contaminant levels, associations between contaminants and egg health must be regarded as suggestive and not conclusive'.

It should be pointed out that a number of other workers have examined petroleum-induced abnormalities of embryos and larvae at a macroscopic level (Hawkes 1980; Kuhnhold 1974, 1977*a*, *b*; Linden 1976; Smith & Cameron 1979; Struhsaker 1977; Tilseth *et al.* 1981). Prominent among the abnormalities found were malformed jaws, flexures of the vertebral column, lower embryonic heart rate, loss of coordination and equilibrium, and degeneration of neurosensory cells. So far, associations of chromosomal and gross abnormalities have not been made.

6. Conclusions

Some tentative conclusions can be reached about an association of petroleum contamination and disease in marine animals, based on available published information.

1. Petroleum and a number of its fractions, in sufficient concentrations, act as environmental chemical stressors, causing pathologies in several organ systems, particularly neurosensory and integumental. Increasing concentrations result in increasingly severe tissue damage.

2. Many of the pathologies, such as fin erosion, ulceration and olfactory cell necrosis are non-specific tissue responses to chemical stressors, and can be duplicated by exposure to other chemical pollutants. They represent classical cell and tissue responses to toxic irritants.

3. Attempts to relate oil contamination of estuarine or coastal waters with certain types of neoplasias in bivalve molluscs have been inconclusive. In one well documented instance neoplasia was demonstrated to result from virus infection, possibly exacerbated by chemical stress from chronic petroleum contamination.

4. Prevalences of chromosomal and cytological anomalies in developing planktonic fish eggs have been shown in one extensive and continuing study to have a statistical relation to degree of contamination of surface waters, including that caused by petroleum.

5. Experimental findings of mortality and developmental abnormalities in fish eggs exposed to petroleum or its components support the hypothesis that pollution acting on sensitive early division stages of embryos may introduce a new and significant factor in determining the survival of fish populations.

6. Because of the complexity of factors both natural and man-induced, that exist in most marine environments, clear association of pathologies in fish and shellfish with petroleum contamination is often not possible.

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Discussion

R. Lloyd (*M.A.F.F. Fisheries Laboratory, Burnham on Crouch, Essex, U.K.*). Dr Sindermann, stated that skin ulceration, fin erosion and hepatic cell damage in fish were non-specific responses to chemical stress. Could these responses also be caused by severe changes in ambient temperature, and nutritional and behavioural stresses?

C. J. Sindermann. Environmental stressors – physical, chemical or biological – can result in a spectrum of abnormalities, in response to induced stress. The particular expression of the response may vary with the form and extent of change, and may be further modified by secondary microbial infection.

S. L. Vader (*University of Tromsö, Norway*). How fast would Dr Sindermann expect the chromosome damage to occur in eggs and larvae? It is already during the first cleavages after petroleum contamination, or after several days, or in the next generation?

C. J. Sindermann. Chromosomal anomalies may be induced very rapidly in developing embryos and larvae in response to chemical toxins.

W. R. P. Bourne (*Zoology Department, Aberdeen University, U.K.*). A large number of other pollutants are also of course released along the east coast of North America, for example PCBs and other more toxic related compounds such as the polychlorinated dibenzodioxins, which have accumulated in large amounts in the Hudson River, where their occurrence and effects are discussed in a symposium published in volume 320 of the *Annuals of the New York Academy of Sciences*. It has also been suggested that they may cause abnormalities, which complicates the issue.

C. J. Sindermann. The complex nature of coastal or estuarine pollution complicates any attempt to associate specific abnormalities with specific pollutants. Some insights can be gained, of course, by experimental exposures to single or multiple chemical contaminants and observing the resulting pathologies at various concentrations approximating those found in the environment.