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Fish disease as a monitor for marine pollution: the case of the North Sea

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Introduction

The possibility that pollution may contribute to the aetiology of disease in marine fish has been of interest to scientists and non-scientists for some time, partly because of concern over the possible contribution of pollution-mediated diseases to fish mortality and observed population declines. But it has also been suggested that fish diseases may be suitable indicators for monitoring biological effects of pollution; their use for this purpose is the subject of the present review.

Fish challenged by environmental stressors, including pollution, respond in a number of specific ways which lead to functional changes in the biochemistry and physiology of blood and tissues, and ultimately to pathological changes at the organismal level, termed diseases (Sindermann *et al.*, 1980; Wedemeyer and Goodyear, 1984). Recent research has provided much information on the toxic and pathological effects of known concentrations of pollutants, and on the mechanisms of pollutant action in aquatic organisms (e.g. Bayne *et al.*, 1988). At the same time, epidemiological surveys have attempted to correlate the health of marine fish populations with the levels of pollution to which they are exposed. Such surveys have been confined largely to the North Sea and associated waters (including the Baltic and Irish Seas), and to the Atlantic and Pacific coastal waters of North America.

In this review, we attempt to summarize the progress of epidemiological research into fish diseases, and by doing so, to reappraise the potential of using diseases of marine fish to monitor biological effects of pollution. After discussing in general terms the criteria for conducting epidemiological surveys, we provide an overview of fish disease research and then focus on the North Sea and associated waters (Fig. 1) as a case study for the purpose of evaluation.

DEFINITIONS

Epidemiology is defined as the study of disease in populations and of factors that define its occurrence (Thursfield, 1986). Fish epidemiology involves observing fish populations and making inferences from the observations.

Throughout the text the word epidemiology is used as a synonym for epizootiology. The term **disease** refers to pathological conditions, including those of unknown aetiology. The concept of disease supposes the impairment of normal function beyond a certain critical level. In practice, however, this has not been established for all observed stages of the conditions normally included in fish disease surveys, which are summarized for the North Sea in Table 1 (further details: Möller and Anders, 1986). For basic principles of veterinary epidemiology the reader is referred to Thursfield (1986).

BACKGROUND TO THE LINK BETWEEN DISEASE AND POLLUTION

The evidence for a link between pollution and fish disease may be classified into three broad categories.

1. Human epidemiology has demonstrated a connection between the development of malignant tumours and exposure to chemicals or other environmental factors.
2. Laboratory toxicity testing has shown that pathological conditions in fish result from exposure to a large array of chemical agents.
3. Many chemical pollutants have toxic, immunogenic, teratogenic, carcinogenic and/or immunotoxic effects on laboratory animals. The concentrations of several of these

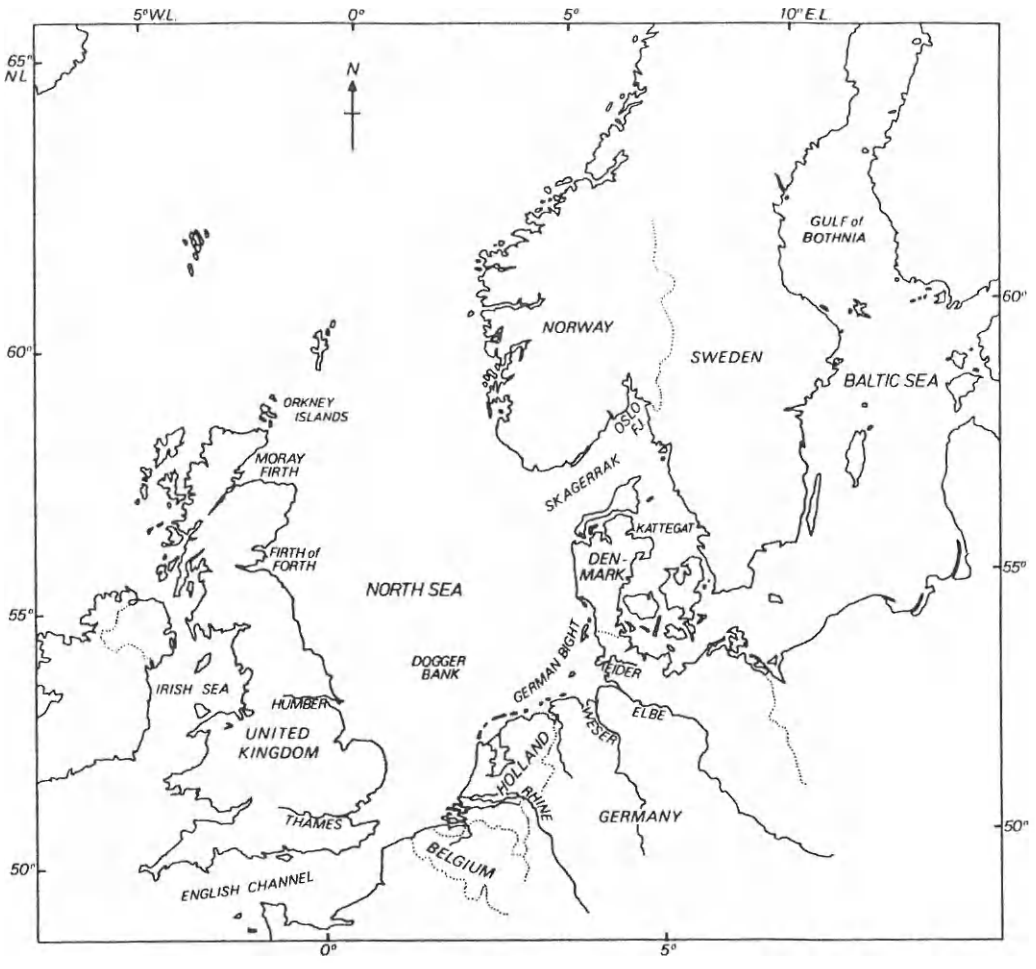

Fig. 1. Map of the North Sea with locations mentioned in the text.

have reached sub-lethal effect levels in estuarine and coastal areas (Dethlefsen and Tiews, 1985).

THE CONCEPT OF A MULTIFACTORIAL AETIOLOGY

Although most diseases were once regarded as having single causes, the multifactorial aetiology of disease is now generally appreciated. The development of disease in an organism has a number of causes, each sometimes classified as direct or indirect, though in practice it is almost always possible to identify intermediate stages in the causal chain. The associations between causes can be viewed in two ways, producing two causal models (Thursfield, 1986).

In the first model, causes are classified into two types: 'sufficient' and 'necessary'. A cause is **sufficient** if, when allowed to operate, it inevitably produces an effect: a sufficient

Table 1. Summary of signs of the important diseases discussed.

Disease name	*Features*	*Main fish species affected*	*Postulated aetiology*
Lymphocystis	White to reddish nodules (1–2 mm in size) or 3-dimensional protuberances on the skin and fins; not infrequently in gills and viscera	Many species, especially flatfish and herring (*Clupea harengus*), the latter in the Baltic	Viral (reovirus); spawning stress; adverse environmental conditions
Spawning papillomatosis	Jelly-like or whitish raised areas on the skin and fins (hyperplasias and papillomas)	Smelt (*Osmerus eperlanus*)	Possibly viral; spawning stress
Pharyngeal granuloma	Silvery white nodules in mouth	Smelt	Detached spines from amphipod prey in mouth tissue
Epidermal hyperplasia/papilloma	Jelly-like or whitish raised areas on the skin and fins (hyperplasias); it is possible that some hyperplasias progress to the papilloma stage	Common dab (*Limanda limanda*), cod (*Gadus morhua*), whiting (*Merlangius merlangus*)	Unknown: possibly viral; heavy metals (Cr); oxygen deficiency
Cauliflower disease	Papillomatous lesions mostly sited near the oral cavity	European eel (*Anguilla anguilla*)	Possibly viral; environmentally dependent
Skin ulcers	Circular lesions, blood-red in the centre and surrounded by a white rim of necrotic tissue	European flounder (*Platichthys flesus*), common dab	mixed aetiology (often associated with bacteria); parasite infestation; net injury; adverse environmental conditions, such as salinity fluctuations, nutrient enrichment, undernourishment and toxicants

Fin rot (fin erosion)	Disintegration of the skin between the fin rays and partial loss of the fin rays	Many species, especially European flounder, perch (*Perca fluviatilis*), ruffe (*Gymnocephalus cernua*)	See skin ulcers
Ulcus syndrome	Dermal papules, ulcerations	Cod	Adenovirus, rhabdovirus; bacterial; nutrient enrichment
Red pest	Red petaechial, cutaneous lesions	European eel	Bacterial; viral; adverse environmental conditions
Spring ulcer disease	Cutaneous ulceration	European eel	Bacterium-associated; cold temperatures; nutrient enrichment
Liver nodules/tumours	(Putative) pre-neoplastic or neoplastic lesions; pre-neoplastic lesions are thought to have a risk of progression to neoplasia	European flounder, common dab, ruffe	Uncertain: probably chemical factors (e.g. PAHs) are involved
Bleaching syndrome	Overall pale appearance; skin may show evidence of necrosis, exposing the muscle as a slimy mass	European flounder, common dab	Unknown
Skeletal deformities	Curvature and compression of jaw, gill operculum and spine	Many species, e.g. cod, fourhorn sculpin (*Myoxocephalus quadricornis*), minnow (*Phoxinus phoxinus*)	Parasites; toxicants; environmentally induced during embryonic and larval stages; impairment of calcium budget
Pseudobranchial tumours	Pseudotumours, single or bilateral on the pseudobranchial organs	Cod	Probably Protista
X-cell disease	Swelling of gill filaments	Common dab	Uncertain: possibly Protista
Glugea stephani	Large white cyst-like pseudotumours in intestine	Flatfish, especially common dab	Protista (*Microspora*)

cause almost always comprises several component causes, and thus disease is multifactorial. A particular disease may be produced by different sufficient causes; if these have a particular component cause in common, it is termed a **necessary** cause. Acting in isolation, however, this necessary cause does not inevitably lead to disease. When diseases are classified by aetiology there is often, by definition, a necessary cause.

The second causal model views direct and indirect causes as a chain of actions, with the latter activating the former. Several factors can act at the same level, and there may be several levels in the hierarchy, producing a web of causation. Again, disease is multifactorial.

The different component causes, or risk factors as they are also termed, can be classified according to the way in which they contribute to disease (Table 2). It should be noted that these factors are frequently assumed or expected to operate but that their operation may not have been demonstrated for particular diseases. Nevertheless, natural variability in disease occurrence is considerable, and epidemiological research must take this into account when attempting to assess the long-term effects of pollution.

The outbreak of many diseases of marine fish depends on the presence of infectious pathogenic organisms such as viruses, bacteria or parasites (Möller and Anders, 1986). But other environmental factors, such as pollution, may be required before the disease can develop. In principle, pollutants may reduce disease resistance by causing physiological stress, or they may increase the number and activity of infectious organisms, causing a higher infection pressure.

Alternatively, pollution-induced stress may lead to damage of a non-infectious nature, such as certain types of tumour, liver or gill abnormalities, and skeletal deformities. The evidence implicating chemicals in the aetiology of some of these conditions is strong, but rarely have infectious agents been discounted as components of their sufficient causes.

General criteria for the use of epidemiological surveys

Fish disease studies can be divided into two major categories based on their principal objectives. The first type addresses the disease and its effects directly, while the second attempts to use disease as an indicator of environmental parameters. The constraints imposed by the different objectives of the two categories are discussed by McVicar (1986).

The present review is concerned only with studies in the latter category, which try to quantify the occurrence of disease (also termed morbidity) and to establish relationships with potential causal factors. As a basic premise, it is supposed that the rate of occurrence of disease in a population (its **incidence**) is related to exposure to pollution of individuals in that population. Field studies then make certain assumptions, two of the most important being that the frequency of a disease in a sample of fish from the population (its **prevalence**) can be used as an indication of its incidence, and that the exposure of fish to pollution can be inferred from measurements of contaminant levels. These assumptions are discussed below.

LIMITATIONS OF EPIDEMIOLOGICAL SURVEYS

Epidemiological research into fish disease is in general far more difficult than in terrestrial animals, particularly as regards access and operation. Some of these difficulties are common to most marine research that requires a vessel, where the sampling environment

Table 2. Some potential factors determining the occurrence of fish diseases.

External environment
Physicochemical and biological factors
Chemical pollutants (heavy metals, organic micropollutants such as PCBs and PAHs) in water, sediment and food
Sediment composition; currents
Temperature, salinity, pH
Oxygen deficiency
Nutrient enrichment (eutrophication)
Bacteria, viruses, other microbes and pluricellular parasites
Algal toxins
Geographic location
Food supply
Fisheries (habitat destruction, net injuries)
Population density
Selection pressure
Competition
Host factors and internal environment
Length/age
Sex
Genetic make-up
Physiological factors (osmotic regulation, growth etc.)
Nutritional condition (dietary patterns)
Movement (spawning or other migrations and local displacement)
Hormonal factors
Immunological factors
Behavioural stress (spawning, feeding, social)
Bioaccumulative capacity (in fatty tissues such as liver)
Processes of detoxification and activation (MFO* enzyme system)
Processes of pathogenesis/carcinogenesis
Temporal factors
Duration and intensity of exposure to disease risks
Latent period of disease
Cohort history

*Mixed-function oxidase (Stegeman and Kloepper-Sams, 1987).

cannot be directly visualized, and where difficulties may be faced in carrying out intricate laboratory techniques (McVicar, 1986).

Special fish disease cruises are very costly, therefore regular epidemiological work often forms part of routine biological surveys (Dethlefsen *et al.*, 1986). Thus there may be restrictions on the choice of the areas and the handling of fish, and a certain loss of control over sampling is often inevitable (McVicar, 1986). Nevertheless, recording of disease during standard stock assessment cruises is potentially useful because the disease

data can then be directly related to other parameters of the population (Egidius, 1983; Banning, 1987; Bucke and Stokes, 1988). Furthermore, Banning (1987) has shown that prevalence values derived from stock assessment cruises agree well with those obtained by special fish disease cruises in the same area.

Another limitation concerns the use of prevalence as a measure of the occurrence of the disease. There is a fundamental distinction between prevalence and incidence (Munro *et al.*, 1983), and it is therefore surprising that some investigators still refer to incidence when prevalence is meant. Incidence measures the rate of occurrence of new cases of disease, while prevalence is a function both of the number of individuals that have become diseased in the past, and of the duration of the signs. Even if the incidence of a disease is low, prevalence may still be high if the signs are long-lasting. Very little information is available on incidences of diseases and on mortality rates and recovery rates; in the absence of this information, the search for links with pollution must be based on prevalence data as an approximation to incidence.

CRITERIA FOR ESTABLISHING CAUSE–EFFECT RELATIONSHIPS

According to Peters *et al.* (1987), several general epidemiological principles can be adapted to fish disease research as follows, the probability that pollution is a cause of disease increasing when two or more of these criteria are fulfilled.

1. There is a correlation between disease prevalence and the distribution of a pollutant within a relatively small area.
2. There are parallel tendencies for changes in disease prevalence and changes in pollution level over a long period of time.
3. The disease occurs regularly in heavily polluted waters, but is seldom if ever encountered in 'clean' waters.
4. Field data are confirmed by long-term experiments.
5. A reduction in the level of pollution results in a decline in the prevalence of disease.
6. Disease prevalence is related to body burdens of pollutants.

Some of these criteria (1, 3, 6) refer to spatial variation in disease prevalence and pollution, others (2, 5, and again 6) to temporal variation. In practice, individual studies may address spatial or temporal variation or both, and may or may not be accompanied by experimental work (4).

Spatial or temporal variation may be inferred from a minimum of two sampling locations or occasions between which disease prevalence differs significantly; if a factor is present at one location or on one occasion, and absent from the other, this is taken as evidence that the factor is causal. Such a comparison (known as the method of difference) suffers from the problem that many factors other than the postulated causal factor may vary similarly. If prevalence is estimated at several locations or on several sampling occasions, a potential causal factor may be found which shows a pattern of continuous variation corresponding to a similar pattern of disease frequency: this (the method of concomitant variation) provides stronger evidence for a causal relationship.

CRITERIA FOR FIELD SURVEYS

In addition to the general limitations described above, specific criteria can be applied to field surveys that aim to relate spatial or temporal patterns in disease prevalence to pollution.

Accuracy and precision of prevalence values

Epidemiology aims to determine the occurrence of disease in a defined study population. In fish disease surveys, the study population is usually the population of a single species inhabiting a given location at a given time. Neither 'location' nor 'time' are formally defined, and the absolute size of the population is frequently unknown. It is usually left to the judgement of the researcher to decide the area fished at each sampling station, while the numbers of tows (and hence the duration of fishing) is often a function of the desired sample size.

The accuracy and precision of prevalence estimates depends on the procedures for sampling and diagnosis as well as on the sample size. Other sources of bias and variability may exist, for example in the analysis of histological samples, but there is no information on their importance in fish disease surveys.

Sampling procedure The catch is a biased sample of the population because the gear is selective and small fish, for example, may not be captured. The effects of mesh size can be accounted for easily by calculating length-specific prevalences, but it has also been suggested that diseased and healthy fish may differ in their catchability, according to the type of gear used (Dethlefsen *et al.*, 1986; Anon., 1989). However, Nielsen and Mellergaard (1984) showed that the use of two different types of rigging on the same trawl had no effect on calculated disease prevalence in plaice (*Pleuronectes platessa*, Pleuronectidae) and common dab (*Limanda limanda*, Pleuronectidae).

There is evidence that disease distribution (as well as host distribution) can be rather patchy, resulting in considerable haul-to-haul variation (Dethlefsen *et al.*, 1984; Nielsen and Mellergaard, 1984). Dethlefsen *et al.* (1984) concluded that acceptable strategies were to carry out multiple sampling at each station, or to sample repeatedly over a number of years; single and unrepeated sampling would give only a very rough estimate of disease prevalence in certain areas. On the other hand, McVicar *et al.* (1988) reported only low haul-to-haul variability in the prevalence of disease affecting several species trawled at locations off Scotland. This subject requires further study (Anon., 1989).

Finally, the catch may need to be subsampled before examination. The subsampling procedure must ensure that diseased individuals are neither more nor less likely than healthy ones to be chosen, whether by virtue of their size or of any other characteristic.

Diagnostic variability Systematic bias in disease diagnosis between observers, arising partly from differences in experience, is known to exist, and the importance of training has been stressed (Dethlefsen *et al.*, 1984, 1986). It has been suggested that supposed long-term increases in disease prevalence could be artefacts arising from increased experience of the investigator, while the validity of comparisons among different authors' data could be doubtful (Möller, 1988).

Random variation due to light conditions, weather, and alertness can obviously occur, even when the same observer is involved. The studies of Dethlefsen *et al.* (1984, 1986) found that a proportion of diseased fish was overlooked during a single inspection; this was higher for lymphocystis than for other more easily detected conditions such as ulcers. Dethlefsen *et al.* (1984) recommended a minimum handling time per fish together with cross-checking procedures to minimize individual differences between observers. More recently, clearly defined criteria ('cut-off' points) for recording certain diseases have been recommended in an attempt to reduce diagnostic variability (Anon., 1989).

Sample size The precision of an estimate of prevalence will improve with increasing sample size. Very large sample sizes may be required to estimate precisely the prevalence of many routinely investigated disease signs that affect only a small proportion of the population. The object of most studies is to detect spatial or temporal differences in prevalence, and therefore statistical guidelines for necessary sample sizes need to incorporate the likely prevalences at each site, the magnitude of the differences one wishes to detect, and the confidence level. In practice, this is seldom done, though some examples for single sites were given by Dethlefsen *et al.* (1986); instead, practical constraints or the judgement of the researcher determine sample sizes. Recommended sample sizes for studies on dab and European flounder (*Platichthys flesus*, Pleuronectidae) in the North Sea area (Anon., 1989) are, for external diseases, a minimum of 100 fish per site in each of two 5 cm length classes, and a minimum of 50 fish per site in a third, larger length class.

Possible causal factors other than pollution

It can be difficult in fish disease studies to eliminate causal factors other than pollution that might equally well produce the observed pattern of disease frequency. Conversely, other (natural) causal factors could obscure weak relationships between disease and pollution. It is unrealistic to attempt to measure all potential causal variables (Table 2) during the course of any study. Instead, an a priori selection of potentially important factors is often made; in addition the design of the sampling programme may ensure that certain factors do not vary.

Measurement of important factors This involves an initial choice – based on experience and on the results of previous studies – of factors for direct measurement. Data analysis can then examine their possible effects. Both host and environmental factors (Table 2) may be measured.

It has been recognized for some time that variation in disease frequency with host size and sex can be great, and that they should be recorded routinely in all studies (Anon., 1989). Other frequently measured host characteristics include population density, nutritional or reproductive status, and age. It has been suggested that age may be a better predictor of the prevalence of some diseases than size (Mellergaard and Nielsen, 1985), and thus differences in age–length relationships could explain some observed spatial differences in disease prevalence (Anon., 1989; Vethaak and Meer, 1991). However, the production of length–age keys is labour intensive and costly, and so far has seldom been attempted in fish disease studies.

Commonly measured environmental and host factors include salinity, water temperature, sediment composition, food supply, condition factor, and net injuries, used by some authors as an index of fishing activities. A provisional list of variables that should be measured in disease studies is given by Anon. (1989).

Design of the sampling programme Most sampling programmes recognize, implicitly or explicitly, the possible influence of certain factors on disease prevalence. Most obviously, sampling is usually limited to selected diseases and host species, the data for which are analyzed separately because fish species differ in their susceptibility to different diseases.

The prevalence of many diseases is known to vary consistently from season to season, with the result that interannual comparisons may be restricted to samples taken at the

same time of year (Dethlefsen *et al.*, 1984; Vethaak, 1985). In studies lasting many years, therefore, sampling should be carried out during a limited period in the same season each year.

It has also been suggested that prevalences may differ in different genetic stocks even if all environmental factors are constant, invalidating direct comparisons of prevalence values over large distances. A corollary of this notion is that, in theory at least, a population with a low 'background' prevalence of disease may be affected by pollution and yet still show a prevalence lower than that in fish from a heavily diseased 'clean' area (McVicar, 1986; McVicar *et al.*, 1988).

More generally, it has been recommended that sampling locations should be chosen to resemble each other as much as possible in terms of biological, chemical and physical parameters that may potentially influence disease prevalence (Dethlefsen *et al.*, 1986; McVicar *et al.*, 1988; Anon., 1989). This presupposes, however, the availability of a considerable amount of information about the sampling locations.

Measurement of exposure of the study population to pollution In certain cases, for example near known point sources of pollution, distance from the pollution source may be used as an indirect index of exposure. Nevertheless, it is clearly desirable to make direct measurements of pollution in any study of fish disease. This practice allows assumptions about exposure to pollutants at the capture location to be verified.

Measurements aimed at quantifying the degree of pollution may be made on the tissues of the fish or, in principle, on the water column, sediments, or other biota. The variables to be measured will depend on the nature of the important sources of pollution: analyses of heavy metals and organic contaminants are frequently included in the list. The concentration of these substances is usually interpreted as a general index of exposure to pollutants rather than as a direct indication of causation by specific toxic chemicals. Recently, more elaborate indicators (termed 'biomarkers') are being used to measure the degree of exposure to, or the effects of, specific xenobiotics: examples are the mixed-function oxidase (MFO) system of the liver (Stegeman and Kloepper-Sams, 1987), the concentration of specific metabolites in the bile (Krahn *et al.*, 1986), the formation of DNA adduct in hepatic cells (Varanasi *et al.*, 1986), and lysosomal stability of hepatic cells (Köhler, 1989).

Few studies have attempted to relate the concentrations of contaminants in individual fish to the presence or absence of disease in those individuals; instead, mean levels in the populations are calculated along with population estimates of disease prevalence. Exceptions include the studies of Dethlefsen *et al.* (1987), Lang and Dethlefsen (1987), Newell *et al.* (1979) and Stork (1983).

The measurement of contaminant levels in tissues of fish from the study population has the major advantages of allowing a direct correlation with the effect (in this case, disease) and of providing a check on the effects of migration. Otherwise, it is necessary to assume that fish captured at a certain location have in fact been exposed to conditions at the location for some time prior to capture. The implications of migration are discussed in more detail in the next section.

Certain chemical pollutants are easier to detect in sediments or in food organisms than by direct measurements in fish tissues: polyaromatic hydrocarbons (PAHs) provide a good example (Malins *et al.*, 1987, 1988). Measurements on sediments or food organisms also provide a direct indication of the environmental concentrations to which the fish are

exposed, without the interfering effects of regulation or other processes. Furthermore, the presence of disease might in itself affect the accumulation of contaminants by fish, for example by impairing the function of the liver. A case can therefore be made for a combination of the above approaches to measuring pollution.

Selection of potentially suitable host species and diseases

Fish species potentially useful in studies of disease and pollution should have the following characteristics.

1. They should be susceptible to diseases whose signs are easily recognized.
2. They should be abundant so that large samples are easily obtainable.
3. A geographically limited distribution of each population is important so that conditions at specific sampling locations can be related to the health of the fish; consequently non-migratory or less migratory host species are the most suitable.
4. Since contaminants are frequently associated with sediments, demersal fish have more potential as indicators than pelagic species.

These characteristics are shown by a number of flatfish species, which are regularly used in disease studies on both sides of the Atlantic. In the North Sea area, dab and flounder have been recommended as indicators (Anon., 1989). Both these species are widely distributed, the latter being confined largely to estuarine and coastal waters. Nevertheless, flounder undertake offshore migrations in order to spawn; individuals are thought to return to the same feeding areas year after year (Veen, 1971). Although dab were previously thought to undergo only restricted migratory movements, recent tagging studies carried out in Dutch and German waters indicate that the species may migrate over considerable distances, and therefore its value as a suitable indicator species should be re-evaluated (A. Rijnsdorp, RIVO, Netherlands, pers. comm.; Vethaak and Meer, 1991).

Because most commercial stocks are migratory, the best potential target species for studying the effects of pollution are of little commercial value; in consequence, knowledge about their biology can be relatively scanty.

Recommendations for the selection of diseases for study in the North Sea area are given by Dethlefsen *et al.* (1986) and Anon. (1989). Ease and reliability of diagnosis are the most important factors, together with the occurrence of sufficiently high prevalences to allow the detection of spatial or temporal variation in samples of limited size.

General overview of research on fish disease in relation to pollution

The first surveys in which fish diseases were examined systematically and intensively were carried out in the 1960s. Despite the lack of quantitative data, however, it is clear that diseases now occurring in North Sea fish populations were already in existence several decades ago, and some observations even date from the last century (Dethlefsen, 1984; Bucke, 1988; Heron *et al.*, 1988). In the German Bight, all the main diseases now studied except liver tumours were recorded as far back as the beginning of the present century (Watermann and Kranz, 1990); these authors claim, however, that the occurrence of restricted areas of high disease prevalence in the open North Sea is a recent phenomenon caused by anthropogenic influences.

The possibility that pollution might be among the factors in the aetiology of fish

disease first gained interest in the United States in the 1960s, where the discovery of high prevalences of tumours in fish led to attempts to correlate their presence with chemical pollution. The earlier studies concentrated on tumours (of skin and liver) and on fin rot.

Fin rot has been the subject of several local studies in the US, in the New York Bight (Mahoney *et al.*, 1973; Murchelano and Ziskowski, 1982; Sherwood, 1982), Washington (Wellings *et al.*, 1976) and along the coast of southern California (Mearns and Sherwood, 1974; Sherwood and Mearns, 1977; Cross, 1985). These studies are good examples of a disease statistically associated with environmental degradation, implicating pollution as a causative factor, but whose aetiology is still unknown.

Many American studies have specifically addressed the phenomenon of neoplastic or cancerous diseases in fish populations, and their association with chemical contaminants in coastal, estuarine and freshwater environments. Mix (1986), in a thorough critical review, identified only a small number of studies whose data were considered to support an association between pollution and neoplasia: Brown *et al.* (1977) on several fish species in the Fox River, Illinois; Kimura *et al.* (1984) on croaker (*Nivea mitsurusii*, Sciaenidae) in Japan; and Malins (see below) on demersal fish in the Puget Sound, Washington. The negative or ambiguous findings of other studies were largely explicable in terms of inadequate survey design and data collection, but in a few cases appeared to be genuine.

Since then, the studies of Malins and co-workers have been continued and expanded, and are worth describing in more detail, as they are generally regarded as the most thorough and comprehensive to have been published. Strong evidence has been produced by this research group to link the presence of liver tumours with chemical contaminants in English sole (*Parophrys vetula*, Pleuronectidae) and other species: the bulk of this evidence suggests a significant role of high-molecular-weight PAHs in the carcinogenesis. It can be summarized as follows:

1. statistical correlations between prevalences of hepatic neoplasms and concentrations of aromatic hydrocarbons in the sediment of different areas (Malins *et al.*, 1985);
2. the capacity of the affected species for uptake and transformation of potentially carcinogenic xenobiotics (Varanasi *et al.*, 1986);
3. correlations between disease prevalences and the levels of certain metabolites of aromatic compounds in the bile of the fish (Krahn *et al.*, 1986);
4. binding of aromatic free radicals to hepatic DNA in English sole from polluted waters, but not in those from reference areas (Varanasi *et al.*, 1986);
5. a similarity between the morphological characteristics of the observed lesions and those of lesions induced in other fish species and in rats by chemical hepatotoxins and hepatocarcinogens in controlled laboratory exposures (Myers *et al.*, 1987).

Other recent American and Canadian studies, though not as comprehensive as these, have attempted to link histopathologically identified lesions in fish to chemical contaminants in coastal and estuarine areas; the following examples provide additional evidence for a causal relationship between pollution and disease.

Epizootic neoplasia in winter flounder (*Pseudopleuronectes americanus*, Pleuronectidae) in Boston Harbour was first described by Murchelano and Wolke (1985); fish from unpolluted sites were not found to have these lesions. Zdanowicz *et al.* (1986) described a variety of histopathological disorders in liver and kidney of winter flounder from 14 estuaries and coastal areas in the north-eastern US. The distribution of some but not all

of these conditions paralleled the distribution of sediment contaminants. In a semi-experimental study, Gardner and Yevich (1988) found that contaminated sediment from Black Rock harbour (Connecticut) induced an array of proliferative lesions in winter flounder and other species.

Huggett *et al.* (1987) found that fish of several species inhabiting the Elizabeth River (Virginia), which is highly contaminated with PAHs, exhibited abnormalities such as cataracts and fin and skin erosion. Later studies showed that the prevalence of these lesions was highest in the most heavily contaminated regions of the river (Bender *et al.*, 1988).

High prevalences (59%) of idiopathic liver lesions, including preneoplastic and neoplastic stages, were found in English sole from an area in Vancouver Harbour (British Columbia) receiving petroleum refinery waste water and other pollutants, with lower prevalences in the surrounding areas (Goyette *et al.*, 1988).

In contrast to the above studies, which are largely histopathologically oriented, fish disease studies in the North Sea area became incorporated into stock assessment cruises at an early stage, probably because of the widespread occurrence and easily visible signs of many of the major epidermal diseases, such as lymphocystis and ulceration. Large-scale surveys which were first carried out in the late 1970s concentrated on these externally visible gross lesions, using an epidemiological approach; by comparison with American studies, they were more superficial in terms of examining the underlying mechanisms, but involved large numbers of fish over wide areas. Although the distinction is not absolute, with epidemiological studies such as those of Couch (1985), McCain *et al.* (1978, 1979) and Ziskowski *et al.* (1987) in America focusing on externally visible gross lesions, it is certainly true that more thorough histopathological and biochemical studies are, with few exceptions, a very recent development in the North Sea area.

Early North Sea surveys concentrated on the German Bight and on Danish and Dutch coastal waters. Since then they have been refined and extended to cover a larger area of the North Sea and associated waters such as the Baltic and Irish Seas. Of particular significance are the extensive baseline studies conducted by Dutch (Banning, 1987) and German (Dethlefsen *et al.*, 1987; Dethlefsen, 1990) workers, and still continuing. More recently, UK workers have commenced a similar programme of studies (Bucke and Stokes, 1988). A comprehensive and specific study on the relationship between fish disease and pollution was initiated in 1983, also by the Dutch (Vethaak, 1985, 1987; Vethaak and Meer, 1991). At the same time, two sea-going workshops organized by ICES (International Council for the Exploration of the Sea) in 1984 (Dethlefsen *et al.*, 1986) and 1988 (Anon., 1989) have addressed the methodology of fish disease surveys and have produced recommendations.

The North Sea is taken as a case study for detailed description and evaluation in further sections of the present review. For previous reviews of the literature, some covering the North Sea area alone, the reader is referred to Bucke and Watermann (1988), Dethlefsen (1988), Malins *et al.* (1988), Mix (1986), Peters (1981), Sindermann (1984, 1989), and Sindermann *et al.* (1980).

Recent epidemiological studies in the North Sea and associated waters

SURVEYS OVER WIDE AREAS

In one of the earliest published studies, Kamp (1977) found that the prevalence of vertebral abnormalities in herring (*Clupea harengus*, Clupeidae) from the North Sea and

NE Atlantic was highest, and had increased slightly over time, in areas where pollution was presumed to be greatest.

Möller (1979), in a review of published literature and of his own data from the southern North Sea in 1977–78, found the principal diseases of dab to be lymphocystis, epidermal hyperplasia/papilloma and ulceration. He concluded that high prevalences of ulcers and lymphocystis in dab in the central North Sea arose from malnutrition, and that pollution might favour the increase of ulcers in cod (*Gadus morhua*, Gadidae) in the Belt Sea (Kattegat), of cauliflower disease in European eels (Anguilla anguilla, Anguillidae) in the Elbe, and of fin rot in dab from the sewage sludge disposal site in the German Bight.

Dethlefsen and co-workers examined a total of 230000 dab from the German Bight and the southern North Sea between 1979 and 1986 (Dethlefsen *et al.*, 1987), in the most comprehensive attempt to date at relating disease prevalence to pollution in the North Sea. Data on dab and cod diseases from the German Bight between 1977 and 1979 were analysed by Dethlefsen (1980), and data for the period 1980–83 by Dethlefsen (1984), while Wolthaus (1984) investigated seasonal fluctuations of disease frequencies of dab in the German Bight. Dethlefsen *et al.* (1987) presented detailed data on dab diseases, and Dethlefsen (1990) briefly summarized this work together with more recent findings.

Initial surveys concentrated on the German Bight, but they were later extended, with an emphasis on lymphocystis, ulcers and epidermal hyperplasia/papilloma in dab. The value of these studies, apart from their extensive nature, lies in their multidisciplinary approach, including measurements of length, age, population density, condition, prey density, the presence of net injuries, and levels of chemical residues in the tissues of dab and in food organisms.

Consistently elevated prevalences of epidermal hyperplasia/papilloma in dab from an area of the German Bight receiving titanium dioxide wastes were interpreted as circumstantial evidence for a causal link (Dethlefsen, 1980, 1984). This view was supported by a correlation between disease prevalence and high concentrations of heavy metals (including chromium, a potential carcinogen) in fish tissues, sediments, and the water column. This evidence has subsequently been the subject of some debate (Möller, 1981; Möller and Anders, 1986; Dethlefsen *et al.*, 1987).

Areas with even higher prevalences of epidermal hyperplasia/papilloma were found off the Humber estuary and on the Dogger Bank (Fig. 1), and an effect of the discharge of heavy metals from sources located along the British coast was suggested. Data on condition factor suggested a possible effect of low food availability on disease prevalence in Dogger Bank dab, but Dethlefsen *et al.* (1987) found no correlation between nutritional status and disease prevalence on a local scale, within the Dogger Bank data set alone. More recent studies on heavy metals and organic residues in liver tissues showed that dab from offshore areas such as the Dogger Bank can be contaminated to an equal or greater degree than those from inshore sites (Büther, 1988; Claußen, 1988).

Dethlefsen (1990) suggested that in certain areas, low dissolved oxygen concentrations could lead to an increase in disease prevalence. Also of interest was the finding that individuals with net injuries were more likely than uninjured fish to show signs of lymphocystis, suggesting that fishing activities could contribute to the high prevalence of this disease in dab from the German Bight (Dethlefsen *et al.*, 1987; Dethlefsen 1990).

In a recent summary of his work, Dethlefsen (1990) concluded that no single underlying cause would explain the spatial pattern of dab epidermal disease over the

whole area studied, but that oxygen deficiency, polychlorinated biphenyls (PCBs) and heavy metals could each be implicated in different specific areas. No consistent long-term temporal trends in disease prevalence over a 10 year period could be identified.

The same group has also investigated disease in cod and other species. Pseudobranchial tumours in cod sampled between 1979 and 1981 occurred at highest prevalences in the central German Bight, coinciding with the titanium dioxide dumping area (Watermann *et al.*, 1982). Marked seasonal and regional differences in the prevalence of skeletal deformities in North Sea cod were found, with highest values in the inner German Bight (Dethlefsen and Lang, 1988). It has been suggested that the majority of fish with this disease stay behind when the population migrates seasonally from the Bight into deeper water, thus explaining the high prevalences (Möller, 1985; Dethlefsen and Lang, 1988).

Watermann and Dethlefsen (1985) described the distribution of epidermal hyperplasia in cod, European whiting (*Merlangius merlangus*, Gadidae) and Atlantic haddock (*Melanogrammus aeglefinus*, Gadidae) in the North Sea and Baltic Sea between 1979 and 1982. Prevalences were highest in whiting, but did not exceed the very low value of 0.15% at any location: an association with other epidermal diseases, and thus possibly with pollution in the German Bight, was discussed in general terms.

General data on the prevalence of selected diseases of dab, plaice and cod in the south-eastern North Sea from 1981 to 1985 (Banning, 1987) illustrate clearly that the presence and prevalence of diseases vary strongly as a function of fish species, area and season. No clear temporal trends were detected. Areas of high disease prevalence (defined as more than 1.5 times the average) were found on the Dogger Bank and near the Danish coast; effects of pollution were not studied, however.

An extensive survey of dab diseases at 52 stations in the central and southern North Sea was carried out in combination with a UK stock assessment cruise in 1987 (Bucke and Stokes, 1988). Skin ulcerations were the predominant lesions, followed by lymphocystis; signs of epidermal hyperplasia/papilloma were infrequent at that time of year. In the Firth of Forth, off the Humber estuary, and on the Dogger Bank, over 25% of fish were found to be affected by one or other of the above diseases, but the authors did not attempt to draw conclusions about the role of pollution in influencing disease prevalence.

The occurrence of a thickening of dab gills, arising from the presence of X-cells, was first discovered in the North Sea during routine monitoring surveys in the early 1980s (Knust and Dethlefsen, 1986; McVicar *et al.*, 1987). An initial study found highest prevalences in the Dogger Bank area, but interhaul variability was high, and the presence of this disease did not appear to be related to that of the other epidermal diseases recorded (McVicar *et al.*, 1987). A more recent extensive survey involved the examination of more than 13000 individual dab: prevalences of X-cells as high as 60% were found in the Moray Firth, but prevalence was low in the southern North Sea, and the condition was not found at all in dab from the west coast of Scotland and the Irish Sea. Of particular interest was the finding that prevalence could vary between 0% and 60% in samples taken from hauls in the same local area (Diamant and McVicar, 1989).

The first attempt to investigate internal organs of flatfish in the North Sea was made during two cruises in 1981, and a single cruise in 1984, covering the German Bight, Dogger Bank, Humber and Thames areas (Bucke *et al.*, 1984). In this baseline histological study of 1400 dab livers and 540 dab spleens, the most significant histopathological changes were melanin deposits, hepatic granulomas, and hepatic hyper-

chromic nodules. Their prevalences were higher in the Dogger Bank area than elsewhere in the southern North Sea. Bucke *et al.* (1984) considered that the basophilic nodules might represent 'stages of early hepatoma', but that in some livers they could well be regenerating areas of hepatocytes replacing areas of focal necrosis.

In the later survey of Bucke and Stokes (1988), neoplastic liver nodules were diagnosed in 23 of 520 large dab examined, and seemed more frequent in fish from the southern part of the study area.

GERMAN, DANISH AND NORWEGIAN STUDIES IN RESTRICTED AREAS

Möller (1981) carried out a survey of epidermal disease in 10 fish species in Danish and German coastal waters in summer 1980, paying particular attention to the Elbe and Weser estuaries and the German Bight titanium dioxide dumping area. High disease prevalences in cod from the central German Bight were attributed to a high population density of fish. In dab, disease prevalence was inversely related to condition factor, and was locally high off south-western Denmark, in the Kattegat and at a reference station on the Dogger Bank. Pollution was not considered to be a significant causal factor, except possibly in the Weser and Elbe.

An extensive case study of several diseases in fish from the Elbe was later carried out by Möller (1984). Disease was most frequent in European smelt (*Osmerus eperlanus*, Osmeridae), eel and flounder. The most frequent diseases in smelt were pharyngeal granuloma, spawning papillomatosis, and skeletal deformities. Eels were affected principally by cauliflower disease, while flounder were found to have lymphocystis, ulceration, bleaching syndrome, and fin rot, the prevalence of the first three conditions increasing markedly with fish size. It was noted that cauliflower disease had been recorded at comparable levels in eels from a clean UK estuary by Hussein and Mills (1982); historical data from the Elbe showed no long-term increase in prevalence of this disease.

The prevalence of most of the above diseases, particularly those affecting flounder, was highest at intermediate stations in the estuary even though pollution levels increased inwards to Hamburg. The major factor triggering disease was considered to be fluctuating salinity in the central estuary, which led, via decreased production and hence a reduced food supply, to starvation of benthic feeders and an associated increase in susceptibility to disease. Further work in 1984–86 revealed that benthic biomass together with condition factor and stomach fullness of flounder were lowest in the central estuary. This confirmed that undernourished flounder are more susceptible to disease, although the possible existence of other relationships was acknowledged, e.g. a detrimental effect of disease on feeding behaviour, or the depletion of prey populations and the spread of infectious disease as consequences of high fish population density (Möller, 1990).

Also in the Elbe, a comprehensive series of studies on liver pathology in smelt and ruffe (*Gymnocephalus cernua*, Percidae) and especially flounder has been carried out, using the neighbouring Eider as a reference area (Köhler and Hölzel, 1980; Kranz and Peters, 1985; Köhler, 1989). Peters *et al.* (1987), in a summary of the work, put forward a strong case for a causal relationship between disease and the high level of pollution, most significantly by chlorinated hydrocarbons, whose concentrations were considered to be sufficient to induce the diseases observed. Data from the period 1980–83 revealed that major liver damage, involving liver cell shrinkage and other degenerative changes, was confined almost exclusively to fish from the polluted river.

Neoplastic liver nodules were found in 32% of sexually mature ruffe from the Elbe, though they did not occur in flounder; unfortunately, no comparison was made with the reference area in this case.

Peters *et al.* (1987) suggested that in the absence of unfavourable natural factors, such as poor nutrition, liver degeneration occurs mostly in heavily polluted waters. Such an association was supported by experimental studies on flounder, which showed signs of liver regeneration when kept in clean water and fed a contaminant-free diet, so that tissue burdens of chlorinated hydrocarbons fell (Köhler, 1989).

Stork (1983), in a study of ulcerated cod in the German Bight, found that concentrations of PCBs were higher in the tissues of diseased than healthy individuals, and suggested that the ulcers might have been caused by pollution.

The studies of Mellergaard and Nielsen (1985, 1987) in the area of the eastern North Sea, the Skagerrak and the Kattegat, showed an increase in the prevalence of lymphocystis and epidermal hyperplasia/papilloma in dab following a major period of oxygen deficiency during summer 1982. An affected cohort of 2-year-old fish was followed in the population for 4 years, indicating that the effects were long-lasting. A similar period of oxygen deficiency in 1986 was accompanied by a twofold increase in disease prevalence (Anon., 1989). These studies provide strong evidence that oxygen deficiency is a causal factor for disease, either (a) by reducing disease resistance or increasing infection pressure directly, or (b) via indirect effects such as depletion of food resources (Anon., 1989).

Jensen (1983) examined data on ulcus syndrome in 26000 cod collected between 1976 and 1979 from four localities along the Danish coast, three of which were contaminated by discharges of industrial waste water containing carbohydrates. At these sites, 23% of individuals were affected, as compared with only 3 of 8500 individuals ($< 0.1\%$) from the reference locality. The data were considered to provide circumstantial evidence for a role of pollution, but the possible involvement of other factors was also discussed (Jensen, 1983). The value of this study is limited by the use of only one single reference site, which may have differed from the others in a number of (unknown) ways. Prevalences declined from 25% to about 5% in 1984. However, it was considered doubtful whether this decline could be ascribed exclusively to a decrease in pollution; instead, a disturbance of recruitment was thought to have led to a reduction in catches of fish of the age range (1.5–2.5 years) that normally shows signs of the syndrome (Bro-Rasmussen and Løkke, 1984).

Spring ulcer disease in eels, showing highest prevalences at low temperatures and in fresh or brackish waters, was studied in Danish fjords by Jensen *et al.* (1983). Important environmental factors in the triggering and development of the disease were considered to include eutrophication, possibly due to sewage and waste water.

Reiersen and Fugelli (1984) examined seasonal and year-to-year variation in lymphocystis affecting flounder from the Oslofjord, Norway, between 1978 and 1982. Although the prevalences (up to 57%) were considered high by the authors, no firm conclusion was drawn regarding the existence of a link between pollution and disease.

DUTCH AND BELGIAN COASTAL WATERS

A 5 year multidisciplinary study of internal and external lesions in flounder and dab was carried out from 1983 to 1987 at several coastal and offshore locations in the Netherlands (Vethaak, 1985, 1987, 1991; Vethaak and Meer 1991). Emphasis was placed on the use

of flounder as a local indicator species in estuaries and coastal waters (Vethaak, 1987). Epidermal hyperplasia/papilloma (dab only), lymphocystis, ulcerations, fin rot, skeletal deformities, *Glugea* infection and liver nodules were the diseases recorded. Preliminary results showed that the prevalences were significantly influenced, not only by season and length or age of the fish, but also by the sex of the fish, an effect which had been ignored in many previous studies. The prevalence of different flounder diseases varied with the degree of salinity (Vethaak, 1991).

Locally, up to 40% of dab and flounder aged 3 years or more were affected with grossly observable liver nodules of a diameter larger than 2 mm, corresponding to histologically identified pre-neoplastic and neoplastic disorders (Vethaak, 1987). The prevalence of these lesions in flounder was correlated with the concentration of PAHs in sediment samples, though not with that of PCBs in the liver tissues of the fish themselves (Marquenie and Vethaak, 1989).

Turning to epidermal diseases in flounder, Vethaak (1987) suggested that pollution is a contributing factor in the development of lymphocystis, skin ulcers and fin rot. Particularly high prevalences of skin ulcers and fin rot were encountered in the south-western Wadden Sea, between 20% and 50% of individuals being affected at some localities, in contrast with much lower prevalences in the eastern Wadden Sea. Those localities with highest prevalences corresponded to discharges of fresh water, though in the freshwater bodies behind the sluices, disease prevalences were very low. The condition factor of affected fish was very poor, with considerable mortality. It was suggested that bacterial infection, osmotic stress, and anaerobic sediments, along with crowding and nutritional deficiencies, could all be involved in disease causation (Vethaak, 1991).

Vethaak and Meer (1991) sampled 6000 dab between 1986 and 1988 at five coastal and offshore locations in relation to the dumping of waste from titanium dioxide production. The most interesting finding was a consistently higher prevalence of epidermal hyperplasia/papilloma at two sites (the dumping ground and an inshore site influenced by direct river discharge) compared with three other 'reference' sites. Disease prevalence was not, however, significantly related to heavy metals associated with dumping. The authors concluded that even though disease prevalence could well reflect pollution, interpretation of a causal link with a specific waste disposal operation was complicated by possible local migratory movements, interference from polluted riverine inputs, and long-distance dispersion of dumped wastes.

Banning *et al.* (1984) collected chemical data from sediments, the water column, and the tissues of diseased and healthy eels from three sites in the Western Scheldt area (Netherlands/Belgium) in 1981–2. Prevalences of the prominent disease, red pest, varied between 25% and 82% at one of the two Western Scheldt sites, but were only 4–9% at the other Western Scheldt site and the reference site in the enclosed Lake Grevelingen. The authors stated that their findings failed to show conclusively a relationship between pollution and the disease. However, a possible indirect relationship was suggested by the presence of bacteria in the blood of 80% of eels in the polluted areas but only 4% at the reference site.

UK EAST COAST AND ENGLISH CHANNEL

Following a preliminary investigation of six fish species in Scottish waters in 1982 (Wootten *et al.*, 1982), more detailed studies in relation to sewage sludge disposal in these waters commenced in 1987 (McVicar *et al.*, 1988). A number of species, including

dab, were examined for disease at the Bell Rock and St Abbs Head disposal sites in the Forth estuary. Two reference areas were chosen, each situated within half a nautical mile of the disposal site study area, but at right angles to the main current direction; another more distant reference site was situated near the Orkney Islands. Significant differences in disease levels were found, even between sites close together; prevalences were higher at the reference areas than at the disposal sites. This survey was repeated in 1988, and yielded similar results (A.H. McVicar, pers. comm.).

Bucke *et al.* (1983b) investigated diseases in six fish species, including flounder and dab, from the sewage sludge dumping area in the outer Thames estuary and from several reference areas in spring 1980. No clear spatial trends in disease prevalence were detected, with the exception of lymphocystis in dab, which was most frequent in one of the control areas. There was considered to be no evidence for a causal relationship between sludge dumping in this area and the particular diseases examined (Bucke *et al.*, 1983a). More recent studies in the same area have drawn a similar conclusion, but detailed results have not been published (Heap *et al.*, 1991).

Newell *et al.* (1979) found similar prevalences of skin necroses in plaice, dab, flounder and Dover sole (*Solea vulgaris*, Soleidae) from a non-industrialized area (Brixham, England) and an industrialized area (Calais, France) in August 1977. Based on this finding, and on an analysis of metal concentrations in tissues of fish from the two areas, the authors concluded that there was no evidence to support an association between pollution and the occurrence of skin necroses.

The crude oil spill resulting from the wreck of the *Amoco Cadiz* in March 1978 provided the impetus for an assessment of the health and rate of recovery of plaice in two heavily oiled estuaries in Britanny during 1978–80. In a total sample of 132 plaice, the predominant epidermal lesions were necrosis of the fin and tail, which occurred at prevalences between 60% and 100% in both estuaries on all sampling occasions except the first (December 1978). Prevalences in plaice from a reference site were only 10–20%. A wide range of histologically identified lesions of gill, liver and other organs were also recorded, and their occurrence was interpreted as evidence suggesting chronic exposure of the fish to petroleum hydrocarbons, which were found in the tissues of oysters from the affected area (Haensly *et al.*, 1982). Disruption of the ovarian cycle of plaice was also reported (Stott *et al.*, 1983).

IRISH SEA

Early studies of epidermal disease in plaice, dab, Dover sole and flounder from the Irish Sea produced little evidence for spatial variability in prevalence (Perkins *et al.*, 1972; Shelton and Wilson, 1973; McArdle *et al.*, 1982). Perkins *et al.* (1972) suggested an association between increases in epidermal disease and the presence of PCBs in some areas, but this interpretation was contested by Shelton and Wilson (1973).

Bucke *et al.* (1983a) examined over 13000 individuals of seven species captured at various locations, including the Liverpool Bay sewage sludge dumping ground, in 1982. No evidence was found to suggest that fish captured near the dumping ground showed higher disease prevalences than those from other locations, but the authors conceded that their spatial comparisons were of limited validity because factors such as fish size were not taken into account.

A more adequately designed study in the Irish Sea was carried out in 1986; this included a histopathological study of selected organs of dab and plaice as well as gross

external anomalies (Bucke and Nicholson, 1987). The prevalence of lymphocystis and epidermal hyperplasia/papilloma in dab, and of lymphocystis and ulcers in plaice, showed significant spatial variation, but that of ulcers in dab did not. Of the organs studied – liver, spleen, kidney, gill – only the liver showed histological changes of possible pathological significance in more than a very few individual fish. However, there was no histological evidence of liver nodules in either species, unlike the 1982 sample where nodules were found in 2 of 208 dab livers examined (Bucke *et al.*, 1983a). The possibility that outbreaks of infectious diseases could be associated with high contaminant levels was not discounted, but no data on body burdens of contaminants were presented; an association of disease with net injuries was also proposed. Finally, it was suggested that there could have been a long-term increase in disease prevalence in the area (Bucke and Nicholson, 1987).

BALTIC SEA

Möller (1979) reviewed published work and his own surveys of fish disease in the south-western Baltic and concluded that no significant effects of pollution could be detected.

Dethlefsen and Watermann (1982) described disease prevalences in cod, flounder, and other species from the SW Baltic in 1978–82. An association between pollution and the prevalence of skeletal deformities, ulcus syndrome and pseudobranchial tumours in cod, and of lymphocystis in flounder, was postulated on the basis of the spatial patterns observed.

Lang and Dethlefsen (1987) determined cadmium concentrations in kidney and liver tissues of cod from the SW Baltic. The prevalence of skeletal deformities was highest in the central part of the study area, whereas mean cadmium concentrations were highest at its eastern end. However, deformed cod had slightly, but significantly, higher cadmium concentrations than healthy individuals, suggesting a possible connection between accumulation of this metal and the occurrence of deformities.

Studies on skeletal deformities in fish from the Swedish coast of the Baltic have been conducted since 1971 by Bengtsson and co-workers. Early work comprised experimental studies in which minnows (*Phoxinus phoxinus*, Cyprinidae) were exposed to various concentrations of metals and developed vertebral abnormalities (Bengtsson, 1974, 1975, 1979). Between 1978 and 1984, Bengtsson *et al.* (1988) examined vertebral defects in fourhorn sculpin (*Myoxocephalus quadricornis*, Cottidae) along the Swedish coast (Gulf of Bothnia). Prevalences varied between 4% and 40%, the highest values being found in the vicinity of a metal ore smeltery. Field data were supported by experimental results indicating that long-term exposure of the fish to simulated smeltery effluent produced similar deformities (Bengtsson and Larsen, 1986). Similarly, fourhorn sculpin showed elevated levels of vertebral deformities in areas polluted by bleached kraft mill effluents, and laboratory studies again confirmed field data (Bengtsson, 1988). However, prevalences were also high (reaching 23%) at some sites known to be relatively unpolluted (Bengtsson *et al.*, 1985).

In a study of fin erosion in perch (*Perca fluviatilis*, Percidae) and ruffe near an effluent discharge from a Swedish pulp mill, Lindesjöö and Thulin (1990) demonstrated a clear gradient in prevalence along a line of stations leading from the discharge to cleaner waters, and prevalences later decreased as the quality of the effluent improved. Furthermore, northern pike (*Esox lucius*, Esocidae) from the polluted area showed both fin erosion and jaw deformities, and perch exhibited deformation of the opercula. Both

types of skeletal deformity were more prevalent in the vicinity of the pulp mill (Thulin *et al.*, 1988, 1989).

Evaluation and discussion

EVALUATION OF NORTH SEA STUDIES

Most published data on fish disease in the North Sea up to about 1980 make no reference even to basic variables such as the length and sex of the fish. Many different fish species were frequently sampled, irrespective of their likely value as indicators. Despite the limited comparative value of the data, these studies provided the impetus for the development of a more adequate survey methodology. More recent studies tend to record length and sex as a matter of course, thus ensuring that differences in length distribution and sex ratios between samples will not obscure spatial and temporal patterns. Unfortunately, exceptions can still be found, such as the extensive study by Ziskowski *et al.* (1987) on external diseases in commercial species along the east coast of North America.

More recent studies also exhibit an increasing tendency to measure other environmental factors and contaminant concentrations. The most thorough published examples are those of Möller (1984, 1990) and of Dethlefsen *et al.* (1987).

The above are general and welcome trends. Turning to specific studies, there are a number of cases where an association between pollution and disease seems to be strongly supported by the evidence.

1. Fin erosion and skeletal deformities in perch, ruffe and pike caused by Swedish pulp mill effluents (p. 21): important features of this work are the existence of a clear pollution gradient in association with a gradient of prevalence, and the decline in prevalence as pollution has lessened.
2. Vertebral anomalies in minnows and fourhorn sculpin caused by Swedish mill and smeltery effluents (p. 21): again, point discharges of pollution are involved, and here the field studies are integrated with a thorough programme of experimental work. It is of some concern, however, that high prevalences of the disease studied were also found at reference sites distant from sources of pollution.
3. Abnormalities of plaice caused by the *Amoco Cadiz* oil spill (p. 20): although the influence of migratory movements was not considered, and the reference area comprised three different localities sampled on three different occasions, the findings of this study appear clear-cut, owing to the particularly severe nature of the pollution.
4. Liver abnormalities of flounder, smelt and ruffe caused by pollution of the river Elbe (p. 17): convincing evidence is provided by the virtual absence of several of the observed conditions from the reference area, and a supporting programme of experimental work.
5. Epidermal hyperplasia/papilloma in dab near the Danish coast, caused by oxygen deficiency suspected to be related to eutrophication (p. 18): here an event occurring at an identified moment was followed by an extensive study over several years, providing an unusual opportunity to investigate the temporal relationship of cause and effect.

In our opinion, the evidence provided by the above studies leaves little room for doubt that pollution is involved in disease aetiology in these specific instances. An additional, special case is provided by the discovery, in several studies, of liver nodules in dab and flounder in inshore as well as offshore areas. In view of the strong evidence linking the

prevalence of such nodules to pollution in American studies of flatfish (p. 13), it is tempting to conclude that a similar link must exist in the North Sea area, even though the causal mechanisms have not yet been elucidated.

Of the other studies described previously, the evidence provided by many in favour of or against a causal relationship is weak: this is due mostly to poor survey design. There remain, however, a number of thorough studies with an adequate design, but whose findings are negative or less clear-cut. A few examples are given below.

1. The negative evidence for the role of pollution in epidermal disease of flounder in the Elbe (p. 17) can be contrasted with the findings on liver anomalies above. Both sets of studies appear to provide a sound basis for their conclusions, and it is tempting to conclude that there may be a real difference here in the suitability of the different diseases as pollution indicators.
2. Prevalences of epidermal hyperplasia/papilloma in dab were higher in dab from German (p. 15) and Dutch (p. 19) titanium dioxide dumping areas than in surrounding areas. However, whereas Dethlefsen *et al.* (1987) found clear correlations between disease and chemical factors, Vethaak and Meer (1991) did not. The fact that both dumping areas are located in the vicinity of heavily polluted rivers makes discrimination between specific pollution sources and the general pollution load of the rivers difficult.
3. The study on the effects of sewage sludge disposal in Scotland (p. 19) is different from all the above, consisting of a single survey lasting only 3 days and addressing spatial variation on a relatively small scale. Its principal interest lies in the fact that statistically significant differences in disease prevalence were observed, but the nature of the spatial variation was contrary to expectations about the influence of sewage sludge pollution, and provides a general warning that statistical correlations cannot be equated with cause–effect relationships. Data on body burdens of contaminants in these fish would have been of interest.

All the above examples are derived from studies related to specific point sources of pollution, or in restricted local areas. Although the latter cannot be distinguished in any absolute way from studies classified in this review as extending over wide areas, the frequency or the importance of certain defects seems to increase in this last category, as follows.

1. Presumably for logistic reasons, the number of hauls per site tends to be low (often one), and the precision of the prevalence estimates is therefore questionable.
2. A lack of chemical data represents a particularly significant omission from wide-ranging surveys, where clear pollution gradients are unlikely to exist.
3. A lack of information about other environmental factors influencing disease prevalence is more significant in wide-ranging surveys as these factors will vary more over a large area.

Despite their shortcomings, many of the studies reached definite conclusions – positive or negative – regarding the role of pollution. Not surprisingly, these findings have subsequently been disputed. However, the findings of more comprehensive studies must also be described as open to debate (Möller, 1987; Bucke, 1988; Bucke and Watermann, 1988; Dethlefsen, 1988). These include the studies of Dethlefsen and colleagues (p. 15), and of Vethaak and colleagues (p. 18) in a more restricted area. Both have suggested a role of pollution in the development of some diseases in certain localities.

The fundamental difficulty of such studies, however comprehensive they may be, is that as Dethlefsen (1990) states, the possibility always remains that there is an "unknown set of factors which have an influence and have not yet been investigated in our studies". The situation with regard to large-scale surveys differs in degree, if not in absolute terms, from studies in which a source of pollution can be precisely located in time and/or space and where other environmental variables are not likely to vary so much over the relatively restricted geographical area examined.

GENERAL DISCUSSION

In the North Sea area, it is evident that specific studies involving identified point sources of pollution have produced more examples of a convincing relationship between pollution and disease than have more extensive studies over wide areas. This distinction may continue in the future: in words of Dethlefsen (1990), "the intensification of studies in the future will not lead to clear answers as to the causation of fish diseases in the North Sea". It is likely, therefore, that the interpretation of data from spatially extensive surveys will always be subject to a certain degree of disagreement. The value of such surveys lies instead in monitoring for long-term trends in disease prevalence, in detecting hitherto unknown diseases and 'hot-spots' of anomalously high prevalence, and in examining the relationship between disease and a complex of environmental variables. Extensive multi-disciplinary studies are very important in that they begin to assign to the very long list of potential causal factors for disease (Table 2) an indication of their relative importance in reality. Three specific examples of important factors are food supply (Möller, 1984, 1990), trauma due to capture and release from fishing nets (Dethlefsen, *et al.*, 1987; Dethlefsen 1990), and salinity fluctuations (Vethaak, 1991).

A feature of several of the most convincing cases of a link between disease and pollution is the accompanying experimental work, as in the studies on vertebral deformities in fourhorn sculpin, of histopathological lesions in Elbe flounder, and – on the other side of the Atlantic – of liver lesions in American east coast flatfish. Many of the more widespread epidermal diseases have not, however, been the subject of any published experimental studies, although such studies are now in progress in some countries bordering the North Sea.

In considering the future development of epidemiological research in the North Sea, the appropriateness for further use of the host species and the diseases presently studied should be discussed. The continued use of dab and flounder as indicator species rests largely on the same aspects of their biology that led to their being recommended originally, since no disease in either has shown a clear relationship with pollution over a wide range of conditions. It is possible, however, that other fish species, including those of no commercial significance, may be equally or even more suitable for monitoring pollution.

The results of American studies suggest very strongly that more attention should be given to liver nodules as a suitable disease for study in the North Sea area (Anon., 1989). Attention was first drawn to the widespread distribution and high prevalence of these nodules in North Sea flatfish by Vethaak (1987), though the original interpretation, that they represent a continuum of neoplastic lesions, including malignant stages, was considered by Bucke and Watermann (1988) to be unsubstantiated. More recently, however, the occurrence of liver neoplasia (but not of malignant tumours per se) in North Sea fish has become more widely accepted (Bucke and Stokes, 1988; Anon., 1989).

Although the need to take and preserve histological samples could be considered an obstacle to the use of this condition in routine monitoring, it is encouraging to note that there appears to be a good correspondence between neoplastic or pre-neoplastic lesions and grossly observable nodules on the surface of the liver (A.D. Vethaak, unpublished data).

There is strong evidence that fin rot and skeletal deformities are causally related to pollution in some fish species, both in the North Sea and elsewhere. There is little doubt that skeletal deformities can have a chemical aetiology (Bengtsson, 1975). However, since they are not always easy to detect, recording them can be costly and labour-intensive; although the method described by Bengtsson and Bengtsson (1983) was considered to achieve a reasonable compromise between cost and the need for large quantities of data, it has not been widely adopted.

The use of fin rot as a diagnostic tool for routinely monitoring the relationship between fish disease and pollution is controversial, because of difficulties in separating the condition from mechanical damage, leading to criticism (Möller, 1987) of published studies and to the exclusion of fin rot from the list of diseases recommended for monitoring by ICES (Anon., 1989). Methods for diagnosing this disease could repay more detailed investigation.

In addition to the considerable methodological problems that persist in epidemiological studies of fish disease, there are also major gaps in our knowledge. These include difficulties in characterizing reference areas and 'natural' background levels of diseases, uncertainty about the dynamics and aetiology of the diseases, lack of knowledge about the biology of the hosts, and the scarcity of accompanying environmental data in most instances (Anon., 1989). It should be further stressed that, whereas information is increasingly available about the behaviour and dispersion of pollutants from major sources, the long-term, possibly synergistic effects of pollutants that are widespread at low levels are far from understood. These pollutants, however, may well have an important effect on fish health.

Finally, it is gradually becoming clear that many types of disease are a direct consequence of an impaired immune system, but studies on immunocompetence and immunosuppression in marine organisms are at an early stage in most countries.

Conclusions and recommendations

On a worldwide scale, the most convincing examples of a causal relationship between fish disease and pollution come from intensive detailed studies carried out in North America, particularly on liver pathology. Despite the existence of a number of convincing examples of an association in the North Sea area, notably in local areas with distinct sources of pollution, negative views on the usefulness of fish diseases in monitoring pollution continue to be expressed. This is partly because fundamental limitations seem to prevent the clear demonstration of cause–effect relationships in more wide-ranging epidemiological surveys. Although such surveys serve important functions, their limitations must be recognized and accepted, and increased emphasis should be given to more intensive and thorough investigations, preferably in localities with distinct sources of pollution and low background prevalences. These studies should concentrate on liver pathology as the most important disease condition, on the measurement of contaminant concentrations or other indicators of exposure or effect, and on accompanying experimental work, while the use

of skeletal deformities as biological indicators merits further development. It is to be hoped that such investigations will provide the detailed information required to establish a solid basis for the use of at least some fish diseases in routine pollution monitoring.

Summary

The use of fish diseases to monitor marine pollution is reviewed and evaluated, with particular reference to the North Sea and associated waters. Criteria for epidemiological surveys are outlined, an international overview of research is given, and recent studies in the North Sea area are described and evaluated.

The basic approach is to identify spatial and temporal patterns of disease prevalence, which can be related to pollution. A major obstacle is to distinguish effects of pollution from those of other variables, especially as most diseases appear to have a multifactorial aetiology. Field studies can be evaluated against a number of criteria: these include the accuracy and precision of prevalence estimates, the extent to which possible causal factors other than pollution are taken into account, and whether or not exposure of the study population to pollution is measured directly.

A distinction can be made between intensive, thorough studies, which frequently use a histopathological approach, and the more extensive surveys of large numbers of fish for grossly observable lesions. Broadly speaking, North American research has emphasized the former approach, and research in the North Sea the latter. Nevertheless, although the most comprehensive evidence for a causal relationship between disease and pollution has been gathered in North America, there are also good examples from the North Sea area, particularly in local areas with distinct sources of pollution. The data from wider-ranging surveys are more ambiguous: while some provide circumstantial evidence for a role of pollution, the apparent complexity of disease aetiology and the limitations of the epidemiological approach may prevent any clear demonstration of pollution as a cause over wide geographical areas. Extensive surveys are nevertheless useful for detecting long-term trends in disease prevalence and 'hot-spots' of anomalously high prevalence, and for examining the relationship between disease and a complex of environmental variables.

For the future, greater emphasis should be placed on the recording of liver lesions, on the measurement of exposure to pollution, and on experimental work.

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