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Foreword
by
The Director.
Marine Laboratory, Aberdeen

Following the publication of a review of monitoring of the UK environment (Department of the Environment (DOE), 1974) a number of monitoring management groups were established to coordinate the various sectorial responsibilities. The Marine Pollution Monitoring Management Group (MPMMG) was set up in July 1974.

Since its formation the MPMMG has published two major reports on its work. The first (DOE, 1977) gave the terms of reference, its basic philosophy and its plans for the first few years work and reported on the results of its early monitoring activities. The second (DOE, 1979) reviewed the progress made since the publication of the first report and outlined the management strategy for future coordinated marine pollution monitoring in the UK.

In 1979 several working groups were set up by the MPMMG with responsibility for handling the priority work specified in the second report. This paper presents the report of one of these groups which was charged with the task of developing guidelines for the use of biological material in first order pollution assessment and trend monitoring. Reports of other MPMMG working groups will be published elsewhere.

Although the report will be made widely available within the UK it is intended primarily for the River Purification Boards (RPBs) and Islands Councils in Scotland and the Regional Water Authorities (RWAs) in England and Wales. The Control of Pollution Act 1974 Pt II extends the responsibility of RPBs, the Islands Councils and RWAs to all coastal waters within 3 miles of low-water mark and it is thought that this report will assist them in fulfilling these and other domestic responsibilities. It will also assist them in satisfying some of the requirements that may stem from EC directives and recommendations made by other international bodies, eg Paris Commission.

The overall aim of the report is to provide a picture of the current “state of the art” of this work together with clear guidelines for this system of monitoring sea water for priority contaminants which is considered by the MPMMG to be relevant and practical in relation to their future marine pollution monitoring activities.

A.D. McIntyre.
Guidelines for the Use of Biological Material in First Order Pollution Assessment and Trend Monitoring

G Topping*

Marine Laboratory, Aberdeen

Introduction

The second report (DOE, 1979) of the Marine Pollution Monitoring Management Group (MPMMG) reviewed its Strategic Plan, the progress made under the first Tactical Plan and sets out directions to be followed under a second Tactical Plan. In this report the Tactical Planning Team made a number of recommendations, two of which were that:

(1) An outline “mussel watch” type programme should be commissioned which would lay down guidelines for contributions to such monitoring in the UK by the interested parties, ie Government Fisheries Laboratories, Regional Water Authorities and River Purification Boards.

(2) A small group of scientists should be convened to examine the problem of trend monitoring and to design a model programme in which selection of species, methodology of sampling, analysis and reporting of data are outlined and approximate costs provided.

Having accepted these recommendations, and recognising that there were many aspects of them which could be handled by the same group of experts, the MPMMG established an appropriate subgroup to implement the two recommendations. The terms of reference of this subgroup are set out in Appendix II and a list of members and their addresses in Appendix I. In addition to these terms of reference the subgroup was asked to discuss, and where appropriate make recommendations on, the following subjects:

(1) The use of sediments in marine pollution monitoring.

(2) The use of mussels and similar organisms in biological effects monitoring.

(3) The use of artificial substrata in marine pollution monitoring.

*This paper is based on a report prepared by a subgroup of the MPMMG. In addition to the author, who chaired the subgroup, the following scientists contributed to this report, B.L. Bayne, G. Bryan, I.M. Davids, W. Halcrow, J.E. Portmann, J.H. Sioner and D. Taylor.
The collection and analysis of samples of sea water as part of a water quality assessment programme can be both expensive in terms of manpower and equipment and very time consuming. In addition, the analysis of the extremely low levels of contaminants normally encountered in sea water \( (10^{-15} \text{ g g}^{-1} - 10^{-9} \text{ g g}^{-1}) \) can pose problems even to the most experienced and competent analysts. There is also no guarantee that the chemical forms of the contaminants measured will be relevant to the subsequent biological fate of these substances. The ability of marine organisms to concentrate contaminants from sea water by a factor of \( 10^3 \) or more enables them to play a key role in water quality assessment by providing the investigator with an initial enrichment or concentration step and a more relevant measure of the role of the individual contaminant. Field and experimental studies have suggested that some bivalve molluscs may be valuable as sentinel organisms for indicating levels of contaminants in coastal marine waters. Several genera of bivalves e.g. *Mytilus*, *Ostrea* and *Crassostrea* are known to accumulate the three main categories of marine contaminant, heavy metals, halogenated hydrocarbons and petroleum hydrocarbons. In an effort to stimulate a uniform and general approach to marine monitoring, Goldberg (1975) proposed a global monitoring programme employing native mussels as indicator organisms — "mussel watch". In 1976 a survey of the water quality of USA coastal regions was initiated using mussels and oysters as proposed by Goldberg (1975). The results of this investigation (Goldberg, Bowen, Farrington, Riseborough, Schneider & Gamble, 1978) and other similar national surveys formed the basis of the workshop "The International Mussel Watch" in November 1978 in Barcelona. The proceedings of the Barcelona Workshop have since been published by the USA National Research Council (NRC, 1980).

**Background to Mussel Watch Type Programmes**

Following the proposal by Goldberg (1975) the UK Government Fisheries Laboratories established mussel watch type programmes for British coastal waters. The Ministry of Agriculture, Fisheries and Food (MAFF) Fisheries Laboratory, Burnham-on-Crouch, carried out a small survey (13 sites) in 1977 along the south coast of England and on the basis of their findings undertook a more extensive survey (80 sites) during July-September 1978 covering the whole of the coastline of England and Wales. The contaminants measured in mussel (*Mytilus edulis* L.) homogenates were heavy metals, halogenated hydrocarbons and petroleum hydrocarbons. The survey of contaminant levels in mussels collected from Scottish coastal waters (128 sites) in March-April 1977 was coordinated by the Department of Agriculture and Fisheries for Scotland (DAFS) Marine Laboratory, Aberdeen who arranged the collection of mussels and analysed them for heavy metals. The DAFS Freshwater Fisheries Laboratory, Pitlochry and MAFF Torry Research Station, Aberdeen analysed some samples of mussels for halogenated hydrocarbons and petroleum hydrocarbons respectively. In February 1979 the Welsh Water Authority (WWA) extended the survey of the Welsh coastal waters by analysing mussels for heavy metals and halogenated hydrocarbons at 36 sites (Severn Estuary – Dee Estuary). In Northern Ireland, a survey of contaminants in mussels commenced during August 1980. This survey was coordinated by the Fisheries Research Laboratory, Coleraine, Department of Agriculture for Northern Ireland (DANI), and initially examined selected metals in samples from 11 coastal sites around the province.

A description of the Scottish surveys together with a discussion of the results of the analysis of the three classes of contaminants are presented elsewhere (trace metals – Davies & Pirie (1980); halogenated hydrocarbons – Cowan (1978) and petroleum hydrocarbons – Mackie, Hardy, Whittle, Bruce & McGill (1979)). The results of the English/Welsh surveys were presented to
the 1980 International Council for the Exploration of the Sea (ICES) Statutory Meeting (Murray & Law, 1980). Although the results of the Welsh survey have not been published they were made available for discussion to the subgroup by its coordinator (W. Halcrow, personal communication). Similarly, unpublished data from the survey of Northern Ireland were made available to the group (J.G. Parker, personal communication).

In view of the large quantity of data obtained in these surveys the subgroup considered that it would be inappropriate to present them in full in this paper. However, it was decided to present a summary of the results, in the form of ranges of values for the three contaminant groups (Table I), and to give a brief account of the overall conclusions.

Table 1
Results of UK “Mussel Watch” Programmes 1977-1979

Range of concentrations of contaminants in *Mytilus edulis*

<table>
<thead>
<tr>
<th>Contaminants</th>
<th>Scotland</th>
<th>England and Wales</th>
<th>N. Ireland</th>
<th>Wales</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metals**</td>
<td>(DAFS survey)</td>
<td>(MAFF survey)</td>
<td>(DAN survey)</td>
<td>(WWA survey)</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.01 – 0.46</td>
<td>0.02 – 0.44</td>
<td>0.03 – 0.45</td>
<td>Not measured</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.18 – 0.94</td>
<td>&lt;0.2 – 6.2</td>
<td>0.12 – 0.62</td>
<td>0.3 – 16.7</td>
</tr>
<tr>
<td>Lead</td>
<td>&lt;0.3 – 4.4</td>
<td>0.5 – 35</td>
<td>0.33 – 3.2</td>
<td>Detection limit – 24.8</td>
</tr>
<tr>
<td>Copper</td>
<td>0.67 – 8.2</td>
<td>0.7 – 6.9</td>
<td>0.78 – 2.7</td>
<td>1.1 – 3.5</td>
</tr>
<tr>
<td>Zinc</td>
<td>6.5 – 47</td>
<td>10 – 90</td>
<td>10.3 – 47.1</td>
<td>14 – 52</td>
</tr>
<tr>
<td>Halogenated Hydrocarbons***</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a-HCH</td>
<td>&lt;1 – 3.0</td>
<td>&lt;2</td>
<td>0.1 – 1.3</td>
<td>Not detected</td>
</tr>
<tr>
<td>β-HCH</td>
<td>&lt;1 – 8.8</td>
<td>&lt;2</td>
<td>0.2 – 2.6</td>
<td>0.4 – 4.8</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>&lt;1 – 405</td>
<td>&lt;2 – 31</td>
<td>&lt;1 – 4.0</td>
<td>&lt;0.4 – 6.6</td>
</tr>
<tr>
<td>TDE</td>
<td>Not measured</td>
<td>Not measured</td>
<td>Not measured</td>
<td>&lt;0.1 – 3.2</td>
</tr>
<tr>
<td>DDE</td>
<td>&lt;1 – 42</td>
<td>&lt;2 – 23</td>
<td>0.5 – 2.8</td>
<td>&lt;0.50 – 3.3</td>
</tr>
<tr>
<td>DDT</td>
<td>&lt;1 – 16</td>
<td>&lt;2 – 60</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>Total DDT</td>
<td>&lt;1 – 33</td>
<td>&lt;2 – 68</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>PCB+</td>
<td>12 –1183</td>
<td>&lt;50 – 150</td>
<td>0 – 2.8</td>
<td>6 – 104</td>
</tr>
</tbody>
</table>

Hydrocarbons++

| Total hydrocarbons     | Not measured | 6.3 – 150 |
| Alkanes                |              |          |
| Muscle tissue          | 0.3 – 9.0 | <0.001 – 2.2 |
| Viscera                | 0.4 – 14.1 |          |
| Aromatic               |              |          |
| Muscle tissue          | 0.015 – 1.0 | <0.001 – 4.0 |
| Viscera                | 0.069 – 8.0 |          |
| Polynuclear aromatic hydrocarbon | 0.054 – 2.8 |

*Unless stated otherwise analytical data refers to measurements on mussel homogenate.

**Concentrations of metals expressed in mg kg⁻¹ wet weight (original WWA data converted from dry weight to wet weight by dividing values by 5).

***Concentration of halogenated hydrocarbons expressed in pg kg⁻¹ wet weight (original DAFS, Freshwater Laboratory, data converted from dry weight to wet weight by dividing values by 6).

++Concentration of hydrocarbons expressed in mg kg⁻¹ wet weight.
Metals

The ranges of concentration of mercury and copper in the four surveys were very similar. The MAFF and WWA surveys tended to find slightly more zinc and considerably more lead and cadmium than the DAFS and DANI surveys particularly in known mineralised areas such as SW England and Wales. The highest concentration of cadmium in mussels from Wales (16.7 µg g⁻¹) is about an order of magnitude higher than the highest concentrations in mussels from England, Scotland and Northern Ireland. On the basis of previous information the surveys produced no major surprises regarding the distribution and concentration of mercury and cadmium in coastal waters of the UK. These surveys however provide a more detailed and coherent picture of metallic containments than was previously available.

Halogenated Hydrocarbons

All MAFF and, to large extent, DAFS samples were found to contain concentrations of α – and γ – HCH below the detection limits, ie <1 and <2 µg kg⁻¹ wet weight respectively. The WWA and DANI surveys of γ–HCH in mussels however produced positive overall ranges of concentration of 0.4 - 4.8 and 0.2 - 2.6 µg kg⁻¹ wet weight respectively. In general the range of dieldrin concentrations found in the DAFS, WWA, DANI and MAFF surveys were similar except that a number of Firth of Clyde stations exhibited (as expected) markedly higher values than the rest of the UK sites. The highest value for dieldrin (6.6 µg kg⁻¹ wet weight) reported by WWA is slightly higher than that reported by DANI but approximately one-fifth of the highest value reported by MAFF (31 µg kg⁻¹ wet weight) and approximately one-sixtieth of the highest value found in the Firth of Clyde (405 µg kg⁻¹ wet weight). The majority (80%) of PCB values reported by MAFF fall below their detection limit of 50 µg kg⁻¹ wet weight. In contrast WWA and DANI were able to report positive values for all their samples; the ranges were respectively 6 - 104 and 0 - 2.8 µg kg⁻¹ wet weight. The highest concentrations of PCBs in mussels were found in the Firth of Clyde and in the Cromarty Firth, namely 374 and 1183 µg kg⁻¹ wet weight respectively.

Petroleum Hydrocarbons

MAFF Burnham-on-Crouch (BOC) measured total hydrocarbons in mussel homogenates using an ultra-violet (UV) fluorescence technique following digestion with methanol-potassium hydroxide mixture and extraction with pentane whereas MAFF Torry Research Station (TRS) measured individual aliphatic and aromatic hydrocarbons in muscle and visceral tissue using gas chromatography coupled to a mass spectrometer (GC-MS) following extraction with chloroform-methanol and separation of alkanes and aromatics by column chromatography. The 1977 survey conducted by MAFF (BOC) also used GC-MS techniques following digestion and extraction as above and the 1978 samples will be processed in the same way. At present only the data from the 1977 survey can be compared with the TRS data.

The UV fluorescence technique provided values of total hydrocarbon concentrations in mussel homogenates of 6.3 - 150 µg kg⁻¹ wet weight; the highest values occurring on the east coast of England at Blyth (130 mg kg⁻¹ wet weight) and Whitstable (150 mg kg⁻¹ wet weight). In contrast the GC-MS procedure produced aromatic values for the muscle tissue of mussels in the range 0.015 - 1 mg kg⁻¹ wet weight (TRS) and 0.001 - 4.0 mg kg⁻¹ wet weight (BOC) whilst those in the digestive gland and gut were in the range 0.065 - 8 mg kg⁻¹ wet weight (TRS). Corresponding figures for the n-alkanes were 0.3 - 9 mg kg⁻¹ wet weight (TRS) and 0.001 - 2.18 mg kg⁻¹ wet weight (BOC) for muscle tissue with 0.4 - 14 mg kg⁻¹ wet weight in the digestive gland and gut (TRS).
Following an examination and discussion of the results of the above surveys the subgroup considered that:

(a) The surveys had provided a reasonable coverage of the UK coastal area in terms of the contaminants of interest.

(b) The surveys had been able to distinguish between known “contaminated” (“hot-spots”) and “clean” or “background” areas and that these findings confirmed and consolidated the results of monitoring programmes previously conducted by the Government Fisheries Laboratories. The “hot spots” were also defined more precisely than was previously possible.

(c) The surveys provided evidence of enhanced levels of some contaminants at a number of sites which had not been revealed by previous monitoring programmes.

The subgroup concluded that the main aims of the exercise had been achieved, namely the identification of contaminated coastal areas around the UK. The subgroup also concluded that there was no need to repeat this type of national survey for at least another 5 years. It recommended that this survey method should be employed on a regional basis to permit more detailed investigations, of selected contaminants in the “hot spots” identified in the national exercises, and that these investigations should incorporate a trend monitoring component.

In view of this recommendation the subgroup felt it appropriate to provide a table giving a list of “hot spots” areas and their contaminants (Table II). Programmes designed to investigate these “hot spots” would be based on guidelines and instructions presented later on in this paper.

<table>
<thead>
<tr>
<th>Hot Spots</th>
<th>Associated Contaminants</th>
<th>Organisations with Responsibility for or Interest in the Area</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scotland</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moray Firth</td>
<td>Pb, PCB, DDT, HC*</td>
<td>Highland RPB, DAFS</td>
</tr>
<tr>
<td>NE Coast</td>
<td>Dieldrin, PCB, DDT</td>
<td>North East RPB, DAFS</td>
</tr>
<tr>
<td>Aberdeen Coast</td>
<td>Dieldrin, PCB, DDT</td>
<td></td>
</tr>
<tr>
<td>Tay Estuary</td>
<td>Hg, Cd, Pb, Cu, Zn, DDT, PCB, HC</td>
<td>Tay RPB, DAFS</td>
</tr>
<tr>
<td>Forth Estuary</td>
<td>Hg, Cd, Pb, Cu, Zn, DDT, Dieldrin, HC</td>
<td>Forth RPB, DAFS</td>
</tr>
<tr>
<td>Firth of Clyde</td>
<td>Hg, Cd, Pb, Cu, Zn, DDT, Dieldrin, PCB, HC</td>
<td>Clyde RPB, DAFS</td>
</tr>
<tr>
<td>Solway Firth</td>
<td>Cd</td>
<td>Solway RPB, DAFS, MAFF</td>
</tr>
<tr>
<td><strong>England</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE Coast</td>
<td>Hg, Cd, Pb, Cu, Zn, HC</td>
<td>Northumbrian WA, MAFF</td>
</tr>
<tr>
<td>Wash</td>
<td>Cu</td>
<td>Anglia WA, MAFF</td>
</tr>
<tr>
<td>South Coast</td>
<td>Cd, Hg, HC</td>
<td>Southern WA, MAFF</td>
</tr>
<tr>
<td>Southampton</td>
<td></td>
<td>South West WA, MAFF</td>
</tr>
<tr>
<td>Devon Coast</td>
<td>Pb, Zn, (poss. Hg, Cu), HC</td>
<td></td>
</tr>
<tr>
<td>SW Coast</td>
<td>Zn, Cd, Cu</td>
<td>North West WA, MAFF</td>
</tr>
<tr>
<td>E Irish Sea</td>
<td>Hg, Cd, Cu, Zn, Pb, Dieldrin, DDT, PCB (possibly HC)</td>
<td></td>
</tr>
<tr>
<td><strong>Wales</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severn Estuary</td>
<td>Hg, Cd, Pb, Zn, Cu</td>
<td>WWA, Severn Trent WA, South West WA, MAFF</td>
</tr>
<tr>
<td>Swansea Bay</td>
<td>Cd, Pb, Cu, Zn</td>
<td>WWA, MAFF</td>
</tr>
<tr>
<td>Cardigan Bay</td>
<td>Cd, Pb</td>
<td>WWA, MAFF</td>
</tr>
<tr>
<td>Menai Straits</td>
<td>Cd, Pb, Cu</td>
<td>WWA, MAFF</td>
</tr>
<tr>
<td>North Wales</td>
<td>Cd, Pb</td>
<td>WWA, MAFF</td>
</tr>
<tr>
<td>Dee Estuary</td>
<td>Zn</td>
<td>WWA, MAFF</td>
</tr>
<tr>
<td>N. Ireland</td>
<td></td>
<td>DANI, DOE (NI)</td>
</tr>
<tr>
<td>Belfast Lough</td>
<td>Zn</td>
<td></td>
</tr>
</tbody>
</table>

*HC = Hydrocarbons
Future Marine Pollution Monitoring in the UK

Marine Pollution monitoring in the UK, carried out on a national regional or local basis, must aim to satisfy the requirements of our various international and national obligations. Although these obligations and the associated organisations with prime responsibility are not presently well defined (DOE, 1979), it is likely that those eventually responsible for this work will be asked to provide answers to the following questions in relation to coastal water quality:

Can we compare the degree of contamination of coastal waters around the UK and indentify the areas ("hot spots") where elevated levels of particular contaminants are present? Is it possible to monitor trends in the level of contamination particularly in areas where it may be necessary to improve water quality to conform to some acceptable standard or guideline? The subgroup consider that the work done to date by MAFF, DAFS, WWA and DAN1 have provided answers to the first question for the three main groups of contaminants. They also believe that the adoption of a similar "mussel watch" type programme, observing the necessary practical guidelines, for "hot spots" will not only provide answers to the second question but will meet some of the requirements referred to above. In the following sections of the report the subgroup present details of the proposed "mussel watch" type monitoring programme including information on the development of guidelines, the selection of suitable organisms and contaminants of interest, the sampling requirements and the frequency of surveys.

Guidelines for a Coordinated "Mussel Watch" Programme

The guidelines presented here are drawn up on the basis of first-hand knowledge and experience of the subgroup and with reference to the discussion and guidelines arising from the Barcelona Workshop (NRC, 1980).

Contaminants of Interest

It is appropriate that future "mussel watch" projects should cater for regional, national and international needs, in relation to the "Black and Grey" substances on the lists of the Oslo and Paris Conventions and the EC Directives (Appendix III). From these a subgroup of priority substances has been identified (Appendix IV).

Halogenated hydrocarbons

Of these compounds, aldrin, endrin, β-HCH, heptachlor, heptachlor epoxide and chlordane are unlikely to be commonly encountered. Attention should therefore be concentrated upon HCB, α and γ-HCH, dieldrin, pp'-DDE, pp'-TDE, pp'-DDT and PCBs.

Metals

Only two metals, mercury and cadmium, are on the "Black" lists. However, the "Grey" lists include a wide range of metals of which arsenic, lead, copper, and zinc are common to all and it is suggested that these should be included in the monitoring programmes; these are also the most common metals found in domestic and industrial wastes.

Hydrocarbons

These substances are not yet classed as priority contaminants by the EC but they do however appear in the "Black" lists of the EC and Paris Commission and as such should merit attention.

There is little doubt that mussels will take up hydrocarbons from sea water and that zones of high contamination may be identified by the analysis of body burden residues. Unfortunately there is evidence that the mechanism of acquisition and depuration is very complex, and varies from compound to compound. The rates of turnover measured can be surprisingly rapid eg the half-life of benzo(a)pyrene in Mytilus edulis was found to be about 14 days, whereas diesel oil exhibited a half-life of 3 days (Dr K. Whittle, personal communication). The inference is that hydrocarbon measurements made on mussels mainly reflect only the comparatively recent exposure history at the time of sampling. In view of this mussels would appear to have a limited use...
in terms of trend monitoring. The readers attention is however drawn to a recent publication by Burns & Smith (1981) in which the authors discuss one possible monitoring strategy for oil pollution in coastal waters. The authors suggest that surveys of indigenous populations of mussels could be used to identify major sources of contamination and areas of special concern and once this has been done the detailed distribution of oil could be pursued through the use of transplants of clean mussels to selected sites.

It should also be noted that the “state of the art” of oil analysis is relatively poorly developed for routine monitoring purposes and only a few intercalibration exercises have been carried out. In addition the high resolution gas chromatography-mass spectrometry required for the identification and quantification of individual aliphatic and aromatic compounds is very expensive and requires well-trained and experienced personnel. Alternative procedures are available which are simple to employ and are less expensive, for example UV fluorescence and Infra Red (IR) spectroscopy. These have severe limitations as a truly quantitative measure of total oil since errors may be introduced by the presence of biogenic hydrocarbons and non-petroleum products and it is difficult to choose the correct oil standard if the source of contamination is unknown of if the oil has undergone weathering.

In view of the above, the subgroup feels that further investigative work is needed on this subject before oil monitoring by “mussel watch” can be successfully incorporated into the proposed regional programmes.

The range of contaminants so far considered represent those currently identified as of particular importance by the UK and international bodies. In addition to these, local organisations may wish to include other contaminants of a particular regional interest. However, recognising the current limited facilities of manpower and money, the subgroup suggests that first priority should be given to those contaminants identified in “regional hot spots” (Table II) which are of wider interest.

Selection of Organism

Butler, Andren, Bonde, Jernelov & Reisch (1971) and Haug, Nelson & Omang (1974) have listed the ideal characteristics of organisms for use as indicators of environmental conditions as follows:—

1. The organism should accumulate the pollutant without being affected by the levels encountered.
2. The organism should be sedentary in order to be representative of the area of collection.
3. The organism should be abundant in the study region.
4. The organism should be sufficiently long-lived to allow the sampling of more than one-year-class if desired.
5. The organism should be of reasonable size, giving adequate tissue for analysis.
6. The organism should be easy to sample and hardy enough to survive in the laboratory, allowing (if desired) defecation before analysis and laboratory studies of uptake of contaminants.
7. The organism should tolerate brackish water.
8. The organism should exhibit high concentration factors, allowing direct analysis without pre-concentration.
9. A simple relation should exist between contaminant residues and average concentrations in the surrounding water.
These conditions restrict the useful organisms to a range of fairly large, abundant, widespread, inter-tidal organisms, mainly macro-algae and molluscs. Filter-feeding molluscs are more likely to reflect conditions in the water, whilst deposit-feeders will respond to sediment chemistry. The sediment chemistry will broadly reflect the contaminant history of the area. The working of the sediment both by organisms and water currents will cause an averaging of relatively short-term variations in contaminant loading. Water chemistry, however, will more closely respond to effluent discharge and dispersal conditions at the moment of sampling. Filter-feeding organisms are therefore more likely to provide the information required to fulfill the objectives laid down. In Table III a list of recommended organisms is presented which can be used for the assessment of contamination by a range of metals and organochlorines under the contrasting conditions found in rocky and muddy inter-tidal areas. In practice the selection of an organism is influenced by knowledge of the usefulness of various organisms as indicators of the contaminants of interest, and the availability of these organisms in the area of investigation.

<table>
<thead>
<tr>
<th>Organisms Recommended for the Assessment of Contamination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Rocky substrata</td>
</tr>
<tr>
<td>Mytilus edulis L.</td>
</tr>
<tr>
<td>Littorina littorea (L.)</td>
</tr>
<tr>
<td>Patella vulgata L.</td>
</tr>
<tr>
<td>Muddy substrata</td>
</tr>
<tr>
<td>Scrobicularia plana (da Costa)</td>
</tr>
<tr>
<td>Macoma balthica (L.)</td>
</tr>
<tr>
<td>Nereis diversicolor Muller</td>
</tr>
</tbody>
</table>

Key: + - Appears to act as good indicators  
? - Doubt about use as indicators

Note: The organisms listed for muddy substrates are all deposit feeders, whilst those for rock substrates are filter feeders or herbivores. It is unlikely that residues from the two groups will reflect contaminant levels in the same part of the environment.

If a single preferred organism had to be nominated, the common mussel, *Mytilus edulis*, would be recommended. Knowledge of the indicator ability, biology and biochemistry of the mussel is relatively extensive. Mussels can be obtained throughout the UK and widely elsewhere, and considerable experience of mussel surveys has already been built up both in the UK and elsewhere (NRC, 1980).

**Sampling**

Sampling should be carried out in such a way that comparison may be made between samples. Residues in many organisms are known to be affected by factors such as age, size and season and programmes should be designed to eliminate, as far as possible, variability arising from these. At the same time, sampling criteria should not be too restrictive, bearing in mind likely limitations in resources, and the intended use of the "mussel watch" approach in first order pollution assessment and monitoring.

The following sections and discussion are concerned with the sampling of mussels, but the principles established could, fairly readily, be adapted to other organisms.

**Time of year**

Seasonal variations in food supply and position in the spawning cycle are known to cause considerable change in total body weight and consequently contaminant residue concentration. Concentrations of metals tend to be highest immediately after spawning, but this is a transitional period for the
animals and for residue concentrations. The most stable period, both for the animals and environmental conditions, is late winter. It is considered that the benefits of sampling in the late winter will outweigh the less than maximal concentrations likely to be found.

*Size or age of animals*

The age of mussels cannot adequately be routinely determined in the field, so one cannot collect animals of the same age, or even within a narrow range of ages. Growth rates vary markedly according to their location (exposure to wave action, availability of food, etc) and no general relationship between size and age can be made. It is recommended that collections be made of the dominant size in the population, and that normalisations of data based on relationships developed by Boyden (1977) be used with caution to eliminate some of the variability.

*Position in the shore*

The period of exposure of mussels to contaminants in the water is governed by the position of the animal in relation to tidal levels. It is recommended that the animals collected are those exposed to the sea for six hours during each tidal cycle.

Where possible animals should be collected from rocks, to reduce the chance of quantities of sediment being found within the animal. Because of potential contamination by preservatives, mussels growing on man-made structures should be avoided if possible.

*Sample size*

The sample size required will depend upon the amount of material needed for the subsequent analysis, the desire to obtain a representative collection, and the resources available for sample processing (which can be time consuming). Treatment or storage of mussels for analysis for different classes of contaminants vary, and a minimum of 25 animals should be collected for each treatment. If it is anticipated that 25 animals would not provide sufficient material, further collection should be made.

*Initial storage*

Samples for metals and pesticide residue analysis should be stored alive, cooled (if necessary), naturally moist, in polythene bags. Any water collecting in the bottom of the bag should be periodically removed.

*Elimination of gut contents*

It is necessary that mussels collected for metal and pesticide residue analysis be allowed a period for the evacuation of the gut which may contain particulate material, since this can significantly add to the apparent body burden of contaminant. This can be accomplished by placing each sample of mussels for 24 hours in about 20 litres of sea water of naturally low contaminant content. This could be undertaken in the field, but would probably be more conveniently performed at the laboratory. Mussel should survive well for 4 - 5 days after collection, allowing time for their transport to a laboratory, provided that recommended storage conditions are maintained.

*Initial processing*

Following elimination of the gut contents the entire soft parts of the mussels should be removed using stainless steel equipment. The bulked soft parts should be weighed, homogenised*, a sample taken for dry weight determination, and the remainder stored deep frozen until analysis. All shells should be measured, to enable an assessment to be made of the effects on residues of the size of the animal.

*One member of the subgroup (Dr Bryan) has experienced metal contamination (Cr and Ni) of biological tissue by stainless steel homogenisers. Investigators of the metals should perhaps bear this in mind in their future studies.*
If pesticide residues are to be determined, provision should be made for measurements of the lipid content of the homogenate.

Information

The sampling position should be precisely noted, to enable the site to be re-sampled in the future. Descriptions of the population, degree of exposure to wave action, immediate environment, industrialisation of the surrounding country, proximity to effluents and freshwater discharges, and any other apparently pertinent information should be recorded at each site.

Selection of sampling sites

The position of sampling sites, and the distance between sites, will control the minimum area of contaminated coastline that will be detectable. Available resources, and the objectives of the survey, will also strongly influence the distribution of sampling sites. The aim of the national exercises already performed by the MAFF, DAFS, WWA and DAN1 was to assess the indicator ability of mussels, and identify fairly large areas of contamination. The mean sampling interval was about 20 - 30km. In producing a broad picture it was necessary that local influences were avoided. Samples were therefore not collected close to obvious sources of contaminants such as rivers and other discharges. Such an approach, however, may not be desirable for detailed regional surveys.

Frequency of surveys

The subgroup envisage that local or regional surveys might be undertaken fairly frequently in response to local requirements, whilst national exercises which require a higher level of organisation would occur at perhaps 5 - 10 year intervals to meet national or international requirements. The local projects would provide a more continuous source of useful data in relation to specific problems.

Whilst departures from the sampling scheme for a single survey (dg in time of year of sampling) might produce consistent data within a small area, it may be difficult to compare such surveys, or combine the data with national survey data.

Summary of main points of sampling scheme

1. Establish number of sampling points in the investigation area
2. Identify accessible populations of suitable organisms in the vicinity of each site.
3. Collect a minimum of 25 individuals of the dominant size at each site. NB the site must be exposed to sea water for six hours in each tidal cycle.
4. Store sample in plastic bag — naturally moist and cooled if possible. Return sample to laboratory within 5 days.
5. Record relevant site information.
6. On return to laboratory carry out depuration procedure.
7. Process soft parts for analysis and measure shell length and dry/wet weight relationship and if need be lipid content.
8. Analyse a suitable sample of the bulked material from each site for metals and/or halogenated hydrocarbons. If possible document the samples for long term storage for future reference.
Mussels are known to exhibit considerable natural variability in contaminant levels and this factor can influence studies which are designed to detect changes in residue levels arising from anthropogenic inputs to the sea. The minimum differences (spatial and temporal) in contaminant level which are detectable by the “mussel watch” type approach will depend on this natural variability and the precision of the analytical technique employed by the investigators. The concentrations of contaminants in mussels may vary within the same population and also between populations and the analytical precision may be poor for some elements or components and very good for others.

Gordon, Knauer & Martin (1980) have investigated trace metal variability in two populations of *Mytilus Californianus* through the analysis of randomly selected individual specimens on two separate occasions. They found mean coefficients of variation in the range 18 - 40% and concluded that a sample of 20 - 100 individuals/site was required for analysis to detect concentration difference of 20% between sites; differences of 40% could be detected by analysing about one-third as many samples. These results may be compared to those obtained by Bryan, Landgston & Hummerston (1980) for *Scrobicularia plana*, (da Costa) which indicate that a difference of 30 - 40% in metal concentration could be detected between samples from two sites by analysing three pooled samples each containing six animals.

A statistical examination has been made of the results of analyses of mussels (*Mytilus edulis*) collected from North Queensferry, Firth of Forth (Dr. I.M. Davies, personal communication). These results (Table IV) based on analyses of two groups of 12 mussels, with size ranges of 56 - 65mm and 66 - 75mm respectively, suggest that relatively small differences (10 - 20%) in residue levels may be detected between populations sampled as homogenates of 25 individuals. In theory this statistical approach is not strictly correct since account must be taken of the precision of the analytical technique employed. It should be noted that the errors from this source will not decrease with increasing sample size. Intercomparison exercises show that analytical precision is commonly of the same order as the calculated variations to be expected between homogenised samples of 25 mussels (Table V). It is likely therefore that detectable difference in concentrations of metals in mussels will be in the range 20 - 40%.

### Table IV

**Analyses of Individual Mussels from North Queensferry, Firth of Forth, Scotland**

<table>
<thead>
<tr>
<th></th>
<th>56 - 65mm</th>
<th>66 - 76mm</th>
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<tbody>
<tr>
<td>Pb</td>
<td>x</td>
<td>C.V.%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.69</td>
<td>28.7</td>
<td>5.7</td>
</tr>
<tr>
<td>Cd</td>
<td>0.5</td>
<td>14.1</td>
</tr>
<tr>
<td>Cu</td>
<td>1.13</td>
<td>25.6</td>
</tr>
<tr>
<td>Zn</td>
<td>33.5</td>
<td>31.1</td>
</tr>
<tr>
<td>Hg</td>
<td>0.24</td>
<td>35.8</td>
</tr>
</tbody>
</table>

### Table V

**Coefficients of Variation of Metal Determinations by DAFS Marine Laboratory for Fish Flour Reference Materials**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>7 - 15%</td>
</tr>
<tr>
<td>Cd</td>
<td>4%</td>
</tr>
<tr>
<td>Cu</td>
<td>7%</td>
</tr>
<tr>
<td>Zn</td>
<td>2%</td>
</tr>
<tr>
<td>Hg</td>
<td>12%</td>
</tr>
</tbody>
</table>
Gordon *et al.* (1980) stress that the first step in a “mussel watch” type programme should be an assessment of the natural variability of the mussel population at each site. This information would then be used to determine the size of the “pooled” sample to be used for subsequent measurements. It should be noted that the guidelines suggested by the subgroup for regional “mussel watch” programmes do not include this preliminary investigation. In the absence of this type of information the investigator will have to assess differences in concentrations of metals in mussels on the basis of the information presented here. Alternatively the investigator can initiate the type of preliminary study described by Gordon *et al.* (1980) but it should be understood that this will increase by an order of magnitude or more the total analytical effort devoted to this work during the first year of its operation.

The Role of Transplanted Metals

In certain areas mussels or other selected organisms may not be found. The subgroup suggest that consideration be given in such circumstances to the introduction of transplanted mussels. Mussels could be obtained from an uncontaminated stock and exposed in moored cages for a fixed period, say one month, in the area of interest. The accumulated contaminants would then be measured and used as indices of environmental quality. This approach has certain advantages in that it may be possible to obtain “cultivated” animals of known age and fairly uniform size. Variability may be further reduced by having control over the timing and duration of exposure. It is unlikely however that direct comparison will be possible between residues in transplanted mussels, and those in resident local mussels, without further research into the kinetics of uptake and depuration in the field.

An example of the use of transplanted mussels is given by Davies & Pirie (1978) who investigated the distribution and concentration of mercury in the Firth of Forth in relation to a known industrial discharge of mercury.

Analytical Considerations

In relation to coordinated monitoring of residues in biological tissue the Tactical Planning Team (DOE, 1979) also emphasised the need for effective calibration of methods of analysis. Analytical methods need careful intercomparison and intercalibration since future participants may have limited experience in the analysis of marine samples. In anticipation of the likely involvement of RWAs and RPBs in marine monitoring the MPMMG set up a subgroup on intercalibration in 1978. Unfortunately for a variety of reasons the subgroup made little progress and was eventually disbanded in 1979. It was recommended that a further effort in this direction be made once the extent of future involvement of RWAs and RPBs in marine monitoring is made clear. In the meantime DAFS and MAFF Fisheries Laboratories can provide advice and guidance on suitable analytical techniques based upon their experience within the framework of ICES intercomparison exercises. They are also prepared to conduct bilateral intercomparison exercises if required. In addition DAFS Marine Laboratory can make available to interested parties samples of the reference materials used in recent ICES intercomparison exercises for heavy metals.

Economic Considerations

The subgroup were asked by the MPMMG to provide some information on the likely costs of “mussel watch” programmes and trend monitoring studies. An account of the main costs is presented in Appendix V.

Biological Effects Monitoring within “Mussel Watch” Programmes

The subgroup recommended that serious consideration be given to carrying out studies of the biological effects of metals, organochlorines and hydrocarbon contamination on mussels, run in conjunction with regional “mussel watch” programmes, as a means of assessing the corresponding biological
impact of recorded contaminant levels in mussel tissue. In this respect the use of mussels has two further advantages:

1. Mussels are tolerant of a wide range of environmental stress; this tolerance is based on a flexible physiology, so that the sub-lethal effects of stress can readily be measured as alterations to physiological and biochemical steady-states.

2. Mussels have primitive detoxification systems which are not fully effective in ridding the individual of contaminants — hence their considerable capacity to accumulate metals and organochlorines. However, the detoxification systems present, though ineffectual, can be used to provide some evidence of the type of pollution that may be causing an observed stress response or decline in the health of the animal.

The causes of variability in the response by mussels to pollution are similar to those affecting residue levels; consequently, a sampling programme that is optimal for residue analysis should suffice equally for biological measurements, i.e. mussels collected in late winter from a standard tidal band on the shore and representing the dominant size class in the population. Analyses carried out at Institute of Marine Environment Research, Plymouth (IMER) indicate that a sample size of 15 individuals suffices for most biological measurements. Transplanted mussels can also be used successfully for effects studies. Animal size is a main cause of variation, but this variation can be reduced by the use of normalisation techniques described in the literature.

Mussels should be returned as rapidly as possible to the laboratory for biological measurements. Experiments have established that some physiological parameters and most cytological states typical of the animals in the field are maintained for 48-72 hours if the animals collected are transferred to sea water in the laboratory at field ambient temperature and salinity.

The various kinds of biological measurements that can be made to indicate the conditions of the animal (and therefore, by implication, the effects of the ambient environmental conditions including pollution) are discussed in recent publications, (ICES, 1978; McIntyre & Pearce, 1980), and in a forthcoming publication (Bayne & Widdows, in press). These include a wide variety of biochemical, histopathological and physiological responses. Some of these measurements require a minimum of sophisticated equipment and training.

The subgroup recognised that biological effects measurements will require skilled personnel and are labour intensive. It is therefore desirable that such measurements be made, at least initially, only where prior chemical analysis has suggested a pollution "hot spot". We therefore recommend that a biological effects study of the kind described, linked to a regional project on residue analysis, be carried out in the short term, to explore more fully the value of such measurements in environmental quality assessment.

The second part of the terms of reference of the subgroup concerns trend monitoring. The "mussel watch" approach discussed above is designed to monitor spatial differences and temporal trends in contamination from the point of view of assessing environmental quality. However, this may not adequately satisfy concern for public health. Mussels and similar organisms may not be fully representative of those marine species commonly consumed by man to allow firm statements to be made about residues in the major commercial species. Sampling of commercial species must therefore be considered. The principles of careful sampling to reduce variability applied in the mussel survey approach are equally applicable when monitoring a wider range of animals for public health purposes. In shellfish, little change from
the scheme developed above need be made but fish introduce some further factors for consideration.

Analysis of fish samples should be made of the edible tissue, usually the muscle tissue or fillet. Residues of metals are known to vary with age, length, weight and sex, whilst pesticide residues are dependent upon lipid content. From the limited number of species and contaminants so far investigated in all these respects it is not yet possible to state which variables generally exert the strongest influence for all combinations of contaminant and species. It is necessary, therefore, to build up for each field situation a fairly complete set of data and make any reduction in the sampling information after sufficient experience has been gained to identify the most important measurements.

A typical programme necessary for work of this kind based on recent work within DAIFS and MAFF fisheries laboratories would be:

(i) Selection of species based on knowledge of consumption patterns and availability.

(ii) Collection of a sample of about 60 fish well distributed over the size range encountered. It may be advisable to establish defined sampling objectives; for example, to divide the size range into 12 equal intervals, and take 5 fish per interval.

(iii) Measure the length and weight of each fish, determine its sex (if possible), and remove scales or otoliths for age determination.

(iv) Using stainless steel equipment remove and skin a fillet of edible muscle, avoiding any strongly coloured tissue adjacent to the skin. After homogenisation sub-sample a suitable quantity from the fillet for analysis.

(v) Determine the concentration of the contaminants of interest, including lipid determination if necessary.

(vi) Examine statistically the relationships between residues and age, length, weight, and sex to determine the sampling strategy necessary for the detection of significant changes in residues. The size of changes required to be detected will depend upon the aims of the programme, environmental quality objectives, public health standards etc. An example of the type of statistical analysis, and the sample size necessary for certain objectives is given in Appendix VI.

The design of trend monitoring programmes is a subject currently under discussion within the framework of the International Council for the Exploration of the Sea (ICES). The subgroup was informed that two of its members were involved in these discussions which had so far taken place principally by correspondence. The results of these discussions were presented to the ICES Statutory Meeting in October 1980 by Dr J. Uthe (Canada) who chaired the relevant subgroup. It is understood that this subgroup, consisting principally of chemists, reported that no agreement could be reached amongst the membership regarding the approach to be taken in relation to statistical treatment of monitoring data and that they recommend that a meeting of statisticians, chemists and biologists should be convened to resolve this problem.

Use of Artificial Substrata in Marine Pollution Monitoring

Previous sections of this report have discussed the use of bivalves in the monitoring of levels of contamination in coastal waters. It was pointed out that in order to make precise comparisons between sampling sites and from one sampling period to another it was necessary to follow specific sampling guidelines since variability of contaminant level in bivalves could be affected by such factors as age, sex, weight, condition factor etc. The replacement of a highly variable organism by a synthetic material, which can absorb the
contaminant from solution in proportion to the concentration might provide the investigator with certain advantages such as uniformity of material, ease of handling and storage, and ability to be deployed in an environment which did not support aquatic life. The use of such material could also result in considerable savings in terms of costs of the monitoring programme by reducing analytical costs. One disadvantage of synthetic materials is that, unlike bivalves, they may not provide relevant information on the biologically important component of the contaminant in question.

The subgroup felt that in view of the current limited “state of the art” they could make no positive recommendation about the use of synthetic material at this stage. It was agreed however, that it would be useful to review this subject (Taylor, 1980) for those organisations who may be interested in pursuing this alternative approach to monitoring.

A review of this subject (Appendix VII), which was prepared for the third meeting of the MPMMG Task Team, compared the advantages and disadvantages of using sediments for contamination monitoring, particularly for trace metals. This paper provides a good summary of the situation and required little further expansion. However, the following points perhaps merit further discussion.

Trend monitoring of contaminants can be broadly divided into two areas:

(i) Monitoring uncontaminated areas to detect increasing concentrations of contaminants.

(ii) Monitoring contaminated areas to assess the rate of recovery or further deterioration.

Sediments have probably little part to play in the former case, since contaminant levels would be low, by definition, and the disadvantages arising from our present lack of knowledge of sediment geochemistry and sediment movement, probably outweigh the advantages to be gained from the ease of sample acquisition and analysis. However, it might be useful to use sedimentary material, preferably in the form of core samples, as an historic archive representing present-day conditions. In future years this collection could be used as a reference for those materials which are not covered by present monitoring schemes. A total of 100 - 200, 1m core samples collected from relatively uncontaminated areas would provide a reasonable cover of UK coastal waters and should not present too large scale a problem of either collection or subsequent storage. A suitable body to undertake this work might be the Institute of Geological Sciences.

Sediments have a much wider application in the monitoring of contaminated areas. In highly contaminated areas, suitable animal species are often absent and transplanted animals may not survive or adapt successfully to the conditions. In such areas, the integrating capacity of the sediments, coupled with their relative ease of acquisition and analysis, makes them more suitable for monitoring purposes than the bulk water phase. In addition, since the anthropogenic component of the metal content of the sediment is usually large in such areas, differential leaching techniques are unnecessary to follow trends.

In conclusion, although the use of sediments in trend monitoring is limited to some extent by a lack of information with respect to transport processes and sediment geochemistry, data from sediments can be extremely useful in trend monitoring to assess the rate of recovery following reduction of a specific input or the impact of a new discharge on a pristine area.
Summary and Recommendations of the Report by the MPMMG Subgroup on “Mussel Watch” and Trend Monitoring

1. The concept of “mussel watch” type programmes has been discussed and an examination made of the work done to date by UK laboratories.

2. It is concluded that these programmes have confirmed known “hot spots” and identified new ones.

3. It is recommended that a “mussel watch” type programme be implemented in the near future on a regional basis to identify trends in the concentrations of contaminants. Priority should be given to “hot spots” identified in the National survey for those contaminants on the priority lists of the EC, the Paris and Oslo Commissions.

4. Guidelines for trend monitoring using “mussel watch” type programmes are presented. Details are given of suitable organisms, selection of sites, number of samples, frequency of surveys, analytical consideration, detection of differences in contaminant levels, use of transplanted mussels and economics of “mussel watch”.

5. Uptake and loss kinetics for hydrocarbons in mussels are highly complex and variable with compound, as a consequence their use in trend studies, especially temporal trend studies, is limited. The subgroup therefore recommends that hydrocarbons should not be included in regional programmes at present. Further investigative work is needed on this subject.

6. Guidelines are also presented for trend monitoring programmes using edible fish. One example of the statistical approach to the selection of sample numbers is presented and an indication of costs is given.

7. The subgroup recommend that biological effects monitoring should be incorporated into “mussel watch” programmes. Initial studies of this kind should be confined to “hot spots”.

8. The role of artificial substrata in marine pollution monitoring is considered. The subgroup were unable to make any recommendations at this stage in view of the current “state of the art”.

9. The role of sediments in trend monitoring is discussed and the subgroup refer to situations where sediment monitoring would provide useful information on changes in contaminant levels.
References


Appendix I. Members of Subgroup

Dr G. Topping — Chairman
DAFS Marine Laboratory, Aberdeen
Dr I.M. Davies — Secretary
DAFS Marine Laboratory, Aberdeen
Dr B.L. Bayne
Institute of Marine Environmental Research, Plymouth
Dr G. Bryan
Marine Biological Association of the UK, Plymouth
Mr W. Halcrow*
Welsh Water Authority, Brecon
Dr J.E. Portmann
MAFF Fisheries Laboratory, Burnham-on-Crouch
Dr J.H. Stoner*
Welsh Water Authority, Brecon
Dr D. Taylor
ICI, Brixham

*Mr Halcrow was initially appointed to represent the interests of the Scottish River Purification Boards. Following his appointment to the Tidal Waters Section of WWA (in February 1980) he replaced Dr Stoner as representative of WWA. In view of the short life of the subgroup he also continued to act as a representative of the Purification Boards.

Appendix II. Terms of Reference for the MPMMG’s Subgroup on “Mussel Watch” and Trend Monitoring

1. Development of a suitable framework within which to implement a “mussel watch” type programme in the UK which will provide guidelines for the use of biological material in first order pollution assessment and monitoring programme and which will include the specification of organism(s) to be used. The guidelines would need to cater for regional, national and international needs and obligations and to allow for appropriate organisations to make a suitable contribution to the execution of a coordinated national programme of this kind (c.f. pages 5 and 6, and p.34 paragraph 2 of the second tactical plan*).

2. Development of a specification for trend monitoring using biological material, especially fish and shellfish, including guidelines as to the limitations required on sample selection and collection in order to minimise extraneous variability (pages 6 and 7, and page 34 paragraph 3 of second tactical plan).


Appendix III. The Main Groups of Compounds or Substances Listed in the Oslo and Paris Conventions and EC Directive Oslo Commission Black and Grey lists

Black
Organohalogen compounds*
Organosilicon compounds*
Carcinogenic compounds*
Mercury and mercury compounds
Persistent plastics and other floating synthetic materials

Grey
Arsenic, lead, copper, zinc and other compounds
Fluorides, cyanides
Pesticides not already banned
Solid bulky objects

*With certain exclusion clauses
<table>
<thead>
<tr>
<th>Paris Commission Black and Grey lists</th>
<th>EC Directive Black and Grey lists</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Black</strong></td>
<td><strong>Black</strong></td>
</tr>
<tr>
<td>Organohalogen compounds*</td>
<td>Organohalogen compounds*</td>
</tr>
<tr>
<td>Mercury and mercury compounds</td>
<td></td>
</tr>
<tr>
<td>Cadmium and cadmium compounds</td>
<td></td>
</tr>
<tr>
<td>Persistent plastics and other floating synthetic materials</td>
<td></td>
</tr>
<tr>
<td>Persistent oils and hydrocarbons of petroleum origin</td>
<td></td>
</tr>
<tr>
<td>*With exclusion clause</td>
<td></td>
</tr>
<tr>
<td><strong>Grey</strong></td>
<td><strong>Grey</strong></td>
</tr>
<tr>
<td>Organic compounds of P, Si, Sn*</td>
<td>Zn, Cu, Ni, Cr, Pb, Se, As, Sb, Mo, Ti, Sn, Ba, Be, B, U, V, Co, Th, Ag and their compounds</td>
</tr>
<tr>
<td>Elemental phosphorus</td>
<td>Biocides not in the black list</td>
</tr>
<tr>
<td>As, Cr, Cu, Zn, Ni and Pb and their compounds</td>
<td>Substances which taint marine products</td>
</tr>
<tr>
<td>Substances which taint marine products</td>
<td></td>
</tr>
<tr>
<td>Non persistent oils and hydrocarbons of petroleum origin</td>
<td></td>
</tr>
</tbody>
</table>

**Appendix IV. Priority Contaminants in Regional “Mussel Watch” Programmes**

In relation to the EEC Directives, the Commission and its members countries have agreed that attention cannot be paid to all the listed substances equally and at the same time it has therefore been agreed that attention be paid first to certain “Priority” substances. These are:

- **First Priority**: Hg, Cd, Dieldrin. (Aldrin and Endrin).
- **Second Priority**: PCBs, HCH (α and γ), HCB, DDT and family.
- **Third Priority**: HCB, Pentachlorophenol and trichlorophenol. Eulan, Mitin.

The same group of substances are likely to be adopted by the Paris Commission.

**Appendix V. Economics of “Mussel Watch” and Trend Monitoring**

The main points to be considered under this heading are the actual time involved in

(a) Desk Studies;
(b) Collection of Samples;
(c) Processing of Samples;
(d) Analysis of Samples; and
(e) Use of transplanted mussel.
Desk Studies

The project leader will be required to spend time on

(1) planning and designing programme — identifying suitable sites, selecting appropriate organisms, drawing up guidance sheets for sampling, processing and analysis; and

(2) evaluating data and writing a report.

It is estimated that these tasks may occupy him for 4 weeks on a full time basis.

Collection of Samples

The collection of mussel samples at a number of sites will involve two people (usually assistants) who may spend 30 - 40 minutes at each site. Bearing in mind that travel time between sites may be in the range 30 - 40 minutes for sites which are separated by 10 - 15 miles and that in any one day the time spent on total sampling will be restricted to 6 hours (±3 hours of Low Water) it is estimated that 4 - 6 Sites can be sampled each day. A typical programme involving 15 - 20 sites would therefore require 6 - 8 man days.

In relation to trend monitoring staff will be required to visit the fish market or contact fishermen to:

(i) Arrange the purchase of specimens; and
(ii) Identify relevant species and select appropriate number of individuals of a specified age/length/weight range.

Within the Government fisheries laboratories these arrangements would follow a well established procedure and would probably require one member of staff to spend ½-1 day at each fish market.

Processing of Samples

Within the Government fisheries laboratories this work is normally allocated to two people (assistants) who take turns to shuck samples, prepare homogenates and record essential data (length, weight, age, shellsize). On the basis of previous work ca 300 - 400 specimens of mussels can be processed by two people in one day. The processing of fish samples can be done by two people at the rate of 60 - 80 fish per day. The ageing of fish is normally done separately by counting the rings on scales or otoliths (ear bones); an experienced person can process 150 - 200 samples each day.

Analysis of Samples

The time spent by the analyst on this work will depend on:

(i) his experience and analytical facilities;
(ii) the total number of samples to be analysed; and
(iii) the range of contaminants to be measured.

Within DAFS and MAFF fisheries laboratories the following rates of analyses currently apply to this type of work:

Metals — ca 40 samples can be analysed for Hg, Pb, Cd, Zn, Cu and As in 1 man week

Halogenated hydrocarbons — ca 6 samples can be analysed for the principal components in 1 man week.

The actual cost of running a "mussel watch" programme is difficult to assess without information on unit costs of administration, transport, analytical facilities and staff. In their report on the US Mussel Watch Goldberg et al. (1978) reported the following costs:
Collection of Samples – $1000 per sample
Analyses of Samples – metals – $50 per sample
– halogenated hydrocarbon
Total cost of
100 samples
$120,000

By contrast the total cost of the Scottish “mussel watch” in 1978 for metals and halogenated hydrocarbons was estimated at £6,500 (= $13,260) based on collection costs of £18 per sample, analysis of metals at £6.50 per sample and analysis of halogenated hydrocarbons at £40 per sample.

Transplanted Mussels

The main costs to be considered in this type of study are:

(i) Purchase of suitable mussels (uniform size and age) from fish farmer plus cost of transporting them to area under study or cost of collecting mature mussels from a “clean environment”. Current cost (1981) of cultivated mussels was ca £5 per 1,000 mussels. The costs of transporting mussels to study area will depend on mileage involved. The cost of collecting mature mussels from a clean environment by laboratory staff can be based on two man days plus transport costs.

(ii) Each mooring system consists of three anchors, one plastic coated wire mesh cage, a buoy and several lengths of rope (Appendix Fig. 1). Current costs of these components are as follows:

- anchor – £15 each, cage – £10 each, and rope – £30 for each mooring.

Three to four moorings can be prepared in one day by one member of staff.

A - Surface float 30-40 inches (0.76-1.0m) circumference
B - Plastic coated wire mesh cage (60 x 40 x 10cm), 1cm mesh size with suitable ropes to allow attachment to float and anchoring system
C - Rope to anchoring system with sufficient slack to allow for tidal height changes, and for the cage to be lifted into a small boat for sampling without disturbing the anchors (length = ca 1½ times depth of water)
D - Light ground chain (length = ca 1½ times depth of water)
E - 20 kg (approx) anchors. The middle anchor may be replaced with a weigh
F - Light ropes to small floats to assist in laying and recovering the mooring (length = ca 1½ times depth of water)

Appendix Figure 1.
Deployment of mussel cage mooring
(iii) Following the transport of the moorings to the study area the moorings are deployed at the specified sampling position from a suitable boat, eg a 13ft (4m) Dory powered by an outboard engine. Assuming that sampling stations are no more than 5 miles apart it is possible to deploy 3-4 moorings per day using three members of staff.

(iv) Each visit to the study area will involve 3 members of staff for 1 day.

(v) Analytical costs — see discussion under (d) above.

Appendix VI.
Trend Monitoring: An Example of a Statistical Approach to Determine Sample Numbers

During 1976 DAFS collected 52 samples of common ling (Molva molva L.), from the North Sea, ranging in length from 50 - 140cm, in weight from 600 - 14,800g and in age from 5+ to 16+. Following the necessary processing a sample of the muscle tissue from each fish was analysed for mercury content. Concentrations of mercury in these samples were plotted against age, length and weight (Appendix Figs. 2, 3 and 4). On the basis of these results it was decided to carry out a multiple regression analysis of the form

\[ y = b_0 + b_1 x_1 + b_2 x_2 + b_3 x_3 \] .......................... (1)

where \( y \) = concentration of mercury

\( x_1 \) = age of fish

\( x_2 \) = length of fish

\( x_3 \) = weight of fish

to identify the most significant variable(s).

Equation (1) was found to explain 74.4% of the total variability in mercury concentration. However, the variable \( x_2 \) alone was found to explain 74.4% of the total variability ie the high correlations between variables indicated that all of them acting together are no more effective than the best of them acting alone. The equation (1) can therefore be reduced to

\[ y = 0.05392 + 0.00002123 x_2 \] .......................... (2)

On the basis of equation (2) it was decided to fit an empirical relationship of the form \( y = a \text{(length)}^b \) or \( \log_e y = \log_e a + b \log_e \text{(length)} \).

The relationship was shown to be

\[ \log_e y = 10.5 + 1.88 \log_e \text{(length)} \] .......................... (3)

and was found to explain 81.7% of the total variability of mercury in common ling.

Having determined the principle variable affecting mercury concentration in a population the next step is to calculate the necessary sample size for trend monitoring.

The sample size required to detect a difference in the means of two samples depends on the size of the difference to be detected and on the variability in the samples. The sample size required for each sample is given by

\[ n = \frac{M \sigma^2}{\delta^2} \] .......................... (4)

where \( n \) = number of samples.
\[ \sigma^2 = \text{variability in each sample (assume to be equal)} \]
\[ \delta = \text{size of the difference to be detected} \]

\[ M \alpha \rho' = \text{multiplier which depends on } \alpha = \text{the significance level of the test to be used and } \rho' = \text{the probability of detecting the difference} \]

Appendix Figure 2.
Mercury concentration in ling against age.

Appendix Figure 3.
Mercury concentration in ling against length (Key as Appendix Fig. 2).
Appendix Figure 4.
Mercury concentration in ling against wet weight (Key as Appendix Fig. 2).

For the purpose of this exercise values of 0.8 and 0.9 for \( p \) and a significance level of 5% \( \alpha = 0.05 \), have been adopted. Map also depends on whether the significance test to be carried out is to be "one or two-tailed". If one is interested in assessing both positive and negative differences; then a "two-tailed" is required. If however one is only interested in an increase of mean concentration then a "one-tailed" test should be carried out.

Using equation (4), and the data used to calculate equation (3), it is possible to determine the samples sizes needed to detect differences in mercury concentration between separate samples of common ling. The results of these calculations are given in Appendix Table I.

It can be seen from the data in Appendix Table I that as many as 900 fish are required to detect an increase or decrease in mercury concentration of 10% with a probability of 0.9, corresponding numbers to detect differences of 20% and 30% are 250 and 125 respectively. If however one constraint is applied to the sampling programme namely the length of fish selected is confined to the range 70-89 cm this has the effect of reducing \( \sigma^2 \) from 0.40 to 0.15, then the required sample numbers is reduced considerably ie as few as 30 samples are required to detect a 30% increase in mercury concentration.

The sections above provide one example of the approach to trend monitoring. MAFF Fisheries Laboratory has described similar studies on blue whiting, flounder and cod (Portmann & Woolmer, 1979) as part of the same study within the ICES framework. In relation to blue whiting these authors report that a sample of 50 fish is required to detect a change of 30% in metal levels at the 5% significance level.
Appendix Table I

Sample Size for Mercury Concentration in Ling

<table>
<thead>
<tr>
<th>( \alpha^2 )</th>
<th>( p' )</th>
<th>1 or 2 tailed</th>
<th>( M_{\text{up}}' )</th>
<th>( d )</th>
<th>( n )</th>
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\[ \alpha = 0.05 \]

\[ n = M_{\text{up}}' \left( \log(e + \frac{d}{100}) \right)^2 \]

Appendix VII.
Monitoring of Contaminants in Sediments (prepared for the 3rd meeting of the MPMMG Task Team, by Dr J.D. Burton, University of Southampton)

The association of pollutants with sediments raises considerations of several kinds in the context of pollution monitoring:

1. Measurements on sediments may be required in relation to the dumping of solid wastes which become mixed with the sediments of the dumping areas.

2. For dissolved or dispersed pollutants which become associated with particulates, monitoring by measurements on sediments offers advantages over water monitoring, independently of any requirement to monitor the pollution of sediments as such. Adequate sensitivity is obtainable with relatively small samples and problems of contamination during sampling and changes in composition during storage are reduced. The transient effects of rapid, short-term fluctuations in environmental concentrations are avoided by the use of sediments which in effect reflect integrated concentrations in the environment concerned. It is also possible to return to a sediment sampling site and obtain further comparable material if it is necessary to confirm or follow up observations of unusual effects. The advantages of measurements...
on sediments are most apparent for heavy metals but could apply to some other pollutants, such as organic substances. For hydrocarbons, fractionation of individual compounds or groups on uptake by sediments can complicate interpretation. Of the nutrient ions, nitrate shows little affinity for sediments while phosphate shows complex sorption reactions which would greatly complicate the use of sediments as indicators of water levels.

(3) Suitable cores of undisturbed sediments may provide a record of pollution history with the possibility of independently determining absolute time scales for the events recorded.

The interpretation of data on sediments requires, however, careful consideration of a number of factors which may influence concentrations. Analytical approaches must also take account of the nature of sedimentary associations. Some important aspects are summarised here with reference to heavy metal pollution.

(a) Sediment transport. The chemical characteristics of sediments in a given area do not necessarily reflect processes in the overlying water column but may be influenced considerably by material transported over considerable distances to the site of deposition. Information on sediment transport pathways and patterns of deposition is necessary if inputs of pollutants to an area are to be related to accumulation in sediments. It must also be borne in mind that pathways may change with environmental conditions, especially in areas such as estuaries, a factor which is especially relevant to the use of sediments as indicators of temporal changes.

(b) Effects of mineralogy and particle size. Elements present in sediments, whether they originate in natural weathering and transport processes or anthropogenically, are associated to differing extents with various minerals. Some constituents such as quartz and carbonates are essentially diluents for heavy metals. Aluminosilicates in particular adsorb metals on their surface and thus fine grained material shows significantly higher concentrations than coarser material in a given region. Wide variations in heavy metal concentrations can arise through these factors and for monitoring purposes normalisation is necessary either by conversion of data to a comparable basis of bulk composition and particle size or by selecting comparable deposits on a basis of mineralogical and sedimentological criteria.

(c) Redistribution of material in the sediment column: mixing and diagenetic mobilization. These factors are especially relevant to the use of sediments as records of temporal changes. Material in the sediment column can become redistributed as a result of the reworking of the deposited particles. This may be brought about by physical processes causing resuspension or by bioturbation. The effects may be recognised by direct evidence of reworking or by the uniformity of “age” of the sediment with depth when geochronological techniques are applied. Redistribution can also be brought about by physiochemical changes in the sediment. Such changes, especially in redox conditions, can modify the distributions between particles and intersitial waters and set up concentration gradients in the pore waters along which diffusion can occur. By this
mechanism considerable changes in vertical distribution may arise.

(d) Sampling and analytical aspects. Sampling of surface sediments needs to take account, among other factors, of possible vertical inhomogeneities and the fact that boundaries such as those in redox conditions may occur at different depths in different areas.

Heavy metals are associated to different extents with the various components of sediments and these associations can be quite different for the natural and anthropogenic material. Thus for a number of heavy metals substantial fractions occur in the lattice positions of minerals. Anthropogenic inputs of these metals will necessarily be associated with non-lattice fractions. Total dissolution of sediments is analytically demanding and for many monitoring purposes sediments are leached to remove the non-lattice held material and, with many of the reagents employed, part of the lattice-held material. Such approaches are probably valid for purposes of comparing levels, provided that adequate criteria in the selection of sediments, as discussed above, are employed. For the purposes of understanding pathways and transfer processes, however, a more detailed knowledge of the association of heavy metals with particular components (e.g., organic matter, authigenic hydrous oxide phases) is needed and some effort has already been devoted to the development of sequential selective chemical leaching techniques to estimate the fractions of metals associated with such bulk phases. These methods are at best selective, not specific, and they lead to operationally defined fractions rather than true phase distributions, but they offer a considerable advance upon the information obtainable by analysis of whole sediments or single leachates obtained using strong reagents.

These comments indicate that while sediments afford analytical advantages for pollution monitoring, a considerable amount of ancillary information and a careful choice of analytical approach are essential if the data obtained are to be susceptible to meaningful interpretation.