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Coastal Zone Information Center

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POLLUTANTS IN THE AQUATIC ENVIRONMENT:

Detection, Measurement and Monitoring

Lectures presented at the Second FAO/SIDA Training Course
on Marine Pollution in Relation to Protection of Living Resources

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LECTURES PRESENTED

at the

SECOND FAO/SIDA TRAINING COURSE ON MARINE POLLUTION IN RELATION TO PROTECTION OF LIVING RESOURCES

Methods for Detection, Measurement and Monitoring of Pollutants in the Aquatic Environment

Gothenburg and Stockholm, Sweden
31 July - 9 September 1973

(SUPPLEMENT TO THE REPORT)

U.S. DEPARTMENT OF COMMERCE NOAA
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PREFACE

Following the FAO Technical Conference on Marine Pollution and its Effects on Living Resources and Fishing (Rome, December 1970), collaboration between the Swedish International Development Authority (SIDA) and FAO was extended to aspects of training in the field of marine pollution and plans were developed for a series of Training Courses on Marine Pollution in Relation to Protection of Living Resources.

So far, four courses of this series have been held.

The first course (Gothenburg, Sweden, May-June 1972) was arranged for senior scientists and research managers from developing countries in charge of planning investigations or developing of control or monitoring programmes. The course provided a broad review on pollutants known to be hazardous to marine life and fisheries, their fate and effects. Related research and monitoring problems and scientific and administrative elements of importance for management measures were reviewed.

The second course, held in Gothenburg and Stockholm in July-September 1973, was arranged for research workers from developing countries (biologists, chemists, technicians, etc.) and was oriented towards planning and conduct of research and monitoring activities giving training in techniques and equipment currently used for the detection and measurement of pollutants in the aquatic environment.

The third course (Lima, Peru, February-March 1975) was arranged for participants from Latin American countries with the purpose of providing a scientific basis for the protection of living resources from pollution and for management of the quality of the aquatic environment.

The fourth course held in Lysekil, Sweden, in October-November 1975 was arranged for scientists from developing countries providing training in, and demonstration of, bioassays and toxicity testing techniques currently used for biological monitoring and establishing of water quality criteria.

This volume contains a selection of the lectures given at the second course. It was completed by the staff of the FAO Fishery Resources and Environment Division, particularly Mr. A. Wenblad. Editorial assistance was given by Dr. R. Vaz, SNU Special Analytical Laboratory, Wallenberg Laboratory, Stockholm, Sweden. The views expressed are those of the individual lecturers and are not necessarily those of FAO or SIDA.

Based on papers on analytical techniques for pollution measurements, presented during the second course, the Manual of Methods in Aquatic Environment Research, Part 1, Methods for Detection, Measurement and Monitoring of Water Pollution has been published (FAO Fish. Tech. Pap. 137).

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PRIMARY PRODUCTION AND NUTRIENTS IN SEA WATER

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1. INTRODUCTION

The biological activity in sea and lake water is regulated by light, transparency of the water, temperature, and availability of nutrients, vitamins, hormones, trace elements and other growth-regulating factors.

The availability of nutrient salts often limits the growth or organic production. Nutrient salts are necessary for the organisms and are taken up by them. When the organisms die, they are decomposed through bacterial breakdown or oxidation. The nutrients are thus brought back into the water in inorganic form. Because the organisms sink when they die the nutrient salts are removed from the surface water and accumulated in the deep water. They are also taken down in convergence areas or through the sinking of cold water during winter. In upwelling areas nutrients are brought back to the surface. In some areas the winter convection may also bring up nutrient-rich water to the surface.

2. UTILIZATION OF NUTRIENTS FOR PRIMARY PRODUCTION

According to Liebig's law of minimum, the growth and yield of a crop is dependent on that nutrient which is present in a minimum. The nutrients which may limit the production in water are phosphorus and nitrogen. Both may reach concentrations close to zero in natural waters. In order to be available for the phytoplankton they have to be in inorganic or very simple organic form, the phosphorus as phosphate and the nitrogen as nitrate, nitrite, ammonia, urea or some amino acids.

In fresh water the availability of phosphorus is generally the nutrient factor that limits production. In the oceans the nitrogen supply is considered to be most limiting. But after a high phosphorus increase, nitrogen and not phosphorus may act as the limiting factor in inland and brackish waters. Inorganic carbon may act as the limiting factor for the phytoplankton production in extremely productive and alkaline waters. Many metals (e.g. iron, manganese, cobalt, molybdenum, zinc and sometimes also magnesium) and chelating substances have such low concentrations (especially in nutrient-poor waters) that they limit the growth of algae.

Elements are removed from the water phase by formation of organic matter through photosynthetic assimilation. These elements are utilized in constant proportions for forming phytoplankton, and also to a smaller degree for higher levels of the food chain. The

elementary composition of living organic matter is for every species quite constant. Carbon, hydrogen and oxygen form the main part of organic matter, roughly in the proportion CH_2O (12:2:16). There are also other elements, important for the organism, present in organic matter in constant proportions. Analysis of large plankton samples which represent the main biomass in the open sea have given quite constant proportions for carbon, nitrogen and phosphorus.

Table I

Atomic Ratios of the Principal Elements in Plankton
(Fleming, 1940)

	C	N	P
Zooplankton	103	16.5	1
Phytoplankton	108	15.5	1
Average	106	16	1

The free oxygen which is produced in the synthesis or is utilized in the decomposition of the plankton should be 212 atoms for each phosphorus atom if only carbon is oxidized. We assume according to Richards (1965) that the biomass has the following general composition and that it decomposes as shown below:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 = 106\ CH_2O + 16\ NH_3 + H_3PO_4$$

$$106\ CH_2O + 106\ O_2 = 106\ CO_2 + 106\ H_2O$$

$$16\ NH_3 + 32\ O_2 = 16\ HNO_3 + 16\ H_2O$$

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 138\ O_2 = 106\ CO_2 + 122\ H_2O + 16\ HNO_3 + H_3PO_4$$

As can be seen, four atoms of oxygen are utilized in oxidizing each atom of nitrogen and the total oxygen consumption is 138 molecules. The O:P ratio will then be 276:1.

These ratios make it possible to estimate the average proportions in which the concentration of nutrients in sea water may be expected to change due to biological activity. The changes observed in the composition of the nutrient concentrations seem to confirm these theoretical considerations. The composition of plankton has here been assumed to be constant, which of course is not always true. The composition of the different species in the biomass is variable and dependent on time and place. Each species may be expected to have a somewhat different chemical composition.

3. SOME FACTORS LIMITING PRIMARY PRODUCTION

3.1 Nitrogen and phosphorus

It has been shown through culture experiments that the chemical composition of unicellular algae changes when the composition of the medium in which they grow is altered. If the concentration of one element is very low in the medium, relative to the need of the organism, cell growth and division can continue for some time. The cells which are produced contain smaller amounts of the deficient elements than the normal cells. When there is an excess of an element in the medium, the cells can increase their content of this element (luxury consumption).

When plankton growth in the sea water increases, available nitrogen and phosphorus dissolved in the water are greatly reduced. In Long Island Sound, according to Riley and Conover (1956), nitrogen seems to be the element available in minimum proportions relative to the needs of the phytoplankton. Its ratio to phosphorus varied there from almost zero to 8:1. The N:P ratio in the plankton was found by Harris and Riley (1956) to be on an average 16.7:1. In spite of the abnormal ratio of N:P in the water, the plants seem to assimilate the elements in the normal ratio of 16:1 until the nutrient concentrations reach extremely low values. When the nitrogen is nearly exhausted from the water phase the phytoplankton seem to be able to continue to utilize phosphorus, which is present in surplus proportions.

Most of the common algae will obtain comparable growth rates with nitrate, nitrite or ammonia. When all three nitrogen sources are present simultaneously in the water, many forms seem to prefer ammonia. According to Vaccaro (1965) this may depend on a toxic effect of the enzymatic reduction of nitrate. It has been shown that starved Chlorella cells given equivalent amounts of nitrogen recovered faster with ammonia than with nitrate. It has also to be pointed out that ammonia is often the only form of nitrogen available in the surface water in measurable amounts during high productive seasons.

It is difficult to explain how phytoplankton can continue to form cells of normal chemical composition when they grow in sea water from which an essential nutrient may be almost absent. According to Redfield et al. (1963), this could be explained in the following way. Mixing processes may transport nutrients from deeper layers up to the euphotic surface water, e.g. eddy diffusion may deliver phosphorus and nitrogen to the surface layer in a higher ratio than normally occurs in this layer. When dead plankton cells decompose, phosphorus and nitrogen are also regenerated in a different ratio than their composition indicates. There is also the possibility that phosphorus for example, is regenerated faster and that nitrogen is retained in the marine humus, which may be quite stable. According to Redfield et al. (1963) the nutrients present in the water represent only the residue of elements, which are not required for the algal cell composition.

There does not seem to be any proof that natural algal populations may form cells with an abnormal composition. In laboratory experiments with algal cultures it has, however, been possible to grow cells with nutrient deficiencies.

Redfield et al. (1963) have also established a norm for surface water regarding nutrients. Thus deviations from the norm can be expressed. As the norm for phosphorus a value based on the conditions in the deeper water of the Antarctic Ocean was used. They derived a nitrogen norm from this value using the ratio 15:1. For the carbon concentration the value given by Sverdrup et al. (1942) was used. The oxygen value was taken as the oxygen saturation value for normal ocean water at +2°C. Table II shows the ratios in which these elements are available in "normal" sea water and those in which they are used for the formation of organic matter. The ratio of availability to utilization is also given.

It can be seen that nitrogen and phosphorus occurring in sea water have almost exactly the proportions in which they are utilized. This was first observed by Harvey (1926) in the English Channel. During the growing season both nutrients disappeared from the surface water.

We can also see that carbon is present in a large excess (ten times the quantity which can be utilized) if phosphorus and nitrogen limit the production. Therefore carbon cannot be a limiting factor in normal sea water. There will always be enough carbon for building up organic matter. Often there are small residues of nitrogen or phosphorus left in the water, depending on the nutrient that has been completely utilized. It is of course clear that local variations may occur in the surface water resulting in differences in the composition of the water.

Table II

Availability of Nutrient Elements in "Average" Sea Water (S = 34.7% T = 2°C) and the Ratios of their Availability and Utilization by Plankton (Redfield et al., 1963)

	Availability in "average" sea water		Utilization by Plankton	Ratio of availability to utilization
	µg-at/l	ratio	ratio	
Phosphorus	2.3	1	1	1
Nitrogen	34.5	15	16	0.94
Carbon	2 340	1 017	106	9.6
Oxygen saturation value	735	320	276	1.16

3.2 Other constituents

In nature, there are often several factors which may influence growth rate. In sea water we have for example, trace metals such as iron, manganese, copper, zinc, molybdenum and cobalt present in such small amounts that they may limit production. Menzel and Ryther (1961) have shown that addition of iron to water where the natural plankton growth has come to an end due to lack of nutrients can stimulate growth. They used surface water from the Sargasso Sea. They did not get the same effect with other trace metals. Harvey (1947) showed that manganese had the same effect for Chlamydomonas in water from the English Channel. Hormones, vitamins and other organic compounds may have similar effects. The main limiting factors for phytoplankton production in the sea seem, however, to be nitrogen and phosphorus. Other factors may locally be limiting, especially in coastal areas, but are not of general importance.

4. POTENTIAL FERTILITY OF SEA WATER

Harvey (1947) has proposed that the concentration of total phosphorus or total nitrogen in the water may be used to determine its potential fertility. The potential fertility is defined as the quantity of organic matter which can be produced by photosynthesis in a unit volume of sea water if it is illuminated until the limiting nutrients are exhausted. Redfield et al. (1963) applied this on the values in Table II. They assume that the carbon of organic matter is 50 percent of the dry weight. When all nitrogen has been exhausted, organic matter with a dry weight of 5.48 mg/l and containing 2.74 mg C/l has been formed. If we assume the dry weight/wet weight ratio is 0.2, the formed plankton would be 28 mg/l or 28 g/ton.

It should be stressed that this is the maximum amount that could be produced in the photic zone if the conditions are ideal. In reality such amounts are never formed. Ryther (1966) estimated that the plankton concentration in ocean water is normally only 1:3 000 000 expressed as wet weight. He assumed that all plankton is formed in the upper 100 metres of the water and that there are 3 g plankton carbon per m^2 of sea surface. From these estimates we can see how low the real fertility of ocean water is compared to the potential fertility. There is only 0.3 g of living plankton matter in 1 ton of ocean water.

5. ANAEROBIC DECOMPOSITION OF ORGANIC MATTER

From Table II we can see that the oxygen reserve of the ocean water is not very large. When there is considerable accumulation of organic matter in an area the dissolved oxygen in the water may be completely used up. This will lead to anoxic conditions in the water. The oxidation of organic matter may however still continue through anaerobic bacterial processes. These processes begin with denitrification where nitrate is transformed into nitrite and then into nitrogen gas or ammonia. When this process is completed, sulphate ions may serve as oxygen donors being transferred into hydrogen sulphide. Finally carbon dioxide may be transferred into methane losing its oxygen to the oxidation process. The reduced products (nitrogen gas, ammonia, hydrogen sulphide and methane) accumulate in the water together with the oxidation products of the organic matter (phosphate, ammonia and carbon dioxide). This accumulation of ions and gases will change the normal proportions of the components in sea water, giving it different characteristics.

Redfield et al. (1963) suggest the following reactions for oxidation of organic matter in the presence and absence of dissolved oxygen with CH_2O representing organic matter.

Oxidation by oxygen (dissolved)

$$CH_2O + O_2 \longrightarrow CO_2 + H_2O$$

$$NH_3 + 2O_2 \longrightarrow HNO_3 + H_2O$$

Denitrification

$$5CH_2O + 4HNO_3 \longrightarrow 5CO_2 + 2N_2 + 7H_2O$$

$$5NH_3 + 3HNO_3 \longrightarrow 4N_2 + 9H_2O$$

Sulphate reduction

$$2CH_2O + H_2SO_4 \longrightarrow 2CO_2 + H_2S + 2H_2O$$

NH_3 is not oxidized

It is also possible to assume that phosphate may be reduced to phosphine.

Phosphate reduction

$$2CH_2O + H_3PO_4 \longrightarrow 2CO_2 + 2H_2O + PH_3$$

According to Soviet scientists phosphine is found in the bottom water of the Sea of Azov.

Finally we have the possibility of carbon dioxide reduction in which methane may be formed.

Carbon dioxide reduction

$$2CH_2O + H_2CO_3 \longrightarrow 2CO_2 + H_2O + CH_4$$

Methane has been found in the deep water of the Black Sea and in some anoxic fjords in Canada. This formation seems to begin before all sulphate is used up. Methane is generally formed in the sediments of stagnant oxygen-free lakes. It is also formed in the bottom mud of shallow salt water lakes or lagoons if the water has a heavy organic load.

The free energy determining the different oxidation steps decreases in the order: oxygen, (dissolved) nitrate, sulphate, carbonate, when these are the hydrogen acceptors. Therefore the free oxygen is utilized first when it is available in the water. Then the nitrate is

utilized. When the nitrate has been exhausted, the oxidation continues with reduction of sulphate. Apparently there is not sufficient free energy left to oxidize the ammonia to nitrogen gas in this step. Therefore ammonia is accumulated during the sulphate reduction. As an intermediate step in the nitrate reduction, nitrite is formed and it is also formed in the oxidation of ammonia to nitrate.

According to Codispoti (1973) denitrification begins when the oxygen concentration is below 0.1 ml/l. This has also been shown for lake water. Nitrite is found in relatively high concentrations in the oxygen minimum layer off the Mexican coast in the Pacific and also in anoxic basins in the border layer between oxygen and hydrogen sulphide. The end product of the denitrification process is, according to Codispoti (1973) most probably free nitrogen gas. The deep water of the Black Sea is supersaturated with free gaseous nitrogen. Richards and Benson (1961) have examined the accumulation of free nitrogen in the anoxic waters of the Dramsfjord and the Cariaco Trench. They compared the observed nitrogen/argon ratios with the ratio in sea water equilibrated with air. The excess quantities of nitrogen found in the anoxic waters corresponded approximately with the amounts estimated to have been produced by the oxidation of organic matter. The changes in the oxygen, sulphide and phosphorus content of the water also supported these results. They found that nitrate and nitrite were present only in trace amounts or were absent from the anoxic water. Ammonia, on the other hand, was present and varied in proportion to the accumulation of sulphide. This shows that sulphate probably does not act as the hydrogen acceptor in oxidizing the ammonia which is liberated from organic matter during the reduction of sulphate.

The most striking change in the ionic composition of anoxic sea water is the occurrence of sulphide ions. Hydrogen sulphide is a poisonous gas which kills all higher life, and only certain bacteria can live in such an environment. It has been established (Skopintsev et al., 1958) that the principal source of the sulphide sulphur in sea water is the reduction of sulphate and not the sulphur from decomposed organic matter.

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REDOX MEASUREMENTS IN NATURAL WATERS AND SEDIMENTS

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1. REDOX POTENTIAL - THEORY

A chemical process in which electrons are lost is called an oxidation. The opposite, a process in which electrons are taken up, is called a reduction. In a chemical reaction an oxidation process is always coupled to a process in the opposite direction, a reduction, and vice versa.

$$Fe^{2+} \leftrightarrow Fe^{3+} + e^- \qquad (1)$$

The reaction above is called a redox reaction. Fe(3) is the oxidized form and Fe(2) is the reduced form. When an Fe(3) ion is reduced for every Fe(2) ion that is oxidized, the reaction is at equilibrium. The redox couple then develops a very characteristic redox potential (E).

The voltage scale is relative and the reaction

$$H_2 \quad 2H^+ + 2e^- \ (0.000\ V) \qquad (2)$$

has been chosen as the zero point in this scale. The redox potential is therefore referred to as E_h. The reaction (1) has according to this scale a potential of +0.771 V. The potential is given according to the international convention. It should be noted that in American books, especially old ones, the potentials are given with the same numerical values, but opposite signs.

The redox potential is measured by using an inert metal electrode (Pt or Au) in combination with a reference electrode (calomel or silver/silver chloride). When immersed in a solution and joined to a circuit they form an electrochemical cell. The EMF or cell potential can then be measured. Each of the electrodes is a half-cell for which the potential is given by the Nernst equation:

$$E = E^o + \frac{RT}{nF} \ln \frac{ox}{red} \qquad (3)$$

E^o = constant for the half-cell
R = gas constant (8.3143 joules/deg. and mole)
F = Faraday's constant (96.487 coulombs/equivalents)
n = number of electrons involved, according to the formula
T = absolute temperature
ox/red = activity ratio of the oxidizing and reducing species.

The potential of an electrochemical cell can be written:

$$E_{cell} = E_{right} - E_{left} \qquad (4)$$

In this case when using a platinum electrode and a reference electrode we can write:

$$E_{cell} = E_h - E_{ref} \qquad (5)$$

What is really measured is the difference in potential between the two electrodes.

The redox potential is an intensity factor like pH and temperature and gives no information on either the oxidizing or reducing capacity.

The potential at the inert platinum electrode is created by an excess or loss of electrons at the electrode surface. A reducing solution has a tendency of giving away electrons and an oxidizing solution has a tendency of taking up electrons.

The reference electrode is constructed to give a constant potential. A calomel electrode consists of mercury in contact with mercurous chloride (calomel) which, when immersed in a solution (KCl) with a constant chloride activity, maintains a stable potential. A glass body surrounds the electrode and contact with the outer solution is provided through a liquid junction.

The electrode reaction may be written:

$$Hg_2Cl_{2(s)} + 2e^- \leftrightarrow 2Hg_{(l)} + 2Cl^- \qquad (6)$$

When changes occur at the electrode due to oxidation or reduction, the electrode potential is kept constant by the dissolution or precipitation of Hg_2Cl_2.

The silver/silver chloride electrode works according to the same principles.

2. REDOX MEASUREMENTS

In natural waters and sediments the redox potential is created by a great number of reactions, none of which are at equilibrium and they are seldom reversible in the strict chemical sense. This is mainly due to biological activity.

These mixed potentials are of limited value for quantitative chemical calculations, (Stumm, 1967). However, the redox potential gives a lot of information on the kind of reactions that take place, both from the chemical and biological point of view. Redox measurements are therefore of great value, being indications useful for ecology and pollution research. Determination of the level of the redoxcline (gradient in the redox state) in a water body or sediment is often more important than measurement of the absolute value of the redox potential.

Fig. 1 shows E_h profiles from two sediment cores. In core RR1 a distinct redoxcline is situated at the 3.5 cm level. In core R4 the redoxcline is situated in the water above and the sediment is reduced from the top layer.

2.1 Equipment

A platinum electrode, calomel reference electrode and a potentiometric instrument, usually a pH-meter with a millivolt scale, are used for E_h measurements.

Commercial platinum electrodes are expensive compared to easily constructed home-made electrodes. A platinum wire (e.g. 25 mm long and 0.5 mm in diameter) is fused into the end of a sodium glass tube or cemented into the end of a plastic tube. The electrode area is adjusted to a suitable size by cutting the wire or enlarging it with a platinum foil. It is thus very

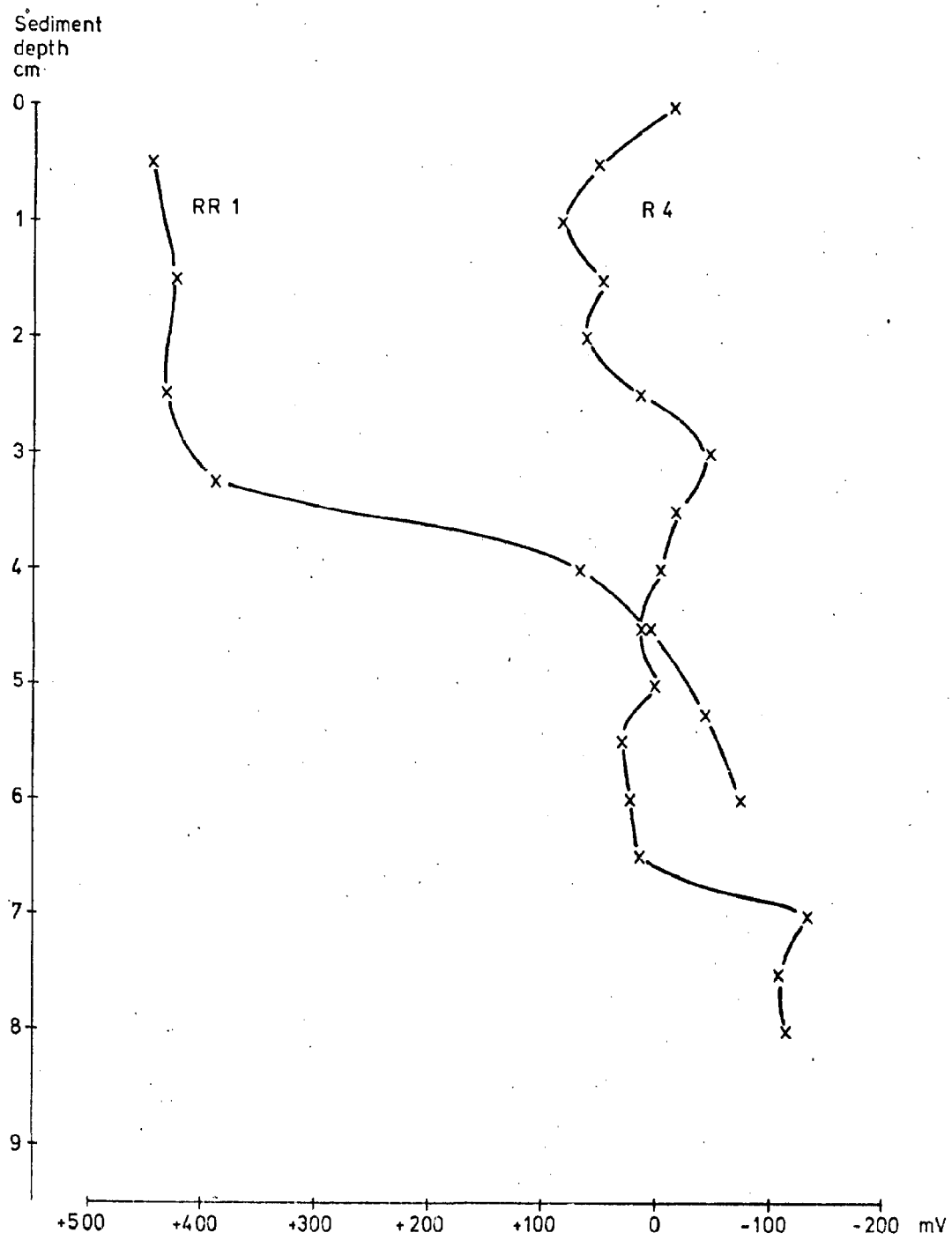

Fig. 1

E_h profiles from two sediment cores

easy to design a platinum electrode for a special purpose. As a rule, for greatest accuracy in measurements, the electrode area should be kept as large as possible within the practical limits for each type of measurement.

Commercial calomel reference electrodes are available with either saturated or 3.5 M KCl solution as the inner solution. Standard potentials for the calomel electrode are given in Table I. The 3.5 M KCl solution is recommended for field work. The saturated KCl solution may cause erratic potentials if KCl is precipitated on the calomel or at the liquid junction due to low temperatures.

Table I

Standard potentials of calomel electrodes (Volt) relative to standard hydrogen electrode (Whitfield, 1971 a)

Temperature °C	3.5 M KCl	Saturated KCl
10	0.2556	0.2543
15	0.2538*	0.2511
20	0.2520	0.2479
25	0.2501	0.2444

* Calculated by the author

2.2 Calibration

The ZoBell solution (0.003 M potassium ferrocyanide, 0.003 M potassium ferricyanide in 0.1 M KCl) is most frequently used for calibrations when measuring E_h. The potential of this solution is +430 mV at 25°C (ZoBell 1946). Unfortunately this solution with a single well poised redox couple cannot detect if the working area of the redox electrode is reduced due to adherent contaminants. The calibration procedure is therefore more concerned with the function of the reference electrode and the instrument.

2.3 Measurements

The platinum electrode surface must be kept as clean as possible. Mechanical cleaning using a mild abrasive or a tissue followed by rinsing with distilled water is a method which is practicable in field work. Chemical cleaning must be used with care because the electrode surface may be damaged.

The liquid junction of the reference electrode may be affected by suspended particles or precipitates, which may change the liquid junction potential. The reference electrode should therefore not be inserted into solutions with suspended particles or in a sediment. The use of a salt bridge between the sample and the reference electrode prevents such errors.

Samples of natural waters and sediments are often poorly buffered with respect to redox potential. It therefore often takes some time to get a stable reading. Electrode drift in four sediment samples at different redoxstates are shown in Fig. 2. Intermediate redox values around the redoxcline (BO 3) are stabilized more slowly due to the unstable redox situation. Fig. 3 shows electrode drift of seven *in situ* measurements made in a Dutch tidal flat sediment. After 30 minutes the electrode was withdrawn 5-10 mm. Stabilization will thereafter be attained at lower values. Stabilization usually takes place within five to ten minutes and an electrode drift of less than 2 mV/min can be accepted as a good reading.

When introduced into a sample the redox electrode always causes some degree of disturbance. Sediment samples are most sensitive because part of the sediment structure is broken and the

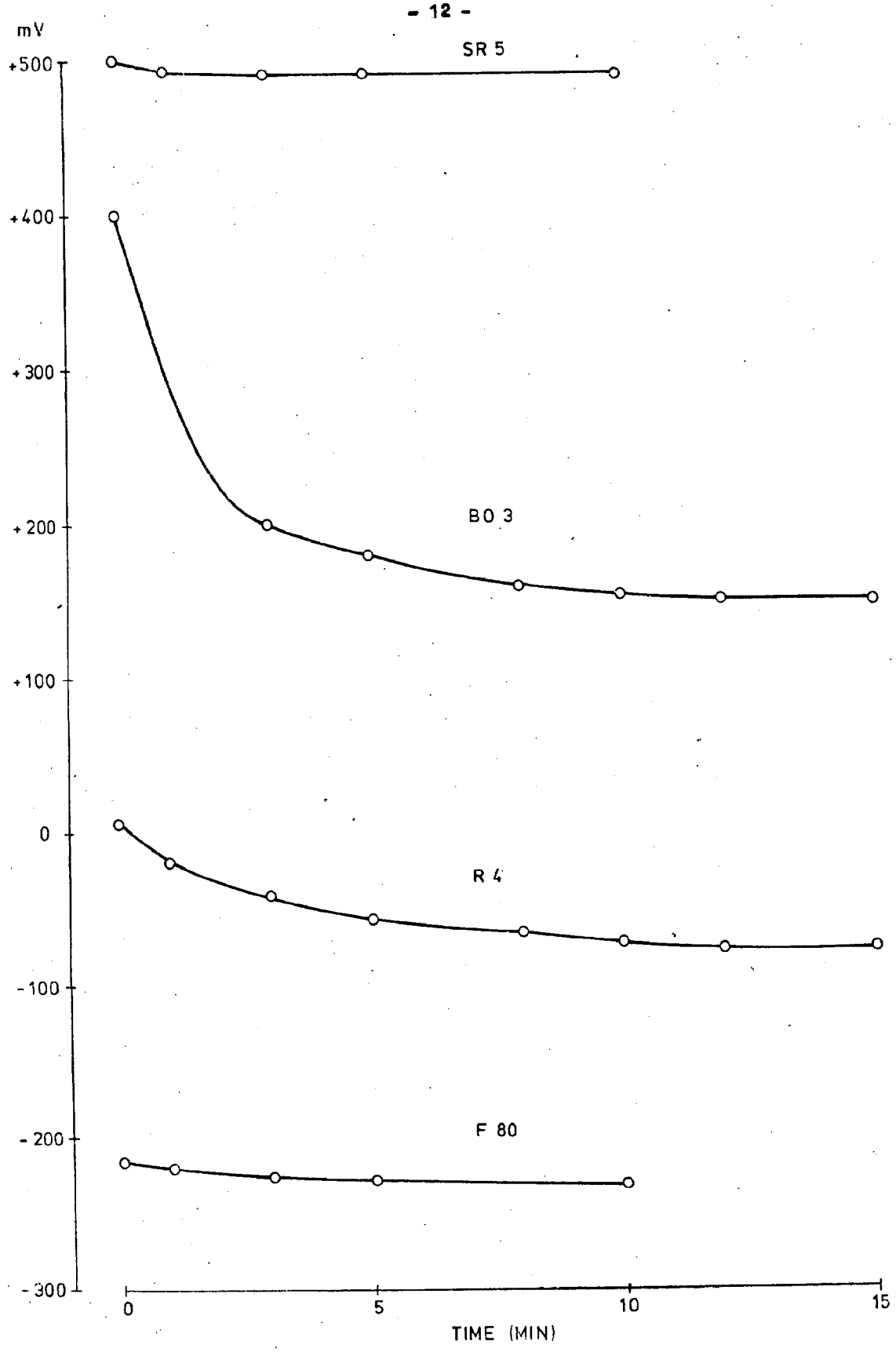

Fig. 2
Electrode drift in four sediment samples at different redoxstates

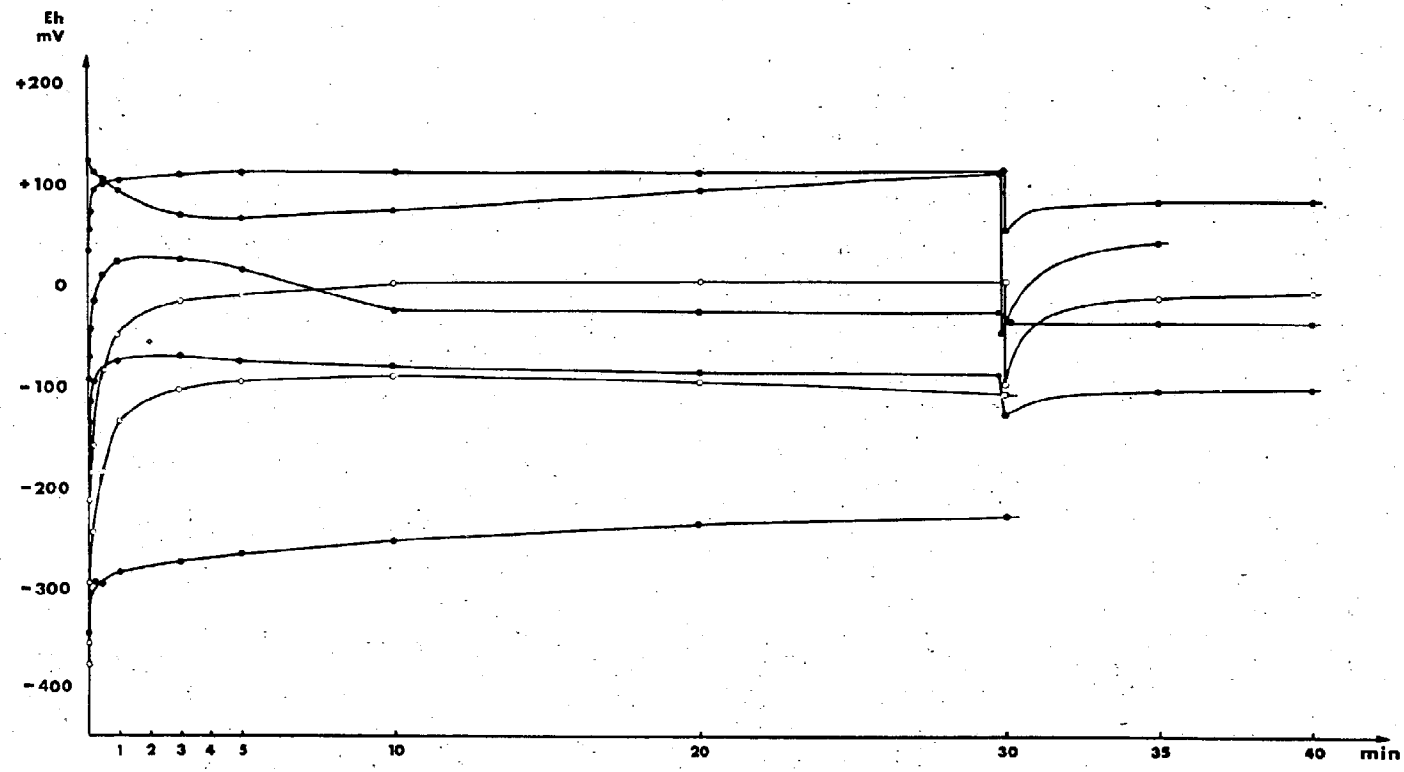

Fig. 3

Electrode drift of seven in situ measurements
in a Dutch tidal flat sediment (from Hallberg, 1968)

interstitial water is pressed away. The shape and size of the electrode must be designed to minimize these effects.

Electrode drift due to introduction of air or gas loss when the electrode penetrates the sediment must also be considered. Absolute stabilization never occurs because of the continuous biological activity.

In situ E_h measurements are most accurate because the natural environment is more or less changed by any sampling technique. In situ probes for E_h and pH measurements have been constructed and used by Mortimer (1941), Manheim (1961) and Whitfield (1971). In situ probes are used for profiling in the water column and the few uppermost centimetres of the sediment. If E_h is measured in samples, the measurements should be performed as soon as possible because long storage of the sample may change the redox potential due to oxidation processes or biological activity to values not representative for the respective natural environment.

The redox potential is dependent on pH, which controls ionic equilibria, solubilities and the formation of complex ions, etc.

The E_h/pH relationship is usually very complicated and a factor to correct E_h readings of the natural samples for a certain pH cannot be given. An average change of about -0.061 V for each unit increase in pH, in the pH range of 3 to 10, has been reported by ZoBell (1946). However, redox measurements are not usually corrected for pH but reported together with the pH value.

Theoretically, the effect of temperature is expressed by equation (3). But in the complex natural systems this effect is as complicated to calculate as that of pH. Temperature affects solubilities, reaction rates, pH, etc. It has been shown in four different marine mud samples that a change in temperature from 0° to $20^{o}C$ caused a change in E_h by between 0.01 to 0.02 Volts (ZoBell, 1946)

The redox capacity of a system is responsible for the reproducibility of the E_h measurements. Natural systems often have a low redox capacity. Therefore E_h measurements can seldom be reproduced to an accuracy greater than ± 0.01 Volts.

A sediment, though appearing homogeneous, contains microenvironments where the redox potential is affected by microbiological activity. Therefore, parallel readings may differ by as much as ± 0.05 Volts or even more.

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INTERLABORATORY STUDY OF METHODS FOR CHEMICAL ANALYSIS OF WATER 1/

by

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1. INTRODUCTION

In accordance with the Environment Protection Act of 1969, standardization of analytical methods and analytical quality control have become subjects of prime importance in Sweden. The central supervising authority for water pollution control is the Environment Protection Board.

A Nordic standardization programme started in 1970, and the aim is to reach the same national standard methods in all the Nordic countries (Funder-Schmidt et al., 1972).

Intercalibration trials or interlaboratory studies have been performed by the Nordforsk (Scandinavian Council for Applied Research) Working Group on Water Analysis and also in the IHD (International Hydrological Decade) programme (Karlgren and Ekedahl, 1971; Henriksen, 1971; Ahl, 1970). But these intercalibrations have been restricted to relatively few laboratories. In 1971 a national Swedish intercalibration programme started with about 60 participating laboratories. This programme has two major goals, the evaluation of analytical methods and the control of work quality in water laboratories. This involves the checking of newly proposed methods, and comparing the results of already existing methods in different laboratories in order to ascertain their levels of accuracy.

In the first intercalibration, June 1971, analyses for specific conductance, calcium, magnesium, sodium, potassium, alkalinity, chloride, sulphate and hardness were requested. The same intercalibration was repeated during November-December 1971. This report summarizes and evaluates the results obtained by the participating laboratories in these two intercalibrations. A more complete Swedish version of the report is available from the Research Laboratory, National Swedish Environment Protection Board. The intercalibration programme has continued during 1972 with intercalibrations of methods for nutrient analyses and methods for determination of metals (Fe, Mn, Al, Cu and Zn).

Experiences from the Nordforsk programme, for example, have shown that duplicate analyses of a single sample in one laboratory give essentially identical results. Other experiences show that the between-laboratory error is the determining factor in evaluating a method (Youden, 1969). For these reasons the intercalibrations were planned according to "the two-sample technique" of Youden (Youden, 1959, Greenberg et al., 1969). Two different but similar samples are prepared and analysed once only. The technique of Youden makes possible a more effective and even more understandable statistical evaluation. This technique concentrates on increasing the number of participating laboratories and reducing the number of determinations per laboratory.

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2. PREPARATION OF SAMPLES AND REPORTING OF RESULTS

Homogeneous and stable water samples are indispensable in intercalibration trials. The samples were taken from natural sources. In the first intercalibration the two samples were waters from Lake Mälaren. In the second intercalibration, sample A was a natural water and sample B the same water with known additions of the constituents to be analysed. The sample waters were left to equilibrate in plastic containers for about a week, then transferred to one-litre plastic bottles and distributed to the participating laboratories as soon as possible. No preservative reagents were added. Laboratories were requested to carry out analyses for calcium, magnesium, hardness, sodium, potassium, alkalinity, chloride and sulphate, and to report the results in meq/l with two decimal places. Specific conductance was to be measured at 20°C and reported as $\mu S\ cm^{-1}$ (equal to $\mu mho\ cm^{-1}$) with three figures. Analytical methods were neither suggested nor specified. The laboratories were asked to treat the intercalibration samples as ordinary routine samples. A report form accompanied the samples and details of methods used were requested.

In the first intercalibration, June 1971, 48 laboratories reported results and 22 made all eight requested determinations. In the second intercalibration, December 1971, the corresponding numbers were 55 and 24. Most participating laboratories were selected from water laboratories approved by the National Swedish Board of Health and Welfare.

3. TREATMENT OF DATA AND INTERPRETATION OF RESULTS

Each parameter is reported in a two-sample chart. The procedure of Youden was used to evaluate the results statistically, with some modification. For each constituent the concentration reported for sample A is the abscissa and that reported for sample B is the ordinate. The pair of results reported for one parameter from one laboratory are used to plot a point, indicated by the coded identifying number of that laboratory. This method can be used to interpret the results with the identity of the laboratories remaining anonymous.

The mean value ($\bar{x}$) and standard deviation (s) were calculated for each sample and constituent. Then, all values outside $\bar{x} \pm 4s$ were rejected (outliers) and the final mean value and standard deviation were re-calculated. The two mean-lines are drawn perpendicular to the corresponding axis, dividing the two-sample chart into four quadrants. The intersection point of the two mean-lines is considered to be the "true value". A line is drawn at an angle of 45° through this point, giving the mean difference between the two samples. The same scale has to be used for both axis.

Results lying close to the intersection point represent a high degree of accuracy. The figures clearly show that the points tend to concentrate in the + + or upper right quadrant or in the - - or lower left quadrant. This tells us that the laboratories tend to get high results for both samples or low results for both samples. The points often fall on a line running from the lower left to upper right (the 45° line) and if there are many points the pattern is that of an ellipse along this line. These patterns demonstrate the dominant role played by the systematic errors. The systematic errors often indicate poor instrument calibration, inaccurate standard solutions or improper working technique. The magnitude of the systematic error can be estimated from the chart. Results in the upper left or lower right quadrants often indicate random errors. Such errors are often due to miscalculations, errors in recording, typing, diluting the samples or some other simple blunder.

The overall precision of the results can also be determined. For each pair of results the difference (D_i) between the two results (sample A and sample B) is calculated. The estimate of the precision can be given by:

$$S_r = \sqrt{\frac{\Sigma D_i^2 - (\Sigma D_i)^2/n}{2(n-1)}}$$

For each laboratory and constituent analysed the systematic error is the same in analysing sample A as sample B. When the difference is taken the systematic error is eliminated. The D_i- quantities are free from systematic errors and contain only random errors.

4. CRITERIA FOR ACCEPTABILITY OF RESULTS

The estimate, S_r, of the overall precision can be used to judge the acceptability of any laboratory's results. The following statistical criteria have been arbitrarily established by Greenberg et al. (1969) and Youden (1959). Points falling between the mean value and $\pm$ 1.552 S_r are acceptable, those between $\pm$ 1.552 S_r and $\pm$ 2.448 S_r are questionable and results falling outside these boundaries are unacceptable. These limits can be visualized in the figures by the construction of concentric circles, centred in the intersection point of the mean-lines, the "accepted true value". The two circles are expected to include 70 percent and 95 percent of the results respectively, if systematic errors could be eliminated. It must be stressed that these relative acceptability criteria are arbitrarily chosen and founded on pure statistical basis. These criteria can be highly deviating for very similar constituents, compare, for example, values for chloride and sulphate in Figs. 7 and 8.

A determination with high precision results in narrow circles. If there are normal systematic errors, a rather large number of results will be classified as unacceptable (see Fig. 7). On the other hand, determination with bad precision leads to a greater spread of results and to wider circles. Strangely enough, this means a greater number of acceptable results (see Fig. 8). For this reason, the limits of acceptability in many cases must be more specifically defined, e.g., in prescribed values given in meq/l or mg/l.

A summary of the results obtained in the two intercalibrations is shown in the Tables I and II. Figs. 1-9 show the two-sample charts, one for each constituent, obtained in the second intercalibration.

5. DISCUSSION

Data on analytical methods used by the laboratories are summarized in Tables I and II. These data can be used to compare different methods for the same constituent. For almost all constituents one method was the predominant choice among the participating laboratories. This fact makes a true comparison rather difficult, since many of the results are not represented by a sufficient number of determinations.

For calcium and magnesium (and total hardness) the most common choice was EDTA-titration or determination by atomic absorption. These two methods were equally precise and accurate. Determination by flame photometry yielded more variable results. For sodium and potassium, flame photometry dominated the picture, obviously being as good as atomic absorption. Alkalinity was determined by titration with a mixed indicator. This titration yielded very good results. An acceptability limit of about $\pm$ 0.05 meq/l and 68.5 percent acceptable results indicate both high precision and accuracy in this determination. Only practical reasons can explain the use of the potentiometric titration technique.

Chloride was predominantly determined by Mohr titration.

The narrow circles in Fig. 7 demonstrate the high precision in this titration. On the other hand, only 21.1 percent acceptable results indicate frequent systematic errors. In Fig. 7 the results are mainly divided into two clusters of points, one in the + + quadrant and one in the - - quadrant. In this titration of chloride with silver nitrate, potassium chromate is used as an indicator. At the end point the slightly soluble red silver chromate is formed and a certain amount must be formed before it is visible. In the titration of dilute solutions the indicator blank should be determined separately and subtracted from the amount of standard solution used. Presumably, some laboratories in the + + quadrant have not done this blank correction and have obtained results that are a little too high.

Table I

Summary of methods used in analysing the samples by the laboratories (intercalibration 1)

Constituent and method	Laboratories using method		Mean ± Stand. deviation (meq/l)		Coeff. of variation	
	Number	Per cent	1	2	1	2
Calcium						
Titr. EDTA	17	51.0	1.16 ± 0.06	1.39 ± 0.04	5.5	3.1
Atomic absorption	9	27.3	1.18 ± 0.04	1.44 ± 0.04	3.7	3.1
Flame	4	12.1	1.19 ± 0.13	1.30 ± 0.62	11.2	47.8
Titr. $KMnO_4$	2	6.1	—	—	—	—
Unknown	1	3.0	—	—	—	—
Magnesium						
Titr. EDTA /Σ(Ca+Mg) —Ca/	15	44.1	0.39 ± 0.11	0.51 ± 0.15	28.7	29.7
Atomic absorption	10	29.4	0.39 ± 0.02	0.46 ± 0.03	6.1	5.9
Titr. EDTA	4	11.8	0.39 ± 0.09	0.51 ± 0.07	23.1	14.4
Flame	3	8.8	—	—	—	—
Colorimetric (titan or brilliant yellow)	2	5.9	—	—	—	—
Total Hardness/Σ(Ca+Mg)/						
Titr. EDTA	25	64.1	1.54 ± 0.13	1.84 ± 0.16	8.7	8.5
Atomic absorption (Ca+Mg)	9	23.1	1.58 ± 0.05	1.90 ± 0.04	3.0	2.3
Combination (Ca+Mg)	5	12.8	—	—	—	—
Sodium						
Flame	22	81.5	0.43 ± 0.05	0.73 ± 0.06	11.2	7.8
Atomic absorption	5	18.5	0.45 ± 0.05	0.71 ± 0.06	10.4	8.1
Potassium						
Flame	23	82.1	0.08 ± 0.04	0.11 ± 0.04	41.7	36.9
Atomic absorption	5	17.9	0.08 ± 0.01	0.11 ± 0.01	13.0	10.9
Alkalinity (HCO^-_3)						
Titr. Mixed indicator	44	93.6	0.89 ± 0.04	1.06 ± 0.06	4.6	5.6
Potentiometric	2	4.3	—	—	—	—
Unknown	1	2.1	—	—	—	—
Chloride						
Titr. Mohr	41	89.1	0.38 ± 0.06	0.69 ± 0.06	15.2	8.0
Potentiometric	2	4.3	—	—	—	—
Gravimetric	1	2.2	—	—	—	—
Colorimetric /$Hg(SCN)_2Fe$/	1	2.2	—	—	—	—
Eel Chloridemeter	1	2.2	—	—	—	—
Sulphate						
Gravimetric	20	47.6	0.74 ± 0.11	0.83 ± 0.16	15.2	19.2
Titrimetric EDTA	7	16.7	0.71 ± 0.14	0.81 ± 0.09	19.6	11.2
Turbidimetric	4	9.5	0.63 ± 0.08	0.76 ± 0.05	13.2	6.7
Titrimetric (Thorin)	4	9.5	0.76 ± 0.04	0.87 ± 0.10	5.6	11.1
Ion-Exchange /Σ(SO_4+Cl)/	3	7.1	—	—	—	—
Colorimetric ($BaCrO_4$)	2	4.8	—	—	—	—
Titrimetric /$Pb(NO_3)_2$/	1	2.4	—	—	—	—
Conductometric	1	2.4	—	—	—	—

Table II

Summary of methods used in analysing the samples by the laboratories (Intercalibration 2)

Constituent and method	Laboratories using method		Mean ± Stand. deviation (meq/l)		Coeff of variation	
	Number	Per cent	A	B	A	B
Calcium						
Titr. EDTA	23	52.3	1.14 ± 0.05	1.30 ± 0.07	4.4	5.4
Atomic absorption	15	34.1	1.18 ± 0.08	1.33 ± 0.11	6.8	8.3
Flame	5	11.4	1.18 ± 0.12	1.35 ± 0.14	10.2	10.4
Titr. $KMnO_4$	1	2.3	—	—	—	—
Magnesium						
Titr. EDTA /Σ(Ca+Mg) −Ca/	22	57.9	0.44 ± 0.09	0.54 ± 0.10	20.5	18.5
Atomic absorption	14	36.8	0.40 ± 0.03	0.49 ± 0.03	7.5	6.1
Flame	1	2.6	—	—	—	—
Colorimetric	1	2.6	—	—	—	—
Total Hardness /Σ(Ca+Mg)/						
Titr. EDTA	36	69.2	1.58 ± 0.05	1.83 ± 0.05	3.2	2.7
Atomic absorption (Ca+Mg)	14	26.9	1.55 ± 0.07	1.78 ± 0.10	4.5	5.6
Combination	2	3.9	—	—	—	—
Sodium						
Flame	25	83.3	0.43 ± 0.07	0.74 ± 0.09	16.3	12.2
Atomic absorption	5	16.7	0.42 ± 0.07	0.69 ± 0.11	16.7	15.9
Potassium						
Flame	25	83.3	0.08 ± 0.01	0.18 ± 0.01	12.5	5.6
Atomic absorption	5	16.7	0.08 ± 0.01	0.18 ± 0.02	12.5	11.1
Alkalinity (HCO^-_3)						
Titr. Mixed indicator	50	96.1	0.88 ± 0.04	1.07 ± 0.04	4.6	3.7
Potentiometric	2	3.9	0.88	1.07	—	—
Chloride						
Titr. Mohr	43	84.3	0.39 ± 0.05	0.58 ± 0.05	12.8	8.6
Potentiometric	3	5.9	0.34	0.53	—	—
Colorimetric /$Hg(SCN)_2Fe$/	2	3.9	0.35	0.54	—	—
Titr. $Hg(NO_3)_2$	2	3.9	0.34	0.55	—	—
Coulometric	1	2.0	—	—	—	—
Sulphate						
Gravimetric	24	48.0	0.77 ± 0.05	1.02 ± 0.07	6.5	6.9
Titrimetric EDTA	11	22.0	0.74 ± 0.06	0.96 ± 0.09	8.1	9.4
Titrimetric (Thorin)	6	12.0	0.78 ± 0.05	1.01 ± 0.07	6.4	6.9
Turbidimetric	4	8.0	0.61 ± 0.20	0.81 ± 0.20	32.8	24.7
Ion-Exchange /Σ(SO_4+Cl)/	2	4.0	0.87	1.12	—	—
Colorimetric ($BaCrO_4$)	2	4.0	0.75	1.03	—	—
Conductometric	1	2.0	—	—	—	—

Table III

Laboratory identification numbers listed by number of constituents analysed and number of unacceptable results reported[1] (Intercalibration 2)

Numbers of Constituents Analyzed	Number of Unacceptable Results Reported						
	0	1	2	3	4	5	6
1	—	60,	—	—	—	—	—
2	—	—	—	—	—	—	—
3	—	—	14,	—	—	—	—
4	6,	—	53,	—	—	—	—
5	—	2, 37, 41, 42,	8,	—	52,	54,	—
6	—	44,	10, 13, 33,	—	—	—	—
7	23, 25, 39,	—	11, 12, 21, 62, 28, 57,	9, 30,	15, 50, 63,	27,	—
8	—	—	22,	—	—	—	—
9	18,	1, 5, 20, 29, 51, 64,	4, 31, 45, 49, 56, 38[3]	17, 24, 35, 46, 43	55,	32[2], 40, 16[4],	19, 34,

1 ΣCa+Mg are in some cases calculated
2 11 results reported.
3 14 results reported.
4 12 results reported.

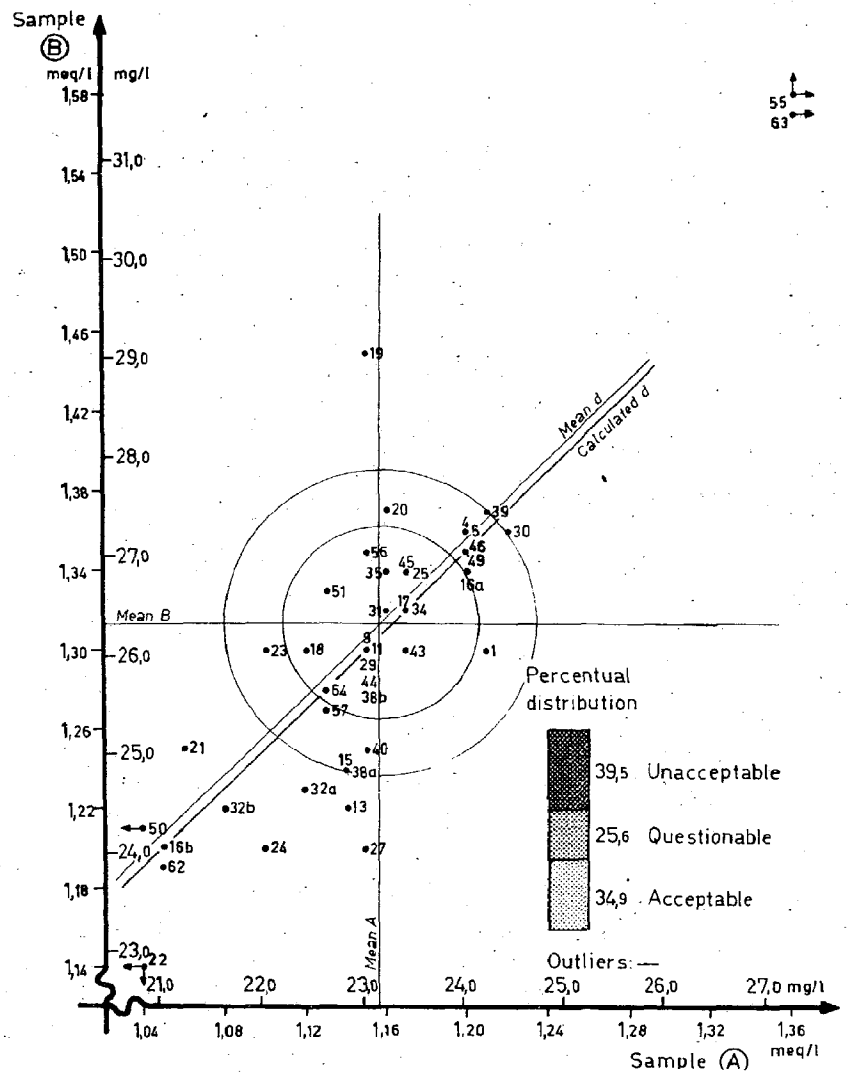

Fig. 1

Laboratory Analysis for Calcium Concentrations

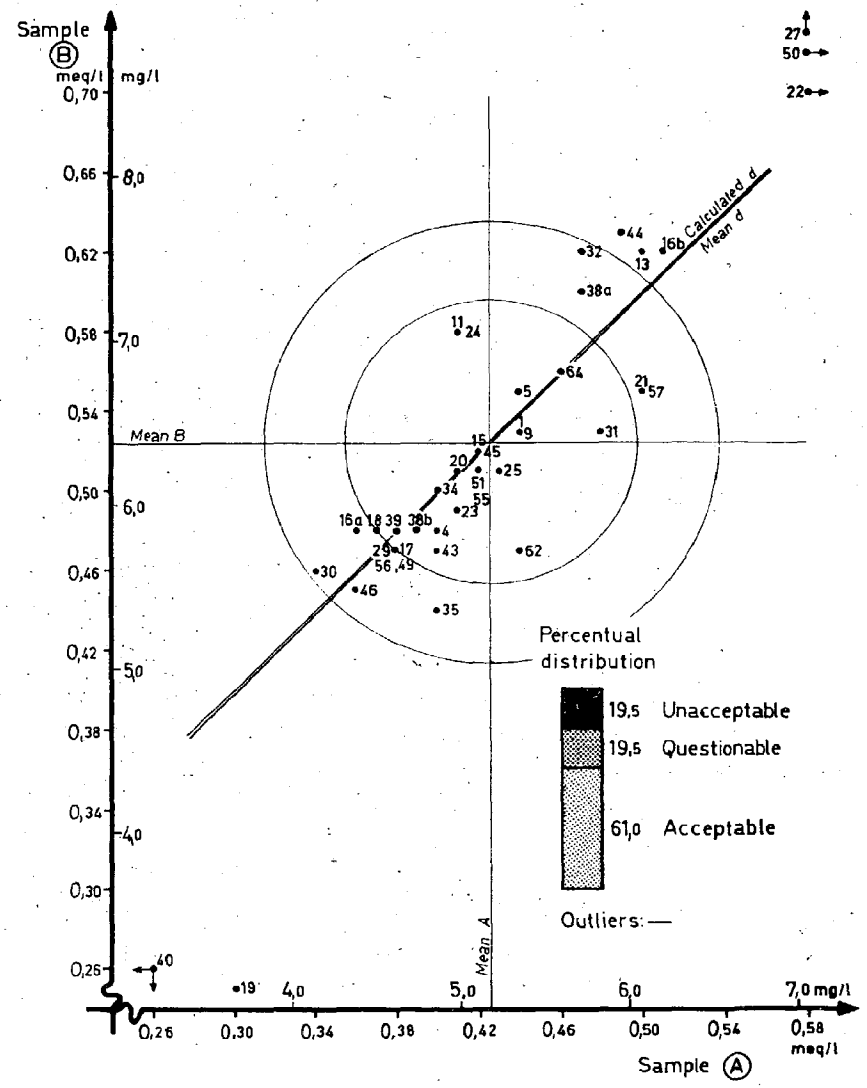

Fig. 2

Laboratory Analysis for Magnesium Concentrations

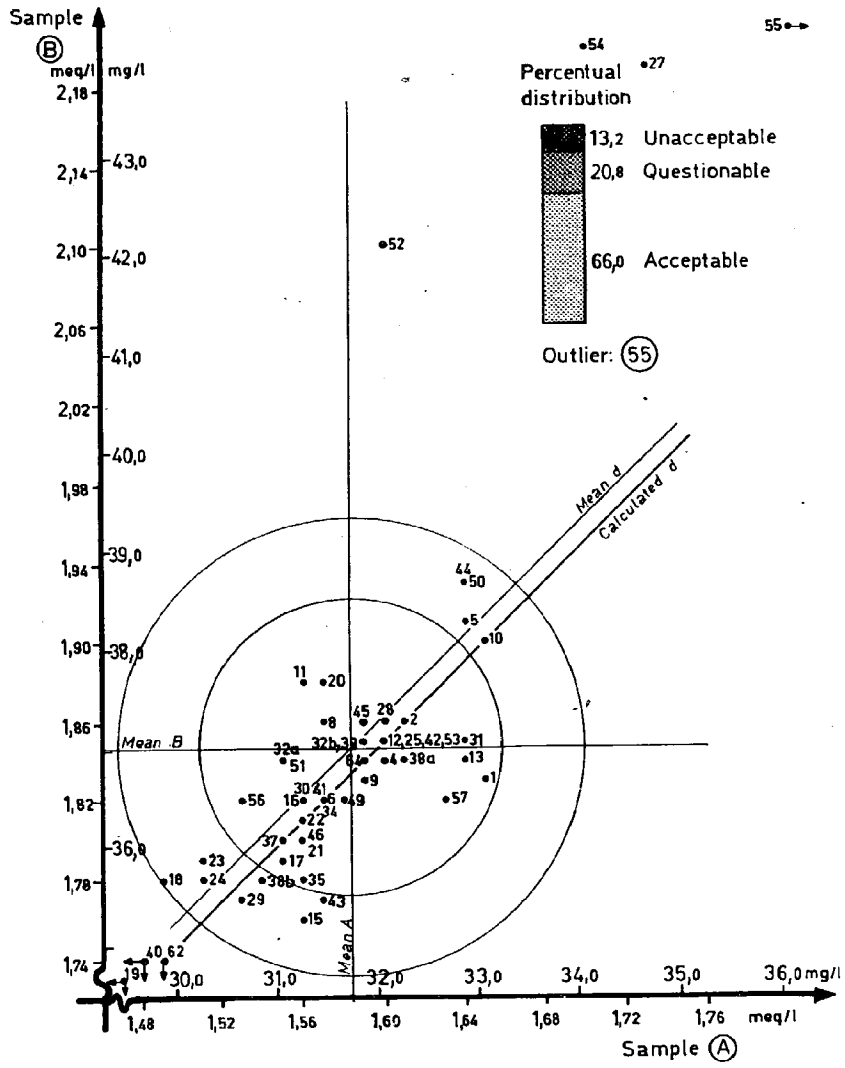

Fig. 3

Laboratory Analysis for Total Hardness $\left[\sum (Ca + Mg)\right]$

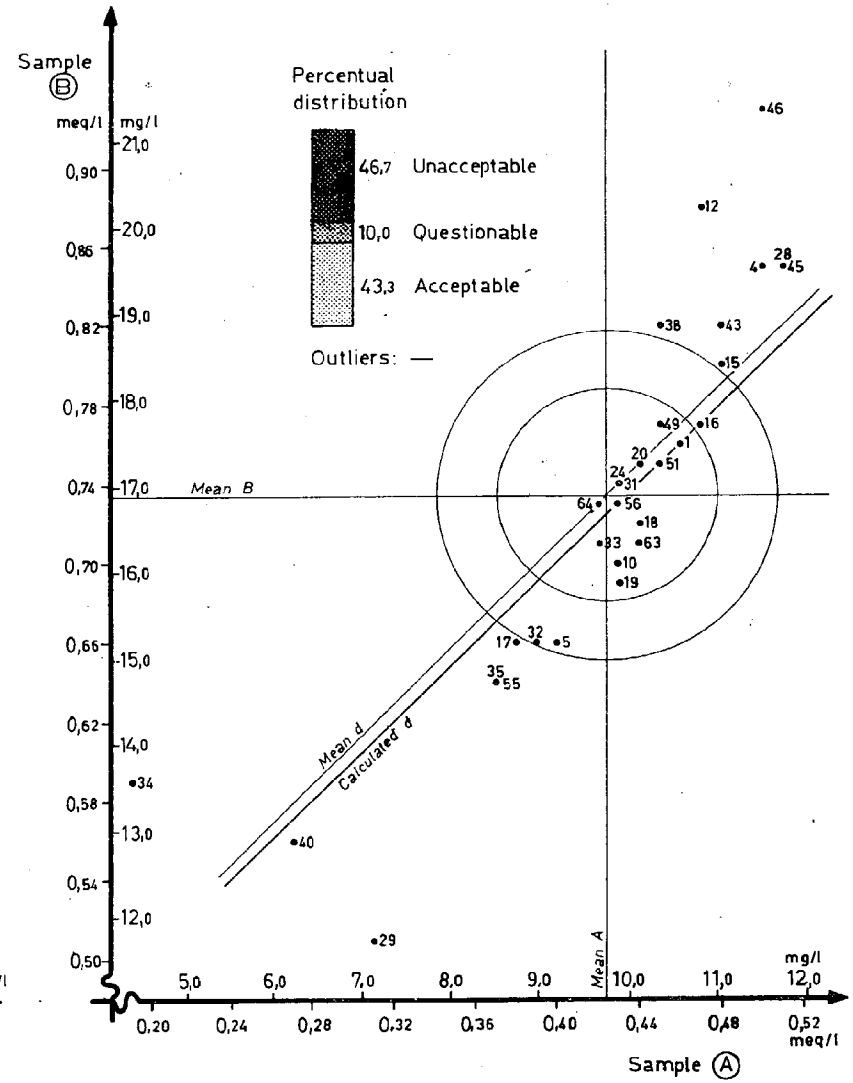

Fig. 4

Laboratory Analysis for Sodium Concentrations

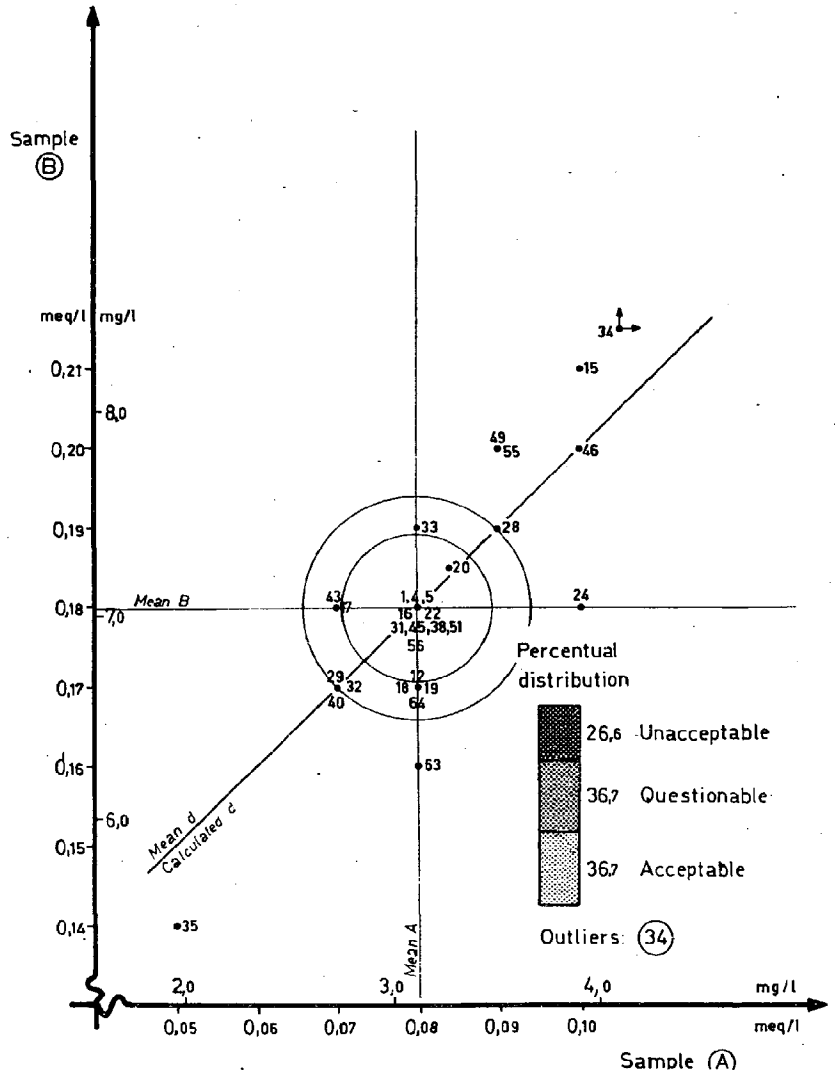

Fig. 5

Laboratory Analysis for Potassium Concentrations

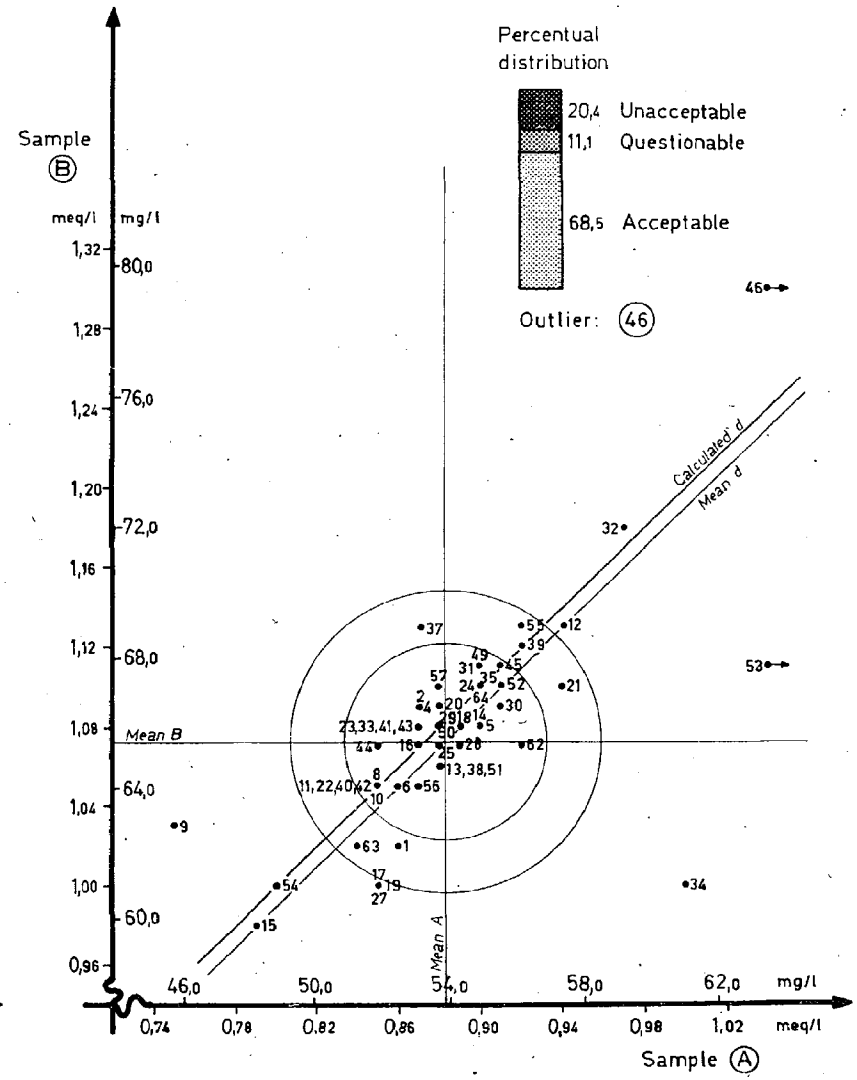

Fig. 6

Laboratory Analysis for Alkalinity

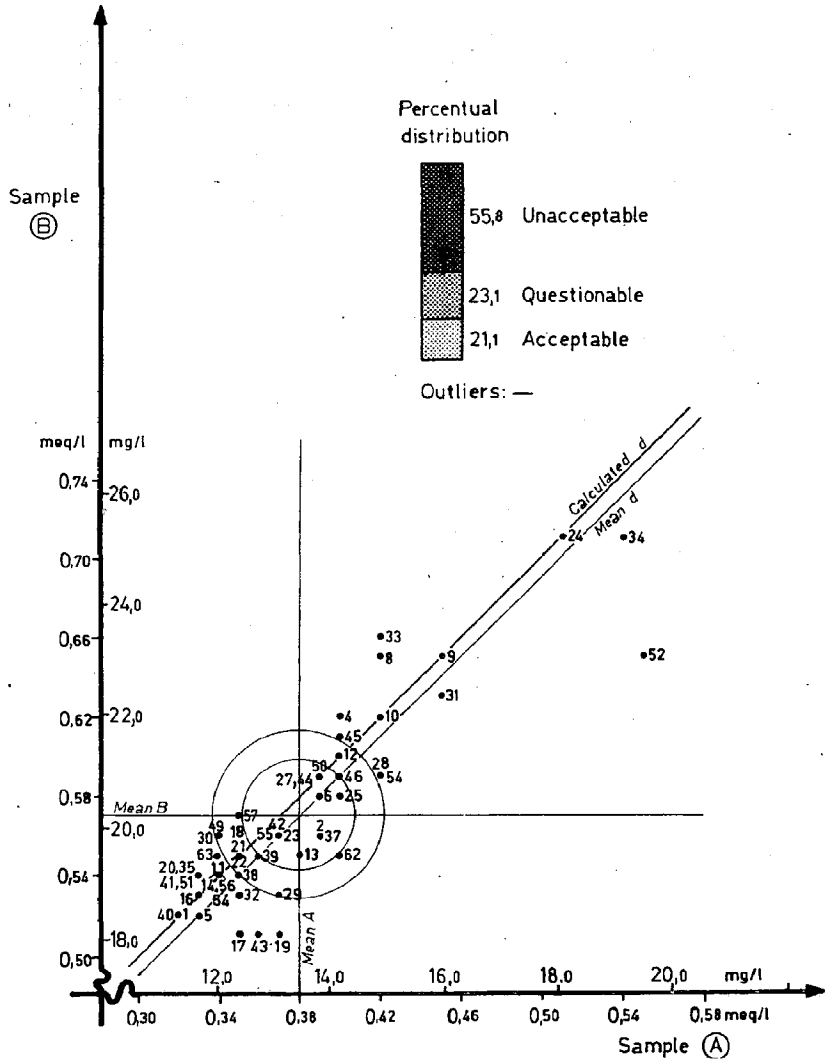

Fig. 7

Laboratory Analysis for Chloride Concentrations

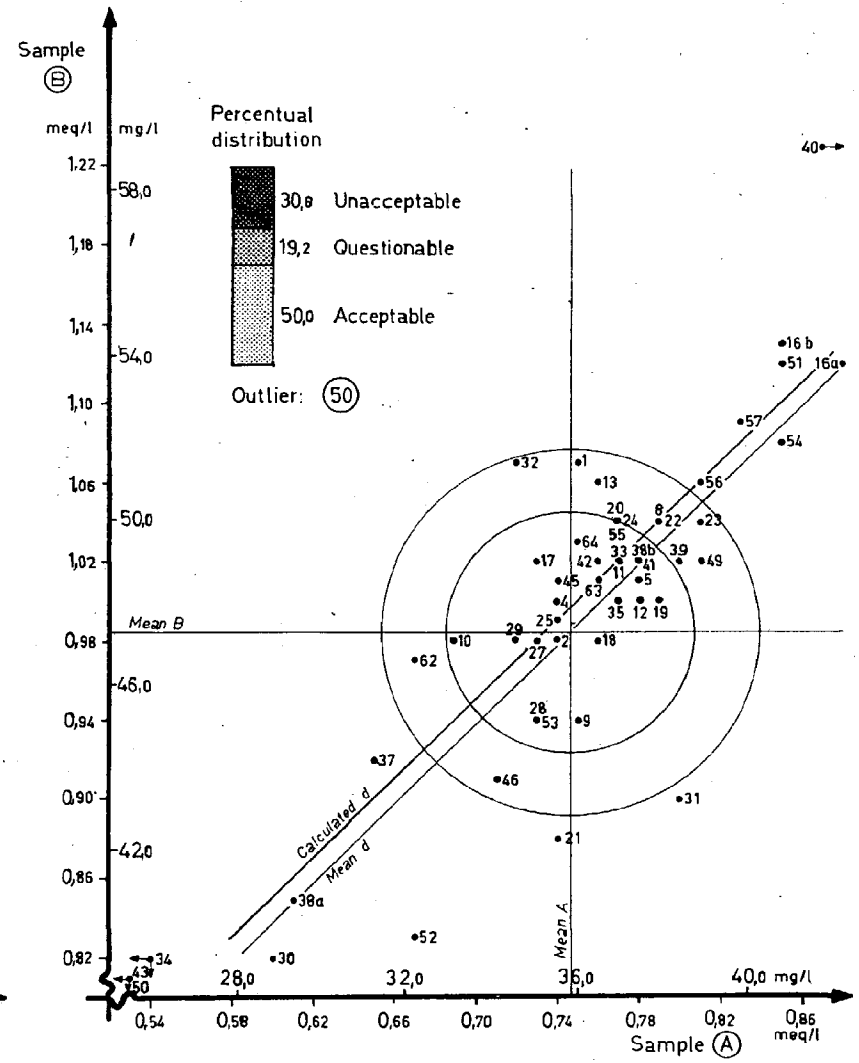

Fig. 8

Laboratory Analysis for Sulphate Concentrations

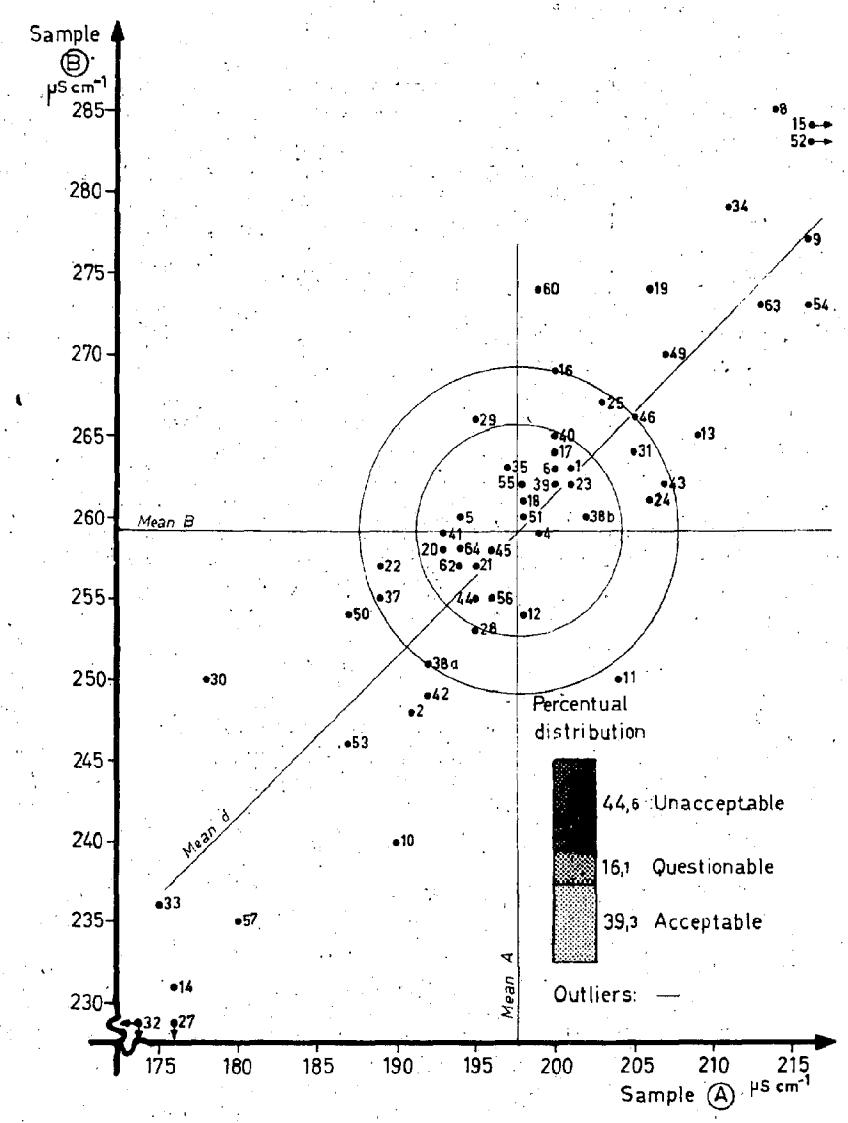

Fig. 9

Laboratory Analysis for Specific Conductance

Some manuals in current use do not stress this blank correction. Finally, this leads to the conclusion that the considered true value, the intersection of the mean-lines, is a little too high.

For sulphate the gravimetric procedure was the most common method, but a rather high number of principally very different methods also have been used. In both intercalibrations the turbidimetric procedure gave lower results than other methods used. For specific conductance there is a general improvement in the results between the two intercalibrations. Presumably, this improvement was due to a better calibration of the instruments.

In all cases there is a good agreement between the found "mean difference" and the "calculated difference". There also appears to have been a general improvement in the analytical work between these two intercalibrations. For calcium, potassium, chloride and sulphate the results indicate an improved overall precision, calculated on differences as described above. This leads to narrower limits of acceptability but to a decreased percentage of acceptable results. For magnesium, hardness, sodium, alkalinity and sulphate there is an increased percentage of acceptable results, the limits of acceptability being the same. This type of improvement points to a correction of systematic errors.

Laboratories making complete mineral analysis of natural waters can profitably control the analytical results by calculating the anion-cation balance (Greenberg and Navone, 1958). This result can also be compared with the specific conductance value. The sum of the anions must theoretically be equal to the sum of the cations. This calculation is the main reason why meq/l is used instead of the more common unit mg/l. The difference between the sums of the anions and cations may not exceed a given percentage - at lower levels five percent of the sum of the anions is frequently used. In the second intercalibration the average sum of the cations (A 2.08 meq/l B 2.75 meq/l) compares fairly well with the anion sum (A 2.00 meq/l and B 2.63 meq/l).

This possibility for controlling the overall acceptability of the results was obviously neglected in many laboratories. In the first intercalibration 22 results, from a total of 289 (i.e., eight percent), deviated considerably. Table III shows the number of unacceptable results reported by each laboratory as a function of the number of constituents analysed (Greenberg et al., 1969; Greenberg, 1961).

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OIL AND OIL DISPERSANTS

by

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1. INTRODUCTION

During recent decades, and especially the last few years, more and more attention has been paid by the authorities and the public in many countries to the increasing problem of pollution by oil. It is evident that the areas of great oil consumption are not always the areas of high oil production (Fig. 1). While U.S.A. and U.S.S.R. are dependent to a minor degree on imports, Europe and Japan have to rely on imports for about 80 percent of their consumption. The main oil transport by sea thus takes place from the Middle East along two routes to Europe and Japan. In 1971, 1 355 million tons of crude oil were transported by sea. This means that 60 percent of all sailed distances were oil transports.

2. OIL POLLUTION FROM SHIPS

Together with the total increase, the character of the transports has changed during recent years. The crude oil used to be refined in the production area and the different products were transported to the consumers in tankers, which nowadays are regarded as very small units (e.g. 16 000 dwt). Today the crude oil is transported to the consumption area for refining. The size of the ships has increased enormously; the VLCC (Very Large Crude Carrier) varies from 100 000-200 000 dwt, or even larger.

The use of larger ships means that comparatively fewer ships have to undertake the transportation. This leads, in one respect, to less risk of collisions and accidents, but on the other hand, these large ships are more difficult to manoeuvre, requiring greater water depths along the routes and in harbours. Especially in areas of intense marine traffic, this increases the risk of collisions and creates a great demand for highly skilled merchant officers and crews, as well as reliable technical equipment aboard the ships.

After a tanker has unloaded its cargo to a refinery, certain amounts of oil remain in the cargo spaces. On her voyage back to the crude oil production area, the ship must take in large quantities of water in the cargo spaces in order to acquire sufficient stability. When discharging this water the oil residues will be discharged too.

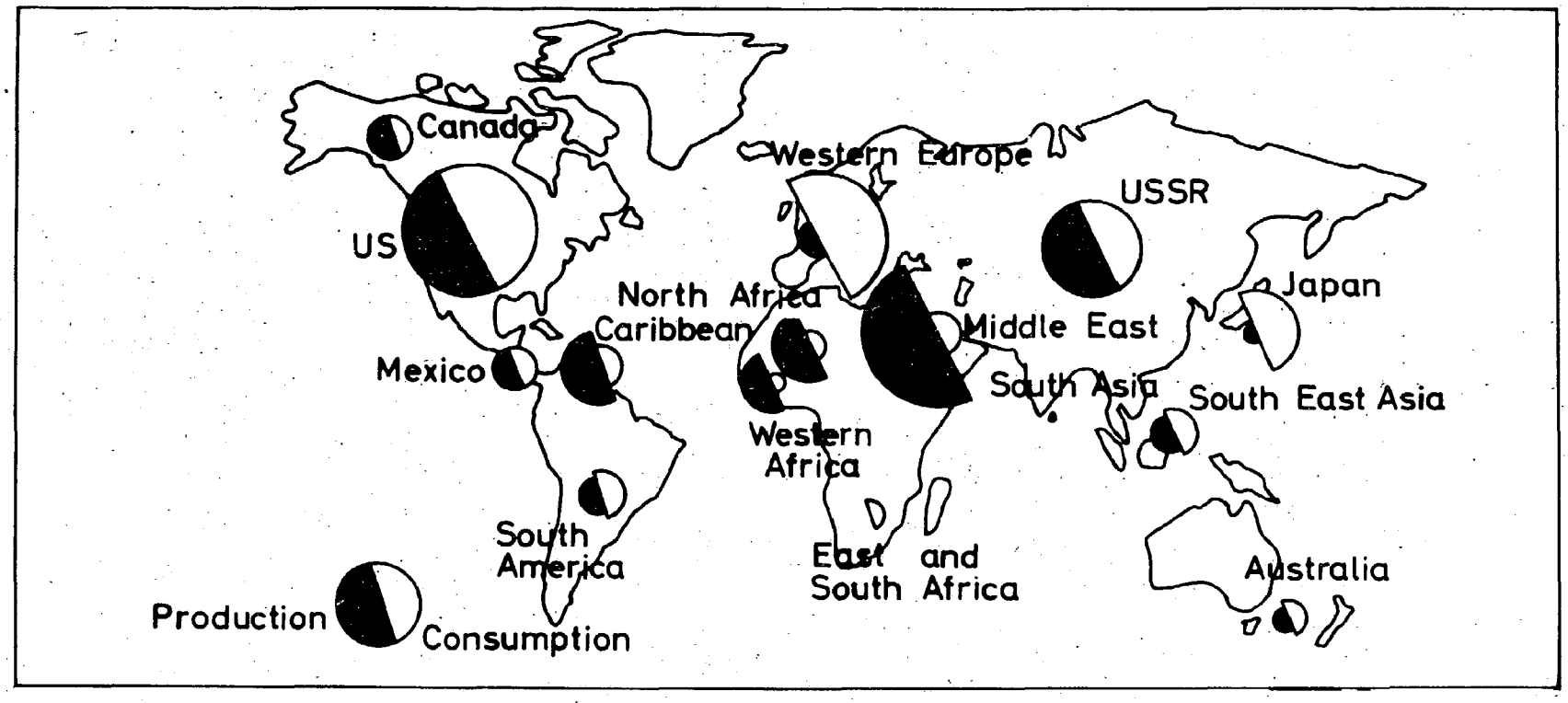

Fig. 1

Demands and supplies of oil 1971 (From: British Petroleum Company, 1971)

The first major step toward preventing deliberate oil pollution was taken in 1954 when 42 countries, including all the maritime powers, drew up the International Convention for the Prevention of Pollution of the Sea by Oil, which came into force in July 1958. This Convention was deposited with the Government of the United Kingdom and was later transferred to the Inter-Governmental Maritime Consultative Organization (IMCO) when it was established in 1959. This Convention (and its amendments of 1962) allows ships to release water containing up to 100 ppm oil in international water within 100 nautical miles off land. Outside this zone the oil content in the water is not restricted.

In 1969 more amendments were proposed, but so far only a few countries have ratified them. According to the proposals the following rules were to be enforced.

(a) Limitation of the total quantity of oil which a tanker may discharge in any ballast voyage to 1/15 000 of the total cargo carrying capacity of the vessel,

(b) Limitation of the rate at which oil may be discharged to a maximum of 60 litres per nautical mile travelled by the ship,

(c) Prohibition of discharge of any oil whatsoever from the cargo spaces of a tanker within 50 nautical miles of the nearest land.

Here it may be appropriate to point out that so far no international legislation or agreements exist concerning the economic responsibility for the consequences of an oil catastrophe. While waiting for this problem to be solved, the companies dealing with oil and oil transportation, as well as the oil owners, have themselves reached two agreements and regulations; TOVALOP (Tank Owners' Voluntary Agreement Concerning Liability for Oil Pollution) and CRISTAL (Contract Regarding an Interim Supplement to Tanker Liability for Oil Pollution). Via these two agreements governmental organizations in countries suffering from oil catastrophies can receive payment for damages and cleaning-up operations.

On the technical side of oil spill prevention, the so-called load-on-top system (LoT) is widely in operation. About 95 percent of the crude oil tonnage is now equipped with LoT.

According to the LoT system one or two cargo tanks are cleaned and filled with ballast water. The oily water resulting from the cleaning is not discharged but led to a slop tank. Then the other tanks are cleaned and may also be filled with ballast water if required. All the cleaning water is collected in the slop tank, where the oil separates from the water during the ballast voyage. When the ballast water is discharged it is virtually free from oil. The water in the slop tank is carefully discharged. When a minor part of the water remains in the tank the amount of out-coming oil increases. The discharge is stopped and the new oil is loaded on top of the oil and oily water in the slop tank.

However, even if the LoT system is a useful tool, other methods must also be used in order to minimize oil spillages. Some harbours are equipped with facilities to receive and treat residues of used and unused oil as well as oily ballast water. The oil can be separated from this water and all the oil residues can be refined and used again, but separation and other treatments are expensive, and thus many harbours are reluctant to offer these facilities.

According to calculations by Kluss (1968), the LoT system every year prevents 1.6 million tons of oil from reaching the sea. It is of course very difficult to estimate how much oil is actually released into the marine environment. Some calculations suggest an amount of 0.7 million tons from the crude oil carriers. However, the product carriers are not always equipped with LoT, and all ships, whether oil tankers or not, release oil to the marine environment in the form of contaminated engine cooling water, leakages at the propeller shafts,

etc. In addition to this, oil drilling at sea and natural seepages from oil wells make their contributions.

It is very difficult to estimate how much oil annually reaches the marine environment. Figures published range from 2.1 million tons (SCEP, 1970) to 5 or 10 million tons (Blumer et al., 1971); with several estimates in between.

3. EFFECTS AND FATE OF OIL POLLUTION AT SEA

Once oil is released into the sea, certain damage takes place regardless of which counter-measures (if any) are applied. Before discussing different methods for oil combatment at sea it is of interest to get an idea of what will happen to the oil if it is left undisturbed. A very useful review on this subject is published by Parker et al., (1971) from which the following is quoted:

3.1 "The likely course of events"

"The possible changes that can occur when crude oil is deposited on the sea are summarized in Fig. 2. At first, the oil will spread rapidly to form a thin, homogeneous slick. At the same time, evaporation will take place (25-30 percent in 2-3 days) so that the oil remaining becomes increasingly richer in the less volatile components and so more viscous. The rates of spreading and evaporation therefore decrease. Depending upon the type of oil and the roughness of the sea, some emulsification may take place due to natural emulsifiers present in the sea and in the oil. Initially, other effects such as dissolution and chemical and biological actions are expected to be small but, as the slick spreads out these effects will be correspondingly accelerated, although their absolute rates may still be slow.

Subsequent changes will depend on the proportions of oil converted to water-in-oil and oil-in-water emulsions. The former are produced when water becomes entrained with viscous oil by wave action. The water content of such an emulsion may increase to 70-80 percent, giving a gel-like mass (the so-called "chocolate mousse"). With large spillages in which thick layers of oil persist for some time, large aggregates of chocolate mousse can be produced. Chemical and biological reactions in such material are likely to be slow because the surface area open to attack is relatively small, although some degradation could take place if suitable anaerobic bacteria were occluded. On reaching the shore, the "chocolate mousse" will pick up sand and debris and the water will evaporate to leave compact tarry lumps in which further degradation will be very slow indeed.

True oil-in-water emulsions may be formed because of the presence of natural emulsifiers or by the application of detergents. In the absence of artificial treatment, it is likely that the bulk of the oil (in a dispersed form) will exist as relatively large droplets formed by the agitation of the waves. In still water these large droplets would coalesce to reform a slick, but in disturbed water the droplets will be dispersed through a large volume of water. Separation will then be slow and the large surface/volume ratio of the oil droplets will permit other forms of attack. Sufficient solar radiation may penetrate the upper layers of the sea to make photochemical reactions possible. The oil may undergo chemical degradation as a result of bacterial attack, it may be ingested by filter-feeding organisms present in plankton, or it may simply stick to plankton, other marine life, or debris and become distributed even more widely."

3.2 Dispersion solution and microbial degradation

The lightest fraction of the n-paraffins in the oils has the greatest solubility. These substances are also regarded as the most harmful together with the aromatic compounds. Boylan and Tripp (1971) have shown that these substances may be dissolved in sea water to a greater extent (about 1.5 mg/l) than the higher paraffins. Also the formation of fine dispersions needs careful consideration as a dispersion containing 1 ppm (1 mg/l) of oil distributed over one km^2 to a depth of one metre amounts to one ton of oil.

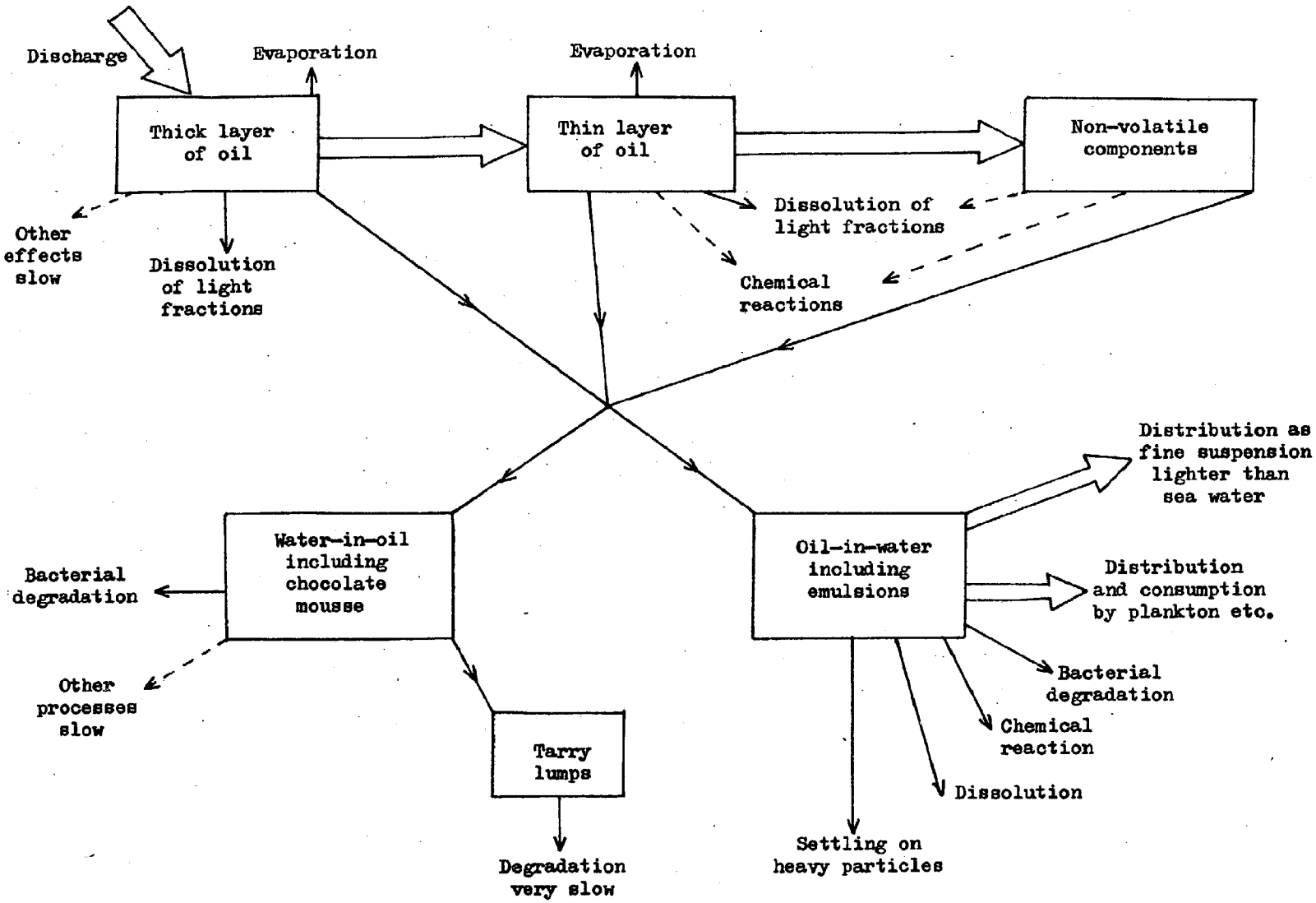

Fig. 2

Processes leading to the consumption of crude oil at sea (Parker *et al.*, 1971)

The microbial degradation of oil is frequently studied and reported in the literature. Yet it is difficult to deduce from these investigations to what extent this degradation plays an important role in the natural decontamination.

4. POSSIBLE WAYS OF DEALING WITH RELEASED OIL

In the open ocean, or where there is a considerable tidal movement, consideration should be given to taking no action on the oil at all. If the number of diving sea birds is small and if the oil is unlikely to be blown by the wind onto a recreation beach, there is little point in wasting effort on mechanical or chemical treatment. The best way may be to let nature solve the problem as none of the presently available counter-measures can completely eliminate the biological damage of oil spills. However, it should be remembered that it is the coastal waters, the most productive areas of the ocean, that receive the heaviest influx of oil.

Suction and Skimming - A good way of dealing with an oil spill is of course to collect the oil and remove it from the water. Obviously this method can only be applied when the amount of oil is reasonable. In order to prevent the oil slick from spreading it must be confined by floating barriers or booms. Once inside this confinement, the oil can be sucked or pumped up if the layer is thick enough. Another mechanical way is to skim the oil off the water. The skimmer consists of a belt forming a loop between two cylinders. The belt (and one cylinder) extends below the oil surface and the oil adheres to the belt. As it rotates, the oil is carried away from the water and at the upper cylinder is scraped off the belt. Considerable amounts of oil (several cubic metres per hour) can be collected with this method. However, at present no booms are capable of confining oil when the speed of the tidal movement or other currents perpendicular to the boom exceed one knot (0.5 m/sec), or when the waves are higher than 0.5-1 m. Thus the method is generally limited to rather sheltered waters or good weather conditions.

Absorbing and Gelling - Several absorbing materials such as straw, sawdust, wood chippings, etc., have been used to absorb oil. Gelling compounds are certain chemical formulations which, after addition to the oil, transform it into a jelly. The disadvantage with all these mechanical methods is that often great volumes of material have to be handled at sea during the operation and ashore after the collection.

Burning - Several attempts have been made to burn the oil when it rides on the water surface. The problem is, however, that the released oil spreads out so quickly that the thickness of the oil layer decreases sufficiently for the cooling effect of the water beneath to prevent ignition. Attempts have been made to overcome this by using different materials to absorb the oil, that then act as wicks, but these trials have not been very successful.

Oil Sinking - One of the methods suggested for removing oil from the surface of the water is to sink it to the bottom. However, before adopting this method, consideration must be given to:

(a) the effect of the oil on the bottom flora and fauna,

(b) long-term effects caused by the bacterial degradation of the oil,

(c) movement of the sunken oil on the bottom and the effect of such oil on the marine life.

This immediately suggests that care should be taken not to sink oil down to the sea-bed in areas which have biological or commercial values, such as shellfish-beds or spawning grounds for fishes. The repeated sinking of oil in any one area of the sea should also be avoided. Sinking of oil is achieved by means of an agent such as a sand slurry, siliconized ash, chalk powder, sulphur, cement, calcium stearate, etc., that is sprayed over the oil slick. Gravity forces the sprayed particles to sink down to the sea bottom.

Dispersion - Dispersants are surface active agents just like ordinary soaps and washing powders. By means of these agents the oil slick is split up into small droplets. The surface active molecules are hydrophilic (water attracting) at one end and hydrophobic (water repelling) at the other. This second end can also be called oleophilic (oil attracting). Thus this part of the molecule is attracted by the oil. In this way the oil is divided into droplets, each one surrounded by the dispersant molecules with the hydrophobic part "attached" to the droplets and the hydrophilic end oriented toward the water. This makes it possible for the dispersant to carry large amounts of oil in the water, even if the oil is not dissolved in the water.

The dispersion of oil is effected by spraying the active substances, dissolved in a suitable solvent, over the oil slick. After that, sufficient agitation of the sprayed area must be ascertained. This is usually done by the propeller of the spraying ship or towing bars or other equipment through the sprayed area. The dispersing and splitting-up of the oil slick implies that the size of the surface area increases enormously as the number of droplets increases. This increase of surface area is a commonly cited motivation for the dispersion of oil, as the microbial attack is expected to increase, thus accelerating the decomposition of the oil; this, however, has not been sufficiently proved. The concentration of the dispersant in the product varies from a few percent to about 30 percent. In some cases the solvent used is water, but the most effective substances are not water-soluble, and then a petroleum fraction is used. This often contains aromatic compounds. How much of the dispersing agent is required to treat a certain amount of oil varies depending on the conditions. However, it is not uncommon that one has to use half as much, or the same amount as the oil to be treated. Thus, in many cases great amounts of petroleum fractions (with aromatic compounds) are discharged into the marine environment in order to "remove" a pollution caused by mineral oil.

The authorities in different countries have very different views regarding the application of the dispersing agents. For example, the Canadian authorities have banned their use and the U.S. Coast Guard restricts their application. They are not allowed to be used in sheltered waters and in shallow areas in the U.S.A. In the TORREY CANYON catastrophe (March 1967) off the south coast of England, 119 000 tons of crude oil were released. In the cleaning-up operations of the water and coastal areas, some 2.5 million gallons (or about 11 million litres) of formulations with dispersing agents were used.

5. BIOLOGICAL ASPECTS OF OIL POLLUTION

It has been shown by Blumer et al. (1971), Ehrhardt (1972) and others, that the substances in mineral oil are taken up by certain marine organisms. However, as the organisms are not capable of metabolizing the components, they may be enriched to a certain degree depending on the rate of excretion. For instance, shellfish polluted by fuel oil still contained considerable amounts of oil six months after they had been transferred to unpolluted water. Thus, as these substances enter the food chain they may be regarded as risks for the higher trophic levels.

Blumer (1969) has also suggested that the long-term effects of low concentrations of oil may be even more dangerous and long-lasting than the more evident short-term consequences. Many predatory fishes find their prey with the aid of their olfactory senses, while others get away from their hunters using the same sense. Migrating fishes find their way home with a very sensitive analysis of the smell of a certain area. With the olfactory sense, extremely small amounts of different substances may be localized in water, oil and its aromatic compounds may completely hide the natural smell in the water. Furthermore there are risks that fishes may be misled by erroneous clues. "This", Blumer says, "may have a detrimental effect on the survival ability of any species in the sea, thus concerning many other species which, through the food-chain, are dependent on the damaged one".

It is often claimed that modern dispersing agents are almost non-toxic with LC_{50} values as high as 3 000-4 000 mg/l. These values are generally the results from tests with adult

fishes. However Kühnhold (1972) has shown that chemically dispersed oil at sub-lethal concentrations considerably prevented the hatching of herring larvae. The hatched larvae were to a great part deformed and mostly died within one day. Furthermore, the chemoreceptors of the larvae seemed to be blocked quickly. The larvae did not avoid the contaminated water when they were given the opportunity to do so.

One other aspect of oil pollution is the ability of oil to concentrate DDT and other non-polar hydrocarbons (whether they are chlorinated or not) which are insoluble or almost insoluble in water.

In a laboratory study, Hartung and Klingler (1970) found that mineral oil in a sediment could concentrate pp'-DDT from water by a factor of $1.08 \cdot 10^6$. Analyses of sediments from the Colorado River confirmed this, yielding a concentration factor of $1.45 \cdot 10^6$.

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CASE STUDY:
CHEMICAL ANALYSES OF A SEA AREA POLLUTED BY MINERAL OIL

by

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1. INTRODUCTION

In January 1973 a field of drifting, polluting oil was detected outside the town of Varberg, on the west coast of Sweden. The Coast Guard Service informed the Fishery Board about the situation and two staff members of the hydrographical department joined the coast guards on a patrol ship in order to make observations and collect samples. The oil field consisted of large, as well as small lumps of thick oil, separated by clear water. Samples of water taken between the lumps and outside the field revealed that the concentrations of non-polar hydrocarbons were below 0.05 mg/l. That only small amounts of hydrocarbons were found even in the water samples within the field can be explained by the fact that the oil was thick and viscous due to the low water temperature.

2. ANALYSES

Fig. 1 shows an IR spectrum of the oil. In the first spectral region the sample was run at two concentrations, thus giving two curves. The three peaks, which are marked with crosses, are present in all IR spectra of mineral oils. The GLC analysis is presented in Fig. 2. The oil contained substances with a wide range of boiling points. This can be seen from the elution temperatures, which range from 70-350°C. There are also two pronounced maxima around 150°C and 300°C. To get an idea of how different oils look in these analyses, a low boiling fuel oil (of the type fuel oil No. 1) is shown in Figs. 3 and 4. In the IR spectrum the three characteristic peaks are found again. The GLC shows that the boiling range of this oil is much narrower, with elution temperatures not exceeding 235°C and with a maximum at 140°C. All peaks are on the same scale. It must be emphasized that this fuel oil No. 1 was not present in the oil spill; it is just shown here for comparison.

The oil was emulsified to some extent, using a petroleum-based emulsifier. This operation was later interrupted however when it was decided that the oil was no longer a threat to the coast.

The IR spectrum of the emulsifier run at two concentrations is shown in Fig. 5 where it is clearly seen that the product is petroleum-based (the three peaks marked with crosses). The upper curve reveals a number of smaller peaks (arrows, discussed below) which are seen better in the lower curve, obtained by running the sample with a higher concentration. The GLC curve in Fig. 6 tells us that the petroleum base is of a low boiling type (in fact it lies in the C_{10}-C_{14} range). Four peaks dominate the chromatogram, which has no components above an elution temperature of 140°C. The emulsifying agent cannot be seen by GLC with the column used in this case.

After the emulsifying operation was carried out, some water samples were collected. The IR spectrum in Fig. 7 shows the extracted substances (this spectrum was run after evaporation of the extract to dryness on a disc of sodium chloride). In the quantitative analysis the extract had to be diluted 40 times in order to be readable in the photometer. The concentration of non-polar hydrocarbons thus found was about 40 mg/l. The three oil peaks are marked with crosses. In Figs. 5 and 7 we can compare the peaks marked with arrows, indicating the emulsifier (surface active agent). At 1620-1640 cm^{-1} a small band is seen which points to the presence of alkyl phenols. At 1100-1120 cm^{-1} a strong ether band is seen; this is characteristic for surface active agents of the non-ionic type. Finally, at 950 and 830-850 cm^{-1} a peak and a band from the ethylenoxide chain in the emulsifier are found. Thus there is no doubt that the used emulsifier is present in the extract from the water. From the IR spectrum in Fig. 7 we cannot say if the oil bands are from the polluting oil or from the solvent of the emulsifier. The GLC curve in Fig. 8, however, tells us that there are no hydrocarbons boiling above 150°C. If this GLC is compared with that in Fig. 6, we can see that even if some new peaks appear, the positions of four peaks - and the size distribution of these are quite similar to that of the solvent for the emulsifier. On the other hand, no traces of peaks from the polluting oil can be seen. This does not imply that the emulsifier is unable to "dissolve" the oil into the water in the form of an oil-in-water emulsion. In this case a very small amount of emulsifier was used and therefore the effect was weak.

What has been said and shown above should of course be compared with the natural composition of the organic compounds which can be extracted from sea water of the area. In Fig. 9 a GLC analysis of such an extract is shown. This sample was obtained from outside the drifting oil field. The total concentration according to the IR measurement was below 0.05 mg/l. Thus it was not possible to run a complete IR spectrum of the sample. When looking at the GLC is should be remembered that the sensitivity of the instrument - and consequently the sizes of the peaks - are often changed during an analysis. If the peaks in Fig. 9 were to be given on the same scale, say that of region B, the peaks in region A and peak C would be five times greater and peak D would be ten times greater than in the figure. Thus the GLC is dominated by three peaks: C, D and one in region A. The total appearance of this chromatogram tells us that it represents products excreted by plankton, probably phytoplankton.

All IR spectra were run in a precision grating instrument, either in solution (carbon tetrachloride) or evaporated to dryness on sodium chloride discs.

The GLC analyses were carried out in an analytical gas chromatograph with a flame ionization detector. The column used was 2.1 m long (all glass) filled with three percent of OV-1 (a silicone oil) impregnated on Chromosorb W 60/80 mesh. The oven was programmed at a temperature rate of 10°C/minute from 50-70°C up to 350°C. The samples were injected (1-10 µl) as solutions in chloroform or carbon tetrachloride.

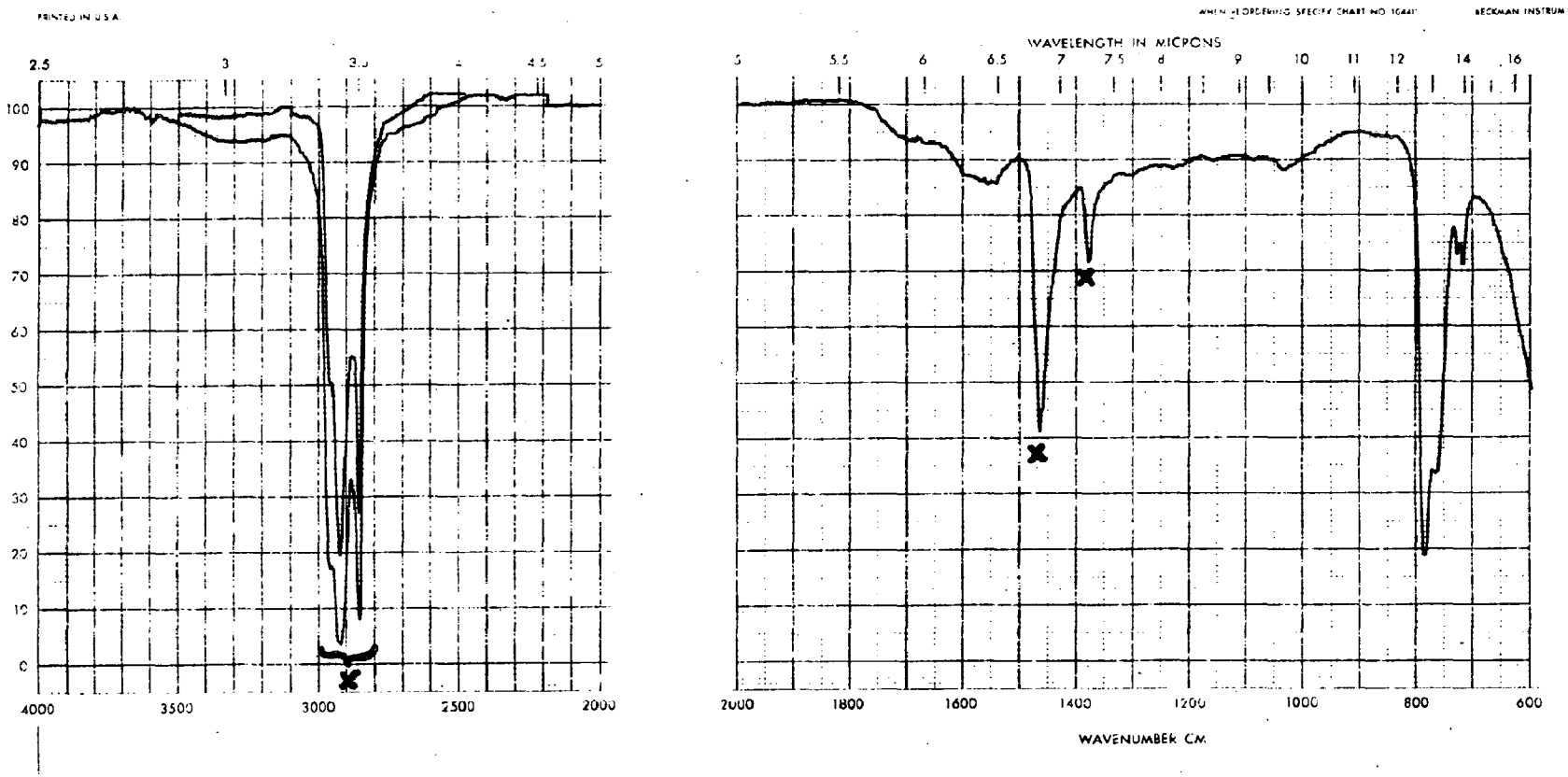

Fig. 1

IR spectrum of an oil lump picked up from the field of floating oil

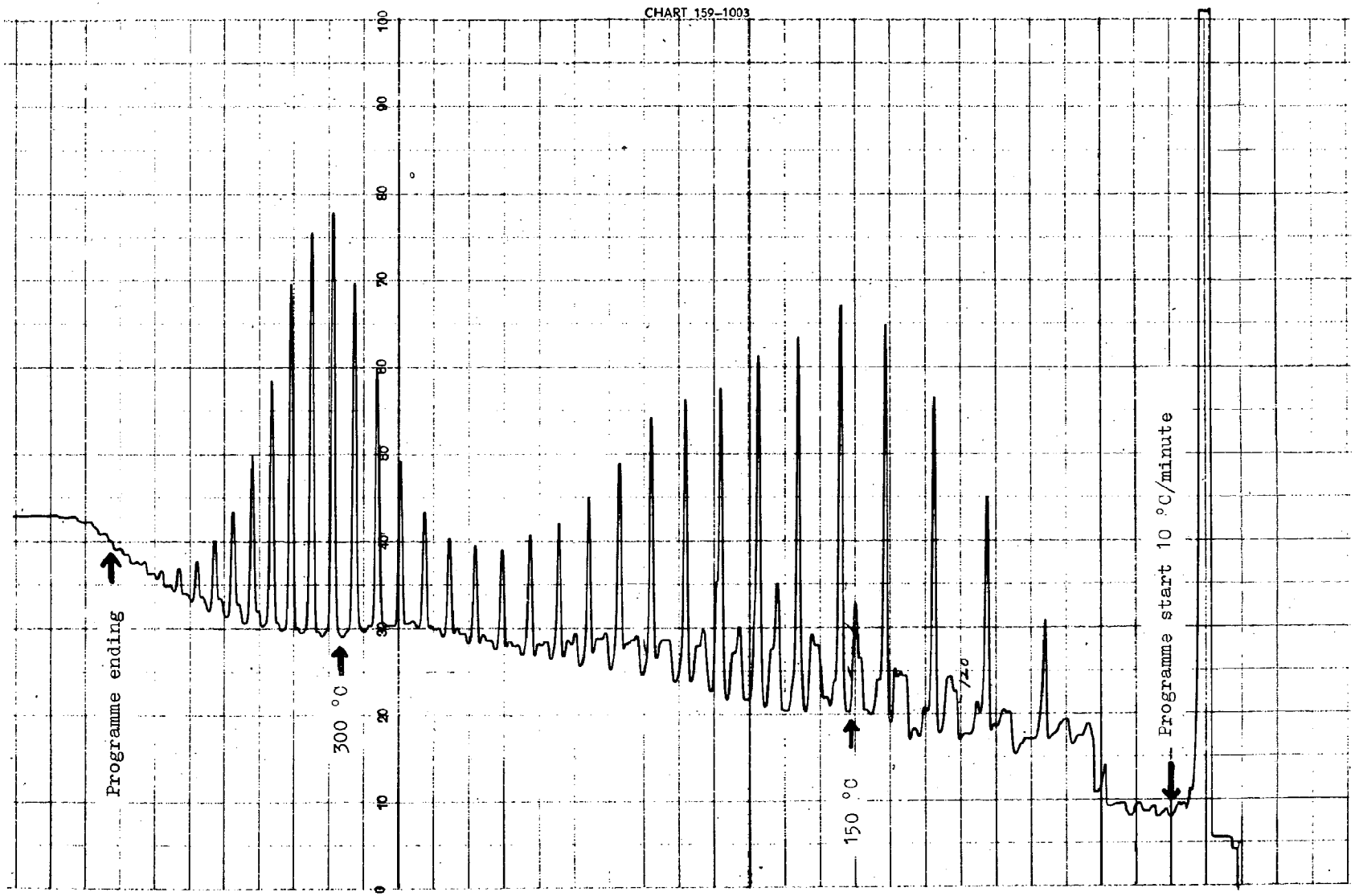

Fig. 2

Gas chromatogram of an oil lump picked up from the field of floating oil

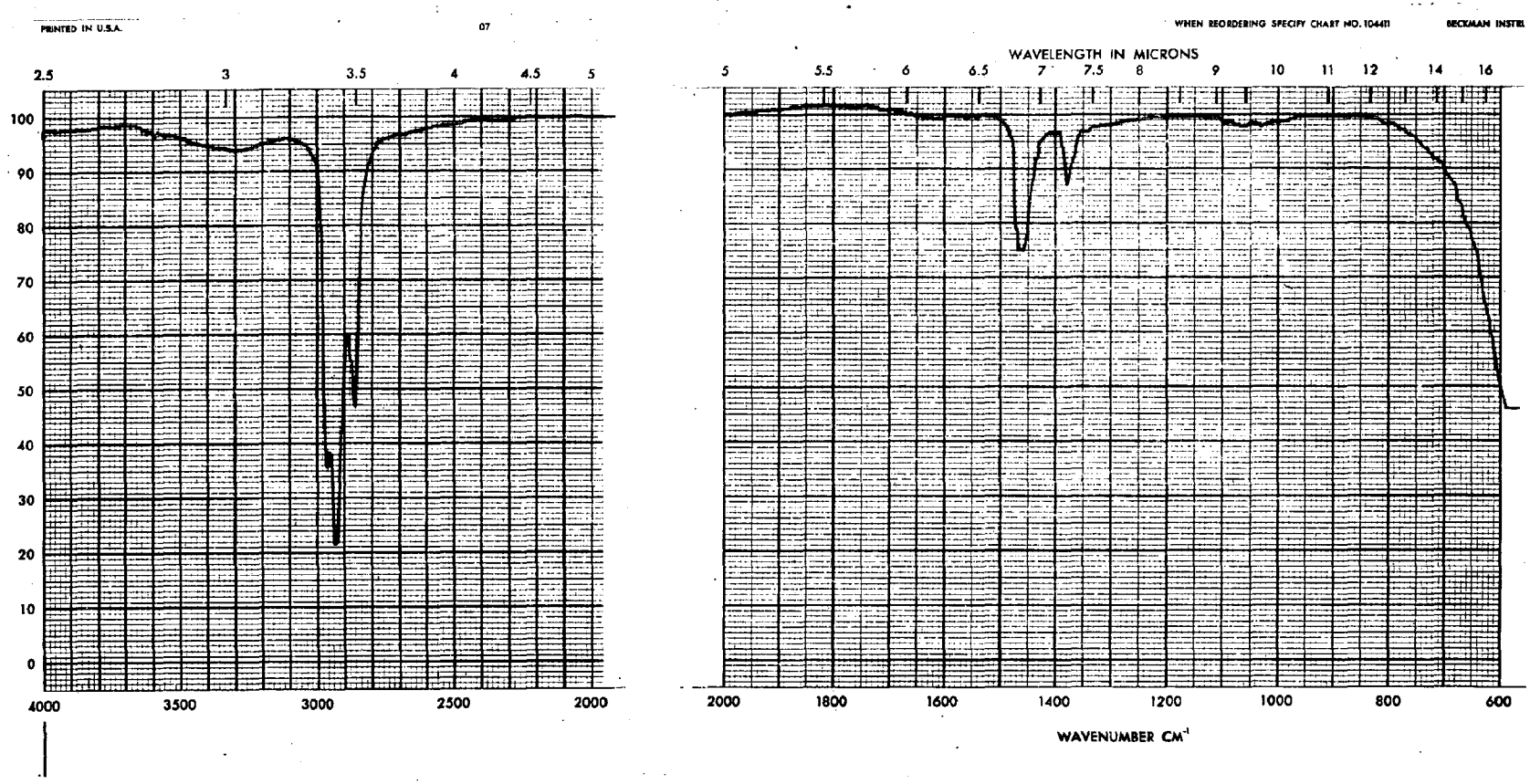

Fig. 3

IR spectrum of an ordinary fuel oil No. 1 of low sulphur content

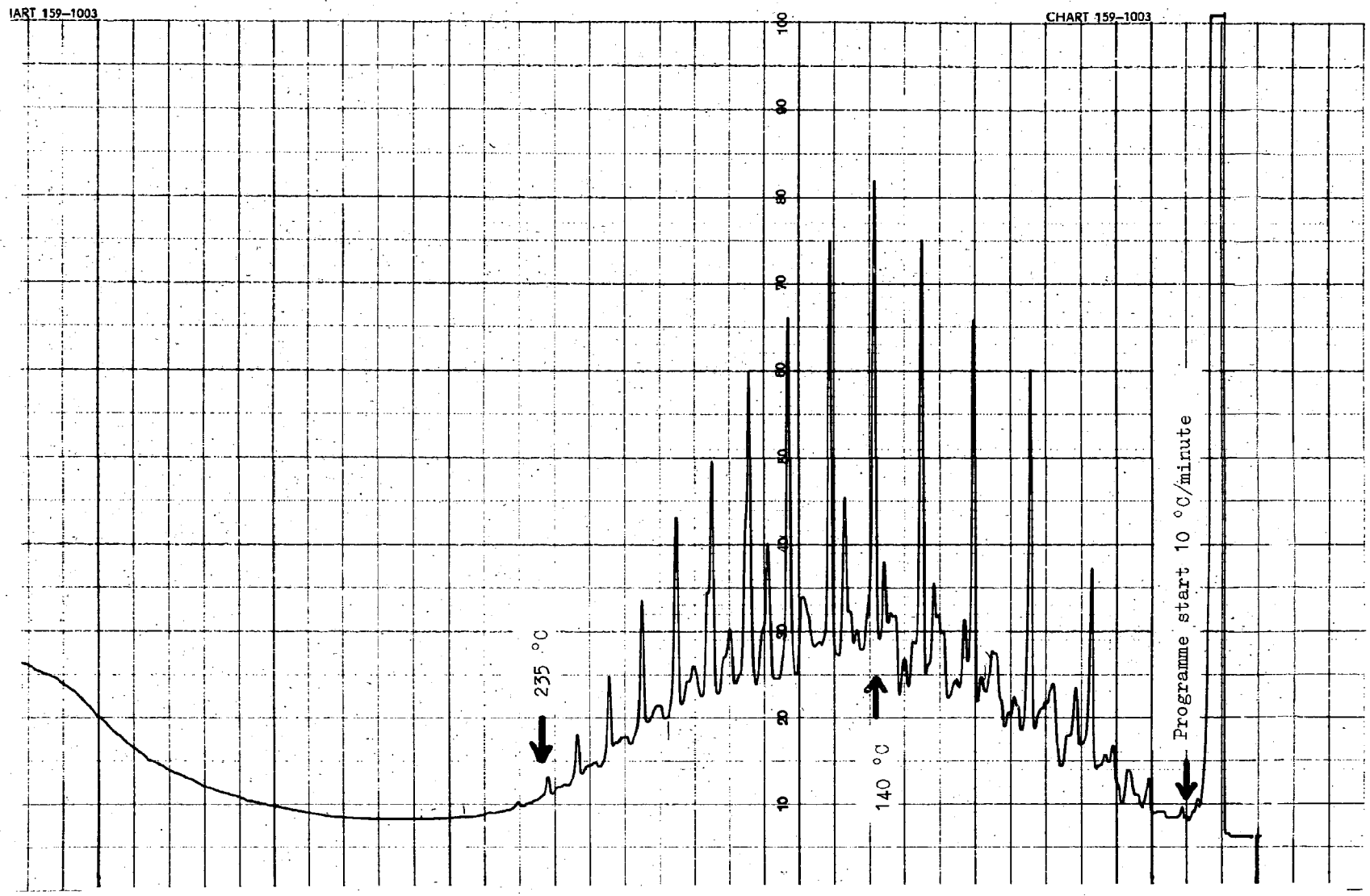

Fig. 4

Gas chromatogram of fuel oil No. 1

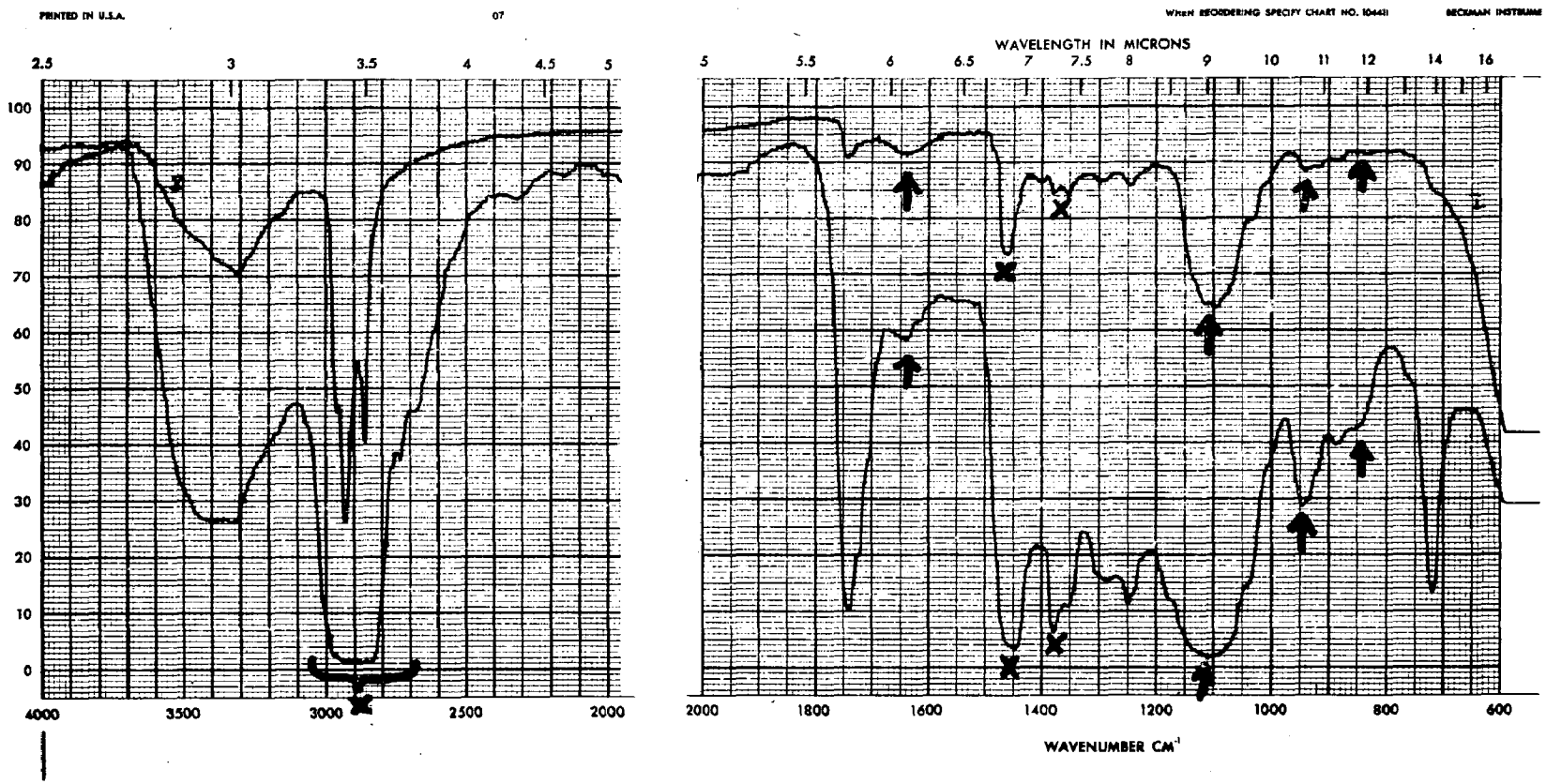

Fig. 5

IR spectrum of the petroleum-based emulsifier run at two concentrations

Fig. 6

Gas chromatogram of the petroleum-based emulsifier

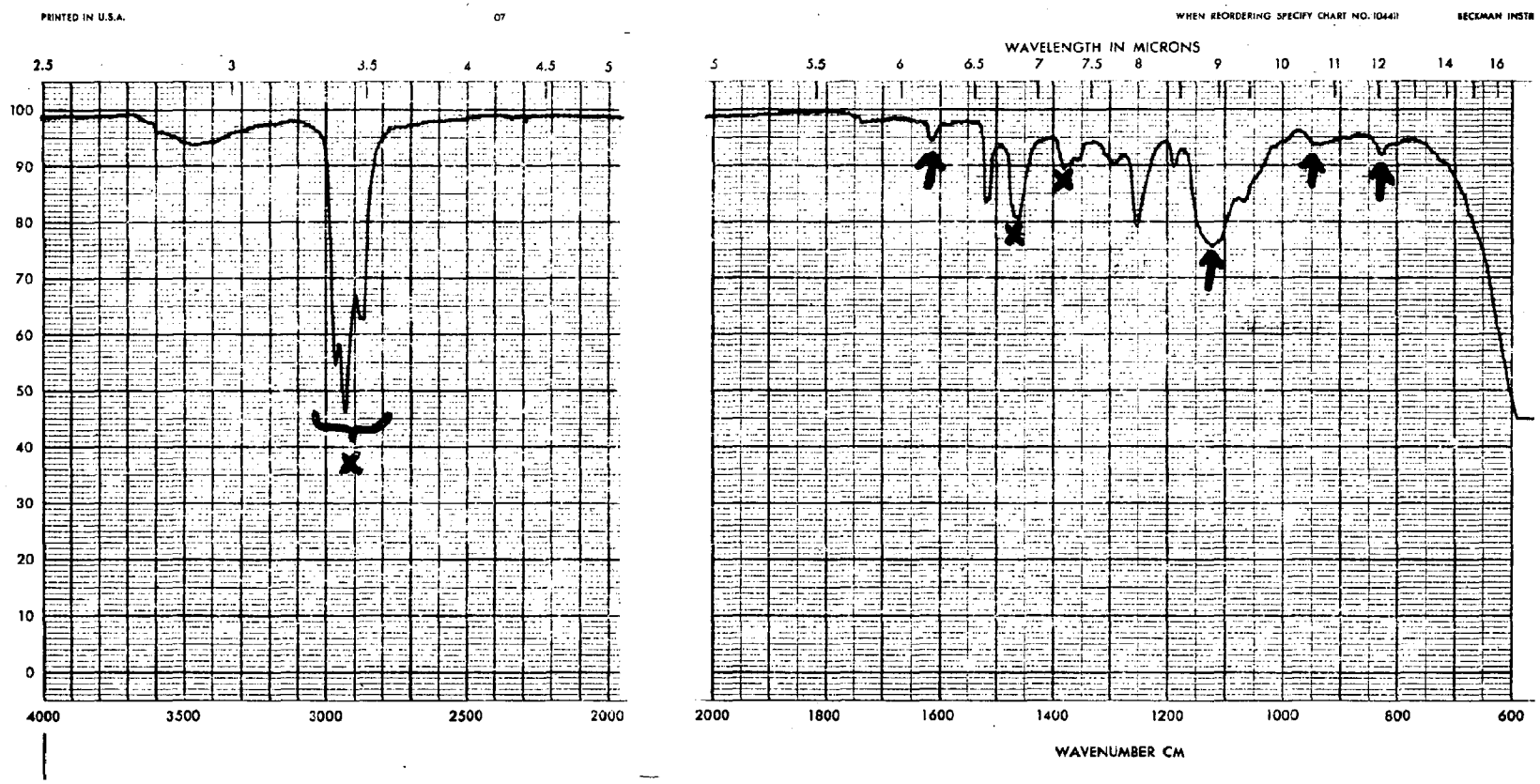

Fig. 7

IR spectrum of a water sample collected after the emulsifying operation

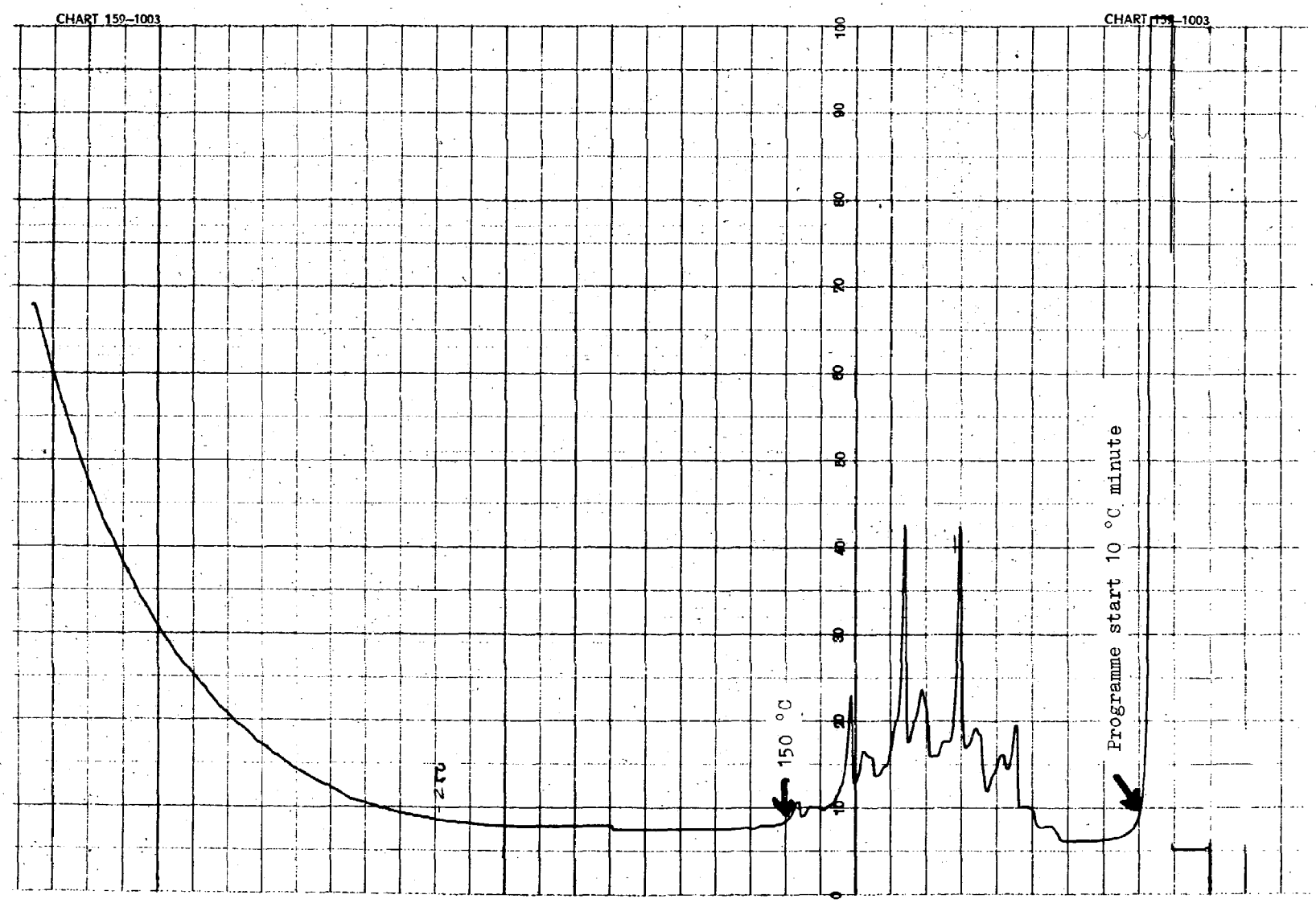

Fig. 8

Gas chromatogram of water sample collected after the emulsifying operation

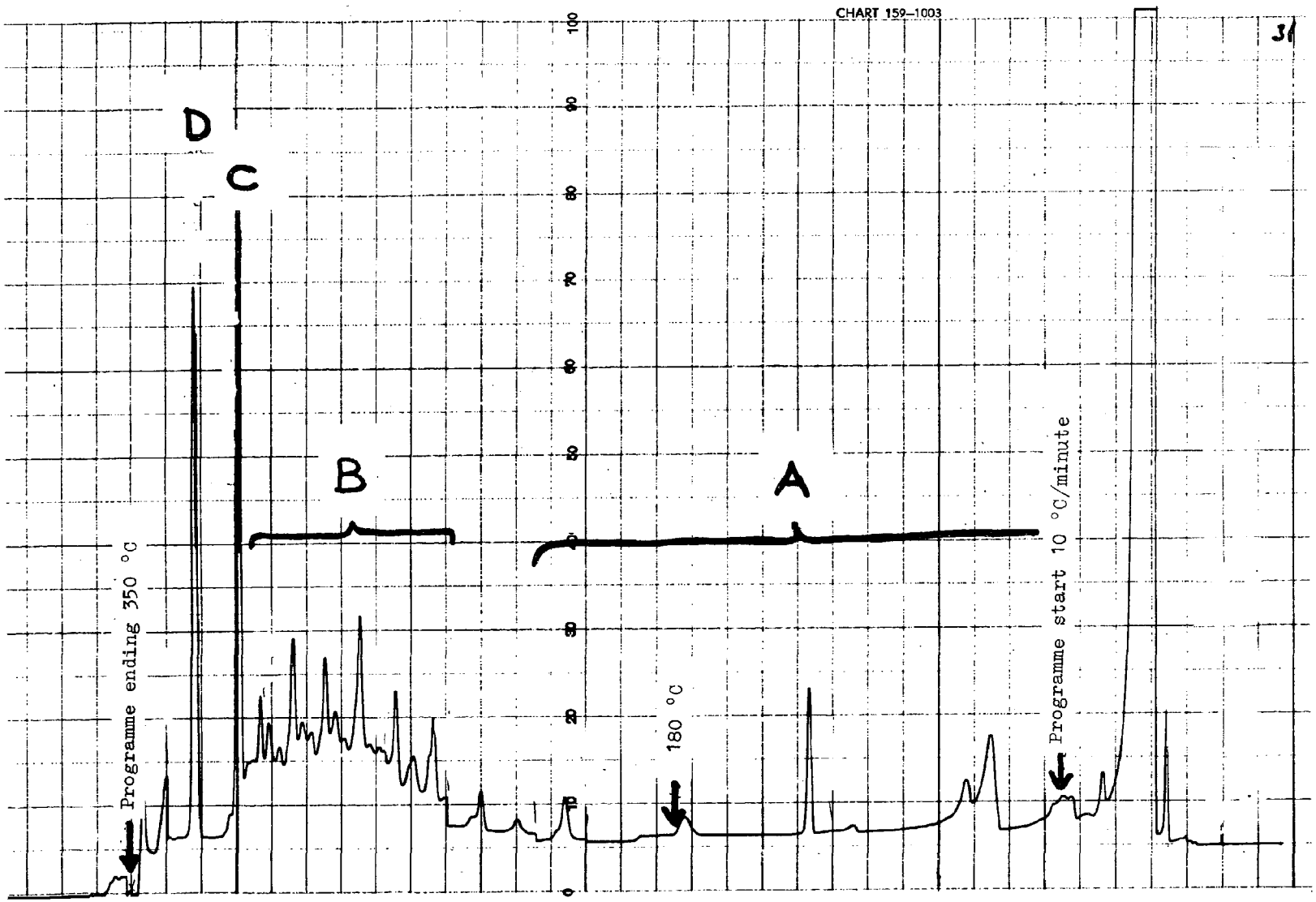

Fig. 9

Gas chromatogram of organic components extracted from a water sample which never had been in contact with the mineral oil

ECOLOGICAL EFFECTS OF MARINE POLLUTANTS

by

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1. INTRODUCTION

A pollutant may for our present purposes be defined as a substance present in the ecosystem, as a result of man's activities, in quantities sufficient to produce undesirable effects. As to effects, let us, in the present context, exclude the possible results in man of consumption of contaminated food, and consider only the changes caused within an ecosystem by the pollutant in question.

Initially, it is necessary to detect the presence of the pollutant and to measure it quantitatively, and the first problem is to decide if any given level measured is in fact "pollution" as defined above. Artificial substances such as some pesticides and PCBs are made exclusively by man and their presence in nature can always be attributed to human activities. However, many other substances which can be toxic if present in sufficient quantity (nutrient salts, hydrocarbons, heavy metals) are always widespread, and it is important to distinguish between the natural levels on the one hand and any input by man on the other, if a realistic approach is to be made to the problems of pollution.

A good knowledge of "baseline" levels is therefore an essential requisite for recognition of conditions which might cause concern. Table I(a) shows levels of naturally occurring metals in sea water and organisms from open water situations and Table I(b) gives values from specific coastal areas where industrial effluents are numerous. Table II shows similar data for some artificial organic substances indicating that levels of organochlorines are greater in an industrial estuary than in the open sea.

Table I

(a) Concentrations of some metals in ppm
(Bowen, 1966)

	Hg	Pb	Cd	Cu	Zn
Water	0.0003	0.0003	0.0001	0.003	0.01
Plants (dry weight)	0.03	8.4	0.4	10.0	150
Animals (dry weight)	0.01-0.5	0.5-10	0.1-3.0	4-5	6-1 500

(b) Concentrations of some metals in organisms from coastal waters in ppm dry weight

	Hg	Pb	Cd	Cu	Zn
Seaweed *Fucus vesiculosus* 1/	-	-	33.0	-	-
Zooplankton *Sagitta* sp. 2/	-	4.5	1.1	5.0	126.0
Mollusc *Mytilus edulis* 3/	2.1	-	-	-	-
Fish *Sprattus sprattus* 4/	-	7.4	0.2	7.0	140.0

1/ Preston (1973) Severn Estuary, England
2/ G. Topping (personal communication) Clyde Estuary, Scotland
3/ Jones *et al.* (1972) Tay Estuary, Scotland
4/ Andersen *et al.* (1973) Inner Oslo fjord

Table II

Organochlorines in ppm wet weight
(A.V. Holden, personal communication)

		Total DDT	PCBs
Plankton	(Open sea)	0.003 - 0.016	0.04 - 0.06
	(Clyde)	0.01 - 0.07	0.05 - 1.1
Herring	(Open sea)	0.04 - 0.09	0.01
	(Clyde)	0.2 - 0.5	0.8 - 1.5

Although we can detect elevated levels of some chemical either in the water or in the sediments, before we are justified in affirming that it is a pollutant with a biological effect, we must show first that it can enter the organisms and second that its presence in the organisms is in some way adverse.

2. ENTRY OF POLLUTANTS INTO BIOTA

Regarding entry to the biota, we can consider three types of substances. First, those which are normally present at low concentrations in the sea and may be taken up by marine organisms at transfer rates proportional to the environmental concentrations, without any threshold. Second, those substances such as nitrogen, phosphorous and silicon whose concentrations vary greatly from month to month in the sea so that a trophic level such as the primary producers will have evolved a range of species adapted to the range of concentrations, and a threshold response might be expected. Finally, there are those artificially produced substances like DDT, not naturally present in the sea and for which only experimental tests can show what is the nature of the transfer from environment to organism.

While entry into the biosphere may take place at any trophic level, transfer from water to organism is probably most efficient in the micro-organisms (e.g., phytoplankton and bacteria) because their surface area to biomass ratio is relatively large and transfers across membranes and into cells are likely to proceed at a more rapid rate or at lower sea water concentrations than for large organisms. Having been incorporated into cells of, for example, phytoplankton, a pollutant may be transferred to the bodies of the animals grazing on phytoplankton and later to those which prey on the grazers. The pollutant, sometimes in an altered form, may thus be transferred up a food chain. There is, therefore, a tendency for persistent substances to accumulate and be concentrated in the higher trophic levels.

3. EFFECTS OF POLLUTANTS

Having shown that the pollutant does in fact enter the organisms we must next find if it has an adverse effect. There are two ways in which pollutant effects can be demonstrated. The first is by detailed field observations which must usually involve long time series of data, and if possible, comparisons of several similar areas, some of which are free of pollution, so that changes in the populations can be detected. The second is by experimental studies on single species, or better, interacting populations over long periods.

3.1 Field studies

3.1.1 Gross effects

Considering first the field aspect, in cases of gross pollution there is no problem of detection. A totally azoic zone or a zone of substantially reduced fauna in the vicinity of an effluent pipe or other point of discharge is sufficient evidence. Surprisingly this seems often to result not so much from highly toxic substances, as from situations where there is a large and rapid input to the bottom of material which, even if completely non-toxic, would have a smothering effect and could destroy the community on which it lies. A well documented case is that of the aluminium works near Marseilles (Bourcier, 1969), where sludge, consisting mainly of oxides of aluminium, iron and silicon are discharged from a pipe at a rate of 85 m^3/hr at a depth of 350 m into a submarine canyon. After about two years of this discharge, an azoic zone was produced, about 2 km wide and stretching down the canyon into deep water for about 6 km, where the sludge covers the bottom to a depth of at least 12 cm. For 2 km or so on each side of the azoic zone, and stretching down the slope for several km more to a depth of about 1 800 m, there was evidence of the deposit but at much reduced thickness and rather patchy. Here the fauna was normal and some of the animals had been ingesting the effluent and maldanid worms were using it to build tubes, suggesting it was not particularly toxic.

Similar examples of blanketing effects could be quoted from other areas, not just from red muds produced in the manufacture of aluminium but also from other inert materials such as fly ash and china clays. The important point is perhaps that they result in azoic zones only as long as the material is rapidly accumulating, but that given time to stabilize and to weather, a new and perhaps different fauna may colonize the ground.

Another type of pollutant which produces effects evident at long distances from the source is oil. This causes obvious loss of amenity on beaches and it certainly kills many sea birds. It may also produce some mortality of other marine biota, particularly on the shore by smothering attached organisms, but in general, apart from the possible effects of low concentrations of soluble components which will be discussed later, the effects of oil on marine ecosystems may be less serious than the visual appearance would lead one to expect. Often more damage is done by the methods used to disperse oil (such as certain detergents) than by the oil itself, and the general advice at present is to try to prevent oil coming ashore, and even if it does, to leave it as far as possible to natural processes which will eventually break it down and assimilate it.

3.1.2 Subtle effects

Apart from such gross and obvious examples of the presence of a pollutant, we are usually faced in the field with detecting effects which may not be at all clear cut. The problem is that environmental conditions are continuously varying on a substantial range of time scales, and causing fluctuations in the populations of the organisms which make up ecosystems. It is thus difficult to distinguish these natural fluctuations from any changes which may have been caused by pollutants. A good example of this difficulty is shown by a long series of observations made on the bottom fauna of a sandy bay in a sea loch on the west coast of Scotland. Fig. 1 shows the changes in population density of the dominant organism of the intertidal benthic populations - a small bivalve mollusc Tellina tenuis. In 1965 the population was high but the numbers declined year by year and there was no successful recruitment. It is of course well known that in many species recruitment is not an annual feature, and indeed that one good brood may dominate a population for several years. But the gradual decline of a population over eight years is shown here and if at any period after 1965 a toxic effluent had been introduced into the bay or if there had been an oil spill, there is little doubt that this would have been blamed for the population decline. Detailed study of this population and of many of the ecological factors involved has shown that in fact natural conditions such as predation and adverse weather can probably explain the decline.

Problems of this kind are particularly relevant in determining the effects of so-called "thermal pollution". With the increasing world demands for power, electric generating stations are increasingly being built and these are frequently sited on the coast where supplies of cooling water are readily available. This may result in heated effluent discharging into the surrounding water often at temperatures considerably above ambient, so that a region of increased temperature is produced. The consequence tends to be an increase in the metabolism of organisms exposed to this, and again it is difficult to detect effects which can clearly be separated from natural fluctuations.

This type of situation has been examined in detail by Barnett (1972) at a power station in the Firth of Clyde. We are not here concerned with effects on organisms which pass through the cooling system - Barnett suggests that this will not harm bivalve larvae, and Fox and Moyer (1973) deduce that dead micro-organisms from this source may have a fertilising effect on outside waters. We are rather concerned with biological effects in the vicinity of the outfall. In the Firth of Clyde, Barnett and his colleagues showed that several of the dominant species living in the sand near the power station were influenced. For example, a population of the gastropod Nassarius reticulatus living at and just below the low water mark was studied in the area of the power station effluent, and compared with a population on a beach several km away. It was shown that the animals near the outfall had significantly thinner shells and that their breeding cycle was considerably modified. Spawning started in late January and early February, some three months earlier than the normal population. One consequence of this is that the larvae would appear in the water before the phytoplankton on which they feed had developed their spring bloom (controlled by light), so that a substantial mortality of the larvae by starvation could be expected. Other animal groups - bivalve molluscs and amphipod and copepod crustaceans were also studied, and in all cases changes attributable to the increased temperature were detected. These changes, however, could be recognised only because the region had been carefully studied for a number of years before the power station came into operation, and because nearby sandy beaches were available for comparison as controls. By these means it was possible to distinguish effects due to the elevated temperatures from natural fluctuations.

Looking at this question of natural fluctuations over a longer time series and a wider range of species, work of the Oceanographic Laboratory at Edinburgh provides information on the plankton of the North Sea over more than 20 years, and indicates a significant decreasing trend in the numbers of several important species (Colebrook, 1972). However, there is a similar trend in the open north Atlantic and it is not possible to provide a causal link between this plankton change and any set of environmental facts for which measurements are available. It was shown, however, that at least ten years of observations would be required before a trend could be statistically demonstrated showing the time scale involved in this type of work.

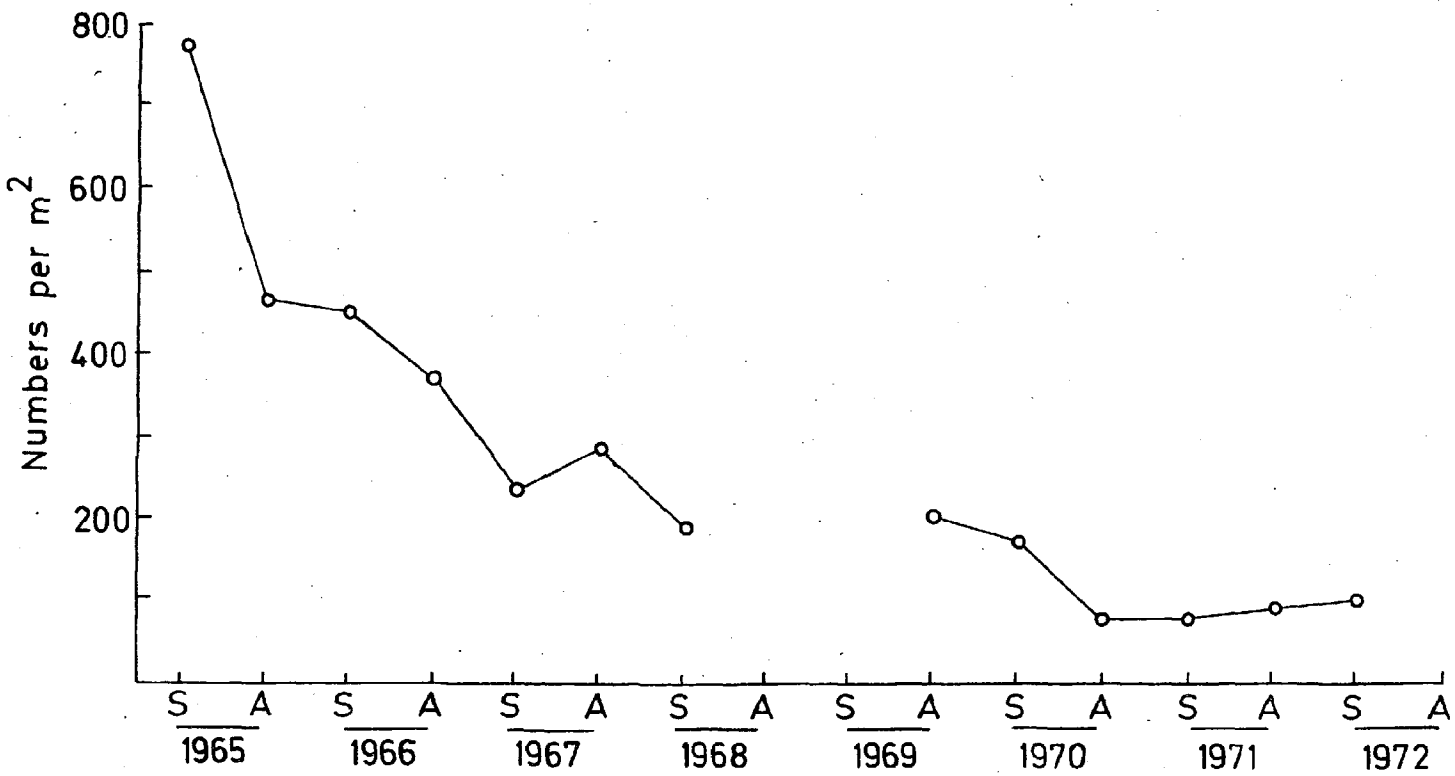

Fig. 1

Population fluctuations of Tellina tenuis spring and autumn 1965-1972

It is relevant that while this decreasing trend in plankton in the North Sea has been shown, in the same period there has been an increase in the yield of commercial fish from that area. Some of this is due to the greater landings of pelagic fish thanks to the introduction of the purse seine net, but the demersal landings also rose and this was due to exceptionally good broods of several species during 1962 and 1967 in particular. Thus, although we can measure increases in the level of pollution in the North Sea and can detect a decrease in the plankton, any corresponding adverse effect on fish must have been masked by some undetermined beneficial factors. Thus, a recent meeting of a working party of scientists concluded that there was no evidence of an effect of pollution on our North Sea resources (Goldberg, 1973). This did not mean that there was no effect but rather that it was not possible to detect one in the face of actual trends.

3.1.3 Eutrophication

Field observations thus seem to provide unequivocal evidence of pollution effects only when these effects are obvious and serious, but there is one aspect on which our knowledge is becoming more precise - eutrophication. Nutrient enrichment can be seen as increasing primary production with some increase in other trophic levels, and is likely to be beneficial as long as the rate of enrichment is not too high. When the rate of enrichment becomes too high however, damaging effects on the environment may occur. As a result of numerous studies we have some idea of permissible rates of nutrient addition for certain types of environment (Ketchum, 1971), and it is sometimes possible to predict the changes when they occur.

At higher trophic levels also we have some idea of the effects of organic enrichment. Much information comes from studies of grounds where sewage sludge has been dumped at sea and the dumping ground in the Firth of Clyde is a good example. Sewage sludge from the city of Glasgow has been dumped on one particular ground (Garroch Head) for almost half a century and the current rate is more than one million tons per year. Studies of this ground and a comparison of its characteristics and fauna with clean grounds nearby show that the dumping has indeed produced an effect. First the mud is black and fibrous and has a foul smell and the organic content (3-8 percent) is considerably increased, while sediment on nearby ground 1.5 km or so away is grey, of fine texture, odourless, and has a considerably lower organic content (0.3-2.0 percent). Even more significant is the difference in fauna. On the clean ground a good mixed fauna of many major groups of molluscs, crustaceans, echinoderms and worms is found, numbering about 1 300 individuals per square metre and with a biomass of about 40 g dry weight. However, as the dumping ground was approached, the species diversity decreased and the number and biomass of a few species increased until on the middle of the ground only two or three species of worms were found, but the numbers of individuals reached almost 50 000/m^2 and the biomass almost 130 g wet weight. However, this marked effect was confined to the centre of the dumping ground, an area of some square kilometers, so that the effect of a long period of dumping was considerably restricted, and it could be suggested that the environmental loss is small compared with the economic convenience.

It is perhaps useful to speculate on what increased load this dumping ground would stand without becoming totally azoic. Experience in New York Bight is relevant here (Pierce, 1972). Sewage sludge from the New York metropolitan area has been dumped there for some 40 years, and recently in quantities almost ten times those in the Clyde. This has resulted in a sediment which in places is three or four times richer in organic matter than in the Clyde and, as would be expected, in a very substantial modification of the fauna. However, there are no large stretches completely devoid of life, and again the total area affected, some 36 km^2 is relatively small.

It may be of interest to consider the different sorts of situation available for sludge dumping, and the areas chosen by Glasgow and London perhaps represent the two extremes. As described above, Glasgow sludge is dumped in water of 90 m depth in a region of little water movement, so that the material is concentrated in a relatively small area which is thus significantly altered. London sludge on the other hand, is dumped in the outer Thames in quite shallow water (15-20 m) where strong tidal currents are found. It has not been possible to detect any substantial effect of this dumping. Surveys (Shelton, 1970), indicate nothing

more than a slight increase in numbers and diversity of the polychaete worms. The choice between deep, still water, where the sludge can be detected and monitored, or shallow, flowing water, where the material is widely dispersed, must be made on the basis of local conditions and fishery considerations, and also in the light of the precise composition of the sludge, which may or may not contain toxic substances (trade and industrial waste) as well as sewage.

3.2 Experimental studies

3.2.1 Short-term toxicity experiments

The simplest of these tests examines the direct transfer of pollutants from water to organisms. This is done by short-term toxicity tests when selected species are kept in a range of concentrations of the pollutant for several days and the concentration for 50 percent mortality is determined. Such tests provide valuable quantitative information on effects, but it is often difficult to extrapolate the result to field conditions and to greater time scales. The major difficulty is that in the sea we are usually dealing with low levels of a wide mixture of pollutants acting over long periods and also that in the sea there is the question of transfer through whole food chains.

3.2.2 Experiments with natural water on individual species

One approach to the problem is to experiment with the effects of the water taken directly from a polluted area. The recent work of Steele et al. (1973) on herring is a good example. Eggs from spawning herring on a small bank in the Firth of Clyde were collected and fertilized artificially from male herring caught in the same area. The eggs were then hatched and the resulting larvae grown in water from four different locations: first in water from the spawning ground (Ballantrae Bank), second in water from the central deep section of the Firth, then from water collected inshore in a polluted coastal region (Irvine Bay) near the spawning bank, and finally, as a control, in clean water from Loch Ewe on the north-west coast. The results show that the time taken for the eggs to hatch, and the mortality after hatching, was significantly different in the four water types. The order was the same in each case, with a short time to hatching related to a high mortality. The mortality rates after hatching were highest in the inshore areas of the Clyde; 72 percent for the polluted coastal region and 33 percent for the spawning bank, with a very low mortality; 6 percent in the clean water from Loch Ewe. These effects may be ascribed to some factor or factors in the different waters, and it is particularly significant that the inshore water had much higher nitrates and had a greater BOD than the others, indicating contamination. It is also known that plankton and herring from the Clyde have higher levels of DDT and PCBs than those from outer waters off the north-west of Scotland (Williams and Holden, 1973).

3.2.3 Food chain experiments

While results of this kind demonstrate that polluted water in the field does have effects on single species at sublethal levels, there is still the question of how whole food chains may be influenced, and this required not short-term tests on single species, but long-term tests on at least simplified food chains. Work of this sort has been done on the ecosystem of sandy beaches (Saward et al., 1972). These beaches are often nursery grounds for flatfish, and in some such communities a favourite food of the fish is the siphons of the small bivalve, Tellina, which are cropped off by the predators, the bivalves themselves feeding mainly on material filtered off from the water. We have therefore a food chain, phytoplankton to bivalves to fish, incorporating the three main trophic levels, primary production, herbivores and carnivores, and after some trials we were able to set up this food chain in large experimental tanks indoors. Using copper as a pollutnat at three different concentrations with a control we studied over a period of ten days the effects of these three trophic levels, having first determined the 96 hour LD50 of copper on Tellina and plaice as 1 000 and 750 micrograms per litre respectively. The level in sea water in one of our industrialized areas in the Clyde is about 3 micrograms per litre and the concentrations used in the experiments were 10, 30 and 100 micrograms per litre.

These experiments showed that the copper added to the water became distributed throughout the entire experimental ecosystem. In the water it was mainly in soluble form and only a small proportion was found in suspended particulate material. The bottoms of the tanks were covered by a layer of sand, and copper was taken up by the layer in amounts reflecting both the level of copper in the water and the duration of exposure - in no case was a plateau concentration reached during the course of the experiments. Considering next the biota, shell and soft tissues of Tellina were analysed separately, and although copper was taken up by both, the accumulation in soft tissues was much greater. Levels increased with both dose concentration and period of exposure. After 100 days the Tellina soft tissue concentrations of copper per unit dry weight were 1 100 ppm, 470 ppm and 270 ppm for dosage regimes of 100, 30 and 10 µg Cu/l respectively. The normal background level of animals collected from the beach was less than 50 ppm. For the fish, there was little change in copper content of body tissue during the experiment, but the levels in the viscera increased considerably. The copper content per unit dry weight of plaice viscera exposed to 100 µg Cu/l for 100 days was 570 ppm, compared with a normal background of 30 ppm.

Corresponding with the evidence of accumulation in the food chain, there was evidence of metabolic effects. Both the chlorophyll a and total pigment levels of particulate matter in the tank water decreased with increased copper dose and the total C^{14} fixalis for treated tanks was lower than the control. For Tellina, condition indices (dry soft tissue weight/shell length) and carbohydrate reserve levels were lower in all treated tanks, and mortality was greater at dosage levels of 100 and 30 µg Cu/l. Considering the fish, growth of plaice in all polluted tanks was lower than in the control and no threshold was indicated. It was of interest that fish biomass in the 100 µg Cu/l tank was higher than in the 30 µg/l tank and this can partly be explained, first in that an algal mat which forms on the sand surface and interferes with fish feeding was much reduced at high dose levels, and second in that withdrawal of Tellina siphons is inhibited again at high dose levels, so that food availability for plaice was increased in these tanks.

The conclusion from these experiments is that copper affects the Tellina/plaice food chain at all levels, not only directly but also in a complex manner due to interaction of ecosystem components.

One important point is that while the viscera of the fish showed some increase in copper levels, there was no increase in the flesh so that we have an effect without the usual chemical evidence. We have thus been able to show that at concentrations not much above those found in the natural environment we have significant effects on the ecosystem and a further justification for extrapolating this to the field is that the levels of copper in our experimental Tellina after 100 days was similar to that found in Tellina in nature near the outfall of one of the main effluent pipes in the industrialized area.

3.2.4 Parent-offspring experiments

We have shown that pollutants can have ecological effects on being transferred to organisms from the water and also through the food chain. One final consideration is effects by transfer from one generation to another of a given species. Relevant work has been described by Menzel et al. (1970). They reared in brackish water calanoid copepods in various DDT concentrations from juvenile to adult, and the nauplii produced were transferred to clean sea water and their growth studied. It was shown that offspring whose parents were reared in concentrations of at and above 0.01 micrograms per litre could grow to the pre-adult stage but could not metamorphose into adults. There has thus been some effect transferred from one generation to another at a DDT concentration that could be found in polluted areas.

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INVESTIGATION OF ACUTE POLLUTION PROBLEMS AFFECTING FISHERIES IN ESTUARIES AND COASTAL WATERS

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1. INTRODUCTION

Since adverse effects of water pollution on fisheries have been studied extensively in the freshwater environment, approaches that have been made to fishery problems there will be discussed for their relevance in identifying and investigating some of the most acute and urgent problems affecting fisheries in estuaries and coastal waters. Questions relating to general ecology and to the open sea will not be raised.

The main pollution problem for freshwater fisheries in the United Kingdom stems from the combined effect on rivers of sewage and industrial wastes in lowering the concentration of dissolved oxygen and raising that of poisons, principally metals (copper and zinc), cyanides, ammonia and phenols. It will be shown that the short-term lethal effects of these conditions on trout can be reasonably well defined from laboratory studies. The question remains of predicting the long-term effects on survival and on other responses under the fluctuating conditions characteristic of polluted streams and of determining their ecological importance not only for trout but also for a variety of coarse fisheries, so that realistic water quality criteria for fisheries can be established. Such criteria can then be used in the management of polluted waters.

The logical long-term approach, alongside appropriate field studies, would be to develop more elaborate laboratory experiments culminating in work on simulated polluted rivers and partially-controlled natural systems and indeed some work of this kind has already been done, but an alternative interim approach is to seek empirical relations between short-term estimates of toxicity to fish and the presence or absence of fisheries in polluted rivers.

2. LETHAL EFFECTS

To define maximum permissible levels of pollutants implies that the causes of pollution should have been identified and that their adverse effects on relevant components of the ecosystem should have been assessed. Clearly, any practical approach to the problem must be limited, at least initially, not only to those factors that have the greatest impact on

fisheries but also to those effects that are most relevant and most easily measured both in the field and in the laboratory. In the United Kingdom the approach during the last decade or so has been to concentrate much of the work on fish themselves, mainly through laboratory studies to assess under various environmental conditions the direct toxicity of the most commonly occurring poisons. The assessment is generally made in terms of concentrations at which times of survival are expected to be relatively long (several months) and at which only a negligibly small proportion of a population of fish would be killed after such a long period of continuous exposure.

2.1 Effect of fixtures of poisons

2.1.1 Pure substances

Much of the published information on the toxicity of substances to fish relates to materials tested singly, whereas generally in polluted rivers, and frequently in effluents that are discharged to them, mixtures of poisons are present. A few early workers, for example Southgate (1932), showed that it was possible to determine the effect of a mixture of similar poisons in water by summation of their individual toxic fractions, and others have suggested that synergism may be important, e.g. with heavy metals (Doudoroff, 1952), but in the last decade a considerable amount of more detailed and longer-term work has been carried out.

In general it has been shown that the combined action of several poisons is approximately additive; if, for example, the concentrations of two poisons that kill 50 percent of the fish in a few days (LC_{50}) are A_t and Z_t respectively, then the concentration of each (A_s and Z_s) required in a mixture to given the same toxicity is such that the sum of the fractions $A_s/A_t + Z_s/Z_t$ equals unity. This relation has been found to hold with rainbow trout (*Salmo gairdneri*) for (i) mixtures of zinc and copper in both hard and soft water (Lloyd, 1961); (ii) ammonia and phenol (Herbert, 1962); (iii) ammonia and zinc in both hard and soft water, and also in the presence of reduced dissolved oxygen (Herbert and Shurben, 1964); (iv) copper and ammonia, and zinc and phenol, though there is a tendency to over-predict the toxicity of these two mixtures the lower the percentage response chosen (Herbert and Vandyke, 1964); (v) copper and phenol (Brown and Dalton, 1970); and (vi) phenol and cyanide in saline waters at up to 20 percent sea water, though at 50 to 70 percent sea water the observed 48-h LC_{50} was 1.7 times the predicted value (Ministry of Technology, 1969).

Mixtures of three substances have been tested together, including (i) phenol, zinc and ammonia, in which there was a tendency to over-predict the toxicity where zinc predominated (Brown *et al.*, 1969), and equitoxic concentrations of (ii) phenol, zinc and copper, and (iii) nickel, zinc and copper (Brown and Dalton, 1970), with which there was good agreement between predicted and observed results.

While the bulk of this work indicates that satisfactory prediction of the toxicity of simple mixtures of the commonly-occurring poisons can be made, there is no theoretical basis for this and no reason to suppose it would be true for other substances or other fish, or for effects other than survival. On the contrary, circumstances can be envisaged when it would not, for example when complexes are formed between metals and cyanide; certainly there are examples of unpredictable toxicity of complex mixtures, particularly pesticide formulations (Alabaster, 1969).

2.1.2 Sewage effluent

The predictive method has been used to estimate the relative importance of the commonly-occurring poisons (ammonia, monohydric phenols, zinc, copper and free cyanide) in sewage effluents in industrial areas in the United Kingdom, samples being taken from a variety of disposal works covering a range of treatment processes and receiving various types of industrial wastes (Lloyd and Jordan, 1963, 1964); the toxicity of the effluents to rainbow trout was measured under controlled conditions and predicted on the assumption that the toxicity of the mixture of all given poisons could be calculated from chemical analyses in a manner similar to that used in laboratory studies. In 13 out of 18 toxic effluents, the predicted

toxicities were within ± 30 percent of the observed values, and 6 effluents were correctly predicted to be non-toxic; only 2 effluents were more toxic than predicted, probably because of the presence of other unidentified poisons.

2.1.3 Field data

The same empirical approach has been applied in field studies of fishless rivers in the Midlands (Herbert, Jordan and Lloyd, 1965), taking into account the effect of environmental factors on the toxicity of individual poisons in the manner recently summarized by Brown (1968), and in general there was reasonable agreement between predicted and observed toxicity. More recent work has been summarized by Brown et al. (1970), in which it was shown that there was an overall tendency to under-predict toxicity by about 35 percent in polluted freshwaters and by about 65 percent in polluted saline waters.

Thus, all the evidence available suggests that much of the short-term toxicity of the commonly-occurring poisons can be satisfactorily predicted, at least for rainbow trout in freshwaters.

2.2 Relation between long- and short-term effects of poisons

With the five common poisons, ammonia, phenol, cyanide, copper and zinc, and mixtures of them, the relation between period of survival of trout and concentration of poison is such that a good indication of the median threshold concentration is obtained within a few days.

Other metals, however, (cadmium, chromium, and nickel), are much more toxic to trout after long periods of exposure than the results of short-term tests might suggest; for these it would not be appropriate to use 48-h LC_{50} values alongside those for copper and zinc when seeking correlation of toxicity with long-term effects on fish populations but to use the best estimate of the median threshold concentration.

2.3 Fluctuations in water quality

Most laboratory studies have been carried out with continuous exposure of the fish to constant environmental factors, whereas conditions in rivers are continually changing. This makes it virtually impossible to predict the survival of fish under field conditions.

With some poisons, e.g. cyanide, exposure of the fish for a short time to a concentration that eventually would have proved lethal, has no apparent adverse effect after the fish are transferred to clean water, and also has no effect on their resistance to subsequent exposure to the poison. Such recovery of the fish is not found with all substances. With DDT and an organo-phosphorus pesticide (Abram, 1967), and an effluent containing pesticide residues (Alabaster and Abram, 1965), under conditions in which short (6-h) periods of exposure to the poison alternated with similar periods in clean water, exposure time is approximately additive, the survival time of the fish being approximately equal to the time they would have survived in the mean concentration had it been applied continuously.

Further work with ammonia, zinc and equitoxic mixtures of the two (Brown, Jordan and Tiller, 1969) has also shown that under conditions in which the concentrations alternated between 1.5 and 0.5 times the 48-h LC_{50} at intervals of a few hours, survival of trout was close to that of fish kept continuously at the 48-h LC_{50}. More recent tests (Ministry of Technology, 1971) have been made with two poisons, phenol and cyanide, to which trout were exposed for alternate 4-h periods, under continuous-flow conditions, one poison gradually replacing the other, and then over the next period being itself replaced, each having a final concentration equivalent to the 48-h LC_{50}. The results showed that the mixtures were only slightly less toxic than predicted, assuming no recovery of the fish from cyanide, and that the toxicity of the system was reasonably well described by the average conditions.

With other poisons, e.g. the herbicide Reglone (Alabaster and Abram, 1965) and cadmium (Ministry of Technology, 1969), fish which had been exposed to potentially lethal concentrations for times far shorter than those at which they would have been killed under conditions of continuous exposure subsequently died after being returned to clean water; for these substances attempts to predict survival from average concentrations would, therefore, tend to under-estimate toxicity.

Long-term exposure of fish to sub-lethal concentrations of some substances has also been shown to affect their subsequent resistance to poisoning. Trout exposed to ammonia for several months became more resistant to lethal concentrations of ammonia and less resistant to lethal concentrations of zinc (Ministry of Technology, 1968). Somewhat similar results have been reported for rudd exposed to sub-lethal concentrations of zinc; they developed an increased resistance to this poison (Ministry of Technology, 1966). On the other hand, trout, after exposure to sub-lethal concentrations of zinc were of similar sensitivity to a detergent (an alkyl benzene sulphonate) as control fish that had not been exposed to zinc, but were slightly more sensitive than the control to a mixture of detergent and zinc (Brown, Mitrovic and Stark, 1968).

These examples serve to illustrate some of the possible effects on direct toxicity of fluctuations in chemical conditions in polluted rivers. More information is required on both the nature of variations in quality that occur in rivers and of their effects on the survival of fish of all kinds before reliable prediction of toxicity can be made.

3. SUB-LETHAL RESPONSES

Generally, experiments with whole animals are more likely to yield ecologically significant results than those involving only organs or tissues, though the latter may be more useful in the long term in providing a basis for understanding the effects of pollutants in ecosystems. It is important, however, that, when *in vitro* tests are conducted, concentrations of pollutant should be used that are similar to those likely to be found in the whole animal when exposed to the pollutant under field conditions.

An example of a potentially useful test relates to the blocking of chemoreception in crustacea which could reduce the foraging success and also possibly interfere with the ability of male lobsters to detect the female pheramones and thus find a mate (IDOE, 1972).

Another example of changes in behaviour induced by environmental stress and appearing to have ecological significance is provided by the reaction of the shrimp (*Crangon vulgaris*) to reduced dissolved oxygen (DO) (Huddart and Arthur, 1971). Normally a proportion of a population swims by pleopod action, rising from the bottom at a shallow angle and then, swimming actively downwards, return to the bottom to burrow where it is sandy. In the presence of lower DO a much larger proportion is free-swimming, rising from the bottom at a steep angle and then sinking passively; with further reduction in DO swift upward escape reactions occur, involving rapid abdominal flexure with the uropods extended, followed by passive sinking. Standing individuals appear to be "on tiptoe" and are unresponsive to stimuli until some time after the restoration of high levels of DO. The consequence of this behaviour in an estuary in which a zone of partly deoxygenated water oscillates with the tide would be for the shrimp to be carried by the water current away from hypoxic conditions. This suggestion is reinforced by the results of a mathematical simulation of the Thames Estuary embracing background swimming, stimulated activity in the presence of reduced DO and exhaustion after 30 minutes of increased activity; these results simulate the distribution and the large rapid reductions in number of shrimp which have been observed in part of the estuary.

On the other hand, many experiments, such as those (Deubler and Posner, 1963) with post-larvae of the American flounder (*Paralichthys lethostigma*), while they demonstrate the ability of the fish to detect and avoid water in which the DO has fallen to about 3 mg/l in a few minutes, give practically no indication of how they would react to the more gradual temporal and spatial changes likely to be encountered in the wild.

Other reactions caused by reducing DO and possibly having survival value for the species would include schooling behaviour which, for the northern anchovy (Engraulis mordax), is altered when DO is slowly lowered to less than 2 mg/l (Moss and McFarland, 1970).

In all these cases there is a need to conduct experiments on as large a scale as possible, to test conclusions using mathematical simulation and to confirm results in the field whenever possible.

4. RESPONSE OF ARTIFICIAL ECOSYSTEMS

One of the most relevant parameters to study is growth of the species because, in conjunction with the biomass it determines production; this, in turn, is related to yield, which is particularly important for commercial species. Growth of a species may be affected by environmental variables directly or indirectly (through effects on its food) or both, phenomena which have lent themselves to elegant elucidation in artificial ecosystems (Warren and Doudoroff, 1971; Warren and Davis, 1971), comprising riffle sections of freshwater streams containing salmonids and sculpin (Cottus perplexus) in the presence of kraft mill effluent, dieldrin, or low DO.

Empirical relationships have been established between the growth rate of fish and their own biomass and therefore between fish production and their biomass, between fish growth rate and food density, and between food density and fish biomass; i.e. fish production is shown to be depedent upon the biomass of both fish and their prey. Such relationships are particularly valuable in making possible a detailed examination of the functions of parts of an ecosystem and yet circumventing the need to rely upon a deep understanding of all the mechanismsm involved, which, although an ultimate goal, is a very distant one even for the riffle system.

Another type of laboratory estuarine microecosystem (Copeland et al., 1972) designed to the hydrological characteristics of Trinity Bay, Texas, and containing representative samples of the natural sediment showed how reduction in freshwater flow reduced net primary production (ratio of photosynthesis to respiration) in all but the seaward segment, and whereas under normal flow numbers of zooplankton were not related to photosynthesis because they were utilizing imported organic nutrients, under drought conditions they were entirely dependent upon photosynthesis. Thus, a large import of polluted freshwater bringing nutrients might offset to some extent, the disadvantages of its toxicity. At the same time, by virtue of its low salinity and its contents of poisons, it would have an impact on the survival of larval fish and oysters and their predators (Walne, 1972). It has been stressed in the context of cycling of metals in estuaries (Wilfe and Rice, 1972) that meaningful evaluation of the ecological stresses imposed by wastes would entail detailed knowledge of the interactions involved, which would require considerable research to quantify the reservoirs and the routes and rates of flux; in the meantime, however, it might be more rewarding to concentrate attention on identifying empirical interrelationships, such as Warren and Doudoroff (1971) and co-workers have done for production in their systems.

It is of interest to note that, as might be expected from the experiments of Warren and Doudoroff (1971), Jensen and Snekvik (1973) have described an increase in growth of trout populations followed by a decrease in abundance and eventual extinction in streams in which the pH has fallen steadily over a number of years.

5. FIELD OBSERVATIONS

Laboratory tests, even those using artificial ecosystems, although of help in understanding some of the complex effects of pollution under natural conditions, are no substitute for observations and experiments in the field. An example of a potentially valuable field experiment is the sub-lethal dosage, with DDT, of tagged salmon smolts and their subsequent release alongside untreated tagged fish; untreated fish showed a markedly better return from the sea as grilse the following year than the treated individuals (Fisheries Research Board

of Canada, 1971). This result is much more meaningful than those of laboratory tests on the effect of DDT on learning ability and behaviour of the fish in temperature gradients.

5.1 Empirical relationships between water quality and fisheries

5.1.1 Freshwater

Intensive studies of catchment areas have been initiated (Alabaster et al., 1972) in close collaboration with the River Authorities which have provided data on the chemical quality of the water at a large number of sampling stations and also made an assessment of the status of the fisheries at each. For each sample the concentration of each of the poisons - ammonia, monohydric phenols, cyanide, copper, zinc and the other toxic metals (nickel, chromium and cadmium) - were expressed as the fractional 48-h LC_{50} to rainbow trout, taking into account the effects on toxicity of environmental factors, including temperature, dissolved-oxygen concentration, pH value and water alkalinity. These fractions were summed for each sample taken and the probability distribution of the summed proportions of the 48-h LC_{50} were calculated for each sampling station. When this was first done in 1968 for stations on the River Trent system, the distributions fell into two fairly distinct groups, one associated with the presence of fish and the other with their absence; the coordinates of the approximate boundary distribution of 48-h LC_{50} between fishless and fish-supporting waters are given in Table I.

Table I

Coordinates of approximate boundary distribution of 48-h LC_{50} between fishless and fish-supporting waters

Per cent probability	1	5	10	25	50	75	90	95	99
Sum of proportions of 48-h LC_{50}	0.07	0.10	0.13	0.18	1.28	0.41	0.60	0.73	1.1

Somewhat similar results have been found subsequently for several other river systems.

By calculating for each station separate distributions for toxicity attributable to one or more poisons, the contribution of each to the total can be easily demonstrated. It underlines the importance of both dissolved oxygen and of metals in determining toxicity over the range where conditions appear to be marginal for fisheries (around 0.28 of the 48-h LC_{50}) and the added importance of cyanide and other poisons where they are worst.

Whether or not the empirical relation found in these studies have any validity elsewhere is not known. It is quite possible that, in attempting to sum small fractions of the 48-h LC_{50} of several poisons, the toxicity of some is underestimated, for example, because of unknown long-term effects, while that of other is overestimated, for example, because of adaptation by the fish. The resulting errors might tend to cancel each other and the same could happen with the relationship derived between toxicity to trout and the presence or absence of another species where the relative sensitivity of two species may be reversed for two different poisons. For this reason alone it would be surprising if the relationships already found were universally applicable.

Clearly, it would be better to make direct measurements of toxicity instead of relying on predictions and to use species appropriate to the fishery of interest instead of trout; much more intensive population studies would also be desirable.

5.2.2 Saline water

Doubts have been expressed about the possibility of detecting the effects of pollution on marine fish stocks by examining commercial catches in view of the large fluctuations brought about by climatic factors and fishing pressure. There is a reasonably adequate theoretical framework for studying, particularly, the exploitation of stocks of some commercial fish, there being good estimates available of recruitment, growth and natural mortality, but estimates are needed for many more species (Gulland, 1971). In particular, more should be discovered about events during the first few weeks or months of life, particularly as the young stages may be the most vulnerable to pollution. It might then be possible to carry out meaningful mathematical simulations of the effects of mortality, perhaps on the lines already attempted by Waller et al. (1971) for the fathead minnow (Pimephales promelas).

Nevertheless, problems are perhaps best identified, as well as being best studied, under field conditions, particularly in estuaries, coastal waters and seas having comparatively little exchange with the open ocean. Special effort ought to be paid to these regions, in view of their importance for shell-fisheries and as nursery and feeding grounds for fish, and also the need to understand the most acute problems first.

Fruh et al. (1972) showed that the diversity of benthic organisms, zooplankton, and fish were related to estimated toxicity in San Francisco Bay and Galveston Bay, but further work is required to identify the main pollutants concerned. The progress already made in that direction in the freshwater environment has depended upon the assumption that the effect of sub-lethal concentrations of five common poisons (ammonia, phenols, cyanide, copper and zinc) is additive when they are present together in a river; this is not necessarily valid for all poisons in all circumstances, especially when very low concentrations are present. For the mummichog (Fundulus heteroclitus), for example, there appears to be synergism between copper and zinc and between these and cadmium (Eisler and Gardner, 1973) and for polychaetes, antagonism between phosphate, nitrate and silicate (Reish, 1970).

6. SUMMARY AND CONCLUSIONS

Freshwater environment studies of river systems have revealed empirical relations between water quality and the presence or absence of fish. By arbitrarily expressing water quality as the fractional 48-h LC_{50} of known poisons it is possible to identify the comparative importance in a given situation of different poisons and environmental factors.

When a single water quality characteristic predominates, an empirical relation between its temporal distribution and the status of a fish population can be used in formulating water quality criteria, though this also requires data on lethal and sub-lethal ecologically significant adverse effects on fish and other aquatic organisms, including reduced growth and fecundity, avoidance of low concentrations of pollutants and other alterations in normal behaviour and physiology. These aspects have so far received relatively little attention, partly because of the difficulty in demonstrating their relevance and partly because they would involve the use of considerable resources that might otherwise be deployed on existing lines of enquiry.

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TOXICITY TESTING AT KRISTINEBERG MARINE BIOLOGY STATION

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1. INTRODUCTION

Increasing coastal water pollution caused by the establishment of industry and increasing population at the coasts has made it essential for the authorities concerned to exercise control of the marine environment and to decide on limits for permissible quantities of pollutants in the sea. Such measures demand knowledge about the reactions of marine organisms to different pollutants and the ecological consequences of their presence in the marine environment.

For these reasons bioassay work has been done at the Kristineberg Marine Biology Station, situated at the Gullmar Fjord on the west coast of Sweden, for several years with marine animals as test organisms. Polluting components in coastal waters are studied, and their effects on the animals determined. The studies are particularly concerned with the toxicity of surface-active agents, frequently present in polluted coastal waters as they are active ingredients in detergents and oil dispersants. Recently, the effects of heavy metals have been included in the investigations. The work is supported financially by the National Swedish Environment Protection Board and the Royal Swedish Academy of Sciences to which the station belongs.

The toxicology studied at Kristineberg can be characterized as an ecological toxicology because it is aimed not only at the necessary determination of the acute toxicity of different contaminants but also at a prediction of possible ecological consequences of their presence in the marine environment.

For these purposes, a great variety of marine animals are used, belonging to different taxonomic groups, such as fish, crustaceans and bivalves, each represented by two or more species from different habitats and with different modes of life. Both adult animals and developmental stages are studied as sensitivity to toxicants varies considerably during the life cycle, the early phases generally being the most sensitive.

Short-term tests are carried out over 96 hours to determine acute toxicity and lethal effects, and long-term tests, running for several months, are used to observe chronic effects of sublethal concentrations, corresponding to those which may occur in coastal waters.

During the tests, mortality and survival times are recorded continuously. A particular interest is taken in the observations of the effects of various biological functions which may be of ecological importance to the animals, such as behaviour, activity patterns,

defence reaction, response to food, etc. These functions serve as early indications of effect, but may differ greatly in sensitivity. Even small changes in such reactions may have fatal consequences for the animals when part of the ecosystems. Effects on growth and development are also studied in the early phases of life.

Attempts are made to understand the general principles for the toxicity of the materials tested, by studying their action on physiological functions such as respiration and osmoregulation as well as their accumulation in tissues and organs.

2. METHODS AND EQUIPMENT

The biotest technique used at Kristineberg is based on the continuous flow principle. Continuous flow aquaria systems are generally considered to facilitate the maintenance of more natural conditions in the physico-chemical environment of the test tanks, i.e. of such factors as temperature, salinity, pH, oxygen content, NH_3-content, bacterial level, etc.

Aquaria systems with running sea water are possible at Kristineberg as the station has a constant supply of clean sea water, being pumped into the laboratory from a 40 m depth of the Gullmar Fjord. At this depth the salinity is fairly constant, between 32-34‰, and the oxygen content is high, 80-90% saturation. The incoming sea water has a temperature variation throughout the year from 4 to 16°C but with no rapid fluctuations. In this region there are no tides.

The biotest equipment (Fig. 1) is principally designed as described by Swedmark et al. (1971). Details are given on p. 75 . It consists of 3 levels of aquaria with a varying number (4-13) of test tanks at each level, of which one is a control tank. The mixing of the standard solution of the test material and the water takes place in each vertical section via its respective funnel. To increase the mixing efficiency, the funnels are equipped with glaff beads on coarse nylon mesh. In order to keep the concentrations of the tested material constant, the standard solution is added to the test aquarium by means of precision dosage pumps (electric piston pumps or microperistaltic pumps). The running sea water is distributed by siphons, valves or by a special dosage apparatus (Granmo and Kollberg, 1972). All tubing is of Tygon or Silicon. Rectangular or cylindrical Perspex plastic aquaria are used. Their volume varies between 10 and 60 l, according to the size of the test animals. Cylindrical vessels are always used for testing eggs and larvae. Generally the number of adult animals in each tank is 10-20, but sometimes less of practical reasons. The equipment described here permits simultaneous testing of several species in a number of different concentrations at uniform environmental conditions and yields a great quantity of data within the test time. It has a simple design and has been shown to be of good reliability.

The standard solution of soluble material (surface-active agents, some oil dispersants, heavy metals) is made by dilution with distilled water. While testing insoluble dispersants and oils, the concentrated, undiluted products are used. These latter materials have proved to be very destructive to the tubing which raises some problems.

The emulsions (mixtures) of different oils and oil dispersants are made up by means of a stirrer in the standard solution tank. In a tight emulsion the droplets are smaller and more homogeneous in size than in a coarse dispersion. For light fractions of refined oil, such as marine diesel oil, the proportions of dispersant and oil were 1:9, to give a tight emulsion. For heavier fractions as fuel oil or crude oil, the corresponding proportions were changed to 1:1 to give a similar emulsion.

By the mixing methods described, surface active agents, dispersants, as well as oil emulsions, were well mixed with sea water. The dispersions formed, generally kept their homogeneity during the passage through the aquaria, with the exception of crude oil, which caused some practical difficulties as a tight emulsion was not obtained. In the tests the oil floated on the surface of the water, or appeared as droplets in a coarse dispersion.

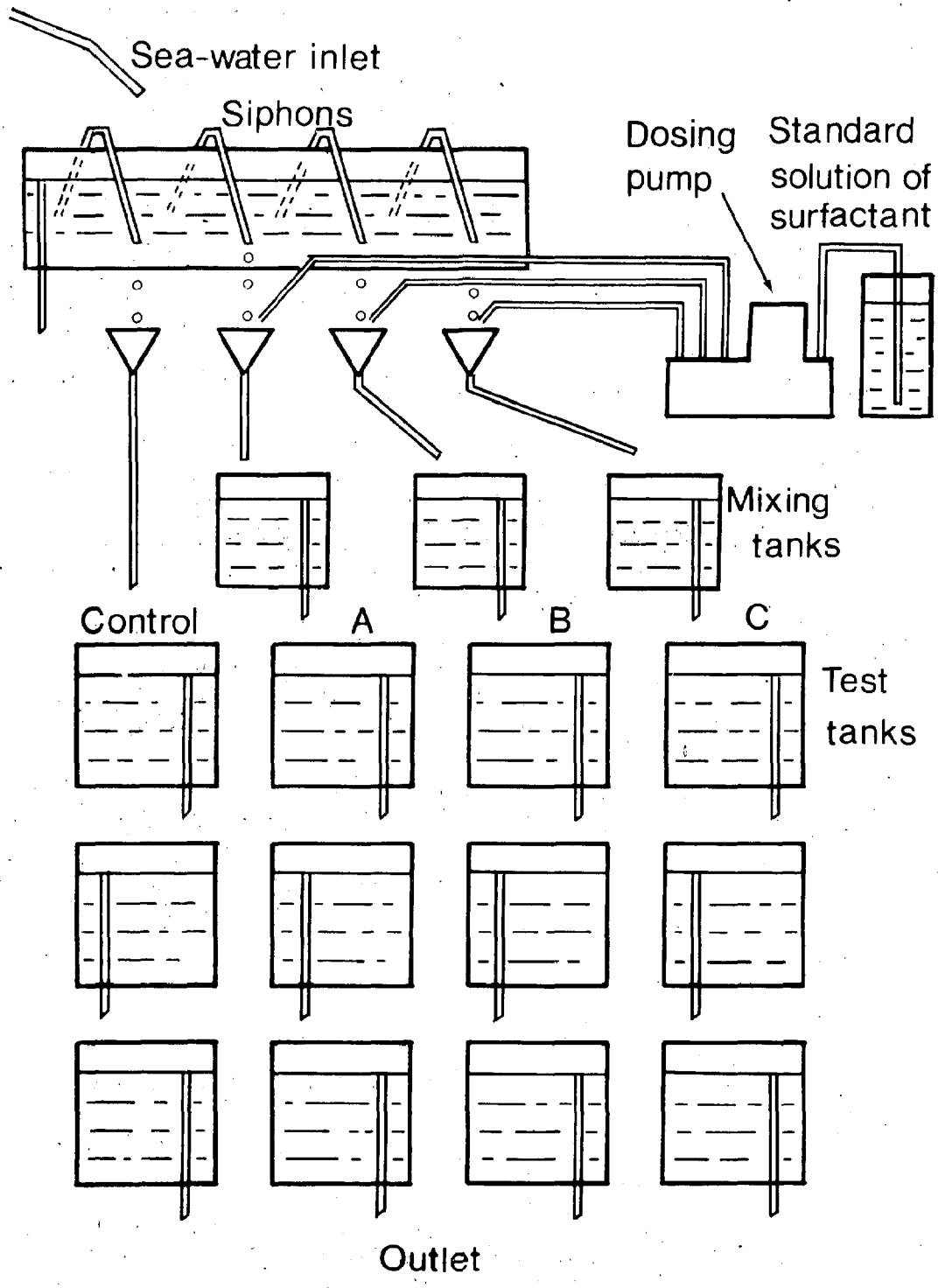

Fig. 1. Equipment for toxicity testing of marine organisms according to the continuous-flow principle. (Swedmark et al., 1971)
(Reprinted with permission from Springer Verlag)

However, this was considered to correspond quite well to the conditions in water of low turbulence and without tides. For this reason, no other mixing method was tried.

Concentrations from 1 to 1000 ppm (mg/l) are used as standard concentrations. As a rule, each product is tested in duplicate series. Substances are tested both pure and in mixtures for the study of joint effects.

The sea water flow is generally kept at 0.5 l/min and there are only small variations in the flow (less than 1%). Regular control of the oxygen content shows that this flow is enough to give a satisfactory oxygenation (more than 70%) in all tanks.

According to our experience, short-term testing is best run at temperatures which do not differ from the normal sea water temperature of the season, as the animals are then exposed to less extra stress. In our region, 6°C corresponds to the mean winter temperature at 40 m, 11°C to spring and autumn temperature and 16°C to the mean summer temperature. However, when necessary, the temperature of the test tanks is kept approximately constant with an immersion heater or a cooler equipment.

As the susceptibility of the animals has been shown to vary with temperature and season, probably in connexion with reproduction periods, it is preferable to make comparative short-term testing during the same season.

Short-term exposure always lasts 96 hours and is followed by a recovery period of at least 48 hours in clean sea water. The recovery period is particularly important when testing bivalves as they often manifest a delayed mortality.

Temperature, salinity and dosage rates are checked daily. The oxygen content is checked once per short-term test, or once a week in long-term tests. Mortality and effects on biological functions are recorded several times a day during short-term testing.

3. CHOICE OF TEST ANIMALS

A variety of marine fish, bivalves and decapod crustaceans are used as test organisms. They all live in the littoral zone (0-40 m) and are caught in the Gullmar Fjord and nearby waters. The test animals have been chosen according to the following criteria:

(a) They should represent different taxonomic groups and also different biological types with regard to locomotion (mobile or sedentary) and nutrition (filter-feeders - carnivorous);

(b) they should include species of economic importance;

(c) they should include species representing different trophic levels;

(d) they should be susceptible and the biological functions studied should be easy to define and observe.

The choice may be limited for practical reasons, such as availability in the region and ease of collection and adaptability to laboratory conditions.

Our standard test species among fish are cod (Gadus morrhua), three-spined stickleback (Gasterosteus aculeatus) and flounder (Pleuronectes flesus), two free-swimming species and one bottom-dwelling. Among decapod crustaceans, prawn (Leander adspersus), brown shrimp (Crangon crangon) and shore crab (Carcinus maenas), i.e. two more mobile and one less mobile species. Sometimes hermit crabs (Eupagurus bernhardus) and spider crabs (Hyas araneus) have also been used. The following bivalves have been chosen: the scallop (Pecten opercularis) a swimming species, the cockle (Cardium edule) which is a superficially burrowing type, the clam (Mya arenaria), a deep-burrowing and relatively stationary species, and the common mussel (Mytilus edulis) which represents a sessile form.

The animals are acclimatized to laboratory conditions for at least one week before testing to retain normal behaviour and physiology. If fish and crustaceans are kept for longer periods, they are fed up to 48 hours prior to, but not during, a short-term test. Even if the animals are fed, they may lose their condition in the store tanks, and thus newly caught animals are generally preferred for the testing. Exceptions are made for species with vertical seasonal migration, such as prawns and crabs, which are caught in shallow water at the end of the summer and kept in store tanks during the winter. In long-term testing all the species, including bivalves, are regularly fed.

Considering the variation of susceptibility during the life cycle, it is preferable to select test animals of the same size in order to obtain comparable results.

For the study of the effects on early phases of the life cycle, including fertilization, eggs and larvae of cod, common mussels and lobsters have been used. The effects on the sensitive phases of the moulting cycle in decapods are studied on prawns.

4. BIOLOGICAL FUNCTIONS STUDIED

To determine mortality different criteria for death, appropriate for each organism, are used. For fish and decapod crustaceans, the cessation of respiratory and body movements are used and for bivalves the cessation of function of the adductor muscles and the complete inactivation of the muscles of the mantle edge. The planktonic eggs of cod and larvae of cod, lobster and mussels are considered dead when laying immobile on the bottom of the tank.

To determine sublethal and chronic effects of toxic materials, the following biological functions of adult animals are studied: locomotory behaviour of mobile species, respiratory movements of fish, response to food by fish and decapods, shell-closure ability of bivalves, burrowing and siphon retraction of cockles and clams and byssal activity of common mussels. The effects on hatching rates and on the development of eggs and larvae are observed.

The effects of surface active agents, including oil dispersants, and heavy metals on the functions studied appear according to certain patterns in the following sequence:

(a) increasing activity (avoidance reactions);

(b) decreasing activity (successively impaired reactions);

(c) immobilization and death.

5. DATA PRESENTATION

The results obtained on acute toxicity and lethal effects of the toxic materials for each species are presented in diagrams, which express the relationship between concentration and survival time. From these toxicity curves the median lethal concentration or LC_{50}, i.e. the concentration that kills 50% of a sample at any exposure time, is interpolated and can be tabulated. The 96 h LC_{50} values are the standard form used for the comparative ranking of the toxic substances. Due to the different susceptibility of the test species, a ranking list is established for each species.

The procedures of plotting toxicity curves and calculating LC_{50} values are given in detail on p . Examples of such curves are shown in Figs 2 and 3, illustrating the toxicity of some oil dispersants to cod and common mussels respectively - one a sensitive species, the other a very resistant one. The exposure time is 96 h, followed by 48 h in clean sea water. Symbols with an arrow indicate that median mortality is not obtained. The distribution of the curves in the diagram shows the different toxicity of the tested material. It is evident from Fig. 2 that the toxicity to cod generally decreases rapidly with decreasing concentrations. Another type of toxicity curve is obtained for common mussels (Fig. 3).

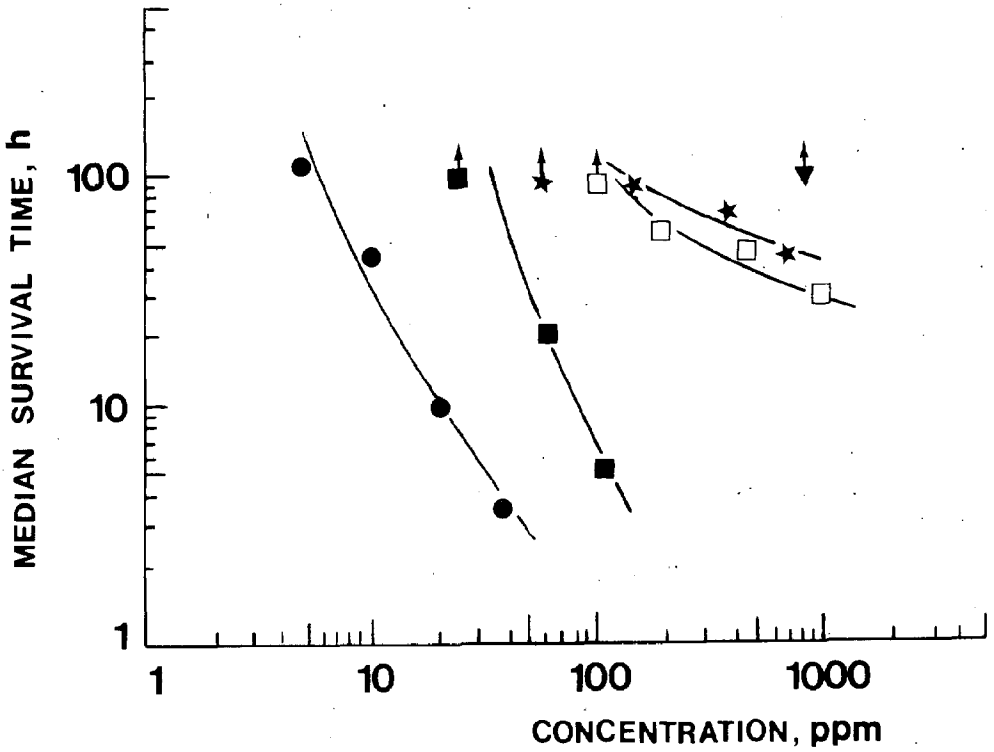

Fig. 2. Toxicity to cod of some surfactants and oil dispersants

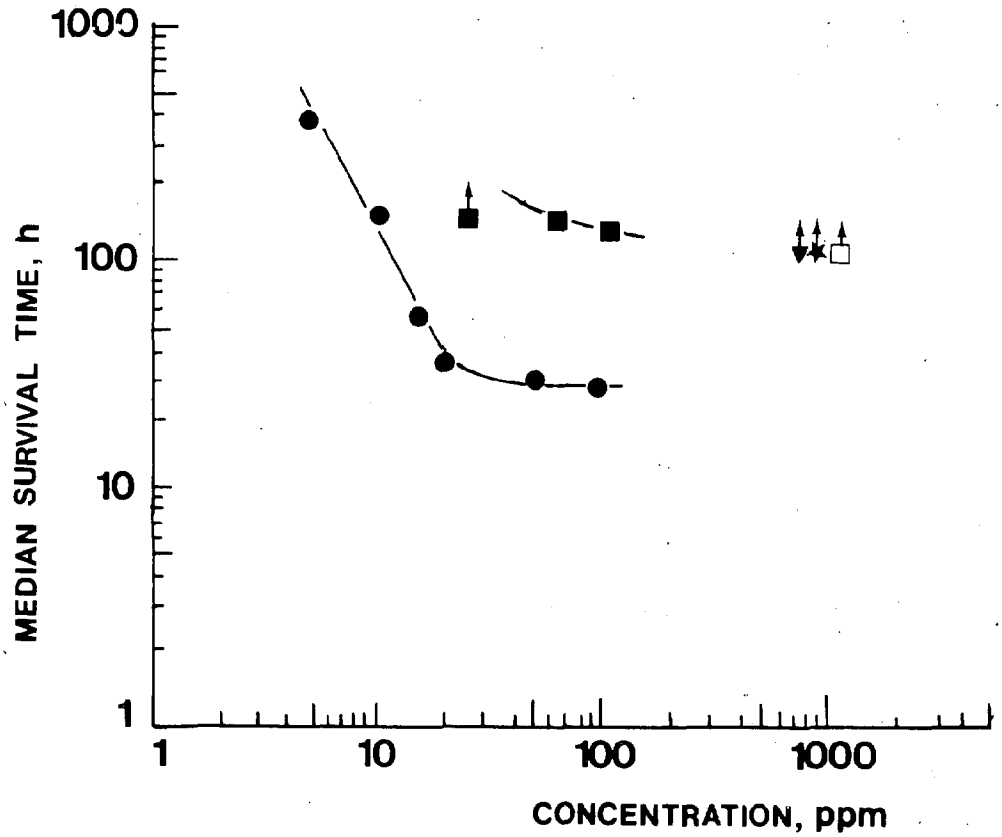

Fig. 3. Toxicity to common mussels of some surfactants and oil dispersants

The toxicity decreases much slower with decreasing concentrations and most of the mortality occurs after exposure. This is characteristic of bivalves which stresses the importance of including a period of clean sea water in the test.

The 96-h LC_{50} values for each product are tabulated as shown in Tables I and II, where the LC_{50} values obtained after 48 h in clean sea water are also included. Due to the different ability of recovery for different species and the often delayed mortality, particularly of bivalves, these values are often lower than the 96-h LC_{50} values. With prolonged exposure time there is also a decrease in the LC_{50} values, i.e. the toxicity increases with time.

The variation obtained in the survival times and in LC_{50} values, probably due to temperature, season and individual condition, has been statistically treated and the 95% confidence limits calculated. For variation in survival times, Litchfield's method (1949) has been used, and for variation in LC_{50} values that of Litchfield and Wilcoxon (1949).

Mortality in controls has been, and should be, rare. When this occurs, the percentage mortality in the test series has been calculated after taking into consideration the control mortality as described by Tattersfield and Morris (1924).

The sublethal effects on the biological functions studied are expressed as the lowest median concentrations of the toxic material that affect activity or reaction patterns. They are called effective reaction concentrations for 50% of the sample, i.e. EC_{50} values, after the proposition by Sprague (1969). In Table III an example of EC_{50} values is given for different phases of the activity pattern observed on the swimming activity of cod, and shell-closure ability and byssus thread formation of common mussels.

From the knowledge of such concentrations for different biological functions, important for the survival of the animals from various ecological habitats, predictions of the possible ecological consequences of pollution in the natural environments can be made.

Table I

Median lethal concentrations (LC_{50}) of some surfactants and oil dispersants for cod

Tested Material	LC_{50} (ppm)	
	96 h	96 + 48 h
Surfactants:		
Linear dodecylbenzenesulphonate (anionic)	1.6	1.0
Nonylphenol ethoxylate 10 (nonionic)	6	5
Tallow alcohol ethoxylate 10 (nonionic)	0.5	0.5
Oil dispersants:		
Fina-Sol SC	30	30
BP 1100	120	80
Corexit 7664	130	
Fina-Sol OSR-2	180	130
BP 1100 X	700	700
Corexit 8666	950	950

Table II

Median lethal concentrations (LC_{50}) of some surfactants and oil dispersants for common mussels

Tested Material	LC_{50} (ppm)	
	96 h	96 + 48 h
Surfactants:		
Linear dodecylbenzenesulphonate (anionic)	100	25
Nonylphenol ethoxylate 10 (nonionic)	12	10
Tallow alcohol ethoxylate 10 (nonionic)	50	15
Oil dispersants:		
Fina-Sol SC	110	90
BP 1100	1000	250
Corexit 7664	1000	
Fina-Sol OSR-2	700	700
BP 1100 X	700	700
Corexit 8666	1000	1000

Table III

Lowest median concentrations of some surfactants and oil dispersants affecting locomotory behaviour of cod, shell-closure and byssus activity of common mussels at 96 h exposure

Tested Material	Concentration (ppm)			
	Cod		Common Mussels	
	Increased activity	Decreased activity	Decreasing shell-closure ability	Decreasing byssus activity
Surfactants:				
Linear dodecylbenzene-sulphonate (anionic)	0.5	0.5		
Nonylphenol ethoxylate 10 (nonionic)	2	4	10	5
Tallow alcohol ethoxylate 10 (nonionic)	0.5	0.5		
Dispersants:				
Fina-Sol SC		26	100	12
BP 1100	40	100	500	40
Corexit 7664	50	100	1000	500
Fina-Sol OSR-2		140	700	110
BP 1100 X	300		700	140
Corexit 8666	950	950	950	65

6. SOME ASPECTS OF BIOASSAY WORK

For the necessary information needed by industry and government bodies as to the relative toxicities of new products, routine testing must be done, the purpose of which is to produce comparative rankings of toxic materials in standard form.

A useful standard form is the median lethal concentration, the LC_{50} value. It must be borne in mind that the LC_{50} values vary considerably according to the test species used, the testing technique (continuous flow or static aquaria systems) and exposure time (24, 48, 96 h or more). Temperature, salinity, season, individual condition as well as the stage of the life cycle of the animals also influence the values. They should, therefore, be considered as an estimation of toxicity and, instead of being arranged in a close rank order, the toxic materials are preferably grouped in categories with respect to their LC_{50} values, for example as follows:

96-h LC_{50}:	below 1 ppm (mg/l)	very toxic
	1- 100 ppm	toxic
	100- 1000 ppm	moderately toxic
	1000-10000 ppm	slightly toxic

The choice of the test species is of great importance as there is a great variation in susceptibility between taxonomic groups and between species, related generally to the mode of life, more active species being more sensitive, as a rule, than less active ones; neither is the susceptibility of a species always the same to different materials. It is, therefore, preferable to use more than one species.

If the results of short-term tests are translated to the field situation at pollution, they may correspond to the acute effects of accidental pollution, often of short duration due to quick countermeasures and rapid dilution.

Predictions of ecological consequences of pollution in aquatic (marine) environments are made possible by giving the bioassay work a biological design. Thus, several species should be used representing different modes of life and activity, different habitats and different taxonomic groups. The lethal toxicity is less essential than the sublethal and chronic effects on biological functions which are important for the survival of the animals in their natural environment. Such effects should be studied at low concentrations and preferably in long-term tests. The functions examined can be locomotory behaviour, defence reactions or food response of adult animals and development and hatching rates of eggs and larvae. The lowest median concentrations which affect activity or reaction patterns, i.e. median effective concentrations (EC_{50}), should then be determined.

The initial effects on the behaviour of an animal are generally an increased activity. The biological significance of this reaction is avoidance, i.e. flight reactions in mobile species or other protective mechanisms in sedentary species such as shell-closure of bivalves. Protective mechanisms seem to be especially well developed in littoral species, naturally adapted to large variations of their environment. These reactions increase the chances of survival of individuals at shorter exposure to pollution and may explain the often mild effects of accidental pollution of limited size.

At a longer exposure, the impairment of biological functions that follows the period of avoidance, implies a danger for the individuals as all reactions - locomotion, response to food, defence, shell-closure, byssal activity, etc. - become weaker. If this happens in the natural habitat, where competition for food and space is important and where the animals are part of a prey/predatory system, the most resistant species are favoured, while the more sensitive species find their chances of survival diminished. This exemplifies how a pollutant present in a concentration that is sublethal for an individual isolated in an aquarium, becomes lethal in an ecosystem, and must be borne in mind in the interpretation of laboratory results when applied to the field situations. It also points to the importance of the determination of the ecological threshold concentrations of toxicity of the pollutants

introduced into the sea. Knowledge of such concentrations is necessary to determine effective limits for permissible concentrations of pollutants in controlled marine environments.

The difference in resistance of species and the successive impairment of biological functions at prolonged exposure may explain why a constant pollution, even in low concentrations, is more serious for the marine fauna and flora than a limited acute pollution. It is well known from many polluted areas (Reish, 1970) that chronic pollution changes the composition of the marine communities and that the biological balance becomes disturbed. An ecological succession of animal and floral communities takes place, which starts with decreasing diversity as the susceptible species disappear and continues with an increasing number of resistant species (indicator species), ending with the replacement of macroscopic organisms by microbes.

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PROCEDURES FOR TOXICITY TESTING IN A CONTINUOUS FLOW SYSTEM

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1. EQUIPMENT

At the Kristineberg Marine Biology Station the apparatus used for toxicity testing is based on the continuous flow principles (Fig. 1). Sea water is introduced at the top (A), and distributed by the water flow system (B), described in detail by Granmo and Kollberg (1972). The water is dosed to the funnels (C) through nozzles (D) in the water flow system. The test solution is distributed from special stock aquaria (G), and is dosed with the aid of a pump (H) to the glass funnels via dosage tubes (I). In the funnels the water and the test material are mixed with the help of glass beads (E). The mixed solution is then distributed in the aquaria, which are placed at several levels (F).

After passage through the equipment, the waste water passes a flocculation unit before discharge into the recipient.

The tubes used must be of a resistant and non-toxic material, as many compounds are corrosive.

It is important to ensure that the circulation in the different aquaria is as good as possible - since non-homogeneous dispersed compounds will give incorrect results. The volume of the aquaria should be chosen according to the size and number of the animals. General rules cannot be given for the volumes, but the American Public Health Association biotest recommendation (1965) gives 1 l/g of animals/day as a sufficient flow, or a minimum flow rate which equals the volume of water in the tank in 6 hours.

Best results are obtained if the test unit is placed where external disturbances are low, as the test animals, especially fish, are easily stressed, which often results in increased sensitivity. Cylindrical vessels are preferred for fish to give them better possibility of swimming.

2. PREPARATION PROCEDURES

To attain the desired water flow from the dosage apparatus, choose nozzles with an appropriate diameter. For more careful adjustments, screw the threaded tube downward or upward depending on whether more or less water is wanted (Granmo and Kollberg, 1972). A simple way to measure the water flow is to use a graduated glass and a stop-watch.

For the dosage of the standard solution, two types of pumps are used, a piston pump and a microperistaltic pump. With the piston pump it is possible to regulate the amount of

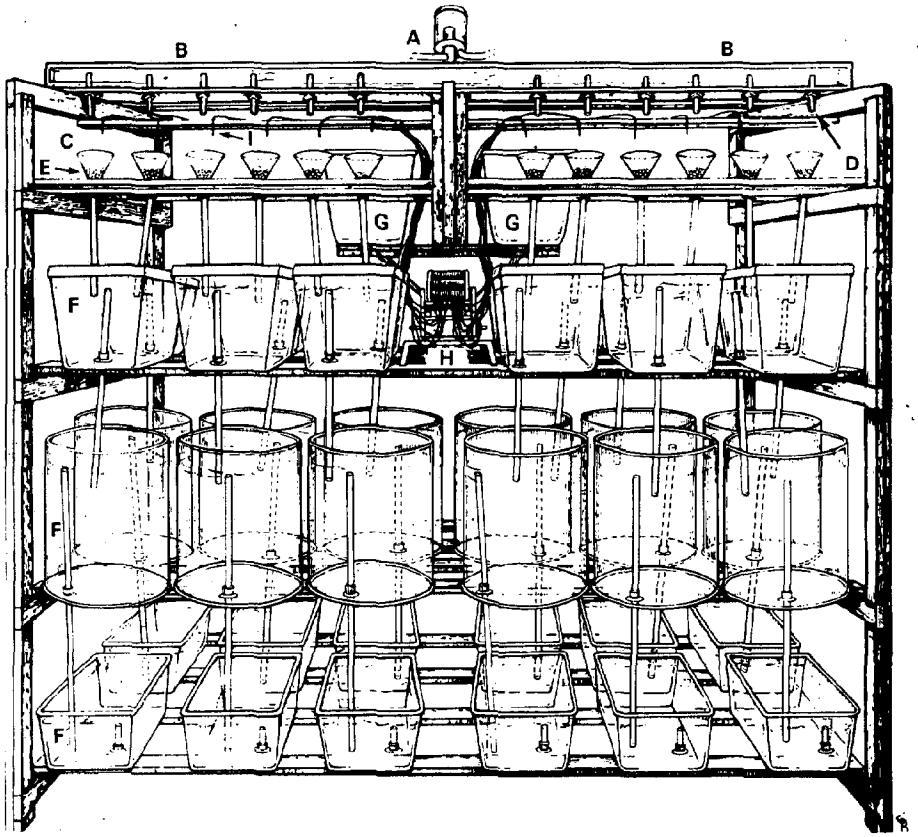

Fig. 1. Equipment for toxicity testing of marine organisms according to the continous-flow system (Swedmark et al., 1973)
(Reprinted with permission from Pergamon Press Ltd.)

standard solution by adjusting the pump. With the microperistaltic pump there is no possibility to correct the volume dosed. With this type of pump the standard solution is distributed through special tubes by the pressure of a roller. By choosing different diameters of the tubes, it is possible to obtain the concentration desired. When joining the tubes from the pump with those from the standard solution and the dosage tubes, connecting glass tubes are used. To protect to tubes against friction damages, grease is rubbed now and then onto the roller in the microperistaltic pump.

The following procedure is used to calculate the concentration of the standard solution wanted:

$$\frac{a \cdot l}{b \cdot 1000} \text{ g/l}$$

or, if the tested material is not 100% of the compound added

$$\frac{a \cdot l}{b \cdot 10 \cdot m} \text{ g/l}$$

where a = water flow, ml/min
b = dose amount, ml/min
l = concentration wanted, ppm
m = percent toxic material

When the concentration of the standard solution is determined, the different concentrations wanted in the test aquaria are easily obtained from the same expression.

Soluble material is dissolved in distilled water to prevent biodegradation, while insoluble products are dissolved in other, preferably low-toxic, solvents or the indiluted material is used.

To get an idea of the materials' toxicity before starting the test, and to facilitate the choice of the most suitable concentration range, a pre-test could be made. This may last for 24 hours with only a few concentrations within a wide range.

According to the aim of the testing, one species or a variety of species of test animals are chosen. Before testing, the animals must be acclimatized to laboratory conditions, which usually takes 3 to 7 days. Furthermore, it is better to keep the animals in the test tanks for one day before an experiment. From a statistical point of view, it is preferable to use as many animals as possible in the aquaria, but, as mentioned above, the number may be limited for practical reasons. The distribution of the test animals in the aquaria ought to be done at random using, for example, a table of random numbers.

3. TESTING

After the start, the dosage rates of standard solution to each aquarium are checked. For a careful control, 2-5 drops are collected from the dosage tube and the time interval for the drops to fall is noted. The drops are then weighed and from this information the concentration can be calculated:

$$\frac{B \cdot 6 \cdot D}{A \cdot 100 \cdot C} \text{ ppm}$$

where A = number of seconds between each drop
B = the weight of one drop in mg
C = water flow in ml/min
D = the concentration of the standard solution, in ppm, using a pure material

Dosage, as well as temperature and salinity, are checked daily, oxygen content and pH weekly.

Observations on the animals. It is important to use parameters which are easy to observe and define. For example, in our laboratory, the following parameters besides death are used:

Fish: Breathing (opercular movement per minute), coughing frequency, swimming behaviour as phases in activity pattern (increased or impaired reactions), or equilibrium disturbances, i.e. side to side rolling and swimming in a vertical position (nose up, tail daown), shoaling, etc.

Bivalves, clams: Shell closure ability and mantle-edge activity and, for common mussels, also byssal activity.

Crustaceans: Swimming activity and locomotory behaviour, food response.

After death, according to the aim of the study, lengths and weights of the animals may be measured, tissues and organs examined and samples taken and fixed for histological examination.

It is important that the animals are observed often, i.e. several times a day in short-term testing. Fixed observation times are preferable, as it is easier afterward to compare the different test results. Observations and calculations are more easily made if data charts, ready to fill up, are prepared before the test. After 96 h exposure the pump is stopped and the test continues another 48 h in clean sea water.

4. CALCULATIONS

4.1 Mortality (lethality)

Note the times from the start of the experiment will death occurs for the individuals in each tank. Then, a percentage/time diagram is established as in Fig. 2. The time scale on the abscissa-axis can as well be a linear one, but in most cases it is preferable to use a logarithmic scale. In the diagram the time to death (survival time) is plotted in a cumulative way for each test concentration. When drawing the curves the points form a straight line (Fig. 2a) or a sigmoid one (Fig. 2b).

Mortality may occur in the control tanks, and is then taken into account by using, for example, the expression of Tatterfield and Morris (1924):

$$X = \frac{(Y - Z) \cdot 100}{100 - Z}$$

where X = calculated lethality in the concentration, in percent
Y = real lethality in the concentration, in percent
Z = lethality in the control, in percent

When the mortality curves for all the concentrations are established, the time (for each concentration) at which 50 percent of the animals are dead (LT_{50}) can be read off. Knowing this, the time/concentration relationship, i.e. the toxicity curve, can be drawn as in Fig. 3. Plot on a double logarithmic diagram your LT_{50} values for each concentration and draw the toxicity curve. To obtain the 96-h LC_{50} value, check on the curve the concentration for 96-h exposure.

Statistical treatment of the LC_{50} values obtained is preferable. The methods of Litchfield and Wilcoxom (1949) or Finney (1952) can be used.

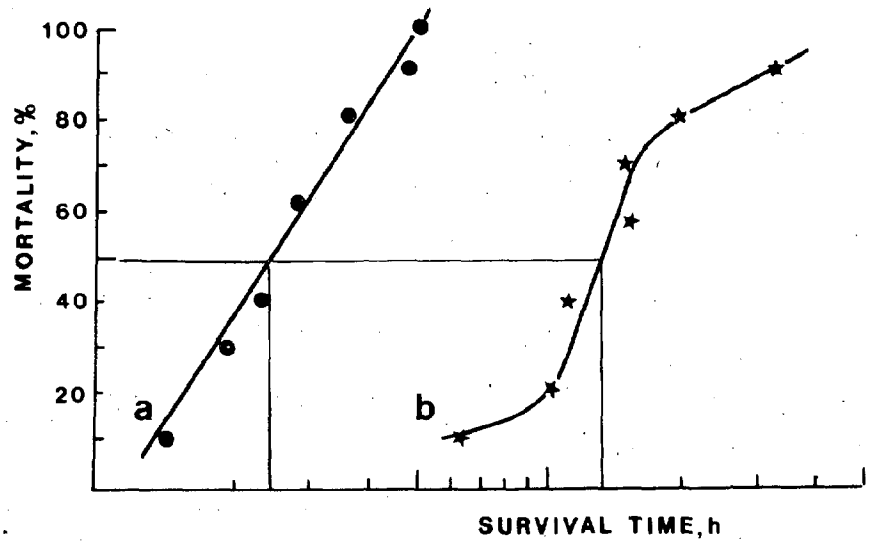

Fig. 2. Curves showing the cumulative mortality in test tanks

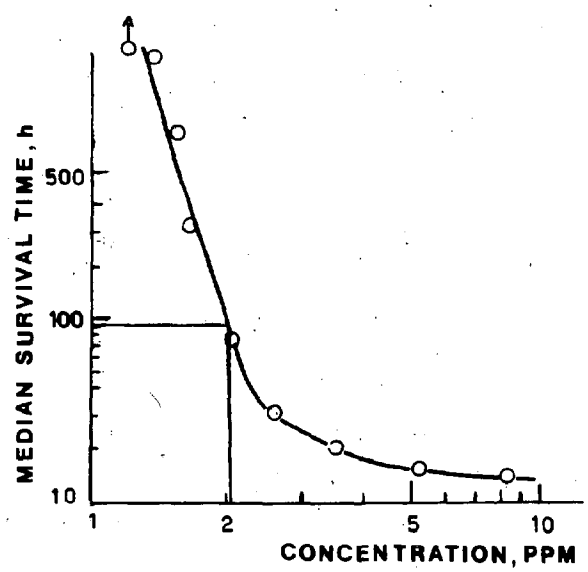

Fig. 3. Example of toxicity curve in a double logarithmic diagram. (Symbol with an arrow indicates that no median mortality was obtained)

4.2 Biological effects

Fish: Make tables of diagrams for coughing frequency, opercular movements, swimming activity, etc.

Bivalves: Byssal activity and shell closure ability can be noted as in Table I.

Crustaceans: Make tables or diagram for swimming activity, etc.

Table I

Number of common mussels with the ability to form new byssus threads after 96-h exposure and subsequent immersion in clean sea water (10 test animals)

Exposure time in hours	Concentration (ppm)					Control
	450	320	174	115	60	
22	3/10	4/10	9/10	9/10	8/10	9/10
43	0/10	5/10	6/10	8/10	8/10	9/10
65	0/10	2/10	2/10	6/10	8/10	9/10
93	0/10	1/10	3/10	5/10	7/10	10/10
117	4/10	6/10	10/10	10/10	10/10	10/10
137	6/9	9/10	10/10	10/10	9/10	10/10

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BIOASSAY METHODS USED BY THE RESEARCH LABORATORY OF THE NATIONAL SWEDISH ENVIRONMENT PROTECTION BOARD

by

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1. LABORATORY INVESTIGATIONS

The purpose of these investigations is to test the toxic effect of new chemical compounds and, to a certain extent also, to check different types of polluted water from industries and communities. The technique for acute toxicity studies is rather simple, using glass aquaria and fish, fingerlings of Salmo salar or Salmo gairdneri. The investigations are usually carried out as short-term tests, over five days. The result is expressed as the median lethal concentration, LC_{50} values. The physico-chemical qualities of the water are registered continuously or controlled with repeated sampling (temperature, pH, specific conductivity, oxygen content, etc.).

The effect of toxic compounds on the hatching of fish eggs and the early development of young fish is studied on cichlids, mostly of the species Cichlasoma biocellatum or Aequidens latifrons (Hasselrot, 1956). These species have been shown to be "normally" sensitive to toxicants and they produce 500-1000 transparent eggs at each spawning. The eggs are deposited on plastic plates which are then transferred to small glass aquaria containing 100 ml of a test solution. The larvae hatch after 3-4 days at a water temperature of 25-26°C. The effect of the tested compound can be observed at the different stages of development from egg to free-swimming fish.

The avoidance reaction of fish, mostly young salmon, in the presence of a water pollutant is studied in a "fluviarium" (Höglund, 1961). In this apparatus a fish is able to choose between different concentrations of a tested compound - if the fish is sensitive to it.

2. FIELD INVESTIGATIONS

To study the effect of water pollution on fish, caged specimens have been stationed in lakes, water courses and coastal waters (Hasselrot, 1964, 1965). The purpose has been to follow the effect of a known discharge or to trace an unknown one at fixed positions in a recipient over a certain time.

2.1 Equipment

Two types of round cages have been used. Both are covered with nylon netting without knots (to avoid injury to the fish by friction) and fitted with rings, mostly of stainless steel. One type of cage has rings 35 cm in diameter, the other 45 cm diameter. The cages are kept in position by means of sinkers, lines and plastic floats.

2.2 Test animals

Fish. For studies of the toxicological effects and the direct intake of compounds by the fish from the surrounding waters, one year old salmon fry are used in fresh waters. In coastal waters, from the area of Öresund, young rainbow trout have been used instead, because of the higher salinity. To study smell and taste-impairing substances, and also intake of metals, older specimens of both species have been used. Salmon and rainbow trout are used generally because of the convenience in having a uniform fish material from piscicultures all year round.

Other test organisms. Since 1972 bivalves have also been used for uptake studies in cages: *Anodonta cygnea* in fresh waters and *Mytilus edulis* and *Macoma baltica* in coastal waters.

To study the concentration of compounds in epiphytes, the laboratory uses a wooden construction consisting of a strip with plates in horizontal and vertical position, connected with a cord to the surface cage at each station.

2.3 Procedure

First, the distribution in the recipient of the effluent from a known source of pollution is determined. The caged fish are then placed in positions estimated to be most representative for the effect of the effluent on the recipient. In each cage of the small type, 30-40 one-year old fry are placed, and, in the larger type, 10-15 two-year old fish. The duration of the investigation varies from five days (for studies on toxicity, taste and smell impairing effects) to one month (for studies on the accumulation of mercury and other heavy metals). The condition of the fish during exposition time is observed and, if possible, various tests are made to check any physico-chemical alterations in the environment of the cages.

At the end of the investigation time the fishes are frozen and taken to the laboratory for analysis of accumulated substances, or to the National Swedish Institute of Public Health for an odour and taste test (organoleptic test). The scale for this test ranges from 1 to 5, where 1 means a fish with a very bad smell and taste, and 5 is an unaffected fish.

Bivalves and samples of epiphytes are also sent to the laboratory for analysis of accumulated substances.

2.4 Results

The reports which follow are given to provide a more concrete presentation of the applicability of the cage investigations as a means of following known sources of water pollution or of tracing an unknown one.

2.4.1 Known sources

Kraft pulp mills. Frövifors Bruk, Frövi. This mill had an annual production of 55 000 t of 90 percent pulp, which was used exclusively for paper making. Steps taken by the mill to prevent water pollution included a well developed fibre recovery system and a scrubber tower for collecting the noxious and evil-smelling sulphuric substances in the digester and evaporator condensates. Before the outlet the waste water passes a big dam. The factory is

situated at a river, Bårsån, with a discharge of 3-30 m^3/s. The river ends in a rather shallow lake, Väringen, which is about 7 km in length.

An investigation of the odour of the water before the exposure of fish in net cages in March 1962 showed that the sulphate waste water was concentrated in the part of the lake between the mouth of the receiving river and the outlet river of the lake (Fig. 1). The waste water was also more concentrated in the deeper layers of the lake. There was a total lack of oxygen below a water depth of 4 m. Only in the upper water layer was the oxygen content sufficient for fish life. The caged fishes were therefore placed only at 1 m levels. The mortality of the exposed salmon fry was total by the end of the exposure time, from Station 2 to Station 5, 6 km downstream from the outlet of the factory (Fig. 2). Also the organoleptical test (made on perch) gave very bad values for the same row of stations and the values were low also at Stations 11 and 12 in the outlet river at a distance of about 10 km from the factory.

In the other part of the lake, outside the current of the polluting river (Stations 6-10) no effect was shown on the fish.

As can be seen in Fig. 2 the concentration of sulphate waste water in the northern part can also be traced in the raised values in the physico-chemical analyses of the water (colour, permanganate consumption, conductivity, contents of sulphate and sodium). The mortality among the salmon fry at Stations 2-5 may especially be explained by the presence of recin acids in the water.

Lövholmens bruk, Piteå. The annual production of this mill in 1965 was 150 000 tons 90 percent pulp. Steps taken by the mill to prevent water pollution were similar to those taken above. The waste water was led into a bay of the Baltic by a tube 1.6 km in length. This investigation was also carried out during late winter, when the influence of the waste water on the surrounding water is greatest because for a long time the water has been isolated by ice and snow from the oxidation effect of the air.

An investigation before the exposure of the fish cages showed that the water in the Baltic had an odour from the waste water even at a distance of 9 km from the mouth of the outlet tube (Station 9b) (Fig. 3). The waste water spread mainly to the south and the odour of the water was usually strongest at a depth of 3-4 m. In the sound between the two islands opposite to the tube, the waste water was concentrated to the surface layers. The test fish was one-year old (2-4 g) to two-year old (30-40 g) salmon. Mortality (100%) occurred only at Station 2 at a depth of 4 m. The organoleptical test on the fish showed low, bad values not only at Stations 2 and 3 near the tube mouth, but also at Stations 4 and 9 and as far south as Station 10, at a distance of 12 km from the tube mouth. The lowest values in both odour and taste were registered for fish from cages at a depth of 3 m. The values obtained from physico-chemical analyses of the environment around the cages can neither be placed in relationship to the effects produced on the fish, nor used to detect the presence of sulphate waste water.

It can be established that the use of direct cage tests in recipients can give a reliable conception of the effects on the fish of discharging waste water from a certain sulphate mill. The cage tests can also serve as a very sensitive method to indicate the presence of sulphate waste water. In this respect the test results from sea investigations suggest these methods are far superior to the physico-chemical examination techniques in current use.

Mercury-discharging industries. Outlets of waste water containing organic or inorganic mercury compounds have caused significant increases of the mercury content in the exposed fish. This has been the case also when no increase of mercury has been observed either in water or in bottom sediment. An example of the latter are the cage investigations in the

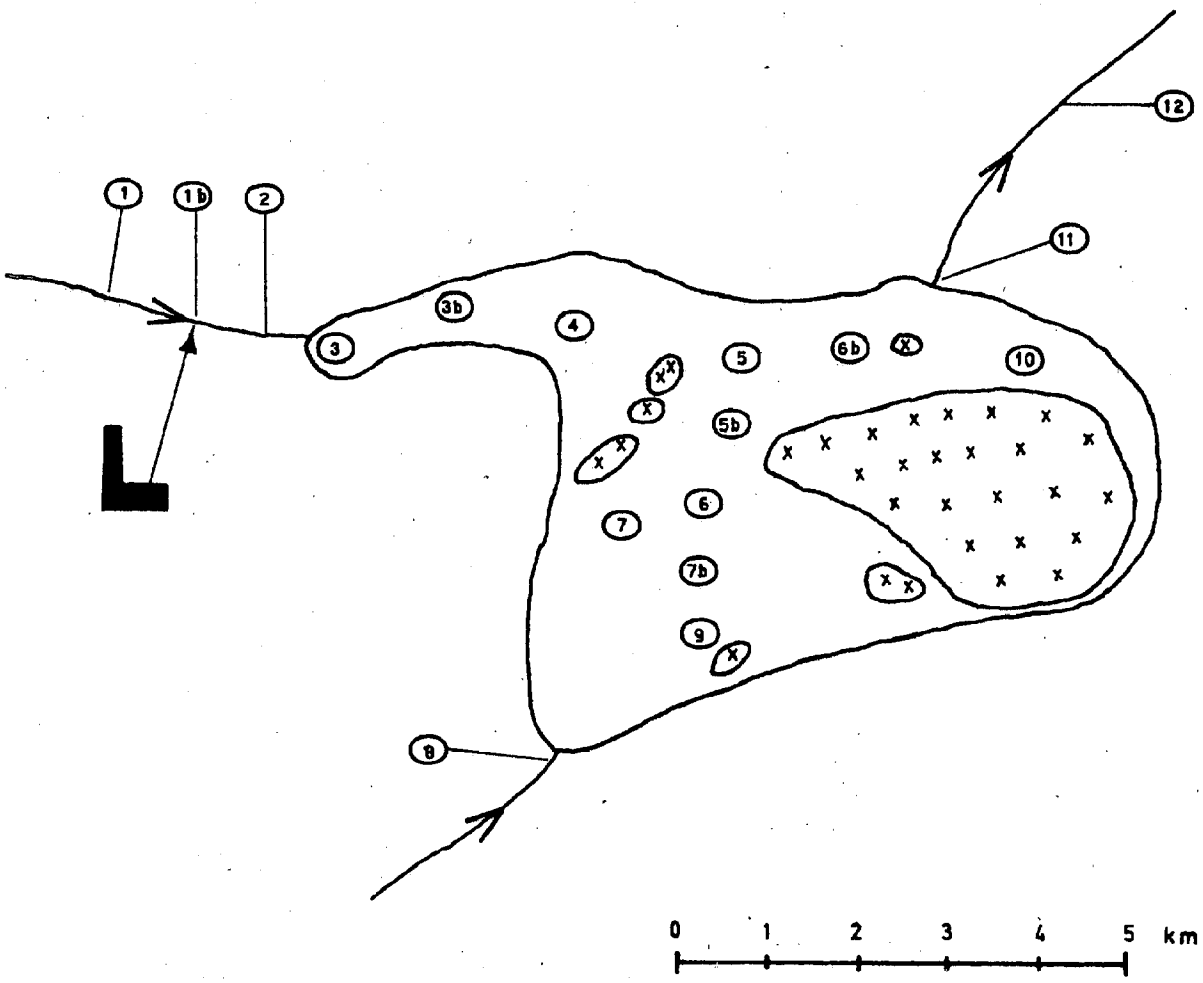

Fig. 1. Lake Väringen with sampling stations downstream from Frövifors bruk

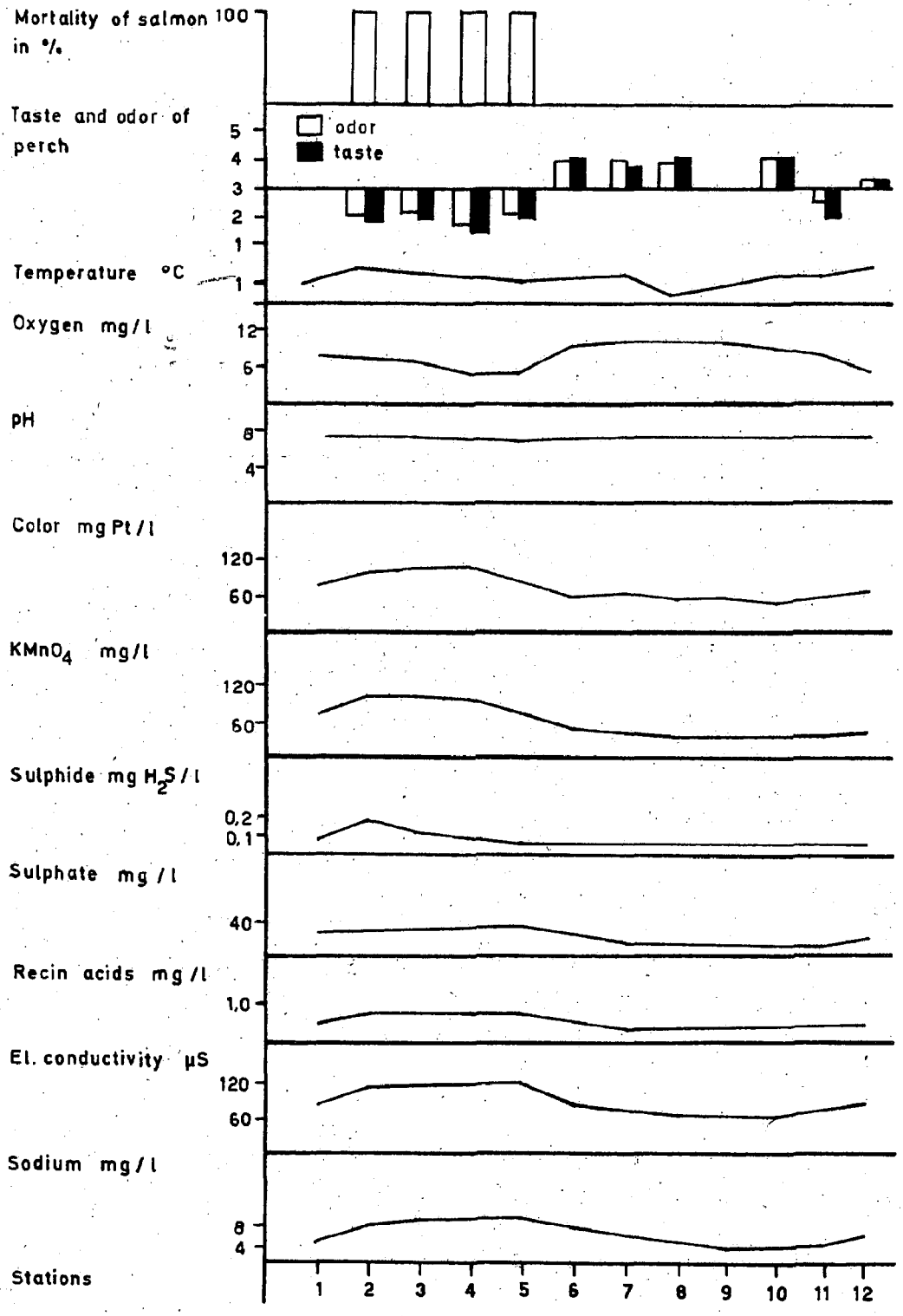

Fig. 2. Results of cage investigations downstream from Frövifors bruk in March 1962

river Göta älv downstream from the wood pulp mill in Göta (Fig. 4). In the autumn of 1967, the waste water from this plant caused an increase of mercury in the river fish to 830 ng/g or 8.8 times the basic value in the hatchery after the month of exposure. The mercury content in water and in sediment of the same station showed no significant increase. The investigations with cages in the river Göta älv are a good example of how, by using this sensitive technique in a water course with a very great discharge (mean discharge = 500 m^3/s), one can observe the presence and the strength of a discharge of mercury, check the effects of taken measures, etc. The series of analysed values from the nearest station upstream, the chlorine alkali plant EKA in Bohus, down to the station of Alelyckan (the intake of water for the city of Gothenburg) is a revealing picture of the extension of mercury emission to the water from this industry. As a result of measures taken at the plant, the concentration in the caged fish decreased progressively from 1967 to 1970.

Exposure of fish in cages has been done every year as a routine check, and that is how a sudden, increased mercury emission in 1971 and 1972 was observed (Fig. 4). New efforts have been made to reduce this registered discharge of mercury.

The highest increase of mercury in liver of one-year salmon exposed for one month, 56 times the basic value in the fish hatchery, is noted downstream from a big wood-pulp mill situated at the river Ume älv, with a mean water discharge of 200-300 m^3/s.

2.4.2 Unknown sources

In the beginning of February 1962, an abnormally high mortality in young salmon was observed in a fish hatchery supplied with water from the river Dalälven. At this spot the river has a mean water discharge of 215 m^3/s during winter and 350 m^3/s for the whole year. It was established that the mortality was caused by a marked anaemia of the fish. Hematocrit values of 5-10 percent (normally about 40 percent) were observed in these fish. Investigations on fish caught in the river itself from March to May showed that the anaemia was limited to the river downstream from Avesta, a highly industrialized town 26 km upstream from this fish hatchery. Even in similar piscicultural establishments at the mouth of the river, 100 km downstream from Avesta, fish taken within a month's interval demonstrated very low hematocrit values. The anaemia gradually ceased at the end of April and the beginning of May.

The primary cause of the fish anaemia could not be disclosed before the water temperature again dropped in late autumn. Net cages with young salmon were then put into the river at many places to ascertain the origin of the anaemia causing agent, which proved to be waste water from a yellow phosphorous producing factory in Avesta. Aquaria tests with waste water from a settling tank in this factory, containing about 0.1 mg P/l, gave within two days a descent in hematocrit values in young salmon from 50 to 11 percent. Still lower concentrations gave low hematocrit values within two weeks. The water temperature was only 1-2°C. When the temperature was raised to 10°C, the hematocrit values increased again.

Other aquaria tests with yellow phosphorous in colloidal form added to water, partly from the river Dalälven upstream from Avesta, partly from the Lake Mälaren, gave the same decreasing effect on the hematocrit values of the test fish. In such a way it has finally been proved that no other agent than yellow phosphorous in the water from the river Dalälven was responsible for the fish anaemia. In the river Dalälven the effective concentration of yellow phosphorous at low water temperatures must have been very small, probably far below ppb. We can thus conclude that the use of caged fish was the only way to solve the problem. The physico-chemical methods in current use for water analyses were not specific and sensitive enough to be of any use.

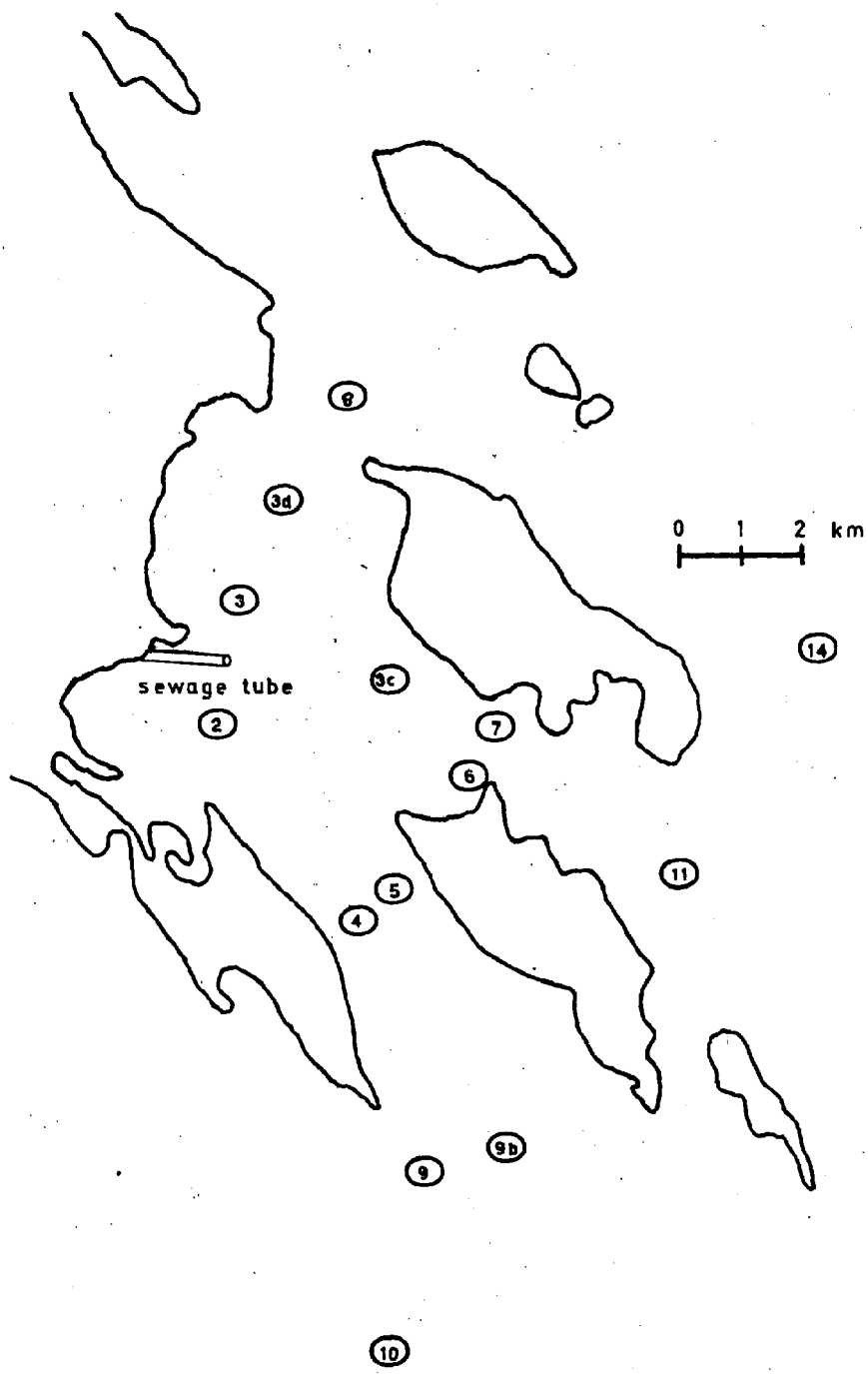

Fig. 3. Sampling stations in the archipelago outside Lövholmens bruk

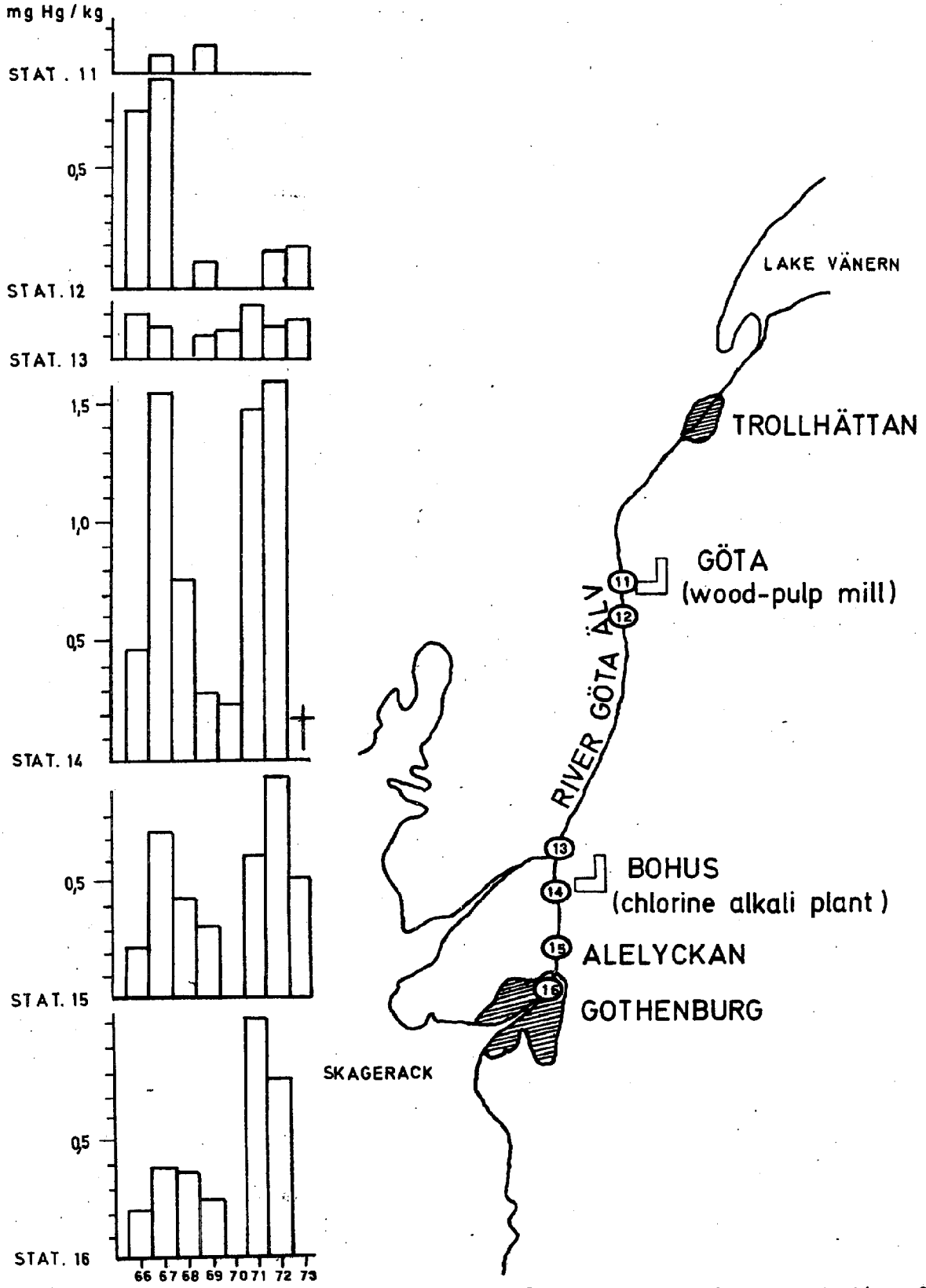

Fig. 4. Investigation area in Göta älv since 1966. Diagrams showing concentration of mercury in test fish
(† indicates that test fish died)

After these surveys the factory in question started to treat the waste water more effectively, e.g. secondary precipitation with milk of lime. Since then no abnormal hematocrit values have been found either in salmon in the fish hatcheries or in caged salmon in the river itself. For safety, there are still (1973) some control fish cages situated in the river just downstream from the outlet of the factory.

In 1962 this dangerous effect of yellow phosphorous was quite unknown. The result of the investigation was first given in 1963 in an official report to the authorities concerned, then in a short report to an OECD Conference in Paris in 1964. In 1970, the same effect of yellow phosphorous on marine fish and crustaceans was reported from Placentia Bay, Newfoundland (Jangaard, 1970, Zitko et al., 1970, Anon, 1970).

2.4.3 Complementary investigations

To check how far out in the sea the smell- and taste-impairing effect of, for instance, sulphate waste water on free-swimming fish could possibly reach, some complementary investigations were done. In one case about 5 000 specimens of one very important local commercial species, vendace, Coregonus albula L., were tagged and put into the Baltic near the mouth of the outlet tube from the kraft pulp mill, Lövholmens bruk. The intention was to follow how far from the tube the polluted fish had swum in a fortnight, which was 30-40 km. A fortnight is a minimum time for a polluted fish to expel the bad smell and taste from its flesh.

Kyrksjön, belonging to the water system of the river Delångersån, is a lake in Sweden containing the most mercury contaminated fish. Here there has also been tagging of fish as a complement to the cage investigations in the lake. On recapture in 1970, in 17 out of 400 tagged pike which had been moved from Dellensjön (an unpolluted upstream lake) to Kyrksjön the year before, very much increased mercury content could be analysed in muscle tissue: 1.3-6.3 mg/kg with a mean value of 2.7 mg/kg, compared to a basic value of about 0.5 mg/kg in pike from Dellensjön (1 mg/kg in fish flesh is the blacklisting value in Sweden). However, more than a thousand salmon fry that had been exposed in cages showed no difference in mercury value between the stations in Kyrksjön and Dellensjön. As the cage investigations give a measure of the direct intake by the fish of mercury from the water environment, the food eaten by the wild fish was undoubtedly of decisive importance for the concentration of mercury in the fish (the industrial mercury discharge was stopped at the turn of the year 1965-66).

3. INVESTIGATIONS AT INDUSTRIES AND WASTE WATER TREATMENT PLANTS

3.1 Industry

Investigations were made in order to find out what types of waste water within a factory caused the greatest effect on the smell and taste of the fish.

3.1.1 Methodology

Containers with 30 test fish each (mostly two-year old salmon) were connected directly to the waste water in question using different dilution degrees. The apparatus functioned according to the continuous water flow principle. Certain chemical compounds could be added to the apparatus, instead of the industrial waste water, in order to find out the effect that compound had on the fish. Each container was cylindrical and made of stainless steel, 70 cm high and 40 cm in diameter. In order to have good water circulation, the horizontal inflow was in the periphery and the outflow in the centre of the bottom. The waste water was pumped first to a special container for mixture and adjustment of the water. The time of the investigations was 5, 10, 15 and 20 days.

The organoleptical test on the fish was made by the test group at the National Swedish Institute of Public Health.

3.1.2 Results

Lövholmens bruk, 1966. To get rid of the smell- and taste-impairing effect on fish, the main discharge from the factory after purification had to be diluted about 1 000 times (see 2.4.1). The uptake of the smell- and taste-impairing compounds was greatest during the first five days. The additional accumulation during the following ten days was only slight. The elimination of these compounds from the fish flesh was also greatest during the first five days. It took, however, a fortnight in clean water for a fish with low organoleptical test values to become satisfactory in this respect. Among the pure chemicals it can be said that methylmercaptan was lethal for the fish in a dilution of 1 : 5 000 000. In a dilution of 1 : 10 000 000 the fish remained alive without any influence on the smell and taste. Dimethyldisulphide (the threshold dilution about 1 : 7 500 000) gave a much stronger, bad effect than dimethylmonosulphide on the smell and taste of the fish flesh.

These results have been of great use for the future plans concerning the improvements in measures taken for the purposes of water protection at that factory.

It can be mentioned that such container tests with fish at another sulphate mill, Gruvöns Pappersbruk, 1963, have shown that the waste water, after finally passing through an activated sludge tank, has practically no adverse effect on the smell and taste of the fish.

At a cellulose factory, Fiskeby bruk, fish in containers have also been used continuously since 1965 as control organisms against sudden appearances of poisonous compounds in the waste water.

A biotest establishment has been in use in Sweden since 1971 at Astra, Södertälje (Höglund, 1972). This industry makes pharmacological products. There have been inspections several times a day to study the condition of the fish. If this is bad, water samples are taken for analysis with an automatic sampler, which is connected to the same water that passes through the containers. This method has been successful in finding the sources of sudden toxic effects in the waste water.

At a mechanical pulp mill, Bowaters Svenska Trämassefabriks AB, Umeå, in 1966, and the chlorine alkali plant EKA AB, Bohus, in 1967, young salmon were also kept in containers (about 30 specimens in each), which for a month were continuously connected to the outlets of waste water of different dilutions. The mercury content of the fish usually increased in the containers with waste water. This could most clearly be seen in the Bowater case, where a mixture of 1 percent industrial waste water and 99 percent water from the river caused, in spite of the low winter water temperatures (0.5-0.8°C), an increase of mercury in the liver to 460 ng/g, which is 2.4 times the basic value in the fish hatchery. The result from the container test at EKA was a little more doubtful. The reason for this was the very uneven mercury content in the waste water. Mercury could, for example, suddenly occur as drops of metallic mercury, a form in which mercury cannot be taken up in fish.

3.2 Waste water treatment plants

In Gothenburg's waste water treatment plant at Rya, a bioassay establishment for fish has just been set up to control the treated waste water before its release to the receiving body of water. This establishment has been arranged under the management of the Research Laboratory belonging to the National Swedish Environment Protection Board in Cooperation with hydrotechnical experts from the Water Treatment Works of Gothenburg.

The establishment consists of two parts. One part is intended for a study of the concentration in fish of heavy metals and biocides. This consists of four cylindrical container of the type described above for the control of outgoing industrial waste water, but these containers are wider and lower. One container is filled with dechlorinated tap water, while the other three have different degrees of waste water dilution (1:1, 1:2 and 1:4) and are continuously connected with the main sewer. There are always in each container 31 two-year old rainbow trouts, which, after exposure for 14 days, are taken up and frozen for sample preparation. The other part of the establishment is an acute-toxic part where the trail fish's inability to swim in a water stream in the presence of a poisonous substance is registered. The water speed (5-15 cm/s) is adjusted by an electrical motor with a rotary propeller. The establishment is connected with the waste water in dilution 1:1. An affected fish is carried backwards by the water flow in the apparatus and is finally registered by photocells at the back of the experiment container.

To prevent accidental registration of a healthy fish, there is a region of strong light which repulses uninfluenced fish. For the same purpose the speed of the water flow is accelerated by a bottom plate, which rises towards the end of the experiment space. There are also front photocells which produce an electron flash when the fish passes. The acute-toxic establishment has partly been made with models from West Germany, Switzerland and France (Juhnke and Besch, 1971; Zahner-Schneider, personal communication; Leynaud-Barbier, personal communication).

By this method, the presence of a poisonous substance can be registered much earlier than with the currently used apparatus, which is based on the death of fish as indicator, and action can be taken at an earlier stage to prevent fish death in the receiving body of water.

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MONITORING OF AQUATIC POLLUTION

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1. INTRODUCTION

The conservation of natural resources, maintaining both the quality of the environment and its living resources, depends to a great extent on man's ability to manage them and to set up and enforce regulations in the best long-term interest. Unrestrained exploitation of the living resources may yield short-term benefits but in the long run is potentially disastrous.

This Course deals with pollution as it relates to the living aquatic resources, and with regard to these resources, the above statement has been proven true by the fact that uncontrolled fishery activities have led, in some regions, to overfishing and to the reduction or even the loss of certain species. Regional fisheries bodies have been established to discuss scientific research and regulations needed to overcome such difficulties.

Similar to unrestrained exploitation, the uncontrolled release of waste through rivers and outlets from municipal and industrial plants, as well as through dumping from ships and aircraft, may lead to ecological disturbances. In addition, it can be a risk to man's health through the ingestion of freshwater and marine food products.

Therefore, monitoring systems have to be established aimed at defining the health of the aquatic environment and the level of contaminants in the various organisms, especially in species of commercial interest. They should also determine possible trends in order to develop, if necessary, a warning system related to pre-set criteria or tolerance limits for critical pollutants.

From the beginning, it should be pointed out, however, that strong efforts are still needed in research to ensure that decisions about monitoring and standards, critera and tolerance limits needed in this connexion are made on a real scientific basis. Until these data are available, judgements have often to be made on the basis of our present knowledge and from results of sometimes inadequate surveys made as investigations for siting of outfalls or dumping into the sea, making at the same time use of a good deal of common sense.

Monitoring, in such cases, has to be started with the specific goal to control the fate of the waste in the environment in order to act immediately in case the basic considerations for releasing the waste are proven wrong.

2. CONCEPT OF AN INTEGRATED MARINE POLLUTION MONITORING PROGRAMME

The concept for an integrated marine pollution monitoring system is represented in Fig. 1. It consists of four main parts:

(a) The network of stations
(b) Elements of national programmes
(c) Elements of regional programmes
(d) Elements of worldwide programmes

2.1 The network of stations

The network of stations listed on the top of Fig. 1 forms the basis for the national and regional monitoring programmes.

A relatively dense network of "impact stations" has to be established in areas where the introduction of pollutants is considerable, such as estuaries, industrial centres or concentrations of population at the coastline with sewage discharge and waste water outlets, and especially in areas where the effects of pollutants are likely to be particularly harmful to living resources, e.g., in major fishing areas in and near the coastal zone.

On the contrary, in areas distant from the direct introduction of pollutants only a few "reference stations" may be needed to ensure that a rise in the degree of pollution in these areas is not overlooked and to show the trend for growing pollution in due time so that measures can be taken to avoid further input of pollution. Ocean weather ships, mid-ocean island stations and fixed buoys in oceanic areas at high latitudes may serve as reference stations.

Extensive and detailed monitoring of either natural or man-made accidental events, e.g., hurricane floods, tsunamis, big oil-well seepages or tanker accidents, should also be organized as part of national and regional monitoring programmes as they can provide valuable and unique information on the response of natural systems to the strong impact of pollutants.

2.2 Elements of national programmes

Research is one of the most important elements in connexion with monitoring programmes. This has been stressed by many working parties, workshops, seminars, etc., which have been convened, especially during the last five years, e.g., in the U.S.A. as part of the International Decade of Ocean Exploration (IDOE) or panels sponsored by the National Academy of Sciences or the National Oceanic and Atmospheric Administration (NOAA) (IDOE, 1972; National Academy of Sciences, 1971; Goldberg, 1972) in the United Kingdom (Cole, 1971), and the Federal Republic of Germany (Kinne and Aurich, 1968). On the international level, the Joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP) and the Food and Agriculture Organisation (FAO), supported by some other international bodies (UNESCO, IAEA, SCOR, WMO), have mainly promoted the discussion on research needed in connexion with monitoring marine pollution (IMCO/FAO/UNESCO/WMO/WHO/IAEA/UN, 1971; FAO, 1971).

The research on a national level has to be related to such problems as establishing "maximum permissible levels" (i.e., accepted level of a pollutant in an organism or in a population or resource to be protected from a specific risk) and "derived working levels" (i.e., maximum acceptable level of a pollutant in specified media designed to ensure that under specified circumstances a primary protection standard is not exceeded), and figures which would be needed for the definition of water quality criteria valid in the various

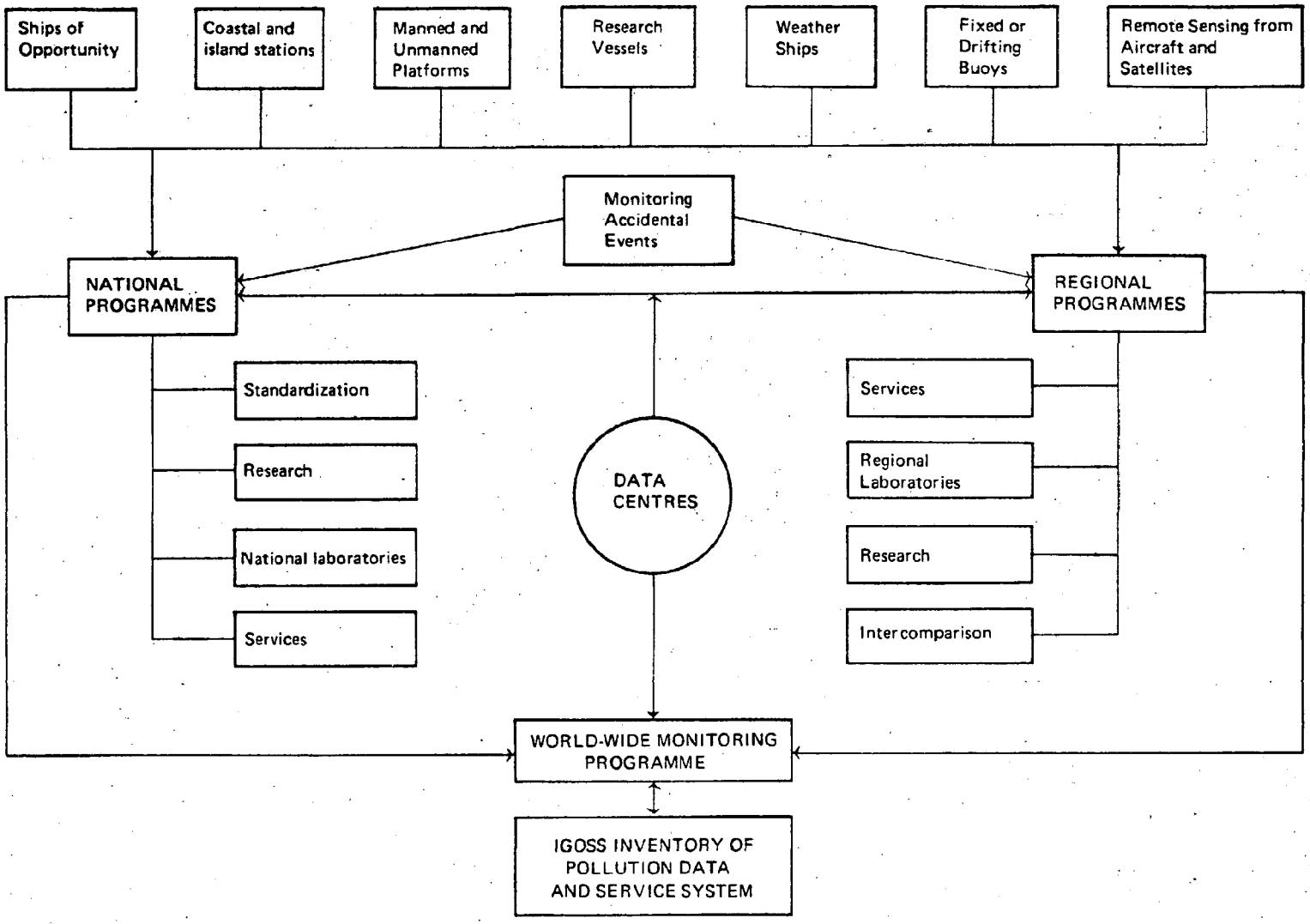

Fig. 1

Concepts of an Integrated Marine Pollution Monitoring Programme

coastal zones. However, there are also research needs in connexion with the standardization of analytical methods for the main pollutants in fresh water and marine environments of the countries; laboratories should also make strong efforts aimed at monitoring biological parameters. So far, monitoring is mostly directed to detecting physical and chemical changes in the natural environment with the underlying philosophy that by measuring parameters that are easy to measure one has taken a shortcut to measuring the health of the sea and its living resources and, thus, biological changes which may be accompanied with them. In fact, such a presupposition needs more profound and detailed knowledge of the relation between environment and living organisms than we have.

On the other hand, direct biological monitoring, e.g., through using indicator species, or making ecological surveys, cannot be used until fundamental ecological research provides a firm basis so that disturbances due to pollution can definitely be distinguished from natural population fluctuations in various marine communities.

Finally, services are included in Fig. 1 as an integral part of national programmes. They will coordinate the monitoring efforts on a national level, act as counterparts for other national services within the framework of regional monitoring systems, and provide facilities for storage and retrieval of the data on contaminants in the environment and in the living aquatic resources.

2.3 Elements of regional programmes

Regional programmes are the second step within a concept of an integrated marine pollution monitoring programme.

In many cases, regional programmes are needed to complete the efforts made on the national level because pollutants introduced at the coastline of one country may be spread, through currents, dispersion and mixing processes, into adjoining coastal waters or into the open sea where fishing grounds may be used by various fishing fleets.

Elements of these programmes are given in Fig. 1. As far as research and services are concerned, the problems are similar to those already dealt with under national programmes. To understand what is meant by intercomparison and regional laboratories, the setting up of a regional programme for the North Sea may be taken as an example.

The countries bordering this area undertook - within the framework of the International Council for the Exploration of the Sea (ICES) - a one year baseline study to determine the level of contaminants in the living resources of commercial importance, and after that time they decided to continue to coordinate their national monitoring programmes through ICES. Problems which came up during their cooperation were the intercomparison of results and availability of laboratories. (ICES, 1974).

Although all participating countries dispose of very well developed oceanographic and fisheries research institutes which should be able to perform the analyses needed in the monitoring programme, they had to limit the programme to the most important pollutants and to a small number of samples due to the lack of personnel. Furthermore, results of the intercomparison - using standard samples for the various contaminants - showed that, in the beginning, there were significant differences, and efforts had to be made to make the results of analyses comparable.

More severe difficulties of this kind may arise if regional monitoring programmes are envisaged in developing countries as the lack of trained personnel and adequate instrumentation will be more grave. For that reason, the establishment of regional laboratories for aquatic pollution research and monitoring will be an important element for the execution of regional monitoring programmes. These laboratories have to be well equipped with the sophisticated instrumentation needed and sufficiently staffed with well trained scientists and laboratory assistants in order to take responsibility for all the analyses which have to be made as part of the specific regional programmes.

2.4 Elements of worldwide programmes

Monitoring programmes with a worldwide scope will depend - as it is indicated in Fig. 1 - on the development of national and regional programmes. Only with the experience gained in national and regional laboratories and with the aid of their well educated and skilled personnel will it be possible to establish systems on a global scale.

Programmes of this kind are envisaged by the Long-term and Expanded Programme of Oceanic Exploration and Research (LEPOR) and, as a major part of LEPOR, in particular by the Global Investigation on Pollution in the Marine Environment (GIPME). Both programmes are being promoted by the Intergovernmental Oceanographic Commission (IOC), which acts as a special joint mechanism of various agencies, including FAO, cooperating through the Inter-Secretariat Committee on Scientific Programmes relating to Oceanography (ICSPRO).

The machinery developed by IOC for oceanographic observations and environmental monitoring is the Integrated Global Ocean Station System (IGOSS)(IOC, 1971) (see Fig. 1). IGOSS has also been designated as the suitable frame for establishing a worldwide marine pollution monitoring system. Plans have already been developed to start a pilot project aimed at monitoring petroleum hydrocarbons, tar balls and oil slicks in the North Atlantic Ocean and adjacent seas, as well as along the main tanker route from the Persian Gulf through the Indian Ocean and around the Cape of Good Hope to Europe. This pilot project became operational on 1 January 1975 and will continue for two years as approved by the General Assembly of IOC in November 1973. Monitoring will be based on observations and sampling made from voluntary observing ships, ocean weather ships, research vessels and suitable offshore platforms, as well as on observations from aircraft and satellites. Working groups will be established to define the methods for sampling and analyses, and to deal with standardized methods for reporting, dissemination, exchange and archiving of data resulting from the project.

With a view to the quickly growing number of data on pollutants in the aquatic environment and in organisms which will result from monitoring programmes at all levels, the archiving of data in a suitable worldwide storage and retrieval system has to be organized very carefully. This is the task of the IOC Working Group on International Oceanographic Data Exchange (IODE) in cooperation with the responsible world and national data centres belonging to the International Oceanographic Data Exchange System (IOC, 1967).

As part of the system the FAO Fishery Data Centre acts as a specialized permanent data centre aimed at collecting and maintaining fisheries data, including data of contaminants in living aquatic resources. An inquiry has already been started in order to get the information needed on institutes which, on a routine basis or as part of special research programmes, are analysing contaminants in aquatic organisms. As a first step, the Fishery Data Centre already compiled a directory of these institutions in order to facilitate the exchange of results and views between scientists working in this field. As a final step, it is envisaged to publish an inventory of data on contaminants in living resources, especially in commercially important fish, available at the relevant institutes in the world (FAO, 1974; 1976).

3. EXISTING MONITORING ACTIVITIES

Monitoring programmes have already been developed at all levels, i.e., on a national, regional and worldwide basis. In principle, we have not only to take into consideration such programmes which are dealing directly with pollutants but we have to realize that monitoring environmental, i.e., physical, chemical and biological parameters is required in order to estimate the rate of input of pollutants to the marine environment as a whole, and their rate of exchange between various water masses and ocean basins, as well as between different phases, such as solution, suspension and biota. Monitoring these parameters may also become important in relation to observed changes in the ecosystem ascribed to pollution but only if, as has already been pointed out earlier, the long-term natural and cyclic changes in the ocean are sufficiently known.

3.1 National monitoring programmes

Table I gives a review of national monitoring programmes based on scientific publications, in particular, on summaries published by the Smithsonian Institution (1970) and more recently, in May 1973, by the Intergovernmental Oceanographic Commission as a result of an inquiry to member states of IOC about their national monitoring activities (IOC, 1973).

Although one can assume that the table contains information about the bulk of activities in this field, the review is most likely incomplete and both corrections and amendments will be needed.

The first three columns of Table I inform about environmental parameters and pollutants monitored by national programmes, the last two columns give some details related to sampling stations and analytical methods used for marine pollution monitoring.

The table contains data from 63 countries, and although there seems to be no need for a specific discussion of its contents, it should be stressed that the number of activities is surprisingly high, also with regard to the analyses of pollutants from samples at sea.

In case the scientific community was able to correctly compare the information available through these activities and to interpret them accordingly, our understanding of the pathways by which the contaminants are distributed, both before and after they are deposited in the ocean, may be facilitated. This could also lead us a step forward in providing a long-term account of the accumulation of chemical contaminants in the marine environment which, in particular, is envisaged by a strategy for a national programme developed recently in the U.S.A. (Goldberg, 1972). I should also like to refer in this connexion to the scientific results obtained within the framework of the already mentioned ICES monitoring activities in the North Sea, e.g., Preston (1973), ICES (1974).

Before we go on to consider existing regional or worldwide monitoring systems I should like now to leave these general considerations on existing programmes and to deal with one specific example of a pesticide monitoring programme in order to demonstrate the carefulness which has to be given to such activities at all stages, i.e., during the preparatory and operational phase and for the evaluation of data.

The programme had been developed by the U.S. National Marine Fisheries Service in order to assess the subtle harmful changes in the marine environment and its living resources which result from the accidental or intentional transport of pesticides into the estuaries along the whole U.S.A. coastline (Butler, 1969).

Proceeding on the assumption that pesticide pollution in estuaries would be intermittent, depending on the seasonal use of pesticides, and that there would only be very low concentrations in the water mass, automated sensing devices could not be used. A suitable bioassay technique was selected on the basis of available studies on the ecology and management of the eastern oyster, Crassostrea virginica.

Typically, a mature oyster is feeding 90 percent of the time and transports about 16 l of water an hour through its gill system to extract the planktonic food. The oyster is physiologically active the entire year throughout much of its extensive geographical range. Most important, it is sedentary and easily handled.

Through suitable experiments it could be shown that oysters remove and store chlorinated hydrocarbon pesticides present in the surrounding water at concentrations as low as 0.1 microgrammes per litre. Oysters continue to build up such residues in their tissues at uniform rates as long as the toxicant is present. This biological accumulation may produce DDT residues, for example, 70 000 times as high as the DDT concentration in the surrounding water. Further, it is an important fact that the oyster flushes these residues out of its tissues at a uniform rate when the water supply is no longer contaminated. By sampling an oyster population regularly, it is possible to determine when the water supply becomes contaminated and when the contamination stops.

Table I

National Monitoring Programmes

COUNTRY	MARINE ENVIRONMENT									POLLUTANTS (RIVER)									POLLUTANTS (COASTAL AREAS)									ANALYTICAL METHOD										SAMPLING STATION							
	Salinity	Temperature	Oxygen	Nutrients	Waves	Stratific.	Currents	Prim. Products	Plankton	Organic	Inorganic	Pesticides	Metals	Ox.Cons. Waste	Dom. Sewage	Heat	Radioact.		Organic	Inorganic	Pesticides	Metals	Ox.Cons. Waste	Dom. Sewage	Heat	Radioact.	Oil	Gas chromatography	Thin-layer chromat.	IR- or UV-spectrom.	Mass spectrometry	Spectrofluorimetry	Atom.abs.spectrom.	Neutron activ.anal.	Colorimetry	Gravimetry	Gamma-spectrometry	Ships of opportunity	Patrol ships	Research ships	Weather of light ships	Manned platform	Unmanned platform	Buoys	Coastal station	Aircraft or satellite
Angola	x	x		x		x	x		x																																				
Arab. Rep. Egypt	x	x	x			x			x																																				
Argentina	x	x	x	x		x	x	x	x	Some dissolved constit.									x		x	x	x	x		x	x	x	x	x			x		x				x						x	
Australia	x	x	x	x				x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x																			
Belgium	x	x		x		x	x		x												x	x		x			x	Sophisticated equipment											x	x	x		x	x	
Brazil	x	x	x	x		x	x																																						
Bulgaria	x	x	x	x		x	x															x	x	x		x	x	Sophisticated equipment											x						
Canada	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x		x	x	x	x		x	x		x			x	x	x	x	x			x	
Chile	x	x	x	x	x	x	x	x			x															x																				
Colombia	x	x	x	x					x																																				
Congo (Braz.)	x	x	x	x		x	x	x	x																																				
Costa Rica	x	x				x	x																																						
Cuba	No information									x				x	x	x																													
Denmark	x	x	x	x		x	x	x	x	x				x	x			x			x	x																								
Ecuador	x	x	x	x				x	x	x				x	x	x																														
El Salvador	x	x				x				x	x	x		x	x																														
Finland	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x					x	x	x	x				Sophisticated equipment										x		x	x		x	x	
France	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x		x		x	x				x	x	Sophisticated equipment										x		x				x	x
Germany (F.R.)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x		x	x	x		x	x	x	x		x	x	x		x		x	x		x		x
Ghana	x	x	x	x			x		x	x				x	x	x																														
Guatemala										Some diss.constit.											x	x																								
Guyana										x				x	x	x																														
Iceland	x	x	x	x	x	x	x	x													x	x						x					x							x							
India	x	x	x						x			x					x									x																				
Indonesia	x	x	x	x		x	x	x	x																																				
Iran										x				x	x	x																														
Ireland	x	x								x	x	x		x	x	x					x	x	x				x	Sophisticated equipment											x							
Israel	x	x	x	x	x	x	x			x	x	x		x	x		x					x				x	x	x		x			x							x							
Italy	x	x	x		x	x	x			x		x		x	x	x	x		x		x			x		x																				

Table I (continued)

National Monitoring Programmes

COUNTRY	MARINE ENVIRONMENT									POLLUTANTS (RIVER)								POLLUTANTS (COASTAL AREAS)									ANALYTICAL METHOD								SAMPLING STATION								
	Salinity	Temperature	Oxygen	Nutrients	Waves	Stratific.	Currents	Prim. Products	Plankton	Organic	Inorganic	Pesticides	Metals	Ox.Cons. Waste	Dom. Sewage	Heat	Radioact.	Organic	Inorganic	Pesticides	Metals	Ox.Cons. Waste	Dom. Sewage	Heat	Radioact.	Oil	Gas chromatography	Thin-layer chromat.	IR- or UV-spectrom.	Mass spectrometry	Spectrofluorimetry	Atom.Abs.Spectrom.	Neutron activ.anal.	Colorimetry	Gravimetry	Gamma-spectrometry	Ships of opportunity	Patrol ships	Research ships	Weather or light ships	Manned platform	Unmanned platform	Buoys	Coastal station	Aircraft or satellite
Ivory Coast	x	x	x			x	x		x	x				x	x	x					x					x	Stand.class.equip.											x						
Jamaica	x	x	x	x		x			x																																			
Japan	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x				x					x	x	x	x		x		
Kenya	x	x				x			x	x				x	x	x																												
Korea	x	x	x	x															x		x		x			x						x	x						x						
Madagascar	x	x	x	x			x	x	x																																			
Malta																		x		x			x																					
Mexico	x	x	x	x		x	x																																					
Monaco								x										x			x	x			x																			
Mozambique	x	x	x	x					x																																			
Netherlands	x	x			x		x			x	x		x	x	x	x	x								x	x						x					x	x						
New Caledonia	x	x		x			x		x																																			
New Zealand	x	x		x				x	x	x	x	x		x	x	x	x			x	x		x			x																		
Nigeria	x	x					x																																					
Norway	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x		x	x	x
Peru	x	x	x	x		x	x	x		Not specified																																			
Philippines	x	x		x				x	x	x				x	x	x		x	x		x	x	x							x						No information								
Poland	x	x	x	x		x	x					x			x					x			x		x	x	Sophisticated equipment											x					x	
Portugal	x	x	x	x	x	x			x																x																				
Senegal	x	x	x	x		x	x		x																																				
South Africa	x	x	x	x	x	x	x		x	Some diss.constit.								x	x	x	x	x	x		x	x	Sophisticated equipment											x	x	x			x	x
Spain	x	x	x	x	x	x	x													x	x					x	Sophisticated equipment										x	x						
Sweden	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x		x	x		x		x	x					x			x			x	x	x		x		x	x
Taiwan	x	x	x	x		x	x		x	x				x	x	x	x								x																				
Tanzania	x	x				x			x	x				x	x	x										x																			
Thailand	x	x	x	x		x				x	x			x	x	x											Stand.class.equip.											x						
Togo														x	x																														
Turkey	x	x	x	x			x			x				x	x	x																													
U.K.	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x																			
Uruguay										x				x	x	x																													
U.S.A.	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x			x	x	x	x	x	x	x	x	x
U.S.S.R.	x	x	x	x	x	x	x	x	x	x				x	x	x		No information																											
Yugoslavia	x	x	x	x		x	x		x	x				x	x	x																													
Zambia										x				x	x	x																													

In order to use the oysters for a monitory programme on pesticides careful attention had also to be given to the sampling and analytical procedures. The main problems which had to be tackled in this connexion were the wide spread of sampling stations from about 50° to 30° northern latitude both at the Pacific and Atlantic coast of the United States and along the coastline in the Gulf of Mexico which meant that assistance and cooperation of many people was required and therefore both sampling and analytical uniformity was difficult to maintain. Besides careful training of sampling personnel it was decided to accomplish all analyses in the same laboratory. This became possible because a technique had been found to prevent spoilage of the samples and degradation of pesticide residues for at least 30 days without refrigeration. Thus, samples could be sent by ordinary mail to the analysing centre. Analyses were made by gas liquid chromatography which allowed to quantify 10 of the most commonly used organochloride pesticides at levels above 0.01 ppm.

Within nearly four years about 5 000 samples have been analysed from 170 permanent stations and it was found that DDT and its metabolites are the most commonly present pesticides; dieldrin, endrin and toxaphene being the next in frequency of occurrence. Normally, the amount of DDT was less than 0.5 ppm and only rarely the residue exceeded 1.0 ppm with a maximum value of 5.4 ppm following a single incident.

In general the DDT residues were not of sufficient magnitude to constitute a human health problem. However, their presence indicates the ubiquity of DDT in the estuarine food web. In particular, it was shown that in estuaries receiving significant amounts of agricultural run-off, the seasonal residue pattern is proportionally higher. In some cases the monitor data could clearly demonstrate the industrial discharge of pesticide waste whose presence had been unsuspected by the state agencies responsible for clean water programmes. Several times the method has been proven sufficient to identify the specific sources of pesticide pollution.

3.2 Regional and worldwide monitoring programmes

With regard to regional and worldwide cooperation, information in literature is only sporadic and Table II, in this case, should only be taken as giving some examples. With regard to joint marine pollution monitoring there are only a few programmes included but it is likely that more joint surveys for pollution investigations are in existence and the table, therefore, needs completion.

4. POLLUTANTS

The pollutants to be monitored have in fact already been selected by the present practice of baseline studies on national and regional levels. Four categories, however, seem to constitute the pollutants that appear to present clear and definable threats to the ocean system, and these groups, therefore, should be taken into account for worldwide monitoring on the basis of experience which may be gained with the pilot project envisaged within the framework of IGOSS; these categories as defined by an *ad hoc* Working Group at the 5th Session of the Joint Group of Experts on Scientific Advice on Marine Pollution (GESAMP) in June 1973 are: petroleum, halogenated hydrocarbons, heavy metals and transuranics.

Petroleum is at present estimated to enter the oceans at an amount of about 2 million tons annually, and with the predicted doubling of oil production over the next ten years this amount may still rise. Although recently some groups argue that harmful effects of oil and its products to living resources have been overestimated, it is a fact that catastrophic oil spills have been followed by mass mortalities of marine organisms and that fish eggs can be damaged with oil concentrations of about 0.1 ml/l (e.g., oil spill at Buzzards Bay, Massachusetts; experiments in U.S.S.R. with sturgeon eggs). Furthermore, the reduction of the amenity of many beaches of the world which are soiled with tar balls and oil slicks is quite obvious. The impact of oil on the marine environment has recently been studied by a working group (IMCO/FAO/Unesco/WMO/WHO/IAEA/UN, 1976).

Table II

Regional and Worldwide Monitoring Programmes

Name	Coord. Body or Countries particip.	Environmental monitoring: Salinity	Temp.	Oxygen	Nutrients	Waves	Stratific.	Currents	Prim. Products	Plankton	Radioact.	Oil	Pollutants monitored	Number of stations	Surveys	Fixed Station	Satell/Aircraft	Data Centre
A. Regional																		
Coop.Studies Kuroshio, CSK	IOC	x	x	x	x	x	x	x	x	x		)	Joint operational pollution programme	Dozens	x	x		x
Coop.Studies Caribbean, CICAR	IOC	x	x	x	x	x	x	x	x	x		)		"	x	x		x
Coop.Studies West Africa CINECA	ICES, IOC, FAO	x	x	x	x	x	x	x	x	x				"	x	x	x	x
Coop.Studies Mediterranean, CIM	IOC, FAO, ICSEM	x	x	x	x	x	x	x	x	x	x	x		"	x	x		x
Mediterranean Sea	IMCO											x		Unknown				-
Mediterranean Sea	OECD (6 countries)												Sewage Bacteria	"				-
Ligurian Sea	France, Monaco, Italy					Project "Ramoge"								Some	x	x	x	
North Adriatic Sea	Italy, Yugoslavia					Project "As-Eros"								"	x	x		
North Sea	ICES	x	x	x	x	x	x	x	x	x	x	x	Pesticides PCBs	Dozens	x	x		x
Baltic Sea	ICES	x	x	x	x	x	x	x	x	x	x	x	Metals Petroleum	"	x	x		x
Antarctic Research Progr., ARP	12 countries	x	x	x	x	x	x		x	x				"	x		x	
West and North Atlantic	Canada, Iceland					Bilateral cooperation								Unknown				
B. Worldwide																		
World Weather Watch, WWW	WMO		x			x								1 000s		x	x	x
Global Radiation Network	IAEA										x			150		x		x
Man and Biosphere, MAB	Unesco				x				x	x				100s	x	x		
Environmental health Monitor	WHO										x		Bacteria			x		x
Long-term and expanded progr. on ocean.research LEPOR	IOC-ICSPRO	x	x	x	x	x	x	x	x	x					x	x	x	x
Global invest. on pollution in the marine environm., GIPME	WMO/IOC-ICSPRO									x	x	x	Main categories of pollutants	IGOSS Network	x	x	x	x
Integr. Global Ocean Station System, IGOSS	IOC-ICSPRO	x	x	x	x	x	x	x	x	x	x	x	Petroleum hydrocarbons, Oil slicks, Tar balls	100s	x	x	x	x

Halogenated hydrocarbons belong to the most ubiquitous pollutants in the oceans, as the above example of monitoring pesticides in the U.S.A. has shown. Polychlorinated biphenyls (PCBs) have been proven to produce a chloracne type of disease upon ingestion by man; DDT and its metabolites, although not affecting human health according to our present knowledge, seem to interfere in hormonal production in higher organisms of the food chain and affect the photosynthetic process in some algae.

From metals, the damaging effects of mercury contained in fish products is well known. However, there is also an urgent need to monitor other elements which are likely to affect life processes, such as cadmium and lead.

Finally, transuranic elements and their compounds which originate from nuclear reactors and devices, are very toxic as regards their chemistry and radioactivity. As their production is growing and their environmental concentrations are expected to increase, programmes of analysis and monitoring in the marine environment should be initiated.

The main characteristics of these pollutants of worldwide importance, as well as those of interest for national monitoring programmes are given in Table III, which summarizes the effects of the various pollutants and discusses possibilities for monitoring them either at the source or in the field. The table further contains information about research which is still needed with regard to monitoring these pollutants and lists most suitable methods of measurement.

5. OUTLINE FOR A MONITORING PROGRAMME IN DEVELOPING COUNTRIES

General survey of possible sources. The first step should be a general survey aimed at finding out the main sources of pollution along the coastline. This sounds simple; however, it requires extensive spade work. At every coastal place industries have to be examined with regard to the composition of their effluents and solid wastes, and analyses have to be made in order to get quantitative estimations for each of the main pollutants. Figures for the biological oxygen demand for the waste coming from municipal and industrial sewage systems should be calculated for each of the big cities and the pollution load of the main rivers has also to be investigated taking into account seasonal variations of the runoff.

Resulting from these estimations a map of the coastline may be designed which can serve as a basis for further steps. This map will contain the main rivers (with figures for their runoff and pollution load in BOD), the various types of industries (indicating the main pollutants), cities and recreation places (giving the number of inhabitants, taking into account seasonal changes of these figures in touristic zones), and the number of treatment plants for domestic and industrial waste (indicating the degree of treatment).

Base-line studies in selected areas. Based on the above maps, areas can be defined in which either the total pollution load is extremely high or a specific pollutant is assumed to be predominant. Pollution investigations may concentrate on these areas first. Systematic analyses of sea water, sediments and/or organisms should be started in these selected zones for a period of at least one year. Guided by the results of such "base-line" studies plans can be made for continuous marine pollution monitoring and these activities may then be extended to the whole coastal zone.

Establishment of a suitable laboratory. Whilst the base-line studies as basic investigations preceding any monitoring activities can be organized with the assistance of university or industrial research institutes, or with the support from foreign laboratories, the real monitoring programme has to be done by a specialized national laboratory equipped with the necessary sophisticated instrumentation and staffed with well trained scientific and technical personnel.

The capacity of these laboratories should be high enough to deal both with all the analyses to be included in the monitoring programme and with basic research needed in this connexion, e.g., toxicity tests for chemicals released from industries, bioassays with suitable organisms.

Table III

Characteristics of Pollutants with a View to Monitoring

Source	Effects on	Monitoring	Methods of measurement	Research still needed
1. <u>Petroleum hydrocarbons</u>				
Ships' traffic, release of ballast water from tankers and tank washing, oil drilling activities, oil well seepage, oil loading in harbours and at platforms, municipal sewage system, oil refineries, etc.	Biological processes (e.g., primary productivity), living resources (especially in early life stages), fishing activity.	Visual observation and use of remote sensing from aircraft and/or satellites to detect polluters - analysing water samples (from surface and deep waters) tar balls, sediments and fish.	Gas chromatography, possibly in combination with mass spectrometry, IR- or UV-spectrophotometry; thin-layer chromatography - airborne and satellite observations.	With regard to doubts expressed recently about significance of detrimental effects to living resources experimental efforts should be strengthened. In particular, verification of assumed carcinogenic effects of petroleum hydrocarbons is still open.
2. Organo-chlorine substances (i.e., pesticides, polychlorinated biphenyls (PCBs) and others which have low water but high fat solubility and accumulate at each trophic level)				
Widespread through agricultural and industrial use.	Living resources and man eating contaminated fish and shellfish.	Not possible at source because the substances are spread through the atmosphere, careless handling of plastics, etc. - in the field they should be monitored by measuring concentrations accumulated in selected indicator species, especially fatty fish.	Gas chromatography, mass spectrometry.	Research on biological significance of concentrations accumulated in fish in order to be able to better interpret the results with regard to effects on man.

Table III (continued)

Source	Effects on	Monitoring	Methods of measurement	Research still needed
3. **Metals** Industrial effluents, dumping of waste, river input to the ocean, atmospheric transport.	Living resources and, at higher concentrations, effects to man eating contaminated fish and shellfish.	At source, surveillance of discharge released from factories aimed, if possible, at reducing the input of metals through suitable waste treatment and/or technical changes in the manufacturing process - in the field higher concentrations can be measured in sea water and sediments (combined with examination of marine organisms), however, chemical analyses of metals at low concentration level in selected indicator species are needed regularly because the build-up of higher concentrations in the tissues will take time.	Neutron activation analyses for multi-element analyses (Cd, Zn, Cu, Ag, Cr, Sb, Se, Hg, As). Atomic absorption spectrophotometry (Al, Cd, Zn, Cu, Hg, Bi). Mass spectrometry (Pb). Spectrophotometry (Fe, Sb, Ti, As). Gas chromatography (organic mercury compounds).	Extensive laboratory investigations to determine short - and long-term effects - as field experiments long-term studies of benthic organisms in areas of discharge are needed - research on accumulation in the food chain.
4. **Radioactivity** From nuclear reactors and devices	Man handling contaminated fishing gear - living resources.	At source, control of disposal of radioactive waste following the regulations of the critical pathway approach - in the field through automated surveillance at some few check-points.	Alpha and gamma spectrometry, gross beta counting, X-ray counting.	Keeping primary protection standards under continuous control.

Table III (continued)

Source	Effects on	Monitoring	Methods of measurement	Research still needed
5. <u>Nutrients</u> Domestic sewage.	Phytoplankton growth, the death of which may lead to extensive areas of de-oxygenation; bloom of certain dinoflagellates (red tides) by which fish are killed and shellfish become poisonous	At source, surveillance of treatment plants to ensure that derived working levels are kept - in the field monitoring of phosphate and nitrate content in sea water, if possible by automated methods. As climatological and biological variables are involved this method will, however, not allow the prediction of plankton blooms - monitoring phytoplankton (see also "Methods of measurement") - bioassay method, i.e., monitoring commercial species and low toxin concentrations in molluscs and bivalves at key stations.	Classical chemical methods - colorimetry - spectrophotometry - Neutron activation analysis - bioassay method, combined with gas liquid chromatography or mass spectrometry.	Investigations on temporal and spatial distribution of phytoplankton - laboratory and field bioassay experiments.
6. <u>Organic waste</u> Industrial effluents and sludge from food processing, paper and pulp mills, distilleries, etc.	Environment (oxygen deficiency). Low oxygen content, for its part, may influence the reaction of fish to toxins which possibly are present, at the same time.	Surveillance of discharge released from factories and control of the degree of treatment - in the field the dissolved oxygen content and the biological oxygen demand should be observed - surveillance of ecology of benthic fauna.	Classical chemical methods for measurement of oxygen and BOD - oxygen probe.	To establish the significance of dissolved oxygen levels on marine species at various life stages - to establish natural tidal and seasonable variations.

Table III (continued)

Source	Effects on	Monitoring	Methods of measurement	Research still needed
7. <u>Other waste from chemical industry</u>				
There is an ever growing number of chemical substances from industry which can be classified either as biodegradable substances or as stable substances, the latter being more dangerous.	Living resources and man eating contaminated fish or shellfish.	Wherever possible, substances well known to become accumulated by species should be controlled at the discharge outlets from factories - for biodegradable substances monitoring the dilution and quick degradation in the field may be sufficient. Stable substances, however, should be carefully monitored in the field with regard to their distribution in sea water, sediments and organisms.	Gas chromatography, mass spectrometry, spectrometry and other techniques which have to be determined for the various chemical substances.	Bioassays and toxicity tests for a high variety of substances. For biodegradable substances their time dependent toxicity has to be established.
8. <u>Physical waste</u> (such as solid waste, suspended matter, heat)				
Municipal and industrial outlets, dumping of waste, heated effluents from power stations, etc.	Biological processes, living resources.	Automated controlling of temperature and content of suspended matter at the points of discharge (e.g., outlet of pipelines) - examination of water masses and the sea bed in areas of dumping of sludge or dredging.	Optical instrumentation, such as scattering meter, fluorimeter. Temperature measurements.	Laboratory experiments aimed at establishing models concerning the dilution of waste under specified hydrographic conditions - relevant field work to prove models by post-discharge surveys.

Design of a network of stations. Some few reference stations and more numerous impact stations, as explained at the beginning of this lecture, have now to be chosen in order to set up the final monitoring programme. Impact stations will mainly be concentrated in areas found to be most influenced by domestic and industrial waste, and special attention will certainly be given to rivers and estuaries. A decision has to be made about the type of sampling stations (see Fig. 1). However, as buoys and unmanned platforms need highly sophisticated instrumentation and are too expensive and because for satellite observations specially trained personnel is needed, most of the sampling activities will, at least in the beginning, be done by small vessels which belong to the pollution laboratory or to which the laboratory has easy access.

Study of suitable sampling technique and frequency. Great attention has to be drawn to the suitable sampling technique in order to reach and maintain the highest standard of quality during collection and analyses of samples. It is often easy to contaminate samples by careless sampling because the contaminants to be assayed occur at very low level. Water samples for measurement of dissolved hydrocarbons, for instance, taken on board a ship can easily be contaminated by the ship's own activities. Metals, on the other hand, are sometimes added to water samples in measurable quantities through contact with metal surfaces in water samplers. Finally, compositional alterations of water samples have to be taken into account and require, therefore, the immediate measurement of these chemical parameters, and, if some pollutants (such as pesticides or some petroleum compounds) occur at a very low level only, which does not allow direct measurement, bioaccumulation in marine organisms has to be used for concentrations of these pollutants.

The sampling frequency is limited, on the one hand, by the laboratory and collection capabilities (upper limit) and on the other hand, by the need to obtain statistically significant data (lower limit). Between these two limits, the sampling frequency has to be chosen so that seasonal changes in the concentration of pollutants arising from changing biological and chemical activity can be defined. As a minimum, four sampling periods in the various seasons are required.

Education and training. Whilst in most countries sufficient scientific and technical personnel are available, in some countries more emphasis should be given to the education of chemists, biologists and oceanographers, as well as to laboratory technicians familiar with problems of marine pollution. These people should be enabled to reach a high standard in the field of pollution analyses and in the evaluation of the results through close cooperation with very experienced scientists in foreign laboratories or through training being organized at home as part of bilateral aid or with the assistance of the UN Specialized Agencies.

The training should cover both sampling and the use of standardized methods of analyses of the most important pollutants in the environment and, at least, the species of commercial value in the area.

Evaluation of the data. The final goal of national pollution monitoring programmes is the provision of suitable data both for scientific research and for the management of the living resources in fresh water and the coastal region. Therefore, the data have to be evaluated in relation to the environmental conditions, such as, temperature and salinity distribution, stratification of water masses, mixed layer depth, tidal streams and currents, i.e., that these additional parameters have to be included in the monitoring activities. They are required to estimate the rate of exchange between different water masses or ocean basins, to estimate the rate of input to the marine environment as a whole and to investigate how they are related to changes in the ecosystem which otherwise may be ascribed to pollution.

Exchange of data. One can envisage that numerous data of contaminants in the environment and aquatic organisms will be available as a follow-up of base-line surveys and monitoring activities.

From the beginning, pollution monitoring programmes should be combined with a national data centre so that outcoming data can readily be handled according to the rules of the "Manual on Oceanographic Data Exchange"(IOC, 1967). This means that data, after careful quality control, will be stored and are ready for retrieval at any time for researchers or desicion makers. This is of particular importance for developing countries in the process of industrialization, when establishing derived working levels for discharge water quality, that is the acceptable pollution level in the waste water which will depend on the already existing pollution load of the recipient water.

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